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Research article

Protecting Unrooted Cuttings From *Bemisia tabaci* (Hemiptera Aleyrodidae) During Propagation

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Abstract

In North America, the sweetpotato whitefly, *Bemisia tabaci* Genn., is an important pest of greenhouse poinsettia. Growers have limited options to control this pest during propagation of cuttings, which are rooted under mist for several weeks. Early establishment of this pest increases the difficulty of managing the whitefly and retaining high aesthetic standard during the remaining crop production phase. We evaluated two neonicotinoids with translaminar activity, thiamethoxam (Flagship 25WG), and acetamiprid (TriStar 70 WSP), for control of *B. tabaci* pre-infested on unrooted cuttings propagated under mist. In an experimental greenhouse, both materials significantly reduced whitefly populations, providing an average reduction of 87.8% and 61.5% total recovered whitefly stages respectively, compared with controls. In another test, dipping cuttings in thiamethoxam (immersion treatment) did not improve control significantly, when compared with foliar sprays applied at label rate. In a commercial greenhouse operation, immersion treatments of thiamethoxam on pre-infested poinsettia cuttings maintained whiteflies at $\leq$ 0.02/plant, compared with up to 0.33/plant in untreated cuttings. Our data suggest that treating unrooted cuttings before or at the start of propagation can be part of an overall strategy for growers to manage whiteflies in poinsettia production.

Key words: poinsettia, thiamethoxam, acetamiprid, propagation, whitefly

The propagation of unrooted cuttings under greenhouse mist systems is a well-established method for nursery stock (Couvillon 1988, Newton and Jones 1993). Although mist propagation systems offer a low cost and reliable approach for many plant species, vegetative cuttings in propagation may be susceptible to pests and diseases. Moreover, the shipment of propagative plant material including cuttings provides a pathway for introduction of pests into greenhouses (Hulme 2009, Navia et al. 2010, Saccaggi and Pieterse 2013). Management of pests early in the production cycle is critical to establishing integrated pest management approaches for many greenhouse pests (Van Lenteren and Woets 1988, Albajes et al. 1999).

In the case of poinsettia (*Euphorbia pulcherrima* Willd. Ex Koltz) propagation of unrooted cuttings under a fine mist of water presents environmental and physiological challenges to management of sweetpotato whitefly, *Bemisia tabaci* Genn. Contact insecticides require thorough coverage of both upper and lower sides of the leaves to be effective against *B. tabaci* (Latheef et al. 2008, Liu and Stansly 1995). Residual control from contact insecticides may be limited by the frequent application of mist; moreover, the lack of a developed root system will limit adsorption (uptake efficiency) from many traditional systemic insecticides (Sicbaldi et al. 1997). *B. tabaci*, however, are unhindered by the presence of mist and continue to lay eggs and develop on the leaves of the unrooted cuttings. If the cuttings are obtained from infested source plants, or if the propagation areas are subject to new whitefly infestations, the new crop of poinsettias will begin their 6-month crop cycle already infested.

Insecticides with translaminar properties provide alternatives to manage pests on cuttings. Translaminar insecticides are readily absorbed and move internally across leaf tissues, offering a strategy to control whitefly during mist propagation. In Canada and Europe, researchers and growers are experimenting with dipping poinsettia cuttings in biopesticides in order to reduce initial infestations of whiteflies on propagated materials (Buitenhuis et al. 2016; Cuthbertson et al. 2009, 2015). However, the use of insecticides with translaminar activity as dipping treatments against *B. tabaci* on poinsettia has not been reported. The objective of this study was to evaluate neonicotinoid insecticides with translaminar activity applied as spray and immersion treatments against *B. tabaci* on poinsettias cuttings under mist-propagation.

Materials and Methods

Insects and Plants

A laboratory colony of *B. tabaci* B Biotype originating from cotton in Brazos County, TX and reared on eggplant in Plexiglass cages maintained at 26 °C 14:10 (L:D) h were used in the first two studies. Adult used in trials came from a one-year-old colony and had a sex ratio ≈ 50% male/female. Adults used in the experiments were of unknown age. Poinsettia "Prestige Red" plants were obtained from a local nursery and grown and propagated at Texas A&M University according to guidelines provided by Ecke et al. (2004).

