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Impact of neonicotinoid seed treatment of cotton on the cotton leaf hopper, *Amrasca devastans* (Hemiptera: Cicadellidae), and its natural enemies

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ABSTRACT

BACKGROUND: Neonicotinoid seed treatments suppress populations of pest insects efficiently, and can enhance crop growth, but may have negative effects on beneficial arthropods. We evaluated effects of either imidacloprid or thiamethoxam on the abundances of a sucking pest, the cotton leafhopper (*Amrasca devastans*), and its arthropod predators under field conditions. We also evaluated the impact of seed treatment on transgenic cotton plant growth, with pests and natural enemies present or absent.

RESULTS: Imidacloprid and thiamethoxam reduced pest abundance, with greater effects when dosages were higher. Treatment at recommended doses delayed the pest in reaching the economic damage threshold by around 10-15 days (thiamethoxam) and 20 days (imidacloprid). Recommended doses also enhanced plant growth under all tested conditions; growth is affected directly as well as via pest suppression. Neonicotinoid applications reduced abundance of beneficial arthropods, with lower populations after higher doses, but negative effects of imidacloprid were not apparent unless the manufacturer-recommended dose was exceeded.

CONCLUSION: Imidacloprid applied at the recommended dose of 5g/kg seed is effective against *A. devastans* and appears to be safer than thiamethoxam for natural enemies, and also enhances plant growth directly. We caution, however, that possible sub-lethal negative effects on individual beneficial arthropods were unevaluated.

Key words: Imidacloprid, thiamethoxam, neonicotinoid seed treatment, cotton leaf hopper, *Chrysoperla carnea*, *Geocoris*, coccinellids, plant growth parameters

1 INTRODUCTION

Modern seed treatment products, focused against insect pests or fungal pathogens, were introduced in the 1970s and 1980s.[21,46] Insecticidal treatment of seeds directly protects crops from early season foliar pests and from seed or root feeders. Seed treatment has become common in agriculture as, compared to traditional foliar application, it has lower financial costs,[52] requires less active ingredient and reduces exposure to non-target organisms.[5,46] Further, seed treatment can provide efficient pest control in situations where crop phenology prohibits foliar applications[30] or in conditions where management timing is crucial but difficult.[8,45]

The development of the neonicitinoid group of insecticides led to increased use of seed treatment in row crops.[15,18] Active ingredients of neonicotinoids are taken up by roots during germination and move systemically within the plant, protecting the growing plant from insect pests.[30,40] Imidacloprid and thiamethoxam are chloronicotinyl insecticides that are agonistic at the nicotinic acetylcholine receptor and interfere with the transmission of impulses in the insect nervous system.[14] Due to their mode of action, they can combat a number of sucking pests on various agricultural crop plants. They have been used successfully against the early pest complex in sugar beet, vegetables, maize and other crops.[25,30,42] For example, imidacloprid and thiamethoxam treatment provides protection against *Amrasca devastans* (Dist.) on okra (*Abelmoschus esculentus* L.)[24] and against *Cerotoma furcate* Forster (Coleoptera: Chrysomalidae) on snap bean (*Phaseolus vulgaris* L.).[23] Field studies have shown that both of these compounds can provide adequate protection against early-season sucking pests of cotton (*Gossypium hirsutum* L.), including *Bemisia tabaci* (Gennadius), *Thrips tabaci* (Linderman), *Aphis gosypii* (Glover) and *A. devastans*.[13,31,52,53] In addition to providing protection against sucking pests, these seed treatment insecticides are reported to enhance plant growth.[29]

