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Toxicity and Efficacy of Chlorantraniliprole on *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae) on Cabbage

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Abstract

Toxicity of chlorantraniliprole was assayed against young (first and second instars) and older larvae (third and fourth instars) of cabbage *Pieris rapae* (Lepidoptera: Pieridae) on cabbage (*Brassicae oleracea*), and persistence of field–aged leaf residue of chlorantraniliprole was assayed with 5-old-day larvae of *P. rapae* on cabbage. Efficacies of chlorantraniliprole and other newer insecticides to *P. rapae* were tested under field conditions for two seasons in Hubei province in China. The LC_{50} value of chlorantraniliprole for early and later *P. rapae* larvae were 7.92 and 11.34 mg/L by contact toxicity, respectively. The LC_{50} value of chlorantraniliprole for early and later *P. rapae* larvae were 0.95 and 4.32 mg/L through ingestion, respectively. The toxicity of field-aged leaf residues of chlorantraniliprole (0-, 3-, 5-, 7-, 10-, 14-, 21-, 25-, and 28-day-old residues) declined gradually under the field conditions. Almost all larvae died on day 5 after feeding on the leaves with 0-21-day residue, and the mortalities were as high as 83.3% and 72.5% for the 21- and 25-day-old leaf residues. Chlorantraniliprole application suppressed *P. rapae* larvae below the economic threshold for 21-28 days. The field efficacy trials show that chlorantraniliprole at 52 mg a.i /L rate was effective against *P. rapae* larvae on cabbage, providing marketable cabbage with three applications per season. In addition, chlorantraniliprole was as effective as indoxacarb and spinosad and significantly more effective than emamectin benzoate.

Keywords: *Pieris rapae*, chlorantraniliprole, reduced-risk insecticides

1. Introduction

The imported cabbage worm, *Pieris rapae* (L.) (Lepidoptera: Pieridae), is one of the most serious insect crop pest in China, costing more than $RMB371 million ($US45 million) in insecticide treatment in 1997 (Wu, 2000). Control of the pest is becoming increasingly difficult due to its resistance to many conventional synthetic insecticides in many areas (Mu et al., 1984; Han et al., 1987; Armes et al., 1997; Li et al., 1999). With the imposed quality restrictions on fresh market vegetables, management of Lepidopteran pests on cabbage has been based on either a low threshold (one larva per three plants) or on scheduled weekly sprays (Cartwright et al., 1987). Thus, some newer kinds of insecticides with a chemistry of the anthranilic diamide, have been introduced as substitutes to control *P. rapae*.

Chlorantraniliprole is a novel anthranilic diamide insecticide discovered by DuPont, also known as Rynaxypyr and DPX-E2Y45, which belongs to a new chemical class of selective ryanodine receptor (RyR) agonists (Lahm et al., 2005; Cordova et al., 2006). Upon ingestion, chlorantraniliprole activates the release and depletion of internal calcium stores in muscles (Bassi et al., 2007). In the target organism, this causes impaired regulation of muscle contraction and leads to feeding cessation, lethargy, paralysis, and death. Differential selectivity towards insect RyRs explains chlorantraniliprole's outstanding profile of mammalian toxicity. It is being developed worldwide by DuPont in a broad range of crop to control a range of pests belonging to the order Lepidoptera and some Coleoptera, Diptera and Isoptera species. It is primarily active on chewing pests by ingestion and secondarily by contact, showing good larvicidal active.

In this study, we tested chlorantraniliprole and several other newer insecticides under laboratory and field conditions for several years in Hubei province in China. We reported the toxicities of chlorantraniliprole on *P. rapae* larvae, toxicities of field-aged leaf residues against *P. rapae* larvae, and the result of field trials compared with other reduced-risk and commonly used insecticides.

2. Materials and Methods

2.1 Pieris Rapae

The *P. rapae* lab colony used in this study were derived from cultures maintained on plotted cabbage (Bailey) without exposure to any insecticide in the greenhouse for > 5 years at 24-30 ^{o}C and 60-80% relative humidity (RH) under a photoperiod of 14:10 h light:dark at the Laboratory of Insect Toxicology, Yangtze University, Jingzhou, China. The emergent butterflies were fed on a 10% aqueous honey solution daily during the experiment. Adults were allowed to mate and oviposit on four small cabbage plants that each had 4-5 fully expanded leaves. The leaves bearing *P. rapae* eggs were detached from the plants, and placed in plastic rearing trays (20 × 40 × 15 cm, F1020-No Holes plastic Flat Tray, T. O. Plastic Inc., Minneapolis, MN). After hatching, the larvae were fed with fresh cabbage leaves as described above.

