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Source: Florida Entomologist, 96(3) : 724-728

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0304

# LABORATORY EVALUATIONS OF LEPIDOPTERAN-ACTIVE SOYBEAN SEED TREATMENTS ON SURVIVORSHIP OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) LARVAE

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ABSTRACT

Two anthranilic diamide insecticides, chlorantraniliprole and cyantraniliprole, were evaluated as seed treatments on soybean, *Glycine max* L., for control of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Bioassays were conducted using 2nd instars and plants grown from the field and greenhouse. In field-grown soybeans, cyantraniliprole and chlorantraniliprole significantly lowered survival of fall armyworm larvae at the V7 growth stage (51 DAP), and, at the R6 growth stage (112 DAP), and survivorship was significantly lower on plants treated with both chlorantraniliprole and cyantraniliprole. In 6 out of 9 total post treatment evaluations, survivorship was significantly lower in chlorantraniliprole seed treatments than in cyantraniliprole seed treatments. Greenhouse grown plants treated with cyantraniliprole and chlorantraniliprole significantly reduced survival at the V3 growth stage, 2, 3 and 4 days after infestation when compared with other seed treatments. These products could be useful in reducing the number of foliar applications required for lepidopteran pests.

Key Words: anthranilic diamide insecticides, early-season insect pests, foliar applications , growth stages

RESUMEN

Se evaluó dos insecticidas diamida antranílicos, clorantraniliprol y cyantraniliprole, como tratamiento de semillas de soja, *Glycine max* L., para el control del gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith). Se realizaron los bioensayos utilizando larvas del segundo estadio y plantas sembradas en el campo y en el invernadero. En la soja cultivada en el campo, cyantraniliprole y clorantraniliprol redujeron significativamente la sobrevivencia de las larvas de gusano cogollero en la etapa de crecimiento V7 (51 DAP), y en la etapa de crecimiento R6 (112 DAP), la sobrevivencia fue significativamente menor en las plantas tratadas con ambos clorantraniliprol y cyantraniliprole. En 6 de las 9 evaluaciones totales hechas pos-tratamiento, la sobrevivencia fue significativamente menor para el clorantraniliprol que en los tratamientos de semillas con clorantraniliprol. Las plantas en el invernadero tratadas con cyantraniliprole y clorantraniliprol redujeron significativamente la sobrevivencia en el período de crecimiento V3, a los 2, 3 y 4 días después de la infestación en comparación con los otros tratamientos de semillas. Estos productos podrían ser útiles en la reducción del número de aplicaciones foliares requeridos para controlar las plagas lepidópteras.

Palabras Clave: insecticidas antranílicos diamida, plagas insectiles de principio de temporada, aplicaciones foliares, etapas de crecimiento

Applying pesticides to seed for control of crop pests is not a new concept. Modern seed treatments began with the advent of organic mercurials used as fungicide in the 1920's (Munkvold et al. 2006). Before 1940, the insecticides used on seed were mainly inorganic substances that were not very effective (Masaitis 1927; Sanderson & Peairs 1931; McDougall 1935; Munkvold et al. 2006). The discovery of chlorinated hydrocarbons (e.g. lindane) provided contact, fumigant, repellent, and limited systemic effects on certain soil- and foliage-feeding insects (Lange 1959). The ability to treat a seed with an insecticide, causing translocation and thus rendering the plant insecticidal, was confirmed in 1952 with organophosphates (Dowdy & Slessman 1952; Ivy et al. 1954).

Many problems were associated with the early insecticide seed treatments, resulting in their limited use. Reductions in germination and emergence were common, as well as seedling phytotoxicity and delayed maturity (Adkisson 1958; Hanna 1958; Bowling 1964; Gifford et al. 1975). Development of insecticides in the 1990's, such as the neonicotinoids and fipronil, as well as improved treatment methods, have eliminated problems associated with earlier insecticidal seed treatments. Thus, seed treatments became a much more valuable method of controlling root feeding and early-season insect pests. A niche market of €155 million (US$ 200 million) for insecticidal seed treatments in 1990 was dominated by carbamates but has increased to a €535 million (US$ 691 million) market, with a share for neonicotinoid insecticides of 77% in 2005 (Elbert et al. 2008). More recently, chlorantraniliprole, an insecticide in the anthranilic diamide class, has proven to be a useful for seed treatments in rice and foliar applications in multiple crops.

