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ScienceDirect

Biological Control 41 (2007) 415–422

Biological Control

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Damage potential of an introduced biological control agent *Agonosoma trilineatum* (F.) on bellyache bush (*Jatropha gossypiifolia* L.)

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Received 5 January 2007; accepted 19 March 2007
Available online 27 March 2007

Abstract

The seed-feeding jewel bug, *Agonosoma trilineatum* (F.), is an introduced biological control agent for bellyache bush, *Jatropha gossypiifolia* L. To quantify the damage potential of this agent, shadehouse experiments were conducted with individual bellyache bush plants exposed to a range of jewel bug densities (0, 6 or 24 jewel bugs/plant). The level of abortion of both immature and mature seed capsules and impacts on seed weight and seed viability were recorded in an initial short-term study. The ability of the jewel bug to survive and cause sustained damage was then investigated by measuring seed production, the survival of adults and nymph density across three 6-month cycles. The level of seed capsule abortion caused by the jewel bug was significantly affected by the maturity status of capsules and the density of insects present. Immature capsules were most susceptible and capsule abortion increased with jewel bug density. Similarly, on average, the insects reduced the viability of bellyache bush seeds by 79% and 89% at low and high densities, respectively. However, sustaining jewel bug populations for prolonged periods proved difficult. Adult survival at the end of three 6-month cycles averaged 11% and associated reductions in viable seed production ranged between 55% and 77%. These results suggest that the jewel bug has the potential to reduce the number of viable seeds entering the soil seed bank provided populations can be established and maintained at sufficiently high densities.

Keywords: Weed control; Woody weeds; Seed viability; Seed production; Capsule abortion

1. Introduction

Bellyache bush (*Jatropha gossypiifolia* L.) is one of the more toxic and aggressive weeds in the dry tropics of north Queensland, Australia (Csurhes, 1999; Parsons and Cuthbertson, 2001). It has the capacity to flower and produce seed year round (Bebawi et al., 2005), with seed production as high as 12,000 seeds/plant/year (Bebawi and Campbell, 2002a). These seeds can remain viable for up to 4 years under natural conditions (Bebawi, unpublished data). This persistence, combined with an ability to tolerate harsh conditions, allows bellyache bush to spread even in dry years. In north Queensland, an infestation near Ravenswood expanded in size by 76% during a 3-year period of below average rainfall (Vogler and Keir, 2005). Lazarides and Hince (1993) suggested that bellyache bush has the potential to spread over 75% of the Australian continent.

Exploration for suitable biocontrol agents in South America and the Caribbean commenced in the 1990s. A suite of insects including stem borers (two *Lagocheirus* spp., *Styloleptus* spp.), root borer (larvae), leaf defoliator (adults) (*Colaspis* spp.), leaf defoliator (*Spodoptera latifascia* Walker), fruit, sap sucking bugs (*Pachycoris klugii* Burmeister and *Agonosoma trilineatum* Fabricius (Scutelleridae) were tested for host specificity in Australia

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doi:10.1016/j.biocontrol.2007.03.007

at CSIRO's Brisbane Quarantine facility. The majority of agents were rejected because they failed to produce enough adults in culture or were not sufficiently host-specific. The seed-feeding jewel bug *A. trilineatum* from Venezuela was selected and approved for release because it passed the host-specificity test requirements (Heard and Chan, 2002).

The jewel bug's took approximately 9 weeks to complete their lifecycle, passing through five nymphal instars. Development of nymphs to the second instar was constrained by low relative humidity or lack of some free water (Heard and Chan, 2002). Adults lived for approximately 10 weeks under laboratory conditions and their feeding completely destroyed bellyache bush seeds (Heard and Chan, 2002). The jewel bug was first released in Australia in the Northern Territory in March 2003 (Vanessa McIntyre, personal communication) and in north Queensland in June 2003 (Heard, 2003; Vogler and Keir, 2005).

