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A comparison of alternative plant mixes for conservation bio-control by native beneficial arthropods in vegetable cropping systems in Queensland Australia

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Abstract

Cucurbit crops host a range of serious sap-sucking insect pests, including silverleaf whitefly (SLW) and aphids, which potentially represent considerable risk to the Australian horticulture industry. These pests are extremely polyphagous with a wide host range. Chemical control is made difficult due to resistance and pollution, and other side-effects are associated with insecticide use. Consequently, there is much interest in maximising the role of biological control in the management of these sap-sucking insect pests. This study aimed to evaluate companion cropping alongside cucurbit crops in a tropical setting as a means to increase the populations of beneficial insects and spiders so as to control the major sap-sucking insect pests. The population of beneficial and harmful insects, with a focus on SLW and aphids, and other invertebrates were sampled weekly on four different crops which could be used for habitat manipulation: Goodbug Mix (GBM; a proprietary seed mixture including self-sowing annual and perennial herbaceous flower species); lablab (*Lablab purpureus* L. Sweet); lucerne (*Medicago sativa* L.); and niger (*Guizotia abyssinica* (L.f.) Cass.). Lablab hosted the highest numbers of beneficial insects (larvae and adults of lacewing (*Mallada signata* (Schneider)), ladybird beetles (*Coccinella transversalis* Fabricius) and spiders) while GBM hosted the highest numbers of European bees (*Apis mellifera* Linnaeus) and spiders. Lucerne and niger showed little promise in hosting beneficial insects, but lucerne hosted significantly more spiders (double the numbers) than niger. Lucerne hosted significantly more of the harmful insect species of aphids (*Aphis gossypii* (Glover)) and *Myzus persicae* (Sulzer)) and heliothis (*Heliothis armigera* Hübner). Niger hosted significantly more vegetable weevils (*Listroderes difficillis* (Germar)) than the other three species. Therefore, lablab and GBM appear to be viable options to grow within cucurbits or as field boundary crops to attract and increase beneficial insects and spiders for the control of sap-sucking insect pests. Use of these bio-control strategies affords the opportunity to minimise pesticide usage and the risks associated with pollution.

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Introduction

Modern crop systems (monoculture crops) are fragile and ecologically unstable, so serious pest problems can be expected (Risch, 1980). The instability of agro-ecosystems can be caused by the vegetational simplification, resulting from the adoption of vast crop monocultures (Tothill, 1958). There is ample evidence that vegetation diversity can have both positive and negative, and direct and indirect effects on populations of not only herbivorous insects, but also on associated natural enemies (van Emden, 1965; Price *et al*., 1980). Densities of natural enemies tend to be greater in polycultures than in monocultures (Andow, 1991), and promotion of biodiversity in agro-ecosystems regularly favours natural enemies, suppresses pests and, in some cases, reduces crop damage (Gurr *et al*., 2000; Landis *et al*., 2000).

Many pest populations may be managed by enhancing the performance and local abundance of the existing community of natural enemies, a practice which was originally recognised by van den Bosch & Telford (1964) and made popular as the title of a book in 1998 (Barbosa, 1998) and recently has been termed 'conservation biological control' (Landis *et al*., 2000). It is perhaps the oldest and most widespread form of biological pest control, with roots in many cultural practices such as vegetation diversity, manipulation of agro-ecosystems intercropping, use of wild plants in and around crops, trap cropping and use of row covers, which all influence the distribution and abundance of natural enemies in crops (Kean *et al*., 2003).

Habitat manipulation, a form of conservation biological control (Landis *et al*., 2000), is an important approach that enhances the environment by making it more suitable for natural enemies, thus improving the probability of successful biological control (Rabb *et al*., 1976). Conservation of natural enemies in integrated pest management (IPM) programmes is enhanced through habitat manipulation (Hopper, 2003). Conservation biological control involves habitat manipulations to enhance the fecundity and longevity of natural enemies (Wratten *et al*., 2003). It includes the maintenance of ecological compensation areas, relying on the increase of plant diversity within or outside crops, and is crucial in enhancing beneficial insects' abundance for pest suppression (Rossing *et al*., 2003).