2.2 Cabbage

We used cabbage as the host plants for both field tests and laboratory bioassay. Cabbage plants for laboratory bioassays were grown Metro-Mix 300 growing medium (Grace Sierra, Horticultural Products, Milpitas, CA) in a greenhouse with 24-30 ^{o}C and 60-80%RH and natural lighting. At the time of planting, a slow-release fertilizer (N:P:K = 12:8:6) was applied to cabbage. Plants used in the experiments were at the 30-35 cm in the height with 10-12 leaves.

2.3 Insecticides

Chlorantraniliprole (Rynaxypyr, 20% purity, recommended field rate 26-52 mg a.i/l, DuPont, USA), indoxacarb (Avatar, 15% purity, recommended field rate 47-63 mg a.i/l, DuPont, USA), spinosad (Tracer, 2.5% purity, recommended field rate 2.0-43 mg a.i/l, Dow AgroSciences Corporation, USA), emamectin benzoate (Banleptm, 1% purity, recommended field rate 2.6-2.9 mg a.i/l, Pharmaceutical Group Corporation, Hebei, China).

2.4 Toxicity Bioassays

2.4.1 Topical Toxicity Test

Topical toxicity test were performed to determine the effective concentration of chlorantraniliprole that would kill larvae *P. rapae* by contact. Larvae were inoculated with 1μl droplet of chlorantraniliprole using a microsyringe at delivered dosages of 1.5, 3, 6, 12, 24 mg/l. Treated larvae were transferred to petri dishes (9 cm diameter and 1.5 cm depth) with four layers of filter paper and held in a chamber maintained at 26±2 ^{o}C, 70±5% RH for 48 h. Each petri dish containing ten larvae were set up for each dose. All treatment had five replications. Mortality, defined as the inability to move when prodded, was recorded by counting the number of dead and moribund larvae after 24 h of exposure.

2.4.2 Ingestion Toxicity to Larvae

Leaf-dip assay was used to evaluate the ingestion toxicity effect of the chlorantraniliprole according to the method of Insecticide Resistance Action Committee (2000). Leaf discs (2.5 cm diameter) cut from cabbage leaves with a cork borer, were dipped for 5s in the chlorantraniliprole or control solutions (sterile distilled water) and dried in the air for 1h at room temperature. Four leaf discs were transferred to Petri dishes (9 cm diameter and 1.5 cm depth) with four layers of filter paper. Ten *P. rapae* larvae were placed alongside the leaf discs at the centre of the Petri dish. Mortality was defined as described above. All experiments were assessed after 24 h. All treatment had five replications.

2.4.3 Toxicity of Field-Aged Leaf Residue to Larvae

Chlorantraniliprole was sprayed at 52 mg a.i/l and untreated plants were used as the control. The insecticides were applied at nine different times to have the residue ages of 1, 3, 7, 10, 14, 21, 25 and 28 days after treatment (DAT). Bioassays were conducted in a laboratory at 26± 2 ^{o}C, 50±5% RH, and a photoperiod of 14 h:10 h (L:D). Bioassays were initiated 2 h after treatment at 0, 3, 7, 10, 14, 17, 25 and 28 DAT. For each date, a single leaf disk (8-9 cm diameter) was cut from one leaf in each plot. The leaves were selected based on location on the plant to increase likelihood of good spray coverage. Each leaf disk was placed in a large clear plastic Petri dish and ten third instars were placed on the leaf disk. Each treatment had six replications. Larvae were fed with freshly cut leaf disks from the treated plants 2 days after initial exposure. Larval mortality was recorded daily for 5 days. Experiments with control mortality more than 20% were discarded and repeated.