This paper reports on a series of shadehouse experiments undertaken to determine the potential of the jewel bug to reduce production of all seeds. Initially, plants containing both immature and mature seed capsules were exposed to various densities of the insect at which the level of capsule abortion and changes in seed parameters (weight, level of damage and viability) were measured. The ability of the jewel bug to survive and cause sustained damage was then investigated by measuring seed production and survival of adults and nymphs across three 6-month cycles.

In this study, the term capsule abortion refers to bellyache bush capsules that shrivel/stop growing while either remaining attached to or dropping off the fruiting stalk. Non-aborted mature capsules generally ripen asynchronously and dehisce, releasing their seeds explosively (Bebawi and Campbell, 2002a).

2. Materials and methods

2.1. Biocontrol agents and host plants

The majority of adult jewel bugs used in the experiments were obtained from a mass rearing colony located at the Tropical Weeds Research Centre, Charters Towers (20°09′S, 146°26′E), north Queensland, Australia. Insects in the colony were reared in containers in a laboratory and supplied with seed capsules on foliage cut from either field sites or potted plants grown at the research centre. The rearing containers were exposed to natural sunlight and temperatures maintained at 26–28 °C. Egg batches collected from adult rearing boxes were positioned on filter paper moistened with distilled water and placed in Petri dishes. The moistened filter paper promotes survival and hatching, though relative humidity was not monitored. Additional adults were obtained from another rearing facility (Northern Territory Department of Natural Resources, Environment and the Arts at Palmerston, Northern Territory, Australia) whenever the local supply was insufficient.

Bellyache bush plants were grown in 40 cm diameter plastic pots filled with garden soil. All pots were placed in a shadehouse and regularly irrigated by adding water until it drained through the perforated base of the pots. Plants were all approximately 18 months old and both flowering and fruiting when the experiments began. Individual potted plants were covered with mosquito netting (75 cm × 230 cm × 680 cm) to contain the jewel bugs, ensure plot integrity and trap seeds released from ripe capsules. Hems of the mosquito nets in contact with the ground were weighed down with 300 cm × 5 cm diameter cylindrical, plastic-mesh sandbags to prevent jewel bugs escaping beneath the net.

2.2. Experiment 1: effect of jewel bug density on seed capsules and individual seeds

A two-factor experiment involving six treatments, replicated five times, in a randomised complete block design was undertaken to determine whether the feeding activity of jewel bugs on bellyache bush capsules had any effect on capsule abortion, seed weight, seed damage level and seed viability. Factor A consisted of three jewel bug densities: control (no jewel bugs/plant), low (six jewel bugs of equi-proportional sex ratio/plant) and high (24 jewel bugs of equi-proportional sex ratio/plant). Factor B comprised two capsule maturity types: immature and mature. Capsules with a radial diameter of <12 mm and which were green and yielded when squeezed were classified as 'immature' while capsules with a radial diameter of >12 mm and which were green and rigid were classified as 'mature'.

Immature and mature capsules were tagged with different coloured wool strips (5 cm long) for identification purposes prior to introduction of jewel bugs onto the plants. Only these tagged capsules were used in this experiment. They were also the source of the immature and mature seeds that are discussed later. The density of immature and mature capsules across all plants averaged 17.3 ± 1.5 and 11.5 ± 2.1 capsules/plant, respectively, when the jewel bugs were added to the netted plants.

The experiment ran for 8 weeks between February and March 2004. After 4 weeks, any additional capsules deemed to be immature or mature were tagged and extra jewel bugs were introduced where necessary to replace the jewel bugs that had died.

The daily temperature range in February was between 22.8 and 33.2 °C and in March was between 20.6 and 33.5 °C (Bureau of Meteorology, 2006). Average relative humidity in February was 72% and in March was 71% (Bureau of Meteorology, 2006).

2.2.1. Capsule abortion, seed weight and seed damage

Capsules were inspected daily during the course of the trial to monitor abortion, and to collect aborted capsules while still attached to the inflorescence stalk and any mature capsules just before they dispelled seeds. Aborted and non-aborted capsules from individual plants were placed in separate paper bags. They were then transferred to the laboratory where capsules were counted and split with forceps to release seeds.