Comparison of Two Insecticides

We compared foliar applications of two neonicotinoid insecticides for *B. tabaci* protection on unrooted poinsettia cuttings propagated under mist in an experimental greenhouse. Thiamethoxam was applied at a rate of 75 mg active ingredient/liter (Flagship 25WG, Syngenta Crop Protection, Inc. at 0.3 g product/liter or 4 oz/100 gal), and acetamiprid at a rate of 59 mg a.i./liter (TriStar 70 WSP, Cleary Chemical Corporation at a rate of 0.08 g product/liter or 1.13 oz/100 gal, 2 soluble bags). These dilutions were selected based on the label rates of active ingredients that would be applied by growers. Water (RO) was used for an untreated control. To establish infestations for the study, 160 poinsettias plants placed on greenhouse benches under Remay cover were exposed to adult whiteflies released at an average of 2.25 whitefly per cutting on two occasions, 5 days apart. Six days later, cuttings containing whitefly eggs were taken from the source plants and stuck individually into 10-cm diameter pots containing potting media (Sunshine Grow Mix #1, Sungrow Horticulture, Agawam, MA) for rooting. Pots were randomly divided into three treatment groups with each one placed on separate mist benches measuring 1.5 × 3m. Benches were covered with a semi-transparent nonwoven polyester fabric (Reemay, Avintiv Old Hickory, TN) fitted over a 1.2 m high PVC frame to maintain humidity. On each bench the 36 potted cuttings were randomized into three groups of 12 plants per the three treatments.

Overhead misting for propagation was provided by Phyto-Mist Brass Nozzles (Phytotronics Inc., Earth City, MO) at 1-m spacing which was programmed to operate for 12 s every 12 min. A decreasing misting cycle intensity was provided to harden the cuttings. Initially the misting cycle operated for 24 h 3 days, followed by operation only during daylight hours (6 a.m. until 8 p.m.) for a further 14 days, and finally operation during only afternoon hours (12 p.m. until 4 p.m.) for a further 8 days. Insecticide treatment applications were made on day 11 of the misting cycle, after the end of the misting period. The three treatments were applied using 1-liter hand held spray bottles, and plants sprayed until runoff. Cuttings under mist were exposed to five additional releases of adult whitefly (at a rate of 1.7 per cutting) on days 4, 8, 11, 16, and 18 of the misting cycle. This was done simulate a worst case scenario, as we have observed whiteflies enter poorly screened greenhouses from adjacent cotton fields following harvest. The study was terminated on day 25, at which time total whitefly counts by stage were made in the laboratory from all leaves with the aid of a dissecting microscope (at 10–40 × *g*).

Comparison of Application Methods

The second study tested the efficacy of two different application methods for thiamethoxam. Treatments were a foliar application, compared with a whole-plant immersion, both using Flagship 25WG at the same label rate used in the previous study (i.e., 75 mg a.i./liter). Control plants were untreated. Infested poinsettia cuttings were obtained from plants exposed to whiteflies as noted above. Plants were stuck into 10-cm pots and arranged on three benches under Remay (as noted above) each containing 13 plants per treatment replicate. For the immersion treatment, plants were individually submerged in a 19 liter (5 gal) bucket for 10 s and then allowed to sit in a 21 °C room for 1 h for absorption of the insecticide before planting.

The misting cycle (12 s per 12 min) was operated continuously for 2 days, followed by daytime operation (8 a.m. until 4 p.m.) for an additional 7 days and afternoon operation (12 p.m. until 4 p.m.) until the experiment was terminated 24 days after the cuttings were stuck. Foliar treatments were applied to "run off" 48 h after planting in the evening when the mist system had ceased for the day. To maintain insect pressure, starting on day 4 of the misting cycle *B. tabaci* adults were released on five occasions (twice per week) at a rate of 1 whitefly per cutting. The study was terminated on day 24 after planting, and number of whiteflies and leaves counted, as noted previously. Environmental conditions monitored with a datalogger (Hobo H8 Series, Onset, Bourne, MA) mounted inside a rain shield revealed an average temperature of 21.3 °C (range 11.8–31.1 °C) during the studies, and 100% RH during misting cycles.

Grower Study

To test the efficacy of cutting treatments under producer conditions, a greenhouse trial was conducted with a local poinsettia grower in Washington County, TX. Approximately 10,000 Prestige Red poinsettia cuttings that contained apparent *B. tabaci* source populations were used and one half randomly assigned to thiamethoxam treatments via immersion, at the same rate indicated in the previous study. The remaining cuttings were untreated. Following treatments, cuttings were placed in rooting media plugs on 12 benches in a mist propagation house. Whitefly counts were conducted weekly on 60 randomly selected cuttings per treatment (i.e., 10 plants per six replicate benches for each thiamethoxam and control cuttings) for 5 wks, until the end of the mist propagation period. Cuttings were replaced but not reused during the study.

Statistical Analysis

Data were analyzed as a randomized complete block design via one-way ANOVA with bench as a statistical block and 10–13 plants per replicate, depending on the study. Means were compared, where appropriate, using Tukey multiple comparison procedures at $P \leq 0.05$ with count data transformed via log $(n+1)$. In the grower study, due to low numbers, whiteflies per bench were totaled over the 30-day propagation period prior to analysis.