2.4.4 Field Efficacy Trials

Two field trials were conducted to evaluate efficacies of four insecticides: chlorantraniliprole (52 mg a.i/l), indoxacarb (63 mg a.i/l), emamectin benzoate (3.9 mg a.i/l) and spinosad (43 mg a.i/l) during 2015-2016 at the research center of pesticide in Yangtze University, Jingzhou, China. For field trials in 2015, cabbage was planted on 10 March 2015. Insecticides were applied four times on 12, 19, 26 May, and 1 June, 2015. For field trials in 2016, cabbage was planted on 10 March 2016. Insecticides were applied on 12 May when *P. rapae* population in some plots reached the economic threshold. Thereafter, the insecticides were applied once per week on 19 and 26 May and 1 June. At termination on 5 July in 2015 and 1 June in 2016, a damage-quality evaluation was made on 10 plants per plot as described below. In general, field plots consisted of two rows of cabbage on 1-m bed with 30-cm within-row plant spacing and 10-m length. All plots were separated with *sorghum* windbreaks and a 1.3-m alleyway. All treatments were arranged in a randomized complete block design with four replications. Insecticide applications were initiated as larval densities exceeded the threshold level of 0.3 larvae per plant as determined in Hubei (Cartwright et al., 1987). Insecticides were applied using a tractor-mounted sprayer. The tractor-mounted sprayer was equipped with three ceramic hollow cone nozzles per row (TX6-red, one over the plant, and one on each side of the row directed into the plant) with a spray pressure of 689.5 kPa and a delivery rate of 280 l ha^{-1} at 3.2 km h^{-1}. To monitor *P. rapae* larval populations, cabbage plants were scouted weekly. Number of *P. rapae* larvae and eggs per plant were counted by checking both the upper and lower surfaces of every leaf on each plant, and 10 plants were examined from each plot. A damage-quality evaluation was made on plants per plot based on the six categories as described by Greene et al. (1969): 0, no apparent damage; 1, minor feeding damage on wrapper outer leaves, or 1% leaf area eaten; 2, minor-moderate feeding damage, or 2-5% leaf area eaten; 3, moderate damage, or 6-10% leaf area eaten, but no head damage; 4, moderate-heavy damage on wrapper and outer leaves with minor damage on head, or 11-30% leaf area eaten; and 5, heavy damage on wrapper and head, or > 30% leaf area eaten.

2.5 Data Analysis

Toxicity of chlorantraniliprole to *P. rapae* eggs and larvae, including LC_{50} and LC_{90} with related parameters, were analyzed using POLO (Robertson & Preisler, 1991). Field-aged leaf residue bioassay data, including percentage of larvae mortality, were transformed to the arcsine square root before analysis to stabilize error variance (K. A. Gomez & A. A. Gomez, 1984). Mean percentage of numbers of *P. rapae* larvae and damages among the treatments were analyzed using analysis of variance (ANOVA) and were separated using the least significant different test least significant different (LSD) following a significant F test at $P = 0.05$. Although all tests of significance were based on transformed data, the untransformed percentages of mortalities are presented. All data were analyzed using the SPSS software package (ver.17, SPSS Inc., Chicago, IL, USA).

3. Results

3.1 Toxicity Bioassays

Based on dose-mortality responses, the LC_{50} value of chlorantraniliprole for early and later larvae ranged from 5.07 to 12.13 mg/l and 7.12 to 19.71 mg/l by contact, respectively (Table 1). The LC_{90} value of chlorantraniliprole for early and later larvae ranged from 22.58 to 122.98 mg/l and 33.10 to 156.09 mg/l by contact, respectively (Table 1). Chlorantraniliprole exhibit high level contact toxicity on *P. rapae* larvae. Similarly, chlorantraniliprole are highly toxic to *P. rapae* larvae through ingestion. The LC_{50} and LC_{90} value for early and later larvae by ingestion were 0.95, 4.32, 7.01 and 19.65 mg/l, respectively (Table 1). The results indicated that chlorantraniliprole is primarily active on chewing pests by ingestion and secondarily by contact, showing good and larvicidal activity. The LC_{50} and LC_{90} values of chlorantraniliprole to young larvae (first and second instars) and older larvae (third and fourth instars) were significantly different, and younger larvae were significantly more susceptible to chlorantraniliprole than the older larvae (Figures 1 and 2).

Table 1. Comparison of toxicity of chlorantraniliprole at 24 h to early and late larvae of *P. rapae* as mean of LC_{50} and LC_{90} through exposure and leaf-dip assay at 95% of fiducial limit

Larval age	Methods	n	LC_{50} (mg/L)[a]	LC_{90} (mg/L)	Slope ± SE	^{2}(df)
Early instars	Exposure	300	7.92(5.07-12.13)	42.92(22.58-122.98)	1.74±0.33	0.76(3)
	Leaf-dip	300	0.95(0.41-2.57)	7.01(2.53-20.34)	1.47±0.63	0.79(3)
Late instars	Exposure	300	11.34(7.12-19.71)	66.42(33.10-156.09	1.67±0.61	1.13(3)
	Leaf-dip	300	4.32(2.79-6.00)	19.65(12.7-44.40)	1.95±0.37	0.53(3)

Note. [a] indicates that the LC values differ significantly between the exposure and Leaf-dip method; LC values in parenthesis indicate the range of toxicities measured.

