Northern Territory Government

Department of Business, Industry and Resource Development

TECHNICAL BULLETIN
NO. 305

THE POTENTIAL WEEDINESS OF TRANSGENIC COTTON IN NORTHERN AUSTRALIA

CSIRO PLANT INDUSTRY

Australian Cotton Cooperative Research Centre

Agdex 151/607 **$8.80 (GST included)** **ISBN 0 7245 4700 2**

EVALUATION OF THE POTENTIAL WEEDINESS OF TRANSGENIC COTTON IN NORTHERN AUSTRALIA

A REPORT PREPARED FOR CONSIDERATION BY THE OFFICE OF THE GENE TECHNOLOGY REGULATOR

NOVEMBER 2002

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ACKNOWLEDGEMENTS

The author wishes to thank Stephen Yeates, Greg Constable, Colin Martin, Gary Fitt, and Danny Llewellyn for their professional guidance and Warren Muller and Mark Hearnden for statistical advice. The technical support from Matt Bolam, Nick Hartley, Heather Emmerson, Greg Aldrich, Uwe Pufke, Erica Eastick, Tony Postle, Ryan Berlowitz, Graham Schultz and the farm staff at Katherine Research Station and Frank Wise Institute, Kununurra, is appreciated. The author also gratefully acknowledges the support for the project from the CSIRO, Department of Agriculture Western Australia, Monsanto, the Northern Territory Department of Business, Industry and Resource Development and the Australian Cotton Cooperative Research Centre.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
APHIS	Animal and Plant Health Inspection Service
blks	blocks
BOM	Bureau of Meteorology
Bt	Bacillus thuringiensis
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAS	Days After Sowing
DBIRD	Department of Business, Industry and Resource Development
DD12	Day Degrees (accumulated heat units for 12 degrees celsius)
DS	Dry Season
FWI	Frank Wise Institute, Kununurra
G0	Conventional Genotype 289
G1	Single gene (Cry1Ac) Sicot 289
G2	Two gene (Cry1Ac and Cry2Aa) Sicot 289
G2X	Two gene (Cry1Ac and Cry2Ab) Sicot 289
GBX	Two gene (Cry1Ac and Cry2Ab) DP50
GLM	Generalized Linear Model
GMAC	Genetic Manipulation Advisory Committee
H	High Population treatment (80 seeds)
Knx	Kununurra
KRS	Katherine Research Station
L	Low Population treatment (10 seeds)
LTA	Long Term Average
NRA	National Registration Authority
NSW	New South Wales
NT	Northern Territory
OB	Open Bolls
OGTR	Office of the Gene Technology Regulator
ORIA	Ord River Irrigation Area
popln	population
Qld	Queensland
S1	Black Seed
S2	Fuzzy Seed
S3	Seed Cotton
trts	treatments
USA	United States of America
USDA	United States Department of Agriculture
WA	Western Australia
WS	Wet Season

PROJECT BACKGROUND

There is a demand from environmental, scientific and regulatory bodies to assess the ecological consequences of the release of genetically modified crop plants. One proposed risk is that transgenic plants may possess an increased potential for weediness if modified with a fitness-enhancing trait, such as insect resistance, as conferred by the addition of an insecticidal gene like the cry genes derived from *Bacillus thuringiensis* (Bt). Genetically modified cotton (*Gossypium hirsutum*) which contained the Cry1Ac Bt gene, conferring resistance to lepidopteran pests like *Helicoverpa armigera*, was the first transgenic crop to be commercially released in Australia, with strict regulatory guidelines restricting production to NSW and southern Qld. There is potential to extend production to northern Australia, where such an industry would need to be based on Bt transgenic plants because of the higher insect pressure compared to the more temperate cotton production regions and the desire to minimise pesticide use. However, there are a number of environmental concerns associated with the potential development of commercial cotton production in northern Australia. Cotton can persist as a perennial plant in tropical areas, where the addition of a Bt gene could provide an ecological advantage compared to the more temperate parts of eastern Australia. Increased fitness due to insect resistance could enhance the ability for improved cultivars to become naturalised, and invasive of non-production habitats. The existence of volunteer Bt cotton populations could provide an avenue for gene dispersal through pollen to naturalised *G.hirsutum* which is naturally a tropical and sub-tropical species, and exists in small isolated populations in northern Australia. The introgression of the Bt gene to these populations could alter their ability to persist and increase.

A number of *Gossypium* species are native to northern Australia. There is concern that the Bt gene could introgress to native *Gossypium* species, and modify their ability to persist and increase. This issue has been discussed and dismissed elsewhere (see Brubaker 2002).

This project aimed to experimentally evaluate the weediness potential of Bt cotton in ways relevant to a scenario where volunteer populations of Bt cotton could establish in non-cropping environments. This was complemented by assessment of the potential for the Bt gene to provide additional fitness in existing naturalised cotton populations. This was necessary prior to any commercial release of Bt cotton in northern Australia.

Only minimal guidelines were available from regulatory bodies on what may constitute a suitable weediness study. However as a minimum, it was considered that the experiment must allow comparison between the transformed and non-transformed cotton plants in habitats ascertained to be at risk from potential invasion. Purrington and Bergelson (1995) note that the USDA APHIS required proof that any transgenic crop intended for widespread unregulated release was no more likely to generate a weed problem than its untransformed counterpart before that crop could be completely removed from regulatory scrutiny.

Weediness of *G. hirsutum:*

To address the issue of increased weediness of transgenic cotton compared to conventional cotton, the characteristics that define a plant as a weed must be quantified. If a weed is defined as a naturalised alien plant, then *G.hirsutum* is listed as a weed, in the form of isolated populations found in national parks (Cowie and Werner 1987), or on Aboriginal land (Smith 2001). A weed is often defined as a plant located where it should not be, with the implication that someone decides if a plant should not be there. From that view, a weed is a purely human construct, not a precisely defined biological category (Williamson et al. 1999), although methods exist for weed risk assessment based on a number of criteria. Integral components of predicting weediness are the taxonomic relationships and weediness of the taxon's relatives, and its history of weediness in the rest of the world (Bergelson et al. 1998; Panetta 1993; Pheloung 1995). There are about 50 species of *Gossypium* (Craven et al. 1994; Fryxell et al. 1992) out of which only one (*G. tomentosum*) is listed as a weed which is found in the USA (Holm et al. 1979). Petitions for non-regulated status of Bt cotton presented by the USDA/APHIS cite that overall the genus seems to be devoid of aggressive weedy tendencies throughout the USA (Payne 1995; Payne 1997).

A list of the world's worst weeds (Holm et al. 1979) has often been used as a primary source by Australian plant introduction assessors (Pheloung 1995). *G. hirsutum* is not listed as a weed in this reference, or as a weed of Australian production systems (Parsons and Cuthbertson 1992; Wood et al. 2000). A report commissioned by the Department of Agriculture Western Australia, concluded that *G. hirsutum* is not considered a weed (Randell, pers.comm, 1997; See Appendix 3), based on a weed

risk assessment as described by Pheloung (1995). This considers factors associated with weediness such as domestication, weediness elsewhere, undesirable traits (e.g. spines, fire hazard, smothering growth habit, toxicity), plant type, reproduction, dispersal mechanisms and persistence attributes. Keeler (1989) considered the weedy characteristics of 20 of the worlds' worst weeds, and of 20 crop plants, to critically evaluate the likelihood of the evolution of plants with weedy phenotypes from genetically engineered crop plants. *G. hirsutum* was conspicuous by its omission considering it was one of the major crops targeted for genetic engineering. Keeler et al. (1996) subsequently discussed cotton in considering weediness of 60 crop plants, concluding that it was not weedy.

Based on its biological properties, there is thus no evidence that *G. hirsutum* is considered a weed in any areas in which it is currently grown. However, there is little predictive power in separating weedy and non-weedy members of closely related plants and the use of taxonomic relationships alone is not sufficient for quantifying the risk of weediness between a transformed and non-transformed crop (Brown 1999).

This study was conducted to determine whether the addition of a single genetically modified trait, i.e.insect resistance, could provide the additional fitness necessary to modify cotton from a plant not considered to exhibit weedy characteristics, to one that does. A methodology for comparative assessment of weediness between conventional and transgenic cotton was required.

Virtue et al. (2001) considered five issues to assess the significance of weeds: (1) current and potential distribution, (2) value of different land uses, (3) invasiveness, (4) impacts and (5) feasibility of successful control. The characteristic most likely to be different between transgenic and conventional cotton is invasiveness, which is defined as the ability to establish, reproduce and disperse within an ecosystem (Virtue et al. 2001). Four invasiveness criteria can be considered: (1) population growth rate; (2) means of dispersal: natural or human; (3) dispersal distance; and (4) susceptibility of land use to invasion (Auld and Coote 1980). We reasoned that means of dispersal and dispersal distance will be no different between transgenic and conventional cotton genotypes, although dispersal attributes would still need to be considered in the evaluation of ability to spread, and any management strategies that may be required. Consequently, population growth rate and susceptibility of land use to invasion were considered to be the two important factors in determining invasiveness of cotton in northern Australia.

Population growth rate (λ) quantifies the changes in the numbers of individuals in a population through time, (De Kroon et al. 2000). It is an appropriate measure of the risk of invasiveness, as it incorporates all of the more detailed information from varying stages in the life cycle of the plant, and provides an absolute value which can be used for comparison between genetic lines (Parker and Kareiva 1996). Identification of the key demographic parameters that contribute to population change, and how these are affected by different environmental conditions, allows a more complete assessment of weediness risk and may indicate how growth stages may be manipulated or managed to minimise the risk of weediness. Key demographic parameters were identified as germination, survivorship after the first dry season, final survivorship, and fecundity, and were components utilised to calculate invasiveness (see Methodology for more detail). Values of $\lambda>1$ (the invasion criterion) predict that the plant population will increase under the given set of environmental conditions; values <1 predict that the population will decline to extinction (Crawley et al. 1993). *"…in the hypothetical case of transgenic plants shown to have a slightly higher rate of increase than the corresponding parental strain. If both parental and transgenic lines exhibit rates of increase far below 1, it may be reasonable to dismiss the risks of the transgenic line regardless of its increased fitness over the parental line".* (Parker and Kareiva 1996).

The second important criterion from Auld and Coote (1980) is "susceptibility of land use to invasions". This is presented here as "susceptibility of habitats to invasion". There are three forms, or seedtypes, in which cottonseed can disperse; black or planting seed (ginned and acid delinted); fuzzy seed (ginned); and seed cotton (unprocessed). The susceptibility of different habitats to cotton seed dispersal would vary between seedtype, as some habitats would not be exposed to certain seedtypes in the course of normal cotton production and processing. (This is further discussed in the Methodology Section under choice of habitats). The experimental introduction of different seedtypes into different habitats allowed assessment of the potential of Bt cotton to establish and reproduce in different environments, both man-modified and natural.

Although there is extensive literature on the potential risks of transgene spread and introgression, the ecological effects of the transgenes themselves have not been widely addressed (Bullock 1999;

Parker and Kareiva 1996). There is less literature on the spread of the transgenic plant into natural habitats (Eastick et al. in press). Very few studies present experimental data relevant to the risk of transgenic crops escaping from cultivation and invading natural vegetation or non-agricultural production areas (Kareiva 1993). This was the prime focus of the ecological assessment presented here. The study was based on field experimental data and observations of plants in the environments which may be exposed to the introduction of the Bt cotton through the spread of transgenic cottonseed.

Linder and Schmitt (1995) note, "*One approach to studying the potential for persistence of a transgene is large-scale demographic experiments, which examine the entire life history of the modified plants over several years*". Another approach uses smaller targeted experiments on specific life history phases. A combination of both large-scale, multi-site demographic experiments and smaller targeted experiments were used to assess the potential invasiveness of Bt cotton. Four experiments were completed, and two qualitative assessments were made of situations that could contribute to the overall understanding of volunteer cotton demographics, both transgenic and conventional cotton, and the potential for such plants to develop to weedy populations. These experiments and assessments were:

1. A large-scale ecological assessment over 20 sites in the NT and WA. This was divided into Experiment 1A; first year sowing over 13 sites, and Experiment 1B; second year sowing over seven selected sites from the previous year.

2. Differences in germination of the three seed types and three genotypes of cottonseed when buried or left remaining on the soil surface.

3. Change in viability of cottonseed when exposed to a native bush habitat over the duration of a dry season.

4. Genotype by nutrition interaction and insect enclosure.

5. Monitoring of existing naturalised populations of *G. hirsutum.*

6. Monitoring of volunteer plants.

These studies will be presented in turn and conclusions concerning weediness of Bt cotton will be discussed primarily in the context of the large-scale ecological study, but will also include implications from the smaller-scale targeted experiments.

EXECUTIVE SUMMARY

INTRODUCTION

As part of the risk assessment process, there was a need to assess the ecological consequences of the release of genetically modified crop plants. It had been speculated that transgenic plants may have increased weediness potential if modified with a possible fitness-enhancing trait. Such a trait could be the insect tolerance provided by the addition of genes derived from *Bacillus thuringiensis* (Bt). Cotton (*Gossypium hirsutum*) genetically modified to contain the Cry1Ac gene from Bt, was the first transgenic crop to be commercially released in Australia, in 1996, with regulatory guidelines restricting production to NSW and southern Qld.

There was potential to extend this production to northern Australia. However, there was insufficient information to determine whether the addition of the Bt gene could provide an ecological advantage in tropical areas, which may increase the risk of weediness over and above that in the more temperate parts of Australia. *G. hirsutum* is naturally a tropical and sub-tropical species, and small naturalised populations exist in northern Australia. In addition a number of other *Gossypium* species are native to northern Australia. There was concern that outcrossing from cultivated *G. hirsutum* could allow introgression of the Bt gene into existing naturalised cotton populations or into native *Gossypium* species and alter their ability to persist and increase. Additional concern was the potential for the Bt gene to enhance the ability for improved cultivars to become naturalised. Potential outcrossing with native *Gossypium* species has been conclusively ruled out due to genetic incompatibility of cultivated and native *Gossypium* species, hence the primary route for dissemination of the Bt gene is transgenic cotton seed. This project was started in June 1999 and field work was completed in December 2001 to assess the potential for seed dissemination and the risk that Bt cotton plants could establish, survive and reproduce better than conventional cotton plants outside cultivated agricultural environments in northern Australia.

METHODOLOGY

How to assess weediness: There was no widely accepted framework of experimental protocols for ecological risk assessment of transgenic crops, and specifically, for evaluation of potential weediness. There was also little literature considering the escape of the transgenic plant into natural habitats and subsequent ecological consequences. Protocols for Weed Risk Assessment are utilised by regulatory bodies evaluating plant introductions into Australia, based on biogeography, historical, biological and ecological parameters. *G.hirsutum* was not considered a weed according to these protocols. However, it was listed as a weed in some literature by the definition that a plant can be considered a weed if found where it should not be, which is a subjective human assessment and not a true biological category. Methodology was required to define biological parameters that could be evaluated as indicators of weediness between transgenic and conventional cotton. A key component of weediness is invasiveness, of which there are four contributing factors. These are means of dispersal, dispersal distance, population growth rate, and susceptibility of land use to invasion. These four contributing factors were included in developing the methodology to assess weediness between cotton genotypes.

Dispersal of the Bt gene could occur in three ways: vegetatively, by pollen, or by seed. Seed was considered the primary route by which the Bt gene could disperse from an original site. Potential or observed dispersal of Bt seed into the environment was documented. Population growth rate (λ) as an indicator of invasiveness is a function of the growth, development and reproduction of the cotton plant throughout its life cycle. We compared critical stages in the demography of Bt cotton with its non-transformed conventional counterpart in selected environments to evaluate parameters contributing to population change. There was a difference in susceptibility of habitats to unintentional dispersal of cottonseed, so invasiveness was evaluated in four non-cotton production habitat categories where cottonseed could potentially disperse.

The major component of the project was a large-scale ecological experiment conducted at a number of sites over two years. Supplementary experiments and monitoring were also included to provide information on critical aspects of volunteer cotton establishment.

Experimental design: The ecological experiments evaluated whether Bt cotton may possess increased fitness in non-crop environments, compared to conventional cotton. Experiments were located near Katherine, NT, Kununurra and Broome, WA. Within these locations, a series of sites were established in a number of habitats where seed could conceivably spread, or disperse. These were categorised as

native bushland, roadside, cattle area, and waterway. Cultivated fields wre not included since control of cotton volunteers in production areas is successfully managed through cultivation and herbicide application. Cottonseed was directly sown in these habitats within three locations in the wet season of 1999-2000, with an additional waterway site sown in Kununurra in the 2000 dry season, resulting in a total of 13 sites. This constituted Experiment 1A and was approved through GMAC as PR89X(2).

Demographic parameters: Methodology was developed to assess demographic parameters based on quantifiable biological properties as indicators of potential weediness of transgenic cotton compared to non-transformed cotton. These parameters were germination, survivorship (based on seasonal changes between the wet and dry seasons), and fecundity, which incorporated seed production and seedling recruitment. Seeds were sown at the commencement of the wet season (November – January) to simulate when a germination event would naturally occur from spillages of whole seed cotton during harvest or transport to gins or transport of fuzzy seed. The planting method employed for our experiments was designed to maximise the probability of germination, by hand-sowing seeds into disturbed ground, covering with soil, and hand-watering. The experiments thus represented the best-case scenario for successful germination as in most cases of physical dispersal the seed would remain on the soil surface. Plants established through the remainder of the wet season and developed to fruiting over the following dry season. Survivorship was assessed at the commencement of the following wet season to evaluate plant mortality and new seedling establishment. Fruit production was recorded throughout the year to determine when maximum seed production occurred and the number of seed available for germination at the commencement of the wet season.

Invasiveness was quantified as the rate of increase of a population (λ), and provided an absolute value that could be compared between genotypes. This was calculated as the proportion of plants surviving after a given time, plus the addition of any new seedlings, compared to the number of plants present initially. This incorporated all of the demographic information gathered throughout the life cycle of the plants, and considered parent plant mortality and new seedling recruitment. A value of λ greater than one indicated that the population was increasing, and would be considered to have the potential to become a weed.

A second series of experiments (Experiment 1B) were established in the 2000-2001 wet season, in which treatments at seven sites selected from those sown the previous year were modified, or repeated. This was to allow inferences about seasonal differences, or to superimpose additional treatments, such as nutrition, on existing factors and to allow the addition of the Cry2Ab gene that had superseded the Cry2Aa gene originally used (approved as an amendment to PR-131X(2)). Experimental design was consistent with that used for Experiment 1A.

Targeted experiments: These experiments were of a smaller scale and examined:

1. Differences in germination of the three seed types (black, fuzzy and seed cotton) and three genotypes (conventional, single gene and double gene) of cottonseed when buried or left remaining on the soil surface (referred to as Experiment 2).

2. Change in viability of cottonseed when exposed to a native bush habitat over the duration of a dry season (referred to as Experiment 3).

3. Genotype by nutrition interaction and insect exclosure study (referred to as Experiment 4).

The results from Experiments 1A, 1B, 2, 3 and 4 allowed for quantitative assessment of the potential weediness of Bt cotton compared to conventional cotton in northern Australia.

There were also opportunities for qualitative assessment of situations that could feasibly contribute to the understanding of volunteer cotton demographics, and the potential for such volunteers to develop as weedy populations. These situations were:

1. Monitoring of existing naturalised populations of *G. hirsutum.* Gene introgression into naturalised populations of *G. hirsutum* is considered possible, so the location of known existing populations was documented and selected populations were monitored.

2. Monitoring of volunteer plants. Recently established volunteer cotton plants were observed in a number of habitats near previous cotton trial sites. Specific individuals were identified and monitored.

Results from all experiments were integrated to provide an assessment of potential weediness of transgenic cotton compared to conventional cotton in northern Australia.

RESULTS AND DISCUSSION

Dispersal: Dispersal of seed from production areas is a physical process so differences between cotton genotypes were not expected. Observations of existing volunteer cotton plants in northern Australia suggest that site and seedtype are the main factors effecting dispersal. Sites observed to be most susceptible were waterways in Kununurra where furrow irrigation was used, and roadsides at all locations. It was observed that seed cotton had the greatest potential for unintentional dispersal, particularly to roadsides during transport, and to drains when seed cotton remained in paddocks after harvest in Kununurra. The potential for dispersal of seed cotton into waterways in production areas where sub-surface irrigation is used (Katherine and Broome) was negligible. Seed remaining in the field at these locations after harvest either rotted, or germinated and was subsequently killed with herbicide or cultivation. Fuzzy seed (ginned) had the greatest potential for intentional spread as cattle feed introduced into non-cropping habitats, but had a low risk of unintentional escape as feed lots were physically restricted, although viable cottonseed can pass through the digestive tract of cattle. Black seed (bagged or planting seed) had the lowest risk of unintended escape.

EXPERIMENT 1A

Germination: Between sites: There was a highly significant difference in germination between sites. The greatest germination was observed in disturbed habitats, Kununurra Cattle No.1, Katherine Cattle No.1 and Creek sites, and Broome dam site. Seeds in the bush sites had relatively low germination rates.

Within sites: Seed type was the dominant factor influencing germination within all sites. Generally, black seed had the highest germination, followed by fuzzy seed, then by seed cotton. This had important implications for seed escape and mitigation strategies since seed cotton had the greatest opportunity for unintentional dispersal but had the lowest chance of germinating. Fuzzy seed had relatively high germination potential. The main use for this form of seed in northern Australia would be as whole seed fed to cattle. Black seed had the least risk of unintentional escape.

Population density, or its interaction with seed type, also affected germination. Generally low population density had a lower germination proportion compared to the higher density.

Microhabitat (niches within each habitat), measured as significant between block effects, also influenced germination percentage at some sites, such as drain and roadside habitats where there were noticeable gradient effects.

There was no significant effect of genotype within 11 of the initial 13 sites. There was a seedtype by genotype interaction at the Kununurra Drain No.1 Site; with the conventional fuzzy seed lower than the respective transgenic treatments. There was a significant effect of genotype at the Kununurra DS Drain, with the conventional genotype lower than the two transgenic treatments.

Seed and dispersal ecology are recognised as major determinants of weed fitness and population growth rate, but the addition of the Bt genes did not enhance dispersal or germination ability of cottonseed in the majority of experimental sites aimed to simulate the escape of cottonseed from production areas.

Survivorship: Between sites: There was a significant effect of site on survivorship (number of plants remaining as a proportion of number of seeds sown) after one year.

Within sites: Only four of the 13 sites had greater than 50% of plots with any surviving plants, corresponding to the Katherine Cattle No.1 and Katherine Creek sites, the Kununurra DS Drain site and the Broome Cattle No.1 site. Eight of the sites had only isolated (<10% of plots with any surviving plants) or no plants surviving after one year.

An ANOVA conducted on these four sites revealed that the interaction between genotype and either seedtype or population was significant, where the two-gene treatment had greater survivorship than the conventional genotype for fuzzy seed only for the Broome Cattle No.1 and Kununurra DS Drain sites after one year. Results were similar for survivorship after two years, indicating that initial survivorship was correlated with subsequent survivorship.

The number of originally sown plants surviving declined over the two years for all sites. Only the Kununurra DS Drain site and Broome Cattle No.1 site had greater than 50% of the plots remaining with any surviving plants, and actual plant numbers were low. The two-year duration for this project did not provide long-term population trends, but indicated that few sites could successfully support cotton plant survival.

There were no consistent results to confirm or deny that cotton with Bt genes had greater survivorship than the conventional cotton. Environmental influences such as nutrition, water availability or excess, interspecific plant competition, herbivory by non-lepidopteran species (e.g. grasshoppers), cattle trampling, and fire all contributed to cotton plant mortality, although the effect of each was not quantified.

Fecundity: Between sites: There was a significant effect of site on fecundity (maximum number of open bolls produced). Fruit production was seasonal, with numbers of open bolls increasing over the progression of the dry season. Over the wet season, plants primarily produced vegetative biomass and squares (flower buds), and seed cotton was dislodged from the plant, leading to seedling recruitment. Plants in most sites displayed slow physiological development. Over the entire duration of the project, plants in eight of the 13 sites did not produce any fruiting structures at all and two sites produced only isolated open bolls (<15 total for the site), all of which had seeds of low vigour. The remaining three sites - Broome Cattle No.1, and the Kununurra WS and DS Drain sites - produced relatively large numbers of open bolls (>150 total for the site). These three were the only sites from which seedling recruitment occurred from the originally sown parent plants.

Within sites: The Broome Cattle No.1 and the Kununurra DS Drain sites were the only ones for which robust data analysis could be conducted. Population was the dominant factor affecting maximum number of open bolls per plot, with the higher density treatment producing greater number of bolls. There was a trend for an increase in boll production with genotype for fuzzy seed only at the Broome Cattle No.1 site, a similar result to that for survivorship, indicating a possible causal relationship. Within the Kununurra DS Drain site, the single-gene genotype produced significantly greater number of bolls than the conventional genotype.

To study open boll production more precisely, removing the factors of seedtype and population could reduce the confounding effects of germination and early survival. This would enable evaluation of fecundity given that a plant has established, excluding other factors.

Invasiveness: Invasiveness, as a determinant of weediness, was calculated as rate of population growth, λ, for each genotype, and was determined for each year (λ_1 and λ_2). The extremely low numbers of surviving plants at the majority of sites required that invasiveness be assessed using a simplistic method (total plants for each genotype across all other factors). All values for λ_1 were less than one, attributable to the high seedling mortality immediately after germination for most sites. Values for λ_2 increased compared to λ_1 for the majority of sites, due to populations approaching an establishment threshold. However, all values, with the exception of the Kununurra WS Drain No.1 site, where significant differences between genoytpes could not be ascertained, remained less than one, indicating that populations were declining and that transgenic cotton was not becoming a weed of these habitats. Values greater than 0.8 were attained for the Kununurra DS drain site and the Broome Cattle No.1 site when compared with values for all other sites as calculated by the simplistic method.

An ANOVA for invasiveness was conducted only on the Kununurra DS Drain, the Katherine Cattle No.1 and the Broome Cattle No.1 sites due to high plant mortality at all other sites. There was no result to indicate that the transgenic genotype led to an increase in the value of invasiveness. There was a significant effect of genotype on λ_1 at the Katherine Cattle No.1 site and on λ_2 at the Kununurra DS Drain site, both for which the mean values for the two-gene was less than the other two genotypes.

The timing of calculation of invasiveness had considerable implications on the resultant values. This project presented values for a two-year period, and no attempt at predicting long-term cotton weed dynamics using population models was made. Results after this time indicated that the addition of the Bt gene did not increase cotton invasiveness. Further verification of these results could be gained from continued monitoring of the experimental sites where volunteer cotton plants have established, namely the Broome Cattle No.1 site and the Kununurra DS Drain site. Fecundity does not necessarily transpose to increased invasiveness due to factors such as competition, space for seedling

recruitment and herbivory by non-Lepidopteran species, consistent with conclusions from a number of similar studies.

***EXPERIMENT 1B*:**

Germination: Direct comparison between the seven sites was not possible for this experiment due to differences in experimental structure, although experimental factors were generally similar to those applied in Experiment 1A. There were significant genotype effects, or interactions with either seed type or population, on germination, but these varied between sites. These differences in viability were possibly due to differences in seed quality as a result of storage or differing plant or seed treatment practices at the source of the seed, as seed was obtained from different paddocks at different times depending on the desired genotype. This differed from Experiment 1A.

There were no significant factors on germination for the Broome Cattle No.2 (no population treatment) and Kununurra Bush No.2 sites (no seed cotton sown).

Trends for the effects of population and seedtype were consistent with results from Experiment 1A.

Survivorship: After the one year, only three of the seven sites had greater than 50% of plots with any surviving plants, and actual plant numbers were low. These were the Kununurra WS Drain No.2 and Cattle No.2 sites and the Broome Cattle No.2 site (after thinning for the cattle sites). There was a significant seedtype by population interaction on survivorship for the Kununurra WS Drain No.2, but no significant factors for the other two sites (attributed to hand-thinning reducing the seedtype effects).

Fecundity: Plants in most sites displayed slow physiological development, as for Experiment 1A. The only sites in which plants developed to produce bolls within the one year were the Broome Cattle Site No.2 and the Kununurra WS Drain No.2 site. For the remaining five sites, three did not produce any fruiting structures at all, whilst the other two produced isolated and immature fruit. There were no significant factors for maximum number of bolls produced for either the Broome Cattle No.2 site or Kununurra WS Drain No.2 site, indicating that the addition of the Bt gene does not provide additional fecundity once the confounding effects influencing plant survivorship are reduced.

Invasiveness: Invasiveness was calculated for consistency with Experiment 1A, but sites persisted for less than one year before completion of the project, so no seedling recruitment could occur. There was no significant effect of genotype for the two sites for which an ANOVA was conducted, and calculation of λ_1 by either this method or the simplistic method resulted in no value greater than 1 for any genotype.

EXPERIMENT 2

Buried seed of all seed types had significantly higher germination than seed remaining on the soil surface for the specific depth of planting versus seed type and genotype experiment. Seed to soil contact was important for optimum germination, which had implications for unintentional seed escape, where probability of germination would be decreased for seed settling on top of soil or vegetation. This also had implications for the large-scale ecological assessment in that all germination values from those series of experiments were likely to be greater than in a realistic situation where seed would only be covered by soil in a chance event causing soil disturbance.

EXPERIMENT 3

There was no effect of seedtype or genotype on survival of seed when exposed on the soil surface of a native bush habitat over the duration of a dry season in Kununurra. There were no viable seeds remaining at the onset of the wet season, indicating that cottonseed dispersed with a considerable time lag until a germination event occurred may be rendered unviable and not eventually germinate.

EXPERIMENT 4

The two-gene genotype produced a greater number of open bolls, and resultant number of seeds, at each of the three nutrition levels, than the conventional genotype, under commercial cotton production conditions when insect pressure was not controlled. This indicated that there may be increased fecundity for transgenic genotypes when nutrition is not limiting, as in habitats such as cattle yards. Nutrition may play a key role in survivorship, which subsequently influences fecundity, and also in expression of the Bt gene. However, as found for the large-scale ecological study, this may not necessarily be transposed to an increase in invasiveness, as other factors such as weed competition,

removed under the commercial conditions imposed on this experiment, may influence population growth. The interaction between nutrition and attractiveness for insect herbivory was not quantified.

Monitoring of Naturalised and Volunteer Populations of G.hirsutum

Naturalised populations of non-transgenic cotton in northern Australia monitored during this study were maintaining a self-perpetuating threshold. In some cases, these populations are derived from introductions of over 100 years ago. Populations generally consisted of isolated clumps of cotton plants within areas of less than 1 hectare, and were primarily in habitats geographically isolated from proposed cotton production areas, so risk of transgene introgression would be extremely low. There appeared to be numerous factors such as water availability, protection from fire, interspecific plant competition, particularly in the wet season when space limits seedling recruitment, and herbivory from non-lepidopteran species, which restricted population growth. This would suggest that addition of the Bt gene would give little advantage to the invasiveness of naturalised cotton populations.

Observations from volunteer plants were consistent with those from the experimental plots. That is, plants observed to reach reproductive potential were those in man-influenced habitats, predominantly roadside and verges adjacent to previous cotton fields, cattle yards and drains. No volunteers were found in undisturbed bush habitats. Fruit production was distinctly seasonal, with seed commencing to mature after the conclusion of the wet season (April-May). This had implications for seed survival during the dry season until a subsequent germination event in the following wet season.

CONCLUSIONS

On the basis of quantitative experiments and observations of naturalised populations, the following conclusions can be drawn:

- Dispersal: Seed cotton and black seed have a very low risk of contaminating natural habitats. Fuzzy seed provides the greatest risk through intentional feeding to cattle.

- Germination: Site and seedtype produced the greatest effects on germination. Generally,

    - Waterway and cattle sites had the greatest germination.

    - Black seed followed by fuzzy seed had the greatest germination, with seed cotton the lowest.

    - Genotype had no effect in the majority of sites.

- Survivorship: Site had the greatest effect. Very few sites had surviving plants after one and two years.

- Fecundity: Site had the greatest effect.

    - Only five sites displayed significant boll set and seed production. These were disturbed sites and were characterised by increased water availability and/or nutrition. It is feasible to consider workable management strategies to minimise risk.

    - At all other sites, there was essentially no successful seed set.

- Invasiveness: No site and no genotype had a value of λ greater than 1, with the exception of the Kununurra WS drain No.1 site, where only one plot of each genotype had remaining plants, so significant differences between genotypes could not be ascertained. The most productive sites, the Broome Cattle No.1 and Kununurra DS drain, still had negative population growth.

- At no site was there conclusive evidence over the duration of this project to support the hypothesis that the addition of Bt genes had enhanced the invasiveness of transgenic cotton.

SECTION 1

EXPERIMENT 1A: LARGE-SCALE ECOLOGICAL ASSESSMENT

Introduction

The major component of the assessment of an increase in weediness potential of Bt cotton was to examine the ability for improved Bt cultivars to become naturalised in non-agricultural production habitats, as compared to conventional cotton. Experiment 1 was designed specifically to assess differences in demographic parameters over a range of habitats.

This was considered a large-scale ecological study, consistent with an approach discussed by Linder and Schmitt (1995), and presented by Crawley et al. (1993) and Crawley et al. (2001). The project aimed to evaluate population change over two years of field experiments to provide a value for invasiveness as an indicator of relative weediness of cotton genotypes.

Methodology

This section presents the experimental methods used in Experiment 1A; large-scale ecological assessment over 13 sites over two years, to evaluate the potential weediness of Bt cotton in north-west Australia, and presents the factors experimentally manipulated, and the parameters measured as indicators of weediness. Greater detail of the rationale behind the development of the methodology and the protocols used are included in the discussion section.

DISPERSAL OF THE TRANSGENE INTO THE ENVIRONMENT

Dispersal was defined as the spread of the Bt gene from its intended site of release. This gene flow can occur through the active or passive dispersal of genes via seed, pollen or vegetative parts of a plant (Saeglitz and Bratsch 2002). Unregulated or unintentional spread of the Bt gene from cotton can occur in several ways: via pollen transfer to related species or to naturalised populations of the crop plant; or directly via the spread of seed through volunteers in subsequent crops, dissemination during transport, or through vegetative persistence after the completion of a field trial or commercial planting.

Cotton does not commonly propagate from vegetative material (Serdy and Berberich 1995) so this is an unlikely route of dispersal away from paddocks in which it is grown, although plants may reshoot (ratoon) and perenniate from stub cotton remaining in the paddock after harvest. These plants can be controlled by husbandry practices (herbicide application, cultivation) performed during paddock preparation for the subsequent crop.

Escape of genes by pollination of native cotton species (e.g. *G.australe*) is considered improbable (Brown et al. 1994; Brown et al. 1997; Brubaker et al. 1999), although the potential exists for pollen transfer to naturalised populations of cotton (*G.hirsutum*) that occur in isolated locations throughout northern Australia. Pollen transfer between commercial transgenic cotton crops and these naturalised cottons is unlikely because of geographical isolation between proposed cropping areas to the majority of naturalised populations. However, this has received some attention in terms of mapping the location of known plants and monitoring demographics of selected populations (refer Section 6).

Consequently, the major avenue of potential Bt gene escape from commercial cotton production systems in northern Australia was considered to be as seed, of which considerable quantities are likely to be disseminated during planting, harvesting, processing and end-product disposal should a commercial industry be established there. This ecological weediness assessment was therefore designed to reflect this, and focused primarily on developing methodology to examine the potential weediness of the transgenic cotton plant compared to conventional cotton introduced into different natural and man-modified habitats as seed.

The different prospective methods of seed dispersal needed to consider the possible sources of the dispersed seed, the route by which it might escape from commercial production, and the associated habitats into which it was most likely to escape and potentially establish as volunteer or weedy plants. All of these issues were addressed in determining the experimental factors to be manipulated, as outlined below.

DEFINITION OF EXPERIMENTAL VARIABLES

Choice of Locations

Potential cotton production areas in northern Australia include Katherine, NT, and the Ord River Irrigation Area (ORIA), Kununurra, and the west Kimberley, near Broome, WA (Refer Figure 1.1). Various research activities into cotton production were already being undertaken in these areas, so the infrastructure was available to assist with the conduct of these weediness studies in what is a relatively remote part of Australia.

Consequently, the sites selected for study were situated at; (1) the Katherine Research Station, Katherine, NT; (2) the ORIA, Kununurra, WA; and (3) Shamrock Station, approximately 150km south of Broome, WA.

Choice of Habitats

Four different categories of habitats into which cotton could disperse were identified, either through observed actual cotton volunteers, or perceived to be at risk of introduction through man's cotton-related activities. These were habitats other than cultivated areas, as control of cotton volunteers in cultivated paddocks is sufficiently achieved through the routine farm husbandry practices of cultivation or herbicide application.

The habitats identified were:

- Roadsides – primarily at risk from seed cotton distributed during module carting.
- Waterways (artificial e.g. drain, or natural e.g. creek)– again at risk predominantly from seed cotton dispersed in runoff from paddocks after harvest.
- Native bush – at risk from seed cotton drifting from road or paddock habitats.
- Cattle habitats – predominantly from fuzzy seed dispersed through feeding to animals in relatively intensive areas.

An experimental site was established in each of these habitats at each of the three geographical locations described above. An additional site was sown in the dry season (June 2000) at the Kununurra Drain habitat. This resulted in 13 sites with distinct climatic, soil, fire and grazing characteristics, as described in detail in Section C: 'Site Descriptions'.

Selected sites were sown to a repeat sowing in the second season (2000-2001), with plots generally in close proximity to those sown in the previous year, so site characteristics remained similar. These sites are considered in Experiment 1B.

Illustrations of selected established volunteers recorded in these habitats are illustrated in Photos 1.1a-c. Note that no volunteer transgenic cotton was found in native bush further than approximately 10 m from a road verge near any of the cotton research sites.

WITHIN SITE FACTORS

Seedtype

Cotton seed can be dispersed in three forms; 1) Black or planting seed that has been acid delinted and generally treated with fungicides and/or pesticides; 2) Fuzzy, or ginned seed (majority of the lint removed); and 3) Seed cotton (unprocessed seed with a dense covering of cotton fibres). These are illustrated in Photos 1.2 a-c. There were differing probabilities of each seedtype escaping into particular habitats (as discussed above). All seedtypes were included in all habitats for a greater number of comparisons and increased confidence in the overall results.

Population Density

Population density was included as a factor in this experiment, at two levels, classified as High and Low. These levels were determined by considering average seed numbers per boll of commercial cotton. A boll was estimated to have five locules, each with about eight seeds, resulting in approximately 40 seeds per boll. Seedcotton escape was considered likely to occur as a clump either from an individual locule (which tends to clump together), or as an entire boll. Germination of volunteer seedlings was generally observed as individual plants, or small clumps of seedlings within a boll. This

rationale was used in the initial GMAC submission, which proposed to use two seeds and 10 seeds as the Low and High population levels respectively. However, a preliminary planting experiment resulted in extremely low seedling numbers at these levels, so the population levels were increased to one locule (equivalent to about 10 seeds) as the Low population level, and 2 bolls (equivalent to about 80 seeds) as the High population level. These resulted in reasonable numbers of seeds germinating in all environments.

Genotype

The commercial transgenic cotton available at the commencement of this project, was INGARD®, containing the Cry1Ac gene from *Bacillus thuringiensis*. It was envisaged that a two-gene Bt cotton would ultimately form the basis of any proposed northern cotton industry because of the high insect pressures in this region and a history of industry failure due to the inability to control insects (see Yeates 2001). This two-gene Bt cotton was initially to be the combination of the Cry1Ac and Cry2Aa genes, and the original submission to GMAC was for assessment of these genes (approved as PR89X(2)). Data from laboratory, glasshouse and field trials in eastern Australia indicated that the two gene material offered greater Lepidopteran pest control than the single gene alone and might therefore be expected to confer a greater degree of fitness in non-cropping habitats (Williams et al. 2001). Three levels of genotype were therefore evaluated in the first year sowings (1999-2000), each in a common varietal background; (1) G0 (Conventional or non-transgenic); (2) G1 (Cry1Ac); and (3) G2 (Cry1Ac and Cry2Aa). All genotypes were derived from the non-transformed parent variety, Sicot 289, and seed for sowing was handpicked from the same paddock growing at Frank Wise Institute, Kununurra (PR89X). A proportion of the seed was ginned on a small laboratory gin to produce fuzzy seed, and some of that was subsequently delinted in sulphuric acid to produce black seed. Laboratory tests for germination were conducted on the black seed to ensure the delinting process had not damaged viability of the seed.

Planting Time

Cottonseed was experimentally sown to coincide with the traditional onset of rains at the commencement of the wet season at each of the three locations (see Figure 1.3; Climate averages), to simulate subsequent germination by rainfall of dispersed seed. The majority of Katherine and Kununurra sites were sown in a period from the end of November to mid-December. The Kununurra Cattle Site No.1 was sown relatively later (February) as water availability was not a constraint due to irrigation, plus cattle were grazing the site until that time. Planting was delayed until cattle were removed to maximise the germination and survival of seedlings. The Broome sites were sown in mid-January because of the later commencement of the wet season in this region compared to the two other more northern locations. Although planting time was not an experimental variable, it may be an important factor because of water availability, and rainfall variability, a feature of the wet-dry tropics (Taylor and Tulloch 1985).

The only modification to time of planting was the sowing of the Kununurra dry season Drain site in June 2000, as water availability for germination in the dry season was not a constraint at such habitats. This was intended to assess seasonal differences in plant phenology from those plants germinating in the wet season.

EXPERIMENTAL DESIGN

The experimental design used was a split-split plot design. For the 13 sites sown in the first season (1999-2000), the main-plot factor was seedtype, sub-plot factor was population and sub-sub-plot factor was genotype. Tables 1.1 to 1.3 detail the experimental factors at each site. A schematic diagram of the experimental design is illustrated in Figure1.2.

Planting Method

Seed was uniformly distributed and hand-placed within a 25 x 25 cm quadrat from which approximately the top 2 cm of soil and vegetation had been cleared, to ensure each seed had adequate seed soil contact. The seed was then recovered with the previously removed soil to prevent desiccation and predation. Plots were hand-watered from sowing until the time of predicted first square, according to the long-term average heat unit accumulation, calculated according to Constable and Shaw (1988). This was equivalent to approximately 540 DD12 or 30 days. Watering aimed to ensure that seeds and resultant seedlings did not visibly appear moisture stressed. The frequency and amount of watering varied among sites, because of differences in soil characteristics, evaporative losses and rainfall. For example, the Broome road and bush sites were watered to saturation every two to three days for the entire period from sowing to predicted first square; the Kununurra Cattle Site

No.1 was watered immediately after sowing, and then natural rainfall negated the need for any continued watering.

Watering stimulated germination to occur immediately after sowing, and reduced the risk of ungerminated seed being carried from the experimental site to an unregulated area where it could potentially then germinate. This then eliminated the need to use netting, which impeded germination.

In summary, the planting method was designed to positively bias the probability of germination, providing a worst-case scenario for the escape and establishment of cotton into the selected habitats.

DEMOGRAPHIC PARAMETERS AS INDICATORS OF WEEDINESS

It was originally intended to measure demographic parameters at times defined by the physiological developmental stages of commercial cotton as predicted from accumulated heat units (Constable and Shaw 1988). This was submitted to GMAC in PR89X2. However, cotton developed much slower in these harsher environments compared to commercial production, and timing of measurements was modified to monthly, and subsequently to longer intervals, due to the slow progress of plant development at the majority of sites. Tables 1.1 to 1.3 specify the actual recording dates for each site. The following parameters were recorded at each time; plant number, plant height, damage rating, and fruit counts of squares, flowers, green bolls and open bolls. Stages of plant growth and development during the project were used to evaluate specific weediness traits. These were germination (maximum number of seedlings that emerged), fecundity (maximum open bolls produced), survivorship 1 (plant number at the end of the first dry season) and survivorship 2 (plant number at the end of the second dry season. The evaluation of invasiveness as a measure of weediness was the cumulative effect of these demographic stages.

Observations on the insects present at each location and site, particularly Lepidopteran species, were noted. To evaluate herbivory, a leaf damage rating on a scale from 0-6, was modified from Brown et al. (1987). As the experiment progressed, it became apparent that leaf damage or leaf loss was not always directly attributable to insect herbivory, but was also due to environmental stress. The rating ultimately was indicative of plant vigour, rather than damage attributable to insect herbivory.

The demographic parameters for which analyses were conducted were:

Germination: Seeds were counted as germinated when green cotyledons were visible, although these may still have been enveloped in lint, as was the case for seed cotton. Germination was calculated as the maximum number of plants emerged as a proportion of the number of seeds sown, and results expressed as a percentage. This occurred within the initial two measurement times, which generally spanned the first two weeks after sowing (designated T1 and T2; Refer to Tables 1.1, 1.2 and 1.3). Analysis was also conducted for the proportion of plants to the number of seeds sown that were remaining at T3, and although the actual time this encompassed varied, plant numbers were always less than those at the two initial measurement times. Imbibed cotton seed normally germinates over a period of four to seven days under ideal conditions but the observation time was extended over this longer period due to the staggered germination of seed cotton in these experiments. Results were therefore evaluated at each of the initial three measurement dates, and cumulatively for the overall period. Considerable attention was given to these discrete initial three measurements because germination is such a critical phase in the establishment of volunteer cotton plants.

Survivorship: The critical time to evaluate survivorship was at the end of the dry season, coinciding with the measurements conducted after the first rains which allowed viable plants to reshoot and produce vegetative matter. This was assessed at two times; Survivorship 1 was the number of original plants per plot that germinated that were still alive after the initial dry season (Surv1), and; Survivorship 2 was the number of original plants per plot germinated that were still alive after the second dry season (Surv2).

Survivorship at each time was calculated in two ways: as a proportion of the number of seeds sown, plus as an absolute value of the number of plants present, as represented by:

$$\text{Surv1 (proportion)} = \frac{\text{No. of originally germinated plants surviving at the end of the first dry season}}{\text{No. of seeds sown}}$$

Surv1 (absolute) = No. of originally germinated plants surviving at the end of the first dry season

$$\text{Surv2 (proportion)} = \frac{\text{No. of originally germinated plants surviving at the end of the 2nd dry season}}{\text{No. of seeds sown}}$$

Surv2 (absolute) = No. of originally germinated plants surviving at the end of the 2nd dry season

Due to low numbers of surviving plants at most sites, survivorship was also calculated as the total number of plants remaining for all treatments as a percentage of the total number of seeds sown (i.e. 3240 seeds for most sites).

Fecundity: This was determined as the maximum number of open bolls produced, and was an indication of reproductive capacity. Actual seed production would have been a more accurate representation, but non-destructive measurements of fruit were required to allow for further development on the plant, so individual seeds could not be counted. It was intended to harvest a proportion of bolls produced (10% of the total number counted per plot, with the rest remaining on the plant to be available for seedling recruitment) to quantify seed numbers per boll and germination viability. The only two sites at which enough open bolls were produced to allow such a hand-harvest were the Broome Cattle Site No.1 and the Kununurra Dry Season Drain Site. Average number of seeds per boll and average germination under controlled conditions was assessed.

The maximum number of open bolls was identified as coinciding towards the end of the dry season, as rains at the commencement of the wet season dislodged the seed cotton from the plant, plus plant phenology shifted to vegetative rather than reproductive growth from this time.

Number of seedlings recruited from the original cohort of plants was also examined.

Invasiveness: This was quantified from calculation of the population growth rate (λ) which incorporated the previously assessed demographic parameters as discussed above. Invasiveness was calculated at two times; at the end of the first year, based on survivorship of original plants plus any seedling recruitment after one year (λ_1) and; at the end of the second year based on continued survival of original plants plus new seedlings (λ_2). These calculations can be represented as:

$$\lambda_1: \frac{\text{No. of original plants remaining after 1st dry season (Surv1) + No. of recruited seedlings (at Surv1)}}{\text{Germination (max. no. of plants T1-T2)}}$$

$$\lambda_2: \frac{\text{No. of original plants remaining after 2nd dry season (Surv2) + No. of recruited seedlings (at Surv2)}}{\text{No. of plants remaining after initial dry season (Surv1 + recruited seedlings at Surv1)}}$$

This allowed differentiation between the initial establishment year, and the second season where some plants perenniated, reaching a more stable population threshold, as all populations declined from the first to the second year.

Similarly to the trait of survivorship, λ_1 and λ_2 were also calculated using a 'simplistic' method due to the low numbers of plants at most sites. A value was calculated for each genotype across all other factors.

DATA ANALYSES

Differences in the demographic parameters between sites were presented as means and standard errors within blocks.

Within sites:

Germination was analysed using a generalized linear model (GLM) on binomial proportions (logits) with Genstat®. Number of subjects was equal to number of seeds sown (10 or 80). Number of successes were equal to the maximum number of seedlings present (Germination; T1 to T2).

Survivorship as a portion of the number of seeds sown was similarly analysed, where number of subjects were equal to number of seeds sown (10 or 80) and number of successes were equal to number of plants remaining (at Surv1 and Surv2).

Absolute survivorship was analysed using an ANOVA with appropriately transformed data of plant number (at Surv1 and Surv2).

Fecundity was analysed using a split-split-plot ANOVA with appropriately transformed data for number of open bolls. Aspects of fecundity were analysed, including total open boll production and production per plant where surviving plant numbers were adequate. This allowed inferences concerning seed production given that cottonseed had dispersed. There was a large number of sites with very few surviving plants, making statistical analysis inconclusive for these sites, so calculation of invasiveness was simplified. It was calculated for each genotype, summed across all other factors. Invasiveness (λ_1 and λ_2) was calculated for each plot for the Kununurra DS Drain site and the Broome Cattle Site, allowing an ANOVA to be conducted.

The specific transformation used for each parameter is provided in the relevant site results (Appendix 2).

Data were analysed considering the effects of site, seedtype, population, and genotype.

Results were considered for each site separately, then consistent trends, or significant anomalies, were collated and compared. Abbreviations used in the results for the factor levels are:

Seedtype:	S1; Black seed	S2; Fuzzy seed	S3; Seed cotton
Population:	H; High (80 seeds / 625cm^2)	L; Low (10 seeds / 625cm^2)	
Genotype:	G0; Sicot 289 conventional		
	G1; Sicot 289i		
	G2; Sicot 289ii (Cry1Ac+Cry2Aa)		

a

b

c

Photos 1.1 a-c. Habitats in which cotton was observed to establish as a volunteer – (a) cattle feeding out areas, (b) roadside and (c) drain

a

b

c

Photos 1.2 a-c. Three different seedtypes used in planting: (a) black seed, (b) fuzzy seed and (c) seed cotton

Table 1.1. Factors and measurement dates for Katherine sites

LOCATION	SITE DESCRIPTION					
	EXPERIMENT 1A				EXPERIMENT 1B	
KATHERINE	ROAD	CREEK	BUSH	CATTLE No.1	CATTLE No.2	BUSH No.2 GxN
SEASON	99 - 00	99 - 00	99 - 00	99 - 00	00 - 01	00 - 01
FACTORS	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype(3); *Genotype(5 Popln (1) 14 trts; 4 blks	Nutrition(2) Population(2) Genotype(4) 16 trts; 4 blks
DATE SOWN	15th Dec 99	9th Dec 99	11th Dec 99	16th Dec 99	5th Jan 00	5th Jan 00
T1	22nd Dec 99	17th Dec 99	17th Dec 99	22nd Dec 99	25th Jan 00	25th Jan 00
T2	30th Dec 99	30th Dec 99	30th Dec 99	30th Dec 99	27th Feb 00	27th Feb 00
T3	25th Feb 00	25th Feb 00	25th Feb 00	25th Feb 00	28th Mar 01	28th Mar 01
T4	4th Apr 00	31st Mar 00	5th Apr 00	4th Apr 00	15th May 01	15th May 01
T5	4th May 00	4th May 00	4th May 00	4th May 00	14th July 01	14th July 01
T6	6th June 00	7th June 00	6th Jun 00	6th Jun 00	8th Nov 01	7th Nov 01
T7	11th July 00	11th July 00	11th Jul 00	12th Jul 00		
T8	11th Aug 00	11th Aug 00	11th Aug 00	11th Aug 00		
T9	5th Jan 01	6th Jan 00	5th Jan 01	4th Jan 01		
T10	29th Mar 01	28th Mar 01	28th Mar 01	27th Mar 01		
T11	7th Nov 01	13th July 01	7th Nov 01	15th May 01		
T12		7th Nov 01		14th July 01		
T13				7th Nov 01		

*No G2X seed cotton sown

Table 1.2. Factors and measurement dates for Broome (Shamrock Station) sites

LOCATION	SITE DESCRIPTION					
	EXPERIMENT 1A				EXPERIMENT 1B	
SHAMROCK STATION	ROAD	DAM	BUSH No.1	CATTLE No.1	BUSH No.2	CATTLE No.2
SEASON	99 - 00	99 - 00	99 - 00	99 - 00	00 - 01	00 - 01
FACTORS	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtyp(3) Genotype(3) Popln (1) 9 trts; 4 blks
DATE SOWN	13th Jan 00	13th Jan 00	13th Jan 00	12th Jan 00	18th Jan 01	18th Jan 01
T1	17th Jan 00	17th Jan 00	17th Jan 00	17th Jan 00	27th Jan 01	27-Jan-01
T2	24th Jan 00	24th Jan 00	24th Jan 00	24th Jan 00	15th Feb 01	15-Feb-01
T3	17th Feb 00		17th Feb 00	18th Feb 00	3rd Mar 01	03-Mar-01
T4	14th Mar 00		14th Mar 00	13th Mar 00	2nd Apr 01	02-Apr-01
T5	30th Mar 00		30th Mar 00	27th Mar 00	2nd May 01	02-May-01
T6	13th Apr 00		13th Apr 00	13th Apr 00	8th Jun 01	08-Jun-01
T7	25th Apr 00		25th Apr 00	24th Apr 00	04-Jul-01	05-Jul-01
T8	16th May 00		16th May 00	17th May 00	3rd Aug 01	04-Aug-01
T9	12th Jun 00		12th Jun 00	12th Jun 00	21-Nov-01	10-Sep-01
T10	11th July 00		11th July 00	11th July 00		19-Oct-01
T11			19th Jan 01	24th Aug 00		20-Nov-01
T12				2nd Oct 00		
T13				18th Jan 01		
T14				2nd May 01		
T15				4th Aug 01		
T16				21-Nov-01		

Table 1.3. Factors and measurement dates for Kununurra sites

LOCATION	SITE DESCRIPTION							
KUNUNURRA	EXPERIMENT 1A					EXPERIMENT 1B		
	ROAD	WS DRAIN No.1	BUSH No.1	CATTLE No.1	DS DRAIN	BUSH No.2	CATTLE No.2	WS DRAIN No.2
SEASON	99 - 00	99 - 00	99 - 00	99 - 00	00	00 - 01	00 - 01	00 - 01
FACTORS	Seedtype(3) Genotype(3) Popln(2) 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype(3) Genotype(3) Popln(2) 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 4 blks	Seedtype(2) Popln(2) Genotype(4) 16 trts; 4 blks	Seedtype(3) Genotype(5)*; Popln (1) 14 trts; 4 blks	Seedtype (3); Genotype (3); Popln (2); 18 trts; 3 blks
DATE SOWN	26th Nov 99	29th Nov 99	3rd Dec 99	2nd Feb 00	30th June 00	14th Dec 00	15th Dec 00	16th Dec 00
T1	04-Dec-99	04-Dec-99	12-Dec-99	07-Feb-00	11-Jul-00	21st Dec 00	22-Dec-00	22-Dec-00
T2	17-Dec-99	29-Jan-00	31-Dec-99	08-Mar-00	06-Aug-00	12th Jan 01	11-Jan-01	11-Jan-01
T3	21-Dec-99	09-Jun-00	14-Mar-00	10-Apr-00	22-Sep-00	6th Mar 01	02-Mar-01	10-Apr-01
T4	04-Jan-00	06-Aug-00	10-Apr-00	09-May-00	14-Nov-00	17-Apr-01	09-Apr-01	19-May-01
T5	10-Apr-00	10-Apr-00	08-May-00	08-Jun-00	22-Dec-00	19-May-01	19-May-01	30-Jun-01
T6	07-Aug-00	30-Jun-01	08-Jun-00	06-Jul-00	11-Apr-01	06-Jul-01	06-Jul-01	04-Aug-01
T7	10-Apr-01	04-Aug-01	07-Jul-00	09-Aug-00	30-Jun-01	26-Oct-01	25-Oct-01	09-Sep-01
T8	25-Nov-01	09-Sep-01	04-Aug-00	12-Dec-00	04-Aug-01			28-Oct-01
T9		28-Oct-01	13-Dec-00	09-Jul-01	09-Sep-01			
T10			17-Apr-01	15-Oct-01	03-Nov-01			
T11			26-Oct-01					

* No G2X seed cotton sown

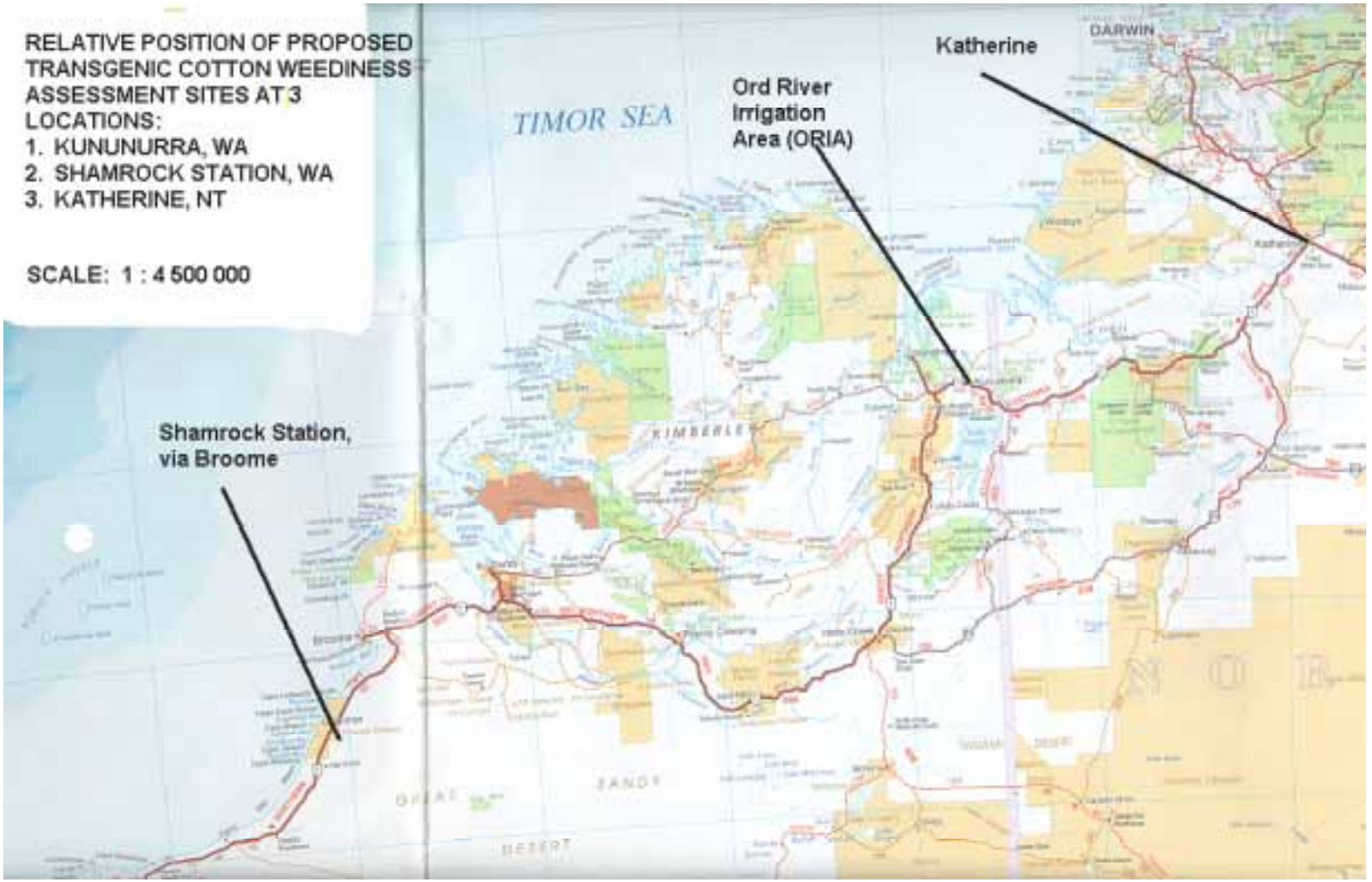


Figure 1.1. Location of the sites across north-west Australia

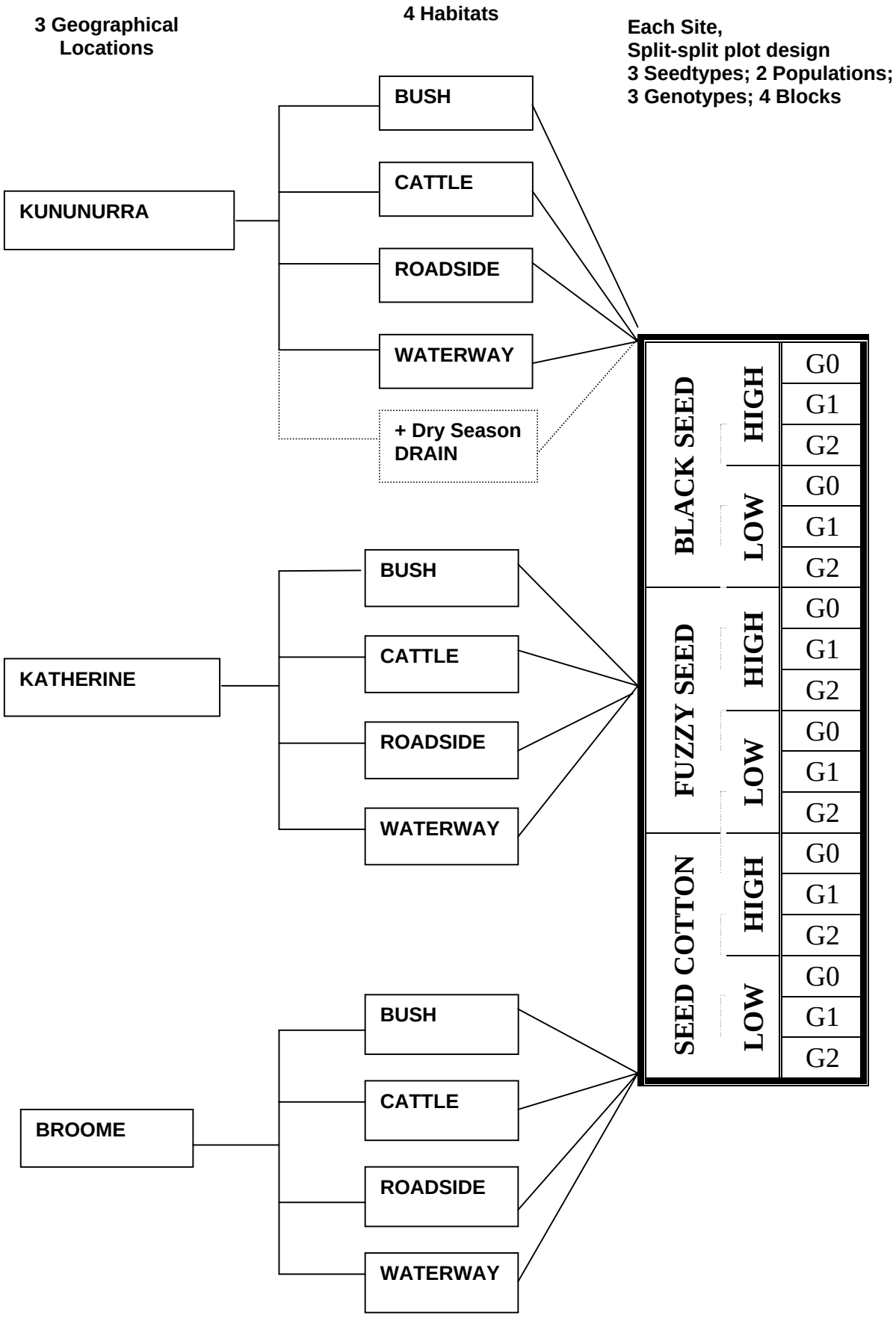


Figure 1.2. Schematic diagram of the experimental design used in Experiment 1

Site Description

This section provides a brief description of each habitat into which cotton was experimentally sown for each location, namely Kununurra, Katherine and Broome. The description includes an outline of vegetation, soil and climate. Sites are described under each habitat category; Bush, Cattle, Road and Waterway. Photos 1.3 a-d, 1.4 a-d and 1.5 a-d illustrate these habitats. Detailed soil chemical analyses are provided in Appendix 1.