Seeds were inspected for damage and weighed prior to germination and viability testing. Damage levels were also determined by using a stereomicroscope to count the number of penetration points caused by insect mouthparts during feeding. Following inspection, seeds were pooled separately for testing based on the capsule maturity and jewel bug density treatments allocated to plants.

For each parameter measured, data were subjected to analysis of variance. Percentage data were arcsine transformed before analysis and later back-transformed. Fisher's protected l.s.d. test was used to identify differences between treatments. Regression analysis was also performed to determine whether there was any correlation between seed weight and seed damage level.

2.2.2. Seed germination and viability

Randomly selected sub-samples (20×5 replicates) of seeds from the three jewel bug density and two capsule maturity treatments were sown at 0.5 cm depth in 300 g (dry weight) of fine river-sand in PVC push caps (11 cm diameter $\times$ 2.5 cm height). The caps were perforated at the base to allow for water drainage. The soil was kept moist by adding water until it drained freely. The PVC caps were placed in natural light in a controlled-environment glasshouse with an alternating 12 h thermo-period (30/20 °C) and arranged in a randomised block design to account for potential lighting differences.

Emerging seedlings were counted and removed daily. Germination was considered to have ceased when no seedlings emerged for 10 consecutive days. The soil was then washed through a 3 mm copper mesh sieve to retrieve non-germinated seeds. Seed viability was tested using the tetrazolium method (Moore, 1985), by placing seeds for 5 days in Petri dishes filled with 10 mL of 1% triphenyl tetrazolium chloride. Seeds that were pink when cut longitudinally with a sharp scalpel were considered viable.

Cumulative viability percentages were calculated on the basis of total seed numbers. Viable seeds were defined as those that germinated in the river-sand plus any ungerminated seeds identified as viable following tetrazolium testing. Statistical analysis using analysis of variance was performed on arcsine-transformed data. Fisher's protected l.s.d. test was used to identify differences between treatments.

2.3. Experiment 2: effect of jewel bug density on seed production

This experiment was similar to Experiment 1 except that a single factor (low and high jewel bug density) was replicated 10 times in a randomised complete block design to determine whether the feeding activity of the jewel bug had any prolonged effect on bellyache bush seed production over three 6-month cycles commencing March 2004. Jewel bug survival was also monitored each month over that period. Natural decline in jewel bug populations dictated the selection of a 6-month cycle. Under laboratory conditions, the longevity of an adult female jewel bug was approximately 2.5 months (10 weeks) (Heard and Chan, 2002). The adoption of a 6-month timeframe allowed time for the insects to complete two generations.

At the commencement of each 6-month cycle, plots were replenished with fresh batches of 6 and 24 adult jewel bugs to ensure continuity of the trial. Daily temperature range in summer/autumn (November–April) was between 17.5 and 35.1 °C and in winter (June–August) between 10.1 and 27.1 °C (Bureau of Meteorology, 2006). Daily relative humidity range in summer/autumn (November–April) was between 59% and 72% and in winter (June–August) between 63% and 65% (Bureau of Meteorology, 2006).

2.3.1. Seed production

Seeds used in this experiment are described as 'ripe seeds' because they were collected from capsules that were allowed to naturally release their seed content in contrast to 'immature or mature seeds' used in Experiment 1 that were physically removed from their capsules. All ripe seeds from control, low and high jewel bug densities were collected and counted at the end of each month and examined under a stereomicroscope for evidence of jewel bug damage. Data were subjected to analysis of variance. Fisher's protected l.s.d. test was used to identify differences between treatments.

2.3.2. Adult jewel bug survival and nymph density

Monthly counts were made to determine adult survival and nymph densities over the 6-month cycles. Adult survival was calculated as a percentage of the initial population still alive at each census.

