Ecotoxicology

Effects of SpinTor (Spinosad) on Cabbage Looper (Lepidoptera: Noctuidae): Toxicity and Persistence of Leaf Residue on Cabbage Under Field and Laboratory Conditions

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ABSTRACT  Toxicity of SpinTor 2 SC (spinosad, a fermentation by-product based compound derived from a naturally occurring soil actinomyces bacterium, Saccharopolyspora spinosa) and its field- and laboratory-aged leaf residues were tested against the 2nd or 3rd instars of cabbage looper, Trichoplusia ni (Hübner), on cabbage. In field-aged leaf residue bioassays, the pyrethroid Karate gave 100% mortality from 0 to 12 d after treatment, whereas SpinTor at all rates was highly toxic to 2nd instars, giving 100% mortality at 0 d after exposure. However, mortalities of T. ni larvae caused by SpinTor at 6 d after exposure reduced to 26.7, 65.0, and 86.3% at 12 d after treatment at 0.31 kg (AI)/ha alone, and 0.051 and 0.105 kg (AI)/ha with a surfactant, Dyne-Amic, respectively. In the laboratory, leaf residue of SpinTor was highly toxic to 3rd instars for at least 36 d. At 4 d after exposure at 36 d after treatment, larval mortalities were still as high as 70–100%, and only SpinTor at 0.52 kg (AI)/ha without adding Dyne-Amic provided lower mortality than the other 3 SpinTor treatments. Toxicities of SpinTor on 3rd instars were determined by 3 bioassays: larvae treated, leaves treated, and both larvae and leaves treated. LC50 and LC90 at 72 h after treatment were not significantly different between only larvae and both larvae and leaves treated. However, when only leaves were treated and the larvae were fed with treated leaves, the LC50 and LC90 were significantly greater than when larvae or both larvae and leaves were treated. These results indicate that SpinTor caused mortality both through direct contact and ingestion, and a combination of contact and ingestion provided greater toxicity to T. ni larvae than ingestion alone.

KEY WORDS Trichoplusia ni, spinosad, biorational insecticide, cole crops

In 1996 and 1997, more than 5,300 ha (13,100 acres) and 4,250 ha (10,500 acres) of cole crops including cabbage, broccoli, cauliflowers, and others were grown in Texas with total values of over $44 and $38 million, respectively (Anonymous 1998). Cabbage looper, Trichoplusia ni (Hübner), and diamondback moth, Plutella xylostella (L.), have been the 2 most important pests on these crops in south Texas (Cartwright et al. 1987), and can be the important production limitation for these crops (Edelson et al. 1993).

Control of these pests is becoming increasingly more difficult because of resistance of these 2 species to many common synthetic insecticides and the imposed damage restrictions on fresh market vegetables. Management recommendations for these lepidopterous pests in cole crops have been based on either a low economic threshold (1 larva per 3 plants) (Cartwright et al. 1987) or scheduled sprays. Growers frequently rely on prophylactic applications of broad spectrum insecticides with >10 applications in a single season (A.N.S., unpublished data). Concurrent with frequent applications of insecticides, control failures for P. xylostella have been reported in south Texas since 1986 (Magaro and Edelson 1990). To combat the pests, growers use synthetic organic insecticides, and some biorational insecticides, including products of Bacillus thuringiensis Berliner (Bt). With the implementation of the Food Quality Protection Act likely to limit the applications of some organic chemical insecticides and the inability of Bt products to provide season-long control of lepidopterous pests (Liu 1999), scientists and growers are seeking alternative materials that are effective against the pests and safe to humans and the environment.

SpinTor 2 SC (suspension concentrate), a new product by Dow AgroSciences (Indianapolis, IN), is one of the recently registered alternatives. It contains the active ingredient spinosad, which is a fermentation by-product based compound derived from a naturally occurring soil actinomycetes bacterium, Saccharopolyspora spinosa, and is a mixture of spinosyn A and spinosyn D (Thompson et al. 1997). As reported, SpinTor has 2 unique modes of action, acting primarily on the insect’s nervous system at the nicotinic acetylcholine receptor, and exhibiting activity at the GABA receptor (Salgado 1997). Spinosad has relatively broad spectrum activity and has been effectively used for control of many species of insect pests in the orders Lepidoptera, Diptera, Coleoptera, and Thysanoptera in various crop systems (Sparks et al. 1995, Bret et al. 1997,

In this study, we report the results from bioassays of field- and laboratory-aged leaf residues to 2nd- or 3rd-instar T. ni. Karate, a pyrethroid, was used as a standard in the field trials and the field-aged leaf residue bioassays. We also bioassayed the toxicity of SpinTor to 3rd-instar T. ni.