The use of insecticide seed treatments to control sucking-pests (e.g. thrips, whiteflies, and aphids) on soybeans, *Glycine max* L., has increased dramatically in recent years. For example, in 2007, only 2% of Mississippi soybeans were treated with an insecticide seed treatment, such as thiamethoxam (Crusier® 5FS, Syngenta Co., RTP, NC), but, by 2011, 75% of Mississippi soybean acreage had insecticide-treated seeds (Musser et al. 2011). Changes in production practices, such as conservation tillage, have resulted in a greater need to protect seeds and seedlings. In addition, the value of soybeans has increased, which has reduced the tolerance for damage from pests. Recent studies in the mid-southern United States have indicated that insecticide seed treatments can increase soybean yields over untreated seed by 235 kg/ha, with a 79% probability of a positive net return above cost (Lorenz, unpublished data).

In this study, 2 anthranilic diamides (cyantraniliprole and chlorantraniliprole) were evaluated for use as soybean seed treatments to control certain Lepidoptera. Although neither are currently labeled for use in soybeans, both have been reported to control a broad range of lepidopteran pests in other crops (Lahm et al. 2012). Chlorantraniliprole (Dermacor X-100™, DuPont Co., Wilmington, Delaware) is labeled as a seed treatment for rice and provides control of grape colaspis, *Colaspis brunnea* (F.), rice water weevil, *Lissorhoptrus oryzophilus* (Kuschel), and true armyworm, *Pseudaletia unipuncta* (Haworth), infestations on seedling rice plants. In addition, chlorantraniliprole controls Colorado potato beetle, *Leptinotarsa decemlineata* (Say), certain species of grubs and leaf miners, as well as termites (Yeoh & Lee 2007; Koppenhofer & Fuzy 2008; Spomer et al. 2009, respectively). Although not currently registered for use on any crop, cyantraniliprole (Cyazapyr™ ) has also been reported to control many piercing-sucking and chewing pests, including key species of whitefly, thrips, psyllids, aphids, plant hoppers, leaf hoppers and miners, fruit flies, and beetles (Portillo et al. 2009; Annan et al. 2010; Stansly et al. 2010).

This study evaluated both chlorantraniliprole and cyantraniliprole when applied as a soybean seed treatment for control of lepidopteran pests. The armyworm complex, comprised of *Spodoptera* spp. and *P. unipuncta*, was the 3rd most damaging lepidopteran pests of soybean in the mid-southern United States (Musser et al. 2011). In 2011, 67% of Arkansas soybean acreage was infested with fall armyworm, *Spodoptera frugiperda* (J. E. Smith), costing growers an average $8.07 per acre (Musser et al. 2011); therefore, we chose to focus exclusively on this noctuid pests in our evaluations.

## Materials and Methods

### Seed Treatments

All pesticide treatments were applied to soybean seeds using a portable 0.11 $m^3$ (16 kg seed/treatment) cement mixer. Treatments (120 mL) were applied to the seeds and tumbled until a consistent uniform seed coating was achieved. Once dry, all treated seeds were stored in individual paper bags at room temperature until planting. Prior to treating with insecticides, all seeds were treated with a fungicide (Apron Maxx RTA + Moly (148 mL/cwt = 148 mL/45.35 kg) (Syngenta Co., Research Triangle Park, North Carolina). In addition, to control sucking pests in the greenhouse and in field plots, thiamethoxam (47.6% ai, 37.9 mL/cwt = 37.9 mL/45.35 kg)) was also applied to seeds treated with cyantraniliprole (100 g ai/L, 89.9 mL/ha) or chlorantraniliprole (50% ai, 130.8 mL/ha). Controls included fungicide-only and thiamethoxam-only treated seeds at the above rates.

### Field Plots and Greenhouse Plants

To conduct bioassays using greenhouse grown plants, soybean seeds (cv. 'Vernal') were planted in May 2011 in 10.2 cm pots containing potting soil at the University of Arkansas Research and Extension Center in Lonoke, Arkansas. All pots were watered frequently to maintain soil moisture. No pesticides were applied to the pots after planting.

Also in May 2011, soybeans (cv. 'Vernal') were planted (160,000 seeds/ha) in research plots in the lower Rio Grande Valley of Texas near Weslaco, Texas. Plots consisted of 16 rows (1.0 m centers), each 15.24 m in length. All plots were arranged in a randomized complete block design, with each variety replicated 3 times (once in each block). All plots were irrigated twice according to local management practices. No pesticides were applied after planting. Soybean growth stages were determined using methods described in Fehr & Caviness (1977).

### Insect Bioassays

Fall armyworms used in these studies were obtained from a laboratory colony maintained at the USDA, ARS, Kika de La Garza Subtropical Research Center in Weslaco, Texas. Larvae were reared on artificial diet for 48 hr before use in all bioassays.

