Research Article

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Seed treatment for managing fall armyworm as a defoliator and cutworm on maize: plant protection, residuality, and the insect life history

Camila Oliveira,[a,b] Silvana M Orozco-Restrepo,[a,b] Antônio CL Alves,[c] Braullio S Pinto,[a] Morgana S Miranda,[a,b] Márcio HP Barbosa,[c] Marcelo C Picanço[a] and Eliseu JG Pereira[a,b*]

Abstract

BACKGROUND: The highly polyphagous and invasive fall armyworm (FAW, *Spodoptera frugiperda*) can feed on different plant parts of host crops, damaging whorls and stalks in early maize growth stages. Systemic insecticide seed treatment (IST) could minimize this damage, although the residual efficacy may vary with the plant tissue damaged. Using damage rating scales and artificial infestation in controlled conditions, we determined the potential of IST against FAW attacking maize whorl leaves or the stalk base.

RESULTS: Chlorantraniliprole, cyantraniliprole, or thiodicarb + imidacloprid IST similarly killed > 80% FAWs for 1 or 2 weeks after plant emergence depending on the plant tissue attacked. The residual efficacy (i.e. time after plant emergence sustaining > 80% larval mortality) lasted from the first to the eleventh day (VE–V3 maize growth stages), while for cutworm on the maize stalk base, it lasted 3–7 days after plant emergence (V1–V2 stages). In terms of damage, the ISTs lasted 15 days after emergence (V4 stage) for FAW on whorl leaves and 10 days (V3 stage) for FAW feeding on the stalk base. The larvae surviving on the seed-treated plants underwent sublethal effects in growth and development, reducing insect fitness.

CONCLUSION: Diamide or carbamate + neonicotinoid seed treatments kill FAW larvae on maize whorls or stalks in favorable edaphoclimatic and insecticide-susceptibility conditions. The cumulative impacts of systemic IST on aboveground insect pests go beyond mortality. The ISTs studied can be valuable against FAW in maize, for instance, to help protect varieties that may not express sufficient insect resistance in maize early growth stages.

Supporting information may be found in the online version of this article.

Keywords: *Spodoptera frugiperda*; *Zea mays*; systemic insecticide; chlorantraniliprole; cyantraniliprole; thiodicarb + imidacloprid

1 INTRODUCTION

The large amounts of crop production losses caused by harmful organisms or diseases perhaps typify the challenge humans have historically had with pests and pathogens.[1–3] Despite the modern technological advances, our contemporary intensive agriculture likely must undergo a paradigm shift to reconcile productivity and sustainability. Seed quality and genetics are voiced as main inputs for increasing the productivity of many crops and even serving in ecological restoration.[4,5] In seed treatment for crop protection, physical, chemical, or biological agents are applied to the seed before sowing, hoping to suppress, control, or repel pathogens, insects, and other pests that attack seeds, seedlings, or plants.[4,5] The idea of treating seeds with materials existed long ago,[6] but it was only in the 1990s that some modern insecticides and fungicides raced for seed treatment.[7] Plant protectants that enhance seed productivity are increasingly adopted, and there is intense research to develop seed treatments against pathogens and arthropod pests.[4,5,8] This method of pesticide application is expected to be increasingly deployed despite some limitations.[9–13]

The intensive agricultural practices worldwide, particularly in warm regions, favor the population growth and resurgence of

* Correspondence to: EJG Pereira, Department of Entomology, Universidade Federal de Viçosa, 36570-900 Viçosa, MG, Brazil. E-mail: eliseu.pereira@ufv.br

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a Department of Entomology, Universidade Federal de Viçosa, Viçosa, Brazil

b Bioagro, National Institute of Science and Technology in Plant–Pest Interactions, Universidade Federal de Viçosa, Viçosa, Brazil

c Department of Plant Science, Universidade Federal de Viçosa, Viçosa, Brazil

phytophagous pest arthropods. For example, the soybean–maize or soybean–cotton rotations in most of the area of arable crops in Brazil[14] provide such high availability of host plants that many polyphagous insect species thrive in these field settings. The armyworm complex of *Spodoptera* spp. stands out among these phytophages, especially the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae). This species is highly polyphagous[15] and has invaded Africa, Asia, and Oceania in the last decade.[16–19] FAW not only is regularly a key pest in maize, but it also infests soybean and cotton or even rice fields, and the populations have a high capacity to evolve resistance to control measures.[14,20,21] FAW and other *Spodoptera* species generally have low inherent susceptibility to Cry Bt toxins in genetically modified soybean and cotton cultivars,[22–24] often requiring supplemental pest management measures.

Sound integrated pest management (IPM) programs should take advantage of reasonable strategically-planned control measures, such as natural biological control, host–plant resistance (either native or transgenic), and seed treatment.[11,13,25–27] The tactic can help protect early plant growth stages when defoliators, stand reducers, and other soil insects attack seeds, seedlings, or plants, compromising crop productivity.[4,26] For example, insecticide seed treatment (IST) may be appropriate in cropping systems in which beneficial conservation practices such as minimal tillage favor soil insects and other generalist insect pests.[11,27,28] Against an aboveground target, the insecticide via seed treatment must be absorbed, translocated by the plant, and ingested by the insect to interact with the biochemical target in an effective concentration.[29,30] Only a few systemic insecticides meet these conditions by having sufficiently high potency and suitable physicochemical properties, including water solubility and partition coefficients.[30,31]

Based on current knowledge, translocation via xylem is a primary route for a pesticide to reach aerial plant parts[32] when applied via soil or seed treatment. The targets of systemic insecticides are often sap-sucking insects,[13,30,33] and sometimes chewers feeding on plant organs belowground or aboveground.[29,34,35] Although not well understood, factors such as the physicochemical properties of the chemical and soil texture/organic matter and moisture influence pesticide uptake and translocation in the plant.[30–32,34] A few studies have addressed the non-target effects of systemic IST on zoophytophagous insects.[36–42] Nevertheless, data are lacking on crucial variables for sound use of systemic ISTs in IPM programs. For instance, despite the increasing worldwide use of ISTs, there are still critical knowledge gaps on their residual efficacy and plant protection (i.e. how long is the time window of plant protection), and their combined, lethal, and sublethal effects on the target insect.[43]

This study assessed the residual efficacy, plant protection, and sublethal effects of ISTs on FAW, a polyphagous, migratory, and invasive noctuid of global interest. In maize, FAW larvae are typically whorl-leaf feeders, but late instars also can feed on the maize plant like a cutworm, especially in early-stage maize of fields preceded by grass-family cover crops or those located nearby areas containing other host crops.[15] In this situation, there is potential for feeding FAW larvae to kill plants like cutworms, thereby reducing stand and potentially yield, making large infestations of FAW a severe threat to maize and other crops. Thus, we tested whether seed treatment could be efficacious against these modes of feeding of FAW. We hypothesized that ISTs would be ineffective in controlling infestations by large late-instar FAW larvae with a 'cutworm feeding behavior' but could be helpful to control FAW infestations by early-instars, generally from eggs laid by the moths on the leaves. This research brings novel and valuable information to guide new studies and assist in using IST in IPM programs.

