Do Multiple Applications of Amino-Feed UV Improve Beneficial Arthropod Abundance and Yield?

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Summary

- Artificial food supplements are used in integrated pest management (IPM) systems to increase predator and parasitoid abundance and rates of predation and parasitism.
- We assessed the efficacy of using nine season-long applications of the commercial artificial food supplement Amino-Feed UV in a cotton field trial. Pest and beneficial arthropod abundance, mortality rates of sentinel prey, fruit counts, crop yield and fibre quality were significantly unchanged by Amino-Feed UV treatment.
- To place these results in context, we used a hierarchical approach ranging from altered foraging behaviour to increased profitability to review 82 trials from 38 publications on artificial food supplements. The level of the work most commonly examined in the publications was the abundance of beneficial (n = 51 trials) and pest (n = 26) arthropods. Although the abundance of the former increased in 34 trials and the abundance of the latter declined in 14 trials, increased profitability was not demonstrated in the four trials where it was examined. The likelihood of a positive outcome in a trial was not significantly affected by single or multiple applications or carbohydrate and/or protein components of the supplement.

Introduction

Many beneficial arthropods exhibit life-history omnivory (Polis & Strong 1996), in that they include honeydew, floral nectar, extra-floral nectar and/or pollen in their immature or adult diet (Hagen 1986; Coll & Guershon 2002). Access to these resources may increase the abundance and rate of parasitism and predation by these arthropods. Carbohydrate and/or protein mixtures, such as Amino-Feed UV®, Envirofeast® and Pred-Feed®, can be applied to cotton crops to act as artificial supplemental food sources for beneficial arthropods. The rationale is to improve the synchrony of beneficial and prey or host populations in time and space (Hagen 1986) by one or more of the following: increased immigration and lowered emigration rates of beneficial arthropods (Evans & Swallow 1993; Evans & Richards 1997; Mensah 1997); consumption of the supplement by beneficial arthropods, leading to higher survival and/or reproduction (McEwen *et al.* 1996); higher parasitism or predation rates (Mensah & Singleton 1999, but see McEwen *et al.* 1996); and reduced number of eggs laid by pest arthropods (Mensah 1996).

Field testing of artificial food supplements in cotton has focussed on targeting a few pest species (mostly heliothis), usually with multiple treatments (range 1 to 13) applied at 7 to 14 day intervals up until flowering or early boll filling growth stages (i.e., until January or early February) (Mensah

1997, 2002a; Mensah & Singleton 2002). The early to middle part of the growing season was often targeted because pest densities were typically low to moderate (which leads to reduced application of disruptive insecticides), beneficial arthropods were perceived to effectively suppress these pest densities (Murray & Mensah 1996), and beneficial arthropod densities were thought to decline from January onwards, regardless of possible food supplement treatments (Mensah 2002a, b; but see Scholz *et al.* 2002).

There is scope to extend the 'application window' of supplements to include a wider range of pest species over the entire season. This opportunity is the consequence of improved development and increased adoption of IPM programmes for cotton (Wilson 2002). It is imperative that proposed changes to the nature of artificial food supplement programmes are economically feasible. In this study we assessed the effectiveness of applying multiple treatments of an artificial food supplement (Amino-Feed UV®) during the growing season to enhance beneficial arthropod densities. We measured beneficial and pest arthropod abundance, mortality rates of sentinel heliothis eggs, fruit counts, crop yield and fibre quality.

Methods

Our study was conducted at Wayne & Liz Thomson's property "RMB 1371" at Byee in the South Burnett region of Queensland. The trial area was located at the end of a 30 ha irrigated solid-plant Ingard® (cv NuPearl Roundup-Ready) cotton field. The trial area was effectively 64 m wide and 300 m long (1.92 ha) and contained six plots, each 32 m wide and 100 m long (0.32 ha). The treatments were multiple applications of Amino-Feed UV and an untreated control, each replicated three times in a randomised-complete block design. Amino-Feed UV contains 24.2 % sugar, 21.3 % crude protein and amino acids, 1.8 % nitrogen and 10 % UV protectant (product label data; Agrichem Manufacturing Industries, Loganholme, Qld). Cotton was grown using standard commercial agronomic practices, but no insecticides were applied. We applied Amino-Feed UV at 3 L per ha in 100 L water per ha by ground rig to the treatment plots on nine occasions at 4 to 24 day intervals between 3 December 2001 and 8 March 2002 (i.e., 47 to 142 days after planting; DAP).

