Effect of Insecticide Drench Applications on Western Flower Thrips, *Frankliniella occidentalis*, Pupae in Growing Media

Yinping Li and Raymond A. Cloyd¹

Department of Entomology, Kansas State University, Manhattan, KS 66506

Nora M. Bello

Department of Statistics, Kansas State University, Manhattan, KS 66506

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Abstract. Western flower thrips, *Frankliniella occidentalis*, is one of the most destructive insect pests of greenhouse-grown horticultural crops. The primary method of managing western flower thrips populations involves applications of insecticides; however, there is no information associated with the effect of the insect growth regulator, pyriproxyfen, or the entomopathogenic fungus, *Isaria fumosorosea*, on western flower thrips pupae in growing media. Therefore, four laboratory experiments were conducted to determine the effect of pyriproxyfen and *I. fumosorosea* applied as a drench to growing media on western flower thrips pupae. Expt. 1 evaluated the efficacy of pyriproxyfen and *I. fumosorosea* on western flower thrips pupae. Based on the results from Expt. 1, Expt. 2 assessed the effect of pyriproxyfen in two growing media (LC1 and BM1) on western flower thrips pupae. Expts. 3 and 4 determined the residual activity of pyriproxyfen in growing media on western flower thrips pupae 3, 5, 7, and 14 days after treatments were applied. The pyriproxyfen treatment resulted in a significantly lower estimated mean probability of western flower thrips adults captured on yellow sticky cards (17%) compared with the water control (59%), untreated check (88%), and two *I. fumosorosea* treatments (46% for 1.0 g and 41% for 2.0 g of Ancora) in Expt. 1. However, for the two growing media in Expt. 2, the estimated mean probability of western flower thrips adults captured on yellow sticky cards was not significantly different between the pyriproxyfen treatment (LC1 = 15%; BM1 = 12%) and the water control (LC1 = 41%; BM1 = 24%). For either the pyriproxyfen treatment or the untreated check, there was no evidence of a significant difference between the two growing media on the estimated mean probability of western flower thrips adults captured on yellow sticky cards. Furthermore, there was no evidence of any residual activity 3 days after drench applications of pyriproxyfen. The results of the study have demonstrated that drench applications of pyriproxyfen are not affecting survival of western flower thrips pupae.

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important insect pest of greenhouse-grown horticultural crops worldwide (Cloyd, 2009; Kirk, 2002; Lewis, 1997a; Reitz, 2009; Robb and Parrella, 1995). Western flower thrips causes direct damage by feeding on and ovipositing in leaves, flowers, and fruits (Childers, 1997; Chisholm and Lewis, 1984; Harrewijn et al., 1996; Hunter and Ullman, 1989). Moreover, western flower thrips cause indirect damage by vectoring the tospoviruses: *Tomato spotted wilt* and *Impatiens necrotic*

spot viruses (Allen and Broadbent, 1986; Pappu et al., 2009). Direct and indirect damage has led to significant economic losses (Goldbach and Peters, 1994; Reitz and Funderburk, 2012).

Tolerance for damage caused by western flower thrips is very low because damage reduces the aesthetic quality and marketability of greenhouse-grown horticultural crops (Loughner et al., 2005; Parrella and Jones, 1987). The primary management strategy used against western flower thrips is applications of insecticides (Herron and James, 2005; Lewis, 1997b; Parrella and Murphy, 1996). Therefore, greenhouse producers routinely apply insecticides to suppress western flower thrips populations (Cloyd, 2009; Kontsedalov et al., 1998; Loughner et al., 2005; Reitz, 2009).