2 MATERIALS AND METHODS

2.1 Insects

Spodoptera frugiperda larvae used in this study were derived from a population provided by the Brazilian Agricultural Research Corporation (Embrapa Maize & Sorghum Research Center, Sete Lagoas, Minas Gerais, Brazil), maintained without exposure to insecticides for over two decades. We used this population to approach a best-case scenario of FAW susceptibility to ISTs. The insects were reared using methods described elsewhere[44] with slight modifications. We placed adult FAWs in cylindrical polyvinylchloride cages (30 cm diameter $\times$ 35 cm height), with the inner walls covered with sulfite paper serving as an oviposition substrate. The moths were fed with a solution of 10% sugar and 5% ascorbic acid. The eggs were collected every 2 days and stored in plastic bags until hatching. Neonates (> 1000) were transferred to plastic 500-mL pots containing an artificial diet.[44] After reaching the third instar (1–1.5-cm size), to avoid cannibalism, the larvae were placed individually in 16-well polyvinylchloride trays (Advento plastics, Diadema, São Paulo, Brazil) having the same type of artificial diet (one larva per well). The rearing procedures were in controlled conditions [27 $\pm$ 2 °C, relative humidity (r.h.) 70 $\pm$ 15% and 14 h:10 h light/dark photoperiod).

2.2 Seeds, treatments, and plant stages

Three insecticides widely used in maize were used as follows: thiodicarb + imidacloprid 450 + 150 g L^{-1} (carbamate + neoicotinoid; Bayer, Belford Roxo, Rio de Janeiro, Brazil), chlorantraniliprole 625 g L^{-1} (diamide; Corteva Agriscience do Brasil, Barueri, São Paulo, Brazil) and cyantraniliprole 600 g L^{-1} (diamide; Syngenta, Paulínia, São Paulo, Brazil). Diamides generally target lepidopterans and certain coleopterans,[35] while the carbamate + neoicotinoid mixture may also target some species of sucking and soil insects.[45] We used the concentration equivalent to the label rate, diluting the insecticide in water according to the manufacturer's instructions. The seeds were from a non-Bt maize hybrid (BRS 3046, Embrapa Maize & Sorghum, Sete Lagoas, Minas Gerais, Brazil). We placed a batch of seeds in 200-mL plastic pots, mixed with the insecticide for 3 min until complete homogenization, and left to dry for 60 to 120 min in the laboratory. The planting was in 2-L plastic pots containing a mixture of local soil and organic substrate (MecPlant, MecPrec Industria e Comercio Ltda., PR, Telêmaco Borba, Brazil) in a 2:1 ratio. Fertilizer application was based on the chemical analysis of the soil, putting 400 kg ha^{-1} nitrogen–phosphorus–potassium (NPK, 4-14-8) (Fertipar Fertilizers and Agricultural Correctives Ltd, Varginha, MG, Brazil). A single seed was placed per pot (5-cm deep) to grow plants of suitable vigor. At each planting date, the pot was irrigated to appropriate moisture content and this was repeated daily in such a manner as to avoid insecticide losses through leaching. There were seven planting dates, starting 33 days before the artificial infestation (see later).

2.3 Experimental setting

Two experiments were conducted in parallel to determine the residual efficacy of the ISTs against FAW larvae feeding as whorlworms or cutworms (Fig. S1). We used plants of seven different ages as follows: 1, 3, 7, 10, 14, 21, and 28 days after emergence, representing the maize growth stages VE, V1, V2, V3, V4, V6, and V8.[46] We arranged the factor growth stage with seed treatment (three insecticides plus one control) in a completely randomized design. We used two glasshouses under controlled conditions (30 ± 5 °C, r.h. 70 ± 15% and 14 h:10 h light/dark photoperiod), located in the campus of the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil. In one experiment, we caged the FAW feeding on the maize whorl leaves whereas, in the other, we caged the larvae on the maize stalk base (Fig. S2).

2.3.1 *Experiment 1: third-instar (L3) larvae feeding on whorl leaves*

We used seven different maize growth stages, obtained from the serial plantings as previously described. There were ten replications (plants), each infested with five third-instar (L3) FAW larvae carefully placed on the maize whorl leaves using a fine paintbrush. Although FAW eggs are often laid on maize plants in the field, we used third instars to reduce the risk of inflating the experimental error associated with the challenging recovery of the neonates on whole maize plants, particularly those in the V6–V8 growth stages. After infestation, the plant was caged with a transparent plastic bag to prevent insect escape. There were pinholes on the cage to maximize ventilation and minimize condensation of water vapor (Fig. S2a). Irrigation was stopped 24 h before the infestation and later maintained intermittently until the end of the experiment. Larval mortality and Davis foliar damage rating scale[47,48] were recorded 96 h after infestation (Fig. S3a). The larvae were considered dead if they did not move when gently touched using tweezers. The surviving larvae were immediately placed in 16-well polyvinylchloride trays (Advento plastics) (one larva per well) and reared in the laboratory using the regular artificial diet (see below).

2.3.2 *Experiment 2: fifth-instar (L5) larvae feeding on the stalk base*

The experiment was similar to the previous one except that we released a single fifth-instar (L5) larva on the stalk base of 21 plants (replications). A layer of lawn grass residues was placed on the substrate at the plant base to simulate a field situation in which maize is grown in an area with desiccated straw, which is common under minimal tillage or in other conservation systems (Fig. S1a). We caged the potted plant in a plastic bag and closed it using a twine tied to the plant stalk 10–15 cm above the soil surface to prevent insect escape. This assembly allowed the plant leaves to stay outside the caged larva, preventing it from climbing and feeding on the leaves. The larvae had access only to the maize plant's stalk base, where they could either cut the plant or bore into the stalk, depending on the plant growth stage (Fig. S2b, c). All plants were infested on the same date using the procedure previously described. After 96 h, we recorded larval mortality and plant damage at the stalk base, considering dead the larva that did not move when touched. An L5 FAW larva damages the early-stage maize plant similar to black cutworm, so we adopted a five-level damage rating scale as follows: 1, no visible damage; 3, stalk rolled on the surface; 5, stalk bored; 7, plant cut-off at the base, and 9, plant killed or stalk tunneled down toward the ground, having the apical meristem destroyed.