We monitored the trial site during the growing season from 35 DAP (see Table 1). We recorded the numbers of beneficial arthropods in the following categories: predatory bugs, predatory beetles, ants, spiders, other beneficial arthropods (mainly anystid mites, lacewings and wasps), and all beneficial arthropods. Numbers of pest arthropods were recorded in the following categories: jassids, true bugs (including mirids and green vegetable bugs), beetles, other pests (mainly aphids and flies), and all pests (note that species such as flies were classified as a 'pest' for convenience, as they may serve an important role as prey). We used sentinel heliothis, *Helicoverpa armigera* (Hübner), eggs on cards to assess predation and parasitism pressure (see Scholz *et al.* 2000). The fate of eggs on cards was assigned to the following categories: hatched; collapsed; predated (chewed, sucked or missing); or parasitised (eggs turned black and adult wasps, mainly *Trichogramma* spp. emerged; the proportion parasitised was calculated from the original number of eggs present on the cards).

Table 1. Monitoring details for a season long Amino-Feed UV study.

Variable	Technique	Further details (intended number, unit-size and frequency per plot)
Mobile beneficial and pest arthropod densities	Beat sheet	5 one-metre samples every 7 days
Aphids and whitefly densities	Part-plant visual inspection	One main-stem leaf from upper-, middle- and lower-third strata location on 10 plants every 14 days
Heliothis egg and larval density	Whole-plant visual inspection	4 half-metre samples every 3-4 days
Heliothis egg mortality rates	Sentinel egg cards	10 egg cards exposed for 48 h every 7 days
Fruit counts	Visual whole-plant inspection	2 to 4 half-metre units every 14 days; first and second position node fruit only
Yield measures	Hand harvest and gin	4 half-metre units at end of season; all fruit positions

To examine the effect of Amino-Feed UV, we compared beneficial and pest arthropod densities, yield measures, fibre quality measures. We predicted that the Amino-Feed UV treated areas would have a higher density of beneficial arthropods and inturn have a lower density of pest arthropods, reflected in increased mortality rates of heliothis eggs on cards. These changes were predicted to result in an increased number of open bolls and higher yield, and possibly enhanced fibre quality compared to the untreated plots (Nemec 2001; Mensah & Singleton 2002). We used a series of ANOVA and contingency table tests to analyse the data.

Results

We found that multiple applications of Amino-Feed UV had no significant effect ($P > 0.05$) on the density of each category of beneficial and pest arthropods across all sampling dates (Figure 1). Multiple applications of Amino-Feed UV had no significant effect on densities of aphids and whitefly (Table 2) or heliothis eggs (Figure 2; analysis not valid for larvae). The fate of sentinel heliothis eggs on cards was not significantly affected by multiple applications of Amino-Feed UV (Figure 3).

The numbers of squares and flowers, closed bolls, open bolls, all fruit and potential fruit, and all fruit as a proportion of potential fruit, in the first and second position nodes were not significantly affected by multiple applications of Amino-Feed UV (Figure 4). All yield (Table 3) and fibre quality (Table 4) measures were not significantly affected by multiple applications of Amino-Feed UV.