Since the introduction of bollworm resistant Bt cotton in 2005, the cotton bollworm (*Helicopverpa armigera* Hübner) has been brought under control in many Asian countries.[37,42] The cotton bollworm is a chewing pest but sucking pests are not susceptible to Bt toxins and thus remain a threat.[37] The cotton leaf hopper, or Jassid, *Amrasca devastans* (Dist.) (= *Amrasca biguttula biguttula* (Ishida))[17] (Hemiptera: Cicadellidae), is one of the most devastating early-season sucking pests of cotton and eggplant (*Solanum melongena* L.),[35,51] with estimated seed-cotton losses averaging 37% in Pakistan.[3] *Amrasca devastans* sucks the cell sap from the underside of the leaves, inducing downward curling and injects phytotoxic saliva into the host plant. Severe damage causes uneven and stunted cotton plant growth, the shedding of squares and bolls along with deterioration of fibre quality.[22,28]

Farmers rely heavily on chemical control to manage *A. devastans*.[4] Direct application of insecticide to *A. devastans* is hindered by the fact that females lay eggs inside host plant leaf veins.[1] Seed treatment is thus an effective method for systemically delivering insecticide to the locality of *A. devastans* eggs. Nonetheless, the sole reliance on insecticides may cause undesired effects in the form of insecticidal resistance by *A. devastans* and/or the mortality of its arthropod natural enemies.[32,38,44] For instance, increased use of neonicotinoid seed treatments has resulted in substantial increases in spider mite (*Tetranychus sp*.) populations across southern Mississippi, USA, by killing natural enemies.[43] Further, in Pakistan, due to over-use of insecticides, *A. devastans* developed resistance against foliar formulations of pyrethroids in the 1990s[2] and some resistance against foliar formulations of neonicotinoids has recently been recorded.[7] Thus, the frequent use of cotton seed treatment insecticides, such as imidacloprid and thiamethoxam, may ultimately affect their efficacy against *A. devastans*.

Insecticidal treatment may also incur side-effects on non-target arthropod predators (beneficial natural enemies) that occur within the transgenic cotton agro-ecosystem[33,34]. Here we evaluate the efficacy of imidacloprid and thiamethoxam seed treatments at different dosages, including the recommended dose rates, for managing *A. devastans* and also their impact on natural enemies. We also evaluate the effect of these insecticides on seed germination and on cotton plant growth in both the presence and absence of *A. devastans*.

2 MATERIALS AND METHODS

Our experiments used seeds of transgenic cotton (Bt-CIM-599). The evaluated insecticides were imidacloprid (Confidor 70 WS, Bayer Crop Science) and thiamethoxam (Actara ST 70 WS, Syngenta). The manufacturer-recommended doses for their application are 5g/kg cotton seed for imidacloprid and 3g/kg seed for thiamethoxam.

2.2 Effect of insecticide dose on arthropod populations

2.2.1 Seed treatment

Each insecticide was tested separately and at four dosages; specifically 0.5×, 1×, 1.5× and 2× its recommended dose. Before insecticidal application, acid delinted (using concentrated H_2SO_4 at 100 ml/kg seed) cotton seeds were soaked in tap water for 30 min, to remove the acid, and then dried on sieves. Imidacloprid or thiamethoxam was then mixed into 200 ml of water in separate containers. Cotton seeds were then placed in bowls and shaken vigorously with an insecticide solution (imidacloprid at 2.5, 5.0, 7.5 and 10 g/kg seed, thiamethoxam at

1.5, 3.0, 4.5 and 6 g/kg seed) for five minutes then spread on plastic sheets to dry. Seeds for a control treatment (without insecticide) were prepared as above but shaken with water rather than an insecticide solution. The 9 experimental treatments were thus the four doses of imidacloprid, the four of thiamethoxam and the control.

2.2.2 Experimental design

Field experiments were conducted in both 2010 and 2011, between mid-May (sowing) and late October (harvest) under semi-arid climatic conditions on silt loam soils at the Central Cotton Research Institute, Multan, Pakistan.

Seeds were planted in the bed and furrow method, via manual dibbling. Seeds were used at a rate of approximately 23 kg per hectare. Experiments were laid out in a randomized block design comprising one replicate plot of each of the nine treatments within each of three blocks. Each plot was an area of 9.15m × 4.57m, with 0.25m between plants and 0.83m between rows within plots. Plots were 1.2m apart and blocks were 3.0m apart, with spaces between plots and blocks left fallow.