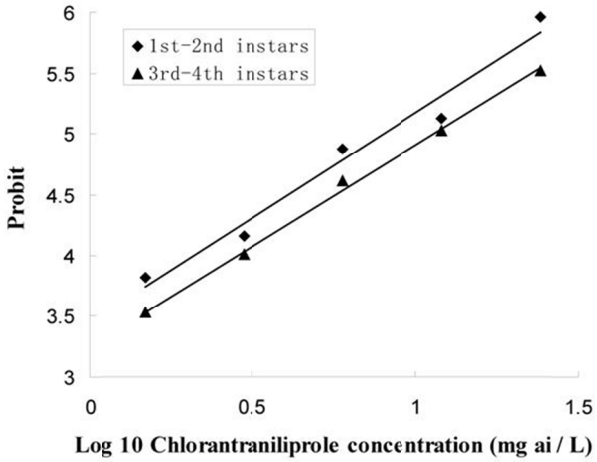

Figure 1. Log dose-probit response lines and slopes for *P. rapae* larvae exposed topically to chlorantraniliprole concentrations

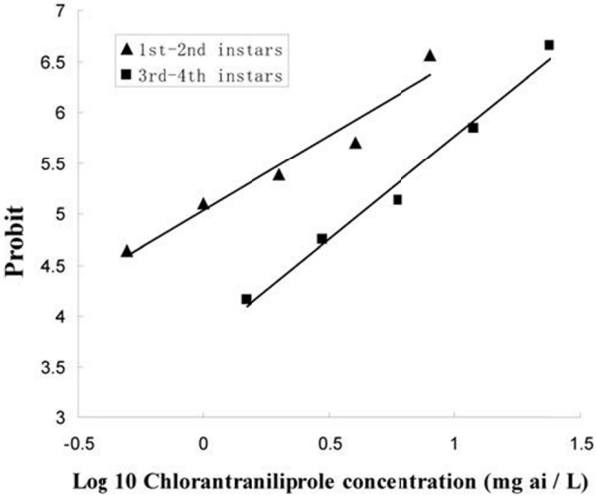

Figure 2. Log dose-probit response lines and slopes for *P. rapae* larvae through ingestion to chlorantraniliprole concentrations

3.2 Field-Aged Leaf Residue Bioassay

Field-aged leaf residue of chlorantraniliprole on cabbage was highly toxic to 5-day-old larvae of *P. rapae*. Percentage mortalities of *P. rapae* larvae were closely related to the ages of leaf residues and the durations of

exposure (Figure 3). Field-aged leaf residues were highly toxic to larvae, resulting in 100%, 100%, 95.0%, 92.3%, 82.5 and 83.3% mortalities at 6 day after exposure after feeding on the treated leaves of 0, 3, 7, 10, 14 and 17 d, respectively. Mortality still reached 72.5% for the residue of 25 d, but it dropped to 52.5% for the residue of 28 d. These results give us a hypothesis about the mortality-time after treatment dependence and show that chlorantraniliprole could be effective against *P. rapae* for at least 17-25 days on cabbage under the field conditions of Hubei province, in China.

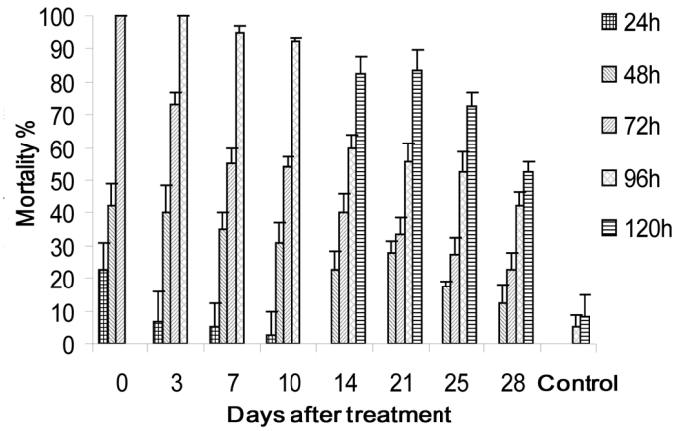

Figure 3. Persistence of filed-aged leaf residue of chlorantraniliprole on cabbage on 5-day-old *P. rapae* larvae