Results

Comparison of Two Insecticides

Insecticide treatment was highly significant ($F_{2,6} = 15.0$, $P < 0.005$). Mean comparisons revealed that both insecticides significantly reduced all the different immature stages of whitefly relative to the control (Table 1). Overall, thiamethoxam-treated plants had a larger reduction, i.e., 87.8% fewer whiteflies stages on plants, compared with 61.5% for acetamiprid. However, the number of whiteflies recovered differed significantly only in the number of eggs. The bench (blocking factor) effect on total whiteflies recovered was not significant ($F_{2,6} = 0.22$, $P = 0.81$).

Comparison of Application Methods

Both application methods of thiamethoxam significantly reduced whitefly infestations relative to controls, but did not differ significantly with respect to each other in reduction of eggs, early, or late instars (Table 2). Overall, plants treated via immersion or spray had a similar reduction, i.e., 91.2% and 91.1% fewer total whiteflies

Table 1. Evaluation of foliar-applied neonicotinoid insecticides against whiteflies, *B. tabaci*, on propagated poinsettia cuttings under mist

Treatment	Total	Eggs	First instar	Second + third instars	Fourth instar/pupae
Control	250.9 ± 45.2a	93.1 ± 11.6a	55.5 ± 14.0a	99.1 ± 16.1a	3.0 ± 0.6a
Thiamethoxam	96.6 ± 21.4b	48.9 ± 7.4b	29.2 ± 3.3b	17.5 ± 3.2b	1.0 ± 0.4b
Acetamiprid	30.9 ± 5.9c	8.3 ± 1.3c	17.9 ± 2.3b	4.4 ± 0.9b	0.1 ± 0.1b

Data are mean ± SEM/whiteflies per cutting from three replicate groups of cuttings; different letters indicate differences according to Tukey's HSD test at $P < 0.05$.

Table 2. Evaluation of thiamethoxam against whiteflies, *B. tabaci*, on propagated poinsettia cuttings under mist according to application method

Treatment	Total	Eggs	First–third instars	Fourth instar/pupae	Exuvia
Control	107.5 ± 4.8a	65.5 ± 2.6a	37.7 ± 5.2a	3.3 ± 1.3a	1.0 ± 0.1a
Immersion	9.4 ± 1.6b	7.1 ± 0.7b	2.3 ± 0.9b	0.0 ± 0.0b	0.0 ± 0.0b
Foliar spray	9.5 ± 5.5b	7.5 ± 4.2b	2.0 ± 1.4b	0.0 ± 0.0b	0.0 ± 0.0b

Data are mean ± SEM/whiteflies per cutting from three replicate groups of cuttings; different letters indicate differences according to Tukey's HSD test at $P < 0.05$.

stages on plants, compared with controls, respectively. Relatively few exuviae were recovered; however, these may have been underestimated as they are transparent and easily missed. The average number of leaves per plant was 6.2 ± 0.5 and did not differ between treatments ($F_{2,6} = 1.99$, $P = 0.22$). The bench (block) effect on total whiteflies recovered was also not significant ($F_{2,6} = 0.004$, $P = 0.99$).

Grower Study

More whiteflies were observed in the untreated bench samples, reaching a maximum of 0.33 live immature whiteflies per plant on day 23, compared with a maximum of only 0.02 whiteflies/plant in thiamethoxam-treated plants (Fig. 1). The decline on day 30 may have been due to adults emerging. However, due to the relatively low number of whiteflies recovered and high variability between benches, this difference approached, but was not statistically significant ($F_{1,10} = 4.35$, $P = 0.063$).

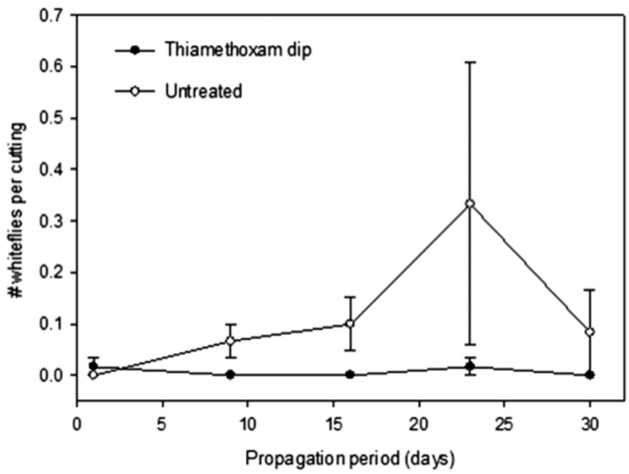

Fig. 1. Counts of whiteflies, *B. tabaci*, life stages (mean ± SEM/plant) on propagated poinsettia cuttings under mist at grower operation according to prior thiamethoxam-immersion.