2.4 Post-exposure effects

In both experiments 1 and 2, we placed the larvae that survived for 96 h on the maize plants according to treatment in polyvinylchloride trays of 16 wells (Advento plastics) containing the same type of artificial diet used for rearing (one larva per well) (Fig. S3d). The trays were brought to the laboratory to record the larval weight, growth, and development (27 ± 2 °C, 70 ± 15% r. h., and 14 h:10 h light/dark photoperiod). We recorded time to pupation, weighed each pupa (< 24 h), and placed them in 50-mL cups containing a moist cotton swab. We calculated the growth rate as the weight increased after IST exposure on the maize plants (L3) or during development (L5) divided by the number of days until pupation. We also recorded the survival rate. Assuming a positive correlation between pupal weight and fertility of the moths,[49] we calculated a fitness index using the following formula: f = proportion survival to pupation × pupa weight ÷ development time.[50] That allows integrating the combined effects on three fitness components (survival, growth rate, and body size) into a variable.

2.5 Statistical analysis

To analyze the larval mortality, plant damage, and other life-history traits, we used generalized linear models composed of the main effects of seed treatment and plant age/stage and their interaction. Depending on the response variable, we used binary, binomial, multinomial, or lognormal distributions and their default link functions, although departures from homoscedasticity and normality were not troublesome. When appropriate,[51] we adjusted for natural mortality in the control treatment (no IST, Supporting Information, Table S1). The residual efficacy was analyzed using non-linear regression ($P < 0.05$) as the insecticidal effect decays over time depending on various processes in the rhizosphere, plant, and insect. The regression model used for L3 mortality data was the logistic dose–response curve ($y = a + b/(1 + (x/c)^d)$, where y is mortality (%), and x is the time (in days) after plant emergence.[52] While the L5 mortality data had a peak so that the Gaussian model ($y = a + b \times \exp(-0.5 \times ((x - c)/d)^2)$ was appropriate, the Gompertz dose–response curve ($y = b \times \exp(-\exp(-(x - c)/d))$ better described the damage by L5 larvae. We defined the residual efficacy as the duration after plant emergence that the insecticide caused at least 80% pest mortality, a usual rate for a pesticide to be considered efficacious. We used a similar analysis protocol for the life-history variables of the insects recovered after 4 days of feeding on the plants. Sigmoidal, peak, and exponential models were used whenever appropriate for regression of these variables on days after plant emergence. The criteria to select the non-linear regression models were their logical description of the underlying phenomenon and parsimony, which seeks to explain the data with a minimum number of parameters or predictor variables.

3 RESULTS

3.1 Experiment 1: third-instar (L3) larvae on plant whorl

The survival of L3 larvae on the control treatment plants was always higher than 85% (Table S1), demonstrating that the natural mortality rate was sufficiently low to detect differences among the ISTs. The adjusted larval mortality differed over time after plant emergence ($F_{6,189} = 98.41$, $P < 0.01$) but not among the ISTs ($F_{2,189} = 1.42$, $P = 0.24$). The ISTs did not differ in their residual efficacy at killing L3 FAW larvae, as indicated by a non-significant interaction between seed treatment and time ($F_{12,189} = 1.28$, $P = 0.23$). As a result, one single regression model sufficiently described the mortality changes over time (Fig. 1(a)). All seed treatments caused more than 80% larval mortality up to 10 days

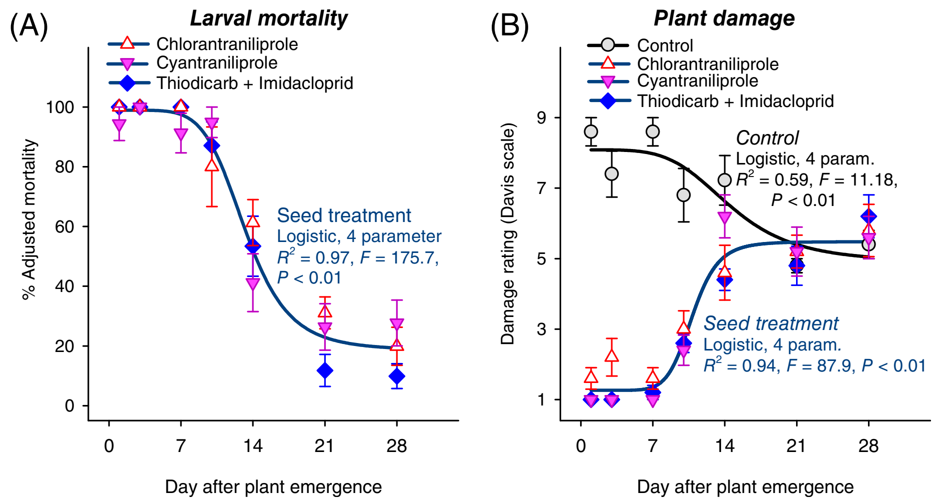

Figure 1. Response to insecticidal seed treatments by third-instar (L3) larvae of fall armyworm (*Spodoptera frugiperda)* on whorl leaves of whole maize plants grown in controlled edaphoclimatic conditions during different days after emergence. Their correspondence to the maize growth stages is 0 = VE, 3 = V1, 7 = V2, 10 = V3, 14 = V4, 21 = V6, and 28 = V8. (a) Curve of mortality adjusted for natural mortality in the control treatment on plants up to 28 days after emergence (VE–V8 growth stages); because of the non-significant difference ($P > 0.05$) among the insecticides in any of the maize growth stages (days after emergence), their effect is described by single regression curve. (b) Foliar injury by L3 larvae on seed-treated or untreated (control) maize plants. Data are means and standard error of ten replications (plants), each artificially infested with five L3 larvae at the maize whorl leaves and dissected 96 h after infestation. Regression coefficients and more information are shown in Supporting Information, Table S2.

after plant emergence (Fig. 1(a)). Afterward, larval mortality decreased to about 20% at 21 days of emergence and remained at the same level until 28 days after plant emergence (Fig. 1(a)).