The site number as allocated in this report for each habitat is presented. There were 13 sites for Experiment 1A, all approved as PR89X(2) (containing combinations of Cry1Ac and Cry2Aa genes).

The Release Site Location details as supplied on the OGTR website as of May 2002 (see www.health.gov.au/ogtr/gmorecord/pdfdir/pr89x2cotton.pdf) are included for each site for ease of cross-referencing.

Table 1.4. Site details of Experiment 1A

Site No.	Geographic Location	Habitat Category	Location Description	Land Use	Vegetation	Soil	OGTR details
1	Kununurra	Bush	Behind Meteorological Station, Frank Wise Institute, Durack Drive	Natural Habitat: Open Woodland	Dominant species: *Lysiphyllum cunninghamii, Carissa lanceolata, Flemingia panciflora, Chrysopogon fallax, Sorghum timorense* Secondary species: *Trichodesma zeylanicum, Iseilema marratherum, Hibiscus panduriformis, Chionachne hubbardiana, Passiflora foetida*	Cununurra clay (alkaline phase) Brownish cracking clays with high pH topsoils	Kununurra Bush: Site Two, PR89(X)2
2	Kununurra	Cattle	Bay 111, Legune Road, Leucaena Farm, Heytesbury Past. Co.	Man modified: Leucaena paddock (irrigated)	Dominant species: *Leucaena leucocephala* Secondary species: *Echinochloa colona, Ludwigia perennis, Aeschynomene indica, Wedelia asperrima*	Aquitaine soil (Bluish phase) Brownish cracking clays with high pH topsoils	Kununurra Cattle Bay 111: Site One, PR89(X)2
3	Kununurra	Road	Quarantine Yards, townside boundary fence, track off Victoria Highway, 5 km east of Kununurra	Man modified: Cleared fenceline track	Dominant species: *Sorghum stipodeum, Aristida hygrometrica, Whitechloa biciliata, Spermacoce exserta, Sida spp*	Cockatoo sand (or possible Pago sand)	Kununurra Road Site: Site Three, PR89(X)2
4	Kununurra	Waterway Wet Season Drain	Hillside Drain No.3 (HD3), Legune Station Rd, Carlton Hill Station	Man-modified: Irrigation drain in disturbed woodland	Dominant: *Ludwigia spp, Hibiscus panduriformis, Brachyachne convergens, Stylosanthes spp, Sida spp, Heteropogon contortus, Aristida spp, Echinochloa colona, Fimbristylis spp* Secondary species: *Lysiphyllum cunninghamii, Digitaria spp, Alysicarpus vaginalis*	Cununurra Clay Cracking clay with some coarse sand	Kununurra Drain Site: Site Four, PR89(X)2
5	Katherine	Bush	Napier Paddock, Katherine Research Station, NT DBIRD	Natural habitat: Open Woodland	Dominant species: *Chrysopogon spp, Heteropogon contortus Erythrophloem chlorostachys, Eucalyptus tetradonta,*	Red Earth, Tippera Clay Loam	Katherine Bush Site 1: Site nine, PR89(X) 2
6	Katherine	Cattle	Front leucaena Paddock, Katherine Research Station, NT DBIRD	Man modified: Leucaena paddock (Dryland)	Dominant species: *Leucaena leucocephala, Urochloa mosambicensis* Secondary species: *Macroptilium lathyroides*	Tippera clay loam	Katherine Cattle Site 1, Front Paddock: Site Ten,PR89(X)2
7	Katherine	Road	Napier Paddock, Katherine Research Station, NT DBIRD	Man modified: Adjoining old Stuart Highway	Dominant species: *Heteropogon contortus,*	Disturbed Red Earth Lateritic gravel	Katherine Road Site: Site Twelve, PR 89(X)2
8	Katherine	Waterway Creek	Bailey Paddock, Katherine Research Station, NT DBIRD	Natural habitat: Savanna woodland, riparian habitat	Dominant species: Sedges, *Timonius timon* Secondary species: *Imperata cylindrica*	Creek levee. Surrounding soil are Loamy Red Earths with scattered limestone outcrops	Katherine Creek Site: Site Eleven, PR 89(X) 2

Site No.	Geographic Location	Habitat Category	Location Description	Land Use	Vegetation	Soil	OGTR details
9	Broome	Bush	Off Road from homestead to Lunguda Yards, Shamrock Station	Natural habitat: Pindan community	Dominant species: *Acacia hippuriodes, A. eriopoda, Plectrachne* spp. Secondary species: *A. colei, Distichostemon hispidulus, Newcastelia spp*	Yeeda Land System Sand plain with deep red sands	Broome Bush Site 1: Site Fifteen, PR89(X)2
10	Broome	Cattle	Wonglie cattle yards, Shamrock Station	Man modified: Non-used yards	Dominant species: *Citrullus lanatus* Secondary species: *Chloris spp, Cenchrus spp*	Wonganut Land system Low-lying sand plain	Broome Cattle Site 1, Wonglie yards: Site Seventeen PR*((X)2
11	Broome	Road	Next to fence on road from Wonglie yard to The Dam, Shamrock Station	Man modified: Cleared fenceline track	Dominant species: *Plaectrachne spp, Acacia spp*	Yeeda Land system Sand plain with deep red sands	Broome Road: Site Sixteen, PR89(X)2
12	Broome	Waterway Dam	The Dam at the north junction of Wonglie paddock and Four Square Paddock, Shamrock Station	Man modified: Cleared and constructed dam	Dominant species: *Cenchrus spp, Acacia spp*	Yeeda Land System Sand plain with deep red sands	Broome Dam Site: Site Eighteen, PR89(X)2
13	Kununurra	Waterway Dry Season Drain	On the opposite bank of the Kununurra Wet Season Drain No. 1 site, so location, vegetation and soils are as for above site	Man-modified: Irrigation drain in disturbed woodland	Dominant: *Ludwigia spp, Hibiscus panduriformis, Brachyachne convergens, Stylosanthes spp, Sida spp, Heteropogon contortus, Aristida spp, Echinochloa colona, Fimbristylis spp* Secondary species: *Lysiphyllum cunninghamii, Digitaria spp, Alysicarpus vaginalis*	Cununurra Clay Cracking clay with some coarse sand	Kununurra Drain Site (Dry Season): Site Eight, PR89 (X)2

a

b

c

d

Photos 1.3. (a) Kununurra bush, (b) cattle, c. waterway – drain and (d) road habitats

a

b

c

d

Photos 1.4. (a) Broome bush, (b) waterway – dam, (c) road and (d) cattle habitats

a

b

c

d

Photos 1.5. (a) Katherine waterway – creek, (b) road, (c) bush and (d) cattle habitats

CLIMATE

The long-term mean daily temperature and mean monthly rainfall data for Katherine, Kununurra and Bidyadanga is presented below in Figures 1.3 a, b and c. Shamrock Station is within 50 km east (inland) of Bidyadanga, which is situated on the coast. These data were used because Shamrock Station has no long-term climatic records, but there are climatic records dating back to 1891 available from the Bureau of Meteorology (BOM) for Bidyadanga. There were observed differences in daily temperatures and rainfall between the coast area and Shamrock Station itself, but the overall trends are similar, and provide an idea of the temperature and rainfall regimes in this area.

Katherine long-term climate data was obtained from the BOM website for the Katherine Council station with data from 1873 to present. Kununurra data was obtained from the BOM website for the Kununurra Aero station with data from 1971 to present.

The long-term climate data for an eastern Australia cotton growing area, Narrabri, is also provided for comparison (Figure 1.3d.).

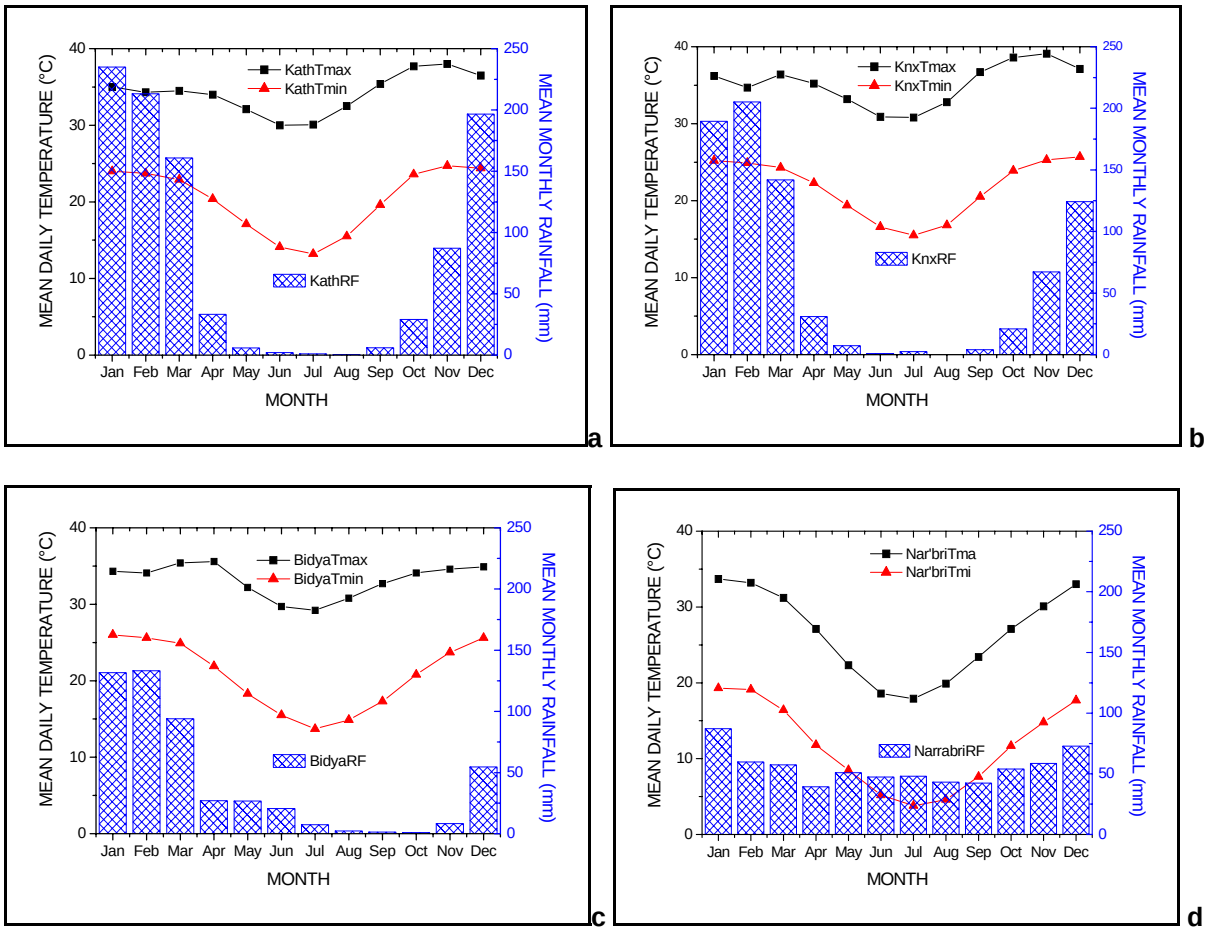
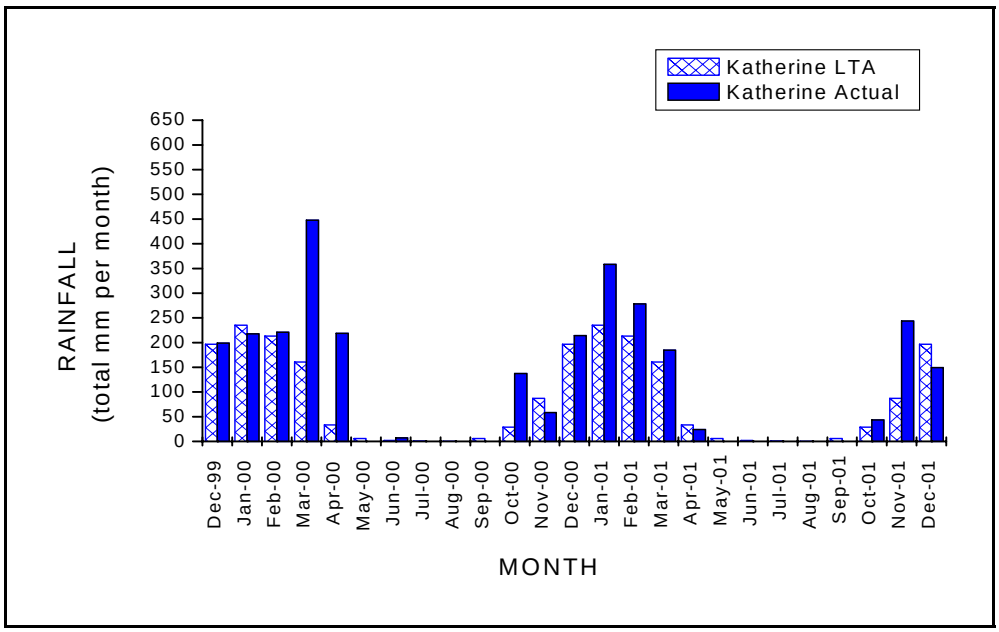


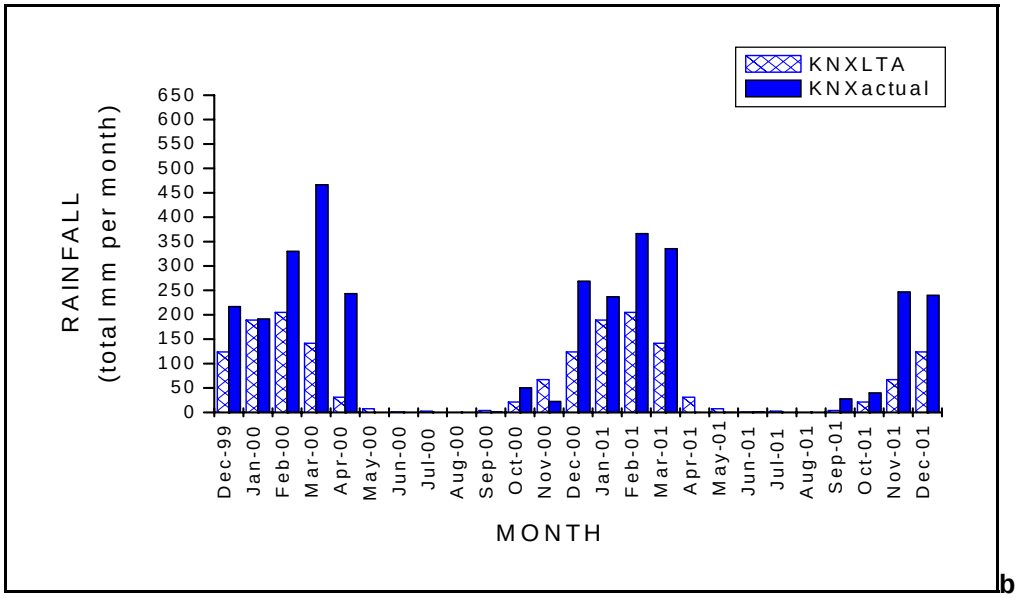
Figure 1.3. Long-term monthly climatic averages for temperature (maximum and minimum) and rainfall (monthly) for 4 locations; a. Katherine, b. Kununurra, c. Bidyadanga, and d. Narrabri

COMPARISON BETWEEN LONG-TERM DATA (LTA) AND ACTUAL VALUES OVER THE DURATION OF THE PROJECT

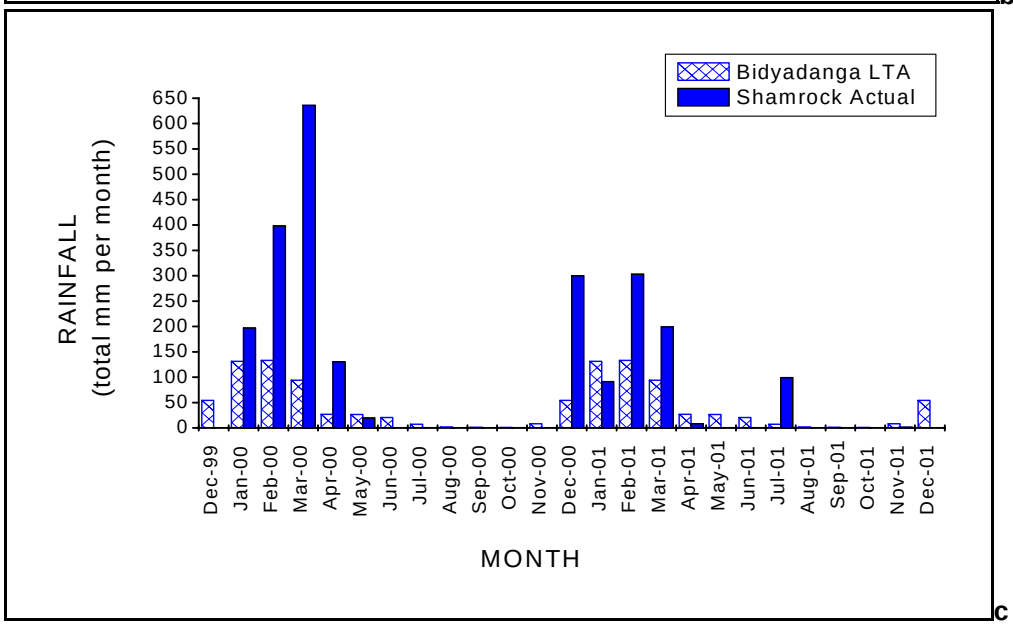
Trends for temperatures were consistent between long-term mean daily and actual values. The long-term mean monthly rainfall was plotted against the actual rainfall for the duration of the project. The two Wet Seasons provided well above average rainfall, as illustrated in Figures 1.4 a, b and c.



a



b



c

Figure 1.4a, b and c. Actual and long term average (LTA) rainfall data for three locations; (a) Katherine, (b) Kununurra and (c) Broome

Results and Discussion

All the 13 sites in Experiment 1A had the same treatments and levels applied within each site, allowing 'site' to be included as a variable in the overall statistical analysis model. Habitats, categorised as cattle, waterway, roadside and bush, were not significantly different when tested against between site variation. Thus, variation between sites for each of the demographic parameters was assessed by a one-way ANOVA (no blocking) with Site as the treatment. The mean values of each block across all other factors within each site was used as the internal replication. This allowed presentation of data for between site differences, with further analysis conducted within site.

Results are presented with significant factors and a brief description of the effect for each site. The demographic parameters assessed were germination, survivorship, fecundity and invasiveness and are presented in summary tables below. A more detailed analysis, results and discussion pertinent to each site are included in Appendix 2: Individual Site Results for Experiment 1A.

GERMINATION

Between Sites: There was a highly significant difference in germination between sites (P<0.001), presented in Fig.1.5. The greatest overall germination was observed in disturbed habitats (modified by man's activities); Kununurra Cattle No.1, Katherine Cattle No.1 and Creek sites, and Broome Dam site (mean=75%). Seeds in the bush sites (undisturbed by man, except in the process of planting) had relatively low germination rates (mean=41%).

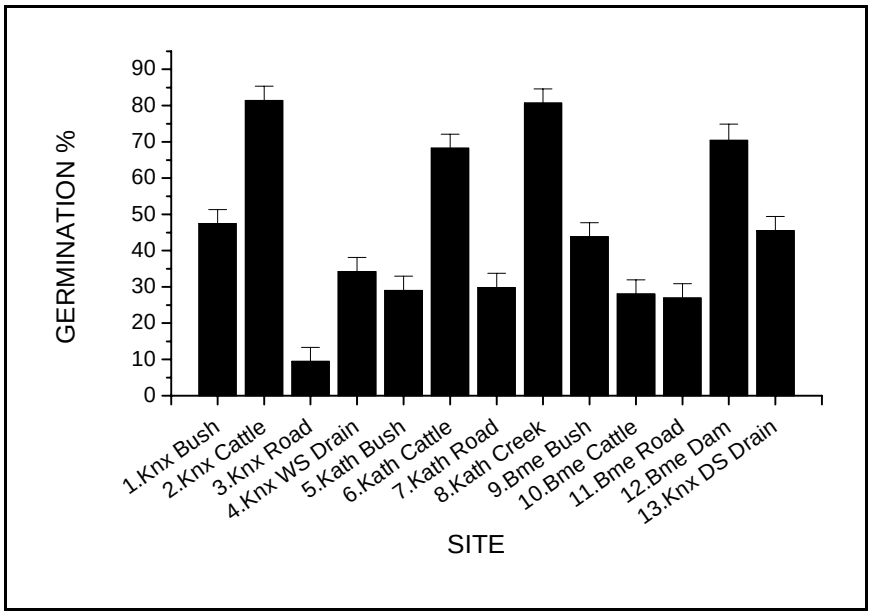


Figure 1.5. Mean germination between sites (error bars are ±s.e.)

Within Site: Table 1.5 presents within site analysis for germination, listing the significant factors and a brief description of the effect for each site.

Seed type was the dominant factor influencing germination within all sites and was highly significant (P<0.001) in eight of the 13 sites (seedtype, or its interaction with another factor was significant (P<0.05) in the remaining five sites). Generally, black seed had the highest germination, followed by fuzzy seed, then by seedcotton (mean across all sites =56.5%, 49.6% and 29.7% respectively). This had important implications for seed escape and mitigation strategies since seed cotton had the greatest opportunity for unintentional dispersal but had the lowest chance of germinating. Seedcotton also exhibited a lag in germination compared to the other two seed types, which may have implications with variability of wet season rainfall patterns between years. Fuzzy seed had relatively high germination potential. The main use for this form of seed in northern Australia would be as whole seed fed to cattle. Feeding out areas are characterised by congregation of cattle, leading to a concentration of dung and hence higher nutritional status of the soil. This had consequences for physiological development after germination, as plants in higher nutrition habitats had a greater ability

to produce fruiting structures (but a greater risk of being trampled by cattle). Black seed was observed to have the least risk of unintentional escape.

Population density, or its interaction with seed type, also affected germination, and was significant ($P<0.05$) in eight of the 13 sites. Generally, the low population density had lower germination than the higher density, suggesting that dispersal of seed as groups or clumps may enhance establishment compared with isolated seed dispersal. There was no significant effect of genotype within eleven of the initial thirteen sites. There was a significant ($P=0.037$) seedtype by genotype interaction at the Kununurra WS Drain No.1 Site; with the G0 fuzzy seed lower than its respective transgenic treatments, although there was no genotype effect for the other two seedtypes. There was also a significant effect of genotype ($P=0.007$) at the Kununurra DS Drain, with the conventional genotype lower than the two transgenic treatments.

Microhabitat (niches within each habitat), measured as significant between block effects, also influenced germination percentage at some sites, such as drain and roadside habitats where there were noticeable gradient effects. Seed and dispersal ecology are recognised as major determinants of weed fitness and population growth rate, but overall the addition of the Bt genes did not enhance dispersal or germination ability of cottonseed simulated to have escaped from production areas.

Table 1.5. Significant effects on germination for Experiment 1A

GERMINATION			
SITE No.	**SITE**	**SIGNIFICANT EFFECTS**	**DESCRIPTION OF EFFECT**
1	Kununurra Bush No.1	SEEDTYPE x POPULATION (P=0.03)	L < H for black seed only, otherwise L > H
2	Kununurra Cattle No.1	SEEDTYPE (P<0.001)	S3<S2,S1
		POPULATION (P<0.001)	L<H
3	Kununurra Road	Results too variable for confidence in results. Subsequently resown.	
4	Kununurra WS Drain No.1	SEEDTYPE x POPULATION (P=0.017)	L= H for black seed only, otherwise L > H
		SEEDTYPE x GENOTYPE (P=0.037)	G0 < G1, G2 for fuzzy seed only
5	Katherine Bush No.1	SEEDTYPE (P<0.001)	S3<S2,S1
6	Katherine Cattle No.1	SEEDTYPE (P<0.001)	S3<S2,S1
7	Katherine Road	SEEDTYPE (P<0.001)	S3<S2<S1
		POPULATION (P=0.009)	L<H
8	Katherine Creek	SEEDTYPE (P<0.001)	S3<S2,S1
9	Broome Bush No.1	SEEDTYPE x POPULATION (P=0.018)	L < H for black seed only, otherwise L = H
10	Broome Cattle No.1	SEEDTYPE (P=0.013)	S3,S2<S1
11	Broome Road	SEEDTYPE (P<0.001)	S3,S2<S1
		POPULATION (P<0.001)	L<H
12	Broome Dam	SEEDTYPE (P<0.001)	S3<S2,S1
		POPULATION (P=0.031)	L<H
13	Kununurra DS Drain	SEEDTYPE (P<0.001)	S3<S1<S2
		POPULATION (P=0.05)	L<H
		GENOTYPE (P=0.007)	G0<G2<G1

SURVIVORSHIP

Between sites: There was no consistent result indicating that cotton with Bt genes had greater survivorship than conventional cotton after one or two years. There was a significant effect of site ($P<0.001$) on Surv1. Only four of the 13 sites had greater than 50% of plots with any surviving plants; Katherine Cattle No.1 and Katherine Creek sites, the Kununurra DS Drain site and the Broome Cattle No.1 site. Means corresponded to 8.86%, 19.84%, 17.46% and 8.58% plants remaining from seeds originally sown for these sites respectively. Eight of the sites had isolated or no plants surviving after one year. Less than 10% of plots in each site had any surviving plants, corresponding to a mean of less than 3% of plants remaining from originally sown seed. Environmental influences such as nutrition, water availability or excess, interspecific plant competition, herbivory by non-lepidopteran species (eg. grasshoppers), cattle trampling, and fire all contributed to cotton plant mortality.

An ANOVA for Surv1 was conducted on each of the four sites where there were greater than 50% of plots with surviving plants. There was a significant interaction ($P<0.05$) between genotype and either seedtype or population for these sites. Results for the ANOVA for these four sites, plus a description of effect for the remaining sites is provided in Table 1.6. Number of plants surviving within each site as a percentage of number of seeds sown is provided in brackets. Only in the Kununurra DS Drain site did the two transgenic genotypes have greater survivorship than the conventional (fuzzy seed only), consistent with the trend for germination at this site, indicating a causal relationship between germination and survivorship.

The number of surviving originally sown plants declined over the two years for all sites. For Surv2, only the Kununurra DS Drain site and Broome Cattle No.1 site had greater than 50% of the plots remaining with any surviving plants, and actual plant numbers were small. There was a genotype effect only within fuzzy seed for these two sites. Results for the ANOVA for these two sites, plus a description of effect for the remaining sites is provided in Table 1.7. Number of plants surviving within each site as a percentage of number of seeds sown is provided in brackets.

The effect of site on survivorship (within site ANOVA means) over one and two years is presented in Figure 1.6.

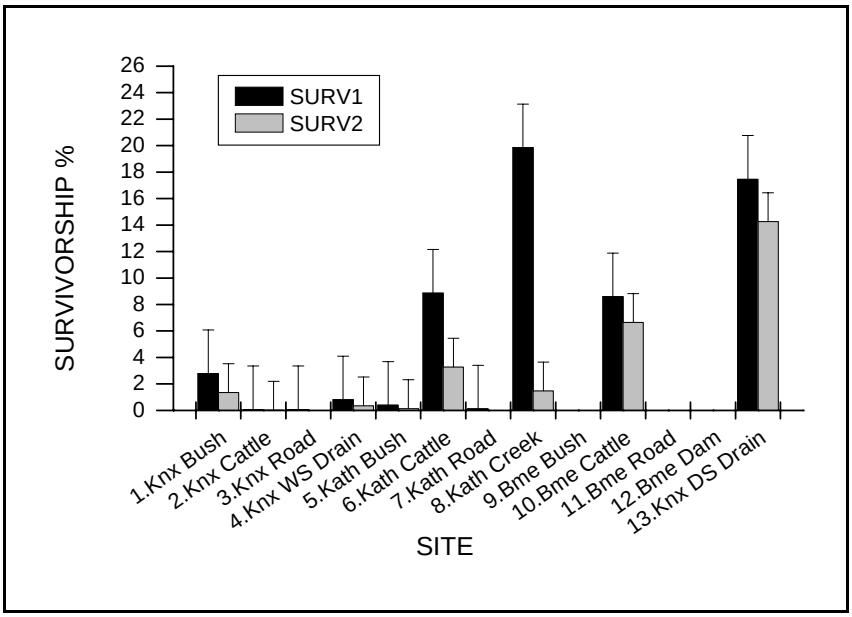


Figure 1.6. Site means for survivorship over one and two years between all sites (error bars are ± s.e. and are for within each time only)

Table 1.6. Significant effects on survivorship 1 for Experiment1A (or description of plants remaining where there were only isolated plots with surviving plants)

SURVIVORSHIP 1 (SURV1) (No. of plants remaining after one year)					
SITE No.	**SITE**	**NO.OF PLOTS WITH SURVIVING PLANTS (Plants as % of seeds sown)**	**DESCRIPTION OF EFFECT (Individual plot data is presented for sites where there were not enough surviving plots to analyse the data)**		
			G0	**G1**	**G2**
1	Kununurra Bush No.1	12 / 72 (3.86%)	P3: 4 plants (S1H) P16: 40 plants (S2H)	P1: 13 plants (S1H) P6: 2 plants (S1L) P10: 7 plants (S3H) P17: 5 plants (S2H) P38: 2 plants (S2L) P44: 46 plants (S1H) P61: 1 plant (S3H)	P2: 3 plants (S1H) P15: 1 plant (S2L) P60: 1 plant (S1H)
2	Kununurra Cattle No.1	3 / 72 (0.09%)	P57: 1 plant (S2H)	P41: 1 plant (S3H) P54: 1 plant (S1H)	-
3	Kununurra Road	1 / 72 (0.09%)	P40: 3 plants (S2H)	-	-
4	Kununurra WS Drain No.1	4 / 72 (1.02%)	P47: 20 plants (S1H)	P46: 7 plants (S1H)	P30: 2 plants (S1L) P48: 4 plants (S1H)
5	Katherine Bush No.1	4 / 72 (0.28%)	-	P40: 1 plant (S3L) P58: 5 plants (S1H) P65: 1 plant (S2L) P70: 2 plants (S3H)	-
6	Katherine Cattle No.1 #	44 / 72 (7.31%)	SEEDTYPE X GENOTYPE (P=0.015) Inconsistent effect of Genotype within Seedtype		
7	Katherine Road	3 / 72 (0.18%)	P28: 2 plants (S3H) P31: 1 plant (S1H)	P29: 3 plants (S3H)	-
8	Katherine Creek #	41 / 72 (24.56%)	GENOTYPE X POPULATION (P=0.01) H>L for G0 only		
9	Broome Bush No.1	0 / 72 (0.0%)	-	-	-
10	Broome Cattle No.1 #	41 / 72 (9.85%)	SEEDTYPE X GENOTYPE (P=0.012) G2 > G0, G1 for fuzzy seed only		
11	Broome Road	0 / 72 (0.0%)	-	-	-
12	Broome Dam	0 / 72 (0.0%)	-	-	-
13	Kununurra DS Drain #	47 / 72 (15.49%)	SEEDTYPE X GENOTYPE (P=0.031) G1, G2 > G0 for fuzzy seed only		

Sites with >50% of plots with surviving plants.

Table 1.7. Significant effects on survivorship 2 for Experiment 1A (or description of plants remaining where there were only isolated plots with surviving plants)

SURVIVORSHIP 2 (SURV2) (No. of Plants remaining after two years)					
SITE No.	**SITE**	**NO.OF PLOTS WITH SURVIVING PLANTS (Plants as % of seeds sown)**	**DESCRIPTION OF EFFECT (Individual plot data is presented for sites where there were not enough surviving plots to analyse the data)**		
			G0	**G1**	**G2**
1	Kununurra Bush No.1	6 / 72 (1.76%)	P16: 21 plants (S2H)	P1: 6 plants (S1H) P6: 1 plant (S1L) P10: 1 plant (S3H) P38: 2 plants (S2L) P44: 26 plants (S1H)	-
2	Kununurra Cattle No.1	1 / 72 (0.03%)	-	P41: 1 plant (S3H)	-
3	Kununurra Road	0 / 72 (0.0%)	-	-	-
4	Kununurra WS Drain No.1	3 / 72 (0.65%)	P47: 12 plants (S1H)	P46: 7 plants (S1H)	P48: 2 plants (S1H)
5	Katherine Bush No.1	1 / 72 (0.03%)	-	P65: 1 plant (S2L)	-
6	Katherine Cattle No.1	19 / 72 (2.16%)	P15: 2 plants (S2H) P20: 1 plant (S1L) P27: 2 plants (S2H) P33: 2 plants (S3L) P58: 1 plant (S2H) P61: 3 plants (S1L) P69: 9 plant (S3H)	P6: 2 plants (S1H) P14: 16 plants (S2H) P23: 2 plants (S1H) P45: 12 plants (S2H) P53: 2 plants (S1L) P60: 3 plants (S2H) P62: 7 plants (S1L) P68: 1 plant (S3H)	P30: 1 plant (S2L) P51: 2 plants (S1H) P59: 1 plant (S2H) P63: 1 plant (S1L)
7	Katherine Road	0 / 72 (0.0%)	-	-	-
8	Katherine Creek	9 / 72 (2.41%)	P44: 16 plants (S1H) P65: 25 plants (S3H) P70: 10 plants (S2H)	P45: 10 plants (S1H) P46: 1 plant (S1L) P50: 3 plants (S3H) P72: 8 plants (S2H)	P66: 1 plant (S3H) P71: 4 plants (S2H)
9	Broome Bush No.1	0 / 72 (0.0 %)	-	-	-
10	Broome Cattle No.1 #	37 / 72 (7.93%)	SEEDTYPE X GENOTYPE (P=0.016) G2 > G0, G1 for fuzzy seed only		
11	Broome Road	0 / 72 (0.0%)	-	-	-
12	Broome Dam	0 / 72 (0.0%)	-	-	-
13	Kununurra DS Drain #	43 / 72 (12.81%)	SEEDTYPE X GENOTYPE (P=0.003) G2 ,G1 > G0 for fuzzy seed only		

Sites with >50% of plots with surviving plants

FECUNDITY

Between sites: There was a significant effect (P<0.001) of site on fecundity (maximum number of open bolls produced per plot). The site means for maximum number of bolls per plot across all factors is illustrated in Fig.1.7. Plants in most sites displayed minimal growth and development. For the duration of the project, plants in eight of the thirteen sites produced no fruiting structures and two sites produced only isolated (<15) open bolls, all of which had seeds of low vigour. The remaining three sites; Broome Cattle No.1, and the Kununurra WS and DS Drain sites, produced relatively large

numbers of open bolls (>150) over the entire site. These three were the only sites from which seedling recruitment occurred from the originally sown parent plants.

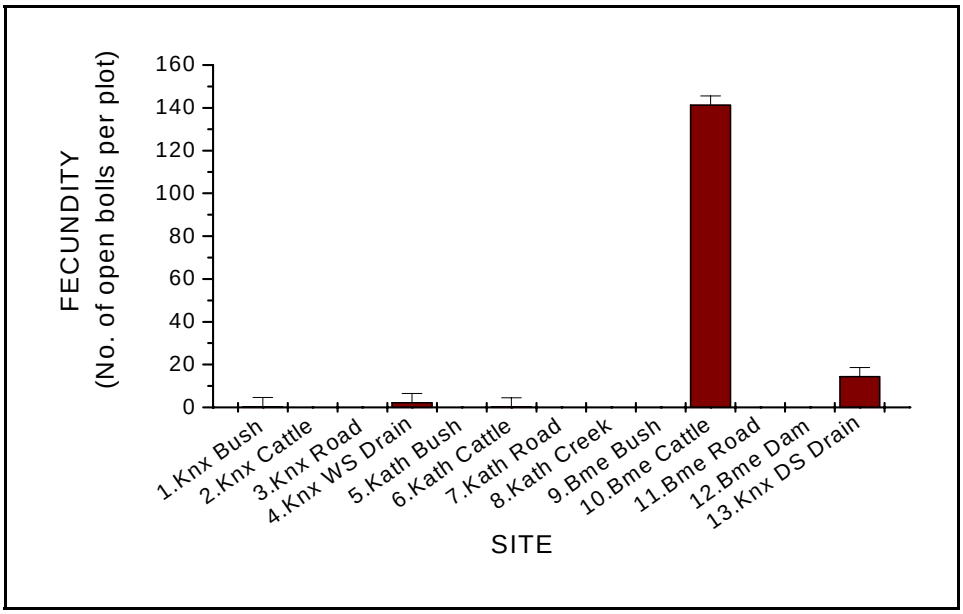


Figure 1.7. Mean maximum number of open bolls per plot across all sites (error bars are ± s.e.)

Within Site: The Broome Cattle No.1 and the Kununurra DS Drain sites were the only ones for which robust data analysis within site could be conducted. Fruit production was seasonal, with numbers of open bolls increasing with progression of the dry season. Over the wet season, plants primarily produced vegetative biomass and squares (flower buds), and seed cotton from the preceding dry season was dislodged from the plant, leading to seedling recruitment.

Results for the ANOVA for maximum number of open bolls for each of these two sites, plus a description of effect for the remaining sites is provided in Table 1.8.

Population was the dominant factor affecting maximum number of open bolls per plot produced at both sites, with the higher density treatment producing greater number of bolls. This is likely due to the importance of population in absolute survivorship and the ultimate establishment of a volunteer populace so plants are available to produce bolls.

There was also some effect of genotype observed. This was only as an interaction approaching significance (P=0.052) with seedtype for the Broome Cattle site, where there was a trend for increase in boll production with genotype (G2>G1>G0) for fuzzy seed only. Within the Kununurra DS Drain site, only the single-gene genotype produced significantly (P=0.005) larger number of bolls than the conventional genotype.

Table 1.8. Number of open bolls produced, and significant effects on fecundity

FECUNDITY (Maximum Number of Open Bolls per plot) (No. of seedlings present are included in brackets for relevant sites)*					
SITE No.	**SITE**	**No.of bolls produced (Total per Genotype across all other factors)**			**SIGNIFICANT FACTORS and DESCRIPTION OF EFFECT**
		G0	**G1**	**G2**	
1	Kununurra Bush No.1	4	10	0	NA
2	Kununurra Cattle No.1	0	0	0	NA
3	Kununurra Road	0	0	0	NA
4	Kununurra WS Drain No.1	42 (8)	106 (7)	9 (0)	NA
5	Katherine Bush No.1	0	0	0	NA
6	Katherine Cattle No.1	0	5	1	NA
7	Katherine Road	0	0	0	NA
8	Katherine Creek	0	0	0	NA
9	Broome Bush No.1	0	0	0	NA
10	Broome Cattle No.1#	4771 (11,6)	2922 (22,10)	2470 (115,58)	POPLN (P=0.009) ; H > L
11	Broome Road	0	0	0	NA
12	Broome Dam	0	0	0	NA
13	Kununurra DS Drain	199 (0,9)	470 (2,9)	357 (48,38)	POPLN (P=0.037); H > L GENOTYPE (P=0.005); G1>G0

*Seedling numbers are those present after 1 year and 2 years respectively
Other fecundity parameters are presented in Appendix 2.

INVASIVENESS, λ

Between Sites: Invasiveness, as an indicator of weediness, was calculated as population growth for each genotype, and was determined for each year (λ_1 and λ_2). Invasiveness values greater than one indicate that a population is expanding and by our definition, may have a risk of weediness. There was a significant effect of site (P<0.001) on both λ_1 (Inv1) and λ_2 (Inv2) illustrated in Fig.1.8.

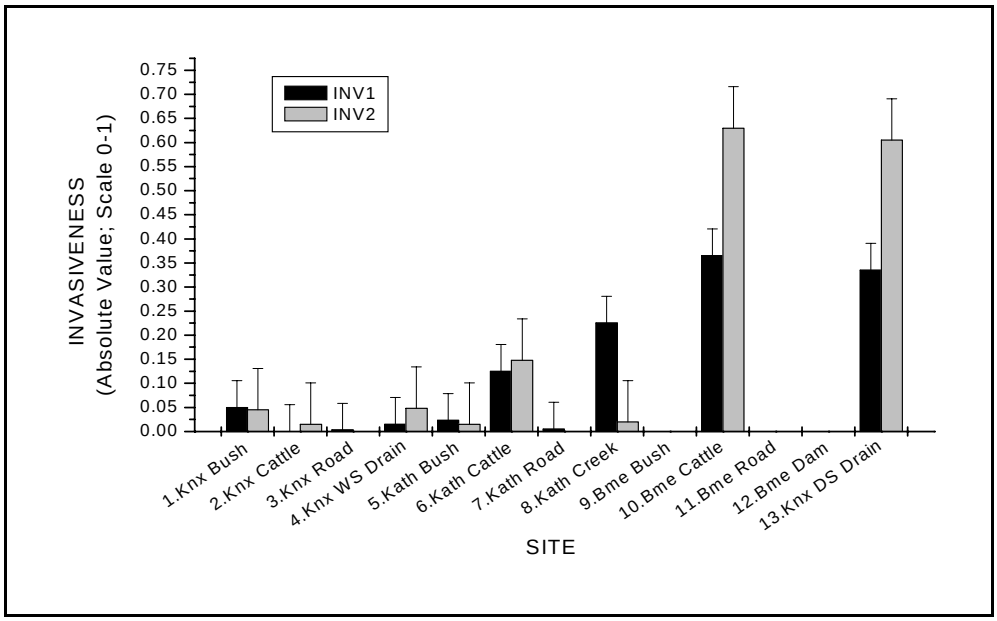


Figure 1.8. Site means for Invasiveness over one and two years (error bars are ± s.e. and are for within each time only)

The extremely low numbers of surviving plants at the majority of sites required that invasiveness within site be assessed using the simplistic method (site means of total plants for each genotype).

All values for λ_1 were less than one, attributable to the high seedling mortality immediately after germination for most sites. Comparable λ_2 values were higher for the majority of sites, although values were still less than one at all sites, with the exception of the Kununurra WS drain site, indicating that populations were declining. Highest values (λ_2 greater than 0.8 as calculated by the simplistic method) were attained for the Kununurra WS and DS drain sites and the Broome Cattle No.1 site. Only one plot with surviving plants of each genotype remained at the Kununurra WS Drain site, so statistical analysis could not determine if there was a significant difference between genotypes.

Results from ANOVA for specific sites where there was adequate plant numbers remaining; Katherine Cattle No.1, Broome Cattle No.1 and the Kununurra DS Drain sites are presented in Table 1.9. All ANOVA calculated means for both λ_1 and λ_2 for these sites were less than 0.8, indicating that all populations were declining. These results are presented in Table 1.9 in italics below the comparative values calculated using the simplistic method.

Within Site: An ANOVA for invasiveness was conducted only on the Katherine Cattle No.1, the Kununurra DS Drain, and the Broome Cattle No.1 sites due to high plant mortality at all other sites.

At no site was there a significant increase in the value of invasiveness for the transgenic lines, G1 and G2, compared to the conventional genotype. Genotype was significant for λ_1 at the Katherine Cattle No.1 site ($P=0.017$) and for λ_2 at the Kununurra DS Drain site ($P=0.002$), where in both cases, the two-gene had lower values than the other genotypes.

Population and fecundity were major influences on invasiveness, although the effects were inconsistent between sites.

The high density treatment produced significantly greater values than the low density treatment for both λ_1 and λ_2 at the Broome Cattle site. However, the reverse occurred at the Kununurra DS Drain site for the λ_1 parameter only.

There was a positive correlation (Spearman's rank correlation; $P<0.001$) between fecundity (maximum open bolls per plot) and invasiveness for both the Kununurra DS Drain and Broome Cattle sites.

Table 1.9. Invasiveness values for each genotype and description of effect if ANOVA was conducted for Experiment 1A *(Numbers in italics below the simplistic values are means calculated by ANOVA for relevant sites)*

INVASIVENESS									
SITE No.	**SITE**	λ_1 **(SIMPLISTIC METHOD: Calculated per Genotype across all other factors)**			**SIGNIFICANT FACTORS**	λ_2 **(SIMPLISTIC METHOD: Calculated per Genotype across all other factors)**			**SIGNIFICANT FACTORS**
		G0	**G1**	**G2**		**G0**	**G1**	**G2**	
1	Kununurra Bush No.1	0.094	0.156	0.012	NA	0.477	0.474	0.0	NA
2	Kununurra Cattle No.1	0.001	0.002	0.0	NA	0.0	0.50	0.0	NA
3	Kununurra Road	0.038	0.0	0.0	NA	0.0	0.0	0.0	NA
4	Kununurra WS Drain No.1	0.069	0.022	0.018	NA	1.0	2.0	0.333	NA
5	Katherine Bush No.1	0.0	0.025	0.0	NA	0.0	0.11	0.0	NA
6	Katherine Cattle No.1	0.135 *(0.175)*	0.163 *(0.151)*	0.038 *(0.044)*	GENOTYPE (P=0.017) G2<G1, G0	0.202 *(0.146)*	0.410 *(0.212)*	0.179 *(0.093)*	SEEDTYPE (P=0.039) S3<S2<S1
7	Katherine Road	0.010	0.014	0.0	NA	0.0	0.0	0.0	NA
8	Katherine Creek	0.417	0.373	0.079	NA	0.135	0.065	0.071	NA
9	Broome Bush No.1	0.0	0.0	0.0	NA	0.0	0.0	0.0	NA
10	Broome Cattle No.1	0.367 *(0.322)*	0.444 *(0.266)*	0.613 *(0.506)*	POPLN (P=0.004) H > L	0.880 *(0.588)*	0.819 *(0.445)*	0.483 *(0.305)*	POPLN (P < 0.001) H > L
11	Broome Road	0.0	0.0	0.0	NA	0.0	0.0	0.0	NA
12	Broome Dam	0.0	0.0	0.0	NA	0.0	0.0	0.0	NA
13	Kununurra DS Drain	0.290 *(0.39)*	0.295 *(0.32)*	0.043 *(0.63)*	POPLN (P=0.02) L > H	0.902 *(0.618)*	0.874 *(0.561)*	0.810 *(0.308)*	GENOTYPE (P=0.002) G2 < G0, G1

SECTION 2

EXPERIMENT 1B: LARGE SCALE ECOLOGICAL ASSESSMENT: SECOND YEAR SELECTED SITES

Introduction

The project to assess weediness potential of Bt cotton in northern Australia was initiated in June 1999, with the initial series of sites sown over the 1999-2000 wet season, designated as Experiment 1, subsequently named Experiment 1A. Results and observations from this initial experiment indicated that there were a number of extrinsic factors that appeared to influence the establishment, growth and development of these experimentally sown cotton plants, both transgenic and non-transformed genotypes.

It was decided that a number of habitats selected from those sown in Experiment 1A, should be repeated to examine seasonal effects, and to incorporate some modifications to treatments applied in the first year. This resulted in seven sites sown over the 2000-01 wet season (designated as Experiment 1B) and consisted of sites approved as either PR89X2 or PR131X2 by OGTR.

A significant modification to the genotype treatment for certain sites was the inclusion of a two-gene genotype, expressing Cry1Ac and Cry2Ab genes. The Cry2Aa gene used in Experiment 1A was superseded by the Cry2Ab gene over the course of 2000. Both two-gene genotypes were included where possible in Experiment 1B to allow a comparison with the results obtained from Experiment 1A.

The Broome Cattle and Bush habitats from Experiment 1A were included in Experiment 1B. Unfortunately due to regulatory constraints and the sowing date required, the new experiments prevented the inclusion of the Cry2Ab gene at these sites.

The Kununurra Drain, Cattle and Bush habitats and the Katherine Bush and Cattle habitats from Experiment 1A were included in Experiment 1B, with modifications to some factors. More detailed description of the sites and experimental factors are provided in the methodology and site description sections for this experiment. The demographic parameters measured remained the same as for Experiment 1A, that is, germination, survivorship and fecundity to enable calculation of invasiveness and conclusions concerning potential weediness between cotton genotypes.

Methodology

This section presents the experimental methods used in Experiment 1B, large-scale ecological assessment and modified second year sowings over seven sites, to evaluate the potential weediness of Bt cotton in northwest Australia. This presents the factors experimentally manipulated, including the modifications made to specific treatments at selected sites repeated from those assessed in Experiment 1A. Methodology was generally consistent with that used in Experiment 1A, so reference to that section provides further detail as required.

DISPERSAL OF THE TRANSGENE INTO THE ENVIRONMENT

Aspects concerning the dispersal of the Bt transgene into the environment remained as for Experiment 1A. That is, seed was considered the major avenue of Bt gene escape, and this series of sowings continued to reflect this.

DESCRIPTION OF EXPERIMENTAL FACTORS

The experimental factors used in Experiment 1B, and an explanation for any modifications from Experiment 1A, are discussed in turn, and summarised in Table 2.1 which presents the treatment levels for within site.

Choice of Locations

The seven sites included in repeat sowings continued to be situated at the three locations studied in Experiment One, namely the Katherine Research Station, Katherine, NT; (2) the ORIA, Kununurra, WA; and (3) Shamrock Station, approximately 150 km south of Broome, WA.

Choice of Habitats

Experiment 1A evaluated the growth and development of cottonseed experimentally sown into four different categories of habitats into which it had been identified that cotton could possibly disperse and volunteer. Results indicated that habitats where cattle dung had modified the soil nutritional status were the habitats where cotton was most likely to establish, survive to maturity and reproduce. Thus, this habitat type was selected at each location for inclusion in Experiment 1B. The "bush" habitat was also selected at each location, due to environmental concern of invasion into native bush habitats.

The "Waterway" habitat category was distinctly different between locations. The drain habitats associated with flood irrigation in the ORIA produced vigorous cotton plants in Experiment 1A, so this habitat was again included in this second series of sowings. Cotton plants did not survive in the waterway habitats at Katherine and particularly Broome, so these habitats were not included in Experiment 1B. Other than the irrigation production area itself, the only waterway habitats within Shamrock Station (Broome) are artificial watering points for cattle, so are more representative of that habitat type. The "Road" habitat at all locations produced plants of extremely low vigour, so this habitat type was not repeated.

This resulted in seven sites with characteristics consistent with their relative sites from Experiment 1A. These are presented in Table 2.2: 'Site Descriptions'.

Seedtype

Cotton seed can be dispersed in three forms; 1) Black or planting seed that has been acid delinted and generally treated with fungicides and/or pesticides; 2) Fuzzy, or ginned seed (majority of the lint removed); and 3) Seed cotton (unprocessed seed with a dense covering of cotton fibres). All three seedtypes were used as treatment levels in Experiment 1A.

For Experiment 1B, modifications were incorporated for selected sites. There were difficulties in obtaining seed cotton of the two-gene seed containing Cry1Ac and Cry2Ab (termed G2X), so this combination was excluded.

Nutrition

Results in Experiment 1A suggested that poor nutrition was a factor in the dramatic site differences observed, particularly for the 'bush' habitat type. Consequently, "Nutrition" was included as a treatment in the Katherine Bush habitat to evaluate this hypothesis. Black seed only was used in an effort to maximise the number of seeds that germinated to allow more robust interpretation of the effect of nutrition on subsequent volunteer cotton growth and development. A similar site was attempted on three occassions at Kununurra but was aborted each time due to extremely low plant establishment. This was attributed to extremely high grasshopper damage which killed the majority of germinated seedlings for the initial sowing, inundation of the site immediately after sowing that appeared to rot all seeds for the second sowing, and lateness of the season by the third sowing . This experiment is discussed briefly as a supplement to Experiment 4 (Section 5).

Population Size

Population as a treatment in Experiment 1A consisted of two levels; High (80 seeds/625 cm^2) and Low (10 seeds/625 cm^2). However, differences in resultant number of seedlings that established did influence demographic parameters. Population level was subsequently modified for the cattle habitat at each location for Experiment 1B, and kept as a constant (50 seeds sown and thinned to 10 plants per 625 cm^2 plot where possible) in an effort to minimise variation in demographic parameters due to differences in number of seedlings established.

Genotype
The commercial transgenic cotton available at the commencement of this project, was INGARD®, containing the Cry1Ac gene from *Bacillus thuringiensis*, although it was envisaged that a two-gene Bt cotton would ultimately form the basis of any proposed northern cotton industry. This two-gene Bt cotton was initially to be the combination of the Cry1Ac and Cry2Aa genes, and the original submission to GMAC was for assessment of these genes (approved as PR89X(2)).

Over the course of the first year of the project, the Cry2Aa gene was superseded by the Cry2Ab gene. As such, the second season of experiments included both the original and new two-gene combinations. Sicot 289 was the common varietal background, but a different variety, DP50 was also utilised in the second season as an additional cultivar background for the Cry1Ac/Cry2Ab genes. Consequently, there were up to five levels of "genotype" as a factor in Experiment 1B (GMAC approved - PR131X(2)): 1) Sicot 289 G0 (Conventional); 2) Sicot 289i G1 (Cry1Ac); 3) Sicot 289ii G2 (Cry1Ac and Cry2Aa); 4) Sicot 289ii G2X (Cry1Ac and Cry2Ab); and 5) Deltapine50 GBX (Cry1Ac and Cry2Ab).

Planting Time
Seed at all sites was experimentally sown to coincide with the traditional onset of rains at the commencement of the wet season at each of the three locations (see Figures 1.3 a-c; Climate averages), consistent with the protocol from Experiment 1A.

EXPERIMENTAL DESIGN

The experimental design within each site was a split-split plot design, based on the structure used in Experiment 1A. Table 2.1 presents the main plot, sub-plot and sub-plot factors within each site.

Planting Method
This was consistent with Experiment 1A, and continued to follow the protocol to maximise germination, providing a worst-case scenario for the escape and establishment of cotton into the selected habitats.

Table 2.1. Description of treatments applied, their levels and allocation in the split-split-plot design

SITE DETAILS	SEEDTYPE LEVELS	POPLN LEVELS	GENOTYPE LEVELS	ADDITIONAL TREATMENTS
Site No.14 Knx Bush No.2	Black Fuzzy	L H	G0 G1 G2 G2X	
Site No.15 Knx Cattle No.2	Black Fuzzy Seed Cotton*	50 seeds sown, then hand-thinned to maximum of 10 seedlings	G0 G1 G2 G2X* GBX	
Site No.16 Knx WS Drain No.2	Black Fuzzy Seed Cotton	L H	G0 G1 G2	
Site No.17 Kath Bush No.2	Black	L H	G0 G1 G2 G2X	NUTRITION N0; none applied N1; fertiliser applied
Site No.18 Kath Cattle No.2	Black Fuzzy Seed Cotton*	50 seeds sown, then hand-thinned to maximum of 10 seedlings	G0 G1 G2 G2X* GBX	
Site No.19 Broome Bush No.2	Black Fuzzy Seed Cotton	L H	G0 G1 G2	
Site No.20 Broome Cattle No.2	Black Fuzzy Seed Cotton	50 seeds sown, then hand-thinned to maximum of 10 seedlings	G0 G1 G2	

*No seed cotton of G2X available.

KEY: Main Plot Factor Sub-plot Factor Sub-sub-plot Factor

DEMOGRAPHIC PARAMETERS AS INDICATORS OF WEEDINESS

The demographic parameters examined were consistent with those used in Experiment 1A, although measurements were conducted for less than an entire season. Tables 1.1, 1.2 and 1.3 specify the actual recording dates for each site.

The demographic parameters for which analyses were conducted were:

Germination: As for Experiment 1A, germination was calculated as the maximum number of plants emerged as a proportion of the number of seeds sown.

Survivorship: This was assessed only at one time; Survivorship 1 was the number of original plants that germinated that were still alive after the initial dry season, corresponding to time of final measurements.

Survivorship per plot was calculated in two ways; as a proportion of the number of seeds sown, plus as an absolute value of the number of plants present, as represented by:

$$\text{Surv1 (proportion)} = \frac{\text{No. of originally germinated plants surviving at the end of the first dry season}}{\text{No. of seeds sown}}$$

$$\text{Surv1 (absolute)} = \text{No. of originally germinated plants surviving at the end of the first dry season}$$

For the Cattle habitat sites, where seedlings were hand-thinned to a maximum of 10, survivorship was evaluated by replacing the number of originally germinated plants with the number of seedlings present after thinning. This effectively maximised survivorship and boosted calculated values for invasiveness as the natural losses after germination were not considered.

Due to the low numbers of surviving plants at most sites, survivorship was also calculated as the total numbers of plants remaining across all factors as a percentage of the total numbers of seeds sown.

Fecundity: This was determined as the maximum number of open bolls produced, and was an indication of reproductive capacity. There was little opportunity to assess seasonality of fruit production as would occur in a perennial plant, as sites were only evaluated for the initial establishment year.

Invasiveness: This was calculated from the population growth rate (λ) which incorporated the previously assessed demographic parameters as discussed above. Population growth rate was calculated at the end of the first year, based on survivorship of original plants plus any seedling recruitment during the year. Calculation of invasiveness after less than an entire season only provides limited opportunity for seedling recruitment to occur, and does not allow for the establishment year to be compared to successive seasons where population may have reached a more stable threshold.

The calculation can be represented as:

λ_1:

$$\frac{\text{No. of original plants remaining after initial dry season (Surv1) + No. of recruited seedlings (atSurv1)}}{\text{Germination (max. no. of plants T1-T2)}}$$

Similarly to Experiment 1A, there were a large number of sites with very few surviving plants, making statistical analysis inconclusive for these sites, so calculation of invasiveness was simplified. The above calculation was applied for each genotype, summed across all other factors.

DATA ANALYSES

Consistent with Experiment 1A.

The only sites with enough surviving plants to conduct robust statistical analysis were the Broome Cattle No.2 and Kununurra WS Drain No.2 sites. Invasiveness results from the simplistic method and calculated via an ANOVA are both presented.

Site Description

This section provides a brief description of each habitat into which cotton was experimentally sown for each location, namely Kununurra, Katherine and Broome. The description includes an outline of vegetation and soil. Climate was discussed in Experiment 1A so is not presented here (refer Section 1.C). Sites are described under each habitat category; Bush, Cattle and Waterway (no Road habitats were resown in the second year).

The site number as allocated in this report for each habitat is presented. There were seven sites for Experiment 1B, approved as either PR89X(2) (containing combinations of Cry1Ac and Cry2Aa genes) or PR131X(2) (containing combinations of Cry1Ac and Cry2Ab genes).

The Release Site Location details and associated PR number as supplied on the OGTR website as of May 2002 (see www.health.gov.au/ogtr/gmorecord/pdfdir/pr89x2cotton.pdf) are included for each site for ease of cross-referencing.

Table 2.2. Site descriptions for Experiment 1B

Site No.	Geographic Location	Habitat Category	Location Description	Land Use	Vegetation	Soil	OGTR details
14	Kununurra	Bush	Behind Meteorological Station, Frank Wise Institute, Durack Drive	Natural Habitat: Open Woodland	Dominant species: *Lysiphyllum cunninghamii, Carissa lanceolata, Flemingia panciflora, Chrysopogon fallax, Sorghum timorense* Secondary species: *Trichodesma zeylanicum, Iseilema marratherum, Hibiscus panduriformis, Chionachne hubbardiana, Passiflora foetida*	Cununurra clay (alkaline phase) Brownish cracking clays with high pH topsoils	Kununurra Bush: Site Two, PR89(X)2
15	Kununurra	Cattle	Bay 8F, Frank Wise Institute, Durack Drive	Man modified: Leucaena paddock (irrigated)	Dominant species: *Leucaena leucocephala, Digitaria eriantha (Pangola)*	Cununurra Clay (alkaline phase) Brownish cracking clays with high pH topsoils	Kununurra Cattle Bay 8F: Site Five, PR131 (X) 2
16	Kununurra	Waterway Wet Season Drain	Higher on the bank than the Kununurra Wet Season Drain Expt. 1 site.	Man-modified: Irrigation drain in disturbed woodland	Dominant species: *Ludwigia spp, Hibiscus panduriformis, Brachyachne convergens, Stylosanthes spp, Sida spp, Heteropogon contortus, Aristida spp, Echinochloa colona, Fimbristylis spp* Secondary species: *Lysiphyllum cunninghamii, Digitaria spp, Alyscarpus vaginalis*	Cununurra Clay Cracking clay with some coarse sand	Kununurra Drain Site 2: Site Seven, PR89(X) 2
17	Katherine	Bush	Napier Paddock, Katherine Research Station, NT&DPIF	Natural habitat: Open Woodland	Dominant species: *Chrysopogon spp, Heteropogon contortus, Erythrophlem chlorostachys, Eucalyptus tetradonta,* Secondary species:	Red Earth, Tippera Clay Loam	Katherine Bush Site 2: Site Nine, PR 89(X) 2
18	Katherine	Cattle	Dryland Leucaena Ganley Paddock Katherine Research Station, NT DPIF	Man modified: Leucaena paddock (Dryland)	Dominant species: *Leucaena leucocephala, Urochloa mosambicensis* Secondary species: *Stylosanthes humilis*	Tippera clay loam	Katherine Cattle Site 2, Back Paddock: Site Fourteen, PR 131 X(2)
19	Broome	Bush	In close proximity to Bush No. 1 site, so location, vegetation and soils are as for above	Natural habitat: Pindan community	Dominant species: *Acacia hippuriodes, A. eriopoda, Plectrachne* spp. Secondary species: *A. colei, Distichostemon hispidulus, Newcastelia spp*	Yeeda Land System Sand plain with deep red sands	Broome Bush Site 2: Site Twenty, PR89(X)2
20	Broome	Cattle	Lunguda cattle yards, Shamrock Station	Man modified: Non-used yards	Dominant species: *Citrullus lanatus* Secondary species*: Cenchrus spp*	Yeeda Land System Sand plain with deep red sands	Broome Cattle Site 2, Lunguda Yards: Site Nineteen, PR89(X)2

Results and Discussion

All the series of sites that constituted Experiment 1A had the same treatments and treatment levels applied within each site, allowing the site to be included in the overall model. However, for the series of sites in Experiment 1B, there were various treatment combinations applied between sites, so results are presented with significant factors and a brief description of the effect for each site. The demographic parameters assessed; Germination, Survivorship 1, Fecundity and Invasiveness are presented in summary tables below. More detailed analysis, results and discussion pertinent to each site are included in Appendix 3: Individual Site Results for Experiment 1B.

Germination

Direct comparison between the seven sites was not possible for this experiment due to differences in experimental design, although experimental factors were generally similar to those applied in Experiment 1A. There were significant genotype effects, or interactions with either seed type or population on germination, but these varied between sites. These differences may be due to differences in seed quality as a result of storage or differing plant or seed treatment practices at the source of the seed.

