Impact of a Nucleopolyhedrovirus Bioinsecticide and Selected Synthetic Insecticides on the Abundance of Insect Natural Enemies on Maize in Southern Mexico

R. ARMENTA,[1, 2] A. M. MARTÍNEZ,[1, 3] J. W. CHAPMAN,[4] R. MAGALLANES,[2] D. GOULSON,[5] P. CABALLERO,[3] R. D. CAVE,[6] J. CISNEROS,[1] J. VALLE,[1] V. CASTILLEJOS,[1] D. I. PENAGOS,[1] L. F. GARCÍA,[1] AND T. WILLIAMS[1, 3]

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ABSTRACT The impact of commonly used organophosphate (chlorpyrifos, methamidophos), carbamate (carbaryl), and pyrethroid (cypermethrin) insecticides on insect natural enemies was compared with that of a nucleopolyhedrovirus (Baculoviridae) of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize grown in southern Mexico. Analyses of the SELECTV and Koppert Side Effects (IOBC) databases on the impact of synthetic insecticides on arthropod natural enemies were used to predict ≈75–90% natural enemy mortality after application, whereas the bioinsecticide was predicted to have no effect. Three field trails were performed in mid- and late-whorl stage maize planted during the growing season in Chiapas State, Mexico. Synthetic insecticides were applied at product label recommended rates using a manual knapsack sprayer fitted with a cone nozzle. The biological pesticide was applied at a rate of 3×10^{12} occlusion bodies (OBs)/ha using identical equipment. Pesticide impacts on arthropods on maize plants were quantified at intervals between 1 and 22 d postapplication. The biological insecticide based on *S. frugiperda* nucleopolyhedrovirus had no adverse effect on insect natural enemies or other nontarget insect populations. Applications of the carbamate, pyrethroid, and organophosphate insecticides all resulted in reduced abundance of insect natural enemies, but for a relatively short period (8–15 d). Pesticide applications made to late-whorl stage maize resulted in lesser reductions in natural enemy populations than applications made at the mid-whorl stage, probably because of a greater abundance of physical refuges and reduced spray penetration of late-whorl maize.

KEY WORDS synthetic pesticides, baculovirus, maize, natural enemies, crop phenology

RESOURCE-POOR MAIZE FARMERS in Mesoamerica routinely apply organophosphate, carbamate, or pyrethroid insecticides to control insect pests of maize (Andrews 1988). The principal pest of maize is the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Larvae feed in the developing leaf whorl causing damage. Insecticide applications to control larvae may be frequent; in Nicaragua, small-scale maize farmers made an average of 6.3 applications of insecticide per growing season (Hruska and Gould 1997).

In this region, synthetic pesticides are often applied without protective equipment (Friedrich 2000), resulting in detrimental effects on the health of farm workers (McConnell and Hruska 1993, Hunt et al. 1999). Application of broad-spectrum insecticides can also result in significant reductions in natural enemy abundance, which may lead to resurgence of pest populations (Croft 1990).

Natural enemies are of major importance in the control of *S. frugiperda* and other pests of maize (Fuxa 1982, Chapman et al. 2000). Van Huis (1981) reported that predation of *S. frugiperda* egg masses by earwigs resulted in up to 57% mortality of field populations. Parasitism may also be important, typically accounting for 15–30% mortality of larvae in Mesoamerica (Wheeler et al. 1989, Martínez et al. 2000). Furthermore, larvae of this species are highly cannibalistic, particularly in late instars, and it is unusual to find more than one larva per whorl (Chapman et al. 1999).

Nucleopolyhedroviruses (Baculoviridae) have shown considerable promise as bioinsecticides, particularly in small-scale, low-technology situations (Caballero et al. 2001). As pest control agents, these pathogens have several advantages over conventional pesticides, in that they are host-specific, simple to apply, amenable to a variety of formulations, and safe

[1] ECOSUR, Apdo. Postal 36, Tapachula, Chiapas 30700, Mexico.
[2] UNACH, Facultad de Ciencias Agrícolas, Huehuetán, Chiapas 30670, Mexico.
[3] Depto. Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain.
[4] Plant and Invertebrate Ecology Division, IACR Rothamsted, Harpenden, AL5 2JQ, UK.
[5] Biology Department, University of Southampton, Southampton, SO16 7PX, UK.
[6] Escuela Agrícola Panamericana, El Zamorano, Honduras.

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to humans (Hunter-Fujita et al. 1998). Limitations to the use of baculoviruses include the costs of production and their relatively slow speed of kill, although the latter may not be an issue of concern for a crop such as maize that can withstand moderate defoliation without significant losses in yield (Hruska and Gould 1997).

In the current study, we employ databases on the effects of pesticides on insect natural enemies to generate predictions on the impact of different classes of chemical insecticides on populations of natural enemies and other insects. These predictions were tested in field trials using carbamate, organophosphate, and pyrethroid insecticides, and the nucleopolyhedrovirus bioinsecticide applied to mid-whorl stage maize. We also compared the effect of applying the bioinsecticide and one synthetic insecticide (chlorpyrifos) on the abundance of arthropods on mid- and late-whorl stage maize, which are the phenological stages that are most frequently sprayed to control *S. frugiperda.*

Materials and Methods

Database Analysis. Information on toxicity to natural enemies of selected insecticides commonly used in Mexico was obtained from two sources. First, we used the results of the IOBC Working Group 'Pesticides and Beneficial Organisms' that are available as a database on the Koppert Biological Systems website (Koppert 2002). The toxicity of each pesticide is given as the percentage reduction of the capacity of natural enemies to control pests in the presence of the pesticide and is classified on the following scale: 1 = <25%; 2 = 25–50%; 3 = 50–75%; 4 = >75% reduction. Toxicity scores were averaged for the predatory or parasitic stages of each type of natural enemy (principally *Amblyseius* spp., *Aphidius* spp., *Chrysoperla carnea* [Stephens], *Encarsia formosa* Gahan, *Eretmocerus* spp., *Hippodamia convergens* Guérin-Méneville, *Orius* spp., *Phytoseiulus persimilis* Athias-Henriot, *Podisus maculiventris* [Say], *Trichogramma* spp.). Entomopathogens, soil-dwelling mites, nonactive life stages (e.g., parasitoid pupae) and natural enemy-pesticide combinations with unknown classifications were ignored. The mean toxicity value (±SE) was then calculated for all natural enemy groups for which information was available in the database (n = 6–14, depending on pesticide). The period during which the pesticide remains harmful to natural enemies, also given in the database, was used to compare persistence of toxic residues of different pesticides. The database information on residual effects is mainly intended for the conditions found in greenhouse crops in the northwest of Europe. In field crops, the residual effect is expected to be shorter but serves as a useful comparative indicator.