3. Results

3.1. Experiment 1: effect of jewel bug density on seed capsules and individual seeds

3.1.1. Capsule abortion

Jewel bug density and capsule maturity significantly affected the level of abortion of bellyache bush seed capsules (Table 1) that were present at the start of the experiment, with mature capsules less likely to abort than immature ones (Fig. 1).

3.1.2. Seed weight

Jewel bug density and capsule maturity significantly affected fresh seed weight of bellyache bush (Table 1). Immature seeds were much smaller than mature ones, averaging 30 ± 2.6 and 56 ± 1.8 mg on control plants, respectively. The introduction of jewel bugs resulted in average weight reductions for both immature and mature seeds (Fig. 2).

Table 1
Summary of observed *F*-values, degrees of freedom (*df*) and tabulated *F*-values between treatments variables investigated in this study

Variables	Treatments	*F*	*df*	*P*
Capsule abortion	Jewel bug density	4.80	2	<.001
	Capsule maturity	6.07	1	<.001
	Density × maturity	2.67	2	<.001
Seed weight	Density	17.72	2	<.001
	Maturity	82.52	1	<.001
	Density × maturity	3.55	2	.043
Undamaged seed	Density	85.40	2	<.001
	Maturity	1.38	1	.263
	Density × maturity	0.49	2	0.624
Seed viability	Density	74.69	2	<.001
	Maturity	18.66	1	<.001
	Density × maturity	2.11	2	0.141
Seed production	Cycles	88.74	2	<.001
	Months within cycles	41.28	5	<.001
	Density	56.04	2	<.001
	Cycles × months	33.50	10	<.001
	Cycles × density	9.17	4	<.001
	Months × density	4.90	10	<.001
	Cycles × months × density	2.30	20	.001
Jewel bug survival	Cycles	32.80	2	<.001
	Months	322.08	5	<.001
	Density	528.00	2	<.001
	Cycles × months	5.98	10	<.001
	Cycles × density	19.17	4	<.001
	Months × density	64.15	10	<.001
	Cycles × months × density	2.41	20	<.001

Fig. 1. Abortion of immature (white bars) and mature capsules (grey bars) of bellyache bush as affected by jewel bug density. Vertical bars indicate the SE of the means.

3.1.3. Seed damage

In contrast to capsule abortion, there was no significant difference in the proportion of seeds damaged (indicated by the presence of penetration points) by the jewel bug within immature and mature capsules (Table 1). Seed damage was, however, significantly influenced by the density of jewel bugs present (Table 1), with the proportion of seeds damaged being two times more than at the higher density (Fig. 3).

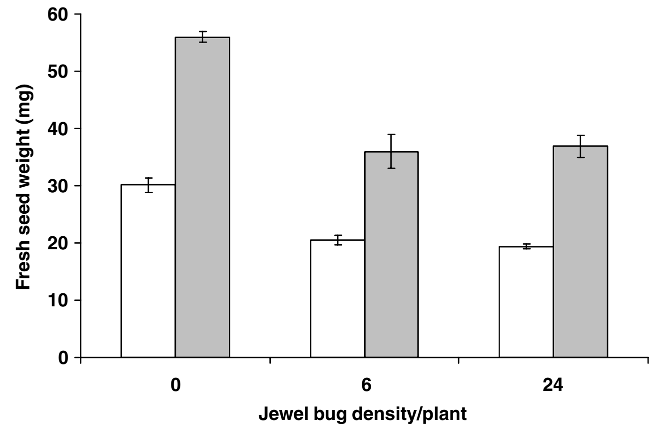

Fig. 2. Mean fresh seed weight of immature (white bars) and mature (grey bars) bellyache bush seeds as affected by jewel bug density. Vertical bars indicate the SE of the means.