For greenhouse grown plants (V3 stage - vegetative, 3rd node), a single leaf from an individual trifoliate node was selected to determine bioactivity against fall armyworms. Three larvae (2nd instars) were placed in a Petri dish with a single leaf from the tallest trifoliate. Each treatment was replicated 10 times using leaves from a different plant for each replication. Survivorship was evaluated every 24 h for 4 days. Evaluations were conducted by prodding larvae with a fine-tip paint brush. If coordinated movement was observed, larvae were rated as 'living'. Larvae that were recorded as 'dead' were not removed from Petri dishes

From the field plots, 20 different plants per seed treatment were identified, and the tallest trifoliate from the canopy was excised from an individual plant, placed into a cooler containing wet ice, and transported to the laboratory. Tissue samples were taken at 51 days after planting (DAP) at the V7 (vegetative, 7th node) stage and 112 DAP at the R6 (reproductive, full seed) stage. A single leaf per trifoliate was selected for bioassays to determine bioactivity against fall armyworms. Individual leaves were placed into a 50 × 9 mm Tight-Fit Lid sealing Petri dish (BD Falcon® #351006, VWR International). Three larvae (2nd instars) were placed in each dish. At 3 days after infestation (DAI), larvae were prodded with a camel-hair brush and considered alive if coordinated movement was observed.

Percent survival by seed treatment was analyzed using analysis of variance (REML-ANOVA), and means were separated according to LSMEANS (Littell et al. 1996; PROC MIXED, SAS Institute version 9.3, 2012) using the Satterthwaite degrees of freedom method, and resulting graphs were generated by JMP 10 software. All survival data were transformed using arcsin(sqrt) to ensure residuals were representative of a more normal distribution. All data were back-transformed for producing the resulting figures.

## Results and Discussion

Soybean seeds treated with lepidopteran-active insecticides reduced survivorship of fall armyworms in laboratory bioassays at the V3 from excised from plants grown in the greenhouse (Fig. 1). Because there was minimal mortality observed in any of the seed treatments at 1 DAI, these data are not shown. At 2, 3, and 4 DAI, there were significant differences ($P < 0.05$) among the various seed treatments, and the difference increased over time, with cyantraniliprole and chlorantraniliprole reducing survivorship to 50 and 20%, respectively, compared with the controls at the end of 4 days. When evaluated at 2 DAI, only seeds treated with chlorantraniliprole caused significantly lower survival of fall armyworms compared with the control and all other seed treatments. There were no significant differences among the lepidopteran-active insecticides cyantraniliprole and chlorantraniliprole. When evaluated at 3 DAI, only seeds treated with chlorantraniliprole caused significantly lower survival of fall armyworms compared with all other seed treatments. At 4 DAI, both cyantraniliprole and chlorantraniliprole caused significantly lower survival of fall armyworms compared with the other seed treatments.

Similarly, soybean seeds treated with lepidopteran-active insecticides reduced survivorship of fall armyworms in laboratory bioassays using leaves collected from plants grown in field plots at both the V7 (51 DAP) and R6 stage (112 DAP) (Fig. 2). Survivorship of larvae exposed to leaves from plants treated with cyantraniliprole or chlorantraniliprole was significantly decreased by about 45% when evaluated at 3 DAI for leaves collected at the V7 and R6 stage. However, while at the V7 stage there were no significant differences in larval survivorship between soybean seeds treated with the lepidopteran-active insecticides, seeds treated with chlorantraniliprole caused significantly lower survival of fall armyworms compared with those seeds treated with cyantraniliprole at the R6 stage.

This study showed that soybean seeds treated with certain insecticides belonging to the anthranilic diamides class can provide good efficacy against certain Lepidoptera during vegetative and reproductive plant growth stages. Clearly, a broader range of lepidopteran pests need to be examined to see if efficacy differences exist across