2.2.3 Population sampling

Sampling for *A. devastans* and its predators began two weeks after sowing. Once *A. devastans* was seen to be present, data were recorded following Razaq *et al.*[36]: every five days and within each sampling site, 10 plants per replicate were randomly selected and one apical leaf, one mid-plant leaf and one leaf from the lower part of each plant were inspected. The random selection of plants was repeated at each visit. The numbers of *A. devastans* per leaf found within each replicate on each visit were used as the estimators of population abundance. Predator abundance was estimated by counting the numbers of predatory arthropods (insects and spiders) present on 5 whole plants from each replicate on each visit.

2.3 Effect of insecticide on cotton germination and growth

2.3.1. Germination

The effect of seed treatment on the probability of seed germination was evaluated by treating seeds with the manufacturer-recommended doses of thiamethoxam (3 g/kg) or imidacloprid (5 g/kg) or with the no-insecticide control. In each replicate, a hundred seeds were wrapped in a paper towel[50] and the number of seeds that germinated was subsequently counted. There were 6 replicates of each treatment.

2.3.2 Field growth

At 30 and 40 days after sowing in the field (with *A. devastans present*), two plants were removed gently from each plot in which seeds had been treated with the manufacturer-recommended doses of thiamethoxam (3 g/kg), imidacloprid (5 g/kg) and from the control plots. In the laboratory, plants were washed with water to remove the soil and then spread on paper. For each removed plant, the number of leaves per plant was counted, and the root length and stem length measured.

2.3.3 Greenhouse growth

We used a greenhouse to obtain plant growth estimates in the absence of *A. devastans*. Seeds were treated with the manufacturer-recommended doses of imidacloprid or thiamethoxam, or were untreated (control) following methods described above. Seeds were then sown in soil (silt loam) in plastic pots, with four seeds per pot and ten pots per treatment. Pots were placed in a greenhouse at CCRI, Multan, in May 2012. Plants were watered daily, as required. Conventional NPK fertilizer was applied to each pot three times during the experiment. After 10, 20, 30 and 40 days, six plants from each treatment were removed gently, washed with water and spread on paper. Root and stem lengths were measured and the numbers of leaves counted. After each observation day, pots containing fewer than four plants were discarded to remove confounding influences of variation in interplant competition.

2.4 Statistical analysis

All statistical tests were carried out using Genstat software (VSN International, Hemel Hempstead, UK). We used general linear models (GLMs)[10,16] to explore effects of dosage of imidacloprid or thiamethoxam on the numbers of *A. devastans* and of beneficial insects present and also to examine patterns of seed germination and cotton plant growth. For analyses of *A. devastans* and predator seasonal totals we treated data on according to the randomized block design (i.e. ANOVAs and ANCOVA's with blocking). Repeated measures ANOVAs and ANCOVAs were further employed for analyses of within-season pest sample data and for analyses of cotton plant growth.

3 RESULTS

3.1 Effect of insecticide dose on arthropod populations

3.1.1 Amrasca devastans

The overall numbers (seasonal totals) of *A. devastans* present were greater in 2010 than in 2011 (4,360 *vs*. 3,394; $F_{1,50} = 19.58$, $p < 0.001$); so further analyses of pest abundance were carried out separately for each year. In both years, around half as many *A. devastans* were present when insecticide had been applied to seeds than when it had not (ANOVA: 2010, $F_{1,23}$ = 48.87, p < 0.001; 2011, $F_{1,23}$ = 145.83, p < 0.001). When insecticide had been applied (i.e. with control treatment data excluded), the dose applied to the seeds influenced *A. devastans* seasonal totals; fewer *A. devastans* were present when doses (g/Kg) were higher (ANCOVA: 2010, $F_{1,19}$ = 47.77, p < 0.001; 2011, $F_{1,19}$ = 49.32, p < 0.001). The type of insecticide applied (imidacloprid or thiamethoxam) had no significant influence on *A. devastans* numbers in 2010 (ANCOVA: $F_{1,19}$ = 1.59, p = 0.223) but in 2011 seasonal totals were lower for a given dose (g/Kg) of thiamethoxam than for imidacloprid (ANCOVA: $F_{1,19}$ = 23.60, p < 0.001). These patterns in seasonal pest totals are illustrated in Figure 1.