Second, we analyzed the "SELECTV Database of Pesticide Side Effects on Arthropod Natural Enemies" described by Theiling and Croft (1988) and available online at the University of Oregon's Phosure website (Phosure 2001). Each entry in the SELECTV database comprises one screening of a pesticide on one natural enemy taxon under conditions described in the source publication. The severity of pesticide effects on natural enemies is classified as a median toxicity rating according to the following scale: 1 = 0%, 2 = <10%, 3 = 10–30%, 4 = 30–90%, 5 = >90% mortality. Only toxic effects that resulted in mortality were considered; sublethal effects on natural enemy performance were excluded. Entries for each of our selected pesticides were searched using the following natural enemy group search terms based on the types of natural enemies usually found on maize in Mexico (Araneae, Chrysopidae, Coccinelidae, Carabidae, Hymenoptera, *Orius* spp., Syrphidae, Tachinidae). No entries for Dermaptera were found for the pesticides used in our study. Mean toxicity scores (±SE) were calculated for all natural enemies taken together. The number of entry sample sizes differed according to the pesticide in question (n = 9–129).

Insects, Virus, and Field Site. A colony of locally collected *S. frugiperda* was maintained on semisynthetic diet (adapted from Mihm 1984) at 25–27°C in the laboratories of El Colegio de la Frontera Sur (ECOSUR), Mexico. Fourth-instar larvae from this colony were used to produce the *S. frugiperda* multinucleocapsid nucleopolyhedrovirus (SfMNPV) originally isolated in Nicaragua and previously characterized by Escribano et al. (1999). Virus-killed larvae were stored at −10°C until required for further processing. To extract viral occlusion bodies (OBs), larvae were thawed, homogenized in 0.1% sodium dodecyl sulfate, centrifuged at 90 g for 5 min and the supernatant centrifuged at 3,000 g for 10 min. Pelleted OBs were resuspended in distilled water and quantified using a bacterial counting chamber (Hunter-Fujita et al. 1998). This suspension was stored at 4°C for no longer than 1 wk before use in field trials.

Field trials were performed in maize fields close to the village of Frontera Hidalgo, 18 km south-east of Tapachula on the coastal plain of Chiapas, Mexico, and 1 km from the border with Guatemala, at an altitude of ≈50 m above sea level. The climate in this region is warm and humid (35°C day, 23°C night) with a mean monthly rainfall of ≈300 mm and a relative humidity >85% during the growing season from May to November.

Trial 1: Single Application of Chlorpyrifos or SfMNPV at the Mid-Whorl Stage. On 11 June 1998, a common local variety of maize (Tacsa-H101) was planted in plots 5 × 5 m at a standard density of 25 cm between plants and 70 cm between rows. Plots were separated by a gap of 5 m, in which four rows of maize were planted. Plots were treated with N–P–K (18:46:00) fertilizer (50 kg/ha) and urea (50 kg/ha) preemergence, and at 30 and 60 d postplanting with urea at the rate of 100 and 150 kg/ha respectively. Weed control was performed manually when necessary.

At 32 d after planting, when maize plants were 35–45 cm tall and with 8–9 leaves (mid-whorl stage), plots were subjected to one of the following treatments: (1) SfMNPV at a rate equivalent to 3×10^{12}

OBs/ha, (2) chlorpyrifos (Lorsban 480EM, Dow Agrosciences, Mexico D.F., Mexico) at the product label recommended rate of 480 g active ingredient (a.i.)/ha, or (3) water control. Treatments were randomly assigned to plots and there were six replicate plots per treatment. All applications were made in a volume equivalent to 800 liters water/ha using a manual knapsack sprayer fitted with a cone nozzle, typical of those used by farmers in the region. An agricultural wetter-sticker (0.02% Agral Plus, AstraZeneca, Mexico D.F., Mexico) was included in all treatments.

At two and 15 d following the application, 20 plants per plot were selected using random number tables and carefully examined. The number of arthropods observed on each plant was recorded. In general, broad groupings were used to classify insects and spiders, e.g., lepidopteran larvae, ants (*Solenopsis* spp.), all types of spiders, *Orius* spp., *Chrysoperla* spp. (all stages), predatory Coleoptera, other Coleoptera (mainly sap beetles, *Carpophilus* spp. [Nitidulidae]), colonies of aphids (comprising a minimum of 20 individuals, lesser infestations of aphids were ignored), other insects (such as thrips and leafhoppers), or other natural enemies (such as syrphid larvae and parasitoids). For analysis, arthropods were grouped into classes according to their feeding habits: natural enemies (such as ants, spiders, and *Orius* spp.) and other insects (such as aphids, thrips, and sap beetles). Analyses of the abundance of each arthropod group were performed using the Generalized Linear Interactive Modeling (GLIM) program (Numerical Algorithms Group 1993) with Poisson errors specified, resulting in changes in model deviance that approximate to a χ^2 distribution. In cases in which minor overdispersion was observed in the data, scaling was performed and the results are presented as F-values (Crawley 1993). In all cases, the validity of models was checked using the model-checking macro present in the GLIM program.

The grain yield from each treatment was determined at 110 d after planting as the weight of grain from 30 plants/plot when the average moisture content was 11.0%. Grain weights were subsequently corrected for moisture content on an individual plot basis and the shelling efficiency was the same for all plots.

The results of this experiment were used to plan the sampling intervals described in the following experiments which ranged from 1 to 15 d postapplication or between 1 and 22 d postapplication in the case of the experiment comparing different synthetic insecticides.

Trial 2: Application of Chlorpyrifos or SfMNPV at Both Mid- and Late-Whorl Stages. This trial involved two applications of bioinsecticide or chlorpyrifos when the crop was in the mid- and late-whorl growth stages. The effect of previous chemical or biological treatments made in the mid-whorl stage on the impact of applications made in the late-whorl stage was also examined. The planting design, fertilizer treatments, and weed management practices for this experiment were identical to those described in the previous experiment except that experimental plots were 8×8 m and were separated by a gap of 8 m in which four rows of maize were planted. The crop was planted on 11 July 1998.

At 32 d after planting, maize plants were at the mid-whorl stage and were infested with ≈400 second instar *S. frugiperda* per plot. Larvae were placed in paper bags and scattered arbitrarily onto maize plants by a person walking through the experimental plots. Subsequent releases of 300 and 100 larvae per plot were made at 39 and 47 d postplanting, respectively.

At 34 d postplanting, one of the following treatments was applied to maize plants: (1) SfMNPV at a rate equivalent to 3×10^{12} OBs/ha, (2) chlorpyrifos (Lorsban 480EM, Dow Agroscience) at 480 g a.i./ha, or (3) water control. All applications were made in a volume equivalent to 300 liters water/ha (+ 0.02% Agral Plus) using a manual knapsack sprayer. These mid-whorl stage virus and chlorpyrifos treatments were applied to 12 replicate plots whereas the control treatment was applied to six plots. Plots were laid out in a fully randomized design.

At 1, 3, 8, and 15 d after the application, 20 randomly selected plants per plot were carefully examined and the number of arthropods observed on each plant was recorded. Any *S. frugiperda* larvae present were placed into plastic cups containing semisynthetic diet and reared in the laboratory until death or pupation. Viral deaths were diagnosed by the presence of viral OBs in Giemsa-stained smears of insect cadavers. Published keys (Cave 1993, 1995) were used to identify emerging parasitoids.