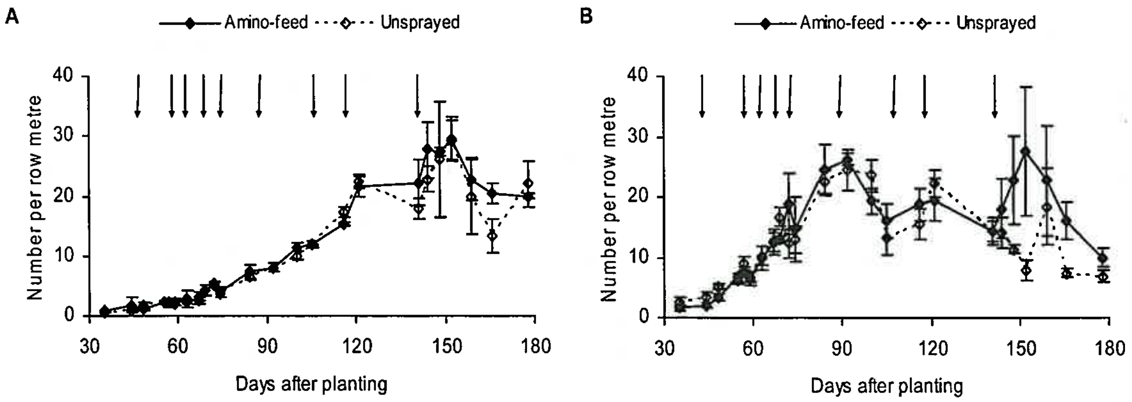

Figure 1. Variation in the mean (± SE) density per metre of all beneficial (A) and all pest arthropods (B) over the growing season for Amino-Feed UV treated and untreated cotton. Vertical downward arrows represent Amino-Feed UV application dates.

Table 2. Variation in mean (± SE) density per main-stem leaf of aphids (winged and non-winged forms combined) and whitefly (nymphs and adults combined) over the growing season for Amino-Feed UV treated and untreated cotton.

Growth stage	DAP	Aphids		Whitefly	
		Amino-Feed UV	Unsprayed	Amino-Feed UV	Unsprayed
Early boll filling	83	1.1 (0.2)	0.4 (0.3)	.	.
Mid boll filling	98	1.8 (0.6)	0.7 (0.2)	0.0 (0.0)	0.0 (0.0)
Mid boll filling	107	1.5 (0.2)	0.4 (0.0)	0.4 (0.1)	0.2 (0.0)
Late boll filling	116	0.5 (0.1)	0.4 (0.1)	0.1 (0.1)	0.1 (0.0)
Late boll filling	121	1.7 (0.4)	0.6 (0.4)	0.2 (0.0)	0.2 (0.1)
Early boll opening	135	1.7 (0.4)	1.3 (0.2)	0.2 (0.1)	0.5 (0.4)
Early boll opening	144	2.2 (0.1)	1.7 (0.8)	0.3 (0.0)	0.3 (0.1)
Mid boll opening	154	2.7 (0.8)	5.1 (1.9)	0.3 (0.1)	0.5 (0.2)
Mid boll opening	166	2.6 (0.7)	8.4 (3.5)	0.2 (0.1)	0.3 (0.0)
Late boll opening	178	3.0 (0.8)	9.9 (2.3)	0.2 (0.0)	0.2 (0.1)

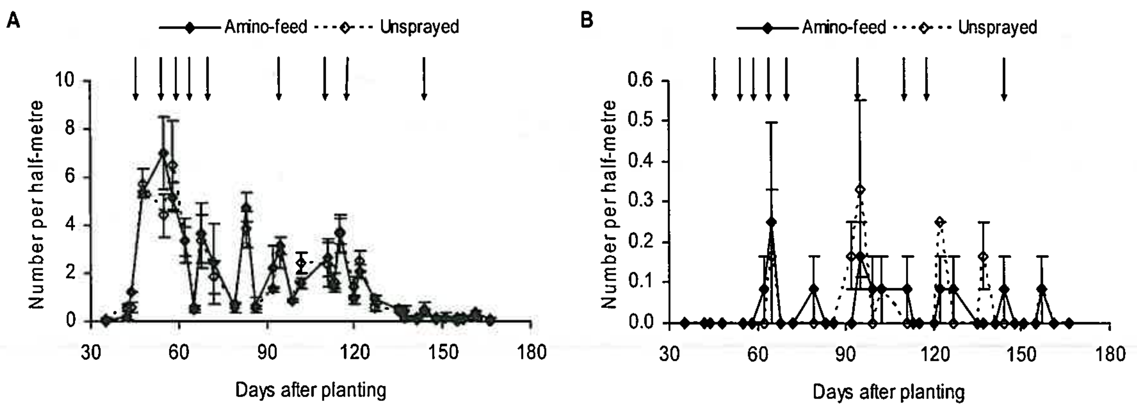

Figure 2. Variation in mean (± SE) density per half-metre of heliothis eggs (A; white and brown stages) and larvae (B; small to large size classes) over the growing season for Amino-Feed UV treated and untreated cotton. Vertical downward arrows represent Amino-Feed UV application dates.