Larvae and adults of western flower thrips feed on the foliage, whereas prepupae and pupae reside in the growing medium and do not feed (Reitz, 2009; Tommasini and Maini, 1995). Most late second instar larvae migrate down the plant stem and pupate in the growing medium or soil (Helyer et al., 1995; Tommasini

and Maini, 1995; Wiethoff et al., 2004). Foliar insecticide applications are the primary means of suppressing western flower thrips larvae and adult populations, whereas less attention has been directed at insecticide use against the pupal stages (Ansari et al., 2008; Belay et al., 2005; Berndt, 2003). The reason being is that the pupal stages are supposedly less susceptible to insecticides (Seaton et al., 1997). Although insecticides have been evaluated against western flower thrips pupae (Helyer and Brobyn, 1992; Ludwig and Oetting, 2001), none are actually labeled for use as drenches against western flower thrips pupae (Ansari et al., 2008; Cloyd, 2009). In addition, some entomopathogenic fungi have been used against the pupal stages of western flower thrips, including *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Brunneum) (Metchnikoff) Sorokin (Ansari et al., 2007, 2008; Helyer et al., 1995; Skinner et al., 2012). Another candidate entomopathogenic fungus, *Isaria fumosorosea* (Wize) (Hypocreales: Cordycipitaceae), infects a wide range of citrus pests and is less harmful to nontarget arthropod natural enemies than conventional insecticides (Avery, 2002; Avery et al., 2008; Sterk et al., 1995a, 1995b). Furthermore, *I. fumosorosea* Apopka Strain 97 is commercially available as a microbial insecticide (Faria and Wraight, 2001; Vidal et al., 1998).

Pyriproxyfen, (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine), is a juvenile hormone analog and a relatively stable aromatic compound (Sullivan and Goh, 2008). Pyriproxyfen mimics juvenile hormone activities by competing for binding sites on juvenile hormone receptors; consequently, larvae do not develop into adults (Sullivan and Goh, 2008). Pyriproxyfen is registered for household, agricultural, and horticultural applications to control many insect pests, including the fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae); the common housefly, *Musca domestica* (Lintner) (Diptera: Muscidae); mosquitoes; the red imported fire ant, *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae); and the silverleaf whitefly, *Bemisia argentifolii* (Bellows & Perring) (Hemiptera: Aleyrodidae) (Cloyd and Dickinson, 2006; Miyamoto et al., 1993; Sullivan and Goh, 2008).

Anecdotal information from greenhouse producers has suggested that growing medium applications of an entomopathogenic fungus or pyriproxyfen, lead to fewer problems with western flower thrips adults. The assumption is that the insecticides are active on the pupal stages; however, there are no quantitative data to confirm these claims. In fact, no scientific studies have assessed the efficacy of *I. fumosorosea* or pyriproxyfen on the pupal stages of western flower thrips when applied as a drench. Therefore, the objective of the study was to assess the effect of *I. fumosorosea* and pyriproxyfen on western flower thrips pupae. Four experiments were conducted to determine 1) the efficacy of *I. fumosorosea* and pyriproxyfen on western flower thrips pupae in growing media, 2) the effect of pyriproxyfen in two growing

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¹Corresponding author. E-mail: rcloyd@ksu.edu

media on western flower thrips pupae, and 3) the residual activity of pyriproxyfen in growing medium on western flower thrips pupae 3, 5, 7, and 14 d after treatments were applied.

Materials and Methods

Insect colony. A western flower thrips colony was maintained under laboratory conditions (20 to 24 °C, 50% to 60% relative humidity, and constant light) in Glad plastic containers [20.4 × 14.4 × 9.4 cm (length × width × height)] (The Glad Products Company, Oakland, CA) with a round hole (9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size: 0.15 × 0.15 mm) (Green-Tek, Janesville, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons, Manhattan, KS), soaked in soapy water [1.5 mL DAWN Ultra dishwashing liquid (Procter & Gamble, Cincinnati, OH) in a 9.4-L plastic container filled with tap water] for 20 min, then triple rinsed with tap water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every 2 to 3 d. Western flower thrips specimens used in this study are deposited as voucher number 237 in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