Materials and Methods

SpinTor. SpinTor 2 SC (spinosad, Dow AgroSciences, Indianapolis, IN) was used at 0.052 kg [ (AI)]/ha (3 oz/acre), and 0.105 kg [(AI)]/ha (6 oz/acre) in the field trials, and at a series of concentrations to achieve high and low larval mortalities for the probit analyses of LC50 and LC90 in the laboratory bioassays. Dyne-Amic (Helena Chemical, Memphis, TN), a blend of nonionic organosilicone and modified spray oil, was mixed with SpinTor in some treatments. Karate (1 EC [emulsifiable concentrate] lambda-cyhalothrin, Zeneca, Wilmington, DE) at 0.028 kg (AI)/ha was used as a standard, and untreated plants were used as controls in the field trials. Dyne-Amic and water were also used as controls in the field trials and in the laboratory bioassays.

Trichoplusia ni. First-instar T. ni (7 d from egg stage) were obtained from Entopath (Easton, PA) and fed on the multiple species diet (Southland Products, Lake Village, AR). The colony had not been exposed to insecticides for >10 yr. The larvae were maintained in a growth chamber at 20 ± 2°C, 50 ± 5% RH, and a photoperiod of 14:10 (LD) h until they were 3rd instars.

Host Plants. Cabbage, Brassica oleracea capitata L. variety ‘Grand Slam,’ was used for both field tests and laboratory bioassays. Cabbage plants for laboratory bioassays were grown in Metro-Mix 300 growing medium (Grace Sierra, Horticultural Products, Milpitas, CA) in an air-conditioned greenhouse. Plants were fertilized with a slow-release fertilizer (N-P-K, 12-8-6) (Diamond R Fertilizer, Winter Garden, FL). Plants used were 30–35 cm high with 10–12 leaves.

Field-Aged Leaf Residue Bioassay 1. This trial was conducted in fall 1998, and the materials used included SpinTor (0.105 kg [AI]/ha) and Karate (0.028 kg [AI]/ha), and untreated plants as the control. Each field plot (5 m long) consisted of 2 rows (1 m bed) of cabbage (precup stage) at 30 cm spacing. All plots were separated with sorghum wind breaks and a 1.3-m alleyway. Treatment plots were arranged in a randomized complete block design with 4 replications. The insecticides were applied on 2 December 1998. Application rates were the same as described above. The leaf disks were replaced 2 d after exposure, and then were replaced daily when larvae consumed more than half of the leaf disk in a single day. For bioassays in the first 3 d after exposure, leaf disks were replaced with ones cut from treated plants. Three days after exposure, leaf disks from the untreated plots were used.

For the 0 and 2 d after treatments, 1 leaf disk was cut from each plot, with the exception of the untreated control treatment in which 1 disk was cut from the untreated row and 1 from the Dyne-Amic treated row. Thus, these 2 bioassays consisted of 6 treatments, and each had 3 replications. The Dyne-Amic control was dropped from subsequent bioassays because of a lack of significant effects, and 2 leaf disks from each plot were bioassayed. Therefore, the 5-, 7-, 9-, and 12-d bioassays consisted of 5 treatments. After dropping Dyne-Amic, each treatment had 6 replications. Larvae were examined daily for 6 d, and classified as live, morbid, and moribund. Percentage morbid plus moribund were used in the analysis and reported.

Laboratory-Aged Leaf Residue Persistence Bioassay. This test was conducted to determine the residue persistence of SpinTor under laboratory condition at 25 ± 2°C, 60 ± 5% RH, and illuminated with fluorescent lights set for a photoperiod of 14:10 (LD) h. Cabbages were seeded individually in plastic pots (10 cm diameter) with standard media. The plants were cut from 1 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 (10 by 1.5 cm), and 10 third instars were placed on the leaf disk. Each treatment had 6 replications. Leaf disks were replaced with freshly cut ones from the treated plants at 2 d after initial exposure. Larvae were checked daily for 5 d and were classified as live or dead (including moribund and moribid).

Field-Aged Leaf Residue Bioassay 2. Five treatments were Karate (0.028 kg [AI]/ha); SpinTor at 0.052 kg (AI)/ha; SpinTor at 0.052 kg (AI)/ha + Dyne-Amic (5 ml/l); and SpinTor at 0.105 kg (AI)/ha + Dyne-Amic (5 ml/liter). Dyne-Amic alone (5 ml/liter) (applied to 1 row of each untreated plot), and untreated plants as controls. The field experimental design and materials applications were the same as described above. The insecticides were applied on 2 December 1998.