Results for the within site effects for germination are presented in Table 2.3. There were no significant factors on germination for the Broome Cattle No.2 (no population treatment) and Kununurra Bush No.2 sites (no seed cotton sown).

Population was significant (P=0.032) for the Katherine Bush No.2 site (seedtype removed as a factor) with the low level having a lower germination compared to the high density treatment. Seedtype (P<0.001), or its interaction with population (P=0.026), was significant for the four remaining sites. The significant influence of seedtype was consistent overall with Experiment 1A, with seed cotton generally having the lowest germination, followed by fuzzy seed then by black seed with the highest. The only anomaly was at the Katherine Cattle site but a high incidence of wireworm was observed in a large number of plots. Lint on the seed cotton may have aided in prevention of predation, which resulted in the relatively higher germination.

This second series of sowings did produce genotype effects that were not evident in Experiment 1A.

The two genotypes containing the Cry2Ab gene (G2X and GBX) had the lowest germination at the Kununurra Cattle No.2 site. This was inconsistent with results for the Katherine Cattle No.2 site, where the G2X genotype had the greatest germination, and the Broome Bush No.2 site where the conventional had the lowest germination. These effects were not observed in the initial year, when all seed was sourced from the same field, and the processes of ginning and delinting and the time until sowing were consistent between genotypes. In the second year, seeds containing the Cry2Ab gene were obtained from different paddocks than those from where the G0, G1 and G2 were obtained. The ginning and delinting requirements for each genotype were determined by the availability of the different seedtypes remaining from the first experiment, resulting in different lengths of storage time for different genotypes and seedtypes prior to sowing. Laboratory tests for black seed only showed that germination of the G2X seed was significantly lower than the other four genotypes. The confounding effects of seed history and source were likely to have contributed to differences in germination within each site, but effects were variable. There were no consistent results to support or deny that the addition of the Bt gene inferred any additional fitness to germination.

Table 2.3. Significant factors on germination for Experiment 1B

GERMINATION			
SITE No.	**SITE**	**SIGNIFICANT FACTORS**	**DESCRIPTION OF EFFECT**
14	Kununurra Bush No.2#	NS	NA
15	Kununurra Cattle No.2 ###	SEEDTYPE (P<0.001) GENOTYPE (P<0.001)	S3<S2<S1 G2X, GBX<G0,G1,G2
16	Kununurra WS Drain No.2	SEEDTYPE (P<0.001)	S3<S2<S1
17	Katherine Bush No.2##	POPULATION (P=0.032)	L<H
18	Katherine Cattle No.2###	SEEDTYPE (P<.001) GENOTYPE (P<.001)	S2, S1<S3 G0, GBX, G2, G1<G2X
19	Broome Bush No.2	SEEDTYPE*POPLN (P=0.026)	H > L treatments for S1 and S3, but L > H for S2.
		GENOTYPE (P=0.04)	G0<G1 and G2
20	Broome Cattle No.2###	NS	NA

No seed cotton was sown in this experiment
Seedtype was removed as a treatment, and all plots were sown to black seed
Seedlings were hand-thinned

Survivorship

Results for the within site effects for survivorship are presented in Table 2.4. After the one year, only three of the seven sites had greater than 50% of plots with any surviving plants. This corresponded to 42.8% of seeds originally sown for the Kununurra WS Drain No.2 site, and 11.9% and 81.7% of the number of seedlings to which the populations were thinned for the Kununurra Cattle No.2 and Broome Cattle No.2 sites respectively (where population had been removed as a factor). An ANOVA was conducted on each of these three sites. There were no significant effects for the Kununurra and Broome Cattle sites (attributed to hand-thinning reducing the seedtype effects).

There was a significant seedtype by population interaction (P=0.026) on survivorship as a proportion of number of seeds sown, for the Kununurra WS Drain No.2. There was an increase in survivorship from the high to low density treatments for fuzzy seed and seed cotton, but a reverse trend for the black seed. Plants derived from seed cotton had the lowest survival at both population levels. For absolute survivorship, there was again a significant seedtype by population interaction (P=0.045) with black seed the greatest survival, followed by fuzzy seed than by seed cotton at the high population density, but little effect of seedtype on number of plants remaining at the low population density level.

Table 2.4. Significant effects on survivorship 1 for Experiment 1B (or description of plants remaining where there were only isolated plots with surviving plants)

SURVIVORSHIP 1						
SITE No.	**SITE**	**NO.OF PLOTS WITH SURVIVING PLANTS (Plants as % of seeds sown) #**	**DESCRIPTION (Significant effects on No. of Plants remaining as a proportion of No. of seeds sown)**			
14	Kununurra Bush No.2	6/64 (0.56)	**G0** P17: 2 plants (S1L) P25: 8 plants (S2H)	**G1** P35: 2 plants (S2L)	**G2** P33: 1 plant (S2L)	**G2X** P9: 2 plants (S1H) P56: 1 plant (S1H)
15	Kununurra Cattle .2#	29/56 (11.9%)	NO SIGNIFICANT FACTORS			
16	Kununurra WS Drain No.2	50/54 (42.8%)	SEEDTYPE*POPULATION (P=0.026) H > L for S2 and S3, but L > H for S1.			
17	Katherine Bush No.2	6/64 (1.2%)	**G0** P42: 1 plant (N0L) P50: 1 plant (N0L) P55: 8 plants (N0H)	**G1**	**G2** P40: 2 plants (N1H)	**G2X** P47: 20 plants (N0H) P54: 2 plants (N0H)
18	Katherine Cattle No.2#	13/56 (8.37%)	**G0** P7: 5 plants (S3) P24: 2 plants (S1) P25: 3 plants (S3) P54: 1 plant (S2)	**G1** P9: 5 plants (S3) P11: 6 plants (S1) P37: 1 plant (S2) P52: 2 plants (S2)	**G2** P13: 4 plants (S1) **GBX** P8: 1 plant (S3) P53: 1 plant (S2)	**G2X** P14: 2 plants (S1) P56: 1 plant (S2)
19	Broome Bush No.2	0/72 (0%)	NO SURVIVING PLANTS			
20	Broome Cattle No.2#	24/36 (81.7%)	NO SIGNIFICANT FACTORS			

N0=No nutrition applied; N1=Nutrition applied
#Survivorship assessed as proportion of the thinned population

Fecundity

Plants in most sites displayed slow physiological development, consistent with Experiment 1A. Numbers of total open bolls produced within each site are presented in Table 2.5. The only sites in which plants developed to produce bolls within the one year were the Broome Cattle Site No.2 and the Kununurra WS Drain No.2 site. For the remaining five sites, three did not produce any fruiting structures at all, whilst the other two, the Kununurra Bush No.2 and the Katherine Bush No.2 (nutrition applied plot) produced isolated and immature fruit. There were no significant factors for maximum number of bolls per plot produced for the Broome Cattle No.2 and the Kununurra WS Drain No.2 sites.

The Kununurra WS Drain site provided an opportunity to assess number of open bolls produced per surviving plant due to the high numbers of plots remaining plants at final measurements. There was a significant effect of both population (P=0.003) and of genotype (P=0.033) on number of open bolls produced per surviving plant (Box-Cox transformation; $z=y^{-0.144}$). There were a greater number of bolls produced per plant from the low population treatment than the high density treatment; means for non-transformed data were 7.77 and 3.72 bolls per plant for the low and high population treatments respectively (s.e.=1.35).

The two-gene treatment produced significantly fewer open bolls per surviving plant than the conventional and single-gene genotypes; means for non-transformed data were 3.92, 6.64 and 6.68 for G2, G0 and G1 respectively (s.e. = 1.51).

Table 2.5. Number of open bolls produced and significant factors on fecundity for Experiment 1B

FECUNDITY (Maximum Number of Open Bolls)					
SITE No.	**SITE**	**Mean Maximum No.of Bolls per plot**			**SIGNIFICANT FACTORS and DESCRIPTION OF EFFECT**
		G0	**G1**	**G2**	
14	Kununurra Bush No.2	0	0	0	
15	Kununurra Cattle No.2	0	0	0	
16	Kununurra WS Drain No.2	375	531	419	NO SIGNIFICANT FACTORS
17	Katherine Bush No.2	0	0	0	
18	Katherine Cattle No.2	0	0	0	
19	Broome Bush No.2	0	0	0	
20	Broome Cattle No.2	328	372	163	NO SIGNIFICANT FACTORS

Invasiveness, λ

Invasiveness was calculated for consistency with Experiment 1A, but sites persisted for less than one year before completion of the project, so no seedling recruitment could occur. Essentially, the calculation of invasiveness was the equivalent of assessment of survivorship as a proportion of seeds that had germinated.

An ANOVA was conducted on the two sites for which there were adequate numbers of plots with surviving plants remaining at the end of the year. There was a significant population by seedtype effect (P=0.032) on the Kununurra WS Drain No.2 site. There was an increase in the value of λ_1 from the high to the low population treatment for fuzzy seed and seed cotton, but a reverse relationship for black seed. At the high population density, plants derived from black seed had the greatest invasiveness compared to the other two seedtypes. However, at the low population density, there was no difference in invasiveness between seedtypes.

For the other applicable site, the Broome Cattle No.2, values for λ_1 were greater than 0.7 for all genotypes, supporting that this habitat is conducive to cotton plant establishment, irrespective of genotype, but there was no significant effect of any factor.

A summary of the results from these ANOVA and the simplistic method for the five sites for which there were only isolated plants remaining are provided in Table 2.6.

Table 2.6. Invasiveness values for each genotype for Experiment 1B (*Numbers in italics below the simplistic values are means as calculated by ANOVA for relevant sites*)

INVASIVENESS							
SITE No.	**SITE**	**λ_1 (SIMPLISTIC METHOD: Calculated per Genotype across all other factors)**					**SIGNIFICANT FACTORS and DESCRIPTION OF EFFECT if ANOVA was conducted**
		G0	**G1**	**G2**	**G2X**	**GBX**	
14	Kununurra Bush No.2	0.015	0.003	0.002	0.004		NA
15	Kununurra Cattle No.2	0.157	0.116	0.075	0.270	0.092	NA
16	Kununurra WS Drain No.2	0.623 *(0.638)*	0.664 *(0.637)*	0.597 *(0.569)*			POPULATION x SEEDTYPE (P=0.032) Increase in λ1 from H to L for fuzzy seed and seed cotton but the reverse for black seed
17	Katherine Bush No.2	0.024	0.0	0.006	0.056		NA
18	Katherine Cattle No.2	0.141	0.156	0.044	0.041	0.027	NA
19	Broome Bush No.2	0.0	0.0	0.0			NA
20	Broome Cattle No.2	0.840 *(0.502)*	0.868 *(0.519)*	0.718 (*0.327*)			NO SIGNIFICANT FACTORS

SECTION 3

EXPERIMENT 2: COMPARISON OF GERMINATION OF THREE FORMS AND THREE GENOTYPES OF COTTONSEED WHEN BURIED OR LEFT ON THE SURFACE OF THE SOIL.

Introduction

This was the first of the large-scale ecological study sites to be sown, originally on 26 November 1999. However, extremely variable germination led to modification of the design for all subsequent sites – such as burying the seed and distributing the seeds evenly in the plot. The need for a second planting presented the opportunity to conduct a more specific experiment; that of buried versus unburied seedtype, and superseded the original sowing.

It was considered that there were three forms of seed by which cotton can escape into the environment. These were black seed, fuzzy seed, or seed cotton. Preceding experiments where seed was buried and watered to provide optimum conditions for germination to provide worst case scenario for weed establishment indicated that there was a significant difference in germination between these seed forms.

It was recognised that seed needed adequate seed/soil contact for maximum germination. In 'escape' conditions, a significant variable was whether the seed was covered by soil, or remained on the soil surface. Subsequent germination was a major determinant of the success of cotton plants establishing as volunteers, with differences in germination a major variable affecting risk assessment associated with the different forms of seed escaping into the environment. In particular, this was applicable to seed cotton that may fall to the roadside during transport between harvest and ginning. Other instances could include fuzzy seed falling to the ground in cattle feeding areas, or black seed falling onto the soil surface at planting time in areas away from intended cultivated areas.

This experiment aimed to compare germination between the three forms of seed, incorporating three genotypes, under two soil coverage conditions.

Methodology

The experimental design was a split-split-plot with seed type as the main plot treatment, genotype as the sub-plot treatment, and depth as the sub-sub-plot treatment. One hundred seeds were hand-sown in a 25 cm by 25 cm quadrat. Soil was removed to a depth of approximately 4 cm, seed pressed gently into the disturbed soil, then covered with the original soil for the buried treatment, or left remaining on the soil surface. Although germination was the key parameter assessed for this experiment, progressive growth and development of the plants was also evaluated.

Seed was hand-watered after sowing, and emerged seedlings were watered to time of predicted first square, to remain consistent with the other sites for Experiments 1A and 1B. Seedling growth, development, fruit production and survivorship were assessed as for the other sites as described in Experiments 1A and B.

Once development occurred, plant counts, and fruit counts were taken every two weeks after sowing until 7 August 2000 (T14), then modified to season (commencement of the wet season; 12 December T15, then end of the wet season; 10 April 2001 T16). It was not intended to conduct another series of measurements until the commencement of the 2001-2002 wet season, but a fire through the site on 30 July necessitated data collection the next day to record what fruit production was occurring. The final measurement was on 25 November 2001 (T18) at which time there was some regeneration of burnt plants.

Data analysis was similar to that conducted for Experiments 1A and 1B.

Results and Discussion

Germination

Seed was sown on 10 February. Initial germination counts were done on 14 February (4 DAS), then plant counts at 11 DAS, 18 DAS, then at less frequent intervals.

There was a significant seedtype by depth interaction (P<0.001) as illustrated in Figure 3.1.

Each seedtype had a significantly greater germination when buried as compared to remaining on the surface. Within the buried treatments, seed cotton had significantly lower germination than the other two seedtypes, which was consistent for the majority of sites in the series of experiments where all seeds were buried to maximise germination.

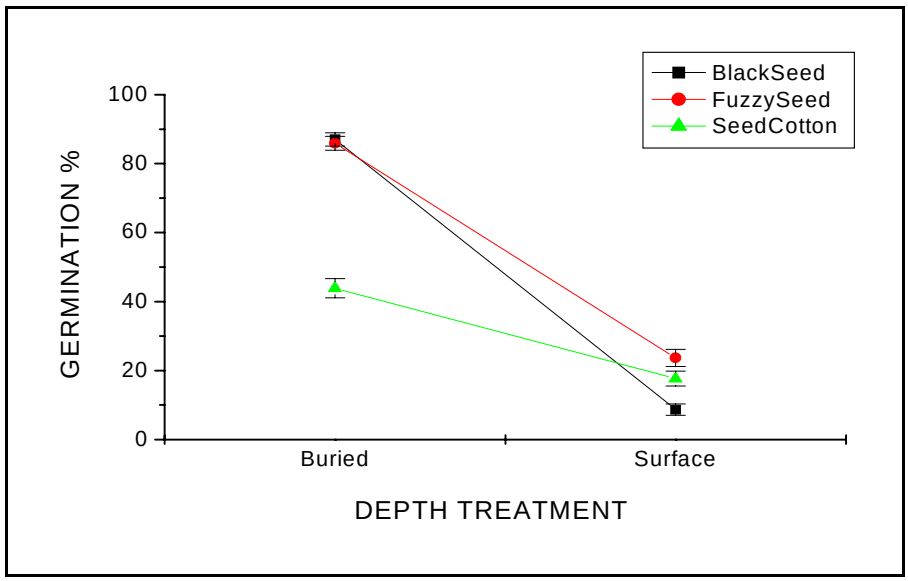


Figure 3.1. Effect of depth by seedtype interaction on germination (error bars are ± s.e.)

Survivorship 1

Measurements for plants surviving after the initial dry season (2000) were conducted on 12 December, 2000 (T15). There were 22 plots with surviving plants (out of a total of 54).

Survivorship 2

There were only two plots, each with only a single plant regenerating, when measurements for survivorship at the end of the second dry season were conducted on 25 November, 2001 (T18). However, the site had been severely burnt on 30 July, prior to which 11 plots had surviving plants. Table 3.1 presents the plots and treatments surviving prior to the time of the fire, and final survivorship. Photos 3.1a,b and c illustrate the site before and after fire, and a regenerating plant from a burnt plot.

Table 3.1. Fruit production and surviving plants (before and after fire)

Plot No.	Treatment B=Buried, S=Surface	No. of plants (Before fire)	Surviving after fire	Fruit production (Open bolls)
17	S1 G0 S	2	0	0
23	S3 G1 B	3	0	0
26	S1 G1 B	16	0	0
32	S2 G0 S	11	0	0
33	S2 G1 S	5	0	2
34	S2 G1 B	56	1	8
45	S2 G0 S	13	0	2
46	S2 G0 B	38	0	2
47	S2 G1 S	6	0	5
48	S2 G1 B	14	1	4
49	S3 G1 S	1	0	0

Fecundity
Six plots within close proximity to each other developed open bolls, with fruit production for the surviving plants described in Table 3.1.

Invasiveness
No second generation seedlings were produced, even though open bolls had been produced prior to the onset of the 2000-01 wet season. Open bolls were present on the plants at the time of the fire, although not burnt thoroughly. Rain had subsequently fallen on the bolls, but no seedlings had germinated to the time of the last measurement (November 2001).

A total of 1,800 seeds (three seedtypes) were sown for each genotype. Calculations were done for the buried treatments only, to allow comparison with the other sites. From the 900 seeds sown for the buried treatment, the number that germinated were 642, 659 and 650 for G0, G1 and G2 respectively. Numbers of plants present for each genotype after the first dry season were 68, 150 and 22, and at the measurement prior to the fire they were 38, 89 and 0. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 = 0.1060; G1 = 0.2276; G2 = 0.0339

λ_2: G0 = 0.5588; G1 = 0.5933; G2 = 0.0

Site Discussion
Results from this experiment indicate a clear reduction in germination of seed on the soil surface. The approach used for the large-scale ecological study was to maximise the probability of cotton plants establishing to provide a worse case scenario of potential weediness, so all seeds were buried. However, the results here suggest that the ratio of buried to surface seed germination (e.g. Fuzzy – seed buried 86%, surface 24%, ratio 0.28) could be used as a correction factor for the germination results for the other sites to achieve more realistic values.

Ideally, a comparison of buried versus surface germination should be duplicated at a number of habitats, which may result in a different 'correction value' for each site. However, it could be confidently assumed that the experimental germination numbers for all sites in the large-scale ecological experiments would be significantly less if the seed had not been buried.

Production of fruiting structures was not expected at this habitat due to the poor water holding capacity and nutrition of the sandy soil type. However, fruiting plants were within a narrow range in the design indicating a suitable microhabitat. It was also suspected that this area had an elevated water table due to the very good wet seasons for the duration of the project, with other areas in the valley reporting unusual perched water tables after the 2000 wet season.

a

b

c

Photos 3.1 a, b, and c. Kununurra seedtype x depth x genotype experiment;
a. before the fire (30 July 2001);
b. after the fire (31 July 2001); and
c. regenerating plant (25 November 2001).

SECTION 4

EXPERIMENT 3: SURVIVABILITY OF THREE FORMS OF COTTONSEED SUBJECT TO ENVIRONMENTAL CONDITIONS OVER A DRY SEASON

OBJECTIVE

To compare the capacity of black, fuzzy and seed cotton to survive under dry season environmental conditions, and evaluate the subsequent germination potential of this seed at the onset of the wet season.

Introduction

Observations from road and paddock verges (1999-2000) indicate that after harvest, seed cotton can germinate over the wet season (October to May), establish as volunteer cotton and develop bolls which may produce mature seed over the duration of the subsequent dry season (May to October). The onset of the dry season in northern Australia corresponds to the commencement of sowing of dry season irrigated crops, and also mustering activities on pastoral properties. Thus, cottonseed may be introduced into the environment at this time as black seed from planting spillage, or as fuzzy seed if feeding cattle. This illustrates the possibility of three forms of cottonseed being introduced into the environment at some stage during the dry season.

The period of time that seed remains exposed to dry season environmental conditions until conditions are conducive to germination at commencement of the next adequate rainfall, is a significant variable, which needs to be considered when assessing subsequent germination potential. This experiment was a modification of protocols in proposal PR89X2 which involved sowing seed into a range of habitats to assess dry season germination. In reality, in the majority of habitats, seed will not have suitable conditions for germination until the following wet season, so this experiment was designed to quantify changes in seed viability over this dry season period.

Evaluating germination potential of seed that may disperse at some time during the dry season, allows some conclusions to be made concerning the probability of establishment of volunteer cotton, and the potential for weediness.

Methodology

The site was in a native bush habitat on a Cununurra clay situated on Frank Wise Institute, Kununurra, amidst the “Bush” Site used for Experiment 1A.

The experimental design was a randomised complete block with three replicates. There were three factors: Genotype, Seed type and “Duration of Exposure”, resulting in a total of 36 treatments.

Genotype:	Seed type:	Duration of Exposure:
1. Conventional, G0	1. Black seed	1. 7 DAS (late July)
2. Single gene, G1	2. Fuzzy seed	2. 28 DAS (late Aug)
3. Double gene, G2	3. Seed Cotton	3. 3 months A.S. (late Oct)
		4. Until Wet Season (mid-Dec)

Seeds were sown after the commencement of the dry season, on 28 July 2000. Twenty-five seeds per plot were placed in nets approximately 10 cm by 10 cm, created from commercially available flywire, illustrated in Photo 4.1. These were placed on a small hand-cleared area, then secured with wire pegs. Plots were 2 m apart.

The nets containing the seeds were collected after the appropriate time period (Time 1 through to Time 4). Seed viability was then evaluated through a laboratory germination test. At each time, a supplementary germination test of the seed treatments (genotype x seedtype) stored in the laboratory (not coolroom conditions) was also conducted for comparison (control; CNTRL).

Results and Discussion

There were no plots with seeds that germinated by the end of the dry season. Seeds appeared to be chewed, primarily by small vertebrates (evidenced from the chewing of the nets). Figure 4.1 shows the decline in germination percentage over time. Photo 4.1 illustrates the seeds in their nets, and damaged observed.

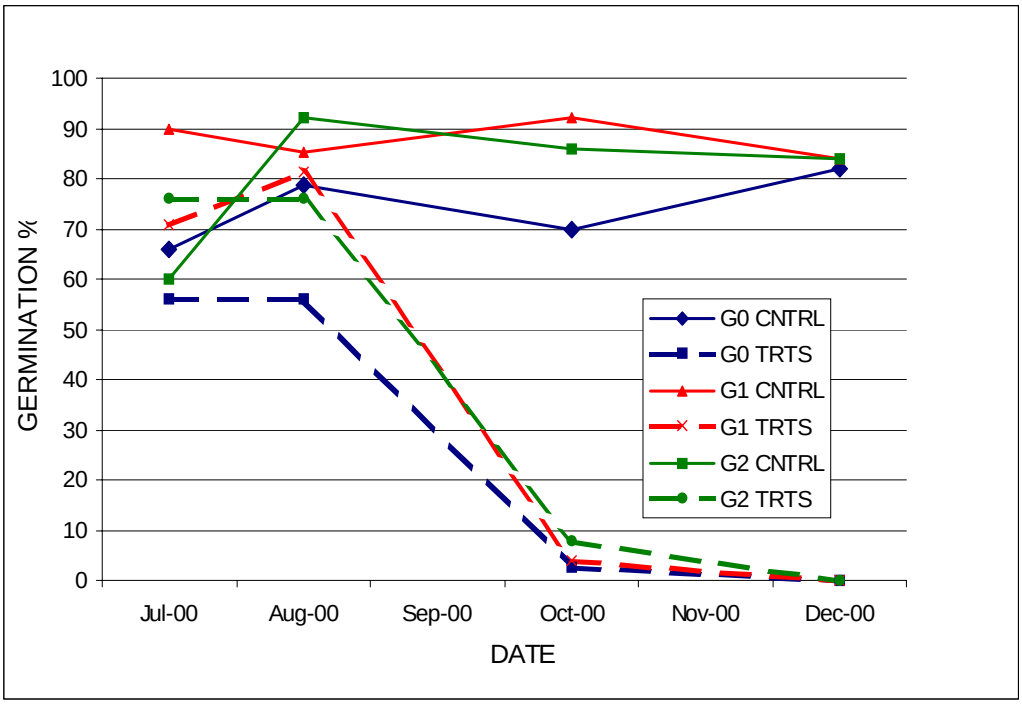


Figure 4.1. Decline in germination of genotypes across three seedtypes when exposed to Kununurra dry season conditions for different durations

The lack of viable seeds remaining by the commencement of the wet season indicated that genotype does not influence the survival of seeds which may be dispersed onto the ground in a native bush habitat over a dry season. This may be due to insect predation or from small animal foraging, prior to the commencement of the wet season.

However, seed remaining on the cotton bush is less susceptible to being eaten, although it was observed to rot in the boll after rain if it was not dislodged to the ground to potentially germinate. These results were consistent with observations from established volunteer cotton plants being monitored as a component of the larger ecological study, as discussed in Section 7.

The habitat in northern Australia most likely to have cottonseed introduced over the dry season is cattle feeding areas, where fuzzy seed is used as a valuable supplementary feed. These habitats may have a different spectrum of vertebrate and invertebrate seed predators than a native bush habitat. Consumption of the seed would be predominantly from the intended species – cattle, although some seed may remain in inaccessible niches, e.g.under a cattle trough, where subsequent germination could occur. These seedlings would be subject to grazing pressure and trampling. There was no evidence to suggest that survival of cotton seed, as a precursor to germination, was enhanced by the addition of the Bt gene(s).

Photo 4.1. Chewed seeds (fuzzy) from dry season seed survivorship experiment

SECTION 5A

EXPERIMENT 4: GENOTYPE VS NUTRITION INTERACTION

OBJECTIVE

To examine the effect of soil nutrient levels on seed production between three cotton genotypes where water availability and weed competition are not constraints to cotton development. This was achieved by comparison of the growth and development between Bt transgenic (single gene and two-gene) and conventional cotton subject to three nutrition levels where insect pressure was not manipulated.

To determine the effect of soil nutrition on the insecticidal efficacy of Bt genotypes.

Introduction

Field ecological experiments conducted at numerous habitats in the 1999-00 wet season (Experiment 1A) demonstrated poor growth and development of both transgenic and conventional cotton. The only habitat from the original 12 sites where cotton developed (phenologically) as predicted according to heat unit accumulation (see Constable and Shaw 1988) was in unused cattle yards (Shamrock Station). This was attributed to a higher level of nutrition relative to other habitats. Also of importance, at this site, was a lack of interspecific plant competition (due to severe grazing pressure from periods when cattle were yarded previously). Observations from other habitats that had higher levels of soil fertility (e.g. leucaena sites grazed by cattle) relative to 'natural' habitats where soil fertility is inherently low, indicated that the increased nutrition benefited the cotton seedlings, but also benefited the grass species present. These grasses rapidly outcompeted establishing cotton seedlings. Habitats most likely to lead to establishment and development of volunteer cotton plants were those with adequate nutrition plus low levels of interspecific plant competition (given that all sites at each location had similar moisture regimes).

Plant nutrition will not only influence cotton growth and development, but may also influence the expression of the Bt gene. This experiment aimed to determine whether the Bt gene will be expressed when the plant is under nutritional stress. This has significant implications for the potential for Bt cotton to become a weed as a result of increased fitness due to the addition of the Bt gene, if the inherently poor nutritional status of northern Australian soils is considered.

Methodology

The experiment was a split-plot design consisting of four blocks within the unsprayed section of Paddock 7A at FWI, Kununurra. The experiment was conducted under commercial cotton production guidelines except no insecticides were used post-emergence (Maize-bait® was applied immediately after sowing to minimise damage to emerging seedlings from wireworms and grasshoppers).

Main plot treatment was nutrition at three rates; residual (N0 - no additional fertilised applied); optimal (N2 - fertilised as applied to bulk growing areas) and sub-optimal (N1 - at one-third standard rate to represent nutrition as may be experienced by volunteer plants away from cultivated paddocks). Fertiliser was applied by commercial rig prior to sowing (7 May 2000). Nitrogen was applied as a side dressing of urea on 16 June 2000. Refer to Table 5.1 for specific composition of fertiliser added.

Sub-plot treatment was genotype - conventional (G0), single gene (G1; Cry1Ac) and double gene (G2; Cry1Ac + Cry2Aa) of variety Sicot 289.

Black seed only was sown (compared to previous work with three seed types) at 10 seeds/m using the Cone Seeder on 13 May 2000. There was unsatisfactory emergence (attributed to ground preparation), so some sections were re-sown on 24 May 2000. Plots were 15 m long by 6 rows (3 x 1.8 m beds). Fertiliser runs were 45 m. The non-treatment 15m each end of the treatment plots was sown with Siokra L23i. The buffer beds (1,2,12,13,23,24) were sown to Siokra V16i with the Max-Emerge® planter, consistent with the remaining bulk area of 7A. Stomp® was applied as a pre-emergent herbicide. Seed was treated with Apron® for protection from *Pythium* and *Phytophthora.* Irrigation was as standard so that no treatment was limited by moisture.

A detailed plan of the experimental layout is provided in Figure 5.1.

MEASUREMENTS

Soil Samples

Soil samples were taken prior to sowing as soon as paddocks were trafficable after the wet season. One sample was taken in each block, corresponding to two samples in the previous lablab area, and two samples in the previous sunflower area, divided into the head ditch and the tail drain ends.

Initial samples were 0-15, 15-30, 30-60 and 60-90 cm. This was to establish the paddock's nutrient status, particularly for the residual treatment. Paddock history; 1999 was lablab/sunflower, and prior to that, paddock was a long-term leucaena cattle grazed paddock.

Bioassays Efficacy

Bioassays were conducted at fortnightly intervals from 1st square to cutout. The first bioassay was conducted on the 10 July, with the final bioassay (number 6) conducted on the 6 September. Five 4th node leaves from the middle four rows were collected from each plot. One neonate *Helicoverpa armigera* larvae was placed on each leaf. Mortality and weight was recorded four days later.

Plant Measurements

Plant measurements were taken at two times during the growing season, corresponding to time of predicted first square, 510DD12 (48DAS; 5 July) and maximum boll number, 1400DD12 (117DAS; 12 September) respectively. Measurements consisted of plant counts, height, numbers of nodes, number of plants tipped, and numbers of squares, flowers, and green bolls. These were taken from 1 m by the two outer middle rows per plot (rows 2 and 5).

Final plant measurements consisted of plant counts, height, numbers of nodes, number of plants tipped, and numbers of squares, flowers, green bolls and open bolls over 1 m by two rows (2 and 5) just prior to harvest (31 October).

Final harvest to determine plot yield was done with an experimental single row picker over two rows (3 and 4) by 12 m of row on the 5 November. A sub-sample of seed cotton was taken (approximately 300 g) and laboratory ginned to determine number of seeds and the weight of 100 seeds (of significance in this experiment compared with lint yield) for each treatment. Twenty-five of these seeds were tested for germination two weeks after harvest. This provided an indication of viability of seed from the three genotypes that would be present to germinate in the field after harvest with oncoming wet season rains. Results were analysed by ANOVA or Regression analysis (logit trasformation for germination and mortality data) using Genstat®.

Table 5.1. Nutrient composition of fertiliser treatments applied

Product applied	N2 : Optimal nutrition		N1 : Sub-optimal Nutrition (1/3 rate)	
	Rate (kg/ha)	Amount of nutrient applied (kg/ha)	Rate (kg/ha)	Amount of nutrient applied (kg/ha)
Urea	330	150 N	110	50 N
DAP	250	44 N, 50 P	83	15 N, 16 P
Sulphate of Potash	96	40 K, 16 S	32	13 K, 5 S
Kieserite	68	17 Mg	23	6 Mg
Boronate	6	1.9 B	2	0.6 B
Telsinogram	2	2 Mn	0.7	0.7 Mn
Essential Minerals	25	1.5 S 3.0 Mg 0.125 B 0.5 Cu 1.5 Zn 0.00125 Mo	8	0.5 S 1 Mg 0.004 B 0.167 Cu 0.5 Zn 0.0004 Mo

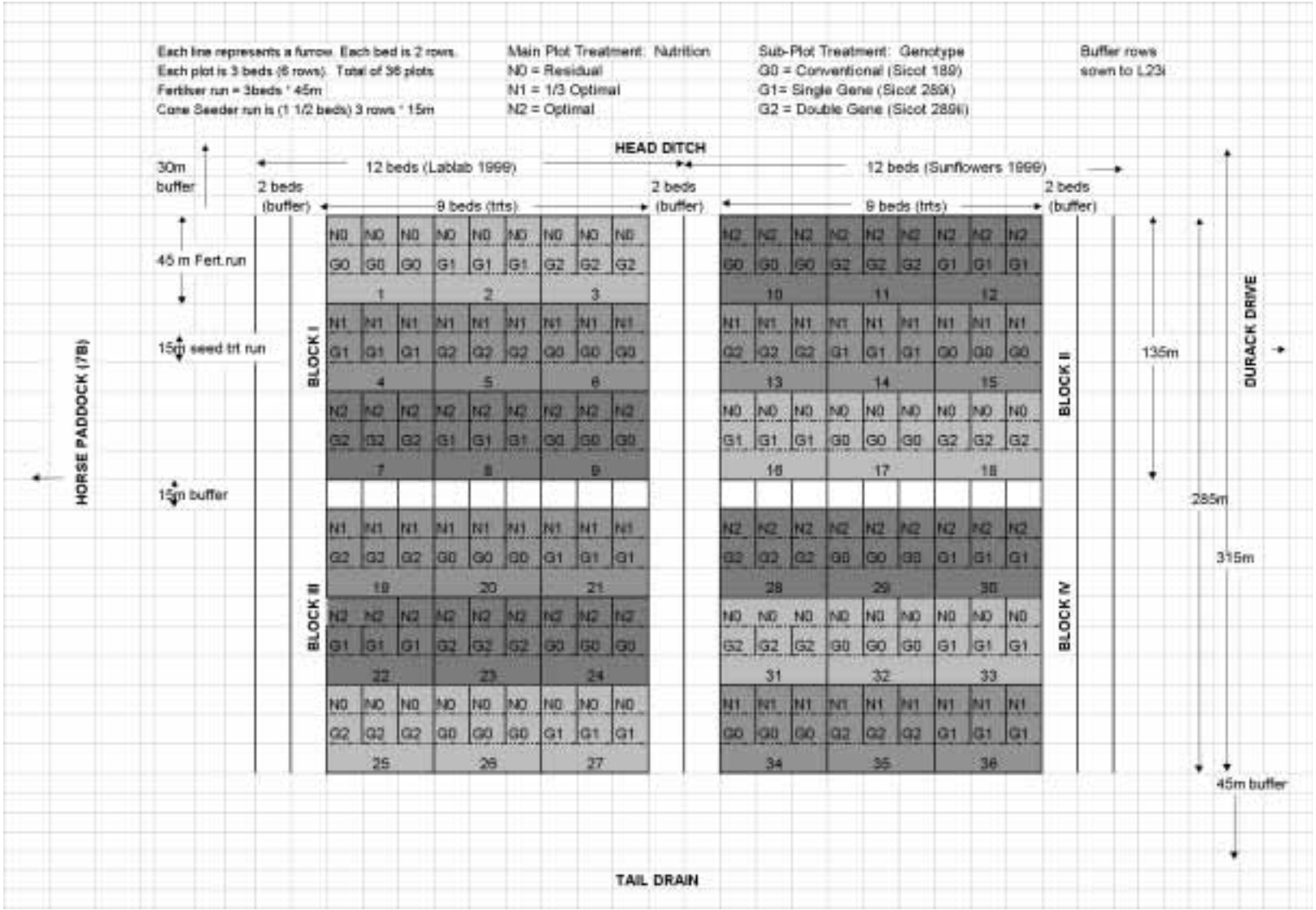


Figure 5.1. Experimental plan for genotype by nutrition experiment, Kununurra

Results

Soil Results
The soil test values below which nutrients may be deficient are listed as:

Table 5.2. Soil nutrient critical levels (taken from McKenzie 1998)

Nutrient	Extraction method	Critical level (mg/kg)
Nitrogen	Nitrate (aq.buffer)	20-25
Phosphorus	Bicarbonate	10-20
Sulphur	Ca dihydrogen orthophosphate	5-10
Iron	EDTA	80
Zinc	EDTA	4
Copper	EDTA	2
Boron	Hot water	0.15
Potassium	Ammonium chloride	150

Results from the soil analysis prior to sowing and fertiliser application are provided in Table 5.3.

Results for the four areas sampled within the paddock indicated that application of nitrogen fertiliser would provide a response in cotton growth, although there was some residual nitrogen present. Phosphorus levels were marginal, so a response to P would also be expected, similarly for sulphur, zinc, and potassium.

There were adequate levels of iron (although this declined with depth), copper and boron.

Table 5.3. Soil analysis results prior to application of fertiliser treatments

	Top (Lab Lab)				Bottom (Lab Lab)				Top (Sunflowers)				Bottom (Sunflowers)			
DEPTH (CM	0-15	15-30	30-60	60-90	0-15	15-30	30-60	60-90	0-15	15-30	30-60	60-90	0-15	15-30	30-60	60-90
TEXTURE (1= S and 3.5 = Clay)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
COLOUR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR	GRBR
NO3 (mg/kg)	17	9	2	1	27	12	4	2	17	7	2	2	15	9	2	3
NH4 (mg/kg)	11	9	6	6	10	6	7	5	12	14	6	5	16	11	7	7
P Cowell (mg/kg)	9	3	2	1	13	14	2	3	15	9	3	3	9	5	1	2
K (mg/kg)	137	135	95	149	166	195	139	152	120	125	121	94	153	97	82	128
S (mg/kg)	3.7	4.3	3.3	9.3	6.4	7.4	6.1	4.1	3.4	4.7	7	8.6	3.9	5.3	5.7	16.1
ORGANIC CARBON (%)	1.03	0.64	0.39	0.25	1.41	1.43	0.4	0.32	1.18	0.67	0.37	0.33	1.14	0.63	0.34	0.3
Conductivity (dS/?)	0.076	0.073	0.149	0.313	0.126	0.11	0.152	0.343	0.088	0.076	0.1	0.161	0.124	0.088	0.157	0.322
PH CACL2	7.3	7.3	7.9	8.3	7.5	7.3	7.9	8.3	7.4	7.4	7.6	7.9	7.3	7.2	7.7	8.1
PH H2O	7.9	8.1	9.1	9.4	7.9	7.9	8.7	9.3	7.9	7.7	8.8	9	7.8	8	8.8	9
DTPA CU (mg/kg)	1.24	1.27	1.06	1.11	1.82	1.89	1.46	1.61	1.51	1.33	1.26	1.2	1.28	1.25	1.2	0.96
DTPA ZN (mg/kg)	1.72	3.3	2.03	1.19	3.98	8.8	1.14	1.35	2.6	3.1	0.53	0.43	1.69	3.2	2.11	2.6
DTA FE (mg/kg)	18.9	17.1	18	18.9	28.8	27	15.3	18	19.8	16.2	16.2	17.1	17.1	18	18	17.1
EXC CA (meq/100g)	20.21	18.21	12.46	8.62	20.54	20.51	18.39	11.21	19.05	17.88	13.72	9.93	19.82	17.62	13.98	11.69
EXC MG (meq/100g)	13.04	13.91	14.59	15.35	13.09	13.09	17.04	21.31	10.45	11.74	13.61	14.01	12.3	14.67	16.24	15.67
EXC NA (meq/100g)	0.66	1.13	4.73	11	0.72	0.76	1.52	6.61	0.3	0.76	3.08	6.5	0.37	0.93	4.69	8.69
EXC K (meq/100g)	0.36	0.35	0.26	0.37	0.47	0.52	0.38	0.39	0.37	0.35	0.31	0.23	0.4	0.27	0.25	0.33
BORON HOT WATER (mg/kg)	0.5	0.4	0.6	2.2	1	0.4	0.3	1.3	0.5	0.4	0.4	0.6	0.5	0.4	0.8	1.9
EDTA CU (mg/kg)	3.85	3.24	2.86	2.64	4.52	4.1	3	2.71	3.63	3.56	2.65	2.74	3.42	2.92	2.35	2.17
EDTA ZN (mg/kg)	3.53	4.5	2.45	1.12	7.28	12.89	1.73	1.88	4.86	5.16	0.52	0.84	3.73	4.25	2.13	3.51
EDTA MN (mg/kg)	364.15	330.46	354.39	430.41	377.66	258.85	140.85	129.48	261.66	357.31	273.32	305.49	308.05	347.55	332.41	366.79
EDTA FE (mg/kg)	100.15	92.71	81.51	73.69	142.63	118.69	48.93	39.61	104.69	92.62	75.33	79.74	97.94	89.17	77.99	69.52

Bioassay Results
There was a significant effect of genotype on mortality (P<0.001) for the first bioassay. The two transgenic genotypes had significantly greater deaths (62.76%; s.e.=4.36 and 75.64%; s.e.=4.10 for the G1 and G2 treatments, respectively) compared to the conventional genotype (0.00; s.e.=0.0). There was no nutrition effect. Another five bioassays were conducted, but there was considerable mortality in the background Helicoverpa colony, even those not used for the bioassays, so these results had to be discarded.

Plant Measurement Results
All plant measurement data are for results from final harvest. There was no difference in plant number at this time.

Open Boll Number
The interaction between genotype and nutrition on number of bolls produced was approaching significance (P=0.087) on log-transformed data. (Nutrition and genotype were each highly significant (P<0.001)). The two-gene genotype produced a greater number of open bolls compared to the conventional cotton treatment at each of the three nutrition levels. The conventional genotype had the least response in boll production to an increase in soil nutrition levels compared to the two transgenic genotypes. Results on the non-transformed data are presented in Figure 5.2.

Seed Number
Results for number of seeds produced are consistent with trends for the number of open bolls produced, with the interaction between genotype and nutrition significant (P=0.02). Post-hoc comparisons within nutrition levels revealed the conventional genotype produced significantly fewer seeds than the two-gene treatment at all nutrition levels. There was no significant difference between the two transgenic genotypes at any of the nutrition levels. This is presented in Figure 5.3. (There was no significant difference between the conventional and the single gene genotypes at the sub-optimal nutrition level, N1).

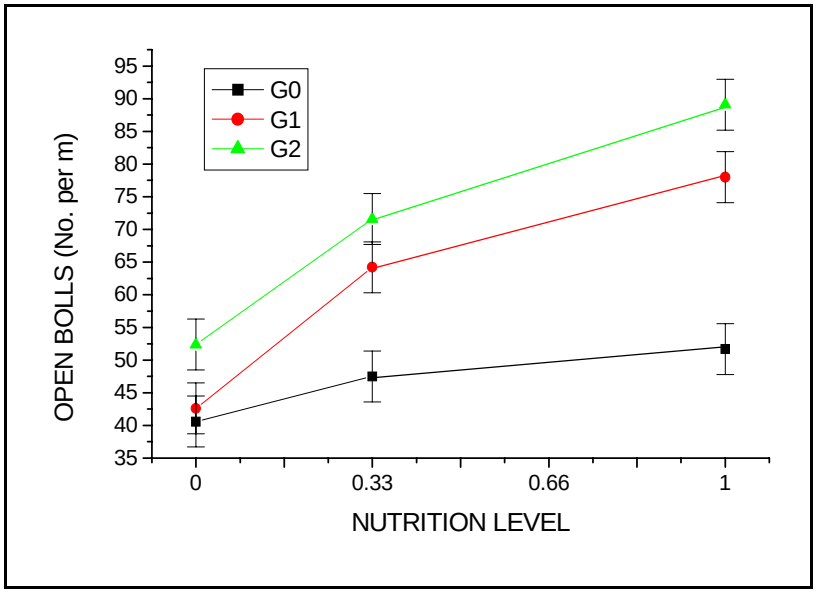


Figure 5.2. Effect of nutrition by genotype interaction on the number of open bolls produced per metre of row (error bars are ± s.e.)

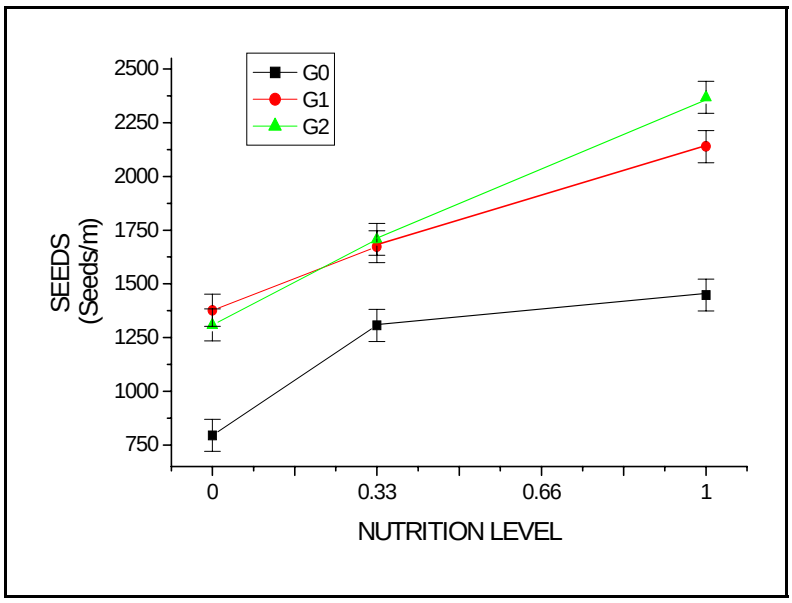


Figure 5.3. Interaction between genotype and nutrition on number of seeds produced per m of row (error bars are ± s.e.)

Seed Weight

There was a highly significant effect of genotype (P<0.001) and significant effect of nutrition (P=0.004) on the 100 seed weight. The conventional seed had the greatest individual weight, followed by the single gene then the two-gene genotype, illustrated in Figure 5.4

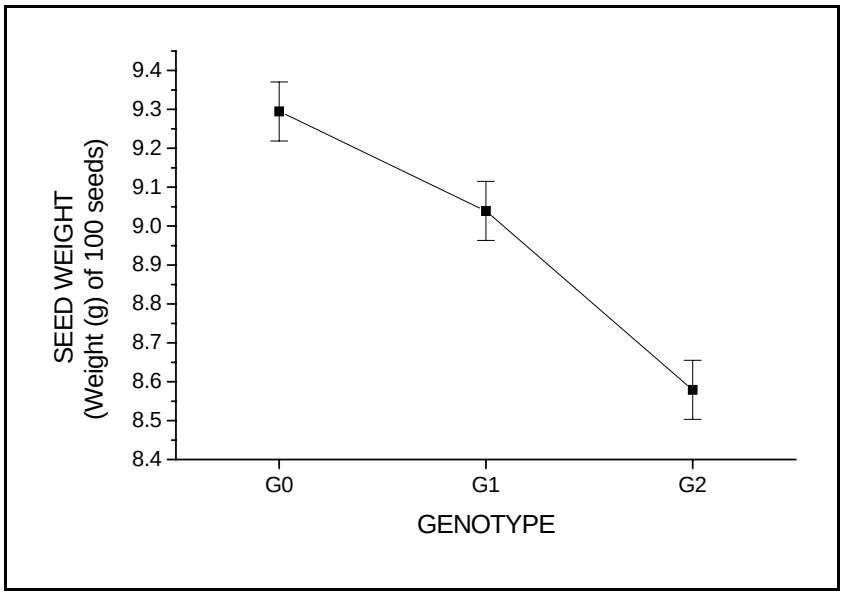


Figure 5.4. Effect of genotype on the weight of 100 seeds (error bars are ± s.e.)

Terminal Damage

There was a highly significant effect of genotype (P<0.001) on the number of plants per metre tipped (terminal shoots chewed off). The average number of plants tipped was 5.0, 1.12 and 0.79 for the conventional, single gene and two-gene genotypes, respectively (s.e.=0.336). The average number of plants per metre at final harvest was 11 (no significant factors), so nearly half of all conventional plants were tipped out.

Germination
There was no difference in the germination percentage of seeds between genotypes or levels of nutrition. Average germination for each genotype was 93.7, 92.7 and 94.0% for the conventional, single-gene and two-gene treatments, respectively.

Discussion

There was a distinct effect of nutrition level on number of seeds produced. The soil fertility levels for this paddock, while still low enough to provide a cotton growth response with application of fertiliser, are considerably higher than levels found in the native bush habitats used in Experiments 1A and 1B for all three locations, Kununurra, Katherine and Broome. Considering the range of habitats used in Experiments 1A and 1B, those of comparable fertility e.g. greater than or close to 10 mg/kg of phosphorus in the upper soil layer (10-15 cm) were; Kununurra Cattle and Drain sites, Broome Dam and Cattle No.1 and No.2 sites, Katherine Cattle No.1 and Katherine Creek sites. This trend was similar for the majority of other nutrients, including nitrogen, sulphur and organic carbon. Under conditions of adequate nutrition, which was displayed by even the zero fertiliser treatment in this experiment, and non-limiting water, the two transgenic treatments produced more seeds compared with the conventional genotype. This indicates that there may be increased fitness with respect to fecundity. However, this experiment was not designed to allow for recruitment of next generation seedlings, as herbicide application and cultivation consistent with commercial practice destroyed all volunteer plants. Thus, there was no assessment of rate of population increase as a component of invasiveness to determine if an increase in seed production transposed to an increase in weediness potential.

It must also be noted that although the soil fertility status of a habitat may be adequate for cotton growth and development, there may be other compounding factors. This was evident for the Broome Dam site, the Kununurra WS Drain 1 and Kununurra Cattle No.1 sites where inundation over the wet season led to a high mortality of the emerged seedlings. That differed from this experiment in which plants were grown over the dry season.

Competition, for resources such as nutrition or water for growth and development, and also for space for seedling recruitment as would be applicable for plants established for greater than one cropping cycle, was not a factor in this experiment as weed management was practised consistent with production guidelines. Crawley et al. (1993) found the more fertile the plots, the more quickly the open ground was colonised by native plants, thereby limiting opportunities for recruitment of the crop species. A subsequent experiment conducted for the purpose of herbicide evaluation in Katherine (a component of the 2002 agronomic experiments) illustrated the poor competitiveness of cotton in the presence of weed competition, even with adequate nutrition and water. Photos 5.1 a and b illustrate the poor growth of cotton in the absence of weed control.

The first bioassay indicated that even at sub-optimum nutrition, the Bt gene was being expressed in the early stages of cotton growth. Unfortunately, disease caused high mortality in the Helicoverpa colony from which the neonates used for the bioassays were selected, and further bioassays were discarded. Thus, any subsequent effect of nutrition, particularly as growing plants depleted the residual soil fertility, was unable to be assessed. It would be difficult to predict the level of expression of the Bt gene in volunteer plants, especially in those greater than a year old, as Bt expression in plants under commercial production declines gradually from first square. It is uncertain whether the Bt gene would provide an increased fitness in mature plants, especially considering phenology of fruit production over the year (differences in the wet and dry season).

The decrease in seed size from the conventional to the single-gene and two-gene is likely an inverse relationship with number of seed produced. Seed size has implications for weediness, in that smaller seeds may be more likely to be subject to desiccation, or quicker utilisation of seed reserves required for germination and extension of the radicle and cotyledon through soil impedance.

The significantly greater proportion of conventional plants tipped out compared to the two transgenic genotypes has implications for open boll production. Tipping out effectively stimulates the plant to produce more vegetative growth, thus increasing the time required to mature fruit. The growing season, or decrease in availability of resources e.g. soil moisture depleting as the wet season ends, as the plant progresses into the dry season, has implications for attainment of weediness. The high proportion of plants tipped out indicates the high insect pressure which may occur in the ORIA.

There were no differences in germinability between genotypes, so there was no increased fitness conferred by the addition of the Bt gene to seed available for germination at the onset of wet season rains. There was no increase in weediness potential for this first of the demographic parameters as determined by lab germination tests.

Conclusion

This experiment indicated that there was a significant interaction for seed production between genotype and nutrition under commercial production conditions when insects were not controlled. As expected, at all levels of nutrition, the conventional genotype produced fewer seeds than the two transgenic treatments. As nutrition level increased, the response in seed production was greater for the two transgenic treatments than for the conventional genotype. Implications for weediness suggest that in habitats for which there is high soil fertility and no interspecific competition, volunteer transgenic cotton may have higher levels of fecundity - although this may not necessarily be transposed to increased invasiveness, as other factors may influence plant survival and seedling recruitment. A subsequent experiment was conducted in an effort to quantify the effect of nutrition as compared to insect herbivory in a non-production area. This experiment is discussed in Section 5B.

Photos 5.1a and b. Cotton development at 10 weeks after sowing (herbicide efficacy experiment). The bottom photo illustrates the poor development of cotton where weeds were not controlled, compared to the top photo where weeds were managed.

SECTION 5B

GENOTYPE VS NUTRITION VS INSECT EXCLOSURE INTERACTION

Introduction

"*Pest attack interacts with crop nutrition not only by masking responses to fertiliser, but because fertilising may increase the susceptibility to insect pests*" (Hearn 1981). Observations and results from earlier experiments (refer Sections 1A, 1B, 4) indicated that volunteer cotton is more likely to produce reproductive structures in habitats of higher nutrition. However increased nutritional status may also result in plants more attractive to insects. It is unclear under non-agricultural conditions what the role of nutrition and/or insect attack may be in the progression of fruiting structures. This experiment aimed to resolve effects of these two constraints.

Methodology

The experiment was a split-split plot design consisting of four blocks within the Bush site at FWI, Kununurra, as was used in Experiment 1A and 1B.

Main plot treatment was protection from herbivory (invertebrates and vertebrates greater than 0.5 mm) at two levels; 1) Enclosure erected around sown seeds; and 2) No enclosure erected. Photo 5.2 illustrates the enclosures used.

Sub-plot treatment was nutrition at two levels; fertiliser applied, and no fertiliser applied. Soil analysis results for this site (refer Appendix 1: Bush habitat) indicated that soil phosphorus and nitrogen levels particularly are below critical levels for cotton. Fertiliser was applied as Thrive ® (N=27:P=5.5:K=9) equivalent to application of 370 kg/ha, effectively applying approximately 100 units of nitrogen per hectare.

Sub-sub-plot was genotype at three levels; conventional (G0), single gene (G1; Cry1Ac) and double gene (G2; Cry1Ac + Cry2Ab) of variety Sicot 289.

The experiment was sown with black seed (25 seeds per 25 cm by 25 cm plot) on 24 January 2001. Emerging seedlings were severely damaged by grasshoppers prior to the enclosures being erected, effectively destroying most populations, indicating there was no selection for nutrition at this early growth stage. Seed was resown on 12 March. Consistent rain over the following week (120 mm) on an already saturated soil rotted the majority of seed resulting in extremely poor germination which necessitated resowing the experiment again on 17 April. Germination was again poor, likely due to the lateness within the season as there was no effective rainfall after this time, so the experiment was eventually aborted.

However, there were isolated surviving plants that did illustrate potential treatment effects, although there were not enough surviving plants to make robust conclusions. Photos (see below) are presented to illustrate the attempt at conducting an insect enclosure study, deemed to be an important component of assessing the effect of an insect tolerance gene on increased fitness, and to provide an indication of possible results. These photos were all taken in mid-October 2001 (nine months after the original sowing).

Results

The enclosures were adequate in excluding the majority of flying insects, although crawling insects, and the occasional flying insect, were found within the nets.

For the original sowing, 47 out of 48 plots produced germinated seedlings; but only nine of these plots had isolated seedlings (remaining before the enclosures were available). At no instance did seedlings of any genotype produce fruiting structures for the low nutrition treatment when exposed to insect herbivory. Seedlings were chewed by grasshoppers and no further growth occurred (Photo 5.3c).

For the plots with elevated nutrition and exposed to insects, only the single-gene treatment (Photo 5.3a) produced one viable open boll. Comparable surviving plants for the two-gene and conventional plots did not produce any bolls. It appeared that the increase in nutrition allowed for greater recovery if plant damage occurred.

In insect excluded (netted) plots, the only plots with plants remaining from the original sowing by the end of the dry season, were conventional (Photo 5.3b) and two-gene genotypes where additional nutrition was applied, and single genotype for the no nutrition applied (Photo 5.3d) treatments. All these plots contained plants that produced open bolls.

There were also obvious differences in plant development between seedlings derived from the three sowing times. Plants surviving from the original sowing were by far the most vigorous when compared to any seedlings which survived from either of the two subsequent plantings. This can be seen in Photo 5.3d – the top seedling has progressed to producing open bolls, whereas seedlings from the subsequent plantings (middle and bottom of photo) did not. This could be attributed to lateness of the wet season, where declining soil moisture prevented seedlings developing further, as compared to those emerging earlier in the season, where there was adequate follow-up rain.

Conclusion

There were not enough surviving plants to make any valid conclusions concerning the relative effect of genotype on open boll production. However, the majority of damage to early seedlings was attributed to attack by grasshoppers, so it is unlikely that the addition of the Bt gene would provide an advantage in the early development stages of volunteer cotton plants.

Photo 5.2. Insect herbivory x nutrition x genotype experiment at Kununurra bush

Photo 5.3a. Nutrition applied. Single gene treatment when exposed to insect herbivory

Photo 5.3b. Nutrition applied, conventional treatment when protected from insect herbivory

Photo 5.3c. No nutrition, single gene treatment when exposed to insect herbivory

Photo 5.3d. No nutrition, single gene treatment when protected from insect herbivory

SECTION 6

MONITORING NATURALISED COTTON POPULATIONS

SUMMARY

There are approximately 60 recorded populations of naturalised cotton in the NT. In order to gather more information on the occurrence and status of this cotton in northern Australia, observations on up to 12 naturalised cotton populations were recorded.

These populations were found to be near high tide marks or the fringe of floodplains and were not generally recognised as invasive or a weed by landholders. It was concluded that the commercial release of Bt cotton in northern Australia poses a minimal hazard to an increase in weediness potential of naturalised cotton populations through introgression of the Bt gene. This is supported by a number of aspects including;

1. Geographic isolation from suitable production areas for the majority of naturalised populations.

2. Ability to eradicate known populations (small areas and plant numbers) which may overlap if production areas are established.

3. Indications that Bt susceptible insects are not a significant constraint to the growth of existing cotton populations.

4. No evidence that existing populations are invasive of their current habitats, to which they have adapted.

5. Indirect evidence from the large-scale ecological assessment which indicated that the Bt gene did not enhance the ability of improved cultivars to become a weed, quantified by calculation of population growth rates between transgenic and non-transformed cotton in a number of non-agricultural production habitats.

Introduction

Additional information has been requested by OGTR and Environment Australia on the occurrence and status of naturalised cotton in northern Australia.

Two points were raised that were seen as relevant to the evaluation of weediness risks associated with GM cotton in northern Australia. Firstly, *G.hirsutum* is naturally a tropical and subtropical species of the New World where it occurs in littoral and riparian habitats. Secondly, naturalised plants of *G.hirsutum* are known to occur in northern tropical Australia. There was the potential for the Bt transgene to introgress to these cotton populations and alter their ability to persist and increase.

It was stated that information that would assist evaluation of this potential hazard would include:

1. Evaluation on the level of potential exposure of these naturalised populations to the Bt transgene. This would encompass; intended areas for extension of use; distribution, abundance and persistence of naturalised cottons - in relevant cropping areas, in nearby and distant river systems and littoral environments.

2. Evaluation on the consequences of transgene transfer to naturalised cultivated cotton species. This would encompass current weed status of naturalised cottons, significance of insect herbivory on survival and reproduction (fitness) of naturalised cottons, and data on potential impacts of Bt transgene introgression on the fitness of naturalised cottons.

There are approximately 60 recorded populations of naturalised cotton in the NT. Members of the public, weed agency personnel and Department of Defence personnel, have also brought five additional undocumented populations to the author's attention. There are only a few isolated plants recorded in Western Australia, none of which are in the ORIA, according to existing herbaria lists. This is significant, in that no plants survived after the collapse of the cotton industry there in the 1970s, even though farmers simply 'walked off' their farms, leaving the cotton crop in the paddocks. Discussion with NT herbarium botanists (Dunlop, pers.comm) concerning the spread of naturalised

cotton populations recorded over sequential visits indicated that these populations are maintained at self-perpetuating levels and provided no indication that the number of plants or extent of the populations were increasing at any site, although quantitative estimates were not conducted.

This section includes observations from visits to selected naturalised cotton populations. These were initially visited primarily because of accessibility and cooperation from the landowner. Selection for further monitoring was on the basis of representation of populations in a range of habitats, encompassing black soil floodplains, coastal shoreline and littoral areas, and exposure to different levels of potential agents of cotton volunteer management such as grazing, slashing, and herbicides as applicable between pastoral properties, public areas and National Parks. NT Department of Business, Industry and Resource Development (NT DBIRD) staff visited a number of naturalised cotton population sites in the NT during May 1998 coinciding with the end of the wet season. A report outlining these observations is provided in Appendix 4 (Schultz, pers.comm). A number of these sites were revisited in late August 1999, coinciding with mid-late dry season. A description of these sites, possible origin of the cotton plants, and possible constraints to significant dispersal of seed cotton from these populations is presented.

Methodology and Results

The sites visited included:

1. Bowen Strait, Cobourg Peninsular
2. Trepang Bay, Cobourg Peninsular
3. Woolner Station, Adelaide River
4. Rapid Creek, Darwin
5. Elsey Station, Mataranka
6. East Arm Port, Darwin
7. Beatrice Hill, Adelaide River

Photo 6.1. Naturalised cotton at Bowen Strait, Cobourg Peninsular, May, 1998

1. Bowen Strait

This habitat was the beach foreshore, with cotton plants growing in beach sand within 50 m of the high tide mark. Plants were scattered along the beachfront, and the population covered less than 1 hectare in area, and probably less than 300 plants in total.

At the time of the initial survey (28 May, 1998), plants had considerable numbers of green and open bolls, and abundant vegetative growth (Photo 6.1). Plants assessed 16 months later were basically ‘sticks’ (Photo 6.2; cotton has red flagging tape) and appeared to have been grazed heavily by vertebrate herbivores, probably buffalo or banteng. Some seed cotton was found entangled in surrounding shrubs along the shoreline, but there was no viable seed within the lint.

Photo 6.2. Naturalised cotton at Bowen Strait, Cobourg Peninsular, September 1999

2. Trepang Bay

This was a similar habitat to the one on Bowen Strait, with cotton plants growing in beach sand within 20 m of the high tide mark. Two large plants (or possibly a number of plants clumped together) were largely intact. This was due to protection from Bundoc Bush (*Caespaelicacea burdoc*), an extremely thorny shrub. These plants had retained lint (some collected), and vegetation/leaf. There was very little visible insect damage. No insects were found upon cursory examination, although accessibility was difficult due to the presence of the bundoc. There was some evidence of grazing on accessible branches, and the surrounding area appeared to be a camp for banteng, with evidence of dung.

Herbarium records show that plants were present at Bowen Strait and Trepang Bay in 1993, although discussion with Aboriginal traditional owners of the area, indicate that the population was present before that time. Historical records suggest that naturalised cotton populations may have originated from initial European settlement, where cotton was grown at Port Essington, and documented as producing the first cotton sold from Australia in 1842 (Curteis unpub.).

For both these beach front populations, no plants were found behind the shoreline into the native bush vegetation, generally a dry monsoonal thicket, indicating that the habitat was unsuitable, probably due to a combination of factors including fire, water availability and competition. It does not appear to be invasive of the habitat, but is considered a weed as defined as an alien plant in a National Park (Gurig N.P.).