Design structure. Four independent laboratory experiments were conducted to evaluate the effect of drench applications of *I. fumosorosea* and pyriproxyfen on western flower thrips pupae in growing medium. Each experiment was set up as a completely randomized design. A single 473-mL deli container constituted a unit of replication. Five deli containers were associated with each treatment-combination in each experiment. The two growing media used in the experiments were LC1 (Sunshine LC1 RSi Professional Growing Mix; SunGro Horticulture Canada Ltd., Seba Beach, Alberta, Canada) composed of 70% to 80% Canadian sphagnum peatmoss, perlite, 0.0001% silicone dioxide, and dolomitic limestone; and BM1 (Berger BM1 All-Purpose Mix; Berger, Saint-Modeste, Quebec, Canada) composed of 75% to 85% sphagnum peatmoss, perlite, and vermiculite. The growing medium used in Expts. 1, 3, and 4 was LC1. Both LC1 and BM1 growing media were used in Expt. 2.

Treatment structure of Expt. 1. The efficacy of *I. fumosorosea* and pyriproxyfen applied as drenches to the growing medium on western flower thrips pupae was evaluated using five treatments: 1.0 g Ancora [*I. fumosorosea* Apopka Strain 97 (Ancora; OHP, Inc., Mainland, PA) at 1.0 g/946 mL], 2.0 g Ancora [*I. fumosorosea* Apopka Strain 97 at 2.0 g/946 mL], Fulcrum [pyriproxyfen (Fulcrum; OHP, Inc.) at 0.14 mL/946 mL], an untreated check, and a water control. The rates for *I. fumosorosea* and pyriproxyfen were the label rates associated with drench applications to the growing medium.

Treatment structure of Expt. 2. The effect of pyriproxyfen applied as drenches to two

growing media on western flower thrips pupae was assessed using a two-way factorial treatment structure consisting of all combinations of three treatments and two growing media. The three treatments were as follows: pyriproxyfen at 0.14 mL/946 mL, an untreated check, and a water control.

Treatment structure of Expts. 3 and 4. The residual activity of pyriproxyfen applied as drenches to the growing medium on western flower thrips pupae was determined using a two-way factorial treatment structure with all combinations of three treatments and two infestation assessments (days) after application of the treatments. The three treatments in each experiment were as follows: pyriproxyfen at 0.14 mL/946 mL, an untreated check, and a water control. Furthermore, there were two infestation assessments (7 and 14 d for Expt. 3, and 3 and 5 d for Expt. 4) after the treatments were applied.

Experimental procedure. All treatment solutions were prepared in water using 946-mL plastic spray bottles (Delta Industries, King of Prussia, PA). Growing medium was prepared as follows: a 6.0-L plastic container (Rubbermaid Home Products, Wooster, OH) was filled with growing medium, which was mixed with ≈200 mL of tap water. Then, the plastic container with growing medium was heated for 25 min in a microwave set at full-power (1200 W output). After the growing medium cooled, 1.2 L of tap water was applied to the growing medium, which was then thoroughly mixed. Approximately 400 mL of the sterilized growing medium was placed into a 473-mL deli container. The deli container was tapped five times to reduce the amount of air space within the growing medium.

In Expts. 1 and 2, 20 western flower thrips pupae were randomly positioned on the growing medium surface of each 473-mL deli container. Pupae are generally located at a depth of 1 to 5 mm in compost that is a freshly steam-sterilized mixture (50:50 by volume) of loam and medium grade sphagnum peat (Helyer et al., 1995). Furthermore, pupae can be located in cracks and crevices in the growing medium (Yinping Li, personal observation). Therefore, no additional growing medium was needed to cover the pupae. Then, 80 mL of each treatment solution was uniformly applied as a drench to the growing medium surface. In Expt. 3 and 4, 80 mL of each treatment solution was initially applied to the growing medium surface of each 473-mL deli container; then, 20 western flower thrips pupae were randomly positioned on the growing medium surface after 7 or 14 d for Expt. 3, or after 3 or 5 d for Expt. 4.