The data on the whorl-leaf damage of control and seed-treated plants demonstrated a significant treatment × time interaction effect (Table 1). When we excluded the control data from the analysis, this interaction and the variation among the ISTs were not significant (Table 1), indicating that the differences in plant damage were primarily between the control and seed-treated plants. The ISTs protected the plants from L3 FAW larvae up to 14 days after emergence (Fig. 1(b)); after this time, there were no differences between seed-treated plants and controls (Fig. 1 (b)). The damage level stabilized close to score 5 in the Davis rating scale, that is, several large elongated lesions greater than 2.5 cm on the whorl leaves (Fig. 1(b), regression equations in Table S2), after 96 h of infestation of five L3 larvae.

3.2 Experiment 2: fifth-instar (L5) larvae feeding on the stalk base

As in the previous experiment, the survival of L5 larvae on the control treatment plants was always higher than 81% (Table S1); thus, the natural mortality rate was sufficiently low, allowing accuracy to detect differences among the ISTs. Regardless of the IST, the adjusted mortality of L5 larvae infested at the base of maize stalk varied over time after plant emergence ($F_{6,416} = 50.66$, $P < 0.01$). There were no significant differences among insecticide treatments ($F_{2,416} = 0.76$, $P = 0.47$); this was indicated by their pattern of residual efficacy over time which showed a non-significant insecticide seed treatment × time after maize emergence interaction effect ($F_{12,416} = 0.68$, $P = 0.77$). The mortality rates were above 80% for all treatments between the third and seventh days after emergence. The highest larval mortality rate occurred on days 6–7 (Fig. 2(a)). After this time, in all seed treatments, there was a rapid decrease in FAW mortality. After day 10, the mortality was not significantly different ($P > 0.05$) until reaching rates between 0 and 20% on day 28 after emergence (Fig. 2(a)).

We also compared the capacity of the seed treatments to provide plant protection against L5 FAW at the maize stalk base. There was a significant interaction of treatment × time (Table 1) when we compared the control data in the analysis, but this interaction effect was not significant when insecticide seed treatments only were contrasted (Table 1). The effect of any of these on the plant damage was not different over time after plant emergence, as indicated by the non-significant interaction of treatment × time (Table 1). The plant damage in all treatments was lower until

Table 1. Results of tests of fixed effects on the plant damage in the experiments with third- and fifth-instar (L3 and L5) larvae of fall armyworm, *Spodoptera frugiperda*. For comparison of insecticidal treatments, the tests were run excluding the control data

	Experiment 1: L3 FAW/maize whorl leaves						Experiment 2: L5 FAW/maize stalk base					
	All treatments			Insecticidal treatments			All treatments			Insecticidal treatments		
Effect	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Seed treatment (ST)	3	108	< 0.01	2	1.3	0.27	3	175.2	< 0.01	2	2.5	0.08
Time (T)	6	27.5	< 0.01	6	59.9	< 0.01	6	16.6	< 0.01	6	56.7	< 0.01
ST × T	18	13.2	< 0.01	12	1.3	0.23	18	25	< 0.01	12	0.8	0.70
Error	250			188			554			416		

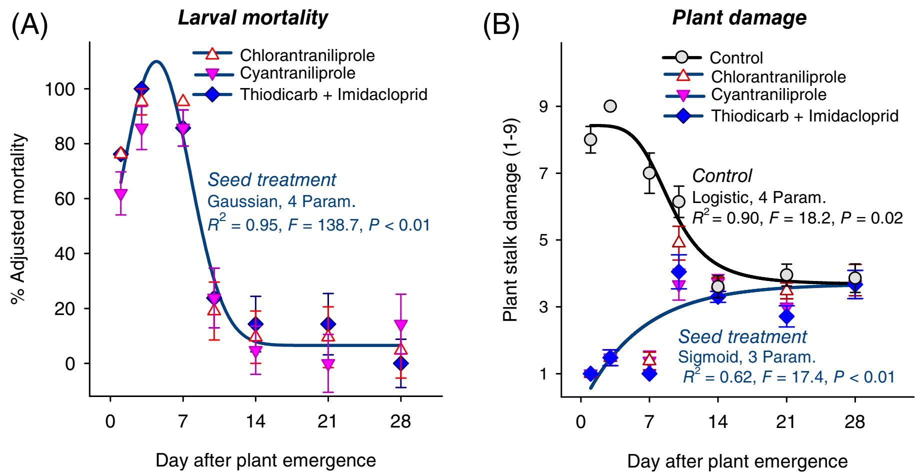

Figure 2. Response to insecticidal seed treatments by fifth-instar (L5) larvae of fall armyworm (*Spodoptera frugiperda)* on the stalk base of whole maize plants grown in controlled edaphoclimatic conditions during different days after emergence. Their correspondence to the maize growth stages is 0 = VE, 3 = V1, 7 = V2, 10 = V3, 14 = V4, 21 = V6, and 28 = V8. (a) Curve of (adjusted) larval mortality on plants up to 30 days after emergence, corresponding to VE–V8 maize growth stages; the single regression curve is because the larval mortality was not significantly different ($P > 0.05$) among the insecticides in any of the growth stages (times) after emergence. (b) Plant injury. Data are means and standard error of 21 replications (plants), each one infested with one L5 larva at the stalk base and dissected 96 h after infestation. Regression coefficients and more information are shown in Supporting Information, Table S3.

10 days after emergence; afterward, there were no significant differences between seed-treated plants and controls (Fig. 2(b), regression equations Table S3). The level of plant damage stabilized around 3–5 in the damage rating scale (1–9), corresponding to plants with the stalk scraped or bored.

3.3 Post-exposure effects

We followed the larvae that survived on the maize plants of ISTs throughout development and compared them to control larvae. In both experiments, with L3 and L5 FAW larvae, there was a significant interaction between seed treatment and time after plant emergence for various life-history traits such as larval and pupal weights, development time, and mean growth rate (Table 2). The control data (i.e. no IST), when excluded from the analysis, resulted in the interaction not being significant (Table 2) in the L3 experiment only but significant in the L5 one (Table 2). The interaction was significant due to the contrasting effects of the ISTs with regards to the control treatment in the experiment with L3 larvae but not in L5 larvae.