Photo 6.3. Naturalised cotton amidst Bundoc Bush at Trepang Bay, August 1999

3. Woolner Station

This habitat was on the fringe of the Adelaide River floodplain, with plants growing in cracking black clay on the margins with dry monsoonal thickets (fringe country) above the floodplain line. This site had the most extensive area of naturalised cotton of those visited, with robust plants on the edge of the floodplain fringe as illustrated in Photo 6.3 below. It was impractical to count individual plants, but scattered clumps of plants were within an approximate area of less than 50 hectares. Photo 6.4 illustrates an isolated clump of plants furthest onto to the floodplain away from the drier upland fringe. Seedling recruitment (<20 for the area) was observed at an initial visit in 1999, with less than half of these surviving at a subsequent visit in 2001. These seedlings were all in immediate proximity to the parent clump of plants.

Photo 6.4. Naturalised cotton plants at Woolner Station, 2nd September, 1999. These plants were at the extreme edge of the range of the population. This population was on the verge of the floodplain water mark (note cattle track on edge of mud).

Population spread across the floodplain appeared to be restricted by depth of water rising in the wet season. The paddock was inhabitated by cattle but there was little evidence of the cotton having been grazed. There was very little insect damage to leaves at this time (end of dry season), but harlequin bugs were in large numbers on the plants closest to water. A low intensity fire had apparently gone through this paddock in previous years.

This site is documented in herbarium records as early as 1988, with the origin of the cotton plants not certain, although possibly introduced from feeding of cotton seed to cattle, although this was not documented. The manager of this pastoral property does not consider the plant as an invasive weed.

4. Rapid Creek

This habitat is on the tidal margin of Rapid Creek, abutting onto mangroves on one side and a median strip next to a major suburban road within Darwin on the other side. A Roman Catholic Mission was started in Rapid Creek in 1884, with records indicating that cotton was one of the crops grown. Populations of cotton at Rapid Creek are recorded in the herbarium records as early as 1967. This population has been decreasing since monitoring was commenced in 1999 due to slashing and control of associated leucaena (*Leucaena leucocephela*) which has also established as a naturalised plant along the creek. There were less than 10 isolated clumps of cotton in the tidal margin along the creek line within approximately 300 m – the largest clump is illustrated in Figure 6.5; mangroves are in the background, leucaena in the left foreground.

Photo 6.5. Naturalised cotton at Rapid Creek, 3rd September, 1999

5. Elsey Station

This site was situated near the homestead buildings of Elsey Station, built on the banks of the Roper River. Cotton plants grew along the bank, and also as isolated clumps next to the homestead buildings. Plants were within a strip approximately 50 m along one side of the bank. No seedlings were observed, but existing plants were extremely robust, and with thick stems, indicating considerable age. Some of these plants had been sawn down to allow easier access to the river, and were producing new growth at the time of inspection (Photo 6.6) in August 2000. There was no evidence of the population encroaching further along the bank.

Photo 6.6. Cotton reshooting after being cut down at Elsey Station. Note the thickness of the cotton trunk, indicating considerable age

6. East Arm

This habitat is located at the East Arm boat ramp, near Darwin. Photo 6.7 illustrates plants growing in the middle of the car park, plus there were isolated clumps within a 50 m radius, totaling less than 30 plants. Plants were mainly vegetative at this time (wet season, end of March). This site was included as it adjoins the proposed East Arm Port, destined to be the major port for Darwin. As such, it is possible that fuzzy seed could be exported to Asian cattle feedlots from this point. Documentation of the existence of these plants prior to any cotton seed export may be necessary in the advent of any future seed dispersal.

The origin of this population is unknown.

Photo 6.7. Naturalised cotton at East Arm boat ramp, Darwin. March 2000

7. Beatrice Hill

This habitat is within 1 km of the Adelaide River, associated with black cracking clay soils. Plants were mainly restricted to the fenceline bordering the Arnhem Highway and a paddock grazed by both cattle and buffalo near Coastal Plains Research Station. Plants were growing in association with a native legume shrub (*Carthormium umbellatum*). Abundant seed on the plant and on the ground was observed at most times during the dry season. Some seedling recruitment was observed after each wet season. Mortality of perenniated plants was difficult to determine.

Number of individual plants was difficult to quantify, but clumps of cotton plants were scattered in a band approximately 10 m wide by 300 m along the fenceline, with three isolated clumps, each containing less than five plants, established within 20 m off the fenceline. These plants were the subject of more detailed monitoring, outlined below.

This site was inundated with water throughout the wet season, which may have contributed to relatively low numbers of seedlings compared to the amount of seed produced, illustrated in Photo 6.8. Population spread was also restricted by vigorous growth over the wet season of annual grasses and forbs on the road verge side, and roadside slashing at the commencement of the dry season. The population is not considered a weedy invader by the manager, and no active weed management is conducted on these plants.

Beatrice Hill was the site of a cotton, coffee and rubber plantation in the late 1800s, and is located close to a current NT DBIRD's Coastal Plains Research Station from which cotton seed could have been used previously for stock feed. Herbarium records document cotton in a similar location from 1984. Anecdotal evidence from long-term departmental staff and the lack of incidence of recruited seedlings except within the immediate proximity of the mature plants suggests that this population is not invasive of this habitat.

The small cotton population is adjacent to several thousand hectares of Mimosa infested floodplain providing a stark contrast of the weediness potential of the two species.

Photo 6.8. Naturalised cotton population at Beatrice Hill, 1 September 1999. Abundance of lint on the ground, but no young seedlings (some were observed the following year).

Insect Sampling

In May 2000 a detailed sampling of insects was made from the Beatrice Hill, Lee Point and Rapid Creek locations both by hand-collecting and D-vac sampling. Results from this collection are provided in Table 6.1. Of the 150 insects collected in total, 24 (16%) were Lepidoptera of which none were confirmed to be a Noctuid. Hemiptera was the dominant insect order found (28% of total insects), suggesting that sucking insects comprised a greater proportion of insect presence and possibly influenced naturalised cotton populations more than did Lepidoptera.

Table 6.1. Insects collected at three existing locations of naturalised cotton populations

Supplementary data – hand collections and D-vac material

	Order	**Family**	**Genus**	**Species**	**Number**
2.v.2000 Beatrice Hill (hand-collected)	Hemiptera	Miridae			1(imm)
		Alydidae	*Riptortus*	*serripes*	1
		Coreidae	*Amblypelta*		2
		Scutelleridae	*Tectocoris*	*diophthalmus*	1
	Coleoptera	Chrysomelidae			1
2.v.2000 Beatrice Hill (Devac 1a) (volunteer cotton)	Blattodea	Blattellidae	*Ellipsidion*	*magnificum*	1
	Hemiptera	Cicadellidae			1
		Alydidae	*Riptortus*	*serripes*	3
	Coleoptera	Chrysomelidae			3
	Diptera	Tipulidae			2
		Ephydridae			1
	Lepidoptera	Geometridae			1 (imm)
		?			3
2.v.2000 Beatrice Hill (hand-collected) (volunteer cotton)	Orthoptera	Tettigoniidae			1 (imm)
	Hemiptera	Miridae			1 (imm)
		Pentatomidae			1 (imm)
	Coleoptera	?			1
		Chrysomelidae			1
	Diptera	Ephydridae			1
	Hymenoptera	Formicidae	*Polyrachis*		1

	Order	Family	Genus	Species	Number
2.v.2000 Beatrice Hill (volunteer cotton)	Araneae	Theridiidae	*Argyrodes*?		1
	Blattodea	Blattellidae	*Ellipsidion*		1
	Hemiptera	Coreidae	*Amblypelta*		1(imm)
		Pentatomidae	*Poecilometus*		1
			sp. 2		1
	Diptera	Tipulidae			1
		Muscidae	2 spp		2
	Lepidoptera				1 (imm)
	Hymenoptera	Eulophidae			1
2.v.2000 Beatrice Hill (Devac 1b) (volunteer cotton)	Araneae	Theridiidae	*Argyrodes*?		2
		Salticidae			3
	Hemiptera	Coreidae			1 (imm)
		Pentatomidae	*Poeciliometis*		1
	Coleoptera	Curculionidae			1 (imm)
	Diptera	Chironomidae			1
		Ephydridae	sp. 2		1
	Lepidoptera	?			3 (2 imm)
2.v.2000 Beatrice Hill (Devac 2b) (volunteer cotton)	Hemiptera	Derbidae	*Proutista*		1
		Lygaeidae	*Arocatus*		1
			Pseudopachyra chius		1
	Coleoptera	Apionidae			1
	Lepidoptera	Lycaenidae			1
	Hymenoptera	Braconidae			1
		Formicidae	*Polyrhachis*		1
2.v.2000 Beatrice Hill (hand-collected) (No. 3)	Araneae	Araneidae			9 (8imm)
	Diptera	Chironomidae	sp. 2		1
		Ephydridae	sp. 2		1

	Order	**Family**	**Genus**	**Species**	**Number**
3.v.2000 Rapid Creek (Devac 1) (volunteer cotton)	Orthoptera	Acrididae			1 (imm)
	Hemiptera	Flatidae	*Siphanta*		1
	Diptera	Platystomatidae	*Riviella*		1
		Ephydridae	sp. 1		1
	Hymenoptera	Bethylidae			1
		Formicidae	*Opisthopsis*		1
3.v.2000 Rapid Creek (Devac 2a)	Blattodea	Blattellidae	sp. 2		1
	Orhtoptera	Tettigoniidae			1 (imm)
		Pyrgomorphidae ?			2
	Hemiptera	Delphacidae			1
		Coreidae			1(imm)
		Colobathristidae	*Phaenacantha*	*australiae*	1
	Diptera	Dolichopodidae			1
	Lepidoptera	?			2
	Hymenoptera	Formicidae	*Oecophylla*	*smaragdina*	1
3.v.2000 Rapid Creek (hand-collected) (volunteer cotton)	Lepidoptera	Arctiidae	*Utestheisa*	*pulchelloides*	1

	Order	**Family**	**Genus**	**Species**	**Number**
3.v.2000 Lee Point (Devac 1b) (volunteer cotton)	Araneae	Salticidae			1
		Araneidae			1
	Blattodea	Blattellidae	sp. 2		1
	Mantodea	Mantidae			1 (imm)
	Hemiptera	Delphacidae	sp. 2		1 (imm)
		Cixiidae			1 (imm)
		Coreidae	*Amblypelta*		1
			sp. 2		1
	Diptera	Dolichopodidae			1
	Lepidoptera	Geometridae			1 (imm)
		Pyralidae			3
	Hymenoptera	Encyrtidae			1
3.v.2000 Lee Point Devac 1c) (volunteer cotton)	Hemiptera	Cicadellidae	sp. 2		1
		Flatidae	*Siphanta*	sp. 3	1
		Colobathristidae	*Phaenacantha*	*australiae*	1
		Coreidae	*Amblypelta*		1
			sp. 2		2
	Diptera	Dolichopodidae			1
	Lepidoptera	?			4 (imm)
		?			3
3.v.2000 Lee Point (hand-collected) (No. 1)	Araneae	Salticidae			1
	Hemiptera	Coreidae			1 (imm)
		Pentatomidae	*Poeciliometis*		2
	Coleoptera	Nitidulidae	*Carpophilus*?		1
		Chrysomelidae	sp. 2		1
	Diptera	Dolichopodidae			1
	Lepidoptera		2 spp.		2 (imm)
	Hymenoptera	Formicidae	*Monomorium*		3

	Order	**Family**	**Genus**	**Species**	**Number**
3.v.2000 Lee Point (Devac. 2a) (volunteer cotton)	Araneae	Salticidae			1
		Araneidae			1
	Mantodea	Mantidae			1 (imm)
	Hemiptera	Coreidae	sp. 2		1
3.v.2000 Lee Point (Devac. 2a) (cont.)	Hemiptera	Pentatomidae			1 (imm)
	Coleoptera	Nitidulidae	*Carpophilus*?		1
		Chrysomelidae	sp. 2		1
		Apionidae			1
	Diptera	Stratiomyidae			1
		Dolichopodidae			2
		Muscidae?			1
	Hymenoptera	Formicidae	*Camponotus*?		1
3.v.2000 Lee Point (Devac. 1a) (volunteer cotton)	Araneae	Araneidae			1
	Blattodea	Blattellidae			1
	Hemiptera	Cicadellidae	sp. 3		1
		Flatidae	*Siphanta*	sp. 1	1
		Coreidae	*Amblypelta*		1
			sp. 2		1
		Colobathristidae	*Phaenacantha*	*austriale*	1
	Diptera	Tipulidae	sp. 2		1
		Ceratopogonidae			1
	Lepidoptera	Pyralidae			1
10.iv.2000	Lepidoptera	Notodontidae			1
8.v.2000 P19 F. Ger..	Lepidoptera	Noctuidae			1

QUANTITATIVE EXPERIMENTS

The cotton population at Beatrice Hill was selected for an insect exclosure study to assess naturalised cotton growth and development in the absence of insect herbivory, as illustrated in Photo 6.10. The exclosures were established at the start of the dry season in May 2001. Twenty-three seedlings were located and individually identified and tagged. Nine seedlings were enclosed in netted cages and the remaining 14 plants were left exposed. Five fruiting branches on the larger plants were selected and encased with netting. Plant heights and fruit counts between the two treatments were conducted during the year. Statistical analysis was not conducted but five of the nine caged plants and seven of the 14 non-caged plants had died by the September 2001 recording. Seedling survivorship and fruit production appeared to be no different between the caged and uncaged plants. Squares and small green bolls were observed on both caged and non-caged seedlings. Squares, green bolls and open bolls were observed on the netted and non-netted branches of the larger plants.

Photo 6.9. Grasshoppers were the dominant insect observed on a visit to the site over the wet season

During a visit to the site in the wet season (January 2002), the caged plants had more intact leaves, and grasshoppers were observed feeding on the vegetative growth on non-protected plants illustrated in Photo 6.9.

Photo 6.10. Enclosure study on naturalised cotton site at Beatrice Hill (near Adelaide River, NT)

Other locations visited where cotton populations were recorded, included:

a. Shoal Bay, on the outskirts of Darwin on Defence Force land. The habitat was along the tidal margin near the beach shoreline. There were isolated clumps totaling approximately 5-10 cotton plants, and within an area less than 50 m by 50 m. No recent seedlings were observed. All plants were due to be eradicated as part of the Defence Force Weed Management Strategy which defined them as an alien species to the native habitat.

b. Lee Point Reserve, on the outskirts of Darwin. There were isolated clumps of approximately 5-10 plants within an area less than 50 m by 50 m. This habitat was where the beach shoreline borders the native bush. Plants were on the edge of the bushline and the watered and mown lawn area of the coastal reserve. No recent seedlings were observed and the population was not invasive of the habitat. It was not subject to weed control by the Darwin City Council responsible for the area. Cotton had been documented at this site in 1972.

c. Elizabeth Downs Station, approximately 180 km south west of Darwin. The habitat was in open woodland that had been recently burnt. There was a clump of less than 30 large cotton plants and some seedlings covering an area of approximately 5 m by 5 m. The population was close to an old Aboriginal mission site.

d. Douglas River, approximately 160 km south of Darwin. There were two distinct populations at this location: one situated on the banks of the Douglas River, consisting of less than 20 large plants within a 30 m band along the river's edge, and the other an isolated clump of plants along a fenceline next to a dirt road within the Douglas River Park. Weed control was not conducted on these populations and managers did not consider them as increasing.

Conclusion and Implications for Weediness

An evaluation of the consequences of transgene transfer to naturalised cotton populations and on the level of exposure of these populations to the Bt gene was the information sought by Environment Australia to assist in the assessment of the hazard of weediness.

Consequences of transgene transfer; current weed status, significance of insect herbivory, and impact of possession of the Bt gene on naturalised populations:

The current weed status of naturalised cotton is not distinct, as the status depends on the definition of a weed. The only locations where the populations are actively controlled are within National Parks, including Katherine Gorge (Nitmiluk), Kakadu and Gurig, and Defence Land, consistent with the definition as given by Cowie and Werner (1987) of a naturalised alien plant. Naturalised populations existing on pastoral properties are not subject to active weed management strategies, as cotton is not considered an invasive plant or one which is reducing the productivity of the property, particularly in comparison to other plants targeted as weeds, such as *Mimosa pigra*. Populations have been recorded at some sites, such as Lee Point and Rapid Creek, for more than 30 years, and there is no evidence that these populations are invasive, as plant numbers appear to be declining or self-sustaining within the specific niche. Recruitment in the populations visited was minimal and sporadic and appeared unrelated to levels of insect herbivory.

Overall, *G.hirsutum* is not considered to exhibit weedy biological characteristics in the non-agricultural production areas where the naturalised populations are currently found.

Herbivory by insects susceptible to the Cry 1Ac and Cry2Ab proteins did not appear to be a significant factor constraining the naturalised cotton populations to the habitats in which they were established. These proteins are essentially Lepidoptera specific with some disputed activity of Cry2Ab against some Diptera. Within the Lepidoptera these two proteins are not universally active. The order Noctuidae (e.g. *Helicoverpa* spp) is the main target group, but even within the Noctuidae some species are not susceptible at all to one or other of the proteins (e.g. the Tobacco looper, Chrysodeixis). The Bt genes used in cotton have been developed to protect the plant against damage to flower buds (squares) and bolls (seed and associated lint) caused by Helicoverpa or similar species which damage reproductive structures. Abundant seed was produced at all monitored sites, suggesting that fecundity was not limiting population growth.

Observations have been made of the level of herbivory and identity of the herbivores at some sites. The major insects observed feeding, particularly over the wet season when plants were mainly vegetative, were grasshoppers, an order not susceptible to these Bt proteins.

Given the available information we believe that the addition of the Bt gene would not confer additional fitness to the existing naturalised cotton populations. This is evident in that they do not exhibit biological weedy characteristics as they currently exist; population growth does not appear to be constrained by Bt susceptible insects; and the complementary multi-site ecological study indicated that the addition of the Bt gene did not enhance invasiveness as evaluated by the rate of population growth.

Based on our observations, none of the significant naturalised populations of *G. hirsutum* are increasing in density or extent. The constraints to naturalised cotton population growth were not quantified, but it was inferred that other factors such as water availability, grazing and fire are more attributable to the confinement of the sites visited than are the dynamics of Bt susceptible insects. Widely fluctuating water height encompassing the coastal sites (e.g. Cobourg Peninsula), tidal edges (e.g. Rapid Creek) and floodplain sites (e.g. Woolner and Beatrice Hill as components of the Adelaide River floodplain) may give rise to a narrow moisture range suitable for growth of cotton plants. There is likely a range where water is not limiting in the dry season, but not waterlogging/submerging plants in the wet season.

In 1999, seven collections from naturalised populations in the NT (Bowen Strait, Trepang Bay, Woolner Station, Rapid Creek, Elsey Station, Point Stuart and Mount Barker) were grown in the field at the Australian Cotton Research Institute at Narrabri. All were found to be similar, and older types of *G. hirsutum*, rather than modern varieties. The plants were glabrous, had high density of gossypol glands, and were daylength sensitive, consistent with cultivars which would have been introduced in the 1800s as part of early attempts to grow cotton. Current commercial types of G.hirsutum grown since the 1970s are not daylength sensitive, are mostly smooth rather than glabrous, and have lower gossypol gland density. This suggests that current commercial cultivars may be less able to establish self sustaining populations.

Naturalised cotton plants were not actively sought as preferred forage by cattle and buffalo, but may possibly have been by banteng (only found in the Cobourg Peninsula). Cattle were observed to graze the cotton plants where they were in a concentrated area and other feed was limiting over the dry season.

Fire is a major determinant of tropical savanna structure (Williams, Duff, et al. 1996), and a large proportion of northern Australia is burnt each season. The restriction of naturalised cotton populations to littoral habitats and water courses is consistent with the documented preferred habitat of the original species, and may also provide protection from fires in northern Australia due to water availability, and reduced fuel load in brackish habitats.

It is concluded that any possible transgene transfer would have little consequence with respect to increased invasiveness of naturalised cotton in the habitats in which it already exists, and in habitats in which it could potentially disperse, as identified in the large-scale ecological assessment. There is anecdotal concern that naturalised cotton populations pose a different level of risk for additional weediness due to transfer of Bt transgene to that of improved cultivars to become naturalised. This is due to the possibility that they have adapted over time to the habitats in which they are currently found compared to the more modern varieties. However, the extremely low level of probability of exposure of these naturalised populations to the Bt gene negates this perceived risk, which is discussed below.

Level of exposure to the Bt transgene; intended areas for commercial production and distribution, abundance and persistence of naturalised cotton.

Monitoring of selected populations, and mapping of known populations, as supplied in Figure 6.1, has provided a qualitative assessment of the hazard of Bt gene introgression from proposed northern commercial production areas. Potential areas for commercial cotton production would be determined by soil and water resource availability. These have been assessed by Yeates (2001), and areas are superimposed on Figure 6.1 to indicate proximity to known naturalised cotton populations. There are few populations that overlap with suitable commercial production areas. These plants could be easily eradicated if future production in these areas occurred. The majority of other populations are geographically isolated (>50 km) from suitable production areas, and as such, are not conducive to the

introgression of transgenes. Existing trials at Katherine are at least 100 km from naturalised cotton populations.

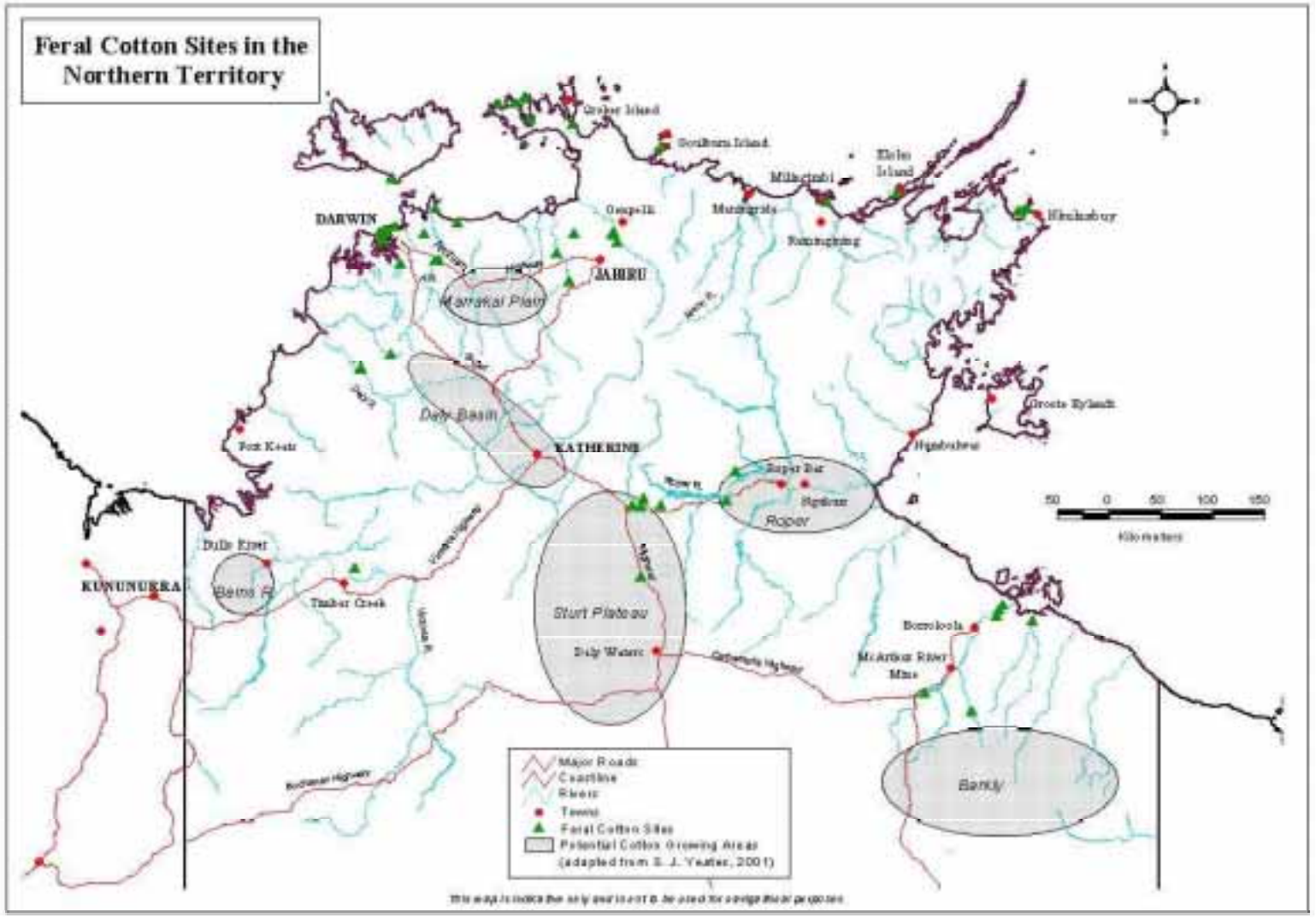


Figure 6.1. Naturalised cotton sites in the NT and potential cotton growing areas

SECTION 7

CHARACTERISTICS OF VOLUNTEER TRANSGENIC COTTON

OBJECTIVE

To monitor the growth and development of transgenic cotton plants that have naturally established in habitats away from cultivated areas to document development, survivability, seed production and population succession.

Introduction

Field experiments to examine agronomic and entomological issues with Bt transgenic cotton had been conducted in Kununurra, Katherine and Broome for at least two seasons prior to the commencement of the weediness project in June 1999. These experiments were conducted under strict guidelines imposed by the Genetic Manipulation Advisory Committee (GMAC), and included the destruction of any volunteers, both within the production paddock, and also in any off-site habitats where plants were observed to establish. Destruction of volunteers, either regrowth from cotton stalks or from seed cotton on the ground after harvest, within production areas was generally achieved consistent with fallow weed management over the wet season. Isolated individuals did establish from seed in non-production habitats. These were:

Kununurra: Roadsides and paddock edges from seed cotton spillage during transport to the gin
Drains from seed cotton dispersal with water flow from paddock after harvest
Cattle yards from spillage of fuzzy seed

Katherine: Paddock edges from seed cotton dispersal after harvest
Cattle yards from spillage of fuzzy seed

Broome: Paddock edges from seed cotton dispersal after harvest

Other: Paddocks where cotton seed had been fed out on commercial cattle properties (illustrated in Photo 7.1).

These volunteers provided an opportunity to evaluate the weediness of Bt cotton under scenarios that would likely occur in the advent of commercial production and are a vital component to long-term evaluation of the potential of volunteer cotton to establish. These plants were subsequently included as additional sites under PR89X(2) as Ecological study sites Numbers 1 to 6.

Photo 7.1. Volunteer cotton plant at cattle feeding out site. Plant had been severely defoliated by grazing.

Methodology

Selected plants at Kununurra only were labelled and protected from destruction that would otherwise have occurred, both intentionally from removal of volunteers under the GMAC guidelines, or indirectly from roadside slashing, spraying of drains, or cattle grazing and trampling in yards.

The plants assessed were:

Plants 1-3: Road verge
Plant 4: Drain edge
Plant 5: Fenceline next to paddock
Plants 6-8: Cattle yard

Fruit counts, plant heights and number of seedlings recruited were recorded at several times throughout each year.

Results

It was necessary to evaluate the cycle of fruit production to assess seasonal factors influencing plant growth and seed production, and probability for seed germination through the wet and dry seasons. General observations indicated that there was very little viable fruit produced over the Wet Season, and damage from leaf eating insects was highly prevalent at this time. Fruiting structures also appeared to rot, as illustrated in Photo 7.2. Competition from other roadside plants was also high, as illustrated in Photo 7.5. Seed cotton that was remaining in the bolls on the plants became susceptible to seed borers and rotting, and had fallen from the bush by the end of the Wet Season.

Fruit set and maturation increased at the beginning of the dry season, and by May, plants had produced considerable numbers of mature bolls. Photos 7.3 – 7.5 illustrate changes in plant growth and fruit over time for Plant 1, with high open boll load evident by July.

There had been some population succession with seedlings produced by all plants except those in the cattle yards, resulting in a high rate of population increase, as an indicator of invasiveness. All seedlings were within 3 m of the parent plant. However, all plants were Bt cotton, so there were no conventional plants for comparison. Results from the large-scale ecological assessment indicated that there was no difference between transgenic and conventional cotton in invasiveness.

Photo 7.2. Damage to fruiting structures over the wet season (7 January 2000) on Bt cotton volunteer plant in the ORIA

Conclusions and Implications for Weediness

Their presence indicates that small numbers of volunteer cotton plants could survive in some environments if protected from normal control measures. As reported elsewhere in this report, there is no indication that the risk of establishment and growth of Bt cotton is greater than that of conventional cotton.

Further monitoring of such volunteers would produce additional information on the risk of weediness under realistic scenarios. Such monitoring would also include a subset of plants which were not protected. It should be acknowledged that most of the plants monitored to date would have been controlled by routine practices. For example, roadside volunteers would be exposed to slashing, drain volunteers to herbicides, and cattle yard volunteers to grazing. A risk assessment and management strategy for commercial production would likely include a monitoring strategy for volunteers and a requirement for further control over the wet season.

Photo 7.3. Cotton volunteer plant in the early wet season (10 November 1999) with lint being dislodged by rain ready for germination

Photo 7.4. 3 July 2000 with mature open bolls

Photo 7.5. Volunteer Bt cotton plant 15 March 2001, at the end of the wet season, with substantial interspecific plant competition from roadside plants

SECTION 8

OVERALL PROJECT DISCUSSION

This risk assessment of Bt transgenic cotton in a range of environments across northern Australia represents one of the first comprehensive attempts to assess the weediness risk of a transgenic plant. This discussion addresses many of the methodological issues covered in the study, summarises the findings and conclusions, and highlights some ongoing issues for consideration.

Development of Methodology

It was acknowledged that a perceived risk of the commercialisation of genetically engineered plants was that transgenes for fitness-related traits may confer an increased potential for weediness in those plants (see various authors in Traynor and Westwood, 1999). Experiments comparing transgenic to non-transformed plants have traditionally compared agronomic and entomological properties within the environment of commercial fields (e.g. Underbrink and Landivar 1999). Few actual experiments had been performed with real genetically modified plants in natural habitats (Kjellsson and Simonsen 1994). There was no widely accepted framework as to what experimental data was required for an adequate risk assessment of transgenic plants (Hails 2000). Ecological biosafety research must focus on the specific inserted trait and the particular types of hazards they may cause to the environment (Kjellsson 1999;Saeglitz and Bratsch 2002) and it is critical to evaluate the impact of the transgene on a case-by-case basis (Schmitt and Linder 1994). Thus, a set of experiments was designed to evaluate the specific transgene/crop combination of Bt cotton (*Gossypium hirsutum*), and its ecological risk with respect to weediness in a specific regional environment, that of north-west Australia.

"*Exactly how ecologists must quantify weediness of a new organism is not straightforward, and posed challenging questions for experimental design and interpretation, the use of models, and statistical inference*" (Kareiva et al. 1996). This was exemplified in the design and implementation of this series of experiments on Bt cotton. Numerous questions must be addressed in assessing the weediness risks associated with the commercial introduction of transgenic crop plants, with relevant methodology required (Crawley 1990). An experimental evaluation of transgenic oilseed rape in natural habitats (Crawley et al. 1993) provided some of the methodology used in this project, where it was necessary to not only identify potential routes of dissemination of the Bt gene, but also to define the plant demographic parameters that would be suitable indicators of potential weediness.

This section discusses the rationale behind the development of the experimental methods and protocols to evaluate the potential weediness of Bt cotton in northern Australia, presents the factors experimentally manipulated, and the parameters measured as indicators of weediness.

Choice of Habitats

Linder and Schmitt (1995) highlighted the need to conduct risk assessment over the range where a transgenic crop will be commercialised, thus it was necessary to establish the experimental sites over the three regions where cotton could be potentially grown, namely Katherine, NT, and Kununurra and near Broome, WA. Performance of a transgenic crop in an agricultural setting may not reflect its capacity to survive in natural habitats (Purrington and Bergelson 1995). Many different habitats in which cotton could potentially occur, were incorporated into the overall weediness assessment. The final choice of experimental sites and the identification of different possible routes of cotton seed dissemination, were derived from observations in northern Australia of previous seed dispersal incidents and the habitats in which volunteers subsequently established. All these volunteers, once identified, were destroyed in compliance with GMAC guidelines. In all of the selected regions, both conventional and transgenic cotton had been grown over the previous few years as part of the on-going assessment of the potential for northern Australia to support a productive and sustainable cotton industry. Based on observations of these volunteer plants, four habitat types were selected for study: waterway, bush, roadside or cattle habitats.

Within Site Factors

Seedtype: Cotton seed can be dispersed in three forms: (1) Black or planting seed that has been acid delinted and generally treated with fungicides and/or insecticides; (2) Fuzzy, or ginned seed (majority of the lint removed); and (3) Seed cotton (unprocessed seed with a dense covering of cotton fibres). This is different to most other crops, particularly canola for which the majority of weediness risk assessment has been conducted. This has implications for overall ecological biosafety assessment as there were differing probabilities of each seedtype dispersing into particular habitats (discussed further

under Dispersal). All seedtypes were included in all habitats for a greater number of comparisons and increased confidence in the overall results.

Population: Crawley (1990) discussed that field experiments on invasiveness should include, among other factors, introductions at different population densities to establish whether there are threshold population sizes below which self perpetuating populations cannot be maintained. Tomiuk and Loeschcke (1993) concluded that there were no general rules on the size of minimum viable populations for establishment and persistence, with numbers specific to the particular species and conditions of release. While our experimental design did not provide the scope to develop estimates of threshold population sizes, population was included as a factor in one series of plantings, at two levels, classified as High and Low. This did provide some indication of population effects, particularly between different sites.

Genotype: A cotton industry in northern Australia would be based on Bt cotton. The commercial Bt cotton available over the duration of this project was INGARD ® containing the Cry1Ac gene from *Bacillus thuringiensis*. It was envisaged that a two-gene Bt cotton would be the basis for a northern cotton industry, so seeds containing Cry1Ac plus Cry2Aa were used in the initial experiment (Experiment 1A). Cry2Aa was superseded over the duration of the first year of the project, so two-gene cotton containing Cry1Ac and Cry2Ab was also included in subsequent experiments (e.g. Experiment 1B).

Planting Method

Cottonseed observed to disperse from research trials appeared to do so in clumps. Initial experiments were planted by placing the seed in handfuls on the soil surface, which had been hand-cleared. Seed was also enclosed in nets, with the uppermost side open to allow cotyledon emergence. However, extremely variable and low germination, likely due to differences in seed to soil contact depending on where in the clump individual seeds were positioned, and mechanical impedance from the netting, required that the planting method be modified if meaningful data was to be gathered in these experiments. One option was to increase seed numbers, but this was considered unacceptable due to potential difficulties in obtaining approval for release of much higher seed numbers into natural habitats. Instead, seed was uniformly distributed and hand-placed within a 25 cm^2 quadrat from which approximately the top 2 cm of soil and vegetation had been cleared, to ensure each seed had adequate seed soil contact. The seed was then recovered with the previously removed soil to prevent desiccation and predation.

It was originally intended to sow the seed, then wait for natural rainfall to stimulate germination. However, to standardise germination between sites, and between seasons, plots were hand-watered from sowing until the time of predicted first square, according to the long-term average of heat unit accumulation. This was equivalent to @540 DD12 (see Constable and Shaw (1988) for method), or @30 days. Watering aimed to ensure that seeds and resultant seedlings were not moisture stressed. The frequency and amount of watering varied between sites, dependant on soil characteristics, evaporative losses and rainfall. For example, the Broome road and bush sites were watered to saturation every two to three days for the entire period from sowing to predicted first square; the Kununurra Cattle Site No.1 was watered immediately after sowing, and then natural rainfall negated the need for any continued watering.

By stimulating germination to occur immediately after sowing, the risk of ungerminated seed being carried from the experimental site to an unregulated area where it could potentially then germinate was minimised. This then eliminated the need to use netting, which was observed to impede germination.

Thus, the planting method was designed to positively bias the probability of germination, providing a worst-case scenario for the escape and establishment of cotton into the selected habitats.

Demographic Parameters

There was no clear understanding of which character or subset of characters would accurately measure or predict the weediness potential of transgenic cotton, but some of the characteristics that have most commonly been discussed include seed production, competitive ability, seed dormancy, germination ability and pollen dispersal (Purrington and Bergelson 1995). Kjellsson (1999) describes tests specific for the inserted trait and include, 1) Plant growth (growth-rate and total plant biomass); and 2) Plant reproduction (seed germination, plant survival, flowering, seed production and seed survival).

Germination: The experiment was designed to maximise germination, to provide a worst-case scenario for weediness, as discussed above in the section on planting method.

Survivorship: Plant numbers were initially recorded approximately monthly from after germination and into the dry season (refer Tables 1.1a,b and c). As the dry season progressed, plants lost vigour and it was not always evident whether the plants were alive or not, and some plants that appeared dead regenerated after initial rains at the commencement of the wet season. Therefore, it was determined that the critical time to evaluate survivorship was at the end of the dry season, coinciding with the measurements conducted after the first rains which allowed viable plants to reshoot and produce vegetative matter.

Survivorship as a proportion of the number of seeds sown illustrated the likelihood of cotton developing as a volunteer given that a certain number of seed had escaped. Statistical analysis of such a proportion was relatively robust, as there were no missing values since all plots had seed sown. As such, these were the results presented. However, in a realistic situation where seed could accidentally disperse, the number of seeds introduced into a habitat would not necessarily be known, and actual numbers would be dependant on man-influenced activities, such as module carting and feeding seed, which could be manipulated by management. The observed establishment of volunteer plants would be evidence that seed had escaped although the percentage these represented from an original seed escape would be difficult to quantify.

Invasiveness: Due to the large number of sites where there very few remaining plants, which made statistical analysis inconclusive, a simplistic method of calculation was developed. This was in consideration with calculations of rate of population change as discussed by Crawley et al. (1993) and Parker and Kareiva (1996). This method of calculation needed to be modified to allow for establishment of a perennial plant plus potential recruitment of seedlings. Crawley et al. (1993) summarised their calculation for λ as the number of seedlings present after generation 2 divided by the number of seedlings of generation 1, but this primarily considered an annual plant, oilseed rape (*Brassica napus)*. This equation was modified for this series of experiments (as cotton is a perennial plant under tropical conditions), to consider the continued survival of the parent plant, plus the recruitment of new seedlings in subsequent generations. This also allowed differentiation between the initial establishment year, and the second season where plants have better established as perennials to reach a more stable population threshold.

The range of demographic parameters studied and the experimental approach adopted here is consistent with the method described by Linder and Schmitt (1995) to examine the entire life history of modified plants over several years. They also described another approach, which used smaller targeted experiments, designed to detect the effect of the transgene on plant performance during life history phases when the transgene is most likely to have a significant impact. This underlying principle was also used in this project to conduct smaller scale experiments assessing specific factors applicable to potential cotton escape.

Our study was initiated to determine whether the addition of a single genetically modified trait, i.e. insect tolerance, could provide additional fitness to Bt cotton that could lead to increased potential to become a weed compared to conventional cotton in northern Australia in habitats other than cultivated areas. A criticism of many previous submissions for commercialisation of transgenic crops was that weed was not explicitly defined (Parker and Kareiva 1996). We examined weed risk assessment protocols to develop a definition of a quantifiable parameter, that of invasiveness. This allowed for weediness conclusions to be made considering an absolute value as compared to a value judgement, as is often used: ‘a weed is a plant in the wrong place’.

A key component of weediness is invasiveness (Virtue, Groves, et al. 2001) for which criteria include means and distance of dispersal, susceptibility of habitat to invasion, and population growth rate (Auld and Coote 1980). Dispersal was not assessed experimentally, but through observations of seed escape and establishment of volunteer plants from cotton experimental areas. Susceptibility of habitats to invasion was considered in development of the methodology and site selection for the series of experiments, as outlined in the methodology section. Population growth rate was the major contributor of invasiveness that could conceivably differ between genotypes. This was experimentally assessed as the cumulative outcome of a series of demographic stages in the lifecycle of the cotton plant, namely, germination, seasonal survivorship, and fecundity. These components of invasiveness, and implications for comparative weediness between conventional and transgenic Bt cotton are discussed.

Dispersal

Three potential routes of gene dispersal from Bt cotton were considered (see Llewellyn and Fitt 1996):

1. Vegetative material. Cotton can reshoot (termed 'stub or 'ratoon' cotton) after harvest from taproot and stem remaining in the field, but this is managed through herbicide application or cultivation. There was little field or documented evidence to support that cotton can establish from plant parts in habitats away from cultivated areas, so this route was not considered a significant avenue of unintentional Bt gene dispersal.

2. Pollen. Gene dispersal via pollen had two aspects. The first was through introgression with native *Gossypium* species. This was considered functionally zero (Brown et al. 1997; Brubaker et al. 1999) and was not examined further in this project. The second component was of gene spread to naturalised populations of cotton, such as exist in northern Australia. This relies on proximity of the two parents. Results presented by Llewellyn and Fitt (1996) indicated that 20 m buffer zones would serve to limit dispersal of transgenic pollen although this was quantified to small-scale field tests. Examination of naturalised populations of cotton and was a lesser component of this project, presented in Section 6.

3. Seed. The primary route for dispersal of the Bt gene was considered to be as seed, and hence was the main aspect considered in this project.

Dispersal of the three seedtypes: Dispersal of seed from production areas is a physical process so was not expected to be different between different genotypes of cotton. Seedtype and site were the main factors effecting dissemination. Seedcotton had the greatest potential for unintentional dispersal, or 'escape'. Observations of seed which had 'escaped' during the course of approved research on cotton production in northern Australia, and establishment of subsequent volunteer cotton plants indicated that location affected seed cotton dispersal in accordance with the irrigation system practiced. Sites most susceptible were waterways in Kununurra where furrow irrigation was used. Volunteer plants in drains and channels were controlled by weed control practices used in these areas, in combination with seasonal monitoring. Proposed tailwater return systems for any future irrigation in the ORIA should restrict the dissemination of seed cotton through water flow to within the production area - where any volunteer plants would be controlled by traditional weed management practices such as cultivation and herbicide.

Escape of seedcotton into waterways from production areas based on overhead (lateral move or pivot irrigators) or sub-surface (drip-tape) irrigation, as is practiced at Katherine and Broome, was a negligible risk. Seedlings were observed to volunteer only within the field or on the perimeter where seed appeared to be constrained by surrounding vegetation as a buffer. This effectively minimised water flow as an agent of dispersal away from the sphere of influence of the production area into any surrounding undisturbed vegetation. Seedcotton remaining after harvest either rotted, or germinated and was subsequently killed by general weed control of herbicide, cultivation or chipping.

There is also increasing research effort evaluating wet season cover crops in northern Australia (Eastick, pers.comm. 2002). Research recently conducted at Kununurra and Katherine has evaluated crops such as forage sorghum, millet, rice and sabi grass (*Urochloa mosambicensis*) in an effort to improve soil health in the 'off-production' season (i.e. the wet season, after harvest is finished). These crops aim to minimise soil erosion from high intensity rainfall over the wet season, improve soil structure through increased organic matter, and to provide suitable mulch for no-till sowing (Katherine) or maintain bed structure (Kununurra) at the commencement of the next cotton production season (end of the wet season). Wet season cover crops are ideally vigorous competitors, and are likely to develop as an essential component of sustainable northern cotton production systems. Seedcotton germinating at the commencement of the wet season would be subject to competition in such a production system, which would decrease the probability for establishment of volunteer plants.

A major avenue of seed cotton dispersal at Kununurra was to roadside habitats during transport of cotton modules from the paddock to the gin. There was no dispersal of seedcotton to roadside habitats at Katherine and Broome due to regulations ensuring all seed was secured and covered prior to transport. It is likely that without such regulations, seedcotton would also have dispersed to the roadside during transport at all three locations. As such, seed would be subject to a wide range of microhabitats, with settling in a suitable niche attributable to chance. Any volunteer plants that did establish would be subject to roadside husbandry practices, particularly slashing at the end of the wet

season, and interspecific plant competition from road verge grasses, such as vigourous *Sorghum* species.

Fuzzy seed had the greatest potential for intentional spread into non-cotton production habitats, introduced as cattle feed, with seed spillage from troughs at feeding. There is also evidence that a proportion of seed can remain viable after passage through the digestive tract of cattle (see Appendix 4), with the additional benefit of germination in a nutrient-rich environment. Feeding areas were generally restricted, such as near cattle yards or within holding paddocks, which would also increase the chance of seedling mortality through trampling, or exposure to foraging in the dry season when other feed becomes limiting. Fuzzy seed had a low risk of unintentional dispersal as spillage after ginning in preparation for storage or packing, which would be within the confines of the processing area (gin yard), and controlled in the course of normal weed hygiene practices.

Cattle production targeting the live cattle export markets in Asia is a major industry in northern Australia. Cattle are fed concentrated feed stuffs such as compressed cubes and pellets whilst on the boats to their overseas destination. There is demand for local sourced high protein as a component of such feeds. A feeding trial was conducted in 2002 examining cattle weight gain on pellets containing cottonseed. Cattle weight gains were promising and observations indicated that the cottonseed was destroyed by physical crushing during the pelleting process. This provides an avenue of use for fuzzy seed from which no volunteer cotton would establish.

Black seed had the lowest risk of unintentional spread, and would be predominantly as spillage at sowing, which would be within the production area, and thus easily controlled in conjunction with other weed control husbandry.

Timing of dissemination: The greatest probability of cotton seed dispersal is as seed cotton during the harvest period, either as residue in the field after picking, drift into drains and paddock verges, or spillage onto roadsides during transport to the gin. Although not manipulated as an experimental variable, sowing time of the 'volunteer seed' aimed to coincide with the onset of the wet season. For example, the Broome sites were sown in mid-January (corresponding to long-term climate maximum mean monthly rainfall) to maximise the probability of germination and establishment. However, a commercial producer in this region would likely sow a crop in May, with the aim to harvest before November. Examination of long-term rainfall data, and for the two years of the study, indicated that there is little significant rainfall prior to December. Seed cotton would be exposed to environmental factors such as high temperatures (mean daily maximum in November of 34.6°C), and predation by both invertebrates and vertebrates, from time of harvest until commencement of the wet season. Similarly, fuzzy seed fed to cattle (often at the beginning of the dry season) would be exposed for a considerable period over the wet season before germinating rains would occur. (This is discussed further in Section 4; Experiment 3 – Seed Survivorship). The relative period between cottonseed dispersal and commencement of rainfall to induce germination will influence the length of time that seed is exposed to environmental factors, which may lead to a decrease in seed viability.

The influence of human activities on dispersal of introduced species has been largely unnoticed by experimental research, although man probably is a major cause for the dissemination of introduced species (Kjellsson 1999). This was certainly the case for cotton, and management strategies should address the effect man has on dispersal of Bt cottonseed.

Germination

Germination of seed in all sites was positively biased to enable optimum germination - simulating maximum volunteer cotton establishment. Results from the first experimental plots to be sown where seed was placed in clumps, left on the soil surface, and germination dependant on rainfall, indicated that germination was extremely low, and was too variable to make valid experimental conclusions. Consequently, seeds were hand-placed into cleared ground, covered with soil, and hand-watered in the early developmental stages, to provide the seeds and seedlings with the best chance of initial establishment. Thus, germination results are exaggerated and allow for evaluation of growth and development of experimentally valid plant numbers, and to provide a worst-case invasiveness scenario.

The significant factors pertaining to cotton germination were site, habitat, seedtype, and to a lesser extent, population. There was no effect of genotype, given that parent seed source was similar.

Effect of Habitat and Site

The significant difference in germination between habitats, and specifically, between sites (e.g. see Figure 1.5) supports the hypothesis different habitats have different levels of risk to the successful establishment of volunteer cotton. The greatest overall germination was observed in disturbed habitats (modified by man's activities), and generally with higher planes of nutrition compared to the bush and roadside habitats; Kununurra Cattle No.1, Katherine Cattle No.1 and Creek sites, and Broome Dam site (mean germination for these sites across all treatments = 75%). Of note, is that seeds in the bush sites, of which transgenic cotton developing as an environmental weed may be considered a concern, had relatively low germination rates (mean = 41%). Germination would occur after initial wet season rains, when there is substantial germination of more competitive and rapidly growing species such as annual grasses and twining legumes. A bush habitat is likely to have few bare ground patches (depending on grazing and fire history), on which seed cotton could fall, so that germination would be even more inhibited. Most plant species require some measure of disturbance before they can recruit from seed into mature perennial vegetation, a condition referred to as 'microsite limitation' (Crawley 1992). These results and observations indicate that germination of cotton in a native bush habitat is highly unlikely. Ecological performance is highly context specific, and the same genotype will give rise to phenotypes with different fitness in different environments (Crawley 1990), consistent with Hails et al. (1997) who found that behaviour of seeds from three genetic lines was highly context specific, with great variability from habitat to habitat. This was exhibited in the site variation in the large-scale ecological study.

The significant effect of block in a number of sites illustrated the importance of microhabitat on germination and indicated that even within a habitat that appeared suitable for cotton volunteer growth, that there were some niches more suitable than others. Such an effect was evident in the Broome Cattle No.1 site, where the spectrum of grass species that regenerated was different between pens and provided an indication of the effect that competitive species may have on the establishment and survival of volunteer cotton, particularly for bare ground space for seedling recruitment. Blocks 3 and 4 were in a pen in which dense stands of Rhodes grass (*Chloris* spp) and buffel grass (*Cenchrus* spp) established after the first wet season. These blocks had significantly less plants surviving compared to blocks 1 and 2 which were in a separate pen where native melons were the dominant species.

This again supports the concept of 'microsite' limitation as discussed by (Crawley 1992), and was particularly evident with habitats expected to possess some sort of edaphic gradient such as a drain or roadside. This has implications for cottonseed dispersal in the role of chance whether cotton seed settles in a suitable niche. E.g. bare ground on roadside.

Effect of Seedtype

The dominant factor influencing germination within sites was seedtype. There is little available literature documenting differences in germination between these three seedtypes under field conditions. This is logical, as cotton is produced for the purpose to remove the lint, plus lint coverage poses obvious practical difficulties in mechanised sowing. Hence, there is no production requirement to sow the seed as seed cotton, and consequently, little research demand to quantify germination of this seedtype. Ecological biosafety research of transgenic cotton seed dispersal is unique in comparison with other transgenic crops assessed to date such as oilseed rape (*Brassica napus*), sugar beet (*Beta vulgaris*), maize (*Zea mays*) and potato (*Solanum tuberosum*), (see Eastick et al. in press; Harding and Harris 1997). There is potential for three distinct forms of seed to escape, each with different levels of probability of occurrence and susceptibility of habitats, with resultant differences in risk of weediness of cotton plants originating from each seedtype.

Generally, black seed had the highest germination, followed by fuzzy seed, then by seed cotton (mean germination across all sites for Experiment 1A = 56.5%, 49.6% and 29.7% respectively). This had important implications for seed escape and mitigation strategies. Seed cotton has the greatest probability of uncontrolled dispersal. This would occur after harvest, coinciding with the commencement of the wet season. Seed cotton would also be the only form from which successive generation seedling recruitment would occur from the originally established volunteer plants. However, seed cotton had the lowest chance of germinating. Wanjura et al. (1969) surmised that perhaps the best early indicator of a seed's ultimate potential is the time it takes for a seed to germinate and emerge from the soil and concluded that those plants emerging earliest had the highest survival rate.

The emergence of the cotyledon from seed cotton was impeded by the abundance of lint, with lint also at times adhering to the soil surface, causing the cotyledon to bend and snap as it developed, or to be more exposed to insect attack. Plant stand success is influenced by soil impedance (the resistance to

root or shank elongation), which determines how hard the seedling shank must push on the cotyledons to move them through the layer of soil. Although some soil impedance is beneficial for shedding the seed coat from the cotyledons, severe impedance can restrict the plant's ability to emerge. In this case, the seedlings energy reserves are depleted by normal respiration (Kerby et al. 1996). It could be theorised that use of seed energy reserves to push the cotyledon through the soil surface would be expended to a greater extent for seed cotton as compared to the other two forms of cottonseed.

Seed cotton also exhibited a lag in germination between the other two seedtypes. This may have implications for realistic rainfall conditions where there may be cycles of wetting and drying, or no follow-up rain, where seed cotton remains in the field and is subject to environmental influences which may reduce its viability. High seed moisture and high temperature accelerate deterioration of cottonseed (Halloin 1975). Germination of acid-delinted cotton seed is reduced by preharvest exposure to weather, particularly if conditions are warm and humid, with seedling field emergence reduced by 11-33% due to weathering (Woodstock et al. 1985). Seed cotton remaining in the paddock after harvest, prior to the commencement of the wet season would experience more weathering compared to the two other seed types (harvested and stored), and thus have greater probability of reduced germination.

Fuzzy seed had relatively high germination potential. The main use for this form of seed in north Australia is as whole seed fed to cattle. Fuzzy seed could potentially escape into roadside habitats from trucks transporting the seed from the gin, but the major form of dispersal is as deliberate release around troughs, or possibly as seed passage through the digestive system. Thus, the combination of a highly germinable seed type into a habitat with high overall germination values, indicates there is a high probability of establishment of cotton volunteers under such a scenario.

Black seed, with the highest germination, conditional upon being buried, also had the least risk of unintentional dispersal. This form was observed to be the most vulnerable to insect attack, particularly by ants (non Lepidopteran, i.e. not susceptible to Bt) and desiccation if remaining on the soil surface.

Effect of Sowing Depth

Seedtype by depth interaction was highly significant for the one targeted experiment conducted to assess the importance of seed being buried or remaining on the soil surface (Experiment 2; Section Three). All seedtypes had a greater germination when buried compared to seed remaining on the soil surface. Within the buried treatments, seedcotton was significantly lower than the other two seedtypes, which was consistent with the majority of sites in the series of experiments where all seed was artificially buried. This illustrated the importance of seed soil contact for germination, as discussed by Kerby et al. (1996) who concluded that intimate seed-to-soil contact was critical, especially in sandy soils, regardless of initial moisture content. This would be of importance early in the wet season, particularly on the sandy soils near Broome where infiltration rates are rapid and soil surface moisture content was observed to decline rapidly. Wanjura et al. (1969) conducted a planting depth experiment, where 2.5cm planting depth treatments were omitted because of erratic emergence due to soil drying. Seeds that germinate at the surface would be less likely to survive than those deeper in the soil because of the greater risk of death by desiccation or disturbance (Colosi et al. 1988). This was the only site this specific depth by seedtype by genotype experiment was conducted. It is acknowledged that germination values would be different at the other sites, due to variability between habitat (as discussed by Hails et al. 1997), but the trend would be consistent. That is, seed remaining on the soil surface would have lower germination compared to if buried, and the experimental germination results for the ecological study sites would have been significantly less if the seed had not been artificially covered. This has implications for germination under natural volunteer conditions of escaped seed, which would predominantly fall onto the soil surface and would require a chance event such as wash by rainfall to be covered by soil.

Effect of Population

The effect of population generally was that the low treatment had lower germination, and greater variability, compared to the higher population. Freckleton and Watkinson (1998) found the most striking effects of temporal variability occurred at low population densities. This may be attributable to the impact of chance events contributing to the death of a seed or seedling in the low population would have a greater impact on the relative proportion of remaining plants as compared to the high population treatment. The interaction between seedtype and population in three sites (e.g. Kununurra Bush site 1) suggested that fuzziness inhibits water uptake. Germination was greater or equal at the low population for fuzzy seed and seed cotton than the high population, but lesser or equal between the low and high population for black seed. This infers that high seed population decreases water

available per seed for imbibition, and that this effect is more marked for fuzzy seeds and seed cotton. At the low population, there is less competition per seed for water to enable imbibition.

This has implications for seed dispersal, where the ability of an individual seed to germinate within a clump of fuzzy seed or seed cotton is relatively higher in a smaller clump than a larger clump, but that isolated seeds may have a reduced chance of establishing as a volunteer plant.

Effect of Genotype

There was no effect of genotype on germination in the initial 12 sites sown. The only site within Experiment 1A to provide some genotype effect was the Kununurra DS Drain Site (genotype by seedtype interaction; fuzzy conventional seed had lower germination compared to transgenic fuzzy seed). Genotype was significant in three of the seven sites for Experiment 1B, but trends were inconsistent. The genotype effect was suspected to be due to either difference in viability of the different seedtypes after storage, or parent seed source husbandry practices. For example, the conventional, single gene (Cry1Ac) and double gene (Cry2Aa) seed for the second year sowings were obtained from an unsprayed cotton paddock. It is conceivable that conventional genotype bolls were subject to greater insect damage compared to the two transgenic genotypes, thus reducing subsequent seed viability.

There was no genotype effect on germination for any of the targeted experiments.

Effect of Time of Germination

The optimum time for seed germination in seasonal climates such as in northern Australia is as early in the growing season as possible to gain resources for growth and reproduction but not so early that survival is unlikely (McIvor and Howden 2000). The ability of seeds to remain viable and dormant in the soil and to germinate in the presence of environmental cues that indicate a locally favourable environment for growth and reproduction can be stronger determinants of fitness than selective pressures during vegetative and reproductive phases (Linder and Schmitt 1995). Dormancy or hardseededness as strategies for seed persistence have been reduced to very low levels in modern cotton cultivars by conscious or unconscious selection (Delouche 1986), and cotton is not considered to possess seed that can persist in the environment for long periods of time (Serdy and Berberich 1995). A targeted experiment (see Section 2, Targeted Experiments; Dry Season seed survivorship) indicated that seed on the ground does not remain viable over the dry season, and was subject to breakdown and predation. Observations (volunteer monitoring) indicated that seedcotton either germinates, or rots over the duration of the wet season, although this is not necessarily all at the first natural rainfall. There appears to be little concern for long-term seed bank accumulation for cotton.

From the hand-watered experiments, where seed was watered to saturation, it would appear that cotton would be stimulated to germinate with the first ‘soaking’ rains. It was not determined what quantity of rainfall (either as a discrete or cumulative amount) was necessary for imbibition of cotton seed, particularly seed cotton. This has repercussions for seed that may escape at the end of the dry season (October –November) and germinate with an early rainfall event, but where lack of follow-up rain (this time lag between early rainfall events was not quantified) may lead to high seedling mortality. There was above average rainfall at all three locations for the 1999-2000 and 2000-01 wet seasons (refer Figure 1.4), which may have provided optimum conditions for cotton establishment compared to a below average rainfall wet season. Temporal variability in weather may profoundly affect weed population numbers (Freckleton and Watkinson 1998). High variance of rainfall events early in the wet season is typical of climatic patterns in northern Australia (Taylor and Tulloch 1985). Further information concerning requirements for seed cotton imbibition, and seedling mortality on the different soil types associated with periodicity of early wet season rainfall events, would enable a more robust predictive model of germination and early seedling survival success associated with long-term climatic trends.

Seed and dispersal ecology are major determinants of weed fitness and population growth rate (Jordan 1999), where seed bank dynamics and seedling establishment may be particularly important for the potential persistence of escaped transgenes (Schmitt and Linder 1994). Germination ability is a major determinant of fitness for which the addition of the Bt gene posed no advantage, and did not contribute to additional weediness potential at this important demographic stage.

Survivorship

Survivorship was highly correlated to germination for all sites, supporting that germination is a vital precursor to establishment of a volunteer cotton population. Site continued to provide a highly

significant effect on cotton demography, influencing survivorship after both one and two years for Experiment 1A. Site was observed to produce differences in survivorship after one year for Experiment 1B, but statistical results could not be presented due to different treatments applied between sites. However, results generally indicated that site, habitat and seedtype had the greatest influence on survivorship compared to population and genotype.

After One Year

For survivorship after one year, eight of the 13 sites for Experiment 1A had less than 10% of the plots with surviving plants, and these generally contained only a small number of plants within each plot. Only four of the 13 sites had greater than 50% of plots with remaining plants, corresponding to the Kununurra DS Drain, Katherine Cattle No.1, Katherine Creek and Broome Cattle No.1 sites. For these sites, there was a significant interaction between genotype and either population or seedtype. In only the Kununurra DS Drain site did the two transgenic genotypes have greater survivorship than the untransformed counterpart (fuzzy seed only), consistent with the trend for germination at his site, indicating a causal relationship between germination and survivorship.

Three of the seven sites in Experiment 1B had greater than 50% of plots with surviving plants, these being the Kununurra WS Drain 2, and the Kununurra and Broome cattle sites, although the cattle sites were evaluated as a proportion of the thinned population. The effect of thinning cotton populations removed confounding factors of seedtype and population, resulting in no significant factors on survivorship at these sites.

The seedtype by population interaction at the Kununurra WS Drain No.2 indicated a resource competition effect when plants are growing vigorously. The decline in plant numbers from the high to low density treatment from seedlings derived from black seed compared to the increase with fuzzy seed and seed cotton supports that competition for resources at higher plant densities, which occurred for the black seed treatments may induce a greater proportion of self-thinning within the population. This was discussed by Crawley (1990) who stated that at some point, population increase is prevented by competition for limiting resources; the population density at which this occurs is known as the density threshold. This suggests that in the advent of a large population of volunteers establishing early, that competition may lead to relatively higher proportion mortality until a density threshold is reached consistent with the available resources for that habitat.

After Two Years

Survivorship after two years was only applicable to the 13 sites sown in the first year. For all sites, the number of surviving parent plants declined over the two years. Nine of the 13 sites had few (<10%) or no plots with remaining plants. Only two of these sites had greater than 50% of the plots with surviving plants. These were the Broome Cattle Site No.1 and the Kununurra DS Drain, with results were similar to those for survivorship after one year, indicating that plants established after one year continued to survive into the second year as the population stabilised.

It should be noted that these habitats were protected from cattle intrusion, so survivorship was positively biased. The cattle habitats in Katherine and Kununurra where cattle continued to graze for the duration of the project, had lower survivorship compared to the protected sites. It should also be noted for the first season, that inter-specific plant competition was minimised as plots were cleared to sow the seed. It was observed that in most habitats, pre-existing vegetation was commencing to recolonise the area, particularly in the cattle yards.

Sites with the greatest survivorship were consistent with a higher plane of nutrition relative to the other sites, (Refer Appendix 1) and/or had adequate water supply. Also to consider, is that habitats in which water was non-limiting over the dry season (creek, drain and dam) are likely to become inundated by water over the wet season. Experimental results and field observations indicated that if seedlings become submerged for an extended period of time, mortality is high. Cotton is not well adapted to waterlogging due to its xerophytic ancestry (Hearn 1994).

It could be deduced that nutrition would have a significant role in survivorship of cotton in selected habitats, such as cattle yards. Increased nutrition may allow the plant to more rapidly establish, thus developing a more pronounced root system, which would consequently enhance survivorship. Also important to consider is that stressed plants, whether from moisture stress, waterlogging or nutrient deficiency, may not express the Bt gene effectively, thus negating any possible weediness benefit from the addition of the gene. A direct test of the hypothesis that a given ecological factor limits the abundance of a particular plant species requires manipulative field experiments, and such experiments are a critical necessity for ecological risk assessment of genetically modified plants

(Schmitt and Linder 1994). This was recognised, and experiments to clarify the role of the theorised limiting factor of nutrition were established, with fertiliser applied as a split-plot treatment in the bush habitats for the second year sowings at Kununurra and Katherine. It had also been theorised that increased nutrition would lead to increased attractiveness to herbivory, so insect enclosures were also included as another factor at Kununurra. However, poor establishment of plants rendered these sites experimentally non-viable although observations of the surviving plants did indicate that increased nutrition and protection from insect herbivory did lead to greater plant vigour and numbers of fruit produced. Plants with higher nutrition were also noted to possess greater ability to recover from grasshopper damage in the Kununurra Bush habitat. Grasshoppers were the most commonly observed insects on the cotton plants across all sites, predominantly in the wet season, and are considered the most important grazing insects in savanna ecosystems (Andersen and Lonsdale 1991). Grasshoppers were noted to chew emerging cotyledons, and seedlings chewed off below the level of the cotyledons did not recover (see Photo A3.1). Grasshoppers are not affected by Cry 1Ac or Cry2 Bt proteins, so would affect conventional and transgenic plants equally.