At 50 d postplanting, when the plants were in the late-whorl stage, a second treatment was applied. Plants that had been previously treated with virus (at 34 d postplanting) were treated with either virus or chlorpyrifos (abbreviated where necessary as "c'fos"). Similarly, plants that had been previously treated with chlopyrifos were on this occasion treated with either virus or chlorpyrifos. Control plants were once again treated with water. The details of the application rates, equipment, volumes of water, and wetter-sticker were identical to those described in the first application. This resulted in a total of five treatments: (1) virus followed by virus (virus-virus), (2) virus followed by chlorpyrifos (virus-c'fos), (3) chlorpyrifos followed by virus (c'fos-virus), (4) chlorpyrifos followed by chlorpyrifos (c'fos-c'fos), or (5) water followed by water (control).

Each of these treatments was applied to six replicate plots; however, two virus-virus plots, one virus-c'fos plot, and one control plot were subsequently excluded from the experiment because of irregular plant growth. Plants were sampled at 1, 3, and 8 d after the second application, as described above. A 15-d postapplication sample was not taken because plants had begun to flower and were no longer suitable for *S. frugiperda* infestation. The yield from each treatment was estimated at 114 d postplanting by determining the dry weight of the grain from 30 randomly selected plants from each plot.

As in the previous experiment, arthropods on maize plants were classified into groups before analysis.

Table 1. Results of analyses on predicted impact and persistence of selected synthetic insecticides on natural enemy populations generated from the Koppert (IOBC) and SELECTV databases

	Type of Insecticide			
	Carbaryl[a]	Chlorpyrifos[b]	Cypermethrin[c]	Methamidophos[d]
IOBC classification[e]				
Mean toxicity rating (±S.E.)	4.00 ± 0.00	3.86 ± 0.14	4.00 ± 0.00	3.88 ± 0.13
Number of natural enemy groups analyzed	6	7	14	8
SELECTV classification[f]				
Mean toxicity rating (±S.E.)	4.04 ± 0.08	4.01 ± 0.26	4.19 ± 0.21	4.39 ± 0.22
Number of records analyzed	129	18	23	9
Persistence of residues				
Mean duration of residues toxic to natural enemies (weeks)[g]	6.2	6.1	10	6.8
Rate of degradation in soil[h]	Rapid	Moderate	Rapid	Rapid

[a] Carbamate.
[b] Pyridine organothiophosphate.
[c] Pyrethroid ester.
[d] Phosphoramidothioate.
[e] Working Group on Pesticides and Beneficial Organisms: 1 = <25%; 2 = 25–50%; 3 = 50–75%; 4 = >75% reduction in control capacity.
[f] Database of pesticide effects on arthropod natural enemies: 1 = 0%; 2 = <10%; 3 = 10–30%; 4 = 30–90%; 5 = >90% mortality. Each record in the database gives the median toxicity value based on between 1 and 36 observations, the mayority single observations.
[g] Values relevant to glasshouse conditions (Koppert, 2002).
[h] Data from Tomlin (2000).

These were *S. frugiperda* larvae, natural enemies, and other insects (Cisneros et al. 2002). Because the number of natural enemies may depend to a large degree on the abundance of prey items available on maize plants (Chapman et al. 2000), these groups were considered as dependent variables and subjected to multivariate analysis of variance with the samples taken after the first and second applications considered separately using the Statistica package (StatSoft 2001). Repeated-measures ANOVA was also performed to determine the significance of changes in the abundance of arthropod groups between sample timepoints. The significance of treatment effects was determined by calculating the F value generated by Pillai's Trace (Pillai 1967). Multiple comparisons between treatments were performed by examination of canonical coefficients and orthogonal contrasts (Winer 1971). The prevalence of parasitoid emergence from *S. frugiperda* larvae was analyzed using contingency tables (χ^2 tests).

Trial 3: Single Application of Methamidophos, Carbaryl, Cypermethrin, or SfMNPV at the Mid-Whorl Stage. The planting design, fertilizer treatments, and weed management practices for this experiment were identical to that described in the previous experiment with plots of maize of 8 × 8 m with 8 m of maize planted between plots. At 31 d postplanting, one of the following insecticide treatments was applied to maize plants at product label recommended rates: (1) methamidofos, 600 g a.i./ha (Tamarón, Bayer); (2) carbaryl, 1200 g a.i./ha (Sevin 80PH, Aventis); (3) cypermethrin 50 g a.i./ha (Arrivo 250 EC, FMC); (4) SfMNPV, 3×10^{12} OBs/ha; or (5) control (water). These products were selected because they were the most commonly used chemicals for control of *S. frugiperda* in maize in southern Mexico. All products were applied in a volume of 300 liters/ha water plus wetter-sticker (0.02% Agral Plus).

There was a natural infestation of *S. frugiperda* larvae in experimental plots (28–34% of plants infested), so that artificial infestation was not necessary. Plots were sampled at 1, 3, 8, 15, and 22 d postapplication. As in the previous experiment, the number of arthropods present on each of 20 plants/plot was recorded and all *S. frugiperda* larvae found were taken to the laboratory and reared on semisynthetic diet until death or pupation. The prevalence of virus infection and parasitism was analyzed in GLIM with a binomial error structure, the results of which are presented in terms of χ^2 statistics (Crawley 1993). The abundance of arthropod groups on maize plants was analyzed using MANOVA procedures as described in the previous experiment.

The presence of ground-dwelling arthropods was monitored by placing five pitfall traps within each plot. Traps consisted of a 500-ml plastic cup with a slit cut in the bottom to allow the drainage of rainwater. Traps were emptied every other day; trapped arthropods were killed by freezing, preserved in formalin, and subsequently counted and classified into orders or to family in the case of Coleoptera. Pitfall capture results were divided into two groups: captures made up to 8 d postapplication and those made after 8 d postapplication. Each capture group was subjected to Kruskall-Wallis nonparametric analysis. Grain yield was determined from a sample of 30 plants/plot at 124 d postplanting.

Results

Database Analysis. All the chemical insecticides selected for study were predicted to produce significant levels of mortality or a marked reduction in the pest control capacity of natural enemies (Table 1). The toxicity values calculated from the IOBC database were at or very close to the maximum value (4 = >75%

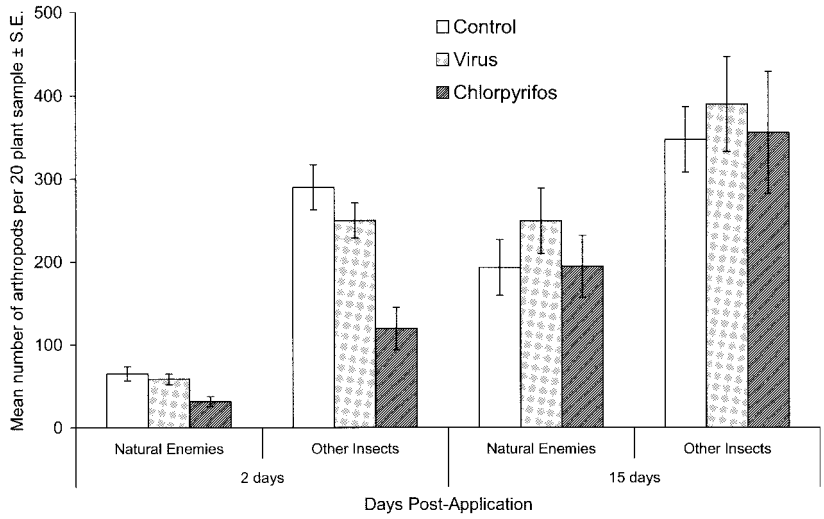


Fig. 1. Mean numbers of arthropod natural enemies and other insects observed in each plot sampled (20 plants sampled/plot) at 3 and 15 d after chlorpyrifos, nucleopolyhedrovirus and water (control) spray applications (Trial 1).