A modified lid with No-Thrips insect screening was used for ventilation and to prevent western flower thrips adults from escaping after eclosion. A half-section of a 7.7 × 10.4-cm (length × width) yellow sticky card (Pestrap Phytotronics, Inc., Earth City, MO) was hot-glued to the inside center of the lid to capture eclosing western flower thrips adults. The 473-mL deli containers had 12 holes perforated with a dissecting probe on

the bottom to allow for drainage and reabsorption. Each 473-mL deli container was placed into a larger petri dish (14-cm diameter) to collect any leachate from the bottom of the deli container. All deli containers for each experiment were prepared using the previously described procedure and placed in the laboratory at 20 to 24 °C, 50% to 60% relative humidity, and a 16:8 (light: dark) hour photoperiod. The numbers of western flower thrips adults captured on the yellow sticky cards were recorded 15 d after western flower thrips pupae were placed on the growing medium. The estimated mean probability of western flower thrips adults captured on the yellow sticky cards was an indirect assessment of pupal mortality (Helyer et al., 1995).

Statistical analysis. For each experiment, a generalized linear mixed model assuming a binomial distribution was fitted to the response variable defined as the number of western flower thrips adults captured on the yellow sticky cards out of the number of pupae initially positioned in the same container. A logit link function was used to estimate the probability of western flower thrips adults captured on the yellow sticky cards. For Expt. 1, the fixed effect in the linear predictor was treatment. For Expt. 2, the linear predictor in the model included the fixed effects of treatment, growing medium, and the two-way interaction. For Expts. 3 and 4, the fixed effects in the linear predictor included treatment, infestation assessment (7 or 14 d in Expt. 3, and 3 or 5 d in Expt. 4) after the treatments were applied, and the two-way interaction. For Expt. 4, to account for overdispersion, a random effect was fitted for the level of observation defined as the cross product of replication, treatment, and infestation assessment after application of the treatments.

In each experiment, overdispersion was determined using the maximum-likelihood based fit statistic Pearson χ^2/DF . There was no evidence of overdispersion. In each experiment, the final statistical model used for inference was fitted using Residual Pseudolikelihood. Degrees of freedom were approximated and estimated standard errors were adjusted using Kenward-Roger's procedure. The statistical model was fitted using the PROC GLIMMIX procedure (SAS Institute, 2012) implemented using Newton-Raphson with ridging as the optimization technique. Pairwise comparisons were conducted using Tukey-Kramer's or Bonferroni's adjustments, as appropriate for main effect or simple effect comparisons, to avoid inflation of type I error due to multiple comparisons.

Results

Expt. 1. There was a significant treatment effect associated with the estimated mean probability of western flower thrips adults captured on the yellow sticky cards ($F = 21.04$; $df = 4, 20$; $P < 0.0001$). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards

was significantly lower for the pyriproxyfen treatment (mean 17%; 95% confidence interval 10.5%, 26.3%; $n = 100$) compared with the other four treatments (Fig. 1). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards for the two treatments affiliated with *I. fumosorosea* Apopka Strain 97 (1.0 g Ancora and 2.0 g Ancora) was significantly lower than the untreated check, but there was no evidence of a significant difference from the water control (Fig. 1).

Expt. 2. Within the LC1 and BM1 growing media, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly lower for the pyriproxyfen treatment [LC1 = 15% (9%, 24%; $n = 100$) and BM1 = 12% (6.7%, 24.5%; $n = 100$)] than the untreated check [LC1 = 91.1% (83.3%, 95.5%; $n = 100$) and BM1 = 85% (76.1%, 91%; $n = 100$)], but not significantly different from the water control [LC1 = 41% (31.4%, 51.4%; $n = 100$) and BM1 = 24% (16.3%, 33.9%; $n = 100$)] (Fig. 2). For the water control, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly higher in the LC1 [41% (31.4%, 51.4%; $n = 100$)] than the BM1 [24% (16.3%, 33.9%; $n = 100$)] growing medium ($t = -2.54$, $df = 24$, $P = 0.018$) (Fig. 3). However, for either the pyriproxyfen treatment or the untreated check, there was no evidence of any significant differences in the estimated mean probability of western flower thrips adults captured on the yellow sticky cards between the two growing media (Fig. 3).