Habitat manipulation also involves methods like trap crops, to reduce the susceptibility to insect pest infestations in a target crop. Habitat manipulation specifically enhances the impact of arthropod natural enemies by providing: (i) alternative host/prey species; (ii) non-prey/host food (e.g. honeydew, pollen, nectar), particularly for parasitoids; and (iii) more favourable micro-climates, including overwintering sites. The use of non-crop habitats within crops which mimic natural habitats can be used to encourage the build-up of natural enemies into fields (Thomas *et al*., 1992). Field boundaries have been recognized for two decades as important reservoirs of predatory arthropod species, stemming from European research by Sotherton (1984, 1985). Habitats, as found in field boundaries, differ in their suitability for predators as some non-crop plants attract more insect pests while others may favour natural enemies (predators and parasitoids) for the reasons mentioned above. Different habitats have been used to increase beneficial predator numbers in agro-ecosystems, for example 'beetle banks' (Collins *et al*., 1997).

The reliance on pesticides in intensively cropped areas has led to uncontrollable situations through the unwitting selection of resistant genotypes, with a high level of resistance readily developed in frequently sprayed contiguous populations (Prabhaker *et al*., 1997; Simmons & McCutcheon, 2001) and the destruction of populations of natural enemies (De Barro, 1995). Due to inadequate efficacy of, resistance to, and increasing environmental concerns with pesticides, there is much interest in maximising the role of biological control in the management of sap-sucking insect pests (Simmons & McCutcheon, 2001). Most research on habitat management has been done in the colder climates of New Zealand, Europe, USA and Canada. According to Dent (1995), conservation of natural enemies is an approach to biological control which had not at that time received sufficient attention. Research in conservation of natural enemies is, however, gaining ground in Australia and New Zealand (Hossain *et al*., 2002; Gurr *et al*., 2005). The conservation and enhancement of predators and parasitoids to suppress arthropod pests is considered one of the most important approaches in modern pest management practices (Landis *et al*., 2000).

Cucurbit crops, such as cantaloupe (*Cucumis melo* L.), cucumber (*C. sativus* L.) and squash (*Cucurbita pepo* L.), have generally been found to be more attractive to sap-sucking insects than other crops (Tonhasca *et al*., 1994). Silverleaf whitefly *Bemisia tabaci* (Gennadius) biotype B (Homoptera: Aleyrodidae) (SLW), as a prime example of a sap-sucking insect pest, has emerged as a key pest of many crops during the past decade. It was first detected in Australia in October 1994 after being recorded in the Berrima region near Darwin, Northern Territory, on both nursery species and horticultural crops belonging mainly to the Cucurbitaceae (Gunning *et al*., 1995). In common with other sap-sucking insect pests, it is primarily a phloem feeder and survives in habitats ranging from temperate through to tropical. It causes damage through direct feeding, which may induce irreversible physiological disorders and crop yield decline, and through excretion of honeydew and virus transmission (De Barro, 1995). Honeydew encourages the growth of sooty mould on the leaves, thereby inhibiting photosynthesis and causing cosmetic damage.

Numerous natural enemies of insect pests are known in different parts of the world and on various crops, but the biology of these natural enemies still requires study, as does their efficacy in aiding the control of sap-sucking insects. Natural enemies of sap-sucking insect pests can be classified into three groups: predators, parasitoids and entomopathogens (Gerling, 1990). Predators are the primary biological control agents in central Queensland due to dry and hot weather conditions of the region, especially during the time of year when cucurbits are cultivated. Only a few groups of insects represent predators of the major sap-sucking insect pests of cucurbits in Queensland, namely whiteflies and aphids: Coleoptera (mainly ladybirds); Heteroptera (bugs essentially belonging to the families Miridae and Anthocoridae); Neuroptera (Lacewings); and Diptera (Gerling, 1990; Vasquez Moreno, 1997). These groups include some well-known generalist predators that prey on sap-sucking insects, including *Chrysoperla* species larvae (lacewings), *Orius* species (minute pirate bugs) and *Geocoris* species (bigeyed bugs). Several coccinellid species are specialist insect predators, such as *Delphastus catalinae* (LeConte) and *Nephapsis oculatus* (Blatchely) (Fasulo *et al*., 1995). Aphids and whiteflies are attacked by several species of predators that can act together to suppress or delay the outbreak of damaging populations (Sechser *et al*., 2003). Populations of insect pests can also be reduced by predation of mites and spiders and some other minor insect taxa.