reduction in performance); the toxicity of cypermethrin and carbaryl was classified as maximal for all types of natural enemies for which information was available. Similarly, the SELECTV database median toxicity ratings were in the range 4.01–4.39 corresponding to a position between the broadest toxicity class (4 = 30–90% mortality) and the highest toxicity class (5 = >90% mortality), for all the synthetic insecticides considered. Only two entries were found in the SELECTV database concerning the impact of nucleopolyhedrovirus on natural enemies; both *Trichogramma cacoeciae* Marchal and *Chrysoperla carnea* (Stephens) suffered 0% negative effects after treatment with *Mamestra brassicae MNPV.*

The persistence of toxic residues under glasshouse conditions was also similar for all the synthetic insecticides (6.1–6.8 wk), with the exception of cypermethrin, for which an average 10-wk period after application was indicated for residue degradation (Table 1). All pesticides were described as being subject to rapid degradation in soil, except chlorpyrifos, which was described as moderately persistent with a 7–15-d half-life for soil applications and a 33–56-d half-life for soil incorporations (Tomlin 2000).

Trial 1: Single Application of Chlorpyrifos or SfMNPV at the Mid-Whorl Stage. Lepidoptera represented only 2.9% and 2.4% of the total number of arthropods observed on maize plants at 2 d (n = 5,015) and 15 d (N = 10,433) postapplication, respectively, probably because of the high densities of natural enemies. Most of the lepidopteran larvae were stemboring *Diatrea* spp. that were grouped with other insects for the purposes of analysis.

At 2 d postapplication, a reduction of ≈50% in the abundance of natural enemies ($F_{2,15} = 6.31$; $P < 0.01$) and other insects ($F_{2,15} = 12.9$; $P < 0.001$) was observed in plots that had been treated with chlorpyrifos, compared with control and virus treatments (Fig. 1). The most abundant predators, the earwig *Doru taeniatum* (Dohrn) and *Chrysoperla* spp., were reduced by 50.2 and 50.6% respectively, whereas foraging ants (*Solenopsis* spp.) were completely eliminated by chlorpyrifos treatment. In contrast, spiders (mostly Anyphaenidae and Gnaphosidae) suffered very little from the chemical insecticide with just a 10.5% reduction compared with the abundance observed on control plants (data not shown). The most abundant members of the other insects group, the nitidulid beetle *Carpophilus* sp. and the staphylinid, *Tachyporus* sp. differed in their sensitivity to chlorpyrifos with reductions of 25.4% and 82.0%, respectively.

The abundance of arthropods in chlorpyrifos-treated plots had recovered in the samples taken at 15 d postapplication and treatment differences were not significant for natural enemies ($F_{2,15} = 0.76$; $P = 0.48$) or other insects ($F_{2,15} = 0.17$; $P = 0.84$). However, chlorpyrifos-treated plots had a higher incidence of *Diatraea* spp. larvae (4.3% of plants infested) compared with control (1.2%) or virus (1.9%) treatments, possibly as a result of the reduction in natural enemy numbers after chlorpyrifos treatment ($F_{1,30} = 15.0$; $P < 0.001$). Fire ants (*Solenopsis* spp.) remained almost completely absent from chlorpyrifos-treated plots at 15 d postapplication but were fairly common (17.5 ± 7.8 per 20 plant sample) in control and virus treatments ($\chi^2 = 6.78$; df = 1; $P < 0.01$). The SfMNPV treatment did not affect the density of arthropods at any sample point compared with control plots (Fig. 1).

The average dry weight yield from all plots was 6.35 ± 0.43 tonnes/ha (mean ± SE). There were no significant differences between treatments ($F_{5,30} = 0.74$; $P = 0.60$).

Trial 2: Application of Chlorpyrifos or SfMNPV at Both Mid- and Late-Whorl Stages. Chlorpyrifos treatment resulted in a significant decrease in the abundance of all arthropods on maize plants at 1 d ($F_{3,25} = 21.1$; $P < 0.001$) and 3 d ($F_{3,25} = 24.8$; $P < 0.001$) after the first application (Fig. 2a). The most severely af-