The influence of the Bt gene on cotton herbivory, specifically certain Lepidopteran species, is the underlying concern for increased fitness and potential for volunteer Bt cotton to become a greater weed compared to plants not possessing the insect tolerance gene. Information on herbivory has only rarely been combined with demographic data in order to assess the effects of herbivory on population dynamics of perennial species (Ehrlen 1995). Herbivory was initially estimated by allocating a damage rating to the cotton plants. This was primarily in the form of leaf damage. Given that the major insects deemed to be potentially affected by transgenic volunteer cotton would be *Helicoverpa* spp, *Earias huegeliana* (rough bollworm), *Pectinophera* spp (pink bollworms) and *Anomis flava* (cotton looper), which except for cotton looper, are fruit feeders, then assessment of foliage damage was not ideal. However, fruit measurements needed to be non-destructive to enable fruit to develop and be available for seedling recruitment the following season, so evaluation of internal damage would have been difficult. As it eventuated, plants at most sites did not reach a reproductive stage anyway. There were no significant factors from analysis of herbivory damage ratings in the initial stages of plant development. As the first season progressed, herbivory could not be attributed to being the major cause of foliage loss, as leaf loss probably occurred due to environmental stress. Herbivory by Lepidopteran species was not considered a major constraint to cotton establishing as a volunteer plant, so Bt genes would not affect establishment.

Fire was also a contributing factor to cotton mortality, with six of the 20 sites affected by fire in the two years of the project, such as illustrated for the Kununurra Road Site (Photos 3.1a, b and c). Fire is considered a major determinant of tropical savanna dynamics (Williams et al. 1996), and it could be expected that fire regimes would also influence dynamics of naturalised cotton populations. It is surmised that existing naturalised populations persist only in niches protected from regular or hot fires (see Section 3: Naturalised Cotton Monitoring).

Competition was also observed to influence cotton plant survival. Invasion ecology suggests that the role of disturbances, in interaction with fertility, are important factors for consideration (Prieur-Richard and Lavorel 2000). This was consistent with observations by Crawley et al. (1993) who found a positive correlation between rape seed production and the rate at which native vegetation recolonised the cultivated plots. The more fertile the plots, the higher the rapeseed production but the more quickly the open ground was colonised by native perennials, thereby limiting the opportunities for rape recruitment. It may be theorised that this would occur in higher nutrition areas where cotton may volunteer, but that regeneration of other species would subsequently limit the opportunities for cottonseed recruitment. This would be in the form of seed cotton, for which penetration through vegetation to achieve contact with the soil would be difficult.

It would be anticipated that plant numbers would continue to decline as available resources, such as residual nutrition, would be depleted, until the population reaches an optimum level from the artificially high seedling numbers originally introduced into the habitat. It was not determined when or what this population threshold would be, and whether there would be a difference between transgenic and conventional cotton. The two years duration for this project, over which a perennial plant was being examined, does not provide such long-term population data trends. Crawley et al. (1993) initially presented differences in transgenic crops in natural habitats. They extended the study and monitored the sites over a 10-year period. The few cases of increased transgenic plant survival that were significant in the short term did not translate into long-term differences in persistence, and cautions against making definitive conclusions on persistence in short-term ecological studies (Crawley et al. 2001).

Kareiva et al. (1996) discussed for a regulator trying to assign risk to a target plant, how many different sites and years should be examined with respect to that plant's capacity for population expansion, or invasiveness? They stated that the magnitude of errors due to reduced sampling effort was substantial, citing canola as an example, where the number of years was more important that the number of sites. They concluded that in general, any sampling effort of less than three years (regardless of number of sites) provided a very poor estimate of canola rate of increase. They also stated that it was hard to imagine that small-scale, short-term ecological experiments would offer accurate predictions regarding invasions, and would provide limited prognostic ability (Ellstrand and Schierenbeck 2000). Taylor and Tulloch (1985) considered that the year to year variation and the time interval between occurrences of a rainfall pattern meant that an ecological study in tropical Australia must continue for at least six to eight years, and that seasonal effects can outweigh treatment effects in manipulative experiments. Greater confidence in predicting the risk associated with a perennial plant such as cotton could be achieved by extending the duration of assessment of population change for those habitats deemed to be at risk. These would likely be areas with adequate nutrition and water availability as exhibited by the Broome cattle yards and Kununurra Drain sites. An assessment of these two sites (September 2002) after the initial project period indicated that the cotton populations are continuing to decline in plant number and vigour, with evidence of heavy grazing by cattle, and competition from species including black speargrass (*Heteropogon contortus*) and three-awn speargrass (*Aristida* spp). There was no obvious difference between genotypes, although quantitative measurements were not conducted.

There was no conclusive evidence supporting the influence of genotype on survivorship. The most consistent trend suggested that plants derived from seed cotton had the lowest survivorship. This may be associated with less seedling vigour due to greater expenditure of energy reserves for cotyledon emergence as discussed with respect to germination.

The initial survival of transgenic organisms depends to a significant extent on pure chance (Tomiuk and Loeschcke 1993) and supports that initial establishment of volunteer cotton plants will be dependant on seed dispersing into a suitable niche, even within habitats conducive to cotton establishment. Environmental influences and extrinsic factors such as nutrition, water availability, herbivory from insects other than those affected by the Bt gene, intra- and inter-specific competition, cattle grazing and trampling, and fire all contributed to cotton mortality. This was implied by the difference in survivorship between sites established experimentally, and also as observed with volunteer cotton dispersed through the course of previous research trials. This was consistent with findings from Raybould et al. (1999) who concluded that in feral rape populations, competition with perennial plants and herbivory by vertebrates seemed far more important to population persistence than did protection from insect herbivory.

Fecundity

There have been few experiments conducted that quantify fruit production between transgenic and non-transgenic crop plants in natural environments. These have primarily focused on rapeseed (considered a high risk for weediness due to weedy relatives; see Eastick et.al., in press), with no equivalent research being conducted on cotton prior to this project. Fecundity was considered as both number of open bolls produced, and also number of seedlings recruited.

Fruit Production
Fruit production between sites (20 sites for Experiment 1A and 1B) is presented in three categories:

1. There were ten sites from which plants produced no fruiting structures (including initial squares):

    - Katherine – Road, Creek No.1, Bush No.1 and Cattle No.2.
    - Kununurra – Cattle No.2, Road
    - Broome - Road, Dam, Bush No.1 and No.2

2. There were five sites from which plants progressed to early fruiting stages, but produced only isolated (<15) and damaged open bolls for the entire site over the two seasons:

    - Katherine – Cattle No.1, Bush No.2 (for one plant; nutrition split-plot treatment).
    - Kununurra – Bush No.1 and No.2, Cattle No.1
    - Broome - None

3. There were five sites from which plants demonstrated substantial reproductive capacity, producing more than 150 bolls total for the site:

    - Katherine – None
    - Kununurra – Drain habitats (Wet Season No.1, dry season, and wet season No.2)
    - Broome - Cattle No.1 and No.2

The majority of sites produced plants that did not develop according to predicted heat unit accumulation, and never reached reproductive stages. If it is considered that an average cotton plant under commercial cotton production systems may produce 10 bolls, each possessing approximately 40 seeds, then 14 of the 20 sites produced seeds the equivalent or less than that of a single commercial cotton plant. Observations on these sites, and a germination test for a specific site (Kununurra Bush Site No.1) indicated that the number and viability of seed produced per boll was less than that of a commercial cotton plant. However, specific habitats where there is a likelihood of conditions similar to that of commercial cotton production bays, may be conducive to cotton reproductive development, as represented by the third category of sites above.

Nutrition was the major factor to which the difference in fruit production between sites was attributed, as supported by the soil nutrient analyses (Appendix 1). Under commercial cropping conditions, nitrogen is considered to limit yield more frequently than any other nutrient, with only insect pests and crop water supply limiting yields to the same degree as nitrogen. The supply of nutrients (primarily carbohydrates and nitrogen) limits the number of fruit that can be matured (Hearn 1981).

The Broome Cattle site grew plants that produced the greatest open boll numbers of all sites. This corresponded to the highest levels of phosphorus, nitrogen and potassium compared to all other sites. The bush site at all three locations had very low levels of phosphorus particularly (<5 mg/kg), and also nitrogen. The fact that no sites at Katherine produced large numbers of bolls was attributable to site selection. It was observed that volunteer cotton plants (subsequently destroyed) in a cattle feeding out area not part of the experimental sites in Katherine, produced mature bolls. It was concluded that mature bolls could be produced at all locations given a suitable habitat, of which adequate nutrition was considered a key factor. Water availability was also inferred to be of importance evidenced by the relatively high fruit production in the Drain habitats. This was further supported by the existence of naturalised cotton populations predominantly in littoral or watercourse habitats. This availability of water, particularly changes between the wet and dry seasons, is considered to contribute to the phenology of perennial cotton development.

Fruit production was distinctly seasonal at each location. Plants were mainly vegetative and actively produced biomass over the wet season, developed bolls towards the end of the wet season, with open bolls being recorded into the onset of the dry season. This trend was also observed in the volunteer plants monitored at Kununurra, and similarly for the monitored populations of naturalised cotton. This is consistent with one of the strategies identified by Fryxell (1986) that wild *Gossypium* species use to enable them to survive arid conditions. Life cycles are adapted to growing vegetatively when water is abundant and deferring fruiting until the start of the dry season followed by dormancy during the rest of the dry season. Hearn (1994) further discussed this specific adaptive response of cotton to the environmental water regime, with fast vegetative growth and facultative fruit shedding in the ‘wet end’ and termination of vegetative growth, shedding of young fruit and maturation of old fruit at the ‘dry’

end. Cotton production in the ORIA in the 1970s was over the wet season compared to the dry season production currently being researched. Wilson et al. (1972) observed that during the rainy season, from late December to March, the shedding of fruiting forms was considerable, owing not only to failure to control insect pests but also to cloudy weather, intermittent waterlogging and difficulties in preventing excessive weed competition. High night temperatures have been shown to reduce pollen production and increase boll shedding (Reddy et.al. 1991). This seasonality of fruit production has implications for weediness. At times when moisture is not limiting, that is, over the wet season, other factors such as high temperatures, waterlogging, low radiation and plant competition may limit fruit production. Plants would require a habitat in which moisture is not limiting as the dry season progresses, to enable filling of bolls.

The Kununurra WS drain No.1 site only had three plots with plants remaining, one of each genotype. All plants were observed to produce large numbers of open bolls, but it was difficult to make any valid conclusions on the effect of genotype. This site was revisited (September 2002) after the project was finalised. There was evidence of heavy grazing by cattle as the fence had been damaged allowing access by cattle. Plants within the two-gene plot had been completely destroyed, and plants in the other two plots had been severely defoliated, providing evidence that cattle grazing may be an effective agent in reducing vigour of established volunteers under certain conditions. This is consistent with the end of the dry season when available feed is limited.

There were significant effects of both population and genotype for the Kununurra DS Drain site. The high population treatment produced greater number of bolls per plot, supporting that the initial demographic parameters of germination and survivorship are essential precursors, as plants must be present to enable the production of fruit. The single-gene treatment produced more bolls per plot than the conventional treatment. However, considering that this was consistent with the effect on survivorship for this site, this may indicate a causal relationship between survivorship and fecundity for bolls produced for the single genotype.

The importance of population was reiterated for the Broome Cattle No.1 site, where similar to the Kununurra DS Drain site, the higher population treatment produced a greater number of bolls. The interaction between population and genotype was approaching significance for the number of bolls produced after the first year, where there was an increase in number of bolls produced between the conventional and transgenic treatments for the high population only, again supporting the importance of a existing plant population to enable fruit production to occur. This was not the case in the second year.

There were no significant effects on boll production per plot for the productive sites in Experiment 1B, the WS Drain No.2, and the Broome Cattle No.2 habitats. The impact of the confounding influences of seedtype and population, and their effect on survivorship, and ultimately boll production were reduced for these sites. This was attributed to the majority of the plants surviving at the drain site negating the factors of seedtype and population. Within the Cattle site, population was removed as a factor, similarly reducing confounding effects.

The low mortality at the WS Drain site No.2 allowed for analysis of boll production per surviving plant, which showed that the two-gene treatment produced significantly less open bolls per surviving plant than the conventional and single-gene treatments. This provides evidence that given a population of cotton volunteers establishes there is no additional weediness potential from Bt cotton than conventional cotton.

There were indications that time of maximum fruit production may vary with genotype and season. This was evidenced by more determinant boll setting of the transgenic plants, and attaining their reproductive capacity earlier than conventional cotton. This is consistent with observations under commercial production where two-gene cotton may ‘cut-out’ earlier than conventional genotype within a season. For some plots at the Broome Cattle No.1 site, maximum open boll production occurred in the first year, but this occurred in the second year for the remaining plots. It was not determined if cumulative boll production would continue to increase for successive seasons. Qualitative assessment of the Broome Cattle No.1 and No.2 sites, and the Kununurra DS Drain site after the project conclusion (September 2002) indicated that fruit production was declining.

Assessment of fruit production was predominantly done per plot, where if there were no plants present, then number of fruit produced were zero. However, a better indication of relative fecundity with respect to weediness, would be fruit production given that a plant has established, as the addition of the Bt gene would be expected, and was experimentally shown, to provide no weediness advantage

to the earlier demographic phases. Fruit production per surviving plant could not be statistically interpreted due to the large number of plant deaths (missing plots). To study open boll production more precisely, the confounding effects of germination and early survival could be reduced. This could be experimentally achieved by removing the factors of seedtype and population, and planting a large number of black seeds, which would tend to maximise establishment, of which the resultant population could then be thinned to a constant number.

There was no consistent evidence to indicate that the addition of the Bt gene led to an increase in number of open bolls produced per unit area. The major influence on this parameter was the population treatment where the high population resulted in a greater number of bolls produced. This was due to a greater chance that there would be plants surviving, and thus able to produce fruit. Fecundity is considered a major parameter contributing to invasiveness, but an increase in fecundity does not necessarily translate into increased invasiveness (Bergelson 1994).

The only sites to produce second generation seedlings were the Kununurra WS Drain No.1 and DS Drain sites, and the Broome Cattle No.1 site. These were produced in isolated plots, so statistical analysis was inconclusive. Recruitment of seedlings as a contributor to an increase in overall population growth was included in the parameter of invasiveness.

Invasiveness

The demographic stages of germination, survivorship and fecundity contributed to evaluation of population growth rate (λ). This was an appropriate measure of invasiveness which provided an absolute value to allow comparison between genetic lines (Parker and Kareiva 1996), and which was a major component of weediness (Virtue, Groves, et al. 2001) . A rate less than 1 meant that the line will go extinct, while a rate greater than 1 meant that a line will increase exponentially in abundance, until a limit is reached, set by competition or some other density-dependant process.

Gidding (1999) discussed an invasiveness model for perennials, where populations could be structured according to age or stage, requiring information on survivorship from time, *t* to *t+1*, for each age or stage. There were obvious differences in population growth for cotton in the initial year, where there was high initial seedling mortality as populations established, to that in the second year where mature plants persisted. Consequently, invasiveness was evaluated for two stages within the project period, corresponding to the initial (λ_1) and second (λ_2) year of cotton population development, as discussed in the methodology. Values for λ_1 for the second year sown sites (Experiment 1B) are indicative of survivorship as a proportion of plants germinated. This is a valuable component in the calculation of invasiveness, but there was no opportunity for additional seedling recruitment by the time of final measurements as little rain had fallen by this time.

This importance in defining the stages to contribute to an invasiveness model for perennials, as discussed by Gidding (1999), was further supported by studies on oilseed rape conducted by Crawley et al. (1993). They found that all treatment genetic lines had $\lambda>1$ on the cultivated plots, based on seeds starting life in a cultivated, competition-free environment. In contrast, the seeds produced by the first generation of the experimental plants were shed into an environment in which the competing vegetation had a full growing season to recover from the cultivation. They computed a new estimate of λ based on the number of self-sown seedlings in year t+1 to the number of seedlings arising from the experimental sowing, similar to our calculation of λ_2. They found the seed sown into undisturbed vegetation never produced values of $\lambda>1$, despite the fact that a few individual plants grew vigorously and set substantial seed under apparently inhospitable conditions. The conclusions were consistent with those for Bt cotton, where the invasion criterion ($\lambda_2>1$) was not observed in any of the habitats, except the Kununurra WS Drain No.1 site.

The simplistic method used to calculate invasiveness for sites with few remaining plants resulted in an invasiveness value greater than or equal to one for the Kununurra WS Drain No.1 site only. This corresponded to a value for λ_2 of 1 for the conventional genotype, and 2 for the single-gene genotype. The two-gene treatment resulted in a value of 0.333. It is difficult to infer conclusions on invasiveness from these values as there was only one plot remaining of each genotype, and number of remaining plants were low. A visit to this site in September 2002, after the project was finalised, revealed that the majority of recruited seedlings observed previously had since died. Quantitative assessment was not conducted, but invasiveness values would be decreased from the previous measurement conducted (λ_2).

Invasiveness by the simplistic method was across both populations, and the three seedtypes. So, in situations where seed cotton could potentially escape, and in second-generation recruitment of seedlings, these figures are likely to be less, without the distortion of the more buoyant influence of black and fuzzy seed on germination.

Treatment effects on invasiveness were statistically analysed if sufficient plant numbers were remaining, corresponding to the Katherine Cattle No.1, Broome Cattle No.1 and No.2, Kununurra DS Drain and WS Drain No.2 sites. Invasiveness values for the second year were generally greater than for the initial year, but at no time were either λ_1 or λ_2 greater than one for the mean of any genotype, although values greater than one were obtained by individual plots. There was a significant effect of genotype on λ_1 for the Katherine Cattle No.1 site, and on λ_2 for the Kununurra DS Drain site. In both instances, the two-gene genotype resulted in a significantly lower value than the other two genotypes. This trend was also observed for λ_2 at the Broome Cattle No.1 site, although differences were not significant. This effect was due to the observed differences in seedling recruitment between genotypes.

Statistical analysis was not done on the number of recruited seedlings due to the low number of plots in which this occurred. Plants from a total of eight plots produced seedlings at the Kununurra DS Drain site. Plants from the two-gene plots produced a total of 48 seedlings in the first wet season, compared to zero and two only from the conventional and single gene treatments. Final recruited seedling numbers by the second wet season were 38, nine, nine for the two-gene, conventional, and single-gene treatments respectively. Similarly, plants from a total of 17 plots produced seedlings at the Broome Cattle No.1 site. Plants from the two-gene plots produced a total of 115 seedlings in the first wet season, compared to 11 and 22 from the conventional and single gene treatments. Final recruited seedling numbers by the second wet season were 58, six, and ten for two-gene, conventional, and single-gene treatments respectively. Although the actual number of seedlings produced was greatest for the two-gene treatments for both these sites, the decline in numbers from the first to the second season resulted in the lowest invasiveness value for each site.

Observations at the Kununurra DS Drain and the Broome Cattle No.1 sites indicated that the two-gene plants from the original cohort of seeds had the least vigour by the time of final measurements, although plants were persisting. This decline in vigour and survivorship may be attributed to the trend that the two-gene plants produced a greater number of bolls than the conventional treatments. Although this was not statistically significant in either site, the biological effect may have resulted in two-gene plants expending greater reserves to produce bolls and greater number of seedlings which then also competed with their parent plants, to effectively deplete resources (nutrition, water) and decrease plant vigour. Continued measurements over subsequent seasons would be able to further quantify effects of genotype on persistence of these populations.

Previous studies to assess seed production to predict whether transgenic plants will have increased weediness potential include that conducted by Raybould et al. (1999). They found that transgenic insect resistant feral oilseed rape produced more seed per unit area than the equivalent unmodified variety, and that significantly more seedlings were produced in the treatment plots compared with the control plots, although absolute numbers were small. They quantified these results by stating this did not mean that insect resistant feral oilseed rape would necessarily cover a larger area or be more persistent than non-resistant varieties. This was due to high seedling mortality by frost, heavy predation by vertebrate herbivores, and competition with perennial plants. Stewart et al. (1997) concluded that where a suitable habitat was readily available, there was a likelihood of enhanced ecological risk associated with the release of certain transgene/crop combinations such as insecticidal rapeseed. However, the greater fitness of increased differential reproduction and survivorship in favour of Bt plants under insect selection pressure (herbivory from diamondback moth, *Plutella xylostela*) was only apparent in the absence of plant competition. These conclusions were similar to ours for Bt cotton.

Trends in the data suggested that the transgenics may have produced more bolls and seedlings than the conventional cotton, but this was not statistically significant and effects were confounded by other factor interactions with genotype. However, our findings were consistent with those discussed by Raybould et al. (1999), Stewart et al. (1997) and Crawley et al. (1993) above, in that any increase in fecundity may not necessarily transpose to increased area, or persistence, as evaluated by our calculations of invasiveness resulting in values less than one in all but one habitat (where there was only one plot of each genotype remaining). The low values for invasiveness were due to a number of constraints to seedling persistence.

Factors contributing to seedling mortality in all genotypes of cotton experimentally assessed were identified as similar to those in the transgenic weediness studies discussed above. These included herbivory by vertebrates and interspecific plant competition (Raybould et al. 1999), plant competition and the externally determined rate of disturbance (Crawley et al. 1993) and space in the vegetation for recruitment (Bergelson 1994).

The timing of calculation of invasiveness had significant implications on the resultant values. Presented values were for the conditions over the two years of the project, and no attempt at predicting long-term cotton weed dynamics using population models was made. The calculation of λ_1 for the second year sown sites (Experiment 1B), and of λ_2 for the first year sown sites (Experiment 1A) did not allow for additional recruitment of seedlings over the following wet season (2001-02), as the project was concluded prior to adequate rainfall to stimulate dislodging of bolls to the ground and the chance for germination. New seedlings were observed at a later visit to the Kununurra DS Drain site. Ideally, population growth rate for perenniated cotton plants should be assessed over greater than two wet seasons, given that in the first wet season the plants were germinating and establishing from the original cohort of seeds. The second wet season allowed for seedling recruitment to occur from the established and perenniated original plants. Results after this time indicated that the addition of the Bt gene did not increase the values of invasiveness, and indications were that the two-gene treatment may have had reduced values, perhaps attributable to biologically higher boll production and seedling recruitment although this effect was not statistically significant. Further support of these results could be gained from quantitative monitoring of the sites where recruited seedlings were recorded (Broome Cattle and Kununurra Drain sites) to provide greater confidence in the assessment of invasiveness of a perennial plant and predictions of the fate of the established plants and recruited seedlings. Population growth rate would consequently be assessed between t_{final} (three years plus), and $t_{established}$ (current λ_1) to better evaluate invasiveness once a population has established. This would effectively exclude the relatively high mortality in the initial year (which may not be observed in a realistic field situation, as it is only the survivors that become apparent).

The need for extension of monitoring to effectively determine invasiveness was discussed by Freckleton and Watkinson (1998), who concluded that estimating λ through detailed monitoring of populations across one or a few seasons would not be adequate for estimating the variance in λ, or even to estimate the geometric mean with great certainty. Saeglitz and Bratsch (2002) suggested that ecological experiments of only a few years duration are limited in their prognostic ability. Crawley et al. (1993) presented initial results from a three-year study examining four different crops (oilseed rape, potato, maize and sugar beet) in 12 different habitats, and recently presented results monitored over 10 years (Crawley et al. 2001). In no case were the genetically modified plants found to be more invasive or more persistent than their conventional counterparts, with populations of maize, rape and sugar beet extinct at all sites within four years of sowing. Results for the initial two and a half years of our study similarly indicated that in no case were the Bt cotton plants found to be more invasive or more persistent than their conventional counterparts. Similarly, populations at all sites appear to be heading for extinction, supported by calculated invasiveness values less than 1, (with the exception of the Kununurra WS Drain No.1 site), and field observations. A visit to the Kununurra drain sites in September 2002 indicated that populations were continuing to decline, although quantitative measurements were not taken. Extension of monitoring would confirm or deny this trend.

Conclusion

Manipulative experiments estimating changes in fitness are still relatively rare, particularly over more than one generation, and both absolute and relative estimates of fitness will vary with ecological conditions, habitat and year. It is always possible to think of a set of ecological conditions that have not been tested (Hails 2000). On the basis of a series of multi-site manipulative experiments on cotton demography, a number of specific studies of life history stages and observations of naturalised cotton populations the following conclusions can be drawn:

1. The inclusion of Bt genes in cotton did not enhance the capacity for seed dispersal.

2. The inclusion of Bt genes in cotton did not enhance germination capacity for seed placed into a number of habitats. Seedtype was the major factor influencing germination, with seed cotton having the poorest germination. Buried seed had a greater chance of germinating than seed remaining on the soil surface, the latter applicable to most cases of cottonseed dispersal.

3. Cottonseed remaining on the soil surface in a bush habitat over a dry season is unlikely to remain viable to the following wet season when the next germination opportunity occurs.

4. There was no consistent evidence that inclusion of Bt genes in cotton enhanced survivorship after one or two years. After two years the populations of original plants had declined markedly at all sites.

5. In all but two habitats, plants did not develop to produce numbers of viable open bolls. Population was the main factor affecting number of open bolls produced, indicating a causal relationship with volunteer plant establishment. For the Broome Cattle No.1 and Kununurra DS Drain sites, trends indicated that there was an increase in fecundity between transgenic genotypes and non-transformed cotton.

6. Decreasing the confounding effects of germination and survivorship by removing the factors of seedtype and population would enable more precise estimation of differences in fecundity between genotype given that a plant establishes.

7. An increase in fecundity did not translate to an increase in invasiveness. For only one site (Kununurra WS Drain No.1) was the invasion criterion satisfied (λ>1), but differences between genotype could not be determined due to only 1 plot of each genotype surviving.

8. Further monitoring of experimental sites where cotton has established would provide more conclusive evidence that the rate of cotton population change is negative, suggesting that the population is heading towards establishment of a threshold based on some density dependant process, or extinction.

9. Major constraints limiting the capacity of cotton as an invasive weed included herbivory by non-Lepidopteran species (vertebrate and invertebrate), interspecific and intraspecific plant competition (space in vegetation for recruitment), waterlogging, water deficit, fire, and poor soil fertility.

10. There is minimal risk that the Bt gene would confer additional fitness to existing populations of naturalised cotton through gene flow. Naturalised populations are not considered to pose a risk of increased weediness because:

    - The majority are in geographic locations isolated from suitable production areas.
    - Our ability to eradicate known populations which may overlap if production areas are established.
    - They are represented by old genotypes not used in modern commercial production.
    - There is no evidence that existing populations are invasive of their current habitats.

11. In the unlikely event of gene transfer, the addition of the Bt gene would not enhance the weediness of existing naturalised cotton populations, because major limiting factors are seed survival and seedling establishment where Bt susceptible insects are **not** a significant constraint.

The study provides no conclusive evidence that the addition of the Bt gene conferred additional fitness to cotton plants in non-agricultural habitats in northern Australia.

REFERENCES

Andersen, A. N. and Lonsdale, W. M. (1991). Herbivory by Insects in Australian Tropical Savannas: a Review. Werner, P. A. Savanna Ecology and Management. 89-100. Oxford, Blackwell Scientific Publications.

Auld, B. A. and Coote, B. G. (1980). A model of a spreading plant population. *Oikos* **34**, 287-292.

Bergelson, J. (1994). Changes in fecundity do not predict invasiveness. *Ecology* **75**, 249-252.

Bergelson, J., Purrington, C. B., and Wichmann, G. (1998). Promiscuity in transgenic plants. *Nature* **396**, 25.

Brown, A. H. D., Brubaker, C., and Kilby, M. J. (1997). Assessing the risk of cotton transgene escape into wild Australian *Gossypium* species. McLean, G. D., Waterhouse, P. M., Evans, G., and Gibbs, M. J. Proceedings of a Workshop: Commercialisation of Transgenic Crops: Risk, Benefit and Trade Considerations. Canberra, Cooperative Research Centre for Plant Science and Bureau of Resource Sciences.

Brown, A. H. D., Hurka, H., Craven, L. A., and Grace, J. P. (1994). Risk of escape of transgenes from cotton into natural populations of Australian *Gossypium* species. Constable, G. A. and Forrester, N. W. (eds.) *In* Challenging the Future: Proceedings of the World Cotton Research Conference. Melbourne, CSIRO Publishing.

Brown, S. L. (1999). Transgenic sugar beet in natural habitats. Amijee, F., Gliddon, C. J., and Gray, A. J. (eds.) *In* Environmental Impact of Genetically Modified Crops. Research Report No.10. Genetically Modified Organisms. London, U.K., Department of the Environment, Transport and the Regions.

Brown, V. K., Gange, A. C., Evans, I. M., and Storr, A. L. (1987). The effect of insect herbivory on the growth and reproduction of two annual *Vicia* species at different stages in plant succession. *Journal Of Ecology* **75**, 1173-1189.

Brubaker, C. L. (2002) (unpub.). An Evaluation of the scientific data bearing on the potential for gene flow between GM cottons and *Gossypium* K-genome species indigenous to Western Australia and the Northern Territory. A Report prepared for OGTR Review Panel.

Brubaker, C. L., Brown, A. H. D., Stewart, J. M., Kilby, M. J., and Grace, J. P. (1999). Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. *Euphytica* **00**, 1-16.

Bullock, J. M. (1999). Using population matrix models to target GMO risk assessment. *Aspects of Applied Biology.* **53**, 205-212.

Colosi, J. C., Cavers, P. B., and Bough, M. A. (1988). Dormancy and survival in buried seeds of proso millet (*Panicum miliaceum*). *Canadian Journal of Botany* **66**, 161-168.

Constable, G. A. and Shaw, A. J. (1988). Temperature requirements for cotton. Agfact P5.3.5. Agdex 151/022. NSW Agriculture and Fisheries.

Cowie, I. D. and Werner, P. A. (1987). Weeds in Kakadu National Park - A survey of alien plants. Report to Australian National Parks and Wildlife Service from CSIRO.

Craven, L. A., Stewart, J. McD., Brown, A. H. D., and Grace, J. P. (1994). The Australian wild species of *Gossypium*. Constable, G. A. and Forrester, N. W. (eds.) *In* Challenging the Future: Proceedings of the World Cotton Research Conference-1. pp 278-281. Melbourne, CSIRO Publishing.

Crawley, M. J. (1990). The ecology of genetically engineered organisms: Assessing the environmental risks. Mooney, H. A. and Bernadi, G. (eds.) *In* Introduction of Genetically Modified Organisms into the Environment. SCOPE No.44. 133-150. Chichester, John Wiley and Sons.

Crawley, M. J. (1992). The comparative ecology of transgenic and conventional crops. Casper, R. and Landsmann, J. (eds.) *In* Proceedings of the Second International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. Braumschweig, Germany.

Crawley, M. J., Brown, S. L., Hails, R. S., Kohn, D. D., and Rees, M. (2001). Transgenic crops in natural habitats. *Nature* **409**, 682-683.

Crawley, M. J., Hails, R. S., Rees, M., Kohn, D., and Buxton, J. (1993). Ecology of transgenic oilseed rape in natural habitats. *Nature* **363**, 620-623.

Curteis, W. M. The Early History of Agriculture and Settlement in the NT. (unpub.) NT Government Technical Report.

De Kroon, H., van Groenendael, J., and Ehrlen, J. (2000). Elasticities: A review of methods and model limitations. *Ecology* **81(3)**, 607-618.

Delouche (1986). Factors affecting seed quality. Mauney, J. R. and Stewart, J. McD. (eds.) *In* Cotton Physiology. Number One. The Cotton Foundation Reference Book Series. @514. Memphis, TN, The Cotton Foundation.

Dunlop, C.R. pers.comm. (2000). Botanist. NT Herbaruium.

Eastick, R. J., Yeates, S. J., and Constable, G. A. (2002) Weediness in Bt crops. 4th Pacific Rim Conference on the Biotechnology of *Bacillus thuringiensis* and its Environmental Impact. (In press).

Ehrlen, J. (1995). Demography of the perennial herb *Lathyrus vernus*. II.Herbivory and population dynamics. *Journal of Ecology* **83**, 297-308.

Ellstrand, N. C. and Schierenbeck, K. A. (2000). Hybridisation as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA* **97**, 7043-7050.

Freckleton, R. P. and Watkinson, A. R. (1998). How does temporal variability affect predictions of weed population numbers? *Journal of Applied Ecology* **35** , 340-344.

Fryxell, P. A. (1986). Ecological adaptations of *Gossypium* species. Mauney, J. R. and Stewart, J. McD. (eds.) *In* Cotton Physiology. Number One The Cotton Foundation Reference Book Series. 1-7. Memphis,TN, The Cotton Foundation.

Fryxell, P. A., Craven, L. A., and Stewart, J. M. (1992). A revision of *Gossypium* sect. *Grandicalyx* (Malvaceaa), including the description of six new species. *Systematic Botany* **17(1)**, 91-114.

Gidding, G. D. (1999). The role of modelling in risk assessment for the release of genetically engineered plants. Ammann, K., Jacot, Y., Simonsen, V., and Kjellsson, G. (eds.) Methods for Risk Assessment of Transgenic Plants. III.Ecological Risks and Prospects of Transgenic Plants, Where Do We Go From Here? A dialog between biotech.industry and science. 31-41. Basel, Switzerland, Birkhauser Verlag.

Hails, R. S. (2000). Genetically modified plants - the debate continues. *Trends in Ecology and Evolution* **15(1)**, 14-18.

Hails, R. S., Rees, M., Kohn, D. D., and Crawley, M. J. (1997). Burial and seed survival in *Brassica napus* subsp. *oleifera* and *Sinapis arvensis* including a comparison of transgenic and non-transgenic lines of the crop. *Proceedings Royal Society of London* **264**, 1-7.

Halloin, J. M. (1975). Solute loss from deteriorated cotton seed. Relationship between deterioration, seed moisture and solute loss. *Crop Science* **15**, 11-15.

Harding, K. and Harris, P. S. (1997). Risk assessment of the release of genetically modified plants: a review. *Agro Food Industry Hi-Tech* **8**, 8-13.

Hearn, A. B. (1981). Cotton nutrition. *Field Crop Abstracts* **34 (1)**, 11-34.

Hearn, A. B. (1994). The principles of cotton water relations and their applications in management. Constable, G. A. and Forrester, N. W. (eds.) Challenging the Future: *Proceedings of the World Cotton Research Conference - 1*. 66-92. Melbourne, CSIRO.

Holm, L., Pancho, J. V., Herberger, J. P., and Plucknett, D. L. (1979). A Geographical Atlas of World Weeds. 1979. Brisbane, Australia, John Wiley and Sons.

Jordan, N. (1999). Escape of pest resistance transgenes to agricultural weeds: Relevant facets of weed ecology. *In* Traynor, P. L. and Westwood, J. H. (eds.) Proceedings of a Workshop on: Ecological Effects of Pest Resistance Genes in Managed Ecosystems. 1999. Blacksburg, Virginia, Information Systems for Biotechnology. pp 27-32.

Kareiva, P. (1993). Transgenic plants on trial. *Nature* **363**, 580-581.

Kareiva, P., Parker, I. M., and Pascual, M. (1996). Can we use experiments and models in predicting the invasiveness of genetically engineered organisms? *Ecology* **77(6)**, 1670-1675.

Keeler, K. H. (1989). Can genetically engineered crops become weeds? *Bio/Technology* **7**, 1134-1139.

Keeler, K. H., Turner, C. E., and Bolick, M. R. (1996). Movement of crop transgenes into wild plants. Duke, S. O. (eds.) *In* Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. 303-330. CRC Press Inc.

Kerby, T. A., JohnsonHake, S., Hake, K. D., Carter, L. M., and Garber, R. H. (1996). seed quality and planting environment. *In* Cotton Production Manual. (eds. T. A. Kerby, S. JohnsonHake, and K. D. Hake.) p. Chapter 16. (Division of Agriculture and Natural Resources: University of California.)

Kjellsson, G. (1999). Methodological lacunas: the need for new research and methods in risk assessment. Ammann, K., Jacot, Y., Simonsen, V., and Kjellsson, G. (eds.) Methods for Risk Assessment of Transgenic Plants. III.Ecological Risks and Prospects of Transgenic Plants, Where Do We Go From Here? A Dialog Between Biotechnology Industry and Science. 185-194. Basel, Switzerland, Birkhauser Verlag.

Kjellsson, G. and Simonsen, V. (1994). Methods for Risk Assessment of Transgenic Plants. I.Competition, Establishment and Ecosystem Effects. Basel, Switzerland, Birkhauser Verlag.

Linder, C. R. and Schmitt, J. (1995). Potential persistence of escaped transgenes: Performance of transgenic oil-modified *Brassica* seeds and seedlings. *Ecological Applications* **5 (4)**, 1056-1068.

Llewellyn, D. and Fitt, G. (1996). Pollen dispersal from two field trials of transgenic cotton in the Namoi Valley, Australia. *Molecular Breeding* **2**, 157-166.

McIvor, J. G. and Howden, S. M. (2000). Dormancy and germination characteristics of herbaceous species in the seasonally dry tropics of northern Australia. *Austral Ecology* **25**, 213-222.

McKenzie, D. C. (1998). SOILpak for cotton growers. NSW Agriculture.

Panetta, F. D. (1993). A system of assessing proposed plant introductions for weed potential. *Plant Protection Quarterly* **8(1)**, 10-14.

Parker, I. M. and Kareiva, P. (1996). Assessing the risks of invasion for genetically engineered plants: acceptable evidence and reasonable doubt. *Biological Conservation* **78**, 193-203.

Parsons, W. T. and Cuthbertson, E. G. (1992). 'Noxious Weeds of Australia.' (Inkata Press: Melbourne, Australia.)

Payne, J. H. (1995). USDA/APHIS Petition 94-308-01p for determination of non-regulated status for Lepidopteran-resistant cotton lines 531, 757, 1076. 31 pages. US Department of Agriculture.

Payne, J. H. (1997). USDA/APHIS Petition 97-013-01p for determination of non-regulated status for Events 31807 and 31808 Cotton. 13 pages. US Department of Agriculture.

Pheloung, P. C. (1995). Determining the weed potential of new plant introductions to Australia. A report on the development of a Weed Risk Assessment System commissioned by the Australian Weeds Committee and the Plant Industries Committee. Perth, Australia, Department of Agriculture.

Purrington, C. B. and Bergelson, J. (1995). Assessing weediness of transgenic crops: industry plays plant ecologist. *Trends in Ecology and Evolution* **10(8)**, 340-342.

Prieur-Richard, A. and Lavorel, S. (2000). Review article. Invasions: the perspective of diverse plant communities. *Austral Ecology* **25**, 1-7.

Raybould, A. F., Moyes, C. L., Maskell, L. C., and Mogg, R. J. (1999). Predicting the ecological impacts of transgenes for insect and virus resistance in natural and feral populations of *Brassica* species. Ammann, K., Jacot, Y., Simonsen, V., and Kjellsson, G. (eds.) Methods for Risk Assessment of Transgenic Plants. III.Ecological Risks and Prospects of Transgenic Plants, Where Do We Go From Here? A Dialog Between Biotechnology Industry and Science. 3-15. Basel, Switzerland, Birkhauser Verlag.

Reddy, V. R., Reddy, K. R., and Baker, D. N. (1991). Temperature effects on growth and development of cotton during the fruiting period. *Agronomy Journal* **83**, 211-217.

Saeglitz, C. and Bratsch, D. (2002). Review article: Plant gene flow consequences. *AgBiotechNet* **4**, 1-8.

Schmitt, J. and Linder, C. R. (1994). Will escaped transgenes lead to ecological release? *Molecular Ecology* **3**, 71-74.

Serdy, Frank. S and Berberich, Sharon A. (1995). Petition for determination of non-regulated status Bollgard (TM) Cotton Lines 757 and 1076 (*Gossypium hirsutum* L.) with the gene from *Bacillus thuringiensis* subsp. Kurstaki. St.Louis, Missouri, Monsanto Company.

Smith, N. (2001). 'Not from here: Plant invasions on Aboriginal lands of the Top End.' (Tropical Savannas CRC: Darwin, NT)

Stewart, C. N., All, J. N., Raymer, P. L., and Ramachandran, S. (1997). Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. *Molecular Ecology* **6**, 773-779.

Taylor, J. A. and Tulloch, D. (1985). Rainfall in the wet-dry tropics: Extreme events at Darwin and similarities between years during the period 1870-1983. *Australian Journal of Ecology.* **10**, 281-295.

Tomiuk, J. and Loeschcke, V. (1993). Conditions for the establishment and persistence of populations of transgenic organisms. Wohrmann, K. and Tomiuk, J. (eds.) *Transgenic Organisms*. 117-133. Basel, Switzerland, Birkhauser Verlag.

Traynor, P. L. and Westwood, J. H. (1999). *Proceedings of a Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems*. Blacksburg, Virginia, Information Systems for Biotechnology.

Underbrink, S. M. and Landivar J. A. (1999). Agronomic differences in growth and yield between *Bt* and conventional cotton. *Proceedings of the Beltwide Cotton Conference*. 1, pages 521-523. National Cotton Council.

Virtue, J. G., Groves, R. H., and Panetta, F. D. Towards a System to Determine the National Significance of Weeds in Australia. Groves, R. H., Panetta, F. D., and Virtue, J. G. Weed Risk Assessment. 124-152. Collingwood, Australia, CSIRO Publishing. 2001.

Wanjura, D. F., Hudspeth, E. B., and Bilbro, J. D. (1969). Emergence time, seed quality and planting depth effects on yield and survival of cotton. *Agronomy Journal* **61**, 63-65.

Wilkinson, M. J., Davenport, J. J., Charters, Y. M., and Jones, A. E. (2000). A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. *Molecular Ecology* **9**, 983-991.

Williams, P. A., Nicol, E., and Newfield, M. (2001). Assessing the risk to indigenous biota of plant taxa new to New Zealand. Groves, R. H., Panetta, F. D., and Virtue, J. G. (eds.) *Weed Risk Assessment*. 100-116. Collingwood, Vic.,Australia, CSIRO Publishing.

Williams, R. J., Duff, G. A., Dowman, D. M., and Cook, G. D. (1996). Variation in the composition and structure of tropical savannas as a function of rainfall and soil texture along a large-scale climatic gradient in the Northern Territory, Australia. *Journal of Biogeography* **23**, 747-756.

Williamson, M., Perrins, J., and Fitter, A. (1999). Can novel weediness be predicted? Amijee, F., Gliddon, C. J., and Gray, A. J. (eds.) Environmental Impact of Genetically Modified Crops. Research Report No.10. Genetically Modified Organisms. 208-216. London, U.K., Department of the Environment, Transport and the Regions: London.

Wilson, A. G. L., Basinski, J. J., and Thomson, N. J. (1972). Pests, crop damage and control practices with irrigated cotton in a tropical environment. *Cotton Growing Review* **49**, 308-340.

Wood, P., Cahill, M., Marlow, G., and Douglas, N. (2000). 'Weeds: the Ute Guide, Northern Grain Belt Edition.' (Qld Dept.of Primary Industries: Qld, Australia.)

Woodstock, L. W., Furman, K., and Leffler, H. R. (1985). Relationship between weathering deterioration and germination, respiratory metabolism and mineral leaching from cotton seeds. *Crop Science* **25**, 459-466.

Yeates, S. J. (2001). Cotton research and development issues in northern Australia: a review and scoping study. *Australian Cotton Cooperative Research Centre and CSIRO Plant Industry*.

LIST OF APPENDICES

APPENDIX 1 SOIL CHEMICAL RESULTS FOR EACH SITE

LOCATION	HABITAT	DESCRIPTION (Depth and Rep)	EC (MS/cm)	Ph	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	P (mg/kg)	S (mg/kg)	TKN %	Zn (mg/kg)	OC %	B (mg/kg)
BROOME	Cattle No. 1	0-10 cm R1+2	0.53	5.6	380	0.24%	490	40.00%	400.00%	89	0.59	7.9	5.2	-
		0-10 cm R2+3	.034	5.9	420	0.22%	390	<25	270.00%	55	0.6	7.4	5.03	-
		10-30 cm R1+2	0.16	6.1	150	260	110	<25	170.00%	13	0.1	1.4	1.13	-
		10-30 cm R3+4	0.09	6.5	210	500	70	<25	150.00%	5	0.08	1.2	0.88	-
	Dam	0-10 cm inner	0.28	4.7	100	610	80	<25	59	121	0.2	11	2.13	-
		0-10 cm outer	0.05	5.2	30	140	<25	<25	24.00%	7.7	0.05	1.7	0.66	-
		10-30 cm inner	0.29	4.5	100	390	60	30.00%	51.00%	124	0.13	5.8	1.49	-
		10-30 cm outer	0.05	6.5	70	220	30	<25	14.00%	7.8	0.03	1.5	0.38	-
	Road	0-10 cm R1+2	0.01	5.9	<25	110	<25	<25	<5	1	0.11	0.6	0.18	-
		0-10 cm R3+4	0.01	6	<25	100	<25	<25	<5	<0.5	<0.01	0.3	0.14	-
		10-30 cm R1+2	0.02	5.8	30	90	<25	<25	<5	2	<0.01	0.4	0.13	-
		10-30 cm R3+4	0.01	5.7	<25	110	<25	<25	<5	0.5	0.01	0.5	0.14	-
	Bush	0-10 cm R1+2	0.01	5.6	40	200	30	<25	<5	1.2	0.02	<0.1	0.45	-
		0-10 cm R3+4	0.01	5.4	<25	150	<25	<25	<5	1.1	0.02	0.2%	0.42	-
		10-30 cm R1+2	0.01	5.5	<25	110	<25	<25	<5	1.1	0.01	0.20%	0.18	-
		10-30 cm R3+4	0.01	5.6	<25	90	<25	<25	<5	1	0.01	0.10%	0.16	-
	Cattle No. 2	0-10 cm R1+2	0.04	6.3	90	330	-	-	81	2.7	0.04	-	0.3	0.2
		0-10 cm R3+4	0.08	5.7	110	620	-	-	120	7.2	0.1	-	1.33	0.3
		10-30 cm R1+2	0.04	6.5	120	260	-	-	62	3.4	0.04	-	0.47	<0.2
		10-30 cm R3+4	0.03	6.3	80	180	-	-	55	2.3	0.02	-	0.24	0.2
	Bush No. 2	0-10 cm R1+2	<0.01	6.3	<25	140	-	-	<5	0.8	0.01	-	0.27	<0.2
		0-10 cm R3+4	<0.01	6.2	<25	90	-	-	<5	0.9	<0.01	-	0.15	<0.2
		10-30 cm R1+2	<0.01	6.6	<25	100	-	-	<5	0.9	<0.01	-	0.09	<0.2
		10-30 cm R3+4	<0.01	6.5	<25	80	-	-	<5	0.6	<0.01	-	0.12	<0.2
KUNUNURRA	Cattle No. 1	0-10 cm R1+2	0.06	6.6	370	0.37%	0.16%	-	19	7.7	0.12	9.7	1.61	-
		0-10 cm R3+4	0.06	6.4	410	0.38%	0.15%	-	17	8.5	0.19	4.4	2.5	-
		10-30 cm R1+2	0.04	7.6	280	0.35%	0.18%	-	<5	2.5	0.05	1.3	0.63	-
		10-30 cm R3+4	0.05	7.6	300	0.34%	0.18%	-	<5	2.4	0.05	1.6	0.65	-
	Drain	0-10 cm R1+2	0.04	7.6	240	0.26%	770	-	<5	3.9	0.06	0.8	0.7	-
		0-10 cm R3+4	0.05	7.3	340	0.14%	410	-	<5	3.2	0.05	0.7	0.49	-
		10-30 cm R1+2	0.04	7.6	200	0.32%	0.11%	-	<5	3.3	0.03	1.2	0.49	-
		10-30 cm R3+4	0.02	6.9	100	0.16%	530	-	<5	3.1	0.03	0.6	0.41	-
	Road	0-10 cm R1+2	0.01	6.6	<25	210	40	<25	<5	<0.5	0.01	0.6	0.20%	-
		0-10 cm R3+4	0.01	7	<25	130	40	<25	<5	<0.5	0.01	0.3	0.19	-
		10-30 cm R1+2	0.01	6.8	<25	220	40	<25	<5	<0.5	<0.01	0.2	0.1	-
		10-30 cm R3+4	0.01	7.2	<25	170	40	<25	<5	<0.5	<0.01	0.3	0.09	-
	Bush	0-10 cm R1+2	0.04	7.3	290	0.42%	0.16%	-	<5	2.3	0.04	3.2	0.61	-
		0-10 cm R3+4	0.04	7.3	280	0.41%	0.16%	-	<5	3.7	0.04	3.9	0.54	-
		10-30 cm R1+2	0.08	7.7	190	0.43%	0.16%	-	7	6.5	0.03	6.9	0.43	-
		10-30 cm R3+4	0.05	7.4	190	0.41%	0.16%	-	<5	0.6	0.03	0.8	0.43	-
	DS Drain	0-10 cm R1+2	0.03	6.5	70	-	-	-	6	4.4	0.02	3.8	0.41	-
		0-10 cm R3+4	0.05	6.3	180	-	-	-	9	8.7	0.06	2.7	0.73	-
		10-30 cm R1+2	0.02	6.3	50	-	-	-	<5	3.2	0.02	2.3	0.33	-
		10-30 cm R3+4	0.1	6.9	170	-	-	-	8	9.3	0.05	6	0.77	-
KATHERINE	Cattle No. 1	0-10 cm	0.03	6.1	170	870	220	<25	10	6.1	0.11	2.3	1.04	-
		10-30 cm	0.01	6	90	860	190	<25	<5	5	0.05	0.3	0.52	-
	Creek	0-10 cm bank edge	0.16	8.6	220	0.58%	0.22%	50	7	10	0.08	22	1.07	-
		0-10 cm @ 5m from bank	0.12	8.2	300	0.90%0.58%	810	<25	15	4.8	0.26	0.4	4.09	-
		10-30 cm bank edge	0.14	8.4	210	0.73%	0.20%	30	8	7.5	0.07	15	1.01	-
		10-30 cm @ 5m from bank	0.13	8.7	90		770	60	8	3.6	0.14	0.3	1.73	-
	Road	0-10 cm	0.06	8.5	40	0.48%	30	<25	<5	1.3	0.02	0.4	0.35	-
		10-30 cm	0.07	8.3	40	0.18%	30	<25	<5	1.1	0.02	0.2	0.2	-
	Bush	0-10 cm	0.03	7.4	150	770	50	<25	<5	1.2	0.04	0.4	0.42	-
		10-30 cm	0.02	7.2	70	640	30	<25	<5	0.8	0.03	0.8	0.25	-

APPENDIX 2

EXPERIMENT 1A: LARGE-SCALE ECOLOGICAL ASSESSMENT: INDIVIDUAL SITE RESULTS. SITES 1-13

SITE 1: KUNUNURRA BUSH SITE NO.1

Date Sown: 3 December 1999

GERMINATION

Plant counts for germination were conducted on the 12 December 1999 (T1), 31 December 1999 (T2), and 14 March 2000 (T3).

Seedtype was highly significant at all measurement times and overall (P<0.001). Germination proportion of all three seedtypes declined over time. Black seed had consistently greater germination at all measurements. There was no difference between fuzzy seed and seed cotton at T2 only, due to seedling mortality of the fuzzy seed treatment by this time, and a catch-up in the lag of germination of seed cotton. At all other measurements, fuzzy seed had a greater germination proportion than seed cotton. This is illustrated in Figure A2.1.

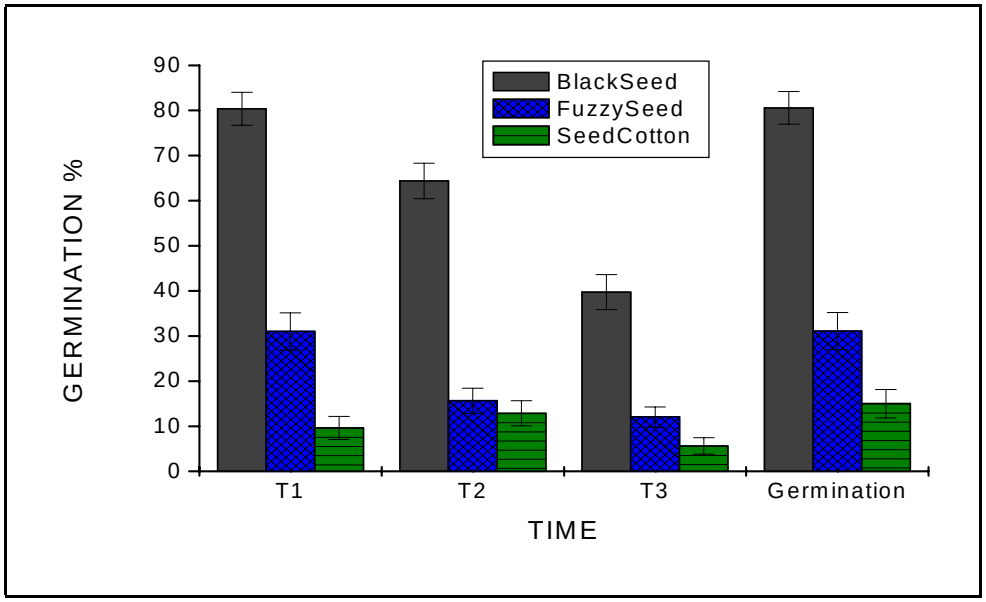


Figure A2.1. Effect of seedtype on germination at each time at Kununurra Bush Site No.1 (error bars are ± s.e., and are for within each time only)

There was a significant seedtype by population interaction (P=0.03) for each time, and for overall respectively, as illustrated in Figure A2.2.

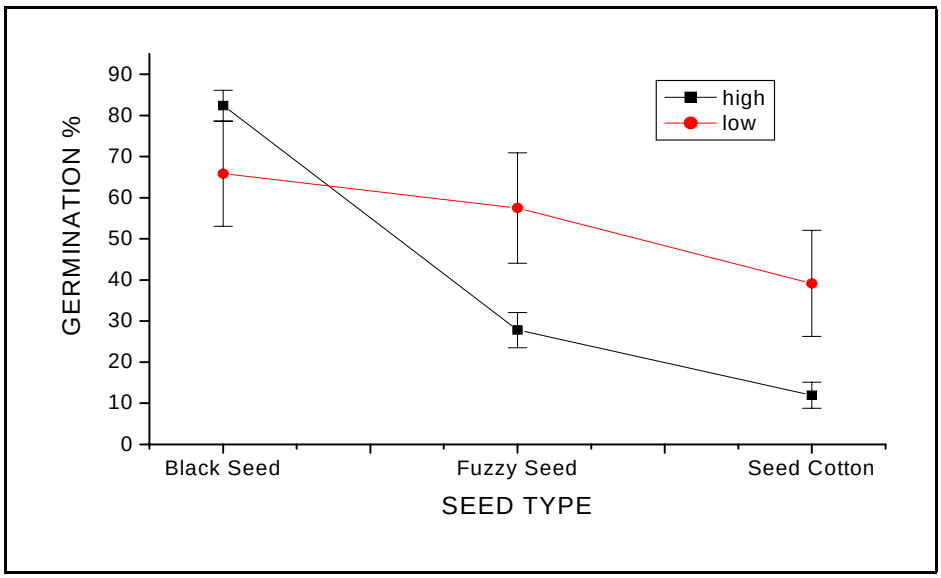


Figure A2.2. Effect of seedtype by population interaction on germination at Kununurra Bush Site No.1 (error bars are ± s.e.)

SURVIVORSHIP 1

Plant counts for survivorship after the first dry season were conducted on 13 December 2000 (T9). There were 12 plots with surviving plants; G0 = 2 plots; G1 = 7 plots; G2 = 3 plots, corresponding to only 3.86% plants from the original seeds sown.

SURVIVORSHIP 2

The final plant count was conducted on 26 October, 2001. There were only six plots with surviving plants, corresponding to only 1.76% plants from the original seeds sown. Plant height ranged from 23–44 cm. Photo A2.1 illustrates the surviving plants in Plot 6 at the final measurement.

Treatments and number of surviving plants for the relevant plots for Survivorship 1 and Final Survivorship are listed in Table A2.1.

Table A2.1. Surviving plots and treatments after one and two (final) years for Kununurra Bush Site No.1

Plot No.	Treatment	No.of plants at T9	No.of plants at final count	Total No. of open bolls produced
1	S1HG1	13	6	1
2	S1HG2	3	0	0
3	S1HG0	4	0	0
6	S1LG1	2	1	3
10	S3HG1	7	1	3
15	S2LG2	1	0	0
16	S2HG0	40	21	4
17	S2HG1	5	0	0
38	S2LG1	2	2	0
44	S1HG1	46	26	3
60	S1HG2	1	0	0
61	S3HG1	1	0	0

FECUNDITY

The sum of the number of open bolls produced per plot over the two years is given in Table A2.1. Only plots 1, 6 (2 bolls) and 44 had bolls remaining at final harvest. A seed count and germination test was

conducted on seeds from these open bolls. Results were: P1 – 16 seeds, 19% ; P6 – 27 seeds (2 bolls), 33%; and P44 – 11 seeds, 37%. All bolls produced were observed to have low seed set and disfigured structure, as illustrated in Photo A2.1.

INVASIVENESS

Four seedlings were recorded from only one plot (No.6), after the commencement of the 2000-01 Wet season, with treatment S1LG1. These seedlings all died over the duration of the following Dry Season.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 466, 486 and 416 seeds for G0, G1 and G2 respectively. Numbers of plants present after the first dry season were 44, 76 and 5, and at the final measurement were 21, 36 and 0 for each genotype. Values from calculations of invasiveness using the simplistic method were:

λ_1; G0 = 0.0944; G1 = 0.1564; G2 = 0.0120
λ_2; G0 = 0.4773; G1 = 0.4737; G2 = 0.0

SITE DISCUSSION

The seedtype by population interaction on germination is possibly explained by availability of water for imbibition. Germination for the high-density treatment black seed was marginally greater than at the low population. For the other 2 seed treatments, the low-density treatments resulted in a higher germination compared to the high population level. This may be that black seed may require less water to successfully imbibe than do the fuzzy seed and seed cotton because lint on the seed would absorb moisture, reducing the amount available for water uptake through the seed coat. Seeds at the high population likely had less available water per seed than the low population, resulting in the more marked decrease in germination from black to fuzzy seed and seed cotton at the high population if moisture was limiting. There is little available literature on imbibition and germination of seed cotton.

Seedling recruitment was extremely low. Observations indicated that at the commencement of the wet season, there was rapid germination and growth of tropical grasses and annual vines and shrubs, such as *Chrysopogon* spp, *Iseilemma* spp, and *Flemingia* spp. These covered bare ground, thus reducing the opportunity for cottonseed to establish soil contact, and decreased available soil moisture to compete vigorously with the low vigour cotton plants.

Photo A2.1. Surviving cotton plants typical of those remaining at the final count at the Kununurra Bush Site No.1

SITE 2: KUNUNURRA CATTLE NO.1

Date Sown: 2 February 2000

GERMINATION

Plant counts for germination were conducted on 7 February 2000 (T1), 8 March (T2) and 10 April (T3).

Seedtype was highly significant (P<0.001) at all three times, and overall. Germination proportion of all three seedtypes declined over the three-count duration. Black and fuzzy seed had consistently greater germination compared to the seed cotton, as illustrated in Figure A2.3.

Population was significant at all times and highly significant overall (P<0.001) with the high density level having consistently higher germination proportion compared to the low density treatment, presented in Figure A2.4.

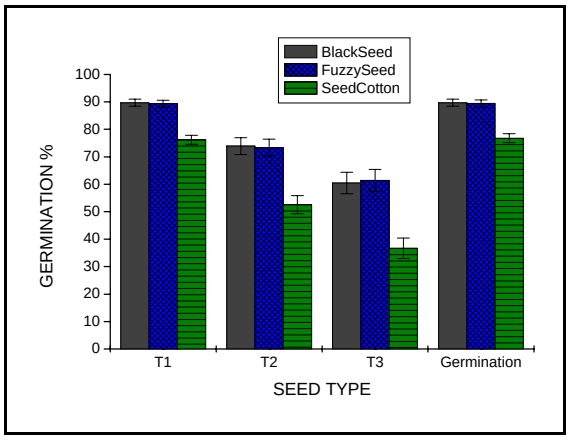


Figure A2.3. Effect of seedtype on germination at each time at Kununurra Cattle Site No.1. (error bars are ± s.e., and are for within each time and overall germination only)

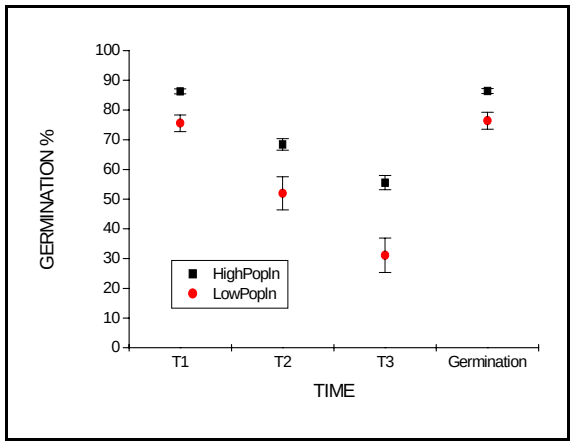


Figure A2.4. Effect of population on germination at Kununurra Cattle Site No.1. (error bars are ± s.e., and are for within each time and overall germination only)

SURVIVORSHIP 1

Survivorship after the first dry season was recorded on 12 December 2000 (T8). There were only three plots with surviving plants; 1 plant (S3HG1); 1 plant (S1HG1); and 1 plant (S2HG0). This was equivalent to only 0.09% plants remaining from seeds sown.

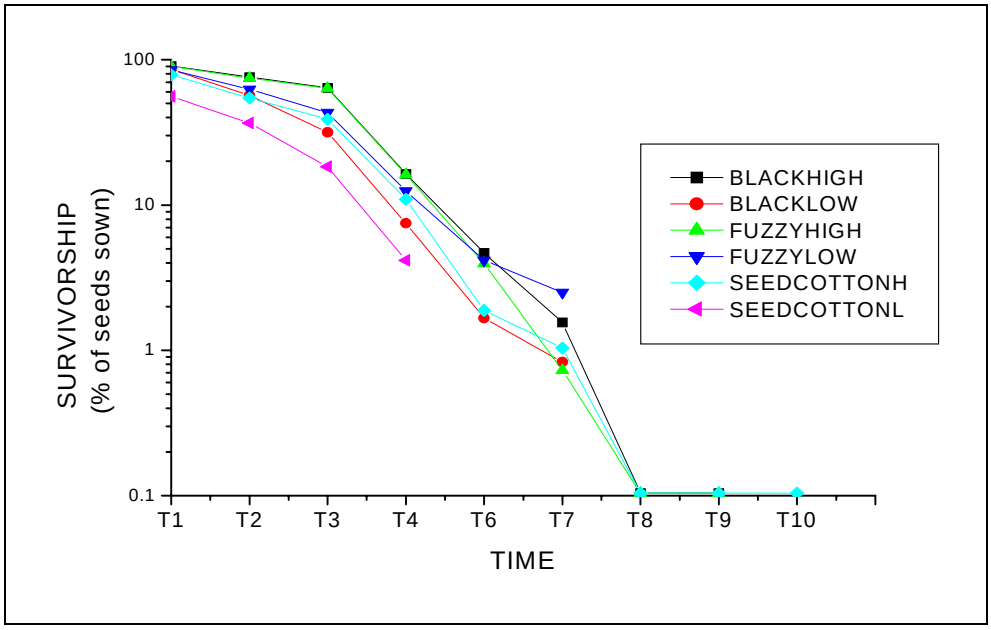


Figure A2.5. Survivorship over time for the Kununurra Cattle Site No.1

SURVIVORSHIP 2

There was only one plant remaining for the final measurement on 15 October, 2001 (T10); derived from the seed cotton, high population, single gene treatment (S3HG1). This was equivalent to only 0.03% plants remaining from seeds sown. Plant height was only 17cm. This is illustrated in Photo A2.2.