This study, therefore, aimed to manipulate the habitat of cucurbit crops in a tropical setting to increase the populations of beneficial insects and spiders, so as to control major sap-sucking insect pests such as SLW and aphids. Further, the habitat was identified that best supported an introduced lacewing (*Mallada signata* (Schneider)) population, commercially available through the Australian company 'Bugs for Bugs' and often used by vegetable growers for bio-control of sap-sucking insect pests. Four potential conservation bio-control treatments were assessed:

(i) 'Goodbug Mix' (GBM) – a proprietary seed mixture produced by the Australian seed company 'Green Harvest'. GBM contains self-sowing annual and perennial flowers, including red clover (*Trifolium pretense* F.); lucerne (*Medicago sativa* L.); sweet alice/Sweet alyssum (*Lobularia maritima* (L.) Desv.); dill (*Anethum graveolens* L.); caraway (*Carum carvi* L.); coriander (*Coriandrum sativum* L.); buckwheat (*Fagopyrum esculentum* Moench); baby's breath (*Gypsophila elegans* Bieb.); Queen Anne's Lace (*Ammi majus* L.); marigolds (*Tagetes patula* L.); and cosmos, (*Cosmos bipinnatus* Cav.), and reputedly enhances pollen and nectar resources utilised by predators and parasitoids.

(ii) Lablab (*Lablab purpureus* (L.) Sweet) – a fast growing, drought-tolerant, annual, summer forage legume. In a crop rotation program, it can significantly improve soil nitrogen levels by nitrogen fixation or by incorporation in soil as a green manure crop. Lablab is tolerant of drought and heat. In NSW, lablab is adapted to slopes and plains with a minimum annual rainfall of 500 mm, and to coastal or irrigated areas. Lablab does very well on a wide variety of soils – from light, sandy soils through to well-drained, heavier-textured soils. Lablab's performance on heavy soils is greatly superior to that of other legumes. Lablab is resistant to phytophthora root rot (Mullen, 1999). Sixteen species of natural enemies belonging to the Trichogrammatidae, Braconidae, Ichneumonidae, Sarcophagidae, Coccinellidae, Chrysopidae and Eumenidae were recorded on lablab in Tamil Nadu, India (Srinivas & Jayaraj, 1989).

(iii) Lucerne (*Medicago sativa* L.) – a medic which harbours a rich arthropod fauna. Unharvested refuge strips of lucerne are known to improve the distribution and activity of natural enemies of sap-sucking insect pests within the field (Hossain *et al*., 2002) by providing suitable microclimates (Pinter *et al*., 1975) and an alternative food source as pollen of lucerne flowers (Kevan & Baker, 1984). Further, lucerne crops in Australia harbour a rich spider fauna (Bishop & Holtkamp, 1982), which respond favourably to unharvested refuge vegetation (Hossain *et al*., 2002); and

(iv) Niger (*Guizotia abyssinica* (L.f.)) – is reported to support high densities of prey insect and provide protective leafy canopies which supply shelter especially during the winter months (Grundy & Maelzer, 2003). Niger has the tendency to be a more successful refuge treatment than some brassicae and legume species. It has an abundance of yellow flowers that were found to be attractive to pollinating insects, serving as supplementary prey on which the predatory bug *Pristhesancus plagipennis* (Walker) were observed to feed (Grundy & Maelzer, 2003).

Materials and methods

Trial design and treatments

Four treatments were arranged on each of three 90-m beds (each treated as a block), 2 m apart, in a randomized complete block design on a central Queensland vegetable farm near Rockhampton, Queensland, Australia (23°22′S, 150°32′E) in 2005–06. Each bed comprised four plots (2 × 20 m) with a buffer of 3 m of hay mulch between plots. The four treatments comprised the potential boundary crops of GBM, lablab (var. High Worth), lucerne (var. Sequel Lucerne) and niger. The GBM mixture consisted 80% by seed number of clover, lucerne and buckwheat and the remainder as the seven other species. Seeds were sown on 10 October 2005 at the recommended rates of 10 kg ha^{-1} GBM, 15 kg ha^{-1} lablab, 15 kg ha^{-1} lucerne and 10 kg ha^{-1} niger and were irrigated with an overhead system. Pumpkin and watermelon crops, both good hosts for SLW, were grown on the east (2 m distance) and south (10 m distance) sides of the trial, while nothing was grown on the north and west sides.

Two hundred second instar green lacewing (*Mallada signata* (Schneider)) larvae from 'Bugs for Bugs', Australia, were released into each plot on 22 November 2005.