Because the response of the individuals exposed as L3 larvae did not vary significantly among the ISTs only ($P > 0.05$, Table 2), a single non-linear model was fit to the data of the seed-treated plants (Fig. 3(a–d), regression equations in Table S2). The larval weight and development time of the L3 larvae feeding on control plants varied significantly according to the time (age) after plant emergence, while the mean relative growth rate and pupal weight did not (Fig. 3(c,d)). The maximum weight gain of the larvae occurred on 3–7-day-emerged control plants, matching the minimum development time on such plants (Fig. 3(a,b)). Within the ISTs, the development time did not vary on differently-aged maize plants (Fig. 3(a–d)). Most larvae died after being recovered from plants 1–7 days after emergence, thus resulting in low larval weight in the ISTs, which no longer differed from control larvae on plants of 21–28 days after emergence (Fig. 3(a)). Interestingly, there was a peak in the relative growth rate and pupal weight of the individuals recovered from the insecticide-seed-treatment plants 14–21 days after emergence (Fig. 3(c,d)). Nevertheless, we did not observe these apparent stimulatory effects in the overall fitness index of the individuals (Fig. 5(a)). The armyworms had fitness values close to zero on the insecticide-treated plants until 7 days after emergence; afterward, their fitness differentials relative to controls were small (c. 20%, $P < 0.05$), close to zero on the plants of 21 days after emergence (Fig. 5(a)). Therefore, the insecticidal seed treatments reduced FAW fitness until this time, which could arguably be the residual effect period.

For the FAWs surviving the ISTs as L5 larvae on the maize stalk base, their life-history traits are shown in Fig. 5(a–d) and details of regression equations in Table S3. Among only the ISTs (i.e. excluding control data), the interaction treatment × time was significant ($P < 0.05$) for the larval weight gain and development time (Fig. 5(a,b), Table 2) but not for the larval growth rate and pupal weight (Fig. 5(c,d), Table 2); therefore, while a model was fit to each IST in Fig. 5(a,b), only a single model was suitable to the ISTs in Fig. 5(c,d).

The larval weight of L5 FAW reached the highest (peak) values on plants of 7 to 10 days irrespective of seed treatment, but after 21 days after emergence, the control larvae weighed less than the control larvae on 1-day-old seedlings (Fig. 5(a)). Larval weight values in the ISTs were generally lower than in control (Fig. 5(a)), although a few larvae recovered from the 7–10-day plants of seed treatment with thiodicarb + imidacloprid or chlorantraniliprole weighed as much as the control larvae ($F_{2,152} = 1.14$, $P = 0.32$) (Fig. 5(a)). A similar pattern was observed for the development time, with minimum values occurring on the 7-day-old control plants (Fig. 5(b)) and differing from the values for the other treatments ($F_{3,233} = 6.35$, $P < 0.01$) (Fig. 5(b), Table 2). Likewise, the values of mean growth rate were higher in the control larvae (feeding on seed-untreated plants) ($F_{3,233} = 8.00$, $P < 0.01$, Table 2), with a peak on the 7–10-day-old plants followed by a declining and then a stabilizing trend in the curve of growth rate (Fig. 5(c)). There was a relatively slight variation in pupal weight, with one heavy pupa in one of the insecticide-treated plants and an overall main effect of treatments ($F_{6,233} = 3.09$, $P = 0.01$) (Fig. 5(d)).

The calculated fitness index demonstrated the cumulative effects of plant quality (as affected by age and seed treatments) (Fig. 4(b)). Consistently, there was a peak in the fitness values

Table 2. Results of the tests of fixed effects on the life-history traits measured to document the post-exposure effects of insecticidal seed treatment in the fall armyworm, *Spodoptera frugiperda*

		Experiment 1: L3 larvae/maize whorl leaves						Experiment 2: L5 larvae/maize stalk base					
		All treatments			Insecticidal treatments			All treatments			Insecticidal treatments		
Variable	Effect	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Larva weight	Seed treatment (ST)	3	21.21	< 0.01	2	1.46	0.24	3	19.09	< 0.01	2	1.14	0.32
	Time (T)	6	41.34	< 0.01	4	3.11	0.02	6	8.98	< 0.01	6	4.70	< 0.01
	ST × T	10	5.19	< 0.01	6	0.50	0.81	14	5.16	< 0.01	8	6.37	< 0.01
	Error	140			77			265			152		
Pupa weight	Seed treatment (ST)	3	7.00	< 0.01	2	0.17	0.84	3	1.71	0.17	2	0.38	0.69
	Time (T)	6	1.90	0.08	4	2.56	0.04	6	3.09	0.01	6	3.30	< 0.01
	ST × T	10	4.46	< 0.01	6	2.08	0.06	12	2.16	0.01	6	1.42	0.21
	Error	524			254			233			129		
Development time	Seed treatment (ST)	3	9.52	< 0.01	2	1.05	0.35	3	6.35	< 0.01	2	0.14	0.87
	Time (T)	6	23.10	< 0.01	4	5.37	< 0.01	6	8.89	< 0.01	6	4.02	< 0.01
	ST × T	10	2.78	< 0.01	6	1.05	0.40	12	3.38	< 0.01	6	3.68	< 0.01
	Error	526			255			233			129		
Mean growth rate	Seed treatment (ST)	3	5.39	< 0.01	2	0.10	0.90	3	8.00	< 0.01	2	0.69	0.50
	Time (T)	6	7.43	< 0.01	4	3.32	0.01	6	5.43	< 0.01	4	2.78	0.01
	ST × T	10	4.69	< 0.01	6	1.08	0.38	12	2.46	< 0.01	6	2.16	0.05
	Error	526			255			233			129		
Fitness index	Seed treatment (ST)	3	59.71	< 0.01	2	9.72	< 0.01	3	133.76	< 0.01	2	5.51	0.01
	Time (T)	6	49.50	< 0.01	4	103.11	< 0.01	6	30.70	< 0.01	6	47.13	< 0.01
	ST × T	10	18.26	< 0.01	6	17.66	< 0.01	12	27.66	< 0.01	6	16.59	< 0.01
	Error	526			254			233			129		

of the individuals of the control treatment recovered from the 7-day-old plants, a trend which fell gradually for plants 10–14 days after emergence and stabilized for older plants, 21–28 days after emergence (Fig. 4(b)). Importantly, all insecticides differed from control ($F_{3,233} = 133.76$, $P < 0.01$, Table 2), leading to lower values of insect fitness index primarily for plants 0–7 days after plant emergence (Fig. 4(b)). The fitness values for larvae feeding on seed-treated and control plants were not significantly different ($P > 0.05$) at 14 days after emergence onwards: this time would be the end of the residual effect period (Fig. 4(a)).