Repeated measures ANCOVAs, excluding control data, confirmed that the numbers of *A. devastans* present varied within each of the two growing seasons (2010: $F_{7,154}$ = 47.52, p < 0.001; 2011: $F_{7,154}$ = 167.69, p < 0.001; Greenhouse-Geisser epsilon = 0.182 for 2010 and = 0.3354 for 2011). Low numbers appeared after 20-25 days after sowing, with first appearances being earlier when sees were untreated (control) or received the lowest does of imidacloprid (2.5 g/Kg) or thiamethoxam (1.5 g/Kg) (Fig. 2). Numbers of *A. devastans* then typically increased over time, peaking after 50 days (2010) and at 55 days (2011). The effect of insecticide dose on *A. devastans* numbers, which is illustrated for seasonal totals in Figure 1, can also be seen in Figure 2: within each year, the numbers of *A. devastans* present were almost always lowest on plants growing from seeds with the highest doses (g/Kg) of insecticide applied (represented by thickest lines), as confirmed by the repeated measures analyses (effect of insecticide dose fitted as a covariate, 2010: $F_{1,19}$ = 47.76, p < 0.001; 2011: $F_{1,19}$ = 49.32, p < 0.001). These analyses also confirmed that in 2010 the type of insecticide applied had no significant influence on *A. devastans* numbers (imidacloprid or thiamethoxam: $F_{1,19}$ = 0.50, p = 0.489, Insecticide type × days after sowing interaction: $F_{7,154}$ = 3.17, p =

0.078) but in 2011 pest numbers were lower when thiamethoxam rather than imidacloprid was applied at a given dose ($F_{1,19}$ = 7.53, p = 0.013) although there was no significant interaction between insecticide type and the number of days after sowing ($F_{7,154}$ = 2.68, p = 0.069).

3.1.2 Predators

There was no difference in the mean number of predators sampled per visit in 2010 and 2011 (exactly 1964 individuals were found in each year: ANOVA: $F_{1,52}$ = 0.00, p = 1.0) and no significant interaction between year and the experimental treatment (Factorial ANOVA: $F_{8,34}$ = 0.47, p = 0.872); so predator data from the two years were analysed collectively.

There were fewer predators present when insecticide had been applied to seeds than when it had not (ANOVA: $F_{1,50}$ = 9.12, p < 0.004). When insecticide had been applied (i.e. with control treatment data excluded), the higher the dose (g/Kg) of insecticide applied, the fewer predators were present overall (ANCOVA: $F_{1,43}$ = 273.11, p < 0.001) and for a given dose, there were fewer predators present when thiamethoxam was used rather than imidacloprid (ANCOVA: $F_{1,43}$ = 150.80, p < 0.001). We separately explored the effects of dose of each chemical on the total numbers of each type of predator: in every case predator numbers declined significantly (p<0.001) with insecticide dose (for imidacloprid: Total, $F_{1,26}$ =109.34; *Chrysoperla*, $F_{1,26}$ =99.37; Spiders, $F_{1,26}$ =105.88; *Orius*, $F_{1,26}$ =91.4; Coccinelids, $F_{1,26}$ =40.11; *Geocoris*, $F_{1,26}$ =45.27; for thiamethoxam: Total, $F_{1,26}$ =326.64, *Chrysoperla*, $F_{1,26}$ =217.85; Spiders, $F_{1,26}$ =262.34; *Orius*, $F_{1,26}$ =330.01; Coccinelids, $F_{1,26}$ =56.22, *Geocoris*, $F_{1,26}$ =48.95). Patterns in seasonal pest totals are illustrated in Figure 3.