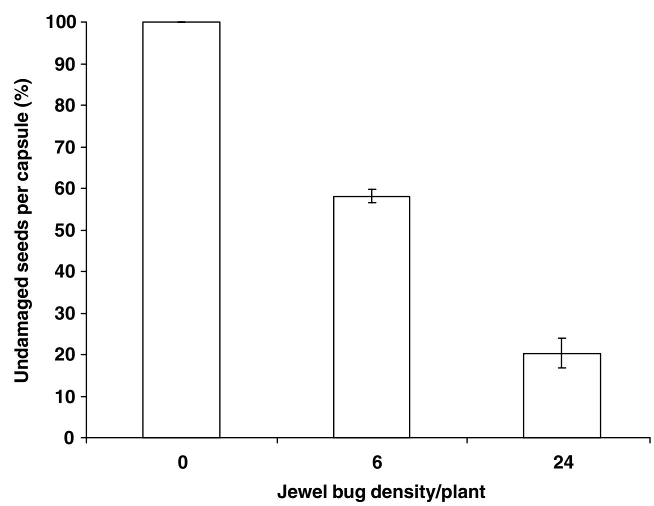

Fig. 3. The proportion of undamaged bellyache bush seeds from combined immature and mature capsules following exposure to a range of jewel bug densities. Vertical bars indicate the SE of the means.

Jewel bugs at the lower density tended to penetrate individual seeds more frequently than at higher densities, with the number of penetration points averaging 1.6 ± 0.24 and 0.6 ± 0.1 per seed, respectively. The maximum number of penetration points recorded on an individual seed was 12.

Physical examination of mature seeds revealed major differences in internal structures between undamaged and damaged seeds. Undamaged seeds had intact endosperm, embryo and cotyledons filling the whole internal cavity. Damaged seeds on the other hand generally had shrivelled endosperms, embryos and cotyledons, resulting in vacant areas.

No significant correlation ($P = 0.05$) was detected between seed damage level and seed weight ($b = 0.15$, $R^2 = 0.10$).

3.1.4. Seed viability

Both capsule maturity and jewel bug density individually affected the viability of bellyache bush seeds, but there was no significant interaction (Table 1). Viability of seeds from mature capsules averaged 40 ± 7% across all jewel bug densities compared to 23 ± 5% for seeds from immature capsules. Jewel bugs reduced the viability of bellyache bush seeds by 79% and 89% at low and high densities, respectively, when compared with seeds from control plants that had an average viability of 71 ± 5% across the two capsule maturity stages (Fig. 4).

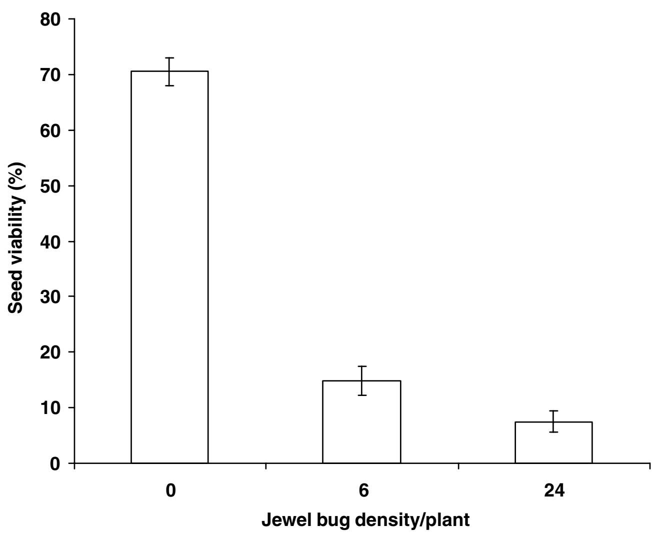

Fig. 4. Changes in the viability of bellyache bush seeds following exposure to a range of jewel bug densities. Vertical bars indicate the SE of the means.

3.2. Experiment 2: effect of jewel bug density on seed production

3.2.1. Seed production

The quantity of ripe bellyache bush seeds released from individual plants was affected by a significant interaction between cycles, months and the jewel bug density present (Table 1). Irrespective of the impact of the jewel bugs, most seeds were produced during the late spring, summer and early autumn periods (Fig. 5), with highest monthly seed production recorded in December 2004 (168 ± 26 seeds/control plant). On average, fewer than four seeds per plant were retrieved on a monthly basis during the cooler winter months (June–August).