Expt. 3. When the growing medium was infested with western flower thrips pupae 7 d after application of the treatments, there was no significant treatment effect associated with the estimated mean probability of western flower thrips adults captured on yellow sticky cards (Fig. 4). When the growing medium was infested with western flower thrips pupae 14 d after the treatments were applied, a significantly higher estimated mean probability of western flower thrips adults was captured on the yellow sticky cards in the untreated check [97.1% (90.9%, 99.1%; $n = 100$)] than the water control, but there was no evidence of any significant differences between pyriproxyfen and the untreated check, or between pyriproxyfen and the water control (Fig. 4).

Expt. 4. There was a significant interaction between treatment and infestation assessment after the treatments were applied ($F = 3.86$; $df = 2, 24$; $P = 0.035$). However, when the growing medium was infested with western flower thrips pupae 5 d after application of the treatments, there was no significant treatment effect based on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards (Fig. 5). When the growing medium was infested with western flower thrips pupae 3 d after application of the treatments, a significantly higher estimated mean probability of western flower thrips adults was captured on the

Fig. 1. Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, adults captured on yellow sticky cards (YSC) associated with each treatment: 1.0 g Ancora [*Isaria fumosorosea* Apopka Strain 97 (Ancora: OHP, Inc., Mainland, PA) at 1.0 g/946 mL], 2.0 g Ancora [*I. fumosorosea* Apopka Strain 97 at 2.0 g/946 mL], Fulcrum [pyriproxyfen (Fulcrum: OHP, Inc.) at 0.14 mL/946 mL], an untreated check, and a water control. Estimated means followed by different letters indicate significant differences ($P < 0.05$) among the treatments.

Fig. 2. Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, adults captured on yellow sticky cards (YSC) for each treatment [pyriproxyfen (Fulcrum: OHP, Inc., Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control] within each growing medium (BM1—Berger BM1 All-Purpose Mix; Berger, Saint-Modeste, Quebec, Canada, and LC1—Sunshine LC1 RSi Professional Growing Mix; SunGro Horticulture Canada Ltd., Seba Beach, Alberta, Canada). Estimated means followed by different lowercase or uppercase letters within each growing medium indicate significant differences ($P < 0.05$) among the treatments.

yellow sticky cards in the untreated check [97.4% (85.3%, 99.6%; $n = 100$)] than the pyriproxyfen treatment; however, there was no evidence of any significant differences between pyriproxyfen and the water control, or between the untreated check and the water control (Fig. 5).

Discussion

This is the first study to evaluate the effects of pyriproxyfen and *I. fumosorosea*

when applied as drench applications to the growing medium on western flower thrips pupae. Otieno et al. (2016) found that azadirachtin, an insect growth regulator that inhibits metabolism of the molting hormone, ecdysone (Ware and Whitacre, 2003), when applied as drenches against the pupal stages of western flower thrips resulted in a 60% reduction in adult eclosion. Helyer et al. (1995) reported that soil applications of chlorpyrifos and malathion, which are both contact insecticides, provided 96.5% and

Fig. 3. Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, adults captured on yellow sticky cards (YSC) for each growing medium (BM1—Berger BM1 All-Purpose Mix; Berger, Saint-Modeste, Quebec, Canada) and LC1—Sunshine LC1 RSi Professional Growing Mix; SunGro Horticulture Canada Ltd., Seba Beach, Alberta, Canada) within each treatment [pyriproxyfen (Fulcrum: OHP, Inc., Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control]. Estimated means followed by different lowercase or uppercase letters within each treatment indicate significant differences ($P < 0.05$) between the two growing media.