3.2 Effect of insecticide on cotton germination and growth

3.3.1. Germination

The overall probability of seed germination was 0.869 (+SE = 0.013, -SE = 0.014) and this did not differ significantly between replicates treated with imidacloprid, thiamethoxam or the control (logistic ANOVA[10,16]: $F_{2,15} = 0.92$, P = 0.422).

3.2.2 Field growth

The lengths of cotton plant roots and shoots and the numbers of leaves on the plants all increased between 30 and 40 days after sowing (repeated measures ANOVAs: Root length: $F_{1,15} = 82.84$, P<0.001; Shoot length: $F_{1,15} = 181.44$, p < 0.001; Number of leaves $F_{1,15}$ = 18.859, p < 0.001, Fig. 4). Roots, shoots and leaves were also affected by seed treatment (respectively, $F_{2,13} = 73.64$, p < 0.001; $F_{2,13} = 458.95$, p < 0.001; $F_{2,13} = 219.30$, p < 0.001); plants treated with the recommended dose of imidacloprid had longer roots and shoots and more leaves than those treated by the recommended dose of thiamethoxam, and untreated plants had the shortest roots and stems and the fewest leaves (Fig. 4) (the numbers of *A. devastans* that were present are shown in Fig. 2). There were also positive interactions between seed treatment and time for shoot length ($F_{2,15} = 13.41$, p < 0.001) and between seed treatment and time for leaf number ($F_{2,15} = 44.63$, p < 0.001) but no significant interaction between seed treatment and time for root length ($F_{2,15} = 0.14$, p = 0.873): plants treated with imidacloprid had notably the longest shoots and most leaves 40 at days after sowing (Fig. 4).

3.2.3 Greenhouse growth

The lengths of cotton plant roots and shoots and the numbers of leaves on the plants all increased between 10 and 40 days after sowing (repeated measures ANOVAs: Root length: $F_{3,45} = 1448.27$, p < 0.001; Shoot length: $F_{3,45} = 1163.82$, p < 0.001; Number of leaves $F_{3,45}$ =

1525.96, $p < 0.001$, Fig. 5). Roots, shoots and leaves were also affected by seed treatment (respectively, $F_{2,13} = 137.84$, $p < 0.001$; $F_{2,13} = 424.63$, $p < 0.001$; $F_{2,13} = 61.36$, $p < 0.001$); plants treated with the recommended dose of imidacloprid or thiamethoxam had longer roots and shoots and more leaves than untreated plants (Fig. 5). There was also a positive interaction between seed treatment and time for shoot length ($F_{6,45} = 17.42$, $p < 0.001$) but no significant interaction for root length ($F_{6,45} = 0.48$, $p = 0.774$) or for leaf number ($F_{6,45} = 2.71$, $p = 0.056$): plants treated with the recommended dose of imidacloprid or thiamethoxam had greater increases in shoot length than untreated plants (Fig. 5).

4 DISCUSSION

Our results re-affirm that insecticidal seed treatments can reduce the incidence of *A. devastans* during the early growth stages of cotton crops.[39,47,48] Dhawan *et al.*[13] found equivalent effects of thiamethoxam and imidacloprid against *A. devastans*: our 2010 data similarly indicate that the overall response of *A. devastans* to insecticide dose is the same for these insecticides. However, our 2011 data indicate that, at a given dose (g/Kg), thiamethoxam has a greater suppressive effect than imidacloprid. In terms of the effects of applying these insecticides at their manufacturer-recommended doses, the 2010 data indicate that imidacloprid would achieve the greater suppression (because the recommended dose is 2 g/Kg higher than that of thiamethoxam) and the 2011 data indicate that the two pesticides would result in similar numbers of *A. devastans* being present during the season overall.