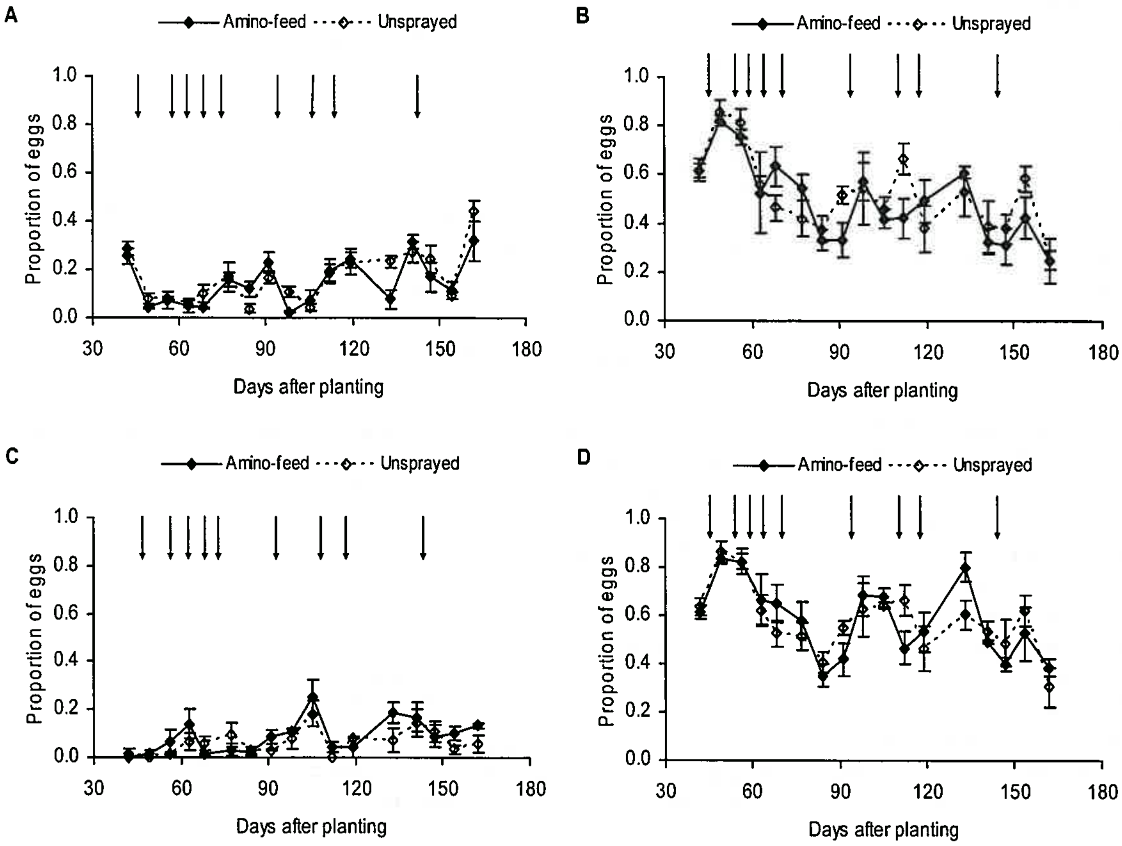

Figure 3. Variation in the mean (± SE) proportion of sentinel heliothis eggs on cards that were hatched (A), predated (B), parasitised (C), or attacked by all beneficial arthropods (predated + parasitised; D) after 48 hours exposure in the field at different times of growing season for Amino-Feed UV treated and untreated cotton. Data not shown for collapsed eggs. Egg card samples each contained a mean of 26.4 eggs (range 8 to 88). Vertical downward arrows represent Amino-Feed UV application dates.