Discussion

The shipment of propagative plant material (cuttings) provides a pathway for introduction of *B. tabaci* into poinsettia greenhouses (Cuthbertson et al. 2011, Frewin et al. 2014). We determined that thiamethoxam and acetamiprid [neonicotinoid insecticides with rapid translaminar absorption and distribution within leaves (Elbert et al. 2008)] applied at label rates provide growers with potential tools to reduce risks of infestation and protect cuttings during propagation under mist systems. We noted foliar thiamethoxam treatments performed better in the second study when cuttings were treated on day 2 of propagation cycle, compared with the first study when plants were treated on day 11 (i.e., 91.1% vs. 61.5% overall whitefly reduction compared with controls, respectively). Thus treatment at the start of the propagation cycle may be beneficial. However, the ultimate success of this approach will depend on the growers needs, and the localized whitefly infestation levels. We did not assess survivorship of whiteflies post propagation, as poinsettia plants are typically exposed to subsequent insecticide or biological control programs and additional pests at this stage (Ecke 2004, Frewin et al. 2014). The reason for the different number of eggs between insecticide treatments in the first study is unknown, but could be attributable to ovipositional preference/avoidance response of the adult female whitefly due to the properties of these insecticides.

We investigated whether immersion of cuttings with thiamentoxam would improve control, relative to spraying to drip. We did not observe any differences between these treatments, suggesting that leaf penetration and bioavailability to whiteflies was sufficient via foliar spray. Currently, immersion or dipping is not an approved label use for this pesticide. However, dipping treatments for new cuttings may have logistical benefits. For example, dipping may result in less pesticide waste compare with spraying, while providing optimal coverage of contact insecticides, which is required against whiteflies (Latheef et al. 2008, Liu and Stansly 1995). As the active ingredients of most neonicotinoids move into leaf tissues within 4 h and surface residues will diminish to low levels within a week (Wise et al. 2006), the use of such materials early in propagation may also be compatible with later use of beneficial arthropods that do not feed directly on the crop (Heinz and Parrella 1994), however this would need to be confirmed.

One issue is that some *B. tabaci* biotypes (notably Q) have developed resistance to neonicotinoids and other insecticides, while overexposure of nonresistant biotypes to pesticides at the propagator may also cause resistance development (Schuster et al. 2010, Castle and Prabhaker 2013). This suggests that biotype monitoring may be needed and that insecticides should immediately discontinued if determined ineffective (McKenzie et al. 2012). Moreover, pending legislation may restrict the availability of neonicotinoids in the future, as various risk assessments concerning environmental impacts of this group of insecticides are currently underway (EPA 2017).

While our results and earlier studies (Buxton and Clarke 1994, Richter 2005) suggest that immersing poinsettia cuttings in conventional insecticides helps control *B. tabaci*, there are issues of worker exposure and waste disposal. Researchers and growers in other regions are therefore experimenting with biopesticides (soaps, oils, and microbial control agents) used individually and in combination as dipping treatments to manage *B. tabaci* on new poinsettia cuttings. In Canada, where access to registered pesticides is limited and insecticide resistant whitefly biotypes occur, researchers have reported that mineral oil (0.1%, v/v) and the combination of insecticidal soap (0.5%, v/v) plus a commercial formulation of *Beauveria bassiana* were the most effective treatments used to dip poinsettia cuttings (Brownbridge et al. 2014, Buitenhuis et al. 2016). Similar results have been demonstrated in the UK (Cuthbertson et al. 2009, Cuthbertson and Collins 2015). Buitenhuis et al. (2016) noted that dipping cuttings was compatible with biological control agents used later in production, i.e., by ensuring that the density of whiteflies entering the production cycle did not exceed control "capacity" of the parasitoids used. However, the rates of oils and soaps used for dipping were decreased from label rates to avoid issues of phytotoxicity. The transmission of plant diseases is another potential concern for growers using dipping treatments.

In summary, the emergence of insecticide resistant whitefly biotypes, increasing restrictions on insecticide availability, and emergence of effective greenhouse biological control strategies all favor movement to preventative rather that curative strategies (McKenzie et al. 2012, Cuthbertson et al. 2011, Frewin et al. 2014). Treatment of poinsettia cuttings with effective materials will help growers knock down any *B. tabaci* populations on plants entering the production cycle to a level at which where they can be more easily managed, in order to produce a clean crop.

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