Sampling

Insect and spider densities were sampled weekly from 2 December 2005 to 3 February 2006 by counting a sample of individuals on a random selection of approximately ten exposed leaves within the canopy of each plot during the early hours of the morning when the insects were least active. Insects and spiders in each plot were counted by carefully turning leaves over and counting the number of adult individuals present (larvae and adults for lacewing) taking two minutes to complete. Also, one minute was spent for visual observation of insects and spiders on the upper side of exposed leaves of each plot so the total time spent on each plot was three minutes. We could not find evidence of parasitism in whitefly nor aphids during the sampling periods.

Data analysis

The total number of each insect or spider species observed across all weekly sampling during the sampling

Table 1. Treatment averages of the numbers of various beneficial insects observed during three minutes of sampling per plot across all the weekly samples during sampling from December 2005 to February 2006.

Habitat treatment	European bees	Lacewing larvae	Lacewing adults	Ladybird beetles	Spiders
	[1]***	***	***	***	***
Lablab	25 b	43 a	28 a	38 a	40 b
Lucerne	21 c	15 b	13 b	19 b	37 b
GBM	45 a	14 b	15 b	17 b	56 a
Niger	21 c	13 b	12 b	18 b	18 c
[2]SEM	1.3	1.1	1.2	2.3	3.6

Means within a column followed by common letters are not significantly different ($P = 0.05$).
[1] Significance of treatment effect; ***, $P < 0.001$ (treatment effect from ANOVA); [2]SEM, standard error of the mean.

period was computed, and the data were analysed by standard analysis of variance. Distributional assumptions for all analyses were assessed by visual inspection of residual and normal probability plots with no major departures being observed, so no transformations were necessary. Pairwise comparison of means was performed using a protected least significant difference test with GenStat 8th Edition (2005).

Results

Beneficial insects

The density of European bees (*Apis mellifera* Linnaeus) differed ($P < 0.001$) among treatments with more European bees for GBM compared with other treatments (table 1). GBM also hosted the largest number of spiders. The population of spiders and European bees remained higher throughout the trial in the GBM than the other treatments (table 1).

There were more than twice as many ladybird beetles (*Coccinella transversalis* Fabricius) and lacewing (*Mallada signata* Schneider) larvae and adults, for lablab compared with all other treatments (table 1; $P < 0.001$), although the population of lacewing larvae in lablab decreased over time, whereas the numbers of lacewing larvae remain relatively constant over time in all other treatments (fig. 1). The population of lacewing larvae and adult and ladybird beetles tended to be relatively higher in the lablab than the other treatments throughout the trial period (table 1). The number of spiders also differed ($P < 0.001$) among treatments with a greater number of spiders for GBM, lablab and lucerne (table 1) compared with niger.

Harmful insects

The density of cotton stainers (*Graptostethus servus* Fabricius), grasshoppers (*Chortoicetes terminifera* (Walker)) and heliothis (*Heliothis armigera* Hübner) differed ($P < 0.001$) among treatments, with more of these insects for lablab compared with other treatments (table 2). The greatest density of aphids (*Aphis gossypii* (Glover)) and *Myzus persicae* (Sulzer)) were observed for lucerne, while the least number was observed for niger (table 2; $P < 0.001$); and the population of aphids in lucerne tended to increase over time, whereas the population of aphids remain relatively constant in all other treatments (fig. 2). The number of vegetable weevils (*Listroderes difficillis* (Germar)) was greater ($P < 0.001$) for niger compared with all other treatments (table 2). In terms of harmful insects, GBM harboured only grasshoppers in any substantial numbers, and the population trend was similar and almost equal to that of lablab (table 2).

Discussion

Lablab hosted large numbers of ladybird beetles and spiders and best supported the population of introduced lacewing larvae and adults with their numbers greater than all other treatments (table. 1). Hence, lablab may be used as a crop to enhance the population of natural enemies for cucurbit pests but requires further field testing. The decline in lacewing larvae numbers in lablab with time may reflect movement out of the lablab into adjacent crops, or it may be due to a less attractive aspect of old lablab plants. Although lablab also hosted large numbers of cotton stainers,