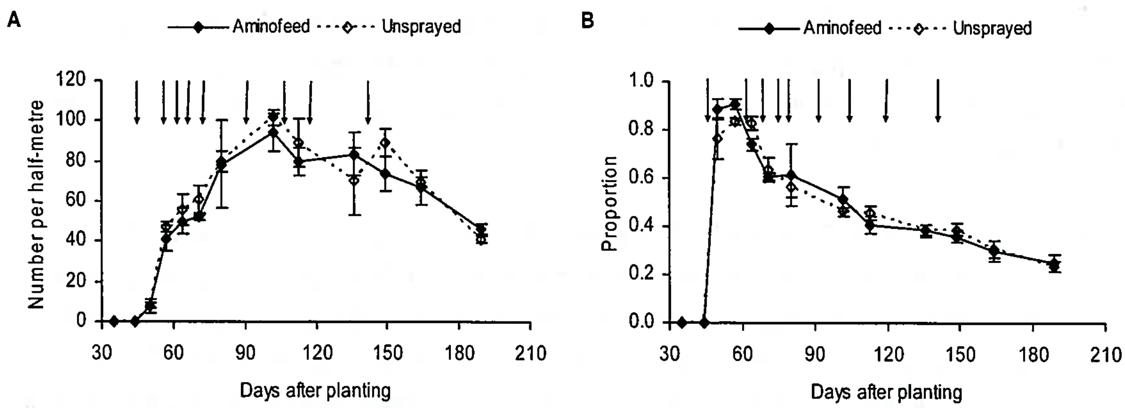

Figure 4. Variation in the mean (± SE) number per half-metre of all fruit (A), and all fruit as a proportion of potential fruit (B) over the growing season for Amino-Feed UV treated and untreated cotton. Data not shown for squares and flowers, closed bolls, open bolls, and potential fruit. Vertical downward arrows represent Amino-Feed UV application dates.

Table 3. Variation in mean (± SE) value per half-metre of various yield measures at conclusion of the growing season for Amino-Feed UV treated and untreated cotton.

Measure	Amino-Feed UV	Unsprayed
Squares + flowers	0.2 (0.2)	0.0 (0.0)
Closed bolls	9.3 (4.4)	4.2 (1.1)
Open bolls	48.6 (4.3)	49.4 (1.7)
All fruit	58.1 (2.8)	53.6 (2.2)
Potential fruit	250.8 (37.6)	238.1 (16.1)
Proportion actual fruit	0.24 (0.03)	0.23 (0.01)
Seed cotton mass (g)	223.9 (30.5)	210.7 (14.0)
Lint mass (g)	98.3 (15.4)	91.0 (5.5)
Proportion lint mass	0.44 (0.01)	0.43 (0.00)
Yield (bales per ha)	8.7 (1.4)	8.0 (0.5)

Table 4. Variation in mean (± SE) value of various fibre quality measures at conclusion of the growing season for Amino-Feed UV treated and untreated cotton.

Measure	Amino-Feed UV	Unsprayed
Length (hundredths of inch)	1.18 (0.02)	1.16 (0.01)
Length uniformity index*	84.3 (0.6)	83.5 (0.4)
Short fibre index†	6.1 (0.6)	7.1 (0.7)
Strength (g/tex)	31.8 (0.7)	32.3 (1.0)
Elongation index‡	4.0 (0.2)	3.9 (0.1)
Fineness√	4.7 (0.1)	4.6 (0.0)
Reflectance colour value (Rd)	80.5 (0.5)	80.8 (0.8)
Yellowness colour value (+b)	7.1 (0.4)	6.9 (0.1)

*ratio of mean length to upper half mean length; †percentage of fibres less than 0.5 inches long; ‡as a percentage of overall length; √fibre mass per unit length (µg per inch)

Discussion

We found that the density of beneficial and pest arthropods, mortality rates of sentinel heliothis eggs on cards, fruit count, crop yield and fibre quality were unchanged in areas treated with nine applications of Amino-Feed UV. The non-significant results indicate the added cost of Amino-Feed UV treatment was not justified. The additional cost of treatment with Amino-Feed UV was calculated at $243 per ha, based on $12 per ha for the product, $15 per ha for ground-rig application, and nine applications. The slightly higher, but non-significant yield in the Amino-Feed UV treatment was attributed to increased plant growth on the edges of the field in two out of three plots for this treatment.