4 DISCUSSION

Data on plant protection and cumulative lethal and sublethal effects of ISTs on target insects are fundamental for sound pest management programs. For the first time to our knowledge, this work shows the efficacy, residuality, and post-exposure effects of three ISTs against FAW. The moths often colonize maize fields by laying eggs on early-stage plants. Mid–late-instar FAW larvae from previous crops or other hosts[15] can also be troublesome. Thus, we hypothesized that IST would be effective only against third-instar or smaller larvae and that there would be differences between insecticides because of their different physicochemical properties. Surprisingly, the insecticides in seed treatment (i.e. carbamate + neonicotinoid and diamides) were equally effective against FAW larvae, causing > 80% mortality or protecting maize plants from FAW as defoliator until 14 days after emergence and cutworm on the stalk base until 10 days after emergence. These effects are credited to the systemic insecticides because the natural mortality in the control plants (no IST) was less than 15% and adjusted accordingly.[51] Consistently, chlorantraniliprole or thiodicarb + imidacloprid was reported reducing FAW infestation 7–14 days after maize emergence.[53] In terms of damage, the ISTs in our study lasted 15 days after emergence for FAW on whorl leaves and 10 days for FAW feeding on the stalk base. During the residual efficacy period, the foliar damage rating was below the value 3 in the Davis scale[47,48] (i.e. leaves with lesions smaller than 1.3 cm), the threshold of FAW sampling endorsed for decision-making for pest management.[48] Therefore, the ISTs were effective against FAW and can be helpful in IPM programs, including those using alternative methods with pheromone and biological control measures.[11,54] The IST may also help protect maize cultivars that may not express sufficient insect resistance in maize early growth stages, a situation in which the IST should be deployed within an IPM framework, only when appropriate.

We used insecticide-susceptible FAW larvae to approach a best-case scenario of the insect sensitivity to ISTs in a controlled environment. Predicting the performance of the ISTs in the field is complex because the edaphoclimatic conditions (e.g. soil organic matter and moisture) and insecticide susceptibility profiles of the FAW populations are variable, perhaps predominantly adverse. The field performance of the ISTs is unlikely to be better than in the present study, especially in regions with the historical use of carbamates, organophosphates, and diamides against FAW. The residual efficacy for the armyworm neonates on maize leaves should be close or only slightly longer than for the third instars used here, given their relative insecticide susceptibility (EJG Pereira, unpublished data). The environmental factors shaping the residual efficacy of ISTs (e.g. ecophysiological and edaphoclimatic conditions) vary in space and time, affecting the insecticide absorption/translocation interactively in the plant and the insect exposure in the feeding site.[8,32] These may be topics for future research to improve our knowledge and predictability of IST effects on the target insects in different scenarios.

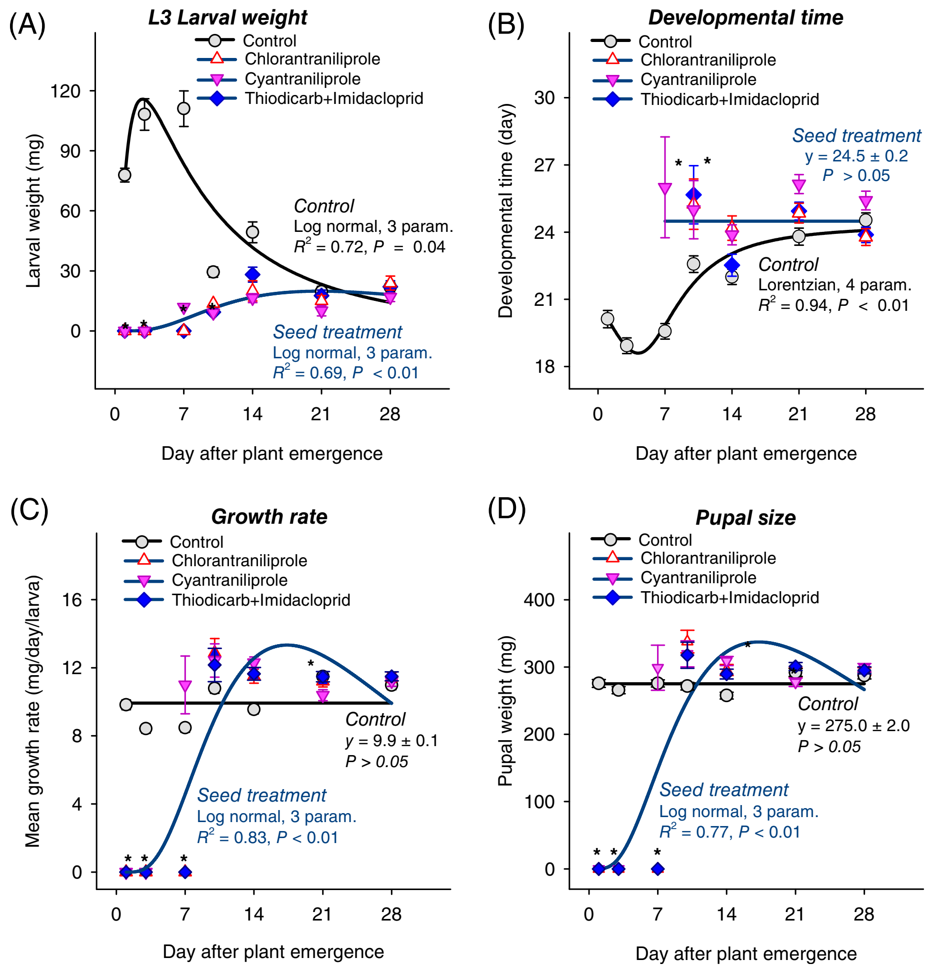

Figure 3. Life-history traits of fall armyworms (*Spodoptera frugiperda)* after 96-h exposure of third-instar larvae (L3) to insecticidal seed treatments on whorl leaves of maize plants in different days after emergence. Their correspondence to the maize growth stages is 0 = VE, 3 = V1, 7 = V2, 10 = V3, 14 = V4, 21 = V6, and 28 = V8. (a) Larval weight after 96 h on the plants. (b) Developmental time of the individuals that survived exposure. (c) Post-exposure mean growth rate. (d) Pupal weight of the survivors. For each panel and in a given day, means and standard errors with an asterisk (*) are not significantly different from the control (P > 0.05, Dunnett's test procedure protected by F test). Regression coefficients and more information are shown in Supporting Information, Table S2.