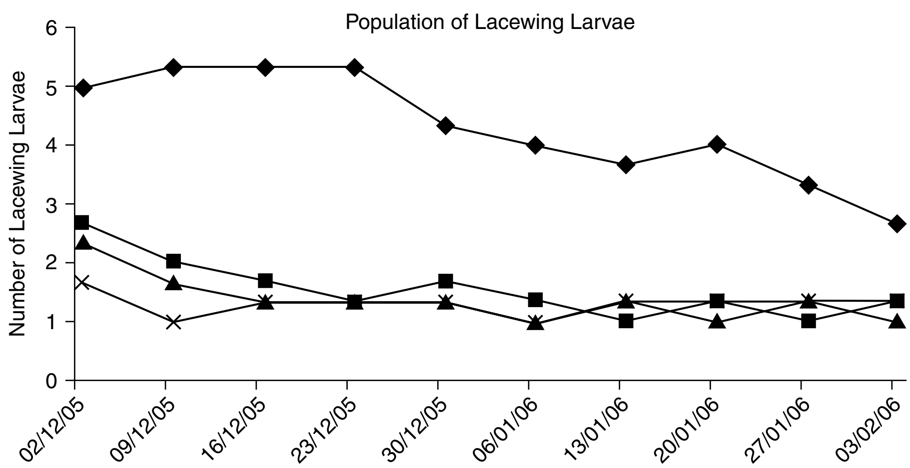

Fig. 1. Number of lacewing larvae observed during three minutes in four treatments (◆, lablab; ■, lucerne; ▲, GBM; and ×, niger) from 2 December 2005 to 3 February 2006.

Table 2. Treatment averages of the numbers of various harmful insects observed during three minutes of sampling per plot across all the weekly samples during sampling from December 2005 to February 2006.

Habitat treatment	Aphids	Cotton strainers	Grass-hoppers	Heliothis	Vegetable weevils
	[1]***	***	***	***	***
Lablab	77 b	46 a	66 a	53 a	30 b
Lucerne	300 a	17 c	39 b	51 a	27 b
GBM	66 bc	23 b	65 a	23 b	29 b
Niger	59 c	14 c	39 b	21 b	131 a
[2]SEM	4.7	1.5	1.4	1.4	2.0

Means within a column followed by common letters are not significantly different ($P = 0.05$).
[1] Significance of treatment effect; ***, $P < 0.001$ (treatment effect from ANOVA); [2]SEM, standard error of the mean.

grasshoppers and heliothis, these are not considered serious insect pests of cucurbits in the central Queensland region. Predators, such as ladybird beetles, are more aggressive, typically longer-lived, may attack more prey and generally have greater consumption requirements (Kean *et al.*, 2003) than parasitoids so may be hosted by lablab due to the availability of food in the form of insect pests present. Predacious lady beetles are known to be one important group of whitefly and/or aphid predators (Hodek & Honek, 1996; Dixon, 2000). Although there may be a short-term increase of insect pests, any short-term increase will lead to a growth and reproductive response of natural enemies (Luff, 1983), resulting in lower densities of the pest insects (Hossain *et al.*, 2000). Once the food source in the crop (refuge species) is decreased, predators such as lacewings, ladybird beetles and spiders will move to adjacent crops (cucurbit crops) in search of food.

The highest numbers of spiders and European bees and similar numbers of other beneficial insects were observed in the GBM mixture compared with the other species. Further, GBM hosted relatively small numbers of harmful insects, although grasshoppers were quite prevalent. The GBM mixture could, therefore, be used as a potential companion or field boundary crop in cucurbits to enhance beneficial insect populations for the control of sucking insect pests, especially SLW. Leite *et al.* (2006) reported that spiders were limiting factors for population increases of SLW.

An important management technique is the provision of floral foods (nectar and pollen) for use by predators and parasitoids (Landis *et al.*, 2000). The Australian proprietary seed mixture, GBM, is also a mixture of colourful flower species developed for the build-up of beneficial insects, spiders and natural enemies of arthropod pests. The GBM mixture hosted the largest numbers of spider fauna. Previously, the role of spiders in population regulation of pests was not fully known (Wheeler, 1973), but they were believed to play a significant role in limiting some herbivore populations (Yeargan, 1975). Spiders have been shown to be one of the aggressive predators and have the ability to move greater distance at a faster pace than other generalist predators (Bishop & Riechert, 1990), and their collective predation lowers pest populations across a number of crop species (Nyffeler *et al.*, 1994).