In an unreplicated trial, Nemec (2001) reported that three applications of Amino-Feed to cotton over a three week period mid-season increased densities of big-eyed bugs, Pacific damsel bugs, pirate bugs, all predatory bugs, spiders, other predators and all predators, but not red-and-blue beetles, lady beetles or all predatory beetles. In two trials, Mensah & Singleton (2002) determined that four or approximately eight (not specified in one trial) applications of Amino-Feed to cotton at 7 to 10 day intervals throughout the early- to mid-season significantly increased densities of red-and-blue beetles and lacewings in both trials, and big-eyed bugs and transverse ladybeetles in one trial, but Pacific damsel bugs and spiders were unchanged. In both of the trials, the density of heliothis eggs was significantly higher, not lower as expected, and larval density was significantly lower in one trial (Mensah & Singleton 2002); this was not found in our study here.

To place the results of our study in context, we used a hierarchical approach to review studies on the performance of artificial food supplements, where success is likely harder to achieve at successive levels of the hierarchy (details of studies reviewed are not listed for brevity). Altered foraging behaviour of beneficial arthropods is the lowest level of success. Consumption of the supplement by beneficial arthropods is the second level and an increase in density is the third. Improved fitness of beneficial arthropods, such as lifespan and fecundity, is the fourth level. Increased predation or parasitism rates comprise the fifth and a reduction in pest densities or plant damage levels is the sixth. Pest densities or plant damage maintained below economic threshold is the seventh level, and finally, increased profitability, the ultimate goal of any biological control programme, is the eighth.

'Not tested' was used to denote where a particular level in hierarchy was not examined or reported (see Table 5). For studies that compared more than one treatment, a successful result was recorded provided at least one of the reported treatments fulfilled the requirements of a given hierarchical level for any beneficial arthropod taxon. An unsprayed or water-sprayed treatment must have been included as a control for inclusion of an article in the review (e.g., work in which sucrose controls were used is excluded), although a conventionally managed treatment may be more appropriate than water for comparing profitability (e.g., Mensah 2002a). Treatments that used food supplements along with insecticides in mixtures or separately were excluded (e.g., Mensah 2002b), unless there was an otherwise identical insecticide programme for comparison, as the effect of the food supplement was confounded, although such treatments may useful in an IPM programme.

We reviewed a total of 82 trials in 38 publications on artificial food supplements. The number of trials that applied artificial food supplements on one (n = 41) or more (n = 40) occasions was similar, and only a single trial compared both applications (i.e., McEwen & Kidd 1995). The distribution of trials does not reflect a view that multiple applications are potentially important (McEwen & Kidd 1995). When food supplements were applied once, 26 trials reported at least one positive attribute across all hierarchical levels, while the remaining 15 were not positive. When food supplements were applied more than once, 33 trials reported at least one positive attribute across all hierarchical levels, while the remaining seven were not positive. A positive response was recorded in the only trial where the two applications were compared. The likelihood of either outcome does not significantly differ between single and multiple application schedules.

The majority of trials tested a mixture of carbohydrate (e.g., sugar) and protein (e.g., yeast) materials (n = 59), followed by carbohydrate only (n = 15), protein only (n = 4) and carbohydrate or protein but not together (n = 4). It may be important to use carbohydrate and protein mixtures such as Amino-Feed®, as Hagen (1986) attributed unchanged densities of beneficial arthropods, reported in Shands *et al.* (1972) and Hagley & Simpson (1981), to the non-combining carbohydrate and protein sources. However, there are several examples where carbohydrate and protein sources were successfully used alone (e.g., Evans & Richards 1997). With carbohydrate alone, there were 12 trials that reported at least one positive attribute across all hierarchical levels, while the remaining three gave no positive result. When protein was used alone, there were three trials that reported at least one positive attribute, while the remaining trial was not positive. When carbohydrate or protein was used there were four trials that reported at least one positive attribute. Finally, when carbohydrate and protein mixtures were used, there were 41 trials that reported at least one positive attribute, while the remaining 18 were not positive. The likelihood of positive and not positive outcomes does not significantly differ between carbohydrate only and carbohydrate and protein mixtures.