The ISTs caused > 80% mortality of fifth-instar larvae feeding on the stalk base from the third to the seventh day after maize emergence, after which the larval mortality decreased and then stabilized. Late-instar FAW are large larvae (*c*. 30 mm or more) and generally insecticide tolerant,[55] but even so, approximately 60% of them died on the seed-treated maize seedlings in the VE–V1 growth stages. Interestingly, the 40% L5 larvae surviving on these plants did not feed on them at all, causing little or no damage (Fig. 2). The insecticide residues in the plant tissues are likely the primary cause of this outcome by mediating feeding avoidance behavior or sublethal intoxication or impairing food ingestion. The larvae had access only to the maize plant's stalk base, where they could either cut the plant or bore into the stalk, depending on the plant growth stage. The larval mortality sharply decreased on plants 8 days after emergence onwards. Although not quantified, the insecticide concentration in the plant tissue must have been reduced by dilution (due to metabolization/degradation and plant growth/development) or by insufficient absorption/translocation of the insecticide molecule to the tissues ingested by the larvae.[32,34] For a systemic insecticide, its water solubility and logK_{ow} (i.e. octanol/water partition coefficient) and the soil characteristics (e.g. percentage of clay and organic matter) normally interact to determine the insecticide uptake and translocation.[29,30] The three seed treatments showed similar performance against insecticide-susceptible L5 FAW, suggesting that the physicochemical properties of the potting soil and insecticides were compatible for their insecticidal action on the above-ground target.

The mortality of L3 larvae caused by the 1-day-emerged maize plant indicates the absorption and translocation of carbamate + neonicotinoid (thiodicarb + imidacloprid) and diamide (chlorantraniliprole, cyantraniliprole) insecticides occurred since the first day of maize emergence. This result with these insecticides is consistent with their absorption and upward translocation[29] and their efficacy against FAW in maize and soybeans.[29,56] The efficacy of thiodicarb and imidacloprid was somewhat surprising given their not-so-favorable physicochemical properties for absorption/translocation.[32,45] The value of diamide seed treatments against lepidopteran and coleopteran insects has been shown for cyantraniliprole against *Agrotis ipsilon* and *Mythimna unipuncta* (Noctuidae) in maize[34,57] and chlorantraniliprole against *Lissorhoptrus oryzophilus* (Curculionidae) and *Diatraea saccharalis* (Crambidae) in rice.[28,35,58] In addition, consistent with our results, seed treatments using anthranilic diamide insecticides were

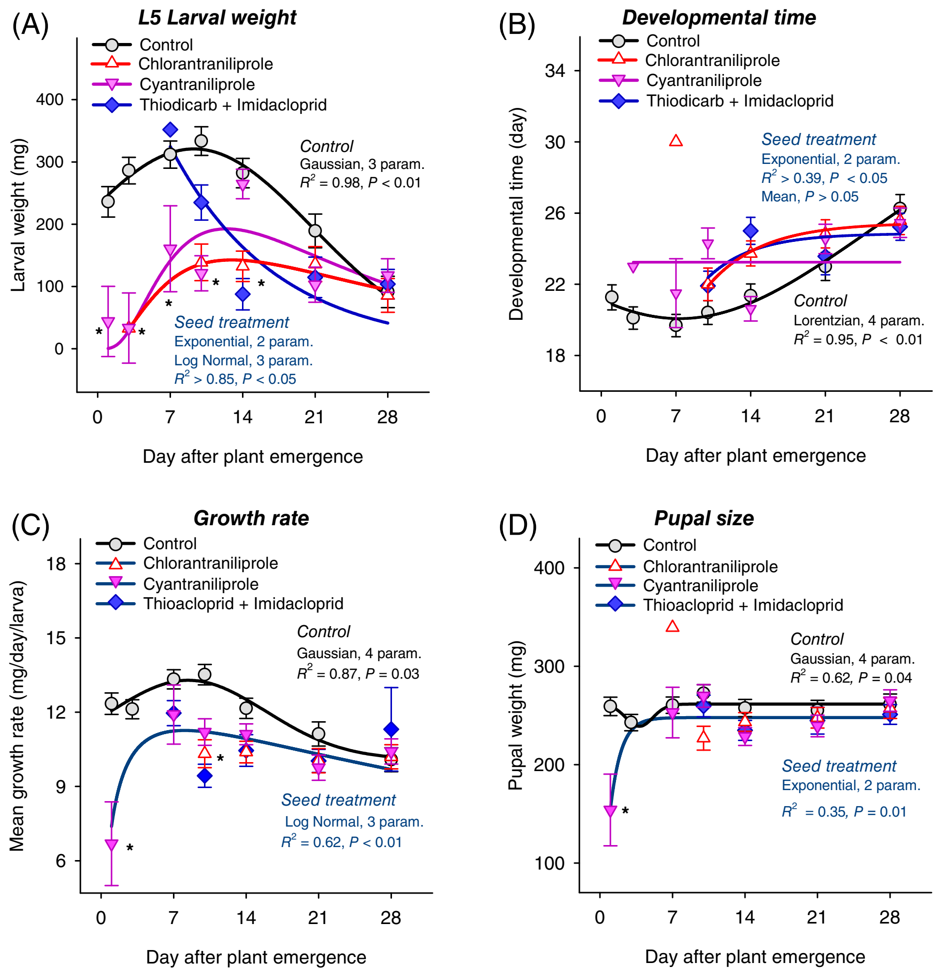

Figure 4. Life-history traits of fall armyworms (*Spodoptera frugiperda*) after 96-h exposure of fifth-instar larvae (L5) to insecticidal seed treatments in the stalk base of maize plants in different days after emergence. Their correspondence to the maize growth stage is 0 = VE, 3 = V1, 7 = V2, 10 = V3, 14 = V4, 21 = V6, and 28 = V8. (a) Larval weight after 96 h. (b) Developmental time of the individuals that survived exposure. (c) Mean growth rate during larval development. (d) Pupal weight of the survivors. For each panel and in a given day, means and standard errors with an asterisk (*) are significantly different from the control (P > 0.05, Dunnett's procedure protected by F test). Regression coefficients and more information are shown in Supporting Information, Table S2.

efficacious against *Delia platura* (Diptera: Anthomyiidae) and *Ostrinia nubilalis* (Lepidoptera: Crambidae) in the snap bean,[59] and these compounds were selectively toxic to several other insect-pest species.[60–66] The residual efficacy obtained here against FAW (*c.* 14 days after plant emergence) was similar to the one against black cutworm,[34] and else the seed treatment was efficacious mainly in the early growth stages of rice and maize.[28,35,57,58]