Laboratory bioassays were conducted from the day of treatment or 0 d after treatment, =2 h after application, and then at 2, 5, 7, 9 and 12 d after treatment. Leaves were selected as described above. After the leaf was selected, a leaf disk (8.5 cm diameter) was cut from the leaf, and 1 leaf disk was used from each plot. Leaf disks were individually placed on the bottom of a clear plastic petri dish (10 by 1.5 cm), and 10 second instars were placed on the leaf disk in each petri dish. Each treatment had 4 replications. The petri dishes were held in an environmental chamber at 26 ± 2°C and a photoperiod of 14:10 (LD) h. The dishes were sealed with parafilm for the 0 d after treatment exposure, and were unsealed thereafter because high humidity inside the petri dishes caused high larval mortality. Leaf disks were replaced 2 d after exposure, and then were replaced daily when larvae consumed more than half of the leaf disk in a single day. For bioassays in the first 3 d after exposure, leaf disks were replaced with ones cut from treated plants. Three days after exposure, leaf disks from the untreated plots were used.

The leaf disk was cut from each plot, with the exception of the untreated control treatment in which 1 disk was cut from the untreated row and 1 from the Dyne-Amic treated row. Thus, these 2 bioassays consisted of 6 treatments, and each had 3 replications. The Dyne-Amic control was dropped from subsequent bioassays because of a lack of significant effects, and 2 leaf disks from each plot were bioassayed. Therefore, the 5-, 7-, 9-, and 12-d bioassays consisted of 5 treatments. After dropping Dyne-Amic, each treatment had 6 replications. Larvae were examined daily for 6 d, and classified as live, morbid, and moribund. Percentage morbid plus moribund were used in the analysis and reported.
maintained in a greenhouse until they grew to 20–30 cm high with 7–10 fully expanded leaves. The plants were then moved to the laboratory. Materials were sprayed using a hand-held sprayer until run-off. All treated leaves, after drying, were used for bioassays. When used, a leaf was detached from a plant, cut to leaf disks of 10–15 cm in diameter, and individually placed in a clear plastic petri dish (15 cm in diameter, and 2.5 cm deep). Four layers of water wettet tissue papers were placed on the bottom of the petri dishes, and a few drops of water were added daily to maintain moisture. Small holes (20–25, 2 mm in diameter) were drilled on the petri dish lids for ventilation. The mortality of the larvae was checked in 5, 8, 10, 12, 18, 24, and 36 d after treatment. The larvae were considered dead if no movement was detected when they were touched with a small brush. Each concentration or treatment had 10 replicates, and each replicate had 10 larvae.

Laboratory Toxicity Bioassays. This test was to determine the contact and ingestion toxicity of SpinTor to 3rd-instar T. ni under laboratory conditions. Three bioassays were conducted: direct treatment of larvae only, treatment of leaves only, and treatment of both larvae and leaves.

Treatment of Larvae Only. Third instars were placed in a plastic insect rearing cup (30 ml). Ten SpinTor dilutions or water (control) were sprayed on the larva to wet using a hand-sprayer. The treated larvae were transferred onto paper towels immediately after being sprayed to absorb extra spray. After air-drying for 30 min, the larvae were transferred onto a clean untreated cabbage leaf disk (10–12 cm diameter) placed with the lower side facing up in a petri dish. Four layers of filter paper were placed on the bottom of the petri dish, and a few drops of water were added daily to provide moisture. Each concentration or treatment had 10 replicates, and each replication had 10 larvae. Mortality counts were made after 1–3 d.

Treatment of Leaves Only. Both sides of cabbage leaves were sprayed with SpinTor dilutions or water (control) to run-off using a hand-held sprayer. After air-drying for 30 min, leaf disks (12–15 cm in diameter) were cut and were placed individually in petri dishes. Four layers of filter paper were placed on the bottom of the petri dish, and a few drops of water were added daily to provide moisture. Ten 3rd instars were placed onto the leaf disk in each petri dish. Each treatment had 5 replications, and each replication had 10 larvae. Mortality counts were made after 1–3 d.

Treatment of Larvae and Leaves. Ten 3rd-instar T. ni were put onto a leaf disk (12 cm in diameter), and were sprayed to run-off. These treated larvae were air-dried for 30 min before they were placed onto the treated leaf disk in petri dishes. One cabbage leaf disk was placed with the lower side facing up in each petri dish. Four layers of filter paper were placed on the bottom of the petri dish, and a few drops of water were added daily to provide moisture. Each treatment had 5 replications and each replication had 10 larvae. Mortality counts were made after 1–3 d.