3.3 Field Efficacy Trials

3.3.1 Field Trial in 2015

P. rapae larval populations were low at the beginning of the season, and increased rapidly 2 weeks after the first application and then exceeded the economic threshold in the untreated check throughout the remaining period (Figure 4). Numbers of larvae per plant among the five treatments were significantly different throughout the season after the first application (Figure 4). Among the insecticides, chlorantraniliprole, indoxacard and spinosad significantly reduced the larval population after the first application and maintained the populations below the economic threshold throughout the season. Larval densities in the treatment of chlorantraniliprole maintained at levels below the economic threshold for 28 days. At harvest time, untreated plants were totally unmarketable with a damage rating of 3.6, whereas the plants treated with chlorantraniliprole, indoxacard and spinosad had no or little damage with ratings of 0.4, 0.6, and 0.5, respectively, which were all marketable. However, plants treated with emamectin benzoate were not marketable with a damage rating of 3.2.

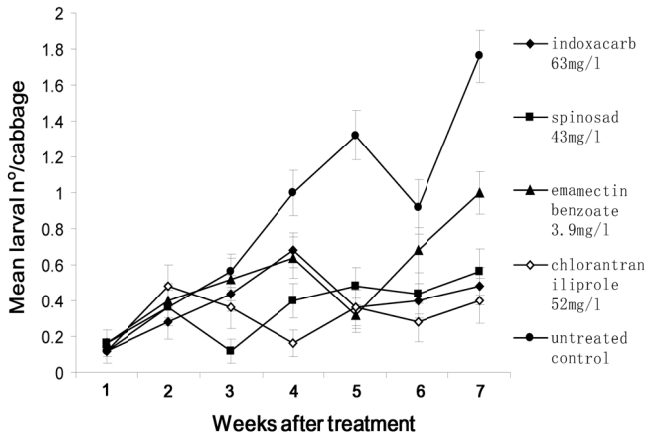

Figure 4. The mean larval number on cabbage plants each week following 4 insecticide application schedules and one control treatment in 2015

3.3.2 Field Trial in 2016

P. rapae populations were slightly higher than those in spring of 2015. All insecticides used were effective against *P. rapae* larva (Figure 5). There were no significant differences in *P. rapae* larval densities on cabbage plants in the first three sampling dates. Although *P. rapae* larval densities on cabbage plants were high with the applications of these insecticides, the overall efficacies of the insecticides were significantly different, and larval density was the lowest on the plants treated with chlorantraniliprole on the last four sampling dates (Figure 5). In two of the four sampling dates, larvae on the plants treated with spinosad were not significantly different from those on the untreated plants. In one of the four sampling dates, larval densities on the plants tested with indoxacard were not significantly different from those on the untreated plants. At harvest time, untreated plants were totally unmarketable with damage rating of 3.9 whereas plants treated with chlorantraniliprole, indoxacard and spinosad had no or little damage with ratings if 0.3, 0.5 and 0.7 respectively, which were all marketable. In contrast, plants treated with emamectin benzoate were rated 2.0 which were marginally marketable depending the demand.

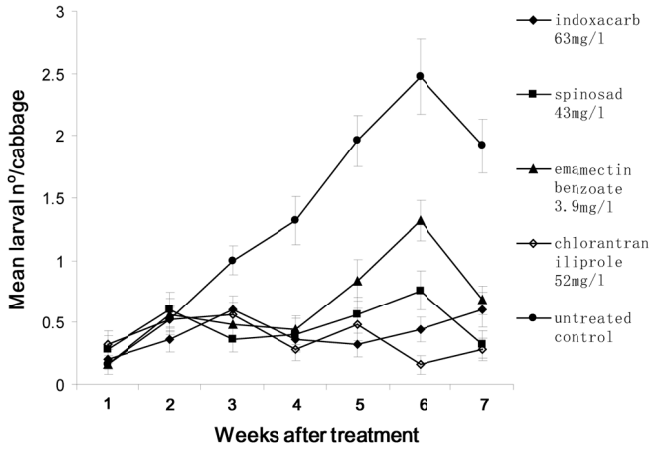

Figure 5. The mean larval number on cabbage plants each week following 4 insecticide application schedules and one control treatment in 2016