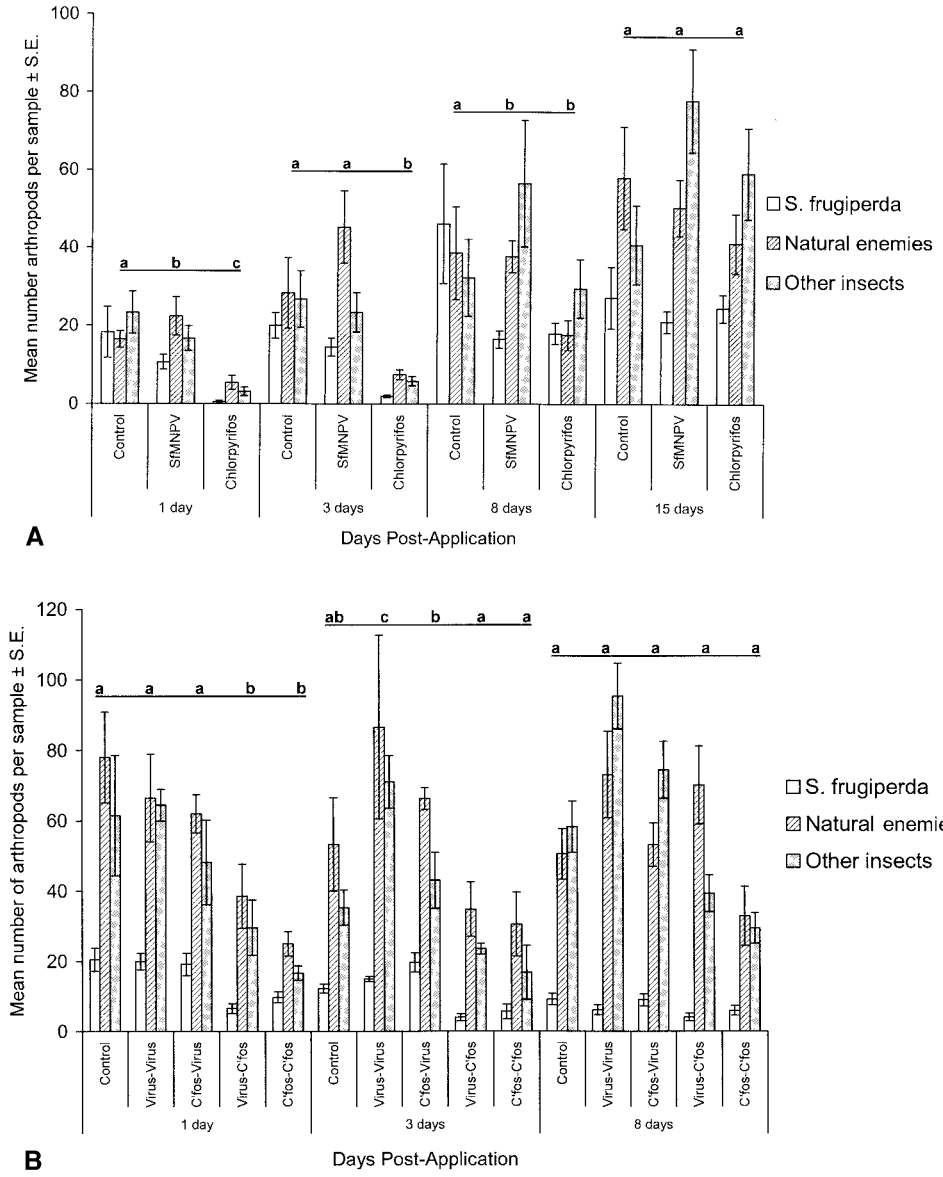


Fig. 2. Mean numbers of *Spodoptera frugiperda* larvae, arthropod natural enemies, and other insects observed in each plot sampled (20 plants sampled/plot) (Trial 2) (A) at intervals between 1 and 15 d after chlorpyrifos, nucleopolyhedrovirus, and water (control) spray applications to mid-whorl stage maize. (B) Plots were subjected to a second application when plants were in the late-whorl stage. Plots that had previously been treated with chlorpyrifos (c'fos) in the mid-whorl stage were either treated with chlorpyrifos (c'fos-c'fos) or nucleopolyhedrovirus (c'fos-virus). Similarly, plots that had previously been treated with nucleopolyhedrovirus were either treated with chlorpyrifos (virus-c'fos) or nucleopolyhedrovirus (virus-virus). Samples were taken at intervals between 1 and 8 d after the second application. In all cases, *S. frugiperda*, natural enemies, and other insects were subjected to multivariate ANOVA as dependent variables. Columns headed by identical letters did not differ significantly for treatment comparisons within each sample time point.

fected group was *S. frugiperda* larvae, which was reduced by 97 and 91% at 1 and 3 d postapplication compared with control plots, respectively. Chlorpyrifos treatment resulted in a 67 and 73% reduction in the abundance of natural enemies and a 86 and 78% reduction in the abundance of other insects at 1 and 3 d postapplication, respectively. The abundance of arthropods in plots treated with virus differed significantly from that of control plots at 1 d postapplication with a 36% increase in the abundance of natural enemies and a 41–28% reduction in the mean number of *S. frugiperda* larvae and other insects, respectively, compared with control plot values (Fig. 2a). However, this difference was not observed in the samples taken at 3 d postapplication. A significant increase in the abundance of natural enemies was observed between

1 and 3 d postapplication ($F_{1,24} = 14.6$; $P < 0.001$), principally because of a high density of fire ants on virus-treated plants at 3 d postapplication.

Significant changes occurred in the density of *S. frugiperda* ($F_{1,24} = 17.4$; $P < 0.001$) between samples taken at 3 d and 8 d postapplication (Fig. 2a). This involved an increase in the abundance of larvae in control and chlorpyrifos-treated plots, reflecting the release of *S. frugiperda* larvae made at 5 d postapplication. In contrast, numbers of larvae in virus treated plots remained static, most likely because of virus-induced *S. frugiperda* mortality in the interval between the 3-d and the 8-d samples that compensated for the artificial infestations made 3 d previously. By 8 d postapplication, the abundance of natural enemies in chlorpyrifos-treated plots was approximately half that observed in control and virus-treated plots. Significant increases in the density of natural enemies ($F_{1,24} = 11.48$; $P = 0.002$) and other insects ($F_{1,24} = 7.62$; $P = 0.01$) were observed in all treatments between 8 and 15 d postapplication. There were no significant differences between treatments at 15 d postapplication ($F_{6,52} = 1.19$; $P = 0.32$) (Fig. 2a).

At 1 d after the second application, there were significant reductions in the density of arthropods in both treatments involving chlorpyrifos: namely chlorpyrifos applied to plots that had previously been treated with virus (virus-chlorpyrifos: $F_{3,20} = 11.32$; $P < 0.001$) or chlorpyrifos (chlorpyrifos-chlopyrifos: $F_{3,20} = 15.0$; $P < 0.001$) (Fig. 2b). Similar to the first application, the greatest effect of chlorpyrifos was observed in *S. frugiperda* larvae at 1 d and 3 d postapplication. However, both chlorpyrifos treatments had a notably smaller effect on natural enemies and other insects at the second application compared with the previous treatment, with reductions of between 50 and 68% at 1 d and 35–43% at 3 d postapplication, compared with control plots. Virus treatments did not result in a reduction in any arthropod group at any sample point after the second application, and numbers of natural enemies and other insects in virus-virus plots were higher than those observed in control plots at 3 d postapplication. Significant reductions in the abundance of *S. frugiperda* larvae were observed between 3 and 8 d postapplication ($F_{1,22} = 14.90$; $P < 0.05$). This difference was especially evident in both treatments involving a second application of virus (virus-virus and chlorpyrifos-virus), presumably because of virus-induced mortality preceding the 8-d sample. By 8 d postapplication the abundance of arthropods was similar in all treatments ($F_{12,63} = 1.38$; $P = 0.20$) (Fig. 2b).

After the first application, nucleopolyhedrovirus infection occurred in 29% and 33% of the *S. frugiperda* larvae that were collected from maize plants at 1 and 3 d postapplication and reared in the laboratory until death or pupation, respectively. This proportion fell to 10–12% in larvae collected at 8 and 15 d. Nucleopolyhedrovirus infections were never seen in larvae collected from plots treated with chlorpyrifos, whereas larvae from control plots suffered a low prevalence (≈0.5%) of nucleopolyhedrovirus infection, suggesting that low levels of virus were naturally present on the maize crop.

For *S. frugiperda* larvae collected 1 d after the second application and reared in the laboratory on semisynethtic diet, the mean prevalence of virus infection ranged from 18% in larvae collected from virus-virus plots ($n = 53$) to 10% in larvae collected from chlorpyrifos-virus plots ($n = 74$). Notably, 12% ($n = 28$) of larvae collected from virus-chlorpyrifos plots succumbed to virus infection indicating that inoculum from the first application had persisted on these plants or that larvae had died after the first application, resulting in an amplification in the concentration of virus on these plants. For samples collected at 3 d postapplication, the mean prevalence of infection was 9% in virus-virus ($n = 55$) and chlorpyrifos-virus ($n = 120$) plots compared with 14% in virus-chlorpyrifos plots, albeit based on a reduced sample size ($n = 35$). Virtually no virus infections were observed in larvae collected at 8 d after the second application. As previously observed, the prevalence of viral infections in larvae from chlorpyrifos and control plots was consistently low (≈1.5%, $n = 332$) in all samples.

The most common parasitoid that emerged from *S. frugiperda* larvae collected after the first application was the braconid egg-larval endoparasitoid, *Chelonus insularis* Cresson, which represented 79.6% ($n = 463$) of parasitized *S. frugiperda.* Other species included the ichneumonids, *Ophion flavidus* Brullé (11.7%), *Pristomerus spinator* (F.) (2.5%), and *Eiphosoma vitticole* Cresson (2.4%), the eulophid *Euplectrus plathypenae* Howard (1.9%), and the tachinids *Lespestia archippivora* (Riley), *Archytas marmoratus* (Townsend), and *Linnaemya comta* (Fallén) (together 1.9%).