Data Analysis. Toxicity of SpinTor to 3rd instars of T. ni, including LC50, and LC90 with related parameters (95% fiducial limits [FL], slope, and standard error), was analyzed using POLO (LeOra Software 1994). The 95% CI of the lethal concentration ratios between every 2 of the 3 treatments that include 1 or do not were used as the criterion to separate the treatments (Robertson and Preisler 1991). Field- or laboratory-aged leaf residue bioassay data, including percent mortality of larvae, were transformed to the arcsine square-root [arcsine (percent mortality/100)] before analysis to stabilize error variance (Steel and Torrie 1980, Gomez and Gomez 1984). Mean percentage mortalities were analyzed using analysis of variance, and were separated using the least significant different test least significant difference following a significant F-test at P = 0.05 (SAS Institute 1995). Although all tests of significance were based on the transformed data, the untransformed percent mortalities are presented.

Results

Laboratory Toxicity Bioassays. LC50 and LC90 values are shown in Table 1, and the lethal concentration ratios and their 95% CI are listed in Table 2. Among the 3 treatments, the lethal concentration values in the treatment of only larvae were significantly different from those in the treatment of only leaves, but were not different from those in the treatment of both larvae and leaves; whereas, the lethal concentration values between the treatments of only leaves were also significantly different from those in the treatment of both larvae and leaves. These results indicated that

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<th>Table 1. Response of 3rd-instar T. ni to SpinTor on cabbage under laboratory conditions 72 h after treatments</th>
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* a mg (AI)/liter.

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<th>Table 2. Comparison of the lethal concentrations (LC) of SpinTor on 3rd-instar T. ni on cabbage among the 3 treatments</th>
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* The lethal concentration of every 2 treatments differs significantly if 95% CI of the lethal concentration ratio does not include 1 (Robertson and Preisler 1991).
SpinTor produced mortality both through direct contact and ingestion, and a combination of contact and ingestion provided greater toxicity to *T. ni* larvae than ingestion alone.

**Field-Aged Leaf Residue Bioassay 1.** Total mortality at 4 d after exposure to the treated residue showed high levels of activity for both Karate and SpinTor treatments (Fig. 1) through 12 d after treatment. SpinTor showed slightly slower and lower activity at the 5 and 12 d after treatment bioassays, as compared with Karate. However, by 4 d after exposure, percent mortalities were not significantly different between the treatments of Karate and SpinTor. Knockdown activity, as represented as activity at 1 d after exposure, was greater for Karate and lower for SpinTor. At 12 d after treatment, only Karate provided significant mortality in all d after exposure. Relatively high mortalities were also experienced in the untreated control at 5 d after exposure in each bioassay. This generally occurred because of depletion of food in the untreated controls after the 2nd d on the replacement leaf disks and resulting cannibalism.

**Field-Aged Leaf Residue Bioassay 2.** Second-instar *T. ni* were highly susceptible to the pyrethroid, Karate, with 100% mortality generally occurring at 0–12 d after treatment for all bioassays (Fig. 2), whereas SpinTor at all rates was highly toxic to the 2nd instars, giving 100% mortality at 0 d after exposure. However, percentage mortalities of SpinTor at 6 d after exposure reduced to 26.7, 65.0, and 86.3% at 12 d after treatment at 0.51 kg (AI)/ha alone, and 0.051 and 0.105 kg (AI)/ha with Dyne-Amic, respectively. The bioassays performed at 7, 9, and 12 d after treatment generally indicated that the high rates provided longer residual effect than the low rates, and that addition of Dyne-Amic also improved residual activity.

The Dyne-Amic treatments may have slightly increased mortality on the day of treatment (0 d after treatment); however, mortality was high even in the untreated control because of excessive moisture in the petri dishes. We think the high mortality was caused by the high humidity in the petri dishes that were sealed with parafilm because the problem did not occur in the subsequent bioassays when parafilm was not used. In some untreated control treatments, high mortalities at 5 and 6 d after exposure were caused by viral infection in the colony.

**Laboratory-Aged Leaf Residue Persistence Bioassays.** Results of this bioassay are summarized in Fig. 3. The leaf residue of SpinTor on cabbage leaves under laboratory condition lasted at least as long as 36 d after treatment. From 5 to 36 d after treatment, 72-h mortality of *T. ni* larvae fed on treated leaves generally approached 100%. The results also indicated that mixing SpinTor with Dyne-Amic under laboratory condition did not increase its activity and efficacy.
which was different from the results found from the field trials where SpinTor mixed with Dyne-Amic significantly increased the efficacy (T.-X.L. and A.N.S, unpublished data). We could not continue the bioassays on the leaf residues beyond 36 d because we depleted leaf tissue.