Maximum reductions in seed production occurred at the highest jewel bug densities and averaged 72%, 55% and 77% at the end of the 1st, 2nd and 3rd cycle, respectively, when compared with control plants (Fig. 5). In comparison, the amount of ripe seeds produced in the low density jewel bug treatment was reduced by 30%, 6% and 55% at the end of cycles 1, 2 and 3, respectively.

Inspection of all ripe seeds for damage caused by the jewel bugs revealed significant differences in the level of damage imposed between cycles and densities of insects present. Damage was higher in cycle 1 than the other two periods, and the higher density of jewel bugs constantly caused a greater proportion of ripe seeds to be damaged. Of the ripe bellyache bush seeds produced in cycles 1st, 2nd and 3rd, 73%, 26% and 34% on average, respectively, were damaged at the high jewel bug density.

Seed production was positively correlated with maximum ambient temperature ($b = 0.07$, $R^2 = 0.66$) but was

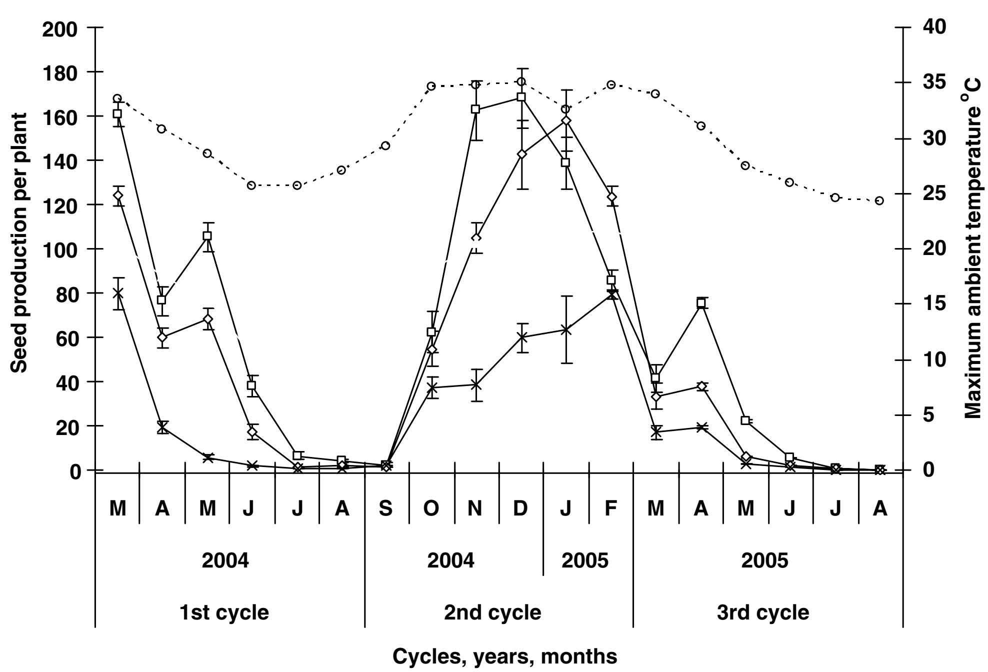

Fig. 5. Production of ripe bellyache bush seeds over three 6-month cycles following exposure to nil (□), low (◇) and high (×) jewel bug densities, and maximum monthly ambient temperatures (○). Vertical bars indicate the SE of the means.

negatively correlated with jewel bug density ($b = -1.7$, $R^2 = 0.99$).

3.2.2. Adult jewel bug survival and nymph density

Significant interactions between cycles, months and jewel bug density were also detected for adult jewel bug survival (Table 1). Maximum monthly adult jewel bug survival averaged 84% in October 2004, irrespective of jewel bug density (Fig. 6). However, jewel bug adult survival declined at the end of each cycle (Fig. 6). Reduction in adult jewel bug survival at the end of the 1st, 2nd and 3rd cycle averaged 92%, 76% and 99%, respectively (Fig. 6). The low jewel bug density treatment had the highest jewel bug nymph density averaging 0.7 nymphs/plant in July 2004, 0.5 nymphs/plant in February 2005 and 1.2 nymphs/plant in May 2005 (Fig. 7). This result may be due to the significant effect of adult density (Table 1).