The prevalence of parasitism differed significantly between treatments at 1 d ($\chi^2 = 6.60$, df = 2; $P < 0.04$), 3 d ($\chi^2 = 7.30$; df = 2; $P < 0.03$), 8 d ($\chi^2 = 13.8$; df = 2; $P = 0.001$) and 15 d after the first application ($\chi^2 = 8.14$, df = 2; $P < 0.02$) (Fig. 3a). The chlorpyrifos treatment contributed to 80% of the χ^2 value at 1 d postapplication but to very little thereafter, although the number of parasitized and nonparasitized *S. frugiperda* larvae recovered from chlorpyrifos-treated plots was significantly reduced compared with control plots in samples taken until 15 d postapplication (Fig. 3a). A moderate reduction in the prevalence and number of parasitized larvae was observed in the virus treatment for larvae collected at 3 and 8 d postapplication wherein many larvae died of virus infection before parasitoid emergence.

The prevalence of parasitized larvae collected after the second application was generally lower than that observed after the first application (Fig. 3b). The braconid *C. insularis* was again the most abundant parasitoid representing 64.5% of total parasitism ($n = 124$), but generally no more than 3–5 parasitized larvae were recovered from each 20 plant sample (replicate plot). The percentage parasitism did not differ according to treatment at 1 d postapplication ($\chi^2 = 6.98$; df = 4; $P = 0.14$), but was significantly different at 3 d postapplication with the absence of parasitism in the chlorpyrifos-chlorpyrifos and virus-chlorpyrifos treat-

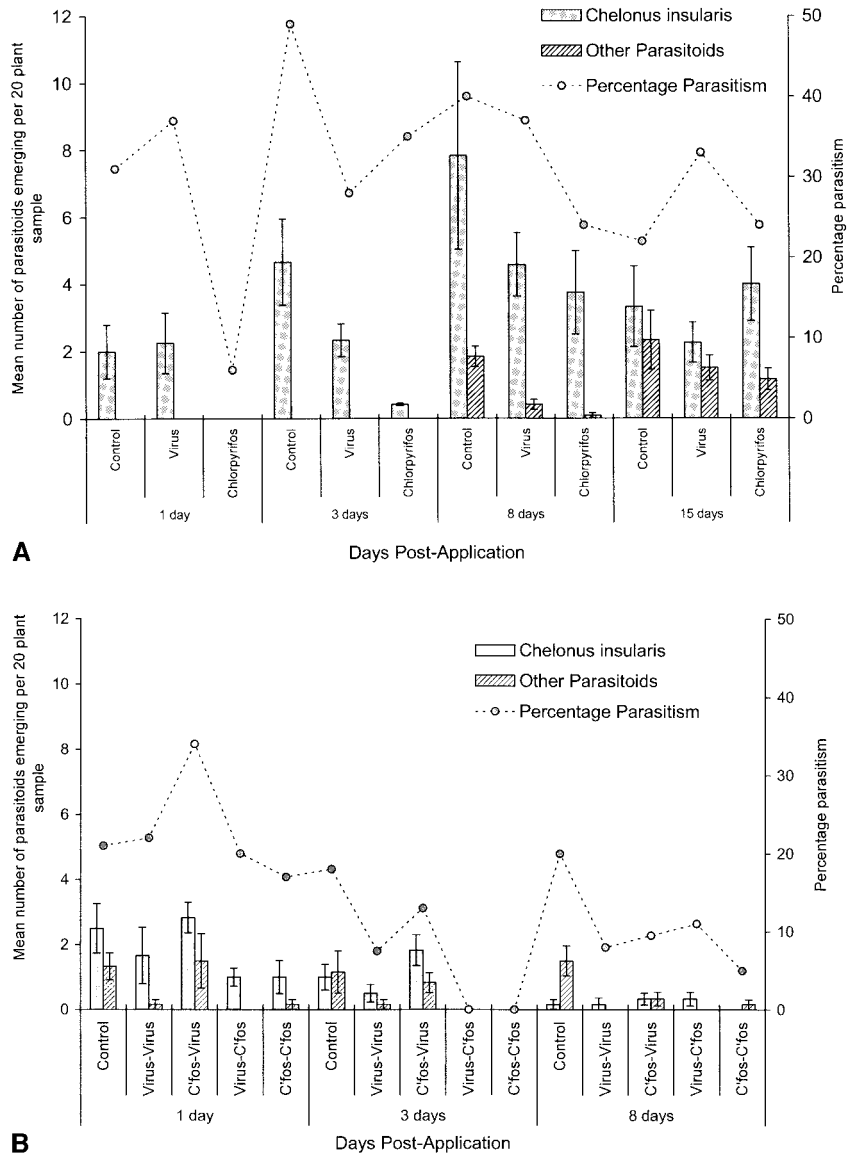


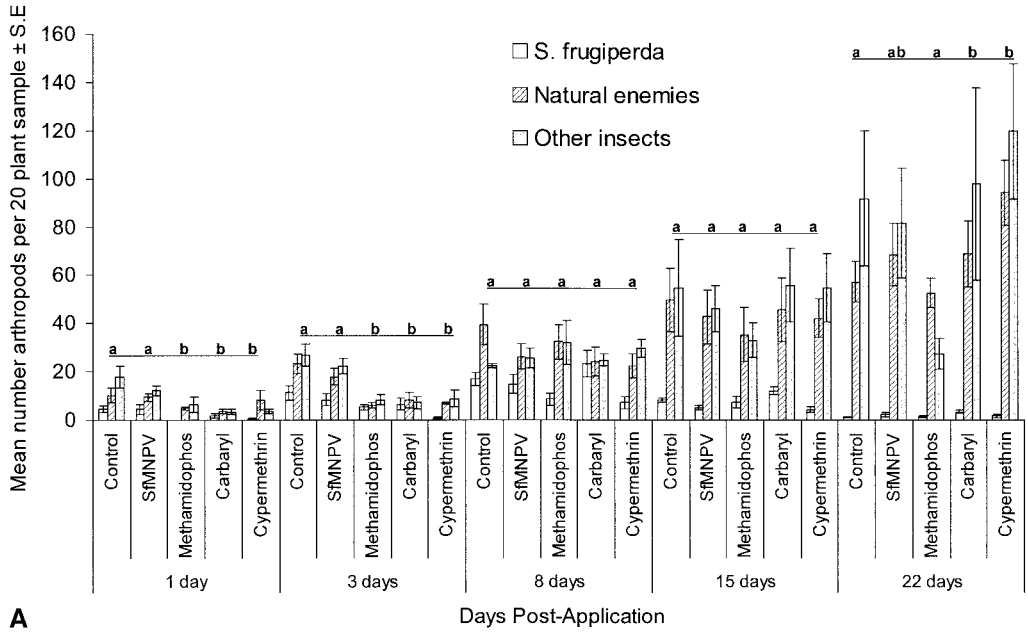
Fig. 3. Mean number of emerging *Chelonus insularis* (Braconidae) and other parasitoids (columns) and percentage parasitism (dots and dotted line) in each plot sampled (20 plants sampled/plot) at intervals between (A) 1 and 15 d after chlorpyrifos, nucleopolyhedrovirus, and water (control) spray applications to mid-whorl stage maize and (B) between 1 and 8 d after a second application when plants were in the late-whorl stage as described in the text (Trial 2).

ments contributing 37 and 19% of the χ^2 value, respectively ($\chi^2 = 10.6$; df = 4; $P = 0.03$). No significant differences in the prevalence of parasitism were detected in the samples taken at 8 d postapplication ($\chi^2 = 4.97$; df = 4; $P = 0.29$), although numbers of larvae were generally very low in all treatments.