Trials that test combinations of carbohydrate and/or protein materials provide a better indication of the importance of food supplement materials. However only five out of 59 trials that use carbohydrate and protein mixtures have adopted this approach, and the results of these studies do not always support each other (Nichols & Neel 1977; Evans & Swallow 1993; McEwen & Kidd 1995). For example, the longevity of adult green lacewings, *Chysoperla carnea* Stephens, was highest with a diet of carbohydrate alone, whilst a carbohydrate and protein diet produced the highest daily and lifetime fecundity (McEwen & Kidd 1995). The materials contained in artificial food supplements need to be critically assessed, as treatment interactions can occur and results may vary between trials, focal taxa and various measurements.

The hierarchical levels most commonly examined in the published work were the abundance of beneficial (n = 51 trials) and pest (n = 26) arthropods. Although the abundance of beneficial arthropods increased in 34 trials and the abundance of pest arthropods declined in 14, there was no change in profitability for the four trials that examined it (Table 5). All levels of the hierarchy have never been simultaneously examined in the one trial or publication. Aspects of the research questions, methodology and results published to date need further consideration to improve the efficacy and utilisation of artificial food supplements. A clear explanation is required of how food supplements function, as there was inconsistency between the aims and expectations of the trials. Is the supplement actually consumed (i.e., acts as an actual 'food supplement'), or does it mediate changes in behaviour without ingestion? Ingestion of a food supplement has only been demonstrated in four trials (Stephen & Browne 2000; Beach *et al.* 2003). Further, is the aim of the supplement to mimic the nutritional value of the prey/host and/or plant resources such as nectar and pollen (e.g., determination of host frass feeding in Beach *et al.* 2003)? Alternatively, is the aim to mimic properties of prey/host that elicit an oviposition or host finding response (e.g., aphid 'juice' used in Ben Saad & Bishop 1976)? If an artificial food supplement stimulates foraging behaviour through contact alone (McEwen *et al.* 1993), but not by feeding, then no immediate nutritional advantage would be conferred, and unproductive searching may occur in areas where food or prey were absent. However, this category of effect may lead to an accumulation of natural enemies where and when required. As pointed out in the description of the hierarchy above, this aggregation may not lead to any impact on prey/host populations.

Table 5. Number of artificial food supplement trials with various outcomes at successive hierarchical levels.

Outcome	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
Positive	6	4	34	7	1	14	2	0
Positive & negative	0	0	5	0	0	3	0	0
Negative	0	0	0	0	1	1	5	4
Non significant	2	0	12	11	1	8	0	0
Not tested	74	78	31	64	79	56	75	78

Methodological problems associated with the use of food supplements have not been analysed fully here, but should be the subject of future analysis and research. These problems can be categorised as 'what', 'when', 'where' and 'how'. 'What' concerns the ingredients in the food supplement; the type, concentration and volume applied. 'When' includes the frequency of application and ideal time of day or part of the growing season to apply the supplement. 'Where' involves placement of the food supplement in relation to vegetational strata and the role of within- and between-field dynamics. Lastly, 'how' concerns aspects of application method and experimental design that warrant closer attention.

The use of artificial food supplements needs to be refined so that positive effects are maximised and deleterious effects are minimised. Potential negative effects include increased pest densities (McEwen & Morris 1998; Mensah & Singleton 2002); decreased prey consumption as the insect 'switches' between arthropod prey and artificial food sources, or because the duration of a life-stage was reduced, which consequently lowered lifetime, but not daily consumption rates (McEwen *et al.* 1996). Crop damage is a possible side-effect, such as stunted growth (Mensah 1997) and leaf fungal diseases (Ben Saad & Bishop 1976; McEwen & Kidd 1995). The lack of tangible evidence for increased profitability may ultimately explain why only 0.03 and 0.14 applications of artificial food supplements are currently applied each growing season to *B.t.* and non-*B.t.* cotton crops respectively (Doyle *et al.* 2002). A better understanding of the effect that artificial food supplements has on beneficial and pest arthropods is necessary to improve the value and utilisation of this IPM tactic. Specifically, manipulative studies will be needed to examine foraging behaviour prior to and after exposure to a food supplement, and the consequences this has on predation/parasitism rates. Until such time, the understanding of the ecological basis of the use of artificial food supplements will remain in its infancy.

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