The route of insecticide application by the seed is generally safer for the environment and the operator,[67] but there are instances of side effects of systemic insecticides to beneficial zoophytophagous organisms.[36–42] Compared with thiodicarb and imidacloprid insecticides, the narrow-spectrum anthranilic diamides may be more relatively selective to non-target organisms.[68,69] There can also be sublethal effects on target and non-target organisms' fitness components and population growth.[43,70] Delayed development and other sublethal effects of chlorantraniliprole or cyantraniliprole have been documented.[60,64,66,71,72] We measured larval mortality and followed the survivors for sublethal effects[73,74] as they can alter life-history traits in the individuals exposed or their progeny.[73,74]

The estimated fitness of L3 FAW larvae not exposed to ISTs was alike on maize plants of ages 1–28 days after emergence (Fig. 4(a)), despite variation in some of their immature life-history traits depending on the plant stage (Fig. 3). The maximal weight gain for the larvae occurred on plants of 3 days after emergence (V1 maize growth stage, Fig. 3(a)). These plants must have provided a relatively suitable microclimate and food quality for the larvae in terms of physical and chemical traits.[75–77] One can make a similar argument for the L5 larvae feeding on the stalk base. On 7-day-old plants (V2 growth stage), L5 FAW larvae had maximal (30% higher) performance, which decreased 30–50% in V6–V8 maize growth stages, 21–28 days after emergence (see Figs 4(b) and 5(a)). The quantity and quality of defensive chemistry/morphology and nutrient content often varies throughout the plant and its life cycle.[78,79] Likely, the leaf and stalk hardness/toughness in maize plants 14 days after emergence (V4 growth stage) or older determined the lower performance of FAW larvae.[77,78,80] The L5 larvae on the stalk base were more restricted to the available plant tissue than the L3 larvae on whorl leaves, maybe explaining why such larvae maintained their performance on plants up to 28 days after emergence (Fig. 4).

For the IST-exposed larvae, the effects of food-plant quality likely interacted with IST residues,[81,82] so that the outcome on the armyworm life history is more challenging to analyze and

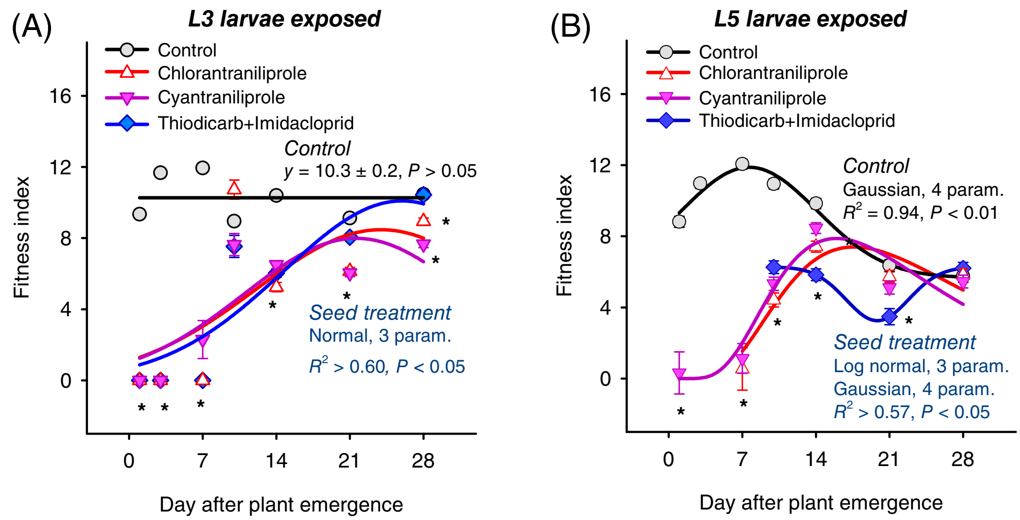

Figure 5. Overall performance of the fall armyworms (*Spodoptera frugiperda*) after 96-h exposure to insecticidal seed treatments on maize plants of different days after emergence. Their correspondence to the maize growth stages is 0 = VE, 3 = V1, 7 = V2, 10 = V3, 14 = V4, 21 = V6, and 28 = V8. (a) Third-instar larvae (L3) on whorl leaves. (b) Fifth-instar larvae (L5) on the stalk base. On a given day, means and standard errors with an asterisk (*) are not significantly different from the control ($P > 0.05$, Dunnett's procedure after F test). Regression coefficients and more information are shown in Supporting Information, Tables S2 and S3.

interpret. Both groups of FAWs that survived on the maize whorl leaves or stalk base of the seed-treated plants changed life-history traits regarding control individuals. The IST residues in the maize leaves possibly induced stress on the L3 larvae, resulting in stimulatory effects[43] in some fitness components (e.g. growth rate and pupal weight, Fig. 3(c,d)). This increased performance regarding the control was approximately 35%, typical of hormetic responses (i.e. a beneficial effect at low concentrations of stressors),[83] a phenomenon which seems to be ubiquitous[73,84–87] and may impose favorable or unfavorable scenarios for plant protection.[73] Interestingly, this type of response (increased performance) was only detected in individual life-history traits and did not translate into an enhanced overall fitness index (variable encompassing survival, pupal weight, and development time). The fitness values of FAWs that survived exposure to IST plants were lower than the control group, even on plants 28 days after emergence (V8 growth stage). This outcome indicates that IST effects on insects go beyond lethality and can negatively impact the individuals more than expected from estimates of short-term mortality.

In summary, this research shows that systemic ISTs can protect the early growth stages of maize plants against FAW as a cutworm or whorlworm. In maize, diamide or carbamate + neonicotinoid seed treatments can kill FAW larvae feeding on leaf whorls or stalks. The residual efficacy/protection of IST against defoliator FAW can last for 14 days, while for FAW as a cutworm, the protection window is from 1 to 10 days after maize emergence, when the FAW population is insecticide susceptible and the edaphoclimatic conditions are favorable for plant uptake and translocation of systemic pesticide. The insects surviving on seed-treated plants underwent post-exposure effects on growth and development, indicating that IST effects on pests can go beyond mortality and may help manage FAW, a globally invasive noctuid. Further studies on pest species of different feeding modes on crop plants (e.g. suckers and other chewers) may be pursued to use IST wisely in pest management programs. Efforts are also necessary to preserve pest susceptibility to systemic insecticides useful in seed treatment and foliar application. Likewise, data on their impact on non-target organisms and knowledge of the integrative and transgenerational effects of ISTs on the target species are relevant for their rational and sustainable deployment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

EJGP, MCP designed the study. BSP, MHPB provided resources; EJGP, CO, SMO-R, ACLA planned experiments; CO, SMO-R, ACLA MSM, MHPB collected data. EJGP, MCP analyzed data. CO, BSP, EJGP wrote the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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