Seedtype and population were the significant factors for germination, so change in plant number was calculated as seedtype and population means. This depletion of plant numbers over time is represented in Figure A2.5.

FECUNDITY

Only one plant from the entire site produced a green boll, recorded on 12 December 2000, which aborted at an early stage. This plant was a fuzzy seed, high population, conventional genotype, treatment (S2HG0).

INVASIVENESS

A total of 1080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 919, 913 and 932 seeds for G0, G1 and G2 respectively. Number of plants present for each genotype after the first dry season was 1, 2, and 0, and at the final measurement was 0, 1 and 0. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 = 0.0011; G1 = 0.0022; G2 = 0
λ_2: G0 = 0.0; G1 = 0.50; G2 = 0

SITE DISCUSSION

The time lag in germination of seed cotton was not as distinct at this compared to the majority of other sites. This may be attributed to better water availability due to sowing well into the wet season, and the hydromorphic soil at this site.

The plants were waterlogged for the majority of the wet season, and at irrigation times during the dry season. The soil was highly compacted from intensive cattle grazing after irrigation over several years. Leucaena (*Leucaena leucocephala*) and barnyard grass (*Echinochloa colona*) growth was vigourous throughout the Wet Season, and competed with the cotton plants. Cattle did not appear to graze the plants, but did cause damage by trampling. There was considerable insect damage to all plots in April (@8 weeks after planting), with grasshoppers observed on the plants. A combination of these factors – waterlogging, soil compaction, interspecific competition, and physical damage (grasshoppers and cattle) appeared to contribute to the high mortality at this site.

Photo A2.2. The only plant surviving at Kununurra Cattle site No.1 after two years

SITE 3 : KUNUNURRA ROAD SITE

Date Sown: 26 November 1999

BACKGROUND

This was the first of the large-scale ecological study sites to be sown. Seeds were sown by hand-placing clumps on the soil surface. This was how it was envisaged that dispersed seed would be likely to settle. However, differences in seed to soil contact, and levels of dessication and insect predation contributed to extremely variable germination. Although this is a meaningful result in itself, it made robust analysis difficult, so methodology was modified (Methodology Section) for subsequent sowings at all other sites. A detailed experiment with depth of sowing imposed as a treatment was then implemented at this site, as discussed in Section 3.

Results for this site are presented as for all the 13 sites, although the sowing technique was different. The germination results for this site provide a more realistic indication of levels of germination for seed that may accidentally disperse. However, the low levels of plant establishment provided difficulties of interpretation for the later demographic stages.

GERMINATION

Plant counts to assess germination were conducted on 4 December 1999 (T1), 17 December 1999 and 21 December 1999 (T3). Seedtype accounted for the majority of the variance (deviance = 162 out of total deviance = 347), although there was a significant ($P<0.001$) three-way interaction between block, seedtype and population, which was difficult to interpret. There were overall trends that the high population level had a lower germination than the low population level, and that fuzzy seed had the greatest germination, followed by seed cotton, with black seed the lowest.

SURVIVORSHIP 1

Plant survival after one year was assessed on 10 April 2001 (T7). There was only one plot with surviving plants (3 plants) corresponding to the S2HG0 treatment; equivalent to only 0.09% plants remaining from seeds sown. Maximum plant height at this stage was 21cm.

SURVIVORSHIP 2

There were no plants surviving when final counts were conducted on 25 November 2001.

FECUNDITY

No plants produced fruiting structures.

INVASIVENESS

No seedlings were ever produced at this site.

A total of 1080 seeds (3 seedtypes) were sown for each genotype. Number that germinated were 80, 50 and 71 seeds for G0, G1 and G2 respectively. There were only three conventional plants remaining after the first year, and no plants by the second year.

Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 = 0; G1 = 0.038; G2 = 0
λ_2: G0 = 0; G1 = 0.0; G2 = 0

SITE DISCUSSION

The extremely variable and low germination results necessitated a change of methodology to all subsequently sown sites; seeds individually placed, and covered with soil. Results we did observe for germination, with the lower population treatment resulting in a greater proportion germinated than the high population level, were attributable to a greater proportion of seeds making soil contact. In the high

population level, seeds were clumped on top of each other, and these ‘perched’ seeds were subject to more rapid drying and greater distance for the radicle to move through the lint to reach the soil.

SITE 4: KUNUNURRA WS DRAIN NO.1

Date Sown: 29 November 1999

GERMINATION

Plant counts for germination were conducted on 4 December 1999 (T1) and 29 January 2000 (T2). T3 was after the wet season (9 June 2000), at which time there were only four plots that had surviving plants.

Seedtype was highly significant at both measurement times, and overall (P<0.001), as shown in Figure A2.6. Black seed was consistently higher than the fuzzy seed, which was higher than the seed cotton.

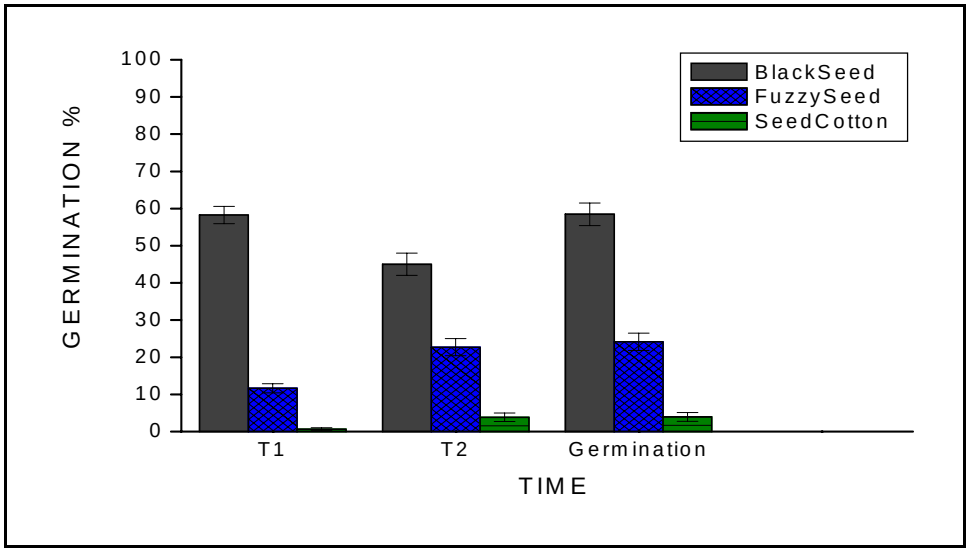


Figure A2.6. Effect of seedtype on germination at each time at Kununurra WS Drain No.1. (error bars are ± s.e., and are for within each time only)

There was a significant interaction between seedtype and population (P=0.017), as shown in Figure A2.7. There was no difference in germination at either population for black seed, but for both seed cotton and fuzzy cotton, germination proportion was greater for the low density treatment compared to the high density treatment.

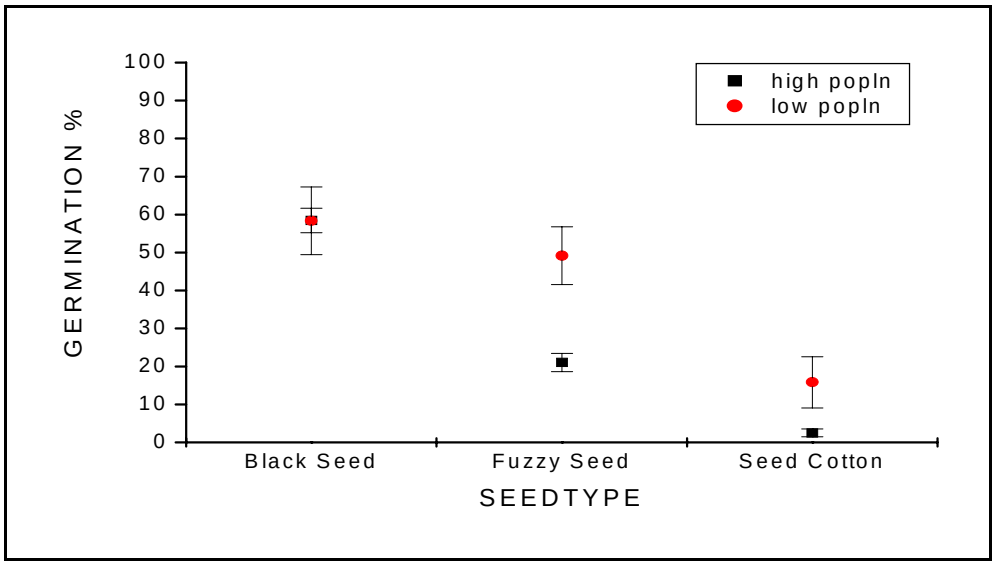


Figure A2.7 Effect of population by seedtype interaction on germination at Kununurra Drain WS No.1 (error bars are ± s.e.)

There was a significant interaction between seedtype and genotype at both initial measurements (P<0.001 and .016), and overall (.037). There was no difference in germination proportion between any of the three genotypes for the black seed or seed cotton. However, the conventional genotype had a significantly lower germination compared to the two transgenic genotypes for the fuzzy seed treatment. The reason for this is uncertain. This result is illustrated in Figure A2.8.

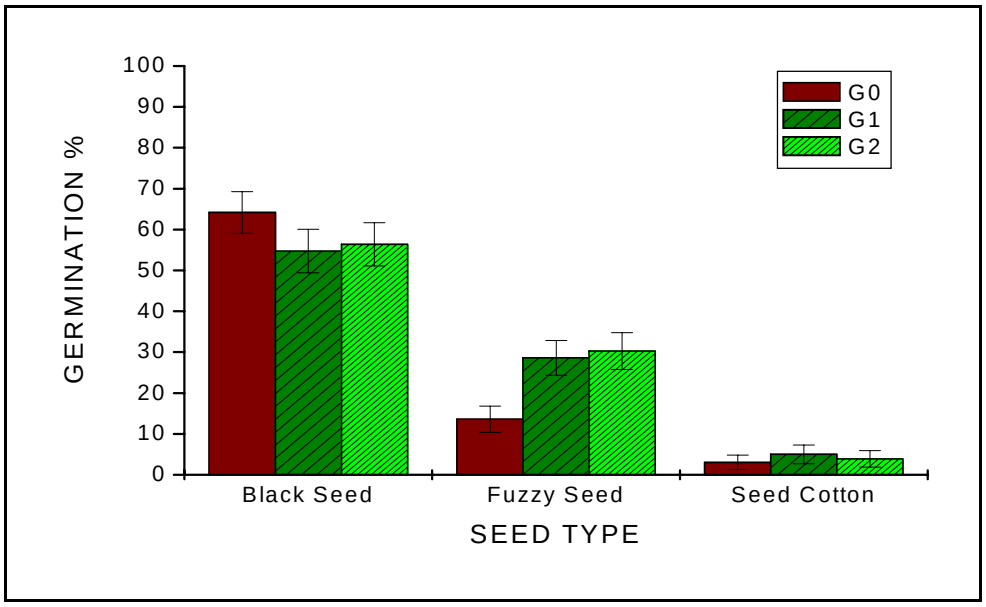


Figure A2.8. Effect of genotype by seedtype interaction on germination at Kununurra WS Drain No.1 (error bars are ± s.e.)

SURVIVORSHIP 1

Water availability was not a limiting factor at this site, so did not influence survivorship over the dry season as at other habitats. The majority of plants died over the wet season after germination. Survivorship was measured after this time, on 9 June 2000 (T3). There were only four plots remaining which had surviving plants. These corresponded to P30: S1LG2 (2 plants); P46 S1HG1 (7 plants); P47 S1HG0 (20 plants) and P 48 S1HG2 (4plants). This was equivalent to 1.02% plants remaining from the seeds sown.

SURVIVORSHIP 2

There were three plots with surviving plots when the final counts were done on 28 October 2001, as included in Table A2.2, equivalent to 0.65% plants remaining from seeds sown.

FECUNDITY

The surviving plants produced a large number of fruit over the 2001 dry season. Rain (@60mm) had fallen on the plots prior to the final count, stimulating some germination of seed cotton. Table A2.2 presents the number of surviving plants and their respective plots and treatments. Fruit production and seedling recruitment is also presented.

Table A2.2. Plant survivorship, open boll production and seedling recruitment for Kununurra Drain Site (WS) No.1

Plot No.	Treatment	No .of plants (final)	No. of open bolls	No. of seedlings
46	S1 H G1	7	106	7
47	S1 H G0	12	42	8
48	S1 H G2	2	9	0

INVASIVENESS

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Numbers that germinated were 291, 318 and 326 seeds for G0, G1 and G2 respectively. Numbers of plants present after the first dry season for each genotype was 20, 7 and 6. At the final measurement, there were 12, 7 and 2 of the originally germinated plants remaining, so with the addition of the recruited seedlings, the population of each genotype totalled 20, 14 and 2 plants respectively. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 = 0.069; G1 = 0.022; G2 = 0.018
λ_2: G0 = 1.0 G1 = 2.0; G2 = 0.3333

SITE DISCUSSION

Blocking was designed down the length of the drain. There was an observable gradation in soil texture from sandier soil at one end of the site to clay at the other end. This appeared to be attributable to the depth that the drain was originally dug, and a rocky outcrop near one end of the drain. The significant difference in germination down the drain length indicates the importance of microhabitat in initial establishment of seedlings, even though the habitat generally is conducive to cotton growth.

The seedtype by population interaction on germination was similar to that of the Kununurra Bush Site. If we consider population change over the second season (Inv2), values indicate that a population of cotton volunteers can increase in favourable niches, but there was no statistical evidence that there was any effect of genotype.

This drain collects the run-off from the surrounding hills and diverts the flow from the irrigation area to a swamp. The high mortality over the first wet season is attributed to the plants being submerged for an extended period. Seeds were initially sown where the grass line commenced on the drain edge. The three vigorous surviving plants appeared to be on a slightly higher patch of ground than the other plots. This site was resown at a higher level on the bank in the following season. These results are discussed for Site No.16; Kununurra wet season Drain No.2 in Experiment 1B.

The low plant number at Survivorship 1 due to high mortality from inundation suggests that volunteer populations which survive near water, may be disadvantaged in above average rainfall wet seasons.

SITE 5: KATHERINE BUSH SITE NO.1

Date Sown: 11 December 1999

GERMINATION

Plant counts for germination were conducted on 17 December (T1), and 30 December 1999 (T2). T3 at this site corresponded to 25 February 2000.

Seedtype was significant at each of the three germination measurement times (P= T1<.001, T2=.01, T3=.012) and for overall germination (P<0.001). Germination of seed cotton was significantly less than black seed and fuzzy seed at all measurement times, as illustrated in Figure A2.9.

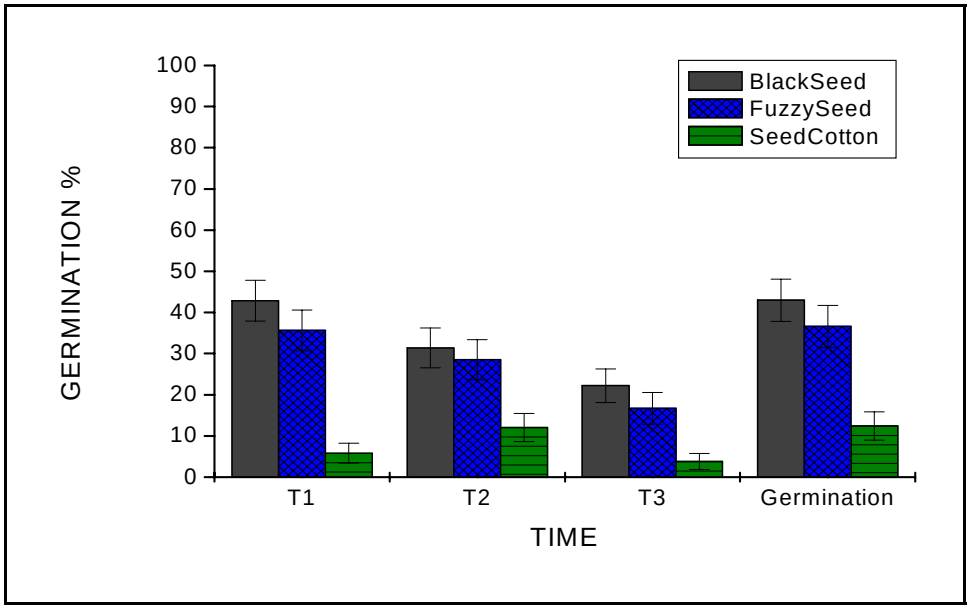


Figure A2.9. Effect of seedtype on germination at each time at Katherine Bush Site No.1. (error bars indicate ± s.e. and are for within each time measurement only)

SURVIVORSHIP 1

There were four plots with surviving plants when measurements were taken at the end of the 2000 dry season, 5 January 2001 (T9). These correponded to P40 – 1 plant (S3LG1); P58 – 5 plants (S1HG1); P 65 – 1 plant (S2LG1); and P70 – 2 plants (S3HG1). This was equivalent to 0.28% plants from seeds sown.

SURVIVORSHIP 2

Measurements for final survivorship were conducted on 7 November 2001 (T10). Rain had fallen on the site by this time (@70 mm), allowing regeneration of shoots if the plant was still viable.

There was only one plant remaining, from P65, as described above. It was only 10 cm in height and with tiny green buds at the cotyledon scars.

Survivorship over time was plotted as a total number of plants for each genotype to assess times of major mortality (Figure A2.10.). Plant numbers declined dramatically immediately after germination, and continued to decrease over the duration of the 2000 dry season, with very few plants remaining by the commencement of the 2000-01 wet season.

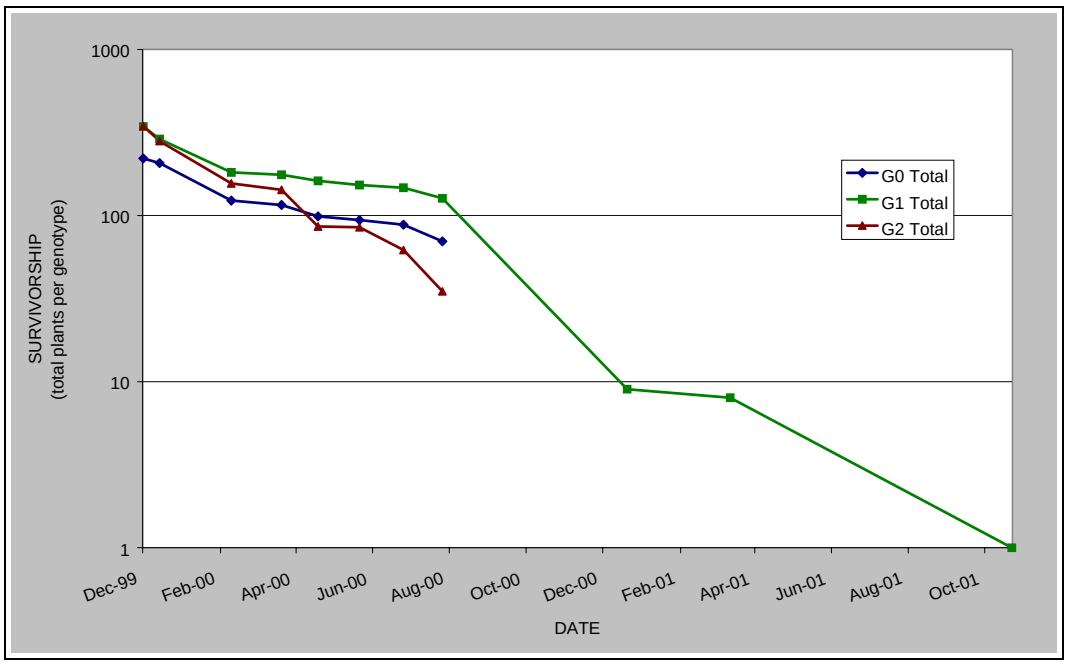


Figure A2.10. Survivorship over time

FECUNDITY

No fruit was ever produced at this site.

INVASIVENESS

No seedlings were ever produced at this site.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Numbers that germinated were 269, 358 and 366 seeds for G0, G1 and G2 respectively. Number of plants present for each genotype after the first dry season was 0, 9 and 0, and at the final measurement was 0, 1 and 0. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 = 0; G1 = 0.0251; G2 = 0
λ_2: G0 = 0; G1 = 0.1111; G2 = 0

SITE DISCUSSION

There was rapid plant mortality immediately after initial germination (T1; 17 December 1999), and then a steady attrition over the dry season, particularly after August as soil moisture became limiting, and temperatures began to increase. Plants were observed to display very poor vigour and yellowing. It was theorised that nutrition may be the major limiting factor in this habitat, so an experiment was designed and conducted in the second season adding nutrition as a factor at this site. This is discussed in the section in Experiment 1B Katherine Bush No.2 (Site 17).

SITE 6: KATHERINE CATTLE NO.1

Date Sown: 16 December 1999

GERMINATION

Plant counts for germination were conducted on 22 December (T1), and 30 December 1999 (T2). T3 at this site corresponded to 25 February 2000.

Seedtype was significant at each of the three measurement times, and for overall evaluation of germination (P<0.001), with seedcotton having consistently lower germination compared to fuzzy and black seed. This is presented in Figure A2.11.

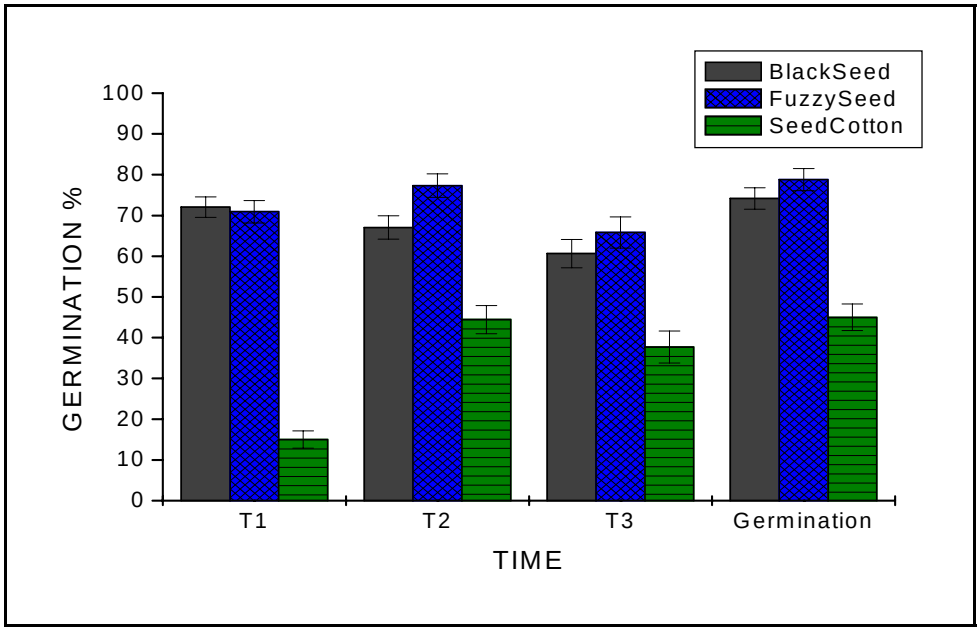


Figure A2.11. Effect of seedtype on germination at each of the initial three measurement times, and overall (error bars indicate ± s.e.and are for within each time measurement only)

SURVIVORSHIP 1

This was calculated at the end of the first dry season, and corresponded to T9 (4 January 2001). 44 plots out of the 72 sown had surviving plants; equivalent to 7.3% plants from seed sown.

Survivorship/Nseeds

There was a significant seedtype by genotype interaction (P=0.015). For no seedtype did either of the transgenic genotypes have a significantly greater survivorship compared to the conventional genotype. For fuzzy seed and seed cotton, the double gene had significantly lower survivorship compared to the conventional genotype. Refer to Figure A2.12.

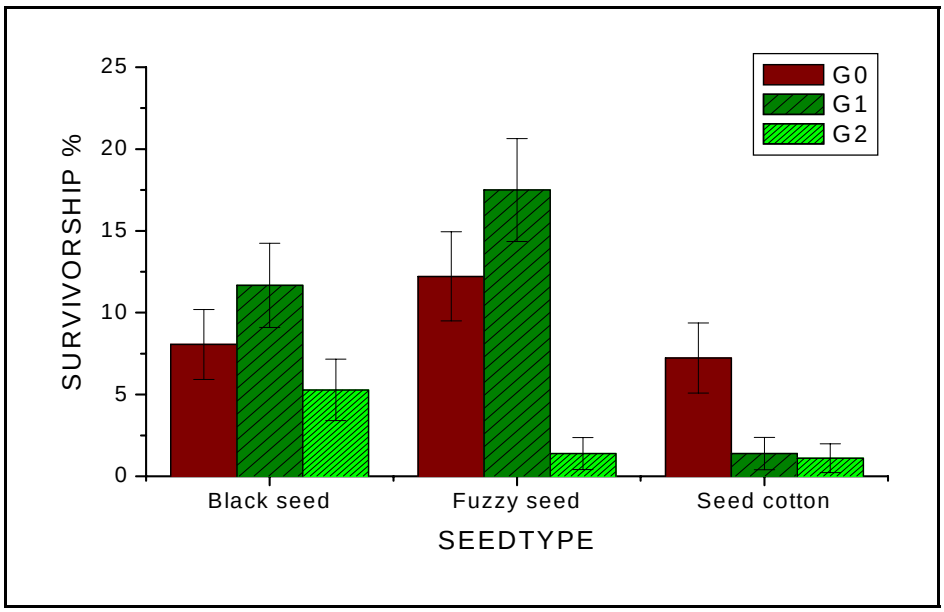


Figure A2.12. Effect of seedtype by genotype interaction on survivorship expressed as a percentage of plants surviving from the original seeds sown (error bars indicate ± s.e.)

Absolute Survivorship

There was a significant effect of both population ($P<0.001$) and genotype ($P<0.001$) on log-transformed data. Values are described for non-transformed data. The low population treatments had significantly less number of plants remaining (1.08 plants) compared to the high population level (5.5 plants; s.e.0.658). The number of two-gene plants present was significantly less than either of the other two genotype treatments (G0=4.12 plants; G1=4.58 plants; G2=1.17 plants; s.e.=0.822).

SURVIVORSHIP 2

Final counts were conducted on 7 November 2001 (T13), prior to which @70 mm of rain had fallen, causing renewed growth (with one plant, P62 – S1LG1 having one square). 19 plots had surviving plants; corresponding to G0=7 plots, G1=8 plots, and G2=4 plots; equivalent to 2.16% plants from seeds sown.

Data analysis on number of surviving plants were difficult to interpret due to the large numbers of missing plots.

FECUNDITY

The only plots to produce open bolls were:

P14 – 1 boll (S2HG1); P45 – 1 boll (S2HG1); P59 – 1 boll (S2HG2); P62 – 3 bolls (S1LG1).

Photo A2.3 illustrates lint on the ground, but seed was observed to disintegrate after remaining on the soil surface. Small invertebrates were observed within the seed.

INVASIVENESS

No seedlings were ever produced.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 731, 677 and 731 seeds for G0, G1 and G2 respectively. Number of plants present for each genotype after the first dry season was 99, 110 and 28, and at the final measurement was 20, 45 and 5. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 = 0.1354; G1 = 0.1625; G2 = 0.0383
λ_2: G0 = 0.2020; G1 = 0.4090; G2 = 0.1785

An ANOVA was conducted on λ_1 and λ_2. There was a significant effect (P=0.017) of genotype on λ_1 with the two-gene treatment significantly less (0.044) than the other two genotypes (G0=0.175 and G1=0.151; s.e.=0.032).

There was a significant effect of seedtype for Inv2 (P=0.039; log-transformed data). Values for non-transformed data are 0.08, 0.132 and 0.238 for seed cotton, fuzzy seed and black seed respectively (s.e.=0.036).

SITE DISCUSSION

There was a significant Block*Seedtype interaction (P=0.006) for germination, again supporting the importance of microhabitat. Differences were observed in subsequent plant vigour, depending on proximity to the leucaena (a nirogen-fixing legume), with greener and more robust plants within 10 cm to the leucaena, and paler plants where surrounded by a higher proportion of sabi grass (Urochloa mosambicensis).

Plants were affected by grazing (indirect damage rather than selective grazing), slashing (the leucaena was slashed to 50 cm high as part of fodder management), competition from sabi grass, particularly in the wet season, and by moisture stress in the dry season. This site was the most productive of all the Katherine sites, probably due to a higher nutrient status. This was consistent with the Broome cattle habitat, although plants at that site were not subject to grazing and trampling.

The effect of population on survivorship after one year, indicated that if there is less dispersed seed initially, then fewer plants will establish, compared to a greater number of seeds dispersing.

Reasons for the lower absolute survivorship after one year of the two-gene are uncertain. One explanation could be the increased use of resources in the early growth and development stages to maximise reproductive potential (which theoretically should be greater in two-gene compared to the other two genotypes when subject to insect pressure given that there are no other limiting factors). These resources were utilised and depleted making subsequent survival more difficult. Another explanation could be possible pleiotrophic effects of the two-gene on normal cotton growth and development.

a

b

c

Photos A2.3a, b and c. Change in plant vigour over time at Katherine Cattle No. 1 for Plot 45 (S2HG1)

SITE 7: KATHERINE ROAD SITE

Date Sown: 15 December 1999

GERMINATION

Plant counts for germination were conducted on 22 December (T1), and 30 December 1999 (T2). T3 at this site corresponded to 25 February 2000.

Seedtype had a significant effect on germination at each of the three germination measurement times, and overall (P<.001, <.001, =.001 and <.001 respectively). Seedcotton had the least germination, followed by fuzzy seed, then black seed, as presented in Figure A2.13.

The effect of population was also significant (P=0.009), with the high level having a lower germination (22.33%; s.e.=1.68) compared to the low level (37.22%; s.e.=5.22).

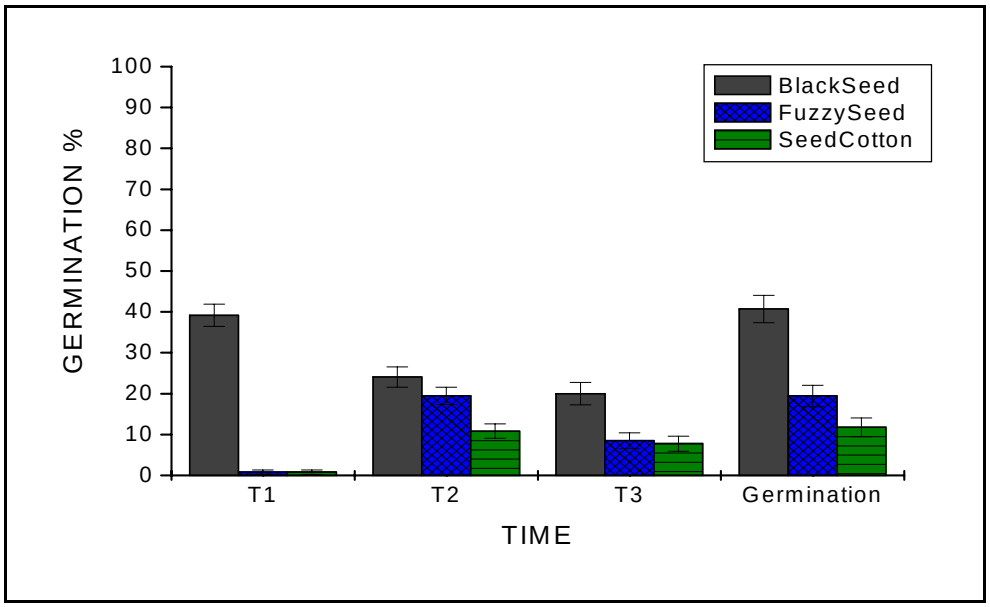


Figure A2.13. Effect of seedtype on germination at each time and overall. (error bars indicate ± s.e. and are for within each time measurement only)

SURVIVORSHIP 1

Only three plots had surviving plants after the first dry season (T9; 5 January 2001). These corresponded to two plants (S3HG0); three plants (S3HG1); and one plant (S1HG0); equivalent to 0.19% plants from seeds sown. Seedlings never reached greater than 15 cm in height, and vigour was always very poor with few leaves and extremely short internode lengths.

SURVIVORSHIP 2

There were no plants remaining when final counts were conducted on 7 November 2001.

FECUNDITY

No fruiting structures were ever produced at this site.

INVASIVENESS

No seedlings were ever produced.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 298, 211 and 268 seeds for G0, G1 and G2 respectively. Number of plants present for each genotype

after the first dry season was 3, 3, and 0, and at the final measurement was 0, 0 and 0. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 =0 0101; G1 = 0.0142; G2 = 0.0
λ_2: G0 =0 0; G1 = 0.0; G2 = 0.0

SITE DISCUSSION

The site was slashed prior to sowing to simulate roadside slashing conditions, but vegetation was permited to grow for the remainded of the experiment. In typical roadside habitats, slashing is generally conducted at the end of the wet season. However, competition was not considered the major restricting factor. Soil unsuitability, both structure and nutrition status, and severe moisture stress appeared to be the major constraints. The seedlings surviving at the end of the first dry season were near a small shrub which had regenerated after slashing. Photo A2.3 illustrates the poor seedling vigour at this site. It should be noted though, that this does not totally exclude vigourous cotton plants surviving and producing viable open bolls on roadsides, as this was observed to occur within the ORIA. (Refer to Section 4: Volunteer Cotton Monitoring).

The interaction between block and seedtype was significant at all three times also. This was not presented in the results, but does support that continuing emergence is subsequently influenced by the 'micro-habitat' surrounding the seed, as there were observable differences within the vegetation and soil of the site along the road edge (parallel to road), and also perpendicular to the road edge. Cotton seed would need to lodge into a suitable niche, such as by water and soil wash from the road edge into a road runoff drain where silt and vegetation may accumulate.

Photo A2.4. Surviving seedlings at Katherine Road site (4 May 2000) at six months after sowing illustrating extremely poor plant vigour (treatment was S2HG1)

SITE 8: KATHERINE CREEK SITE

Date Sown: 9 December 1999

GERMINATION

Plant counts for germination were conducted on 17 December (T1), and 30 December 1999 (T2). T3 corresponded to the 25 February 2000.

Seedtype was the only significant factor (P<0.001). Overall seedtype effect was consistent with the discrete analysis conducted at each of the three times – seedcotton has significantly lower germination compared to both fuzzy seed and black seed (Figure A2.14).

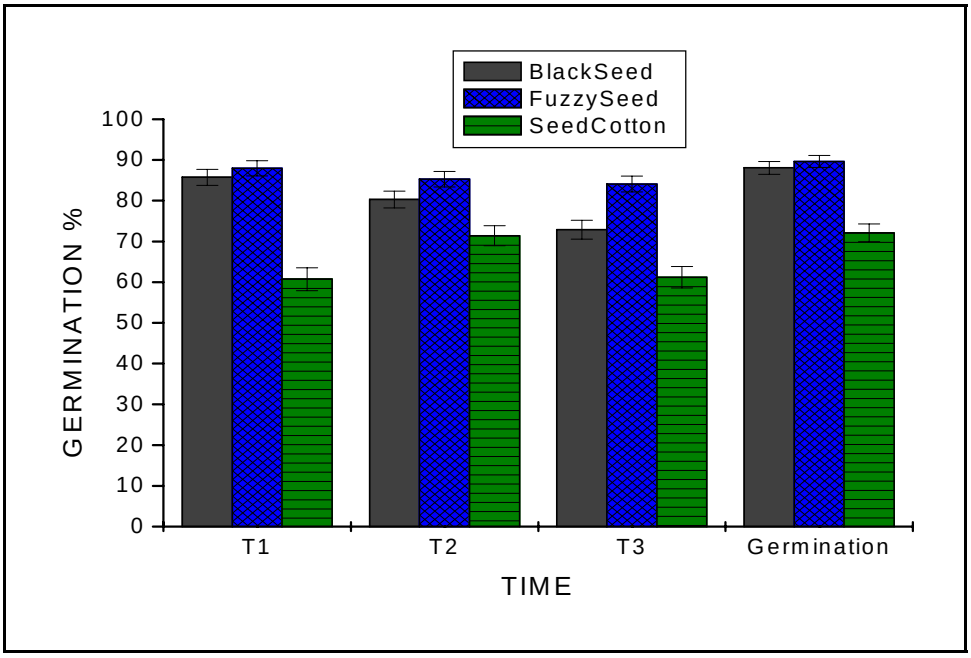


Figure A2.14. Effect of seedtype over three measurement times, and for maximum germination for Katherine Creek site (error bars are ±s.e. for within times only)

SURVIVORSHIP 1

Survivorship was examined at the conclusion of the 2000 Dry Season, corresponding to T9 (6 January 2001). There were 41 plots from 72 with surviving plants; equivalent to 24.26% plants from seeds sown.

Survivorship (Plants at T9/Nseeds)
There was a significant interaction between population and genotype (P=0.01) presented in Figure A2.15.

There was a population effect only for the conventional genotype.

Absolute Survivorship

There was a significant interaction between population and genotype (P<0.001), as illustrated in Fig A2.16. There were less plants of the two-gene genotype than the single-gene and conventional genotypes for the high population level only.

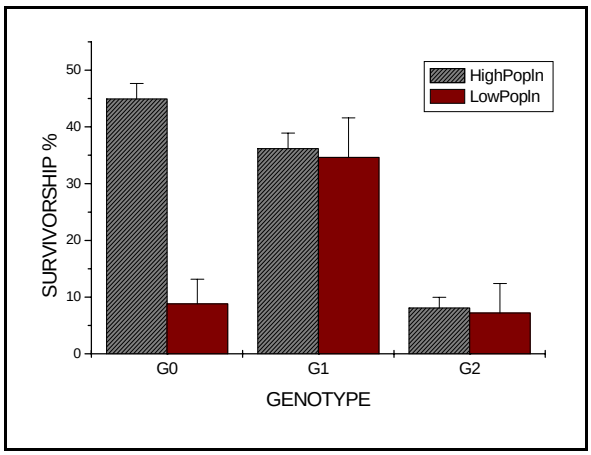


Figure A2.15. Population * genotype interaction at Katherine Creek site for survivorship expressed as a percentage of plants surviving from the original seeds sown (error bars are + s.e.)

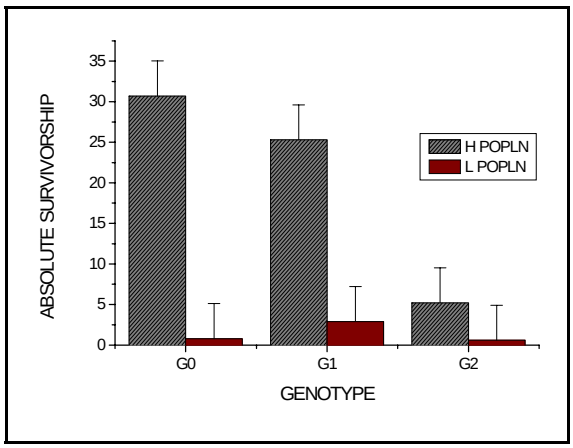


Figure A2.16. Population * genotype interaction at Katherine Creek site for absolute survivorship, expressed as total number of plants remaining. (error bars are + s.e.)

SURVIVORSHIP 2

Final counts were conducted on 7 November 2001 (T12). There were 9 plots that had surviving plants, corresponding to the treatments presented in Table A2.3; equivalent to 2.41% plants from seeds sown. Plot number was included to indicate that survivor plants were generally in close proximity to one another within a treatment combination. Plant height ranged from 10-18 cm.

Table A2.3. Final surviving plants at Katherine creek site

Plot No.	No. of surviving plants/plot	Treatment
44	16	S1 H G0
45	10	S1 H G1
46	1	S1 L G1
50	3	S3 H G1
65	25	S3 H G0
66	1	S3 H G2
70	10	S2 H G0
71	4	S2 H G2
72	8	S2 H G1

FECUNDITY

No plants at this site ever produced any fruiting structures.

INVASIVENESS

No seedlings were ever produced.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 904, 908 and 886 seeds for G0, G1 and G2 respectively. Number of plants present after the first dry season was 377, 339 and 70 for each genotype, and at the final measurement was 51, 22 and 5. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 =0.4170; G1 = 0.3734; G2 = 0.0790
λ_2: G0 =0.1353; G1 = 0.0649; G2 = 0.0714

SITE DISCUSSION

There was a block effect due to differences in waterlogging along the creek line. This supports that even within a habitat, that there are more suitable niches compared to others.

Leafminers were observed at one stage to predominantly be found on conventional plants, but these appeared to only cause minor leaf damage, and it was doubtful that these contributed to the increased mortality of the G0. This increased mortality only at the low population level may indicate that only isolated instances of factors leading to a small number of plant deaths (fungus, insect, water-logging) may have a large relative effect for the low population treatment compared to the high population treatment.

This site appeared to be a potential cotton volunteer haven when site selection was conducted in mid-1999. However, soil analyses results after sowing indicated very high pH, and the site was waterlogged for most of the year also. There was the likelihood that the soil was compacted as cattle were observed to traffic the surrounding area when wet. So, although moisture was not limiting, in fact it may have been too wet, with anaerobic soil conditions unsuitable for viable cotton development. Photos A2.4 a, b and c illustrate the poor development of cotton at this site. There were no observable differences in plants between treatments, so Plot 44 (S1HG0) was used as it displayed good earlier vigour, and was one of the final surviving plots.


a


b


c

Photos A2.5a b and c. a. Plot 44 (S1HG0) at one month, b. at six months and c. at two years illustrating the poor development at this Katherine Creek site

SITE 9: BROOME BUSH SITE NO.1

Date Sown: 13 January 2000

GERMINATION

Plant counts for germination were conducted on 17 January (T1), 24 January (T2) and 17 February 2000 (T3).

The interaction between seedtype and population was significant for germination overall (P=0.018) and at T3 (P=0.012), approximately four weeks after sowing, as illustrated in Figure A2.17.

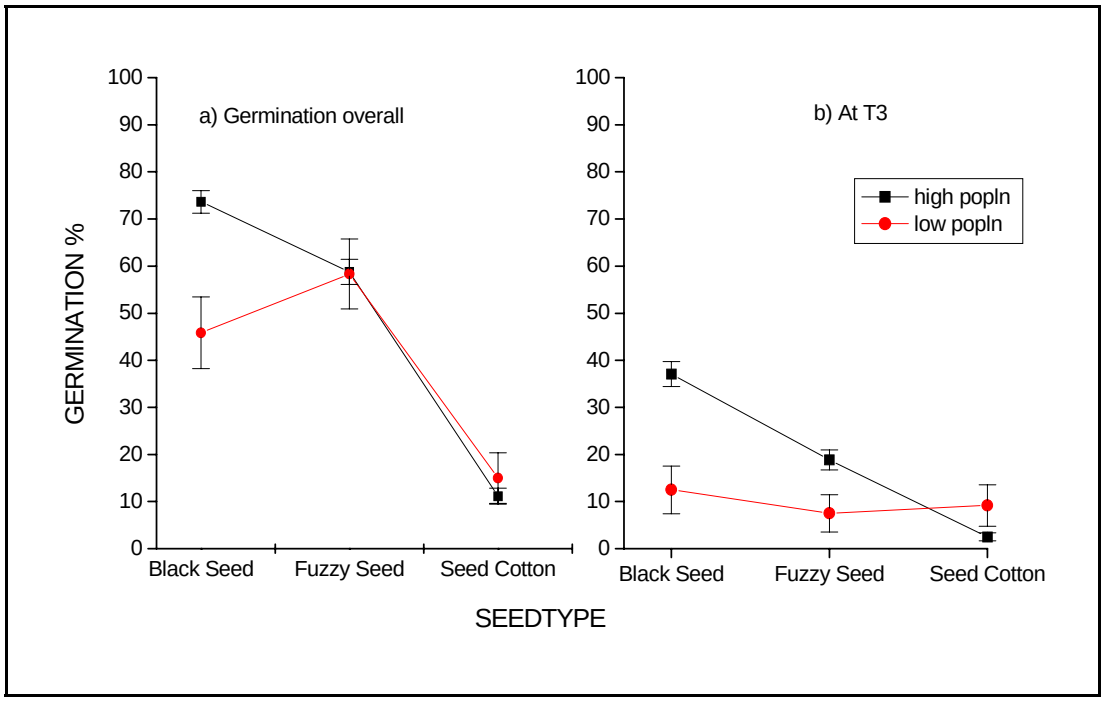


Figure A2.17. Effect of seedtype by population on germination and at T3 at Broome Bush Site No.1. (error bars are ± s.e)

SURVIVORSHIP 1

No plants were surviving after the dry season.

SURVIVORSHIP 2

Zero

FECUNDITY

Plants at this site never progressed to producing any reproductive structures.

INVASIVENESS

No seedlings were produced.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 479, 507 and 535 seeds for G0, G1 and G2 respectively. With no surviving original plants and no seedlings produced, invasiveness for all genotypes was zero.

SITE DISCUSSION

Low nutrition and low water availability, consistent with the soil type dominant in the area (Yeeda and Wonganut land systems) were hypothesised to be the major limiting factors to cotton growth at this site. It is improbable that volunteer plants in this Pindan environment would survive away from the sphere of influence of the irrigation and cultivation area. Volunteer plants from previous research trials were found on the verges of cultivated areas and the bushline. One particularly productive plant, determined to be at least two years old, and with a large number of open bolls, was near irrigated mangoes between the cotton bays, and surrounded by the legume, Stylosanthes spp.

A third major influence was that of fire. It was decided to cease plant counts after the July measurements as the plants were leafless twigs and it was difficult to determine if they were actually dead, or would recover at the onset of the wet season. It was intended to record plant survivorship at that time, but the site was burnt before the measurements could be conducted at the onset of the wet season. Observations before the fire, indicated that the plants were dry brittle twigs less than 15 cm in height and probably dead. Photo A2.5 illustrates the poor plant development at this site.

Photo A2.6. Broome Bush Site No.1 plant development May 2000 (at four months after sowing)

SITE 10: BROOME CATTLE SITE NO.1

Date Sown: 12 January 2000

GERMINATION

There was a significant effect of seedtype (P=0.013). Black seed (36.29 %, s.e=3.71) had greater germination than both fuzzy seed (25.2%, s.e.=4.17) and seed cotton (21.82%, s.e=3.15).

SURVIVORSHIP 1

Survivorship after the 2000 dry season was recorded on 18 January 2001 (T13) after initial rains had fallen. There were 41 plots with surviving plants (total sown 72). This corresponded to 9.85% plants surviving from seeds sown.

Survivorship/Nseeds
There was a significant (P=0.012) interaction between genotype and seedtype, illustrated in Figure A2.18.

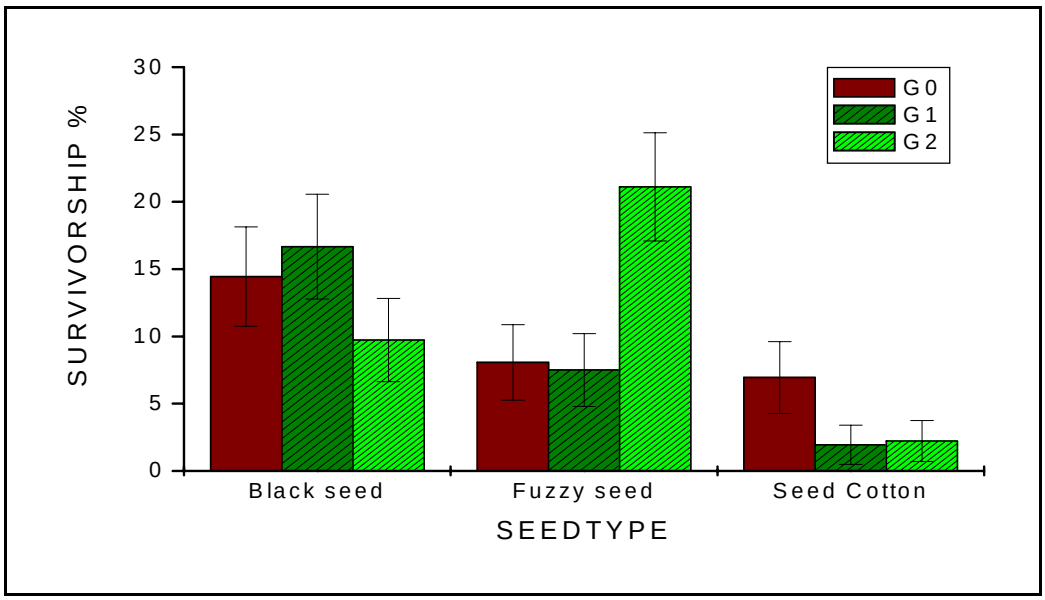


Figure A2.18. Effect of seedtype by genotype interaction on Survivorship1 expressed as a percentage of plants survivng fromoriginal seeds sown at Broome Cattle Site No.1. (error bars are ± s.e)

Absolute Survivorship 1
There was a significant effect of both population (P<0.001) and of seedtype (P=0.042) on number of plants remaining per plot after one dry season from seeds originally sown.

Means for non-transformed data were 0.69 plants and 8.17 plants remaining for the low and high levels respectively. (Log-transformed data values were 0.332 and 1.760 for the low and high population levels respectively; s.e.=0.133).

For the effect of seedtype, means for non-transformed data corresponded to 6.13 plants, 5.5 plants and 1.67 plants for the black seed, fuzzy seed and seed cotton respectively. (Log-transformed data values were 1.395, 1.090 and 0.653; s.e.= 0.1572).

SURVIVORSHIP 2

Final measurements were conducted on 21 November 2001 (T16). The site at this stage had not yet received any rain, which made conclusive determination of mortality difficult. There were 37 plots with remaining plants. This corresponded to 7.93% plants surviving from seeds sown.

/Nseeds
There continued to be a significant interaction between seedtype and genotype (P=0.016), with the trend the same as that presented for survivorship after the first dry season (refer to Figure A2.18).

Absolute Survivorship 2
The two factors population (P<0.001) and seedtype (P=0.022) continued to provide a significant effect on number of plants per plot remaining after the second dry season, with similar results to those from the first year. Means for non-transformed data corresponded to 0.5 plants and 6.64 plants for the low and high levels respectively. (Log-transformed data values were 0.247 and 1.563; s.e.=0.1554).

For the effect of seedtype, means for non-transformed data for the black seed, fuzzy seed and seed cotton, corresponded to 5.54 plants, 3.87 plants and 1.29 plants respectively. These values were all lower than the corresponding values for the previous year. (Log-transformed data values were 1.395, 1.090 and 0.653 for the black seed, fuzzy seed and seed cotton respectively; s.e.= 0.1572).

/Germinated
An analysis was conducted to assess the number of plants that survived as a proportion of those that germinated. There was a significant effect of genotype (P=0.02), illustrated in Figure A2.19. Surviving plants are expressed as a percentage of the number of plants that germinated.

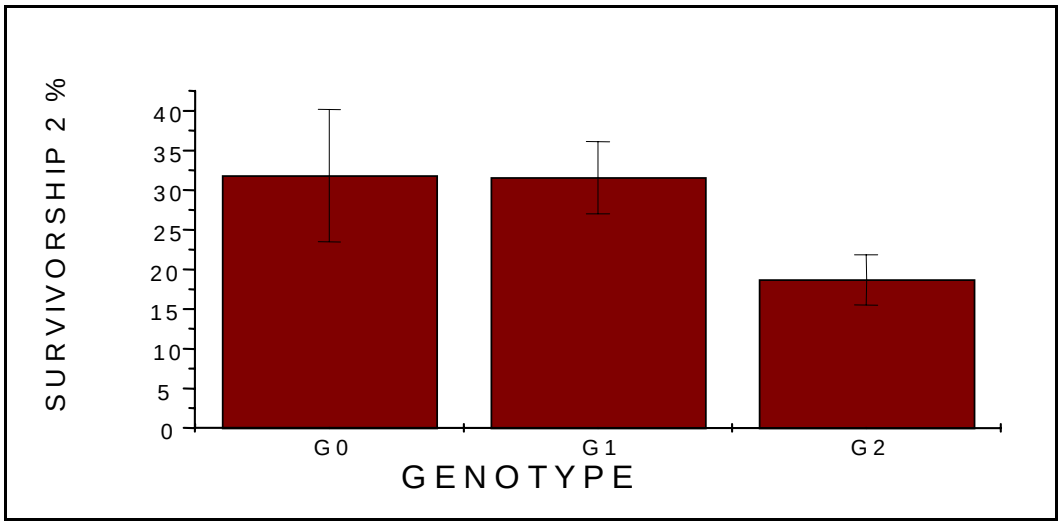


Figure A2.19. Effect of genotype on final survivorship as a proportion of plants that germinated at the Broome Cattle Site No.1 (error bars indicate ± s.e.)

Seedtype was also significant (P=0.004) with results similar to the previous season, indicating that seedlings derived from seedcotton had less survivorship compared to the other two seedtypes (Figure A2.20.)

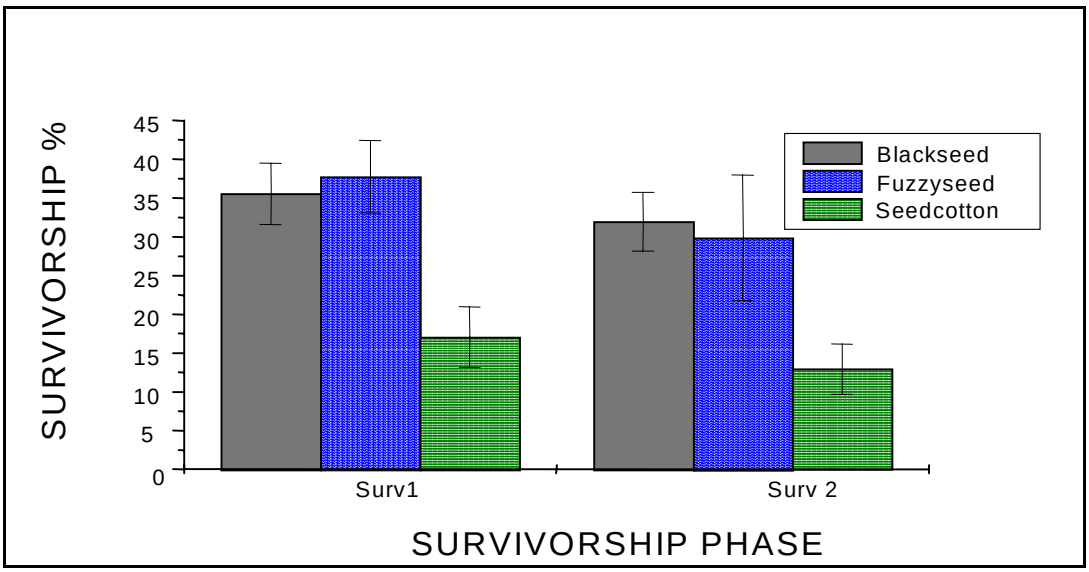


Figure A2.20. Effect of seedtype on survivorship after 1 and 2 seasons given that the plant had germinated (error bars indicate ± s.e. and are for within each survival phase only)

FECUNDITY

Fruit production over time was plotted to examine phenology of fruit production throughout the year (wet and dry seasons) (Figure A2.21).

Plants were mainly vegetative over the course of the initial wet season (T3-T8; Feb-May 2000) with high numbers of squares and green bolls relative to open bolls. This also occurred in the second Wet Season (T13-T14; Jan-May 2001). At the progression of the dry season from May in both years (T8 and T14), the number of squares and green bolls steadily declined as bolls opened. Open bolls consequently increased, reaching a peak towards the end of the dry season (T12, and T16). Number of open bolls then declined over the course of the wet season (T12-T13; Oct-May) as they rotted and fell from the plants.

Fecundity was evaluated as the maximum number of open bolls produced per plot over two years, whether there were surviving plants or not. There were plots where maximum open boll production occurred at the end of the first dry season (T12), but for the majority of the plots, this occurred after the second year.

Due to the differences in time of maturity, open boll production was also assessed at the end of each dry season (T12 and T16) to better understand phenology of fruit production in perennial cotton.

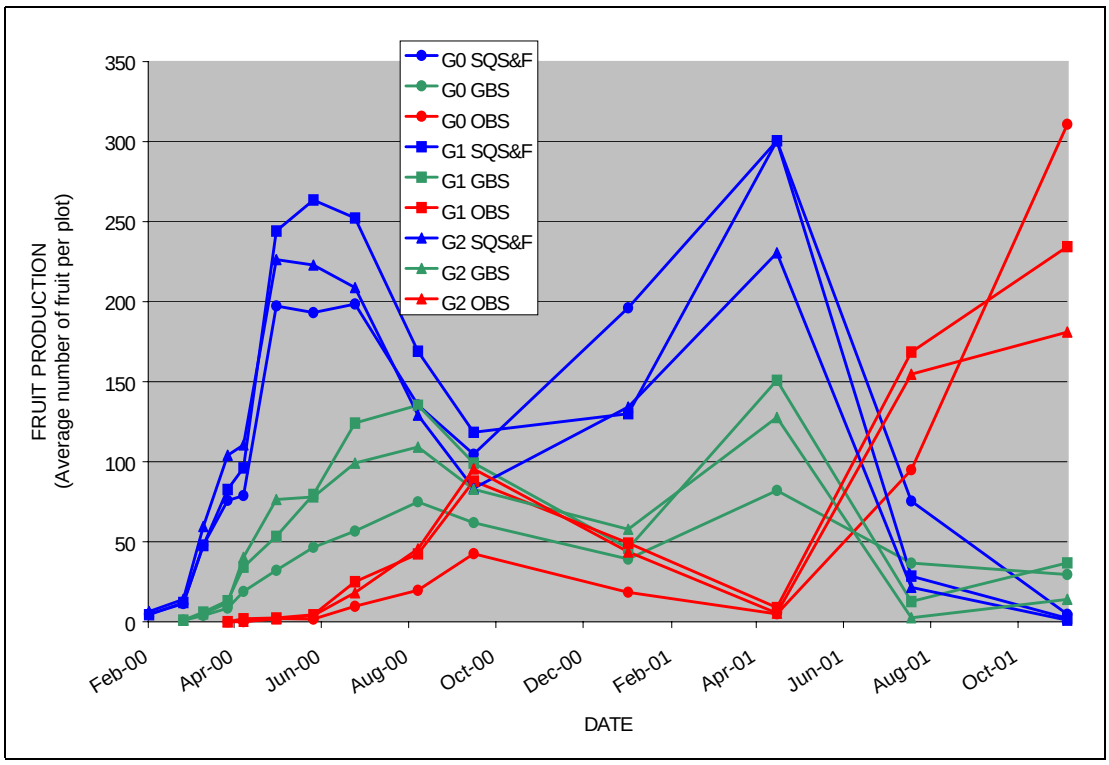


Figure A2.21. Fruit production over time for each genotype mean for Broome cattle site No.1

Maximum Open Bolls

There was a significant effect of population (P=0.009) on maximum number of open bolls produced per plot over the two years, with a greater number of bolls produced for the high population density than the low density (mean = 222 and 60 bolls for the high and low levels respectively; s.e.=34.7).

The interaction between seedtype and genotype was approaching significance (P=0.052), with number of open bolls increasing with genotype (G0<G1<G2) for the fuzzy seed only. Number of open bolls declined with genotype for the black and seed cotton treatments. Although this was not statistically significant, the result is presented in Figure A2.22 as the effect of genotype is of particular interest.

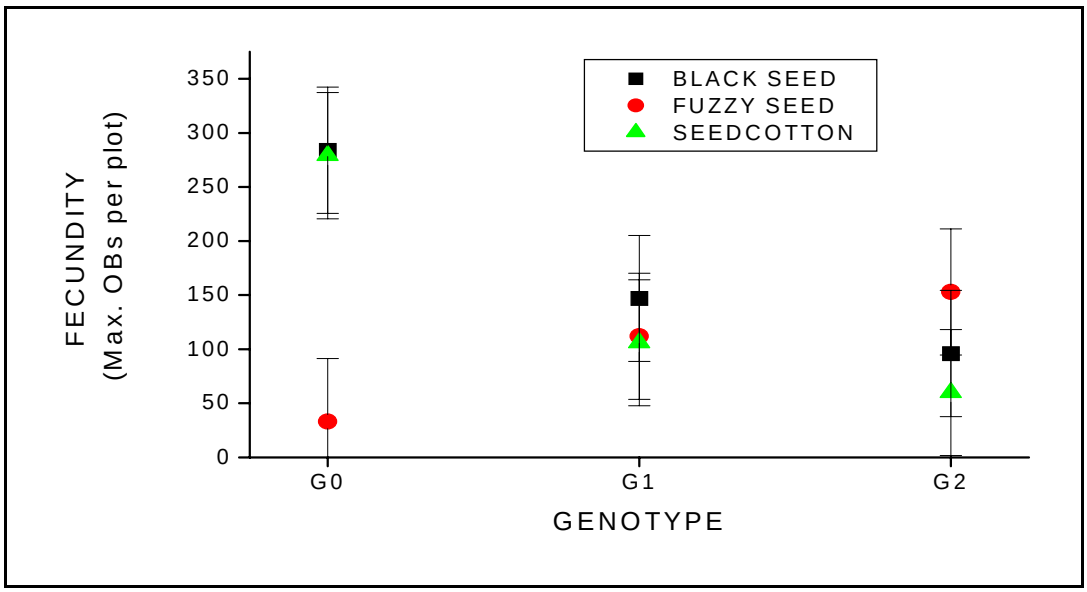


Figure A2.22. Seedtype by genotype interaction (not significant) on mean maximum number of open bolls (OBs) produced per plot (error bars are ± s.e.)

OPEN BOLLS T12 (OBT12)

Seedtype was significant (P=0.016), with number of open bolls produced per plot by the end of the first year greatest for plants derived from black seed than fuzzy seed and seed cotton (means = 61.7, 38.9 and 23.4 respectively; s.e.=6.44).