Pest abundance increased throughout the growing season in both years and exceeded the economic threshold level (ETL) for damage (one *A. devastans* per leaf)[3] before harvest in both years and under all experimental treatments. Treatment did, however, affect the time taken for the ETL to be reached, with duration of protection increasing with increasing insecticidal dose (as also reported by Nault *et al.*[30]). *Amrasca devastans* numbers on untreated (control) plants, and on plants treated with the lowest doses of thiamethoxam (1.5 g/Kg) or imidacloprid (2.5 g/Kg), reached the ETL at around 25 days after sowing in both years. Treatment with the recommended dose of thiamethoxam (3 g/Kg) resulted in the ETL being reached after around 30 days and the recommended dose of imidacloprid (5 g/Kg) suppressed *A. devastans* below the ETL until around 40 to 45 days after sowing. Our results support the recent report from Egypt that imidacloprid has a greater potential than thiamethoxam to control *A. devastans* during the early growth stages of cotton plants.[53] Differences in the effect of these insecticides are potentially due to the development of greater resistance by *A. devastans* to thiamethoxam than to imidacloprid but we know of no direct evaluations of this. For instance, tobacco thrips (*Frankliniella fusca*) have developed resistance to thiamethoxam, but applications of imidacloprid still provide effective management in Arkansas and the mid-south of the USA.[27] The differences in pest populations between the two years in which the field experiment was carried out further indicate that many environmental, especially meteorological, factors may influence the degree of pest control that insecticidal application can provide.[52]

We found that insecticidal application to seeds affected the subsequent abundances of beneficial predatory arthropods in the cotton crop. It is unlikely that this result is due to avoidance of seed-treated plants because systemically present neonicotinoids appear to be undetectable to predators[33]. Moreover, thiamethoxam and imidacloprid are known to be toxic to many predatory invertebrates, including species of *Geocoris*, *Orius* and coccinellids.[5,33,34] The most likely mechanism of exposure is consumption of leaf hoppers that have themselves consumed a neonicotinoid,[34] although exposure to plants grown from treated seeds can also be lethal for coccinellids and *Orius* that feed directly on leaf tissue as well as acting as predators.[5, 33] In general, higher doses of insecticide led to lower populations of predators but the negative effects of imidacloprid were not apparent unless the manufacturer-recommended dose (5 g/Kg) was exceeded. In contrast, the recommended dose of thiamethoxam (3 g/Kg) reduced the abundance of beneficial arthropods to approximately two-thirds of the numbers observed in plots untreated with pesticide. This accords with the findings of Seagraves and Lundgren[39] that thiamethoxam, but not imidacloprid, application was associated with a reduction in a community of generalist predators in the soybean agro-ecosystem. Even when application of insecticide does not affect the abundance of natural enemies (e.g. doses of imidacloprid $\leq$ 5 g/Kg) there may be indirect negative effects on predators via a reduction in the abundance of their prey and also via sub-lethal effects on the performance of individual predators.[19,26,33,34]

Treating seeds with the manufacturer-recommended doses of imidacloprid and thiamethoxam did not affect seed germination rates, showing that these insecticides are not phytotoxins. Similar findings have been reported when these chemicals have been applied to oil palm (*Elaeis guineensis* Jacq.) seeds,[9] and in rice thiamethoxam can enhance the proportions of seeds that germinate.[6] Moreover, we found that application of thiamethoxam and imidacloprid enhanced the subsequent growth of cotton plants in the field, similar to prior reports for cotton growth after imidacloprid application[11,20,29], and for rice with thiamethoxam applied.[6] Such enhancement could result indirectly from the reduced presence of *A. devastans* and/or as a direct effect of the neonicotinoids on plant growth. The fact that cotton plant growth was also enhanced by thiamethoxam and imidacloprid application under greenhouse conditions, where no pests were present, shows that these chemicals affect plant growth directly. Thiamethoxam has previously been reported to enhance plant growth by enhancing ionic transport, which increases mineral nutrition, and by promoting enzymatic activity leading to increased amino acid production.[6] Under greenhouse conditions, the growth of plants following seed treatment with thiamethoxam or with imidacloprid was very similar, whereas in the field plants growing from seed that had had imidacloprid applied were larger at 30 and after 40 days after sowing than those treated with thiamethoxam; likely due to the longer time taken for *A. devastans* populations to reach the ETL when imidacloprid was applied.