No significant treatments differences were detected between grain yields, which ranged from 6.35 to 6.99 tonnes/ha (dry weight) ($F_{4,25} = 0.37$; $P = 0.82$).

Trial 3: Single Application of Methamidophos, Carbaryl, Cypermethrin, or SfMNPV at the Mid-Whorl Stage. Repeated measures analysis of changes in arthropod abundance over time indicated that the abundance of natural enemies ($F_{4,17} = 55.5$; $P < 0.001$) and other insects ($F_{4,17} = 43.9$; $P < 0.001$) increased in all treatments during the course of the experiment (Fig. 4a). Changes in the abundance of *S. frugiperda* larvae during the experiment were treatment-dependent; control, virus, and carbaryl treatments resulted in differences compared with the cypermethrin and methamidafos treatment results over time (treatment*time interaction $F_{16,80} = 2.05$; $P = 0.019$).

Application of SfMNPV had no effect on the abundance of any arthropod group at any sample point compared with the control treatment (Fig. 4a). Application of synthetic insecticides significantly re-



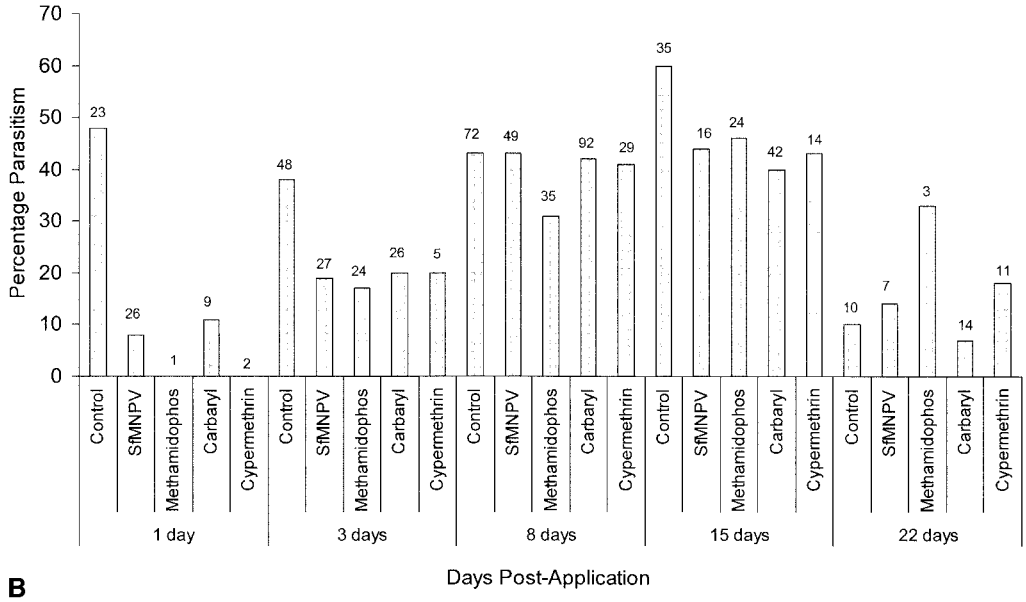


Fig. 4. (A) Mean numbers of *Spodoptera frugiperda* larvae, arthropod natural enemies, and other insects observed in each plot sampled (20 plants sampled/plot) at intervals between 1 and 22 d after application of methamidophos, carbaryl, and cypermethrin at product label recommended rates compared with applications involving nucleopolyhedrovirus and water (control) spray made to maize plants at the mid-whorl stage (Trial 3). Numbers of *S. frugiperda*, natural enemies, and other insects were subjected to multivariate ANOVA as dependent variables. Columns headed by identical letters did not differ significantly for treatment comparisons within each sample time point. (B) Mean percentage parasitism in each plot sampled (20 plants sampled/plot) at intervals up to 22 d after chemical and bioinsecticide applications. Numbers above columns indicate total number of *S. frugiperda* larvae collected in each treatment.

duced the abundance of arthropods at 1 d postapplication ($F_{12,60} = 1.91$; $P = 0.05$) and 3 d postapplication ($F_{12,60} = 2.31$; $P = 0.017$) compared with control and virus treatments. This effect did not persist, however, and significant differences were not observed at 8 ($F_{12,60} = 1.50$; $P = 0.15$) and 15 d postapplication ($F_{12,60} = 1.26$; $P = 0.26$), presumably because of colonization of treated plots by arthropods from sur-

rounding untreated maize plants. In the final sample at 22 d postapplication, the abundance of arthropods, particularly the other insects group in carbaryl- and cypermethrin-treated plots, was significantly elevated compared with other treatments ($F_{12,60} = 2.30$; $P = 0.017$). This effect was mainly because of a proliferation of thrips and aphids in carbaryl and cypermethrin-treated plots.

Virus infections were only observed in *S. frugiperda* larvae collected from virus-treated plants and subsequently reared in the laboratory on semisynthetic diet, except for a single infected larva collected from a control plot at 8 d postapplication. The prevalence of virus infection decreased from 31% in larvae collected at 1 d postapplication to 16% at 15 d postapplication and 0% at 22 d postapplication.

As in the previous experiment, the most prevalent parasitoid was *C. insularis*, which represented a total of 79.7% of observed parasitism ($n = 232$ parasitized hosts). Application of chemical insecticides or virus resulted in a significant decrease in the prevalence of parasitism observed in larvae collected at 1 d postapplication ($\chi^2 = 12.9$; df = 4; $P = 0.01$) (Fig. 4b). The prevalence of parasitism in larvae collected from virus-treated and chemical-treated plots at 3 d postapplication was about half that observed in the control treatment, although only five larvae were collected in the cypermethrin treatment. No significant differences were observed at 8 and 15 d postapplication and too few larvae were recovered in the final sample to permit analysis.

The most abundant predator caught in pitfall traps was the carabid *Calosoma calidum* F., which represented 33% of arthropods captured up to 8 d postapplication ($n = 231$) and 42% of arthropods captured thereafter ($n = 440$). Application of methamidophos resulted in an 89% reduction in the capture of *C. calosoma* up to 8 d postapplication compared with the control treatment (Kruskal-Wallis: $\chi^2 = 9.88$; df = 4; $P = 0.04$). None of the other treatments resulted in significant reductions in *C. calosoma* captures and captures of other ground dwelling groups of arthropods were not significantly affected by any treatment, either before or after 8 d postapplication.

Discussion

The impact of synthetic pesticides on insect natural enemies has been well established (Croft 1990) although information was not available for a considerable number of natural enemy-pesticide combinations, even for continually updated databases, such as that developed from the IOBC Working Group publications (Sterk et al. 1999). Not surprisingly, given the broad spectrum of toxicity of the chemical insecticides selected for study, all synthetic compounds were expected to produce a high prevalence of mortality or a marked reduction in the pest control capacity of natural enemies.