Lucerne hosted relatively larger numbers of spiders as predators; but, at the same time, lucerne hosted the highest numbers of aphids, and these numbers increased over time (fig. 2). The greater number of spiders may be due to the availability of sufficient food in the form of aphids, for aphid numbers per leaf increased over time. Lucerne has been reported to harbour a rich arthropod fauna. As many as 600 arthropod species were recorded in the state of New York, USA (Pimentel & Wheeler, 1973) and 250 species in New South Wales, Australia (Bishop & Holtkamp, 1982). Most of these arthropods are predators and parasitoids, while only a few of these species, including *Heliothis* spp. (Bishop, 1984), are pests that seriously affect lucerne production (Anonymous, 1985). In particular, of all the crops assessed, lucerne hosted the largest number of aphids, which are one of the most harmful insect pests of cucurbit crops. They also characteristically colonise a range of other horticultural crops and cotton. Lucerne and lablab hosted the highest

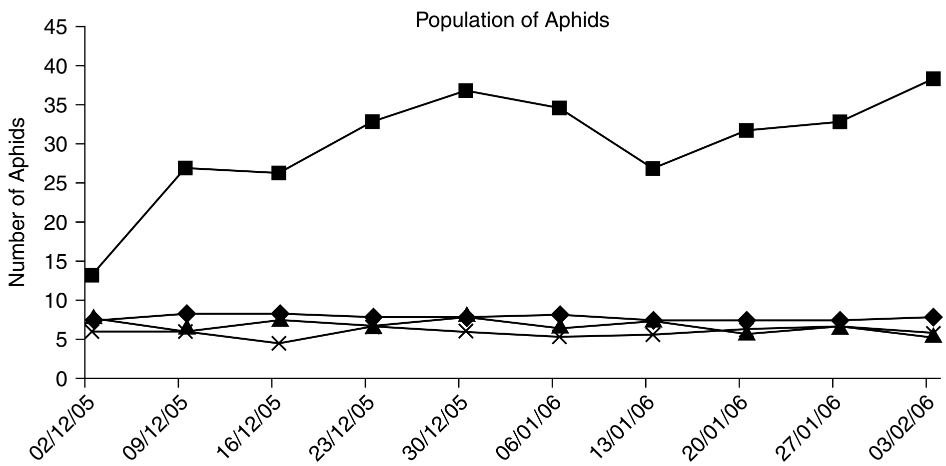

Fig. 2. Number of aphids observed during three minutes in four treatments (◆, lablab; ■, lucerne; ▲, GBM; and ×, niger) from 2 December 2005 to 3 February 2006.

numbers of heliothis than the other two crops, which is why lucerne alone may not be the best option for habitat manipulation for the enhancement of natural enemies in cucurbit crops.

Niger showed little promise in hosting beneficial insects and spiders, and hosted the highest numbers of vegetable weevils throughout the trial (tables 1 and 2) although Grundy & Maelzer (2003) recorded significantly higher ($P < 0.05$) numbers of adults of the predatory assassin bug, *Pristhesancus plagipennis* (Walker) (Homoptera: Reduviidae), on niger than on canola (*Brassica napus* L.), red salvia (*Salvia coccinea* P.J. Buchoz ex Etlinger), linseed (*Linum usitatissimum* L.), lupins (*Lupinus angustifolius* L.) and lucerne (*Medicago sativa* L.).

Modern pest management practices include the encouragement and enhancement of predators and parasitoids to suppress arthropod pests (Landis *et al*., 2000). If the full potential of natural enemies is to be realised in an integrated pest management programme, it is necessary to understand their population dynamics over time and the factors that influence them, including the role of refuges (Wratten *et al*., 2000) and of the interference through intraguild predation (Lang, 2003). Although habitat manipulation can take various forms, van Emden & Dabrowski (1997) suggested that focus should be on provision of non-host foods for natural enemies.

Therefore, lablab could be the best option to grow alongside cucurbits or as a field boundary crop to host beneficial insects and spiders, especially generalist predators such as lacewings, ladybird beetles and spiders to control sap-sucking insect pests such as SLW and aphids. The population of introduced lacewing adults and larvae was greater in lablab than in other treatments, suggesting that it could be used to encourage survival of the predator species. The GBM mixture may be used as an alternative for better habitat manipulation in cucurbit crops. Further field studies are needed to evaluate the effectiveness of promotion of predators and suppression of sap-sucking insect pests, especially SLW and aphids, in cucurbit crops grown alongside lablab before widespread use is recommended.

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