4. Discussion

The present study showed that the knockdown activities of chlorantraniliprole to *P. rapae* larvae were very quick, with high mortality within 1 d after exposure. We also observed inhibition of insect feeding occurs rapidly (minutes to a few hours after ingestion) and death normally occurs within 24-72 hours. In addition to larvicidal activity, chlorantraniliprole has been found to have significant ovicidal activity among other Lepidopteran pests (Lahm et al., 2009). Similarly, field-aged leaf residues of chlorantraniliprole knocked down the larvae within 72 h, causing mortality quicker to young larvae than to older ones, indicating that older larvae are more tolerant to chlorantraniliprole than younger ones. The similar result has been reported in Liu et al. (2003) that older *Plutella xyllostella* larvae were more tolerant to indoxacarb and λ-cyhalothrin than younger ones. To achieve control, chlorantraniliprole should be applied with proper timing when most larvae are relatively young (first and second instars). However, chlorantraniliprole will provide good control of older larvae given adequate time. In addition, chlorantraniliprole should be applied thoroughly to ensure good coverage on all plant surfaces, including the underside of the leaf surface where the larvae are located and feed.

We also observed the larvae rapidly stops feeding, becomes paralyzed, subsequently developed curved or discolored bodies and ultimately dies after they were exposed to treated cabbage leaf disks. However, less intoxicated larvae stopped feeding and remained alive for several weeks before they died. When late-instar larvae were fed with chlorantraniliprole-treated cabbage leaves, some *P. rapae* completed their larval development but did not pupate properly. Some larvae could not make cocoon before pupation, whereas some could spin a cocoon, but it was very loose. Some larvae, when pupating, could not molt properly, with exuvia remaining attached to the body surface, or only a portion of body becoming a “pupa”. Some adults were able to emerge, but almost all were abnormal with wings that were either twisted, or not well developed. Some adults

even lacked appendages, missing antennae or mouthparts, and these adults could not fly. These results clearly indicated that chlorantraniliprole has significant growth regulating effects on *P. rapae* larvae, pupae and adult formation.

The data from the field-aged leaf residues of chlorantraniliprole showed that 21-day-old residue was still highly toxic to *P. rapae* larvae. In addition to its quick knockdown, within 1-3 days after exposure and feeding with treated leaf material, it provide excellent cabbage protection up to 3 weeks or longer under field conditions in Hubei. Our data were consistent with Knight and Flexner (2007), who reported that exposure to chlorantranilipole residues applied to sleeve cages and apple foliage effectively disrupted mating for at least 3weeks under field conditions. Chlorantranilipole showed similar effectiveness in bioassays against field-collected larval populations of codling moth exhibiting a five-fold range of tolerance to azinphos-methyl, and season-long field trials have demonstrated that it can be used effectively to manage codling moth with applications timed every 21 days. It has been reported that chlorantraniliprole has no or little effects on birds, fish, invertebrate, earthworm, honeybee, wasp parasitoid, and predatory mite (Larson et al., 2012). In addition to the indicated species above, several field tests have confirmed minimal to no impact upon beneficial arthropods (Bassi, 2007). Therefore, chlorantraniliprole provides a much safer alternative to currently registered organophosphates, pyrethroids, carbamates, and other high risk conventional insecticides (Bassi, 2007). Although chlorantraniliprole has no cross-resistance with other insecticides, the risk of resistance development has been considered from beginning. We recommended for chlorantraniliprole using with a restricted number of applications per season, within spray programmers that include other effective insecticides with different modes of action.

5. Conclusions

Based on both larval densities and plants damage evaluations, chlorantraniliprole, indoxacarb and spinosad were the most effective insecticides against *P. rapae* on cabbage. Although emamectin benzonate significantly reduced larval densities below the untreated check, they often did not perform as well as the other new products being evaluated. In conclusion, chlorantraniliprole is highly toxic to *P. rapae* larvae not only through ingestion but also through the cuticle. Its effectiveness under field conditions persisted up to 25 d after treated, and the residue will likely last longer than 25 d. Although biological and other bio-rational methods could play important roles in managing *P. rapae*, it is normally difficult to produce cabbage and other leaf vegetable for fresh market, with the necessary cosmetic quality and low cull rate, without using insecticide to control pests. Chlorantraniliprole and the newer insecticide evaluated in our field trials represent valuable new chemical control tools that provide growers with alternative to currently used insecticides.

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