Undoubtedly, the principal cause of the reductions in natural enemy abundance observed after insecticide applications was the toxic effects of the chemical insecticides, although simultaneous reductions in the abundance of other insects means that surviving natural enemies may have emigrated from insecticide-treated plots because of a lack of suitable prey. In general, however, synthetic pesticides had a lower impact on natural enemy populations than that predicted from database analyses, and recolonization of chemical-treated plots was rapid.

The prompt return to levels similar to those observed in control plots was most probably because of movement into treated plots from adjacent untreated maize plants. The relatively small size of experimental plots may have been partially responsible, but, in any case, it was clear that plants were suitable for natural enemy foraging by 8–15 d postapplication for all the synthetic insecticides tested. The intense sunlight, heavy daily rainfall during the growing season, and the rapid increase in foliage area during the mid- and late-whorl stages of maize plant growth are also likely to have quickly diluted or degraded toxic pesticide residues. Other field studies on the impact of a diversity of pesticides on natural enemies in maize and sorghum have reported similar findings, with natural enemy survival or abundance similar to control plot values within 1–2 wk of treatment (Williams et al. 1999, Al-Deeb et al. 2001).

Clearly, caution is required when making assumptions about pesticide impact on beneficial or nontarget organisms based on databases that depend heavily on dose-response relationships obtained in laboratory bioassays (Stark et al. 1995). Moreover, most laboratory studies only consider a single route of natural enemy exposure to pesticides, whereas in natural situations multiple routes of exposure are likely to affect the pesticide dose acquired by a natural enemy (Banken and Stark 1998).

After application of chlorpyrifos, the abundance of natural enemies was immediately reduced by ≈50–70% compared with control plots. This contrasts with predicted mortality of >90% envisaged from direct treatment of natural enemies with chemical insecticide sprays. This difference may be because of the position of the natural enemies on the maize crop; important predators such as earwigs (*D. taeniatum*) and *Chrysoperla* spp. tend to be found nestling in the spaces between the plant stem and leaf axils or on the underside of lower leaves. As such, many would have been protected from direct contact with the chemical spray although contact with contaminated plant surfaces while moving over the plant would still represent an important route of exposure to residues. Similarly, spiders on maize plants almost exclusively inhabited the underside of the leaves closest to the ground and were often observed inside silken tents. As a consequence, they probably had behavioral refuge from insecticidal sprays or residues, an effect also observed in previous studies with chlorpyrifos and spinosad (Méndez et al. 2002).

The phenology of the crop had a clear effect on the degree to which natural enemy populations were affected by chlorpyrifos treatments. Application at the

mid-whorl stage caused a more severe reduction in the abundance of natural enemies and nontarget insects than did application at the late-whorl stage. Presumably, the increased total leaf area of late-whorl stage plants resulted in a dilution of pesticide deposits and reduced spray penetration of the crop canopy allowing greater survival of beneficial arthropods. There was, however, no detectable effect of chemical or bioinsecticide treatments made at the mid-whorl stage on the outcome of treatments made at the late-whorl stage, with the exception of carry-over of SfMNPV inoculum which continued to cause *S. frugiperda* deaths in larvae infesting late-whorl stage maize.

Chlorpyrifos treatment resulted in a marked reduction in the recovery of parasitized *S. frugiperda* larvae. Of the other synthetic insecticides, it appeared that all had a similar degree of toxicity to pest and natural enemy populations alike. Methamidophos was toxic to the most abundant carabid predator, *C. calidum*, with reduced captures up to 8 d postapplication, but not thereafter, possibly as a result of the highly mobile habits of this insect and the relatively small plot size. In all cases, the density of natural enemy populations and the prevalence of *S. frugiperda* parasitism returned to levels comparable to those in control plots by 8 d postapplication and remained similar to those of control plots until the final sample taken at 22 d postapplication.

In contrast, the nucleopolyhedrovirus insecticide had no detrimental effects on populations of natural enemies or other insects at any time. This reflects the very high specificity of baculoviruses that typically infect a few closely related species (Gröner 1986). SfMNPV is very host-specific with only two other *Spodoptera* species known to be susceptible when administered high doses of SfMNPV inoculum (Murillo et al. 2003). The virus caused ≈30% infection in the *S. frugiperda* larvae collected from virus-treated plots, despite the application of a relatively high concentration of virus (3×10^{12} OBs/ha). The poor efficacy of aqueous sprays for control of *S. frugiperda* has been highlighted by Martínez et al. (2000), but can be significantly improved by including viral synergists or phagostimulant substances in the formulation (Cisneros et al. 2002, Castillejos et al. 2002). However, these trials were not designed to test virus efficacy against *S. frugiperda*, so the low prevalence of infection was not an issue of concern.

The interaction between nucleopolyhedrovirus and insect predators has generally been reported to be neutral for the predator and advantageous for the virus because, after feeding on a virus-infected lepidopteran larva, the predator may disseminate the viral OBs on its body surface or in its faeces (Fuxa et al. 1993, Vasconcelos et al. 1996). This is possible because the gut of predatory insects is acidic and does not cause the breakdown of the viral occlusion body, whereas the gut of phytophagous Lepidoptera is highly alkaline allowing viral OBs to dissolve, thus releasing infective virions into the gut cavity (Castillejos et al. 2001).

Nucleopolyhedroviruses do not kill adult parasitoids but may significantly reduce the survival of immature parasitoids that develop in infected hosts because of premature death of the host or the production of toxic viral proteins (Kaya and Tanada 1971, Brooks 1993). In general, the outcome and severity of the interaction depends on the interval between virus infection and parasitism (Escribano et al. 2000). We observed a reduction in the prevalence of parasitoid emergence in *S. frugiperda* larvae recovered from SfMNPV-treated plots and reared in the laboratory in both experiments in which this parameter was measured. This may have been a result of the relatively high prevalence of parasitism (30–50%) that occurred in these experiments. Previous analyses have generally failed to detect a significant reduction in parasitoid emergence from *S. frugiperda* larvae collected from virus-treated plants when the prevalence of parasitism averaged ≈20% (Martínez et al. 2000, Castillejos et al. 2002). Natural variation in field data may reduce the probability of virus-parasitoid interference being detected by routine statistical procedures when the prevalence of parasitism or virus infection is low.

Of course, human health is an issue of concern when applying synthetic insecticides, especially in the absence of protective equipment, as routinely occurs in many tropical countries (Friedrich 2000). In contrast, the nucleopolyhedrovirus has very low human health risks, limited mainly to the possible development of allergies or microbial contamination of virus preparations by potential human pathogens (OECD 1996).

The overall results of these studies were clear. The biological insecticide based on *S. frugiperda* nucleopolyhedrovirus had no adverse effect on insect natural enemies or other nontarget insect populations. The carbamate, pyrethroids, and organophosphate insecticides tested all resulted in reduced abundance of insect natural enemies, but for a relatively short period (8–15 d). Pesticide applications made to late-whorl stage maize were less harmful to natural enemy populations than applications made at the mid-whorl stage, probably because of a greater abundance of physical refuges and reduced spray penetration of late-whorl maize.

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