There was a positive correlation between adult jewel bug survival and maximum ambient temperature ($b = 0.1$, $R^2 = 0.68$).

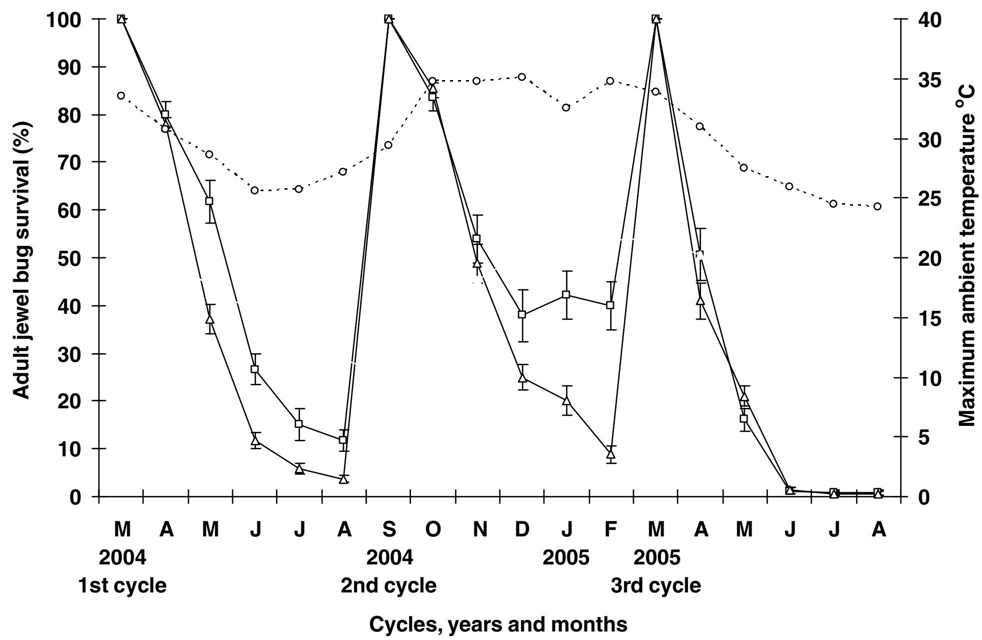

Fig. 6. Survival of adult jewel bugs over three 6-month cycles within low (□) and high (△) density treatments, and associated maximum monthly ambient temperatures (○). Vertical bars indicate the SE of the means.