Discussion

For most insecticide applications, coverage and deposition are critical to obtaining maximum performance, and this is especially true for insecticides that the insects must consume or contact. T. ni, P. xylostella, and most other lepidopterous larvae feed from the underside of the leaf surface. SpinTor has excellent contact and ingestion activity; however, the insect larvae must contact the treated substrate or ingest the active ingredients on the treated foliage for activity to occur. Therefore, SpinTor should be applied with proper application techniques that help ensure thorough spray coverage on all plant surfaces, including the underside of the leaf surface where the larvae are located, to obtain optimum control. Redding and Nead (1998) emphasized the importance of application...
techniques for Tracer, which has the same active ingredient as SpinTor, on cotton. They found that using hollow cone nozzles (TX6) with 413.7 kPa (60 psi) for application of Tracer provided better coverage/control as compared with the same type of nozzles at lower pressure (275.8 kPa or 40 psi) or different nozzles (TX18 and 8003 flat fan) at the same or lower pressures. Although SpinTor resists wash-off after drying for 1–2 h, application to the undersides of the leaf surfaces should also prolong its activity by avoiding exposure to the sunlight and rain.

In the field-aged leaf residue bioassays, the results (Figs. 1 and 2) were slightly different from those from field efficacy studies (T.-X.L. and A.N.S, unpublished data). In general, the laboratory result favored SpinTor in the 1st few days of each bioassay because its
mode of action results in rapid effects but slow mortality. The excellent efficacy of the pyrethroid (Karate) in this bioassay may be at least partially a reflection of the T. ni colony used in the bioassay, which has not been selected for resistance, whereas the T. ni population in south Texas has exhibited signs of low level pyrethroid resistance (A.N.S., unpublished data). Field selection has not occurred with SpinTor and it would be expected that the laboratory colony and field population would respond more similarly with this product. This likely explains the slight superiority of Karate over SpinTor in the laboratory bioassays and the opposite in the field trials.

The addition of adjuvants or surfactants may not increase the activity of SpinTor. In an early study on cotton, Larson (1997) did not find any significant response with several adjuvants, including Silwet (silicon super wetter), Sunspray oil, Agridex oil, Intact (blend of polymer and copolymer), and Kinetic (surfactant). In this study, the addition of Dyne-Amic, a surfactant and activator, to SpinTor in the laboratory-aged leaf residue bioassay did not significantly increase the efficacy of SpinTor. However, in our field trials, the addition of Dyne-Amic to SpinTor improved the efficacy and reduced damage in the field conditions (T.-X.L. and A. N. S, unpublished data).

In the laboratory and other protected environments, leaf residue of SpinTor could be toxic to T. ni larvae longer than 36 d with relatively slow activity. Because photolysis is the primary route of spinosad degradation, longer residual effect on the foliage in the laboratory compared with that in the field condition is expected (Sanders and Bret 1997). Larvae exposed to SpinTor quickly stopped feeding but mortality was expected (Sanders and Bret 1997). Larvae exposed to treated leaves after 24 h. As shown in Figs. 1 and 2, most mortality occurred within 2 d after exposure, but additional mortality did occur by 3 d after exposure to the treated leaves. This indicated that the larvae should be exposed to treated foliage at least 72 h for accurate bioassay results in the laboratory.

In the laboratory, SpinTor has excellent contact and ingestion activities on the T. ni larvae. Thorough spray coverage may optimize activity of SpinTor by a combination of direct contact by the larvae and ingestion of the active ingredient by the larvae. Laboratory toxicity bioassay also indicated that larger larvae of T. ni were likely more tolerant to SpinTor than smaller larvae. Sparks et al. (1995) reported that in a drench bioassay of 1st-instar T. ni, the LC50 was 0.28 mg (AI) /liter (95% CI: 0.14–0.46 mg/liter), which was >10-fold smaller than the 3.81 mg (AI)/liter (90% FL: 3.17–4.54) we found for 3rd-instar T. ni.

SpinTor is a valuable new chemical for management of lepidopterous pests on vegetables. However, when applied with thorough spray coverage, the resulting efficacy and potential residual activity against early instars could rapidly select for resistance. Thus, additional efficacious materials, such as Avant (indoxacarb), Proclaim (emamectin benzoate), Alert (chlorfenapyr), Confirm (tebufenozide), and many Bacillus thuringiensis products, should be used in a manner to sustain long-term product efficacy, and rotated with these products with different modes of action in an integrated management program.

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