The interaction between population and genotype was approaching significance (P=0.053). This effect is discussed because of the importance of genotype as a factor of interest. There was no genotype effect for the low population density, but number of open bolls increased with genotype for the high population density. This is illustrated in Figure A2.23

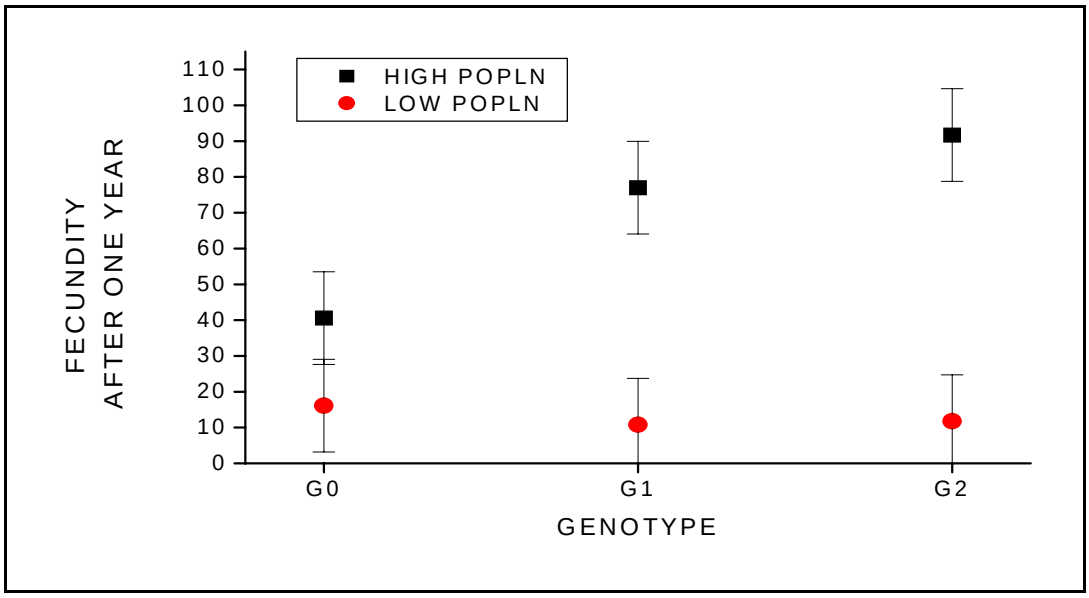


Figure A2.23. Population by genotype interaction (not significant) on mean number of open bolls per plot at T12 (error bars are ± s.e.)

OPEN BOLLS T16 (OBT16)

Population was highly significant (P<0.001; box-cox transformation), with the high density treatment producing more open bolls than the low density treatment (202 and 54 bolls respectively; s.e.=34.5). There was a significant interaction between seedtype and genotype (P=0.032) illustrated in Figure A2.24 with the trend consistent with results for Maximum open bolls.

These differed from the results for OBT12. Although there was no significant interaction between seedtype and genotype at this time, the effect is presented in Figure A2.25 for comparison to illustrate the trend for open boll production with genotype between the two years (T12 and T16). The important trends are; 1) increase in the number of open bolls produced from T12 to T16 for the conventional genotype for black seed and seed cotton; and 2) the decrease from T12 to T16 for the black seed transgenic treatments relative to the black seed conventional treatment.

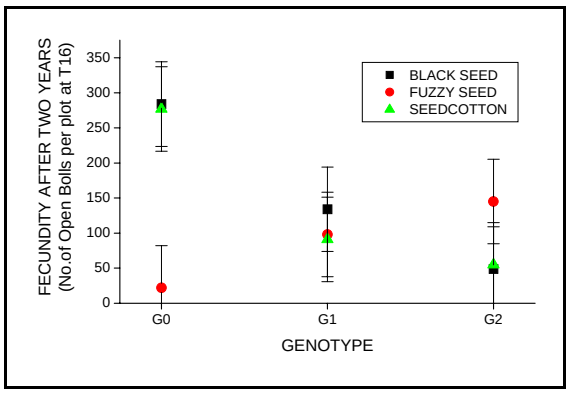


Figure A2.24. Seedtype by genotype effect on open bolls produced per plot at T16 (error bars are ± s.e.)

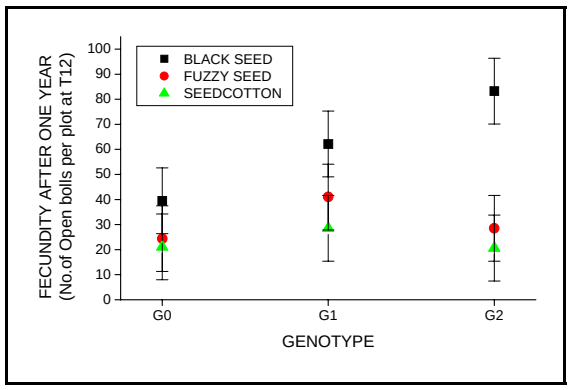


Figure A2.25. Seedtype by genotype effect (not significant) on open bolls produced per plot at T12 (error bars are ± s.e.)

Maximum Open Bolls Per Plant

There was a highly significant effect of population (P<0.001; box-cox transformation), with the high population producing greater number of open bolls per plant than the low population density. The interaction of population with genotype is presented in Figure A2.26 to illustrate the effect, to include genotype as the factor of interest with respect to weediness.

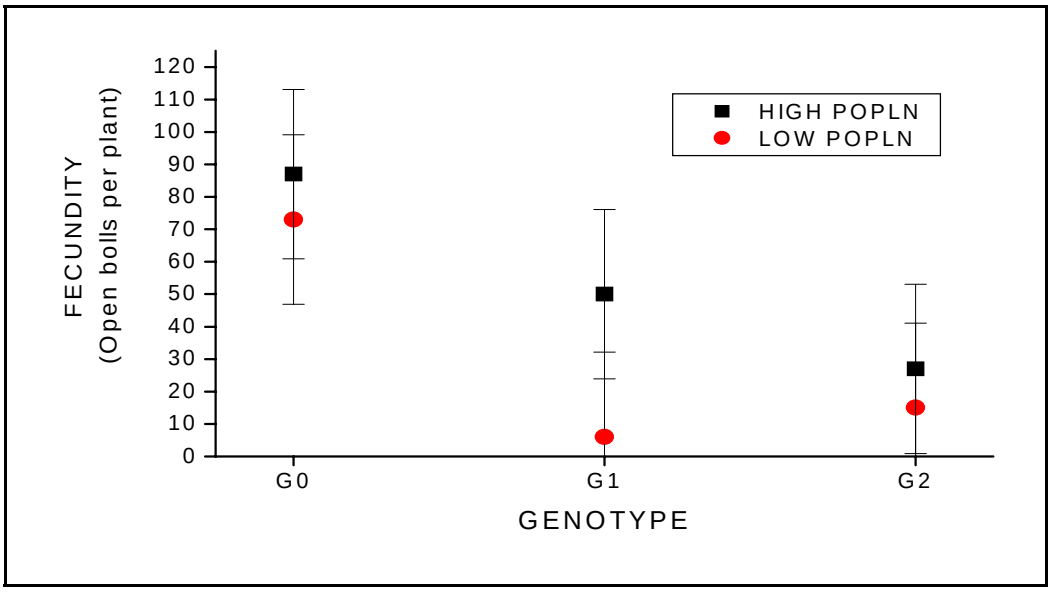


Figure A2.26. Population by genotype interaction (not significant) on No. of open bolls produced per plant (error bars are ± s.e.)

Number of Plants Present at Time of Maximum Boll Production

There was a significant effect of both seedtype (P=0.033) and of population (P<0.001; data box-cox transformed). There were less plants derived from seed cotton present than plants from fuzzy seed or black seed (means = 1.42, 5.08 and 5.75 plants respectively; s.e.=0.86).

There were less plants present from the low population treatment (0.64) than the high density treatment (7.53; s.e.=0.88).

Plants from a total of 17 plots produced recruited seedlings. This corresponded to four, six and seven plots for G0, G1 and G2 respectively. Actual numbers of seedlings is discussed in the section for the invasiveness parameter below. There was a positive correlation between maximum open bolls produced and number of seedlings produced (Spearman's rank correlation = 0.553; n=72, P<0.001) indicating a causal relationship.

INVASIVENESS

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 319, 261 and 382 seeds for G0, G1 and G2 respectively. Number of originally sown plants remaining after the first dry season for each genotype were 106, 94 and 119. Number of recruited seedlings totalled 11, 22 and 115 respectively, resulting in a population of 117, 116 and 234 plants for G0, G1 and G2 respectively. At the final measurement, number of originally sown plants surviving were 97, 85 and 75, so with the addition of the recruited seedlings (6, 10 and 58 remaining), the population of each genotype totalled 103, 95 and 133 plants for G0, G1 and G2 respectively. Calculations of invasiveness using the simplistic method resulted in:

λ_1: G0 = 0.3667; G1 = 0.4444; G2 = 0.6126
λ_2: G0 = 0.8803; G1 = 0.8190; G2 = 0.4829

λ_1
An ANOVA of λ_1 revealed that there was a significant effect of population (P=0.004; Box-cox transformation), with the high density treatment greater than the low density treatment (means = 0.431 and 0.299 respectively; s.e.=0.105).

λ_2
There was a significant effect of population (P<0.001), with results consistent with those from λ_1 (means = 0.722 and 0.171 for the high and low density levels respectively; s.e.=0.051).

There was a trend for an effect of genotype (P=0.09) with the conventional genotype producing the greatest value (0.588) compared to the single gene (0.445) and the two-gene (0.305).

Figure A2.27 illustrates the ANOVA means of the two invasiveness times for each genotype.

There was a positive correlation between maximum open bolls per plot and λ_1 and λ_2 (Spearman's rank correlation = 0.82 and 0.84; n=72, P<0.001).

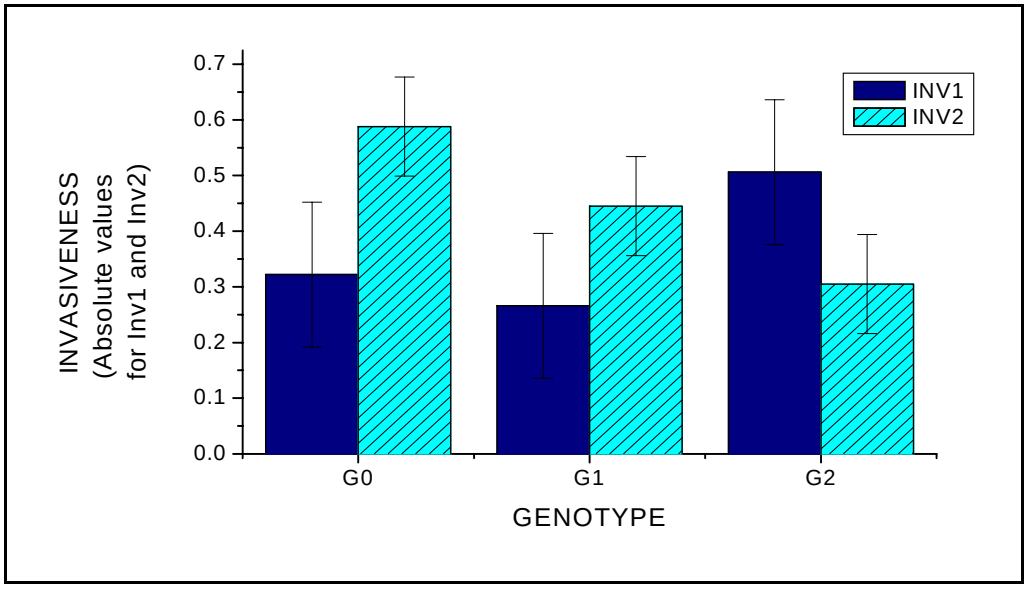


Figure A2.27. Mean values for invasiveness as calculated through ANOVA for the three genotypes. There were no significant genotype effects for either time (error bars are ± standard error and for within each time only).

Table A2.4. Summary of results; significant effects for each demographic parameter (# effect not significant but reported on due to factor of interest – genotype)

BROOME CATTLE SITE NO.1		
DEMOGRAPHIC PARAMETER	**SIGNIFICANT FACTORS**	**DESCRIPTION OF EFFECT**
GERMINATION	SEEDTYPE (P=0.13)	S1>S2,S3
SURVIVORSHIP 1 /NSEEDS	GENOTYPE X SEEDTYPE (P=0012)	G2>G1 and G0 for fuzzy seed only
ABSOLUTE	POPULATION (P<0.001)	H>L
	SEEDTYPE (P=0.042)	S1,S2>S3
/GERMINATED	SEEDTYPE (P=0.006)	S1,S2>S3
SURVIVORSHIP 2 /NSEEDS	GENOTYPE X SEEDTYPE (P=0.016)	G2>G1 and G0 for fuzzy seed only
ABSOLUTE	POPULATION (P<0.001)	H>L
	SEEDTYPE (P=0.022)	S1,S2>S3
/GERMINATED	SEEDTYPE (P=0.008)	S1,S2>S3
	GENOTYPE (P=0.02)	G0,G1>G2
FECUNDITY MAXIMUM OPEN BOLLS	POPULATION (P=0.009)	H>L
	GENOTYPE X SEEDTYPE (P=0.052)#	Increase with genotype for fuzzy seed only
OBT12	SEEDTYPE (P=0.016)	S1>S2>S3
	POPULATION X GENOTYPE (P=0.053)#	G2,G1>G0 for H population only
OBT16	POPULATION (P<0.001)	H>L
	GENOTYPE X SEEDTYPE (P=0.032)	Increase with genotype for fuzzy seed only
MAX OB / PLANT	POPULATION (P<0.001)	H>L
PLANTS AT MAX OB	POPULATION (P<0.001)	H>L
	SEEDTYPE (P=0.033)	S1,S2>S3
INVASIVENESS INV1	POPULATION (P=0.004)	H>L
INV2	POPULATION (P<0.001)	H>L

SITE DISCUSSION

Habitat Characteristics

This was by far the most vigorous of the sites in this experiment. The high nutrient levels, possible leaking water trough, and little interspecific plant competition provided an extremely good habitat for cotton volunteer plants to flourish. This was an unusual cattle yard habitat, as cattle were not permitted in the pens, which enabled maximum cotton development. In reality, it is more likely that yards used for feeding in one year – the time that allows the cottonseed to escape, will also be used for feeding the following year. Cattle would then have access to grazing the cotton bush, plus to trample the plants, effectively reducing invasiveness. Seed fed to cattle has the potential to escape as spillage, and passage through the digestive system. A feeding trial examining such passage indicated that rates of passage were highly variable and influenced by quality of other feed consumed (Appendix 4). Although this probability would vary with cattle and other fodder characteristics, seedlings establishing from such seed would be emerging in a high nutrition environment, which would increase their potential for establishment.

Microhabitat

The significant block effect for the majority of demographic parameters was evident in the field. Microhabitat difference may be due to location of a possibly leaking underground water pipe, or to differences in history between the individual pens within the yard itself. Supporting evidence was the presence of green grasses over the dry season, and differences in the spectrum of grass species that regenerated in different pens. Blocks 3 and 4 were in a pen in which dense stands of Rhodes grass

(*Chloris* spp) and buffel grass (*Cenchrus* spp) established after the first wet season. These blocks had significantly less plants surviving compared to blocks 1 and 2 which were in a separate pen. This indicates the effect that competitive species may have on the establishment and survival of volunteer cotton, particularly for bare ground space for seedling recruitment.

Germination
The effect of seedtype on germination was black seed greater than fuzzy seed greater than seed cotton, consistent for the majority of sites.

Survivorship
The greater absolute survivorship (1 and 2) at the higher sowing density has implications for establishment of volunteer cotton populations from unintentional seed dispersal. If individual or small numbers of seeds disperse, there would be less chance of plants existing after one or two years than dispersal of greater seed numbers.

The influence of seedtype on survival is an important one –seed cotton, which has the greatest chance of uncontrolled dispersal, gave much lower seedling survival than black and fuzzy seed. There was little evidence to support a Bt gene enhancement of survivorship of cotton plants.

Fecundity
There were important differences in open boll numbers between genotypes towards the end of the dry season. In the first season (Sept – Oct 2000), the two transgenic treatments had higher open boll numbers than the conventional genotype. Open boll numbers declined over the wet season. The same trend was observed in the second dry season (Aug 01). However, this relationship changes by the next measurement at the end of the dry season (Nov 01) where the conventional treatment is higher than the two transgenic treatments. This possibly also may have occurred in the previous season, but may have not been determined since there was no subsequent measurement until after the wet season. This highlighted that determining the time corresponding to maximum boll numbers may be different between genotypes and may also be different between seasons. The transgenic plants may be more determinant due to more rapid boll setting, and hence attain their reproductive potential earlier in the season compared to the conventional genotype. Observations in commercial production areas had indicated that double gene cotton did 'cut-out' (or finish development of harvestable bolls) before the single gene cotton. If the wet season commences before the bolls on the conventional plant have time to develop later in the season, then it may not be actually total numbers of fruit which is different, but timing of fruit production in relation to germinating rains. So, time of maximum boll production may vary with genotype and season. Photos A2.7; a,b and c illustrate differences between genotypes for black seed high population plots. These were in August 2001 and illustrate different boll loads, and different amounts of vegetative growth.

This habitat does illustrate the ability of cotton plants to establish as volunteers when conditions are suitable. Population was the dominant factor influencing numbers of open bolls produced, related to the importance of this factor in absolute survivorship of plants. There was some indication that the transgenic plants had the potential to produce more seed then the conventional plant with the effect of genotype approaching significance only as an interaction effect with either population (OBT12; first year production) or with seedtype (max OB over 2 years). The large proportion of missing values (plots with no surviving plants) made robust analysis of number of bolls produced per surviving plant difficult. Given that genotype did not provide significant effects on the early growth stages, ideally, to study boll production more precisely, the factors of seedtype and population could be removed to minimise the confounding effects of germination and early survival prior to the plants reaching reproductive maturity. Black seed only could be sown to a constant number of seeds. Seedling numbers could be then thinned to a constant population and reproductive development assessed to more fully examine the effect of the Bt gene on fecundity.

Invasiveness
Genotype alone did not lead to an effect of an increase in bolls produced; population was the dominant factor influencing fecundity, and was the only factor to influence invasiveness. The importance of this factor for both fecundity and invasiveness was supported by the positive correlation between open boll production and invasiveness at each time. There was no indication that the addition of the Bt gene caused a significant increase in population growth, or invasiveness.

The timing for calculation of invasiveness is critical. λ_1 included seedling recruitment from the first year, so enabled a robust calculation of population change for this time. However, no rain had yet

fallen on the site by the time of final plant counts conducted in November 2001, so λ_2 allowed for mortality of the cohort of first year recruited seedlings, but did not allow for any additional seedling recruitment. Ideally, seedlings recruited after the second wet season would have been included for a more equable calculation of invasiveness.

Observations at the final measurement were that conventional plants were the most vigorous, and the double gene less so. This observation was not totally consistent between genotypes, but does highlight the fact that for a perennial plant, to determine rate of population change, that greater than two years may be necessary. Invasiveness may be related to perenniality, which is difficult to investigate in two seasons. This may be more applicable in sites where high nutrition may allow the transgenic to reach their reproductive potential, which may decrease their energy reserves and possibly lead to increased mortality, but where seedling recruitment also may occur due to the increased fruit production. This would enable calculation of invasiveness as population change between an established population (i.e. plants remaining after an initial establishment year) and a population where at least one full cycle of seedling recruitment and mortality had occurred.

Photo A2.7a. Double gene treatment Broome Cattle Site No. 1 Aug 2001

Photos A2.7b and c. b (top) is conventional genotype – where green bolls subsequently developed; c = single gene genotype (green leaves in the foreground are actually recruited seedlings)

SITE 11: BROOME ROAD SITE

Date Sown: 13 January 2000

GERMINATION

Plant counts for germination were conducted on the 17 January (T1), 24 January (T2) and 17 February (T3), 2000.

Seedtype was highly significant (P<0.001) at all three measurement times, and for overall germination. Germination proportion of all three seedtypes declined over time. There was a distinct time lag in germination of seedcotton compared to the other two seedtypes. This accounted for the seedcotton having a higher germination compared to fuzzy seed for T2 and T3, by which times, seedlings from the fuzzy seed were dying, similarly with the black seed at T2. Black seed had the highest germination, followed by the fuzzy seed then the seed cotton (Figure A2.28).

Population was highly significant on germination (P<0.001) with the high density treatment having higher germination (37.19%; s.e.=1.91) compared to the low density treatment (16.67%; s.e.= 4.28).

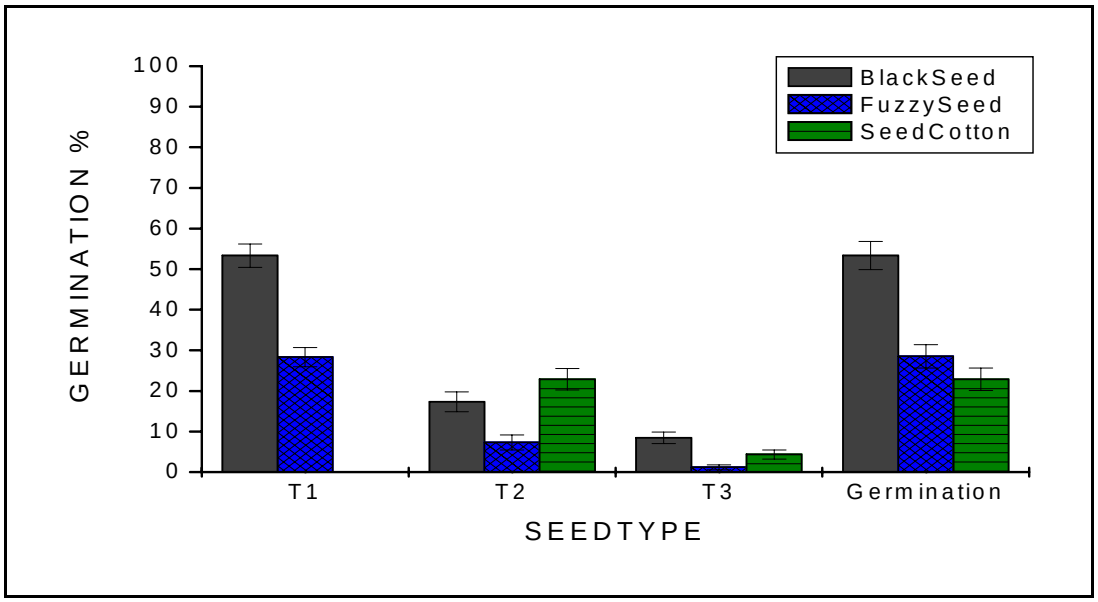


Figure A2.28. Effect of seedtype on germination over time at Broome Road Site (error bars are ± s.e. and are for within each time only)

SURVIVORSHIP 1

There were six plots with surviving plants (T10; 11 July 2000); equivalent to 0.56% plants from seed sown. All plants had the maximum damage rating (6) at this time, as none had leaves, and maximum height was 9 cm. Corresponding treatments and number of plants for these plots were:

1 plant; S2HG0
3 plants; S1HG1
1 plant; S1HG2
10 plants; S1HG2
1 plant; S1HG1
2 plants; S2HG1

This was the last measurement before a fire totally destroyed all remaining plants leaving none by the end of the 2000 dry season.

SURVIVORSHIP 2

Zero

FECUNDITY

No fruiting structures were ever produced at this site.

INVASIVENESS

No successive seedlings were produced.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 364, 334 and 433 seeds for G0, G1 and G2 respectively. With no surviving original plants and no seedlings produced, invasiveness for all genotypes was zero.

SITE DISCUSSION

Plants at this site displayed poor vigour after initial germination, as illustrated in Photos A2.7; a and b. They grew no higher than 10 cm, displayed little leaf development, and developed no fruit.

Although there were surviving plants before fire destroyed the plots, it is doubtful that these plants would have survived until the subsequent wet season. It is certain that they would not have produced any fruiting structures.

The variation of results with block again shows the importance of micro-habitat. At this site, seedlings which received some shade from shrubs on the fenceline, appeared to be more vigourous.

Grasshoppers were observed to eat the newly germinated seedlings.

Similarly to the Broome bush site, poor soil nutrition, lack of water availability and fire were major constraints to cotton volunteer growth at this site.

Photos A2.8a and b. Watering the Broome road site, note the poor vigour of the seedlings

SITE 12: BROOME DAM SITE

Date Sown: 13 January 2000

GERMINATION

Plant counts for germination were conducted on 17 and 24 January 2000 (T1, T2). No other measurements were conducted until after the wet season (May 2000), at which time there were no surviving plants.

Seedtype was highly significant (P<0.001) at both times, and overall, with germination of seed cotton consistently less than fuzzy seed and black seed, as shown in Figure A2.29.

Population was also significant (P=0.031), with the low population consistently producing a lower germination proportion (65.18 ± s.e.4.58) compared to the high population level (75.56 ± s.e.1.42).

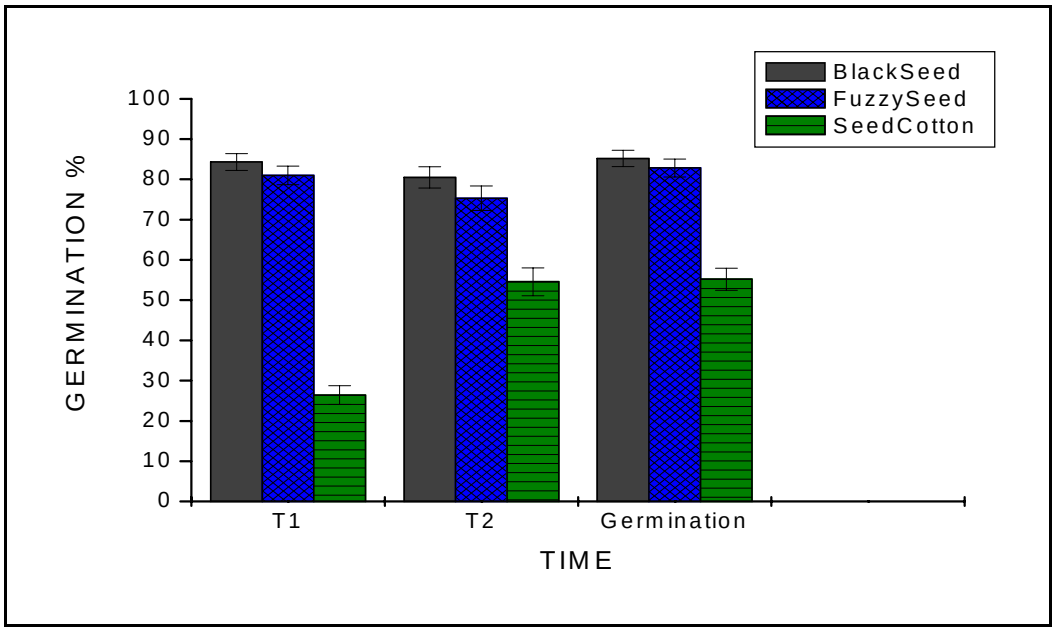


Figure A2.29. Effect of seedtype on germination over time at Broome Dam Site (error bars are ± s.e., and are for within each time only)

SURVIVORSHIP 1

Plants grew well initially, but were killed by submergence in water for over a month during the 2000 Wet Season. There were no surviving plants after the wet season.

SURVIVORSHIP 2

Zero

FECUNDITY

No plants produced fruiting structures. This was due to early death from submergence, rather than lack of physiological development.

INVASIVENESS

A total of 810 seeds (three seedtypes) were sown for each genotype. Number that germinated were 611, 577 and 620 seeds for G0, G1 and G2 respectively. There were no surviving plants, so invasiveness of each genotype was zero.

SITE DISCUSSION

It was very difficult to find a site in this area that had water over the dry season, such as the creek habitat in Katherine, or drain habitat in Kununurra. The dam site was chosen as it was expected to have some available water persisting into the dry season. Seeds were sown into a dry dam bed, and early plant growth was vigourous, consistent with the relatively high nutrient status of the site. However, a well above average wet season filled the dam for considerable time, drowning all plants. A modified experiment (using only one seedtype and a constant population) could have been conducted around the surrounds of the dam, but in hindsight, the habitat has similar characteristics to the cattle habitat (a congregation area), and management strategies to reduce risk of seed dispersal would have been similar.

The site does however emphasise the lack of habitats in this region with consistent water availability for volunteer cotton growth in contrast to those further north possessing more extensive creek and river systems, or drainage systems (such as in ORIA.).

As observed at a number of sites, there was a significant block by seedtype interaction. This was consistent with the physical layout of the site and soil variability, again supporting the influence of microhabitat on germination and subsequent establishment of volunteers.

Photo A2.9. Cotton seedlings grew well initially at the Broome Dam site, shown here at 17 February 2000, but all died over the wet season due to inundation

SITE 13: KUNUNURRA DRAIN SITE: DRY SEASON

Date Sown: 30 June 2000

GERMINATION

Plant counts for germination were conducted on 11 July 2000 (T1), 6 August 2000 (T2) and 22 September (T3). Each of the three within site experimental factors was significant; Seedtype (P<0.001), Population (P=0.05), and Genotype (P=0.007).

Seed cotton had consistently the lowest germination, as illustrated in Figure A2.30. Fuzzy seed had a higher germination compared to black seed, which was the reverse to the majority of other sites.

The high population treatment produced consistently greater proportion of number of seedlings emerged (51.49; s.e.=1.95) compared to the low population level (39.44; s.e.=5.29).

The conventional genotype had a lower germination than the two transgenic treatments. This trend was consistent across all time measurements for germination, as shown in Figure A2.31.

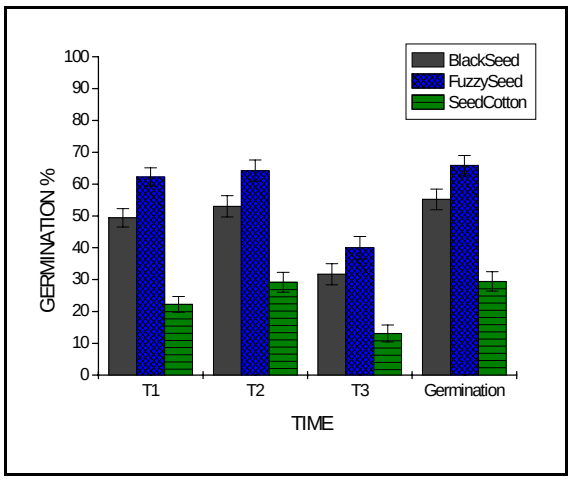


Figure A2.30. Effect of seedtype on germination at each time at Kununurra Drain Site; dry season sown (error bars are ± s.e., and are for within each time only)

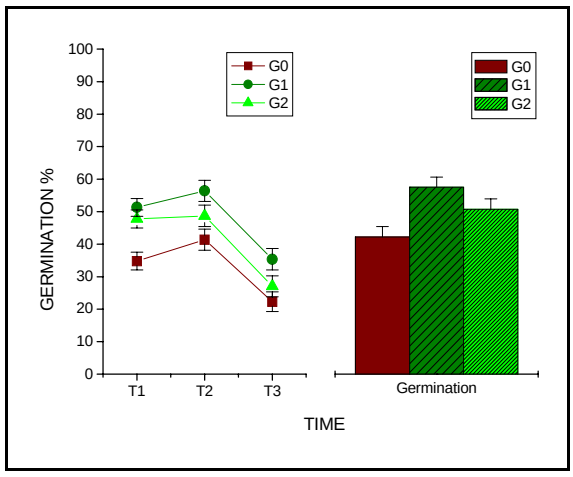


Figure A2.31. Effect of genotype on germination at Kununurra DS Drain Site (error bars are ± s.e. and are for within each time only)

SURVIVORSHIP 1

Seeds were sown at this site in the mid-dry season, so surviving plants were only six months of age when assessment at the end of the first dry season was conducted (T5; 22 December 2000).

Survivorship as a Proportion of Seeds Sown

There were 47 plots (from 72 sown) with plants remaining, corresponding to 15.49% plants remaining from the original cohort of seeds sown. There was a significant seedtype by genotype interaction (P=0.031) where G1 and G2 were greater than G0 only for fuzzy seed (Figure A2.32).

Absolute Survivorship

There was a significant effect of both seedtype (P=0.05) and Population (P=0.004) for log-transformed number of plants present. Seedling number from seedcotton was significantly lower than the other seed types at the end of the first dry season; non-transformed mean values are 3.21, 6.96 and 10.75 plants present for seedlings derived from seed cotton, black seed and fuzzy seed respectively.

There was a significantly greater number of plants remaining from the high population than from the low population; non-transformed mean values were 11.94 and 2.00 plants present for the high and low levels respectively.

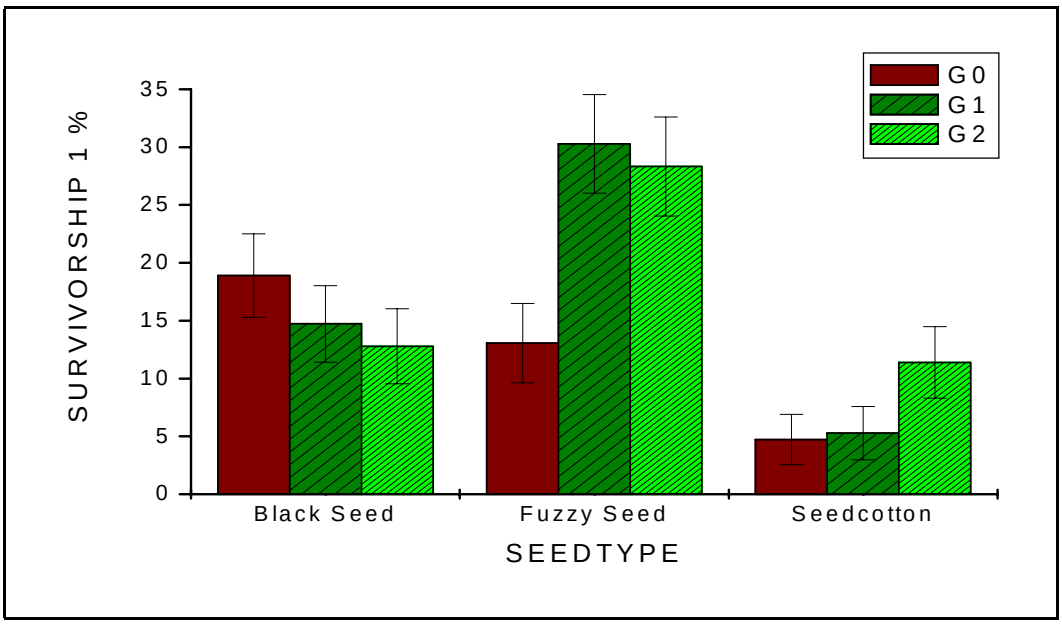


Figure A2.32. Effect of genotype by seedtype interaction on Survivorship 1 expressed as a percentage of seeds sown at Kununurra DS Drain Site (error bars are ± s.e.)

SURVIVORSHIP 2

Plant survival at the end of the second dry season was assessed on 3 November 2001 (T10), with plants approaching 18 months of age. There were 43 plots with surviving plants, corresponding to 12.81% plants remaining from the original cohort of seeds sown.

Survivorship as a Proportion of Seeds Sown
The significant interaction between genotype and seedtype (P=0.003) was similar to those for survivorship in the previous year, although the number of plants surviving for all treatments had decreased.

Absolute Survivorship
There was a significant effect only of Population (P=0.016;) on the total number of plants present in each plot, shown as a greater number of plants remaining from the high population compared to the low population; non-transformed mean values were 9.92 and 1.61 plants remaining for the high and low levels respectively. (Values for square-root-transformed data; H=2.714; L=1.587; s.e.=0.2892).

FECUNDITY

Fruit production over time was plotted to assess plant phenology over the project duration and determine periods of maximum open boll production. This was calculated as mean number of fruit per plot for each genotype, and is illustrated in Figure A2.33. Number of open bolls was approaching a maximum at the end of the dry season (T10; 2 November 2001) for the majority of plots. For the first dry season, number of plots (12) with open bolls and number of bolls per plot were low, so analysis of fruit production was not conducted at this stage.

There was a distinct seasonal effect on cotton fruiting patterns. Square production commenced by mid-August (mid dry season; approximately eight weeks after sowing), continued into the wet season, then declined as the subsequent dry season progressed. Green boll production coincided with square development unti the mid-dry season; numbers then decreased as the green bolls matured to open bolls. Numbers of open bolls increased over the duration of the second dry season, reaching a maximum towards the end of the dry season. Rainfall at the commencement of the wet season helped to dislodge cotton from the bracts, and initiated germination of next generation seedlings.

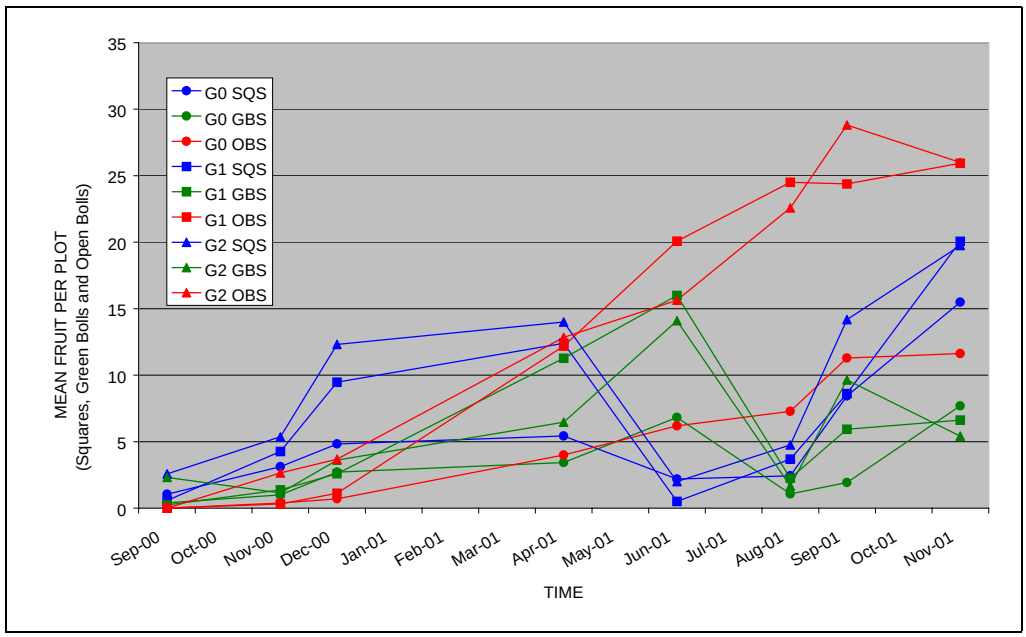


Figure A2.33. Fruit production over time for Kununurra DS Drain Site

The maximum number of open bolls produced per plot were analysed (independent of whether there were surviving plants present or not).

There was a significant effect of both population (P=0.037) and of genotype (P=0.005) on the number of total open bolls per plot. The high population density treatment produced a greater number of open bolls per plot (19.4) compared with the low density treatment (9.1; s.e.=2.95).

A posthoc comparison using the Dunn-Sidak transformation showed the only significant difference was the lower boll number per plot of the conventional compared with the single gene (Values are shown in Figure A2.34.)

There was a positive correlation between maximum open bolls produced and maximum number of recruited seedlings produced per plot. (Spearman's rank correlation = 0.45; n=72, p<0.001), indicating a causal relationship.

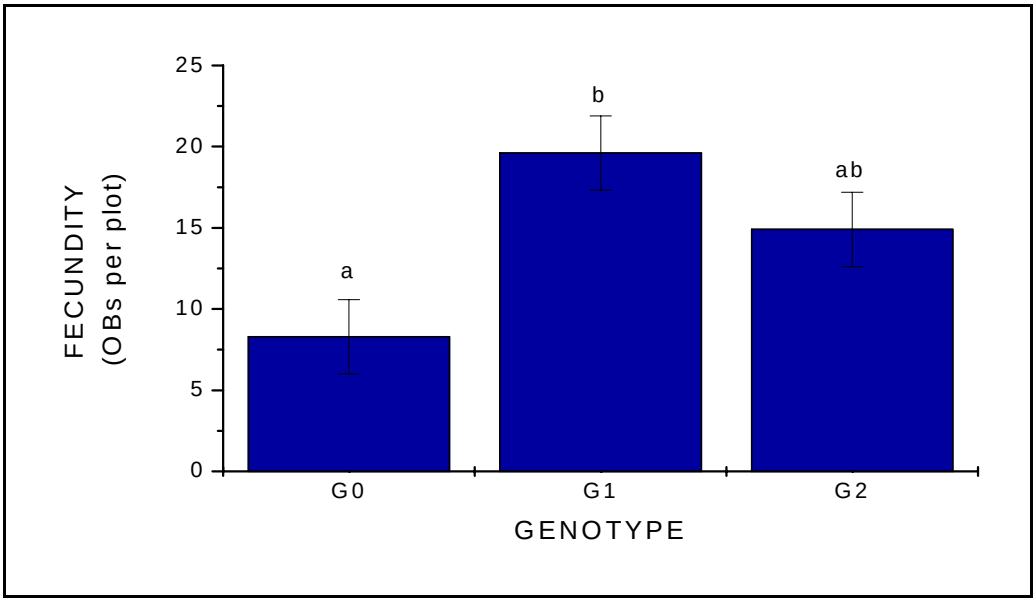


Figure A2.34. Effect of genotype on mean maximum open boll production per plot at Kununurra Drain Site; dry season sown (error bars are ± s.e.)

INVASIVENESS

Three plots had produced seedlings by the time measurements were conducted for Surv1 (T5), corresponding to Plot 56; S3LG2 (14 seedlings), Plot 57; S3LG1 (two seedlings) and Plot 68; S2HG2 (34 seedlings), for which there was some mortality over the following dry season.

There had been some additional seedling recruitment by the time of final measurements (T10, November 2001). There were eight plots with seedlings remaining at the final count (T10). Seedling numbers in these plots are presented in Table A2.5.

Table A2.5. Plots and respective treatments that produced second generation seedlings at Surv2 at Kununurra DS Drain

Plot No.	Treatment	No. of seedlings
44	S2 H G1	7
56	S3 L G2	13
57	S3 L G1	1
58	S3 H G2	3
59	S3 H G1	1
61	S1 H G0	5
68	S2 H G2	22
69	S2 H G0	4

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number that germinated were 456, 621 and 548 seeds for G0, G1 and G2 respectively. Numbers of originally sown plants present for genotypes G0, G1 and G2 respectively after the first dry season (T5; 22 December 2000) were 132, 181 and 189; seedlings totalling 0, 2 and 48, resulting in totals of 132, 183 and 237 plants.

At the final measurement (Surv2), number of originally sown plants remaining were respectively for G0, G1 and G2, 110, 151 and 154 plants. Recruited seedling numbers were 9, 9, and 38, to give totals of 119, 160 and 192 for each genotype.

Values from calculations of invasiveness using the simplistic method were:

λ_1: (=Surv1 + Seedlings 1)/Germination
G0 = 0.2895; G1 = 0.2947; G2 = 0.4325

λ_2: (=Surv2 + Seedlings 2)/(Surv1 + Seedlings 1)
G0 = 0.9015; G1 = 0.8743; G2 =0.8101

An ANOVA was conducted on the two invasiveness parameters.

There was a significant (P=0.020) effect of population on λ_1 (reciprocal root transformation), with the low density treatment having significantly higher value compared with the high density treatment. (Means for the non-transformed data corresponded to 0.65 and 0.25 for the low and high population levels respectively; s.e.=0.162).

There was a significant genotype effect (P=0.002; s.e.=0.162) for λ_2, with the two-gene treatment producing a significantly lower value (0.308) compared to the single gene (0.561) and the conventional genotype (0.618). A posthoc comparison using the Dunn Sidak transformation revealed no significant difference between the means of the single gene and conventional genotypes. Figure A2.35 illustrates the resultant ANOVA mean values of λ_1 and λ_2 for the three genotype treatments.

There was a highly significant correlation between maximum open bolls produced and invasiveness values at each time (Spearman's rank correlation = 0.73 for both times; n=72, P<0.001).

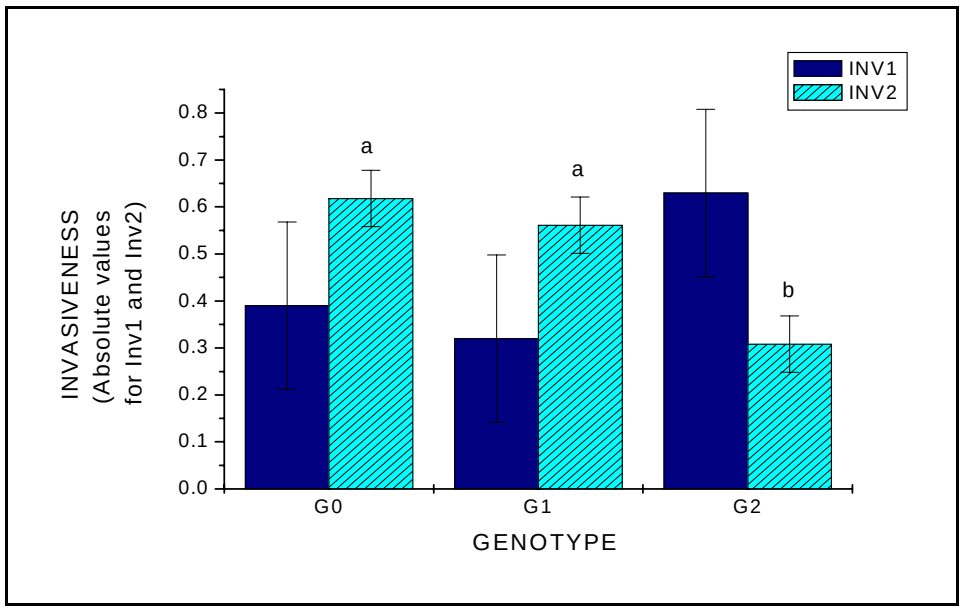


Figure A2.35. Mean values for invasiveness for the three genotypes from ANOVA. There were no significant genotype effects for Inv1 (λ_1). Different letters indicate significantly different genotype effects on Inv2 (λ_2). (Error bars are ± standard error and are for within each time only).

SUMMARY OF RESULTS

Table A2.6. Summary of significant effects for demographic parameters for Kununurra DS Drain site

KUNUNURRA DRY SEASON DRAIN		
DEMOGRAPHIC PARAMETER	**SIGNIFICANT FACTORS**	**DESCRIPTION OF EFFECT**
GERMINATION	SEEDTYPE (P<0.001)	S1,S2>S3
	POPULATION (P=0.05)	H>L
	GENOTYPE (P=0.007)	G1 ≥G2 ≥G0
SURVIVORSHIP 1 /NSEEDS	GENOTYPE X SEEDTYPE (P=0.031)	G2,G1 > G0 for fuzzy seed only
ABSOLUTE	POPULATION (P=0.004)	H>L
	SEEDTYPE (P=0.05)	S1,S2>S3
SURVIVORSHIP 2 /NSEEDS	GENOTYPE X SEEDTYPE (P=0.003)	G2,G1 > G0 for fuzzy seed only
ABSOLUTE	POPULATION (P=0016)	H>L
FECUNDITY MAXIMUM OPEN BOLLS	POPULATION (P=0.037)	H>L
	GENOTYPE (P=0.005)	G1>G0
INVASIVENESS INV1 (λ_1)	POPULATION (P=0.02)	L>H
INV2 (λ_2)	GENOTYPE (P=0.002)	G0,G1>G2

SITE DISCUSSION

Habitat Characteristics

There was a highly significant effect of block (P<0.001) on germination, which could be explained by soil type. Plots were sown along the length of the drain, which had sandier soil at the upper end, graduating to black clay at the lower end. Plant vigour and survivorship was related to soil type, with low survivorship and fruit production on the sandier soil, and vigorous plants and high survivorship on

the black clay. Photos A2.10; a and b illustrate this effect for the black seed high population single gene treatment for plots at each end of the drain. This illustrates the importance of microhabitat within a habitat that may be considered conducive to cotton volunteer growth.

Germination

Fuzzy seed had a higher germination than black seed, in contrast to the majority of other sites (sown in the wet season). This may be due to lower atmospheric humidity increasing the rate at which black seeds dry out. Fuzzy seed may have enough lint to retain some additional moisture.

Genotype produced a significant effect on germination (P=0.007), with the conventional genotype having a lower germination than the two transgenic treatments. This effect may be spurious, as it was not observed at any of the twelve originally sown sites. It may be due to differences in susceptibility of damage to seed during storage. The seed was kept in a non-insect proof area for six months of storage as compared to seed sown in the initial wet season planting where seed was hand-picked then immediately sown. Black seed, if damaged as fuzzy seed in storage prior to acid delinting (which was done immediately prior to sowing), would be screened out during the delinting process, as these would be likely 'floaters' and would be discarded from sowing. Seed cotton would be suspected to have the greatest protection from insects due to its lint coverage, so may have had the least degradation. Fuzzy seed would be relatively susceptible to insect attack, and not screened out during the pre-sowing treatments, so there may have been differences between genotypes in susceptibility to insect predation during storage, although this was not substantiated.

Photo A2.10a. Microhabitat effect at Kununurra DS Drain site – black clay end of drain

Photo A2.10b. Microhabitat effect at Kununurra DS Drain site – sandy soil end of drain

Survivorship

The greater chance of absolute survivorship (1 and 2) for plants derived from a higher initial seed population has implications for establishment of volunteer cotton populations from unintentional seed dispersal. If individual or small numbers of seeds disperse, there is less chance of plants existing after one or two years compared to if a greater number of seeds dispersing. The influence of seedtype on survival is an important one – seedlings derived from seed cotton, which has the greatest chance of uncontrolled dispersal, had less chance of subsequently surviving. There was little evidence supporting that the addition of the Bt gene enhanced survivorship of cotton plants.

Fecundity
This habitat does indicate that under suitable conditions, Bt transgenic cotton may produce a greater number of open bolls per unit area compared to its conventional counterpart, although this relationship was statistically significant only for the single gene treatment.

The seedtype by genotype interaction for survivorship complicates inferences made concerning the increased boll production. The greater establishment after two years of the seedlings derived from the transgenic fuzzy seed provides for greater opportunity for bolls to be produced, as compared with the conventional genotype.

The difference in boll production does provide evidence that transgenic may be more fecund.

Invasiveness
There was no indication that the addition of the Bt gene caused a significant increase in population growth, or invasiveness. The single gene treatment produced the greatest number of open bolls, but this did not transpose to an increase in the invasiveness, although there was a positive correlation between open boll production and invasiveness at each time. The two-gene had the lowest value for invasiveness, and at no time the value greater than one for this site for any of the genotypes (although some individual plots did produce values greater than one). This was consistent with Crawley (1993) who found that rapeseed never produced values greater than one despite the fact that a few individual plants set substantial seed.

There was further recruitment observed by December, but data was not collected at this stage (due to project timeframe), and these numbers were not included in the calculation of invasiveness 2. Invasiveness 2 allowed for mortality of the cohort of first year recruited seedlings, but did not allow for successive recruitment. Ideally, seedlings recruited after the second year would have been included for a more equable calculation of invasiveness.

This site was burnt (unintentionally) after the final measurements (November 2001). Observations after this time indicated that the newly recruited seedlings were destroyed, but that small proportions of the established plants from the original cohort of seeds were producing new vegetative growth. This supports observations from other fire-affected sites, in that fire does reduce cotton population density, with older established plants possessing the greatest potential for regrowth as compared to young seedlings. Fire is a frequent occurrence in northern Australian habitats, and may influence the persistence of naturalised cotton plants in habitats exposed to seasonal fires.

This site was the only site to be sown during the dry season, as water availability could induce germination if seed dispersed to such a site at such a time, although this would be highly unlikely. Cotton displayed similar phenology at this site as compared to other fruiting sites, in that open bolls developed over the dry season, and vegetative growth was dominant over the wet season. One important advantage of germinating at this time was that seedlings were not subject to the sustained inundation experienced in the wet season sown drain site, where there was extreme mortality of the seedlings after germination.

Photo A2.11a illustrates the potential for open boll production at this habitat. There was no evidence that this was transposed to increased weediness of Bt transgenic cotton compared to its non-transformed counterpart as evaluated by a higher rate of population growth over the duration of the project.

Photo A2.11b is also presented to illustrate the damage and some regeneration of plants after fire (after the initial project duration had been completed).

Photos A2.11 a and b. Potential boll production Kununurra DS Drain site (above) and regeneration after fire after the commencement of the 2001-02 wet season

APPENDIX 3

EXPERIMENT 1B: LARGE SCALE ECOLOGICAL ASSESSMENT: SECOND YEAR SELECTED SITES. INDIVIDUAL SITE RESULTS. SITES 14-20

SITE 14: KUNUNURRA BUSH NO.2

Date Sown: 14 December 2000

TREATMENTS

This site was modified from the previous year's bush habitat sowing. Seedcotton was excluded due to seed availability of the different genotypes, and the attempt to maximise number of seedlings to germinate. The additional double gene of Cry 1Ac and Cry2Ab was included for comparison with the double gene (Cry1Ac and Cry2Aa) sown the previous season.

GERMINATION

Plant counts for germination were conducted on 21 December 2000 (T1), 12 January 2001 (T2), and 6 March 2001 (T3).

There was no significant effect of any factors on germination overall.

SURVIVORSHIP 1

The final plant count was conducted on 26 October, 2001. There were only six plots with surviving plants, equivalent to 0.56% plants of seeds sown. Treatments and number of surviving plants for these plots corresponded to:

2 plants; S1HG2X
2 plants; S1LG0
8 plants; S2HG0
1 plants; S2LG2
2 plants; S2LG1
1 plant; S1HG2X

FECUNDITY

Plants at this site never progressed to retaining any viable seed. A single plant (S1HG2X) produced two very small green bolls, but these aborted prior to maturity.

INVASIVENESS

No seedlings were ever produced at this site.

A total of 720 seeds (two seedtypes) were sown for each genotype. Number of seedlings present after germination equalled 661, 664, 645, and 675 for G0, G1, G2 and G2X respectively. Number of plants present for each genotype at the final measurement was 10, 2, 1, and 3. Values from calculations of invasiveness using the simplistic method resulted in the values:

G0 = 0.0151; G1 = 0.0030; G2 = 0.00155; G2X = 0.0044

SITE DISCUSSION

This site exhibited consistently high germination (>80%) for all treatments, due to the exclusion of the seed cotton treatment. In mid-March, the area was invaded by grasshoppers (yellow-winged and spur throated were observed on the plants) which proceeded to lop the seedlings, mostly at just above ground level. The only seedlings that survived appeared to be those chewed above the cotyledon, the axils from which shoots subsequently regenerated, as illustrated in Photo A3.1.

This was different to seedling establishment in this habitat in the previous season. This raises the issue of different insect pressure, applicable not only with grasshoppers, but also other species, according to different seasons. An irregular but high insect herbivory pressure, and interactions with suitable germination season, will have implications for rates of population change for potential volunteer cotton.

Photo A3.1. Plot 9 (S1HG2X) from Kununurra Bush Site No.2 demonstrating high mortality due to chewing, and surviving plants chewed above the cotyledon

SITE 15: KUNUNURRA CATTLE NO.2

Date Sown: 15 December 2000

TREATMENTS

Factors for this site were modified from the previous year's design for the Cattle habitat. Population was removed as a factor. All plots were sown to 50 seeds, then hand-thinned to a maximum of 10 seedlings per plot after the initial germination counts were conducted. The factor of genotype also included 289G2X (double gene containing Cry1Ac and Cry2Ab for variety Sicot 289), and DP50BX (double gene containing Cry1Ac and Cry2Ab for variety DP50), in addition to the three genotypes previously assessed.

Seedtype was again included, although seed cotton of 289G2X was not available, resulting in a total of 14 treatment combinations.

GERMINATION

Plant counts for germination were conducted on 22 December 2000 (T1), 11 January 2001 (T2) and 10 April (T3).

Seedtype was highly significant (P<0.001), with seed cotton having the lowest germination (32.96; s.e.=2.68), followed by fuzzy seed (62.58; s.e.=2.41), and black seed the highest germination (74.56; s.e.=2.2).

Genotype was also highly significant (P<0.001) with the two genotypes containing the Cry2Ab gene producing a lower germination compared to the other three genotypes, as illustrated in Figure A3.1.

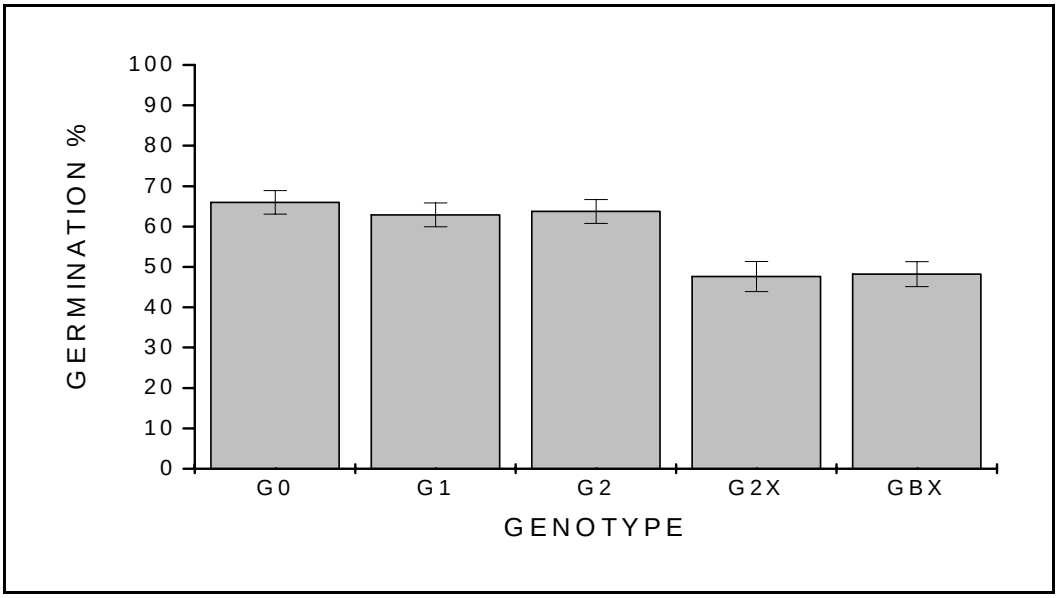


Figure A3.1. Effect of genotype on germination at Kununurra Cattle Site No.2 (error bars are ± s.e.)

SURVIVORSHIP 1

Final measurements were conducted on 25 October 2001 (T7). Survivorship was calculated as the number of plants at this time as a proportion of those established after thinning (T2). There were 29 plots from the original 56 sown that had surviving plants. This was equivalent to 11.9% of plants surviving from those remaining after thinning. There was no significant effect of any factor on proportion of plants surviving at this time.

Absolute
There was no significant effect of any factor on total number of plants surviving at this time.

FECUNDITY

No plants at this site ever developed to produce any fruiting structures.

INVASIVENESS

No seedlings were produced.

A total of 600 seeds (three seedtypes) were sown for each genotype, except for G2X which only had 400 seeds (two seedtypes). Number of seedlings present after germination equalled 386, 367, 372, 231* and 279 and after thinning at T2 equalled 115, 112, 93, 63* and 98 for G0, G1, G2, G2X and GBX respectively. Number of plants present for each genotype at the final measurement was 18, 13, 7, 17 and 9. Values from calculations of invasiveness using the simplistic method resulted in the values in the following table:

Table A3.1. Invasiveness values for each genotype

Genotype	Invasiveness value
G0	0.1565
G1	0.1161
G2	0.0753
G2X*	0.2698
GBX	0.0918

*No seed cotton sown

SITE DISCUSSION

The plants were waterlogged for the majority of the wet season, and at irrigation times during the dry season, although water and cattle management were more controlled at this site as compared to the leucaena cattle site utilised in the previous year. Leucaena and pangola grass growth was vigourous throughout the wet season, and competed with the cotton plants. Cattle did not appear to graze the plants, but did cause damage by trampling. A combination of these factors – waterlogging, soil compaction, interspecific competition, and physical damage appeared to contribute to the mortality at this site. Photo A3.2 illustrates the poor seedling development, and the grass competition at the last recording time.

The difference in germination between the genotypes is probably more attributable to parent seed source rather than the influence of the Cry2Ab gene, although this is not certain. The G0, G1 and G2 seeds were sourced from one paddock, the G2X from another paddock, and the GBX from yet another paddock. This again highlights the importance of conditions under which the parent plant produces seed – differences in insect management, agronomic practices or weathering of seed at harvest, are likely to have more of an impact on germination compared to differences between genotypes.

Photo A3.2. Surviving plants (S1G2X) at Kununurra Cattle Site No.2 at final measurement (error bars are ± s.e.)

SITE 16: KUNUNURRA WET SEASON DRAIN NO.2

Date Sown: 16 December 2000

GERMINATION

Plant counts for germination were conducted on 22 December 2000 (T1) and 11 January 2001 (T2). T3 was after the wet season (10th April 2001), so was not included in calculations to determine germination, but was included in Figure A3.2, which illustrates there was no significant change in plant numbers over the duration of the wet season. This was different to the previous wet season sowing at this habitat, where there was a high mortality due to inundation.

Seedtype was highly significant at both measurement times, and overall (P<0.001), as shown in Figure A3.2. Black seed was consistently higher than the fuzzy seed, which was higher than the seed cotton. There was a significant interaction between seedtype and population at T3 only. The high density treatment resulted in the greater number of seedlings for black seed, but for the other two seedtypes, the low population density resulted in greater seedling number. A similar trend was observed at the final counts for survivorship (as presented in Figure A3.3).

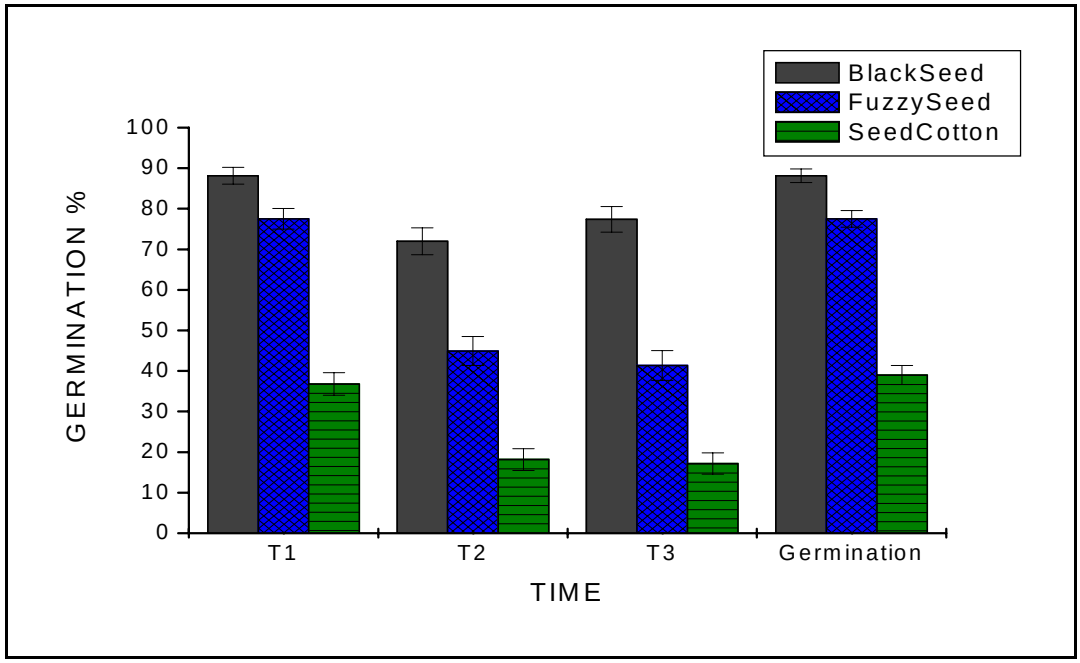


Figure A3.2. Effect of seedtype on germination at each time at Kununurra WS Drain No.2 (error bars are ± s.e., and are for within each time only)

SURVIVORSHIP 1

This site only existed for approximately one year, so plants were approaching 11 months of age at the final measurement. The final counts (T8) were conducted on 28 October 2001, with 50 plots having surviving plants out of the 54 sown, corresponding to 42.8% plants remaining from seeds sown.

Survivorship/Number of Seeds

There was a significant (P=0.026) interaction between seedtype and population, illustrated in Figure A3.3.

There was an increase in survivorship from the high to low density treatments for fuzzy seed and seed cotton, but a decrease for the black seed.