4.1 Conclusions and caveats

Treating cotton seeds with thiamethoxam and imidacloprid has a suppressive effect on the subsequent abundance of the cotton leaf hopper, *Amrasca devastans*. These insecticides not only protect cotton plants from this sucking pest but also enhance plant growth directly. However, both chemicals, and especially thiamethoxam, can have detrimental effects on the populations of beneficial arthropods that are the natural enemies of *A. devastans*. At the manufacturer-recommended dose of 5 g/kg of seed, imidacloprid provided effective control of *A. devastans* for at least 40 days after sowing and had little effect on the seasonal abundances of natural enemies. Despite this, when growing seed-treated cotton, agriculturalists should still carry out routine checking for *A. devastans* throughout the season because the growing season for cotton is relatively long and *A. devastans* populations may increase suddenly mid-season, as seen in 2010. Under such circumstances foliar application of insecticides can be considered as a remedial measure.

While our data suggest that moderate doses of some neonicotinoids, especially imidacloprid, applied to cotton seeds may not have detrimental effects on natural enemy abundance, it is important to consider that we have not evaluated any longer-term effects on individual natural enemies nor have we evaluated effects on further beneficial invertebrate species in and around the cotton agro-ecosystem[34]. Given that there has been recent and substantial concern about sub-lethal but detrimental effects of neonicotinoids, including imidacloprid and thiamethoxam, on agriculturally beneficial insects[12,19,26,33,34,49] we cannot advocate their usage without due caution.

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Figure legends

Figure 1. Effects of pesticide and dose on seasonal total numbers of *A. devastans*. Data points are total *A. devastans* sampled per leaf per replicate in each year. Fitted regression lines are from separate log-linear analyses[10,16] for 2010 and 2011 and do not include data from the control treatment (no insecticide applied). Parsimonious statistical descriptions were obtained by removing sequentially from a maximal model[10] but as information on blocking was excluded, regression lines are presented for informal illustration only. In 2010 the response to dose was curvilinear and there was no difference in effect between the two pesticides. In 2011 the dose response was not curvilinear (i.e. it was a straight line on the log scale) and imidacloprid had a greater suppressive effect than thiamethoxam.

Figure 2. Impact of seed treatment on mean abundance of *A. devastans* per leaf at different time intervals after sowing. Doses are expressed in g/Kg and are 0×, 0.5×, 1×, 1.5× and 2× the manufacturer recommended dose for each insecticide.

Figure 3. Effects of pesticide and dose on predator populations. Data are pooled across the two study years. Fitted regression lines are from separate log-linear analyses[10,16] of the total numbers of predators and for each predator taxon separately. All regressions, except for *Chrysoperla*, *Geocoris* and the Coccinelids treated with thiamethoxam, include a polynomial term. As information on blocking was excluded, the regression lines are presented as informal illustration of analytical results presented in the text.

Figure 4. Effect of treatments on cotton plant size under field conditions (insects present). Seeds were treated with imidacloprid or thiamethoxam at manufacturer-recommended doses or were untreated (control). The standard error of the difference is denoted by s.e.d.

Figure 5. Effect of treatments on cotton plant size under greenhouse conditions (insects absent). Seeds were treated with imidacloprid or thiamethoxam at manufacturer-recommended doses or were untreated (control). The effective standard error is donated by e.s.e.