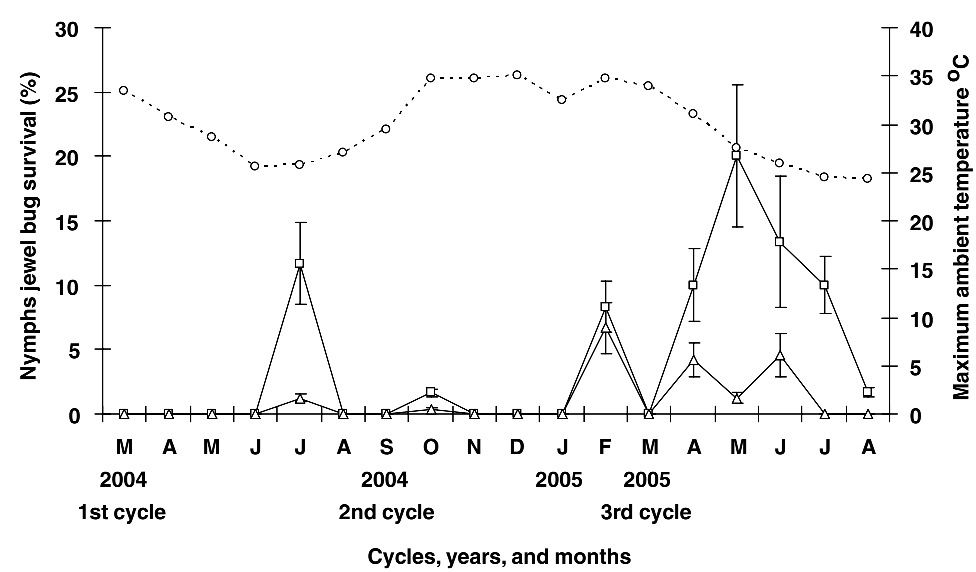

Fig. 7. Density of jewel bug nymphs within low (□) and high (△) jewel bug density treatments and associated maximum ambient temperatures (○). Vertical bars indicate the SE of the means.

4. Discussion

The results from these shadehouse studies suggest that if the jewel bug is present at sufficiently high numbers it has the potential to reduce the quantity of viable bellyache bush seeds entering the soil seed bank through promotion of capsule abortion or through mortality of individual seeds held within capsules. Grimm (1997) observed a similar occurrence on *Jatropha curcas* where a fruit-feeding bug, *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae), caused up to 100% capsule abortion.

The differential response observed in capsule abortion between immature and mature capsules could possibly be due to the jewel bugs having a direct preference for immature capsules. Conversely, capsules may become more resistant to feeding damage by the insect as they mature. The fact that loss of seed viability was similar between immature and mature seed capsules in Experiment 1 suggests that the latter may be the more plausible explanation.

Whether similar results to those found in these shadehouse studies would occur in the field cannot be verified at present, as the jewel bugs have yet to establish and persist at any field sites in Australia since commencement of releases in 2003. Maintaining a population of jewel bugs proved difficult even within the shadehouses. While the exact reasons for the poor egg hatching that was observed and the failure of jewel bugs to persist were not identified, we suggest that insufficient humidity or the lack of available free moisture for egg batches could possibly be contributing factors. If this is the case, there could be implications for establishment and persistence of insects under field conditions in the dry tropics during periods of low relative humidity. However, the restrictions placed on adult jewel bugs in the cage situation did lead to oviposition in exposed and dry artificial sites which may not happen under field conditions.

If populations of the jewel bug could be established in the field and the level of damage that they imposed was similar to that found in the current study, it is not clear whether bellyache bush populations would be adversely affected in the long-term. A review of the literature by van Klinken (2005) revealed limited examples of the impacts of biocontrol agents on seed bank dynamics. Harper (1977) suggested that a reduction in the seed crop by 50% would halve the number of seeds reaching any point in the dispersal range. In an evaluation of two biological control agents on the annual herbaceous weed parthenium (*Parthenium hysterophorus*), which has a relatively long lived seed bank, Dhileepan (2003) suggested that ongoing damage by the insects would result in a decline in the density of populations within 6–7 years.

Like parthenium, bellyache bush is a prolific seeder, particularly if it has an abundance of soil moisture to grow and reproduce. Individual plants have the potential to produce 12,000 seeds annually (Bebawi and Campbell, 2002a) and soil seed banks in excess of 10 million seeds per hectare have been recorded within dense infestations (Bebawi and Campbell, 2002b). Bellyache bush plants also survive for many years and seeds can remain viable for up to 4 years under natural conditions (Bebawi, unpublished data), so the jewel bug would need to maintain prolonged damage if replenishment of the seed bank were to be minimised.

Recommencement of a biological control program for bellyache bush will occur in the near future, with the intent of finding additional agents to attack identified weaknesses in the plant's lifecycle.

Acknowledgments

The authors thank the Queensland Department of Primary Industries and Fisheries, the Department of Natural Resources, Environment and the Arts, Darwin, Northern Territory, and the CRC for Australian Weed Management for providing financial support. We also thank Dr T. Heard (CSIRO), Dr D. Panetta (NR&W), Dr B. Grace (NRETA), Mr J. Vitelli, Dr B. Palmer and Dr S. Campbell for reviewing the manuscript.

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