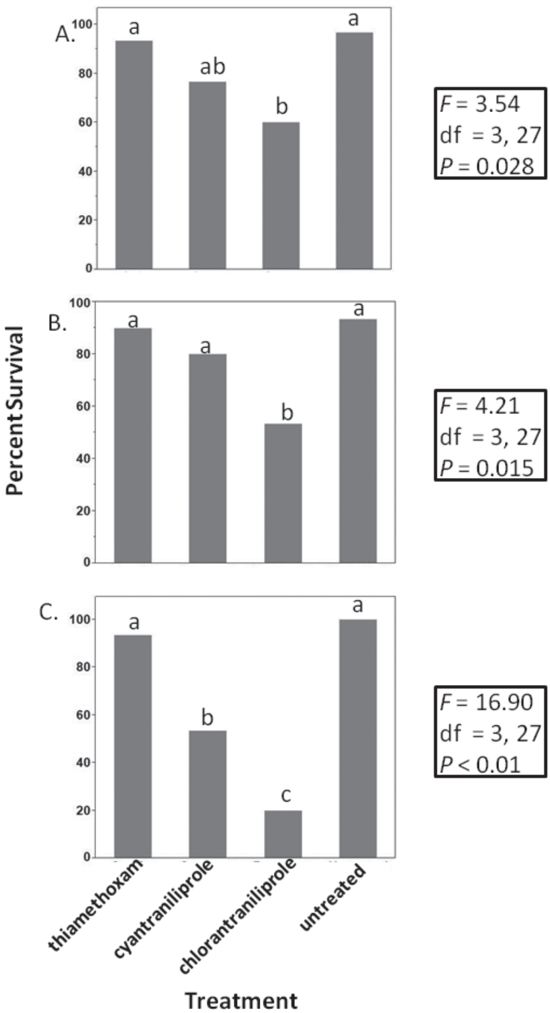


Fig. 1. Efficacy of various soybean seed treatments against fall armyworm larvae at (A) 2, (B) 3, and (C) 4 days after infestation (DAI) from leaves excised from plants at the V3 (vegetative) growth stage. Plants were grown in a greenhouse environment at the University of Arkansas, Lonoke, Arkansas. Untreated seeds contained only fungicide. Bars (means) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS (MIXED PROCEDURE).

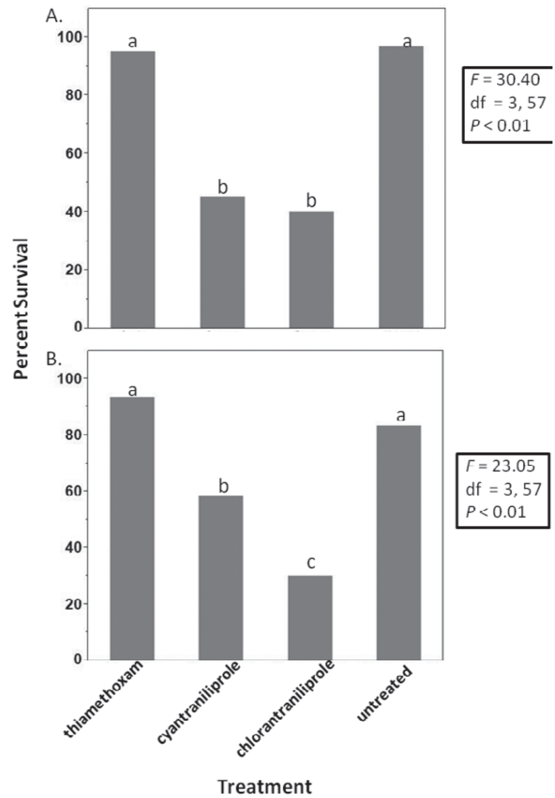


Fig. 2. Efficacy of various seed treatments against fall armyworm larvae at 3 days after infestation from leaves excised from plants at the (A) V7 (vegetative, 7th node) growth stage at 51 days after planting (DAP) and the (B) R6 (reproductive, full pod) stage (112 DAP). Plants were grown in field plots near Weslaco, Texas. Untreated seeds only contained fungicide. Bars (means) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS (MIXED PROCEDURE).

species. In addition, we only examined leaf material; fruit structures (e.g. flowers and pods) need to be examined as well. Nevertheless, we believe this is the first study to show that these insecticides, when used as a soybean seed treatment can essentially provide season-long efficacy against certain Lepidoptera. In 2011, lepidopteran pests alone accounted for US$ 41/acre (US$ 101.31/ha) in yield losses and control costs in the midsouthern United States (Musser et al. 2011). Furthermore, using these seed treatments may reduce the number of foliar insecticide applications to control these pests and, consequently, lower overall input costs for growers.

The residual control and high efficacy of chlorantraniliprole and cyantraniliprole are already showing their worth in cotton when applied as a foliar application. These products could allow producers to grow conventional cotton in areas with high heliothine pressure (Fortner 2012). Chlorantraniliprole also currently provides a control option for the pyrethroid resistant tobacco budworm, *Heliothis virescens* (F.), in cotton (Patman 2012). This chemical has also been observed in experiments to provide 1 month of residual control of corn earworm, *Heliocoverpa zea* (Boddie), in soybeans (G. M. Lorenz, unpublished data).

It both the greenhouse and field plot studies, higher efficacy was observed for chlorantraniliprole compared with cyantraniliprole. One possible explanation could be due to differences in solubility. The solubility of chlorantraniliprole and cyantraniliprole in water is 1 and 18 ppm, respectively (DuPont, personal communication). The lower solubility of chlorantraniliprole could explain the extensive length of its residual control; however, more research is needed to determine the effect of water stress (e.g. drought and water-logging) on the activity of both systemic insecticides.

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