Seedlings derived from seed cotton had the lowest survival of the three seed types at both population densities.

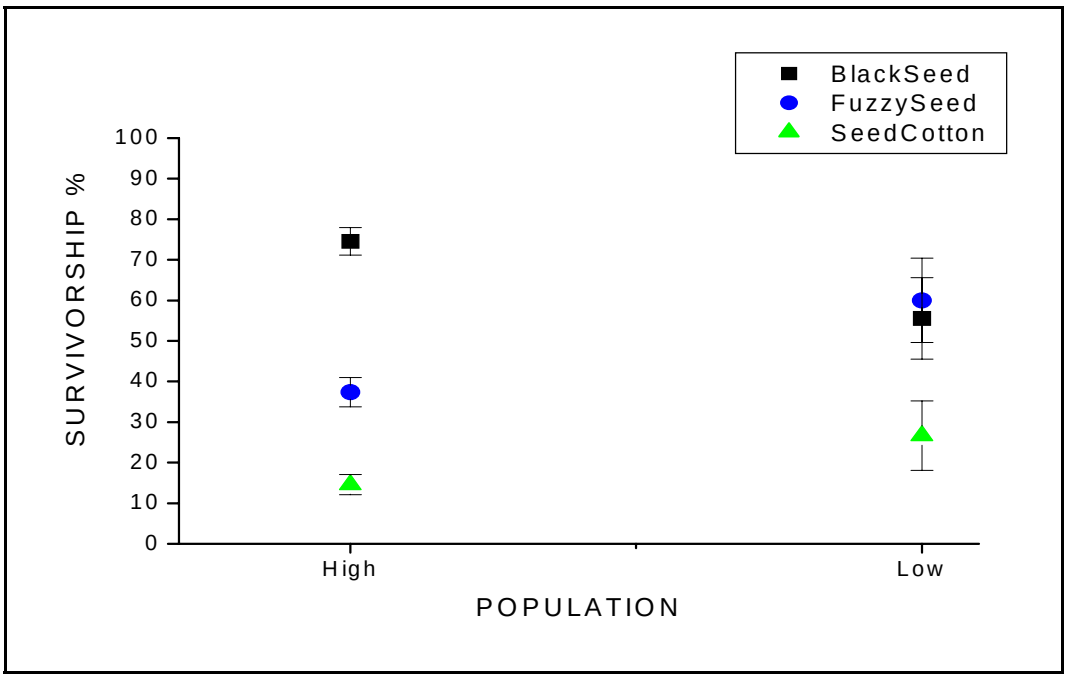


Figure A3.3. Effect of population by seedtype interaction on survivorship expressed as a percentage of seeds sown at Kununurra Drain Site No.2 wet season 2000-01 (error bars are ±s.e.)

Survivorship/Absolute

There was a significant interaction between seedtype and population (P=0.045 on log-transformed data) on survivorship as assessed by the total number of plants remaining at time of final count. Results are presented in Figure A3.4 on non-transformed data. There was a distinct seedtype effect at the high population but not at the low density treatment.

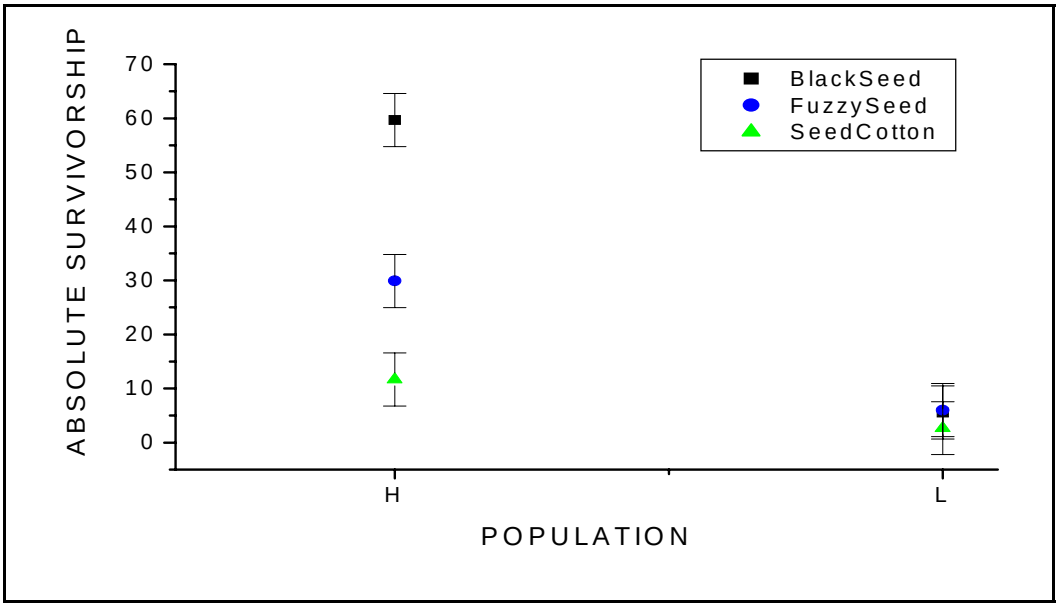


Figure A3.4. Effect of population by seedtype interaction on survivorship, expressed as absolute number of plants remaining at Kununurra WS Drain Site No.2. (error bars are ±s.e.)

FECUNDITY

Assessing fruit production over time for genotype indicated that open boll numbers were increasing at the final count, with seed available for the onset of the commencing wet season.

An ANOVA on the number of open bolls per plot produced no significant results.

There was a significant effect of both population (P=0.003) and of genotype (P=0.033) on number of open bolls produced per surviving plant (Box-Cox transformation; $z=y^{-0.144}$).

There were a greater number of bolls produced per plant from the low population treatment than the high density treatment; means for non-transformed data were 7.77 and 3.72 bolls per plant for the low and high population treatments respectively (s.e.=1.35).

The two-gene treatment produced significantly less open bolls per surviving plant than the conventional and single-gene genotypes; means for non-transformed data were 3.92, 6.64 and 6.68 for G2, G0 and G1 respectively (s.e.=1.51).

There was also a significant effect of population on the number of open bolls per seed sown (reciprocal root transformed data, P=0.002); means for non-transformed data were 2.64 and 0.28 for the low and high density treatments respectively. The population effect on the number of open bolls per surviving plant and per seed sown is illustrated in Figure A3.5 on the non-transformed data.

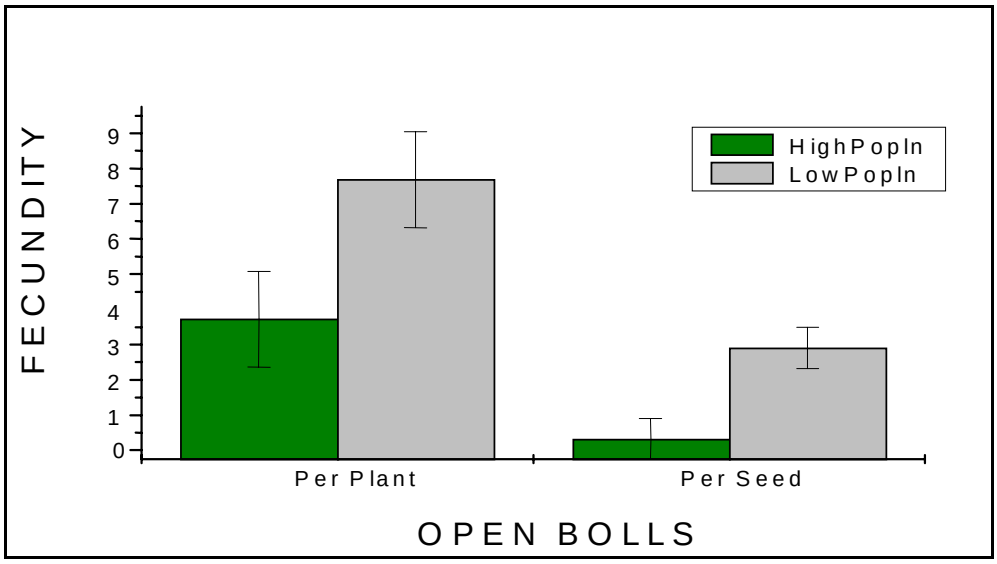


Figure A3.5. Effect of population on fecundity, expressed as mean number of open bolls at Kununurra Drain Site: wet season 2000-01 (error bars are ±s.e. and are for within the per plant or per seed analysis only)

INVASIVENESS

There was some opportunity for seedling recruitment as rain (60 mm) had fallen on the plants prior to the final counts, and lint was observed on the ground (within 1.5 m of the parent plant) at a number of plots. However, no seedlings were observed at this time. Some seed did subsequently germinate prior to all plants being removed in mid-December 2001, confirming the capacity for population recruitment at this site.

A total of 810 seeds (three seedtypes) were sown for each genotype. Germination occurred in 549, 529 and 580 seeds for G0, G1 and G2, respectively. For each genotype at the final measurement there were 342, 351 and 346 plants present. Values from calculations of invasiveness using the simplistic method were:

λ_1: G0 = 0.6230; G1 = 0.6635; G2 = 0.5966

The high numbers of plots with remaining plants allowed an ANOVA to be conducted for Inv 1 at this site. There was a significant seedtype by population interaction (P=0.032), as illustrated in Figure A3.6. Due to no additional seedling recruitment by the time of final measurement, this calculation was equivalent to number of plants surviving as a proportion of those germinated. There was an increase in Inv1 from the high to low population for fuzzy seed and seed cotton, but a decrease for black seed.

There was no difference in the invasiveness value at the low population between the three seedtypes.

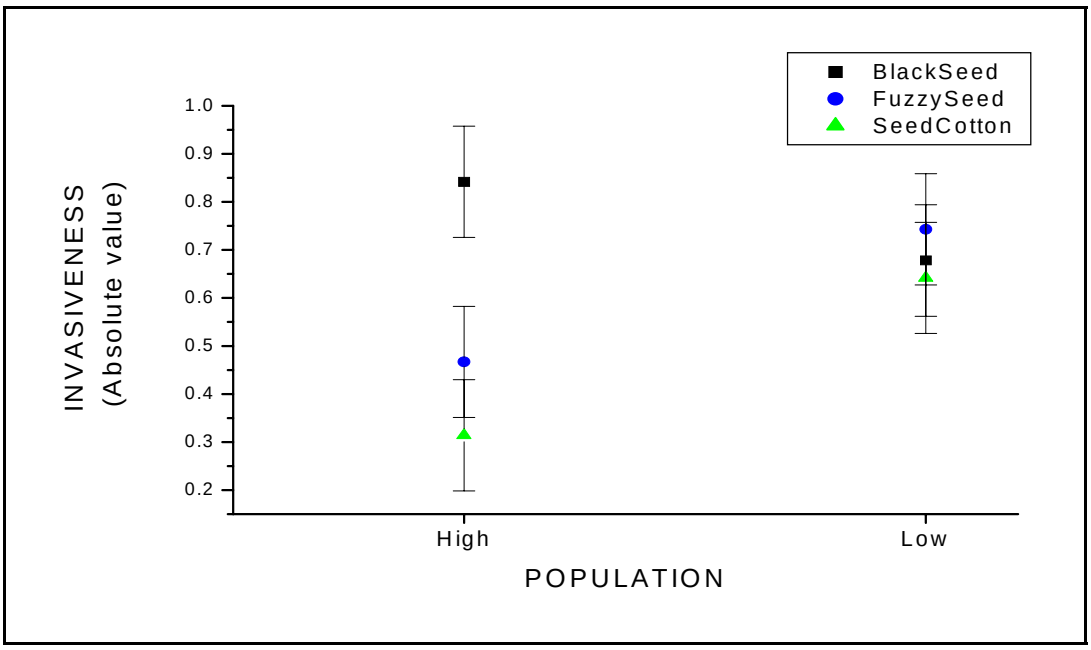


Figure A3.6. Effect of population by seedtype interaction on invasiveness (absolute value) at Kununurra Drain Site No.2: wet season 2000-01 (error bars are ±s.e.)

SITE DISCUSSION

Germination: The interaction between seedtype and population at T3 suggest that as resources become limiting, competition begins to effect plant stands by this time, and the higher density plant stands, corresponding to the black seed treatments, begin some type of 'self-thinning' process, resulting in the decline with the population treatment.

The significant effect of block and its interactions again supports the importance of microhabitat on plant demographic development (both at germination and survivorship).

Survivorship: The results from plant measurements at T3 as discussed above, indicate a casual relationship with survivorship as a proportion of seeds sown, where similar effects were obtained. The decline in plant numbers from the high to low density treatment for seedlings derived from black seed compared to the increase with fuzzy seed and seed cotton supports that competition for resources at higher plant densities may induce a greater proportion of self-thinning within the population. This was further evidenced by the results for absolute survivorship. There was a decline for all seedtypes from the high to low population treatment, with the greatest rate of decrease for the black seed, and no seedtype effect on absolute numbers of plants surviving within the low population density.

Fecundity: Large numbers of bolls were produced at this site, indicating the suitability of this habitat for cotton volunteer establishment. However, there was no evidence to support that the inclusion of Bt gene(s) conferred additional fitness to contribute to weediness.

Population as the major factor influencing number of bolls produced per plant, and per seed sown, has implications for unintended seed escape. Seed is likely to escape in clumps, with only a proportion of seed from each clump able to establish seed soil contact. The germination of a low proportion of seed from a larger number, which may escape, will produce a relatively higher number of open bolls, conducive to a higher risk of weediness. This ability of the cotton plant to compensate at low populations to produce larger boll numbers per unit area, is well-appreciated in commercial production.

Invasiveness: The lack of seedling recruitment at the final counts is likely due to the lack of substantial rain required to both knock the lint from the bracts to the ground, and to saturate the lint as required for seed imbibition. Counts of maximum boll number were deliberately done as close to the onset of the wet season as possible, but before the commencement of significant rains. This was to enable

more accurate counting of the open bolls, as once lint falls to the ground and the bracts rot from the branches, counts become more difficult. This site was revisited in December after additional wet season rains. Numerous seedlings were observed, but were not included in calculations as final measurements and data collation had occurred in accordance with project deadlines. All plants were hand-pulled and remaining lint and mature plants were removed and burnt. Ideally, recruited seedlings would have been included in calculations after the wet season for a more complete indicator of invasiveness.

The assessment of invasiveness without the opportunity to include additional seedling recruitment essentially represents survivorship as a proportion of seedlings that germinated. These results were consistent with those for survivorship as a proportion of seeds sown. The importance of population for all demographic stages has implications for weediness in that low numbers of established plants may not exhibit as greater rate of population decline as populations established with higher numbers of seedlings. These surviving low numbers of plants with less interspecific competition are then capable of compensating by producing relatively larger number of bolls.

SITE 17: KATHERINE BUSH SITE NO.2

+ NUTRITION

Date Sown : 5 January 2001

TREATMENTS

Experimental treatments for this site were modified from those applied in the preceding year. Nutrition was included as a main plot factor, with fertiliser (Thrive®) applied to half the plots at three times after sowing. Seedtype was removed as a factor, and all plots were sown to black seed. The genotype factor included an additional double gene genotype containing the Cry2Ab and Cry1Ac genes.

GERMINATION

Plant counts for germination were conducted on 25 January 2001 (T1), 27 February (T2) and 28 March (T3).

Population was significant at all three times and overall (P=0.034, P<0.001, P<0.001 and P=0.032 respectively), with the high density treatment (80 seeds) resulting in a higher germination than the low density treatment. The effect of population at each of these three times is presented in Figure A3.7.

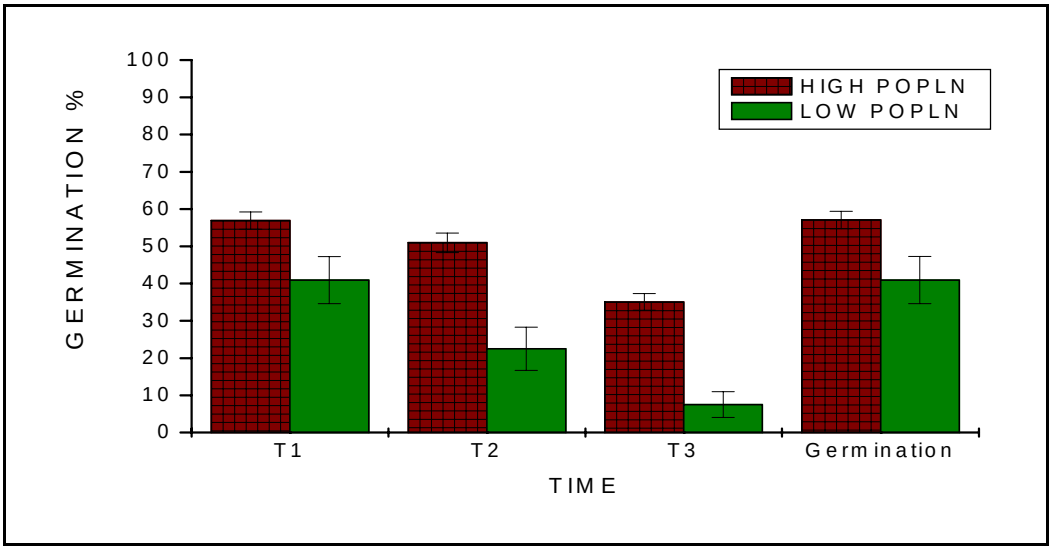


Figure A3.7. Effect of population on germination (error bars indicate ± s.e. and are for within each time measurement only)

SURVIVORSHIP 1

There were only six plots with plants remaining when the final counts were conducted on the 7 November 2001, corresponding to 1.18% plants from seeds sown. Results are presented in Table A3.2 (N0 = No nutrition applied; N1 = Fertiliser applied). Photo A3.4 illustrates the most vigourous plot at the time of final measurements (P47 – N0HG2X)

Table A3.2. No. of plants remaining at time of final measurement

No. of plants	Treatment
2	N1 H G2
1	N0 L G0
20	N0 H G2X
1	N0 L G0
2	N0 H G2X
8	N0 H G0

FECUNDITY

Only two plants produced any fruiting structures. These coincided to P30 (Nutrition applied, Low population, Single genotype) producing one square, and P40 (Nutrition applied, High population, Double gene – Cry2Ab) producing two squares and one green boll. All fruit was aborted before reaching maturity.

INVASIVENESS

No seedlings were ever produced at this site.

A total of 720 seeds (1 seedtype) were sown for each genotype. Number of seedlings present after germination equalled 421, 440, 335, and 396 for G0, G1, G2 and G2X respectively. Number of plants present for each genotype at the final measurement was 10, 0, 2, and 22.

Calculation of invasiveness using the simplistic method resulted in the following values:

Table A3.3. Invasiveness for each genotype

Genotype	Invasiveness
G0	0.0238
G1	0
G2	0.0060
G2X	0.0556

SITE DISCUSSION

It is possible that an increase in nutrition made the plants more attractive to insect attack, particularly grasshoppers, resulting in less numbers of plants surviving from the higher nutrition level treatments. This led to the development of an insect enclosure experiment conducted in conjunction with the Kununurra Bush Site No.2 (See Section 5: Genotype by Nutrition Experiments). Even with an increase in nutrition, it appeared that lack of water availability over the Dry Season contributed to the high mortality at this site. However, there also appeared to be seedling death due to fungal disease, although this was not confirmed.

Photo A3.3. Most vigourous plot at Katherine Bush Site No.2 at time of final measurements (7 November 2001)

SITE 18: KATHERINE CATTLE NO.2

Date Sown: 5 January 2001

TREATMENTS

Factors for this site were modified from the previous year's design for the cattle habitat. Population was removed as a factor. All plots were sown to 50 seeds then hand-thinned to a maximum of 10 seedlings per plot after the initial germination counts were conducted. The factor of genotype also included 289G2X (double gene containing Cry1Ac and Cry2Ab for variety Sicot 289), and DP50BX (double gene containing Cry1Ac and Cry2Ab for variety DP50), in addition to the three genotypes previously assessed.

Seedtype was again included, although seed cotton of 289G2X was not available, resulting in a total of 14 treatment combinations. This was similar to Site 17.

GERMINATION

Plant counts for germination were conducted on 25 January 2000 (T1), after which seedlings were thinned to a maximum of 10 per plot.

There was a highly significant effect of seedtype ($P<0.001$) with seedcotton having the highest germination (49.47±s.e.3.68) and fuzzy seed (29.28±s.e.3.19) and black seed (23.29±s.e.2.86) having lower germination.

There was also a significant effect of genotype ($P<0.001$) with double gene G2X producing a greater germination compared to all other genotypes, presented in Figure A3.8.

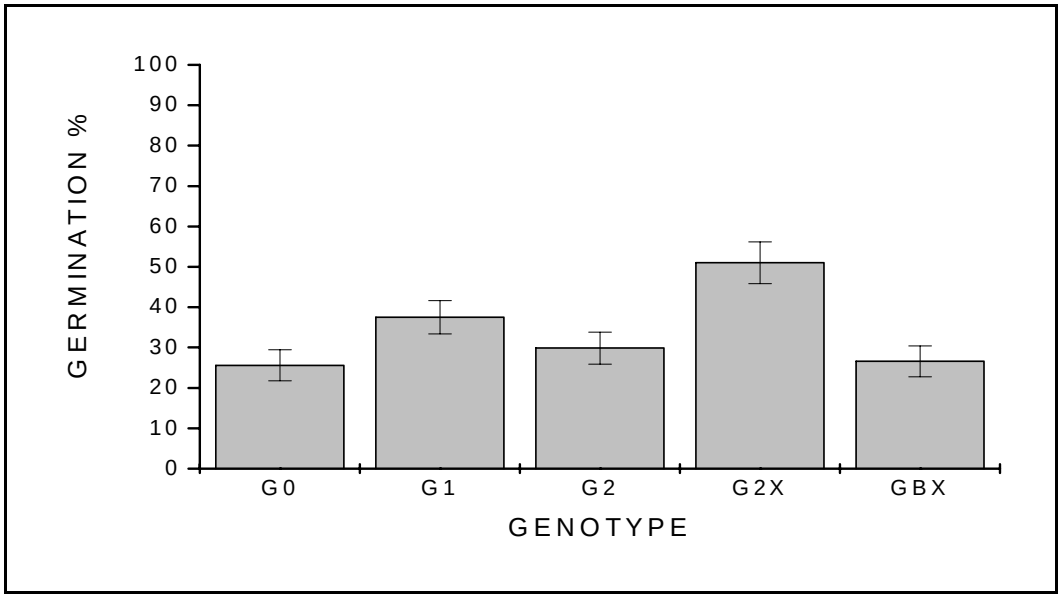


Figure A3.8. Effect of genotype on germination (error bars indicate ± s.e.)

SURVIVORSHIP 1

Final plant measurements were conducted on the 7 November 2001, at which time there were only 13 plots with surviving plants, corresponding to 8.37% of plants remaining after thinning. Plant height ranged from 11-22 cm and all plants displayed low vigour. This is illustrated in Photo A3.4 (P14 – S1G2X) at the time of last measurement date. Plots, numbers of plants and their relevant treatments are described in Table A3.4.

Table A3.4. Description of surviving plants, and their corresponding plots and treatments at Katherine Cattle No.2 site

Plot No.	No.of surviving plants	Treatment
7	5	S3 G0
8	1	S3 GBX
9	5	S3 G1
11	6	S1 G1
13	4	S1 G2
14	2	S1 G2X
24	2	S1 G0
25	3	S3 G0
37	1	S2 G1
52	2	S2 G1
53	1	S2 GBX
54	1	S2 G0
56	1	S2 G2X

FECUNDITY

No plants ever developed to producing any fruiting structures.

INVASIVNESS

No seedlings were produced.

A total of 600 seeds (three seedtypes) were sown for each genotype, except for G2X which only had 400 seeds (two seedtypes). Number of seedlings present after germination equalled 160, 232, 186, 178* and 166 and after thinning at T2 equalled 78, 90, 90, 73* and 75 for G0, G1, G2, G2X and GBX respectively. Number of plants present for each genotype at the final measurement was 11, 14, 4, 3 and 2. Values from calculations of invasiveness using the simplistic method resulted in the values in Table A3.5.

Table A3.5. Invasiveness values for each genotype

Genotype	Invasiveness
G0	0.1410
G1	0.1556
G2	0.0444
G2X*	0.0411
GBX	0.0267

* No seed cotton sown

SITE DISCUSSION

This site produced a different seedtype effect compared to all other sites (except Broome Cattle No.2) in that seedcotton had the highest germination. A high incidence of wireworm was observed in this habitat at the time of the germination counts, possibly associated with the high grass component of the paddock. It is hypothesised that the greater mortality of the black seed was due to greater predation by wireworm, and possibly other insects, compared to seedcotton which was provided some protection due to the large amount of lint surrounding the seed. The black seed had no chemical seed treatments applied as would normally be present on black planting seed.

The G2X genotype had significantly greater germination that the other genotypes. This was different to the results from the corresponding Kununurra site, where seed containing the Cry2Ab genotype had lower germination. Laboratory germination tests conducted on the black seed after small sample delinting showed GBX to be lower then the other genotypes, but there was no difference between the other four genotypes. It is hypothesised that the high germination of G2X may have been in some part,

attributed to the seed treatment (phostoxin – insect preventative quarantine requirement) imposed on this seedcotton before being transported interstate, which was then subsequently returned to WA to be utilised in this experiment, although this does not explain the contrasting result for the Kununurra site.

It is difficult to make conclusions concerning similarities and differences between the double gene treatments due to differences in parent seed history, but it is evident from observation that habitat had a greater influence on the establishment of cotton volunteers as compared to genotype.

Photo A3.4. Surviving plants at Katherine Cattle Site No.2

SITE 19: BROOME BUSH SITE NO.2

Date Sown: 18 January 2001

GERMINATION

Plant counts for germination were conducted on 27 January 2001 (T1), 15 February (T2) and 3 March (T3).

There was a significant interaction between seedtype and population (P=0.026), with a slight increase in germination between the high and low population density treatments for the black seed and seedcotton, but the reverse trend for fuzzy seed, as illustrated in Figure A3.9.

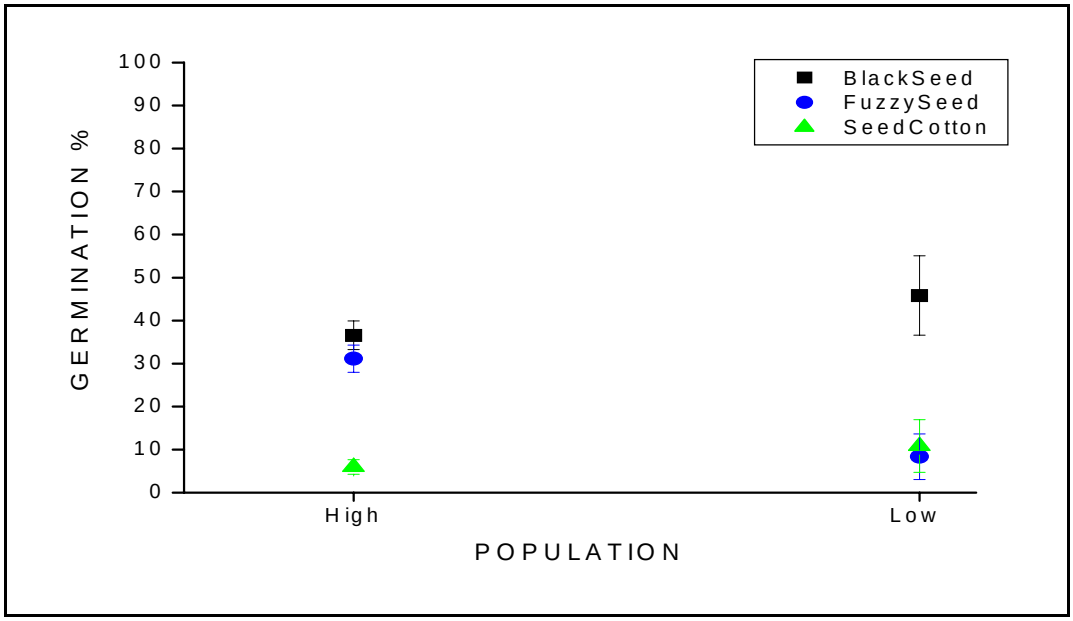


Figure A3.9. Effect of population and seedtype interaction on germination at Broome Bush Site No.2. (error bars are ± standard error)

There was also a significant effect of genotype (P=0.04), with the conventional genotype producing a lower germination than the two transgenic treatments, illustrated in Figure A3.10.

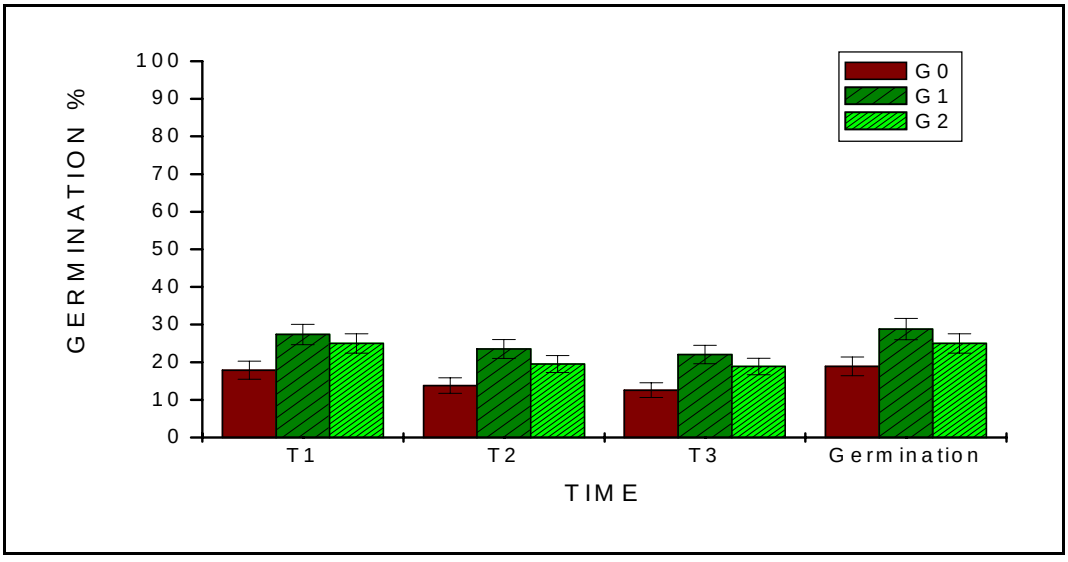


Figure A3.10. Effect of genotype on germination over time for Broome Bush Site No.2 (error bars indicate ±1s.e. for within each time only)

SURVIVORSHIP 1

No plants were surviving after the dry season.

FECUNDITY

Plants at this site produced no reproductive structures.

INVASIVENESS

No seedlings were ever produced at this site.

A total of 1,080 seeds (three seedtypes) were sown for each genotype. Number of seedlings that germinated were 204, 311 and 270 for G0, G1 and G2 respectively. Number of plants present for each genotype at the final measurement was 0, 0 and 0, so invasiveness for each genotype was zero.

SITE DISCUSSION

This site was destroyed by fire in mid-November 2001. There had been surviving plants at the prior count (T9; 10 September), with maximum plant height across the plots of 14 cm, and damage ratings extreme. Observations during October indicated that the plants had died. Ideally, a rain event to stimulate reshooting of any viable plants would have confirmed whether the plants were dead.

The significant effect of genotype may be attributable to seed storage conditions or parent seed source factors. Germination tests had been conducted on the black seed prior to planting to ensure that the process of acid-delinting did not damage the seed. The process of delinting would have screened out unviable black seed. However, fuzzy seed and/or seed cotton which was not viable was not subject to any germination screening. A likely reason for less viable conventional seed was that this seed was sourced from an unsprayed paddock containing each of the three genotypes. Indications from other experiments suggest that the conventional seed is exposed to greater insect damage compared to the transgenic seed, thus reducing its subsequent viability. This was an experimental oversight, but has inadvertently provided another factor to be possibly considered in the issue of weediness – the insect management strategies used in the parent conventional crop. If the crop remains unsprayed, as may be required as a refuge for resistance management strategies, then this seed may have lower fitness compared to the transgenic seed. This may not be the instance if the conventional crop is managed for optimum yield (i.e. is sprayed when insect pressure warrants it necessary).

SITE 20: BROOME CATTLE YARD NO. 2

Date Sown: 18 January 2001

TREATMENTS

This experiment was modified from the design used in the previous season. Population was excluded as a factor. All plots were sown to 50 seeds, then hand-thinned to a maximum of 10 seedlings per plot after the germination counts were conducted on 27 January. The factors of seedtype (three levels) and genotype (three levels) remained as for the previous season.

GERMINATION

There was no significant effect of any factor on germination at this site (seedtype; P=.096). Of note was that black seed did have the lowest average germination (6.2 %), followed by fuzzy seed (12.8%) and seedcotton (15.3%) which is in contrast to the trend in the majority of sites.

SURVIVORSHIP 1

Final measurements were conducted on 20 November 2001 (T11) with survivorship evaluated as a proportion of plants remaining to those present after thinning (T2). This corresponded to 81.7% plants remaining as a proportion of the thinned population. There was no significant effect of any factor.

FECUNDITY

Fruit production over time was plotted to determine critical cotton development stages for specific analysis. An unusual rainfall event (90 mm) occurred in mid-July resulting in an atypical vegetative spurt and associated square production. It is rationalised that as soil moisture then declined, squares and young green bolls aborted, and larger bolls nearing maturity opened. Ideally, maximum fruit production would be measured as coinciding to the end of the dry season, start of the wet season, which would be in December – January, but time constraints for completion of the project had to be managed.

An ANOVA was conducted on maximum number of open bolls produced per plot and also per plant (number of bolls equalled zero if there were no surviving plants). There was no significant effect of any factor on either parameter.

INVASIVENESS

A total of 600 seeds (three seedtypes) were sown for each genotype. Number of germinated seeds equalled 85, 65 and 56, and after thinning at T2 equalled 50, 53 and 39 for G0, G1 and G2 respectively. Number of plants present for each genotype at the final measurement was 42, 46 and 28. Values from calculations of invasiveness using the simplistic method were:

G0 = 0.840; G1 = 0.868; G2 = 0.718

Data analysis by ANOVA showed no significant effects of any factor.

SITE DISCUSSION

This site was unusual in that there were no factors that significantly effected germination, particularly considering the significance of seedtype at the majority of other sites.

It was raining as we were planting. This may have made differences in the wetting/drying cycles and imbibition of less importance between the three seed types. This raises implications for weediness with respect to time of the initial germination event, and frequency and intensity of follow-up rains for initial survival of newly germinated seedlings.

Also, black seed for this sowing was not treated with any of the commercially used seed treatments, as compared to last season, and this may have contributed to the higher black seed mortality compared to the other two seed types.

There was considerable variability observed in fruit production between plots at this site. No significant effects of any factor on survivorship or fruit production may be due to no effect of seedtype on germination, and no population factor, which minimised the complexities of interactions. The initial period of this project allowed for identification of numerous factors that influenced cotton germination, growth and development. Further small scale targeted manipulated experiments examining only isolated factors would allow for greater confidence in drawing conclusions concerning genotype effect without the interactions of other factors.

Calculation of invasiveness at the final measurement on 20 November does not allow for any addition to the population via seedling recruitment as no rain had yet fallen on the site. However, it still enables attainment of a value of overall population change after seedling establishment (numbers at thinning) for each genotype.

This site was also damaged by fire in early November 2001, just prior to the final counts, shown in Photo A3.5. New growth, including squares, had been burnt off, although the green and open bolls were still remaining. It was not determined whether these plants would survive. This may be influenced by length of time until the next rainfall event occurred.

Photo A3.5. Fire caused damage to surviving plants at Broome Cattle Site No.2 (21 November 2001)

APPENDIX 4 FEEDING STUDY WITH FUZZY COTTON SEED

AGRICULTURE WESTERN AUSTRALIA RESEARCH INFORMATION SYSTEM

RESEARCH ACTIVITY DOCUMENT ENTRY SHEET

Title: THE PASSAGE OF FLUFFY WHOLE COTTONSEED IN CATTLE FED HAY OF VARYING QUALITY
Project#: MCE
Activity#: 97KU8
Start-Date: October 1997
Finish-Date: October 1998
Personnel: Bolam MJ, Hadden D
Location: Frank Wise Institute of Tropical Agriculture, Kununurra
Data-Reference: File 7725 EX
Map-Reference: 468507, 8269473, Zone 52. Datum WGS84

SITE DETAILS

Trial undertaken in cattle yards.

BACKGROUND

The developing cotton industry in the Ord River Irrigation Area (ORIA) offers cattle producers an excellent opportunity to obtain a high quality cattle feed without the severe penalty of freight cost from the southern or eastern states. This feed is fluffy whole cottonseed (WCS), a byproduct of the cotton ginning process, and it is high energy and protein for ruminants.

However, the crops grown in the ORIA are experimental and the plants are genetically modified to give the cotton plant resistance to insect pests. As a part of the protocol for the conduct of these trials by Agriculture WA and CSIRO, there is a requirement to destroy the seed produced from the ginning process.

The experiment was a part of the combined efforts of the potential users of WCS to establish a protocol to allow beef and dairy producers in the area to access the benefit of WCS and meet the requirements for destruction of the seed. (see Appendix 1 - The use of Ingard whole cottonseed for cattle feed in the Kimberley)

AIM

To establish the amount of whole cottonseed passed in the faeces of cattle fed three differing qualities of hay

To establish the viability of whole cottonseed passed in the faeces

TREATMENT

Feeding Period A
Two groups of four steers each (average liveweight of 200 kg) were fed a basal diet of either low quality sorghum stubble or medium quality Pangola grass hay. The groups were supplemented with fluffy whole cottonseed recently ginned in Kununurra. Hay was fed at 2% of liveweight per day and WCS was fed on an *ad lib* basis for nine days.

Feeding Period B
Four groups of six cows each and one group of five cows (average liveweight of 380 kg) were fed low quality sorghum stubble and supplemented with whole cottonseed. The hay and the WCS were fed ad lib for 18 days.

MEASUREMENT

Feeding Period A
After introductory feeding period of 21 days, samples of faeces were collected from the yards and assessed for their WCS content each day for 12 days. Faecal samples were washed in a fine seive and seeds collected for counting, weighing and germination testing. Two grab samples of cottonseed as fed, were also germinated using this procedure.

Feeding Period B
On Days 11-16, faecal material was collected morning and evening from the yards. Faecal samples were washed in a fine seive and seeds collected for counting, weighing and germination testing. Two grab samples of cottonseed as fed, were also germinated using this procedure.

RESULTS

Feeding Period A
A total of 70 kg of cottonseed was consumed by the eight steers over the collection period (750 g/hd/day; 0.4% initial liveweight) and a total of 62 seeds were detected in faecal samples. Of these 62 seeds, two seeds germinated in the standard germination test undertaken. The number of seeds/kg of cottonseed were counted, and the mean value of three samples was 11,000 seeds/kg. Therefore, an estimated 770,000 seeds were fed, and only two viable seeds were detected in faeces. It was impossible to make total collections of faeces, however between 50 and 80% of faeces present in the yards each morning were estimated to be collected. The germination percentage of the WCS as fed was 72%. There was no significant difference in the amount of seed passed between the two groups of steers. These steers were slow to adapt to eating fluffy whole cottonseed.

Feeding Period B
The 29 cows consumed 289 kg of cottonseed over the collection period (2 kg/hd/day; 0.5% initial liveweight) and a total of 302 g of seed (2,807 seeds) was recovered from 1,207 kg of faeces (wet weight). Germination testing was undertaken on 500 of the recovered seeds (five separate germ tests of 100 seeds each) and 41% of seeds tested germinated. The germination rate of the seed as fed was 81%. It is estimated that 80% of the faeces passed was collected and assessed for seed passage.

CONCLUSIONS

A preliminary conclusion was made after the first feeding period, that the passage of viable cottonseed in young steers, under these experimental conditions was negligible. The percentage of seed fed that was passed and subsequently germinated was estimated at 0.0003%. As a worst case scenario, if only 50% of faeces passed was collected, this would indicate a viable passage rate of 0.0006%. However, it was considered that this work should be repeated to support such a conclusion, particularly due to the small number of passed seeds available for germination testing.

The second feeding period allowed a greater number of seeds to be germinated. In this case, the percentage of seed fed that was passed and subsequently germinated was estimated at 0.04%. This is a factor in the order of 100 times greater than the preliminary trial suggested. The different class of cattle fed in the second period is likely to have contribute to this result, but I do not believe that this explains a difference of this magnitude.

This trial has demonstrated the variability that may be expected in the passage of viable whole cottonseed when fed to cattle consuming poor and medium quality forages.

RELATED ACTIVITIES

97KU4, 97KU7, 99KU3

PUBLICATIONS

Nil

FUNDING SOURCE

Meat Program, Agriculture Western Australia

APPENDIX 5 WEED RISK ASSESSMENT FORMAT

Pre-entry weed risk assessment

				Outcome:	More Information
				Score:	0
					Gossypium hirsutum
	A.	**Biogeography/ historical**			Upland Cotton
C	**1**	*Domestication/ cultivation*	1.01	Is the species highly domesticated?	
C			1.02	Has the species become naturalised where grown?	
C			1.03	Does the species have weedy races?	
-	**2**	*Climate and Distribution*	2.01	Species suited to Australian climates (0-low; 1-intermediate; 2-high)	
-			2.02	Quality of climate match data (0-low; 1-intermediate; 2-high)	
C			2.03	Broad climate suitability (environmental versatility)	
C			2.04	Native or naturalised in regions with extended dry periods	
-			2.05	Does the species have a history of repeated introductions outside its natural range?	
C	**3**	*Weed Elsewhere (interacts with 2.01 to give a weighted score)*	3.01	Naturalised beyond native range	
N			3.02	Garden/amenity/disturbance weed	
A			3.03	Weed of agriculture	
E			3.04	Environmental weed	
C			3.05	Congeneric weed	
-	**B.**	**Biology/Ecology**			
C	**4**	*Undesirable traits*	4.01	Produces spines, thorns or burrs	
C			4.02	Allelopathic	
C			4.03	Parasitic	
A			4.04	Unpalatable to grazing animals	
C			4.05	Toxic to animals	
C			4.06	Host for recognised pests and pathogens	
N			4.07	Causes allergies or is otherwise toxic to humans	
E			4.08	Creates a fire hazard in natural ecosystems	
E			4.09	Is a shade tolerant plant at some stage of its life cycle	
E			4.10	Grows on infertile soils	
E			4.11	Climbing or smothering growth habit	
C			4.12	Forms dense thickets	
E	**5**	*Plant type*	5.01	Aquatic	
C			5.02	Grass	
E			5.03	Nitrogen fixing woody plant	
C			5.04	Geophyte	
C	**6**	*Reproduction*	6.01	Evidence of substantial reproductive failure in native habitat	
C			6.02	Produces viable seed.	
A			6.03	Hybridises naturally	
C			6.04	Self-compatible or apomictic	
C			6.05	Requires specialist pollinators	
A			6.06	Reproduction by vegetative fragmentation	
C			6.07	Minimum generative time (years)	
A	**7**	*Dispersal mechanisms*	7.01	Propagules likely to be dispersed unintentionally (plants growing in heavily trafficked areas)	
C			7.02	Propagules dispersed intentionally by people	
A			7.03	Propagules likely to disperse as a produce contaminant	
C			7.04	Propagules adapted to wind dispersal	
E			7.05	Propagules water dispersed	
E			7.06	Propagules bird dispersed	
C			7.07	Propagules dispersed by other animals (externally)	
C			7.08	Propagules survive passage through the gut	
C	**8**	*Persistance attributes*	8.01	Prolific seed production (>2000/m2)	
C			8.02	Evidence that a persistent propagule bank is formed (>1 yr)	
A			8.03	Well controlled by herbicides	
A			8.04	Tolerates, or benefits from, mutilation or cultivation	
C			8.05	Effective natural enemies present in Australia	
				Outcome:	More Information
				Score:	0
	Statistical summary of scoring			Biogeography	0
			Score partition:	Undesirable attributes	0
				Biology/ecology	0
				Biogeography	0
			Questions answered:	Undesirable attributes	0
				Biology/ecology	0
				Total	0
				Agricultural	0
			Sector affected:	Environmental	0
				Nusiance	0

A= agricultural, E = environmental, N = nuisance, C=combined

Taken from Pheloung (1995), and referred to in text as being used to conduct a WRA of *G. hirsutum* by Randall (1997)

APPENDIX 6 FERAL COTTON REPORT

Graham Schultz

THE INCIDENCE OF FERAL COTTON (*Gossypium hirsutum*) IN THE NORTHERN TERRITORY

INTRODUCTION

The production of *Gossypium hirsutum* (Upland cotton) has had a chequered development in the Northern Territory. Cotton was sown by Holtze at the Botanical Gardens in Darwin and other identified sites in the 1890s. Commercial production did not develop from this, even though a small gin was built in Darwin in the 1920s. Cotton was the main crop grown on the Ord Irrigation Area (OIA) of Western Australia between 1963 and 1974 with a maximum area of 3,861 ha in 1966. The CSIRO Research Station in Katherine started cotton trials in 1947.

The development of transgenic cotton containing the active constituent *Bacillus thuringiensis* var *kurstaki* (Bt) delta endotoxin has led to renewed interest in cotton for the Northern Territory. The National Registration Authority (NRA) on 30 January 1998 warned that to extend the registration of Bt cotton outside its current commercial use in NSW, Queensland south of latitude 22^{o}S and east of 140^{o}E will require information on feral cottons. They have identified a need to "assess the potential for emergence of insect resistance to the Cry1A (c) toxin, potential for feral *G. hirsutum* to become a weed as a result of possession of the cry(c) transgene, and the potential for cry1A(c) transgene transfer to native *Gossypium* species".

The objective of this project was to review the incidence of feral cotton in the Northern Territory, collect seed and assess the next generation of these plants for their suitability in breeding programs.

ORIGIN AND DISTRIBUTION

Gossypium L. is a Malvaceae and includes about 30 spp. of annual subshrubs, perennial shrubs or small trees distributed in the tropical and subtropical regions of Africa, Asia, Australia and America. Hutchson, Silow and Stephens (1947) reported by Purseglove (1968) as having considered the genus from the evolutionary standpoint and found 20 spp. in eight sections. They recognised four spp. in cultivation, the diploid Old World cottons *G. arboreum* and *G. herbaceum* and the tetraploid New World cottons of *G. barbadense* and *G. hirsutum*. They state that crosses between sections are difficult to make and in some cases impossible. The F1 hybrids are usually sterile. Crosses within sections are possible but individual species tend to retain their identity in consecutive generations.

The origin of the species of *Gossypium* has had considerable study and controversy. The wild lintless diploid species occurs in arid regions of Africa, Asia, Australia and America. It is believed that they are African in origin and arrived in Australia by Wegener's theory of continental drift. These include the section *G. sturtiana* and the species *G. robinsonii* F. Muell. collected from Western Australia and *G. australe* F. Muell. which has been collected widely in the Northern Territory including around Katherine.

The Old World linted cottons include *G. herbaceum* L. and *G. arboreum* L. both of which were introduced by Holtze into the Northern Territory in the 1800s. The New World linted cottons are believed to originate in tropical America although some authors believe India is their centre of origin. The first is *G. barbadense* L. (syn. *G. peruvianum* Cav.) from tropical South America and northern Peru. The annual habitat was established in seed from the West Indies introduced into South Carolina in 1786 and gave rise to "Sea Island' cotton. Holtze also introduced this species. *G. hirsutum* L. is believed to have originated in Central America from Guatemala and northern Brazil.

Purseglove (1968) indicates that *G. barbadense* spread in post-columbian times from eastern South America and the Caribbean to West Africa giving rise to Isham cotton in Nigeria and then along the trade and slave routes to the Sudan and Egypt. Jumel in 1820 established the Egyptian cotton on these perennials and in 1850 they were crossed with "sea Island" cotton to produce the very fine "Pima" cottons. In the early 19th century *G. hirsutum* was introduced and crossed in the United States. They were grown in the uplands for "homespun" to distinguish them from the black-seeded Sea Island cottons of the coast. With the invention of the saw gin by Whitney in 1793 and the rise of the Lancashire cotton industry, upland cotton expanded. The American civil war (1861-65) threatened this

supply so that cotton was introduced into most tropical and subtropical countries of the world including Australia.

SPREAD IN THE NORTHERN TERRITORY

Cotton was considered as a suitable crop in the early days of settlement in the Northern Territory. Its growth was first recorded in 1882 at the now Darwin Botanical Gardens. A list of plants in the gardens in 1887 included *G. arboreum* (Egyptian), *G. herbaceum* (Sea Island), *G. hirsutum* (Upland) and *G. religiosum* (Peruvian). A report to the Administrator by Holtze in 1888 stated "Cotton only requires cheap labour to make its cultivation here pecuniarily a success". In 1895 Holtze received a consignment of about 12 varieties of cotton and in his report for that year stated " Cotton is one of the few plants that have escaped from cultivation about Palmerston". A report from a sugar, cotton expert identified cotton as growing on the Alligator River during this time even though access to that area by land was almost impossible. A Mr Jaensch, an electrical telegraph officer from Powell's Creek reported cotton being grown as a weed due to seed being blown from Holtze's plots in the gardens at Powell's Creek. Captain J Bradshaw planted cotton on the Goyder and Victoria Rivers and reported that "On old Delamere Station, some plants set by Mr Giles, the explorer, in 1878 have so multiplied that they cover several hundred acres of country and from the surrounding hills present a snow white appearance over a vast area".

In 1886 cotton, coffee and Indian rubber were sown at a Beatrice Hills Plantation. A customs station was established on the Bowen Strait near Cobourg Peninsula in the 1890s to collect taxes from Macassan trepang boats. Reports indicate that they often paid in kind by supplying rice and tobacco. Cotton was observed in the area in 1998.

Until 1920, cotton growing was mainly restricted to small experimental plots at the Botanic Gardens but small crops were grown elsewhere. A government guarantee of 5.5d. per lb. and assisted freight charges were introduced in 1922.

Mataranka Experimental Station recorded a yield of 1,300 lb seed cotton per acre in 1923 under a 35 inch rainfall. Other crops grown yielded from 215 lb at Stapleton to 1904 lb on the Roper River. A small ginnery was established at Darwin in 1924. This encouraged production and in 1925 crops totalling 7,000 lb seed cotton were grown at Stapleton, Grove Hill, Daly River, Pine Creek, Mataranka, Borroloola and the lower reaches of the Roper River. A cotton pool was established in Darwin in 1926 but the ginnery could not handle the production. In 1940 three farmers planted 175 acres at Katherine. No yield information was found. CSIRO Research Station at Katherine started cotton experimental plots in 1947. Yields ranged from very disappointing to 2,207 lb of seed cotton per acre.

In 1998 the Parks and Wildlife Commission of the NT Herbarium Database had records of the following Gossypiums. Not all are from the NT and how many are linted types is still to be determined.

1.	*Gossypium nelsonii*	Fryxell.
2.	*Gossypium nobile*	Fryxell, Craven and J.M. Stewart.
3.	*Gossypium pilosum*	Fryxell.
4.	*Gossypium populifolium*	(Benth), F.Muell.
5.	*Gossypium robinsonii*	F.Muell.
6.	*Gossypium hirsutum*	L.
7.	*Gossypium marchantii*	Fryxell, Craven and J.M. Stewart.
8.	*Gossypium bickii*	Prokh.
9.	*Gossypium costulatum*	Tod.
10.	*Gossypium cunninghamii*	Tod.
11.	*Gossypium enthyle*	Fryxell, Craven and J.M. Stewart.
12.	*Gossypium exiguum*	Fryxell, Craven and J.M. Stewart.
13.	*Gossypium hirsutum*	var hirsutum.
14.	*Gossypium australe*	F.Muell.

CURRENT DISTRIBUTION OF *G. HIRSUTUM*

Since its introduction to the NT in the 1880s cotton has managed to spread widely and establish where environmental conditions have proved suitable. The main requirement would appear to be areas of suitable soils, moisture and limited bush fires. Due to the wide distribution of feral cotton any

area selected for development will require a careful inspection. Most sites where cotton has persisted are either coastal areas behind beaches or on the banks of permanent water courses. Cotton was identified from 57 sites in the P&W data base. Some duplication probably occurs but more plants are being reported as interest in this species develops. Five areas were selected for further study. These were from Borroloola on the Gulf, Elsey and Larrimah, Cobourg Peninsula in Arnhem Land and around Darwin.

Borroloola was selected as it was a site of cotton development in the 1920s and is isolated from other areas. Cobourg was the site of early British settlement and Macassan trading. Darwin is the site nearest the Botanical Gardens.

Three plants were found at Larrimah but no seed. Elsey Station at Mataranka was the site of early cotton production and cotton is known to grow along the banks of the Roper River.

In the Borroloola area a cotton plant has been growing in a house yard at the local store since the 1940s. It finally got so big that it pulled down the fence and was cut off in 1997. Seed was collected off the fence where this plant once stood. Cotton is widespread in the area. One explanation for this is that the local Aboriginal people used cotton lint originally from the town bush for decoration in corroborees. Therefore cotton can be found widely where conditions of sandy soils and water exist near corroboree sites. It was reported to be on the gulf islands from this practice.

Although the site of Gurig National Park is still relatively isolated, cotton has been growing in the area for many years and could be from the old Victoria Settlement. I could not find cotton at the actual settlement site as it is being maintained by P&W rangers who remove any weeds from the area. Cotton does grow at the Black Point Ranger Station and was collected from two other sites. One on Trepang bay is difficult to reach except by boat or aircraft and the other on Bowen Strait. At both these sites it would appear that the amount of cotton is increasing. This is most likely due to the lack of fires. It is not likely to spread beyond these protected areas.

Cotton grows at several sites around Darwin. Two sites were selected. The first at Rapid Creek and the second at Beatrice Hill.

Many other sites could have plants that may be suitable for investigation. The most important would be the Gove Peninsula and Cape Hotham at the mouth of the Adelaide River. Both these areas are isolated so that any cotton plants would have been there for a long period of time. Cape Hotham is the site of Escape Cliff a British settlement. These sites will be inspected after the next wet season. The original sites will be reinspected before the 1998-99 wet to see if the collected plants are actually perennials or reproducing each year from seed.

Results

A. A field trip was undertaken to the Borroloola area between the 11-15 May 1998. At Larrimah only three plants could be found near the old railway area. They were very small under 30 cm high and in long grass. I would not expect them to survive another season. No seed could be collected but a botanical specimen was taken. This appears to be about the correct time to collect cotton from feral cotton plants in the Borroloola area. They had dropped an early crop of seed and lint but were carrying plenty of lint and had some bolls that may develop if moisture continues to be available. Cotton is widespread in the gulf area and many locals could state where they had seen the plant. It was here that I was told of the Aboriginal custom of using cotton lint for corroborees. At Manangoora Station, cotton was collected from such a site where it was growing under a group of trees near a billabong.

Elsey Station is owned by Banisi Pty Ltd, an Aboriginal community. They are very interested in diversifying their agricultural enterprises. A follow up inspection of soil types indicated although they have considerable available water and black soil areas, the soil is not a self mulching type. Self mulching types are there but occur closer to the river so that the manager is not keen to develop them for crop production. Feral cotton has spread along the banks of the Roper River. Large numbers of cotton seed bugs were on plants in the area.

Table A6.1. Sites selected for collection of *G. hirsutum* in the Gulf Region

Date Originally Collected	Latitude	Longitude	Local Name	Date Collected	OBSERVATIONS	
					Number of Plants	Comments
29/04/93	14.57.394 S	133.19.905 E	Elsey	14/5/98	4	Behind buildings on river bank
19/05/85	15.34.686 S	133.12.894 E	Larrimah	12/5/98	3	In drain no seed available
20/06/76	16.84.393 S	136.18.414 E	Borroloola Mini Mart	14/5/98	1	Seed on fence
2/05/77	16.03.279 S	135.48.595 E	Borroloola	13/5/98	10	M. Baker place on river bank
13/08/91	16.02.738 S	136.50.457 E	Manangoora	13/5/98	20	Near billabong on road

Table A6.2. Climatic data for selected *G. hirsutum* sites

Borroloola	Lat 16.4 S Lon 136.18 E	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**	**Yrs of Rec**
Mean Daily Max Temp (ºC)		35.5	34.5	33.7	33.8	31.6	29.6	29.6	31.9	34.1	36.5	37.5	36.7	33.8	17
Mean Daily Min Temp (ºC)		24.3	24	22.9	20.1	15.8	12.6	11.8	14	16.3	20.2	23.2	24.1	19.1	17
Mean Mth Rainfall (mm)		190	193	163	45	11	9	1	1	3	12	44	118	790	81
Mean No Rain Days		12	11	10	3	1	1	0	0	0	1	4	8	51	81
Larrimah	Lat15.35 S Lon133.13 E	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**	**Yrs of Rec**
Mean Daily Max Temp (ºC)		35.5	34.3	33.7	33.8	31.4	29.2	29	32.1	34.7	37	37.7	36.9	33.8	21
Mean Daily Min Temp (ºC)		24	23.6	22.5	19.6	16.2	12.8	12	14.7	17.9	21.6	24.1	24.3	19.4	21
Mean Mth Rainfall (mm)		201	191	154	33	14	5	4	*	5	27	65	113	812	33
Mean No Rain Days		15	15	11	3	1	0	1	0	1	3	7	10	67	33
Elsey	Lat 14.57 S Lon133.19 E	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**	**Yrs of Rec**
Mean Mth Rainfall (mm)		211.5	190.3	137.4	37.7	10.9	4.2	4.1	0.5	1.4	22.1	61.1	150.5	773.9	
Highest Monthly Rainfall (mm)		468.9	366.6	450.4	534.9	108.7	88.6	91.6	8	15.6	251.8	354	460.8	1346	
Lowest Monthly Rainfall (mm)		32	26	0	0	0	0	0	0	0	0	0	14.1	301.9	

B. The second field trip was to the Cobourg Peninsula in North West Arnhem Land. This area was the site of Victoria Settlement and a point of contact for Macassan prau searching for trepang along the Australian coast. During the period of British settlement they introduced a wide range of plant and animal species into the area. These included water buffaloes, banteng, samba deer and prickly pear. Cotton has been recorded from six sites on the peninsula and one further inland at Murgenella. No cotton was found at the Victoria Settlement site. The Parks and Wildlife Commission staff who manage Gurig National Park have been developing the original site so that any introduced plants were removed. Cotton was harvested at Black Point ranger station despite the rangers carrying out control operations. Two other sites were also identified and samples collected. The first was at Trepang Bay on the western side of Cobourg. This site is isolated along a beach front and appears not to have been burnt for many years. I would estimate that the cotton is slowly spreading along the beach. The second site was on the Bowen Strait and here also cotton would appear to be spreading. Although a helicopter was necessary to find the sites both could be reached by ground or boat now that an accurate GPS reading has been recorded.

Although insect damage was difficult to identify large numbers of the male cotton harlequin bugs were found at the Bowen Strait site.

Table A6.3. Sites selected for collection of *G. hirsutum* on the Cobourg Peninsula

Date Originally Collected	Latitude	Longitude	Local Name	Date Collected	OBSERVATIONS	
					Number of Plants	Comments
29/03/88	11.23.18 S	132.35.14 E	Bowen Strait	21/5/98	200	Behind beach front
20/4/93	11.09.281 S	132.08.606 E	Black Point Ranger Stn	19/5/98	50	In gardens at Ranger Stn
20/5/98	11.13.16 S	131.54.18 E	Trepang Bay	21/5/98	50	Behind Beach Front

Table A6.4. Climatic data for nearest site to Cobourg Peninsula

Cape Don Lat 11.19 S Lon 131.46 E	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**	**Yrs of Rec**
Mean Daily Max Temp (ºC)	31.2	30.8	30.8	31.4	30.4	28.7	28	28.8	30.2	31.5	32.3	32.1	30.5	29
Mean Daily Min Temp (ºC)	25.3	25.2	25	25.1	24.1	22.5	21.5	22.1	23.3	24.7	26	26.1	24.2	29
Mean No Rain Days	18	17	18	10	3	1	1	0	1	3	8	14	94	69

C. Two sites were selected from the Darwin area. The first was Beatrice Hill on the Adelaide River. This was the same area used in 1886 for cotton coffee and Indian rubber. Cotton grows in the area along Beatrice lagoon and Arnhem highway in a band of native *Cathormiun umbellatum*. There is no way of knowing if this cotton has survived from the original material planted 112 years ago. It is possible cotton seed meal was used as a stock feed during that time on the adjacent Coastal Plains Research Station. Cotton does not exist at the botanical gardens where Holtze planted it but was collected at Rapid Creek.

Table A6.5. Sites selected for collection in the Darwin area

Date Originally Collected	Latitude	Longitude	Local Name	Date Collected	OBSERVATIONS	
					Number of Plants	Comments
08/07/83	12.39.511 S	131.19.858 E	Rapid Creek	21/5/98	200	At Bridge on Trower Road
5/06/98	12.39.507 S	131.19.860 E	Beatrice Hill	5/06/98	50	Along Arnhem Hwy

Table A6.6. Selected climatological data for Darwin Airport. Lat 12.25 Lon 130.52

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual	Yrs of Rec
Mean Daily Max Temp (ºC)	31.7	31.3	31.8	32.6	31.9	30.4	30.3	31.2	32.4	33	33.1	32.6	31.9	45
Mean Daily Min Temp (ºC)	24.7	24.6	24.4	23.9	22	19.9	19.2	20.6	23	24.9	25.2	25.2	23.1	45
Mean Mth Rainfall (mm)	409	353	316	99	17	2	1	6	18	72	142	224	1659	46
Mean No Rain Days	21	20	19	9	2	0	1	1	2	6	12	16	109	46

CONCLUSION

The production of commercial cotton has had a chequered development in the Northern Territory. This tropical plant was recognised as having potential in the late 1800s but has joined a range of other plants that started with such promise and then ceased commercial production. The large numbers of sites of feral cotton would indicate that it has the capacity to survive under a range of NT wet seasons.

The wet season around Larrimah appears to be the minimum required for survival. At an average of 812 mm over 67 rain days it is difficult for cotton to survive. The niche of a table drain has allowed it to just maintain itself. Minimum temperatures and almost a guarantee of rain-free days could see cotton being grown in the area under irrigation during the dry season. The gulf country has large areas of suitable soils and cotton survives here provided there are no dry season fires. Rainfall is lower than Larrimah but temperatures are higher. This would increase insect pressure although collected plants had only limited insect damage.

Cotton on the Cobourg Peninsula has shown an ability to tolerate salt with its ability to survive and spread behind the coastal dunes. The increased possibility of rain each month, higher minimum temperatures and coastal winds would also assist in this spread. There is no reason for cotton to have been taken to this area apart from the initial settlement. Therefore it is a high probability that cotton in the area has been selected from these old varieties.

Cotton in the Darwin area could have been introduced from a range of sources so that it is impossible to define its type without DNA testing.

Therefore a selection of these lines has been sown at BARC to compare their growth and insect resistance characteristics.

The range of native *Gossypium* does not appear to be any threat as its genetic code would appear to have a different chromosome number. This would stop the possibility of natural crossing.

Therefore it is recommended that the following needs to take place

1. Feral cottons continue to be collected when they are available. Particular preference given to the Gove and Cape Hotham areas as it is likely that this material has resulted from natural selection from the original introductions in the 1880s.

2. That any feral cotton in the DDRF, Katherine areas and along the Victoria Highway be collected and then the plants eradicated.

3. That any area selected for commercial cotton production, surveyed and feral cottons be collected and then eradicated.

4. That feral cotton lines be grown and selected as future breeding material.