Fig. 4. Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, adults captured on yellow sticky cards (YSC) for each treatment [pyriproxyfen (Fulcrum: OHP, Inc., Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control] 7 and 14 d after treatment application. Estimated means followed by different lowercase or uppercase letters within each day after treatment application indicate significant differences ($P < 0.05$) among the treatments.

97.5%, respectively, mortality of western flower thrips pupae. In the current study, drench applications of pyriproxyfen to the growing medium resulted in 17% ($n = 100$) of western flower thrips adults captured on the yellow sticky cards in the first experiment. However, the estimated mean probability of western flower thrips captured on the yellow sticky cards was not significantly different between the pyriproxyfen treatment and the water control for the two growing media in the second experiment. Therefore, it appears that pyriproxyfen is not affecting the pupal stage of the western flower thrips in the growing medium. However, further studies

are warranted to determine why the water control resulted in mortality of the western flower thrips pupal stages.

The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* (*brunneum*), are reported to negatively affect the pupal stages of western flower thrips in the growing medium or soil (Ansari et al., 2007, 2008; Helyer et al., 1995; Skinner et al., 2012). For instance, Ansari et al. (2007) found that western flower thrips pupal mortality ranged from 70% to 90% when exposed to growing medium mixed with *M. anisopliae* (*brunneum*). Skinner et al. (2012) observed a 90% reduction in the mean total number of

western flower thrips per plant using myco-tized millet grains containing *B. bassiana* in the soil. However, in the current study, there was no evidence that drench applications of *I. fumosorosea* were significantly different from the water control based on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards. Therefore, *I. fumosorosea* is not an effective microbial insecticide against western flower thrips pupae when applied as a drench. The reason for the discrepancy among the entomopathogenic fungi against western flower thrips pupal stages is not known at this time; consequently, further investigation is warranted.

Studies have demonstrated that growing medium or soil type can affect survival of soil-dwelling life stages of insect pests (Chen and Shelton, 2007; Holmes et al., 2013; Hulthen and Clarke, 2006; Pietrantuono et al., 2015; Varatharajan and Daniel, 1984). Varatharajan and Daniel (1984) reported that the highest number of *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) pupae were found in loose soil with a layer of organic debris on the surface, rather than in clay soil. However, in the current study, there was no evidence that growing medium type in the untreated check treatment affected the estimated mean probability of western flower thrips adults captured on the yellow sticky cards. Furthermore, growing medium or soil type may also influence the effect of drench-applied insecticides on soil-dwelling pests. For instance, Cowles and Villani (1994) reported that soil pH and organic matter affected the efficacy of the insecticides: carbaryl, bendiocarb, and chlorpyrifos against Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae), larvae. Nevertheless, in the current study, there was no evidence that growing medium type associated with the pyriproxyfen treatment affected the estimated mean probability of western flower thrips adults captured on the yellow sticky cards. Therefore, the results demonstrated that the two growing media may not influence the survival of western flower thrips pupae or the efficacy of pyriproxyfen on western flower thrips pupae in the growing medium investigated.

Pyriproxyfen degrades rapidly in soil under aerobic conditions (Fathulla, 1994), which is supported by the results from the third and fourth experiments as the estimated mean probability of western flower thrips adults captured on the yellow sticky cards for the pyriproxyfen treatment was not significantly different from the water control 7 and 14 d posttreatment for the third experiment, or 3 and 5 d posttreatment for the fourth experiment. Therefore, there was no evidence that drench applications of pyriproxyfen had any residual activity on western flower thrips pupae in the growing medium 3 d after application of treatments. Based on the parameters of our study, pyriproxyfen and *I. fumosorosea* do not negatively affect western flower thrips pupae that reside in the growing medium.

Fig. 5. Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, adults captured on yellow sticky cards (YSC) for each treatment [pyriproxyfen (Fulcrum: OHP, Inc., Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control] 3 and 5 d after treatment application. Estimated means followed by different lowercase or uppercase letters within each day after treatment application indicate significant differences ($P < 0.05$) among the treatments.

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