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Considerations for the use of neonicotinoid pesticides in management of *Bactericera cockerelli* (Šulk) (Hemiptera: Triozidae)

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ABSTRACT

Bactericera cockerelli is a pest on multiple solanaceous crop plants and is the sole vector for the bacteria *Candidatus* Liberibacter psyllaurous. When the pathogen is present, feeding by these psyllids results in 'vein greening' disease in peppers and tomatoes, and "zebra chip" disease in potatoes. Currently, management is based entirely on the application of pesticides, including two neonicotinoid compounds. Populations of *B. cockerelli* collected in southern Texas in 2006 and 2012 were examined for reduced susceptibility and behavioral responses to imidacloprid.

Tests comparing imidacloprid and thiamethoxam demonstrated that both can reduce nymph numbers in the field, but retention and effective periods vary among application methods and compounds. In addition, imidacloprid and thiamethoxam are both sensitive to the amount of water applied during irrigation. Collectedly, these results suggest that imidacloprid is unlikely to be effective in controlling *B. cockerelli* in south Texas. Moreover, its use needs to be carefully considered in other locations even where resistance has not yet been detected. Finally, thiamethoxam may be useful, but careful attention must be paid to irrigation and rainfall level, application method, and timing of application.

1. Introduction

Bactericera cockerelli (Šulc) (Hemiptera: Triozidae), is a known pest of solanaceous crops in North and Central America (Butler and Trumble, 2012a,b; Liu and Yang, 2009), and the sole known vector of the bacteria *Candidatus* Liberibacter psyllaurous (CLP, aka *C.* L. solanacearum). CLP infection results in 'zebra chip' (ZC) disease in potato (*Solanum tuberosum*) and a similar disease called 'vein greening' in tomato (*Solanum lycopersicum*) and bell pepper (*Capsicum annum*) (Abad et al., 2008; Crosslin et al., 2011; Hansen et al., 2008; Liefting et al., 2008; Lin et al., 2009; Munyaneza et al., 2007a, 2008). Symptoms of CLP infection potatoes include: chlorosis, stunted plant growth, aerial tubers, reduced tuber quality, and a striped 'zebra' pattern that is prominent after frying (Butler and Trumble, 2012a,b). Symptoms in tomato and pepper are similar and can include chlorosis and reduced fruit size and quality. Additionally, severe infection can result in death of the plant (Goolsby et al., 2007; Munyaneza et al., 2007b, 2008). To date, this vector–pathogen complex has resulted in substantial losses, particularly to the potato (Abad et al., 2008) and tomato industries (Liu and Trumble, 2005).

Currently, management of CLP is achieved exclusively through management of the psyllid. This is primarily achieved through the frequent use of a small group of insecticides with various modes of action that are applied following a calendar-based rotation. This group of commonly used pesticides includes two neonicotinoid compounds, imidacloprid (Admire Pro®, Bayer Crop Science, Research Triangle Park, NC) and thiamethoxam (Platinum®, Syngenta Crop Protection, Greensboro, NC). Typically, these neonicotinoid compounds are applied at or around planting in order to provide early protection against the psyllid, although they are labeled for multiple methods of application, including foliar (Žežlina et al., 2013), soil drench (Faulkenberry et al., 2012) and chemigation (Byrne and Toscano, 2006). Thiamethoxam is also available as a component of the seed treatment CruiserMaxx® (Syngenta Crop Protection). Studies suggest that for both imidacloprid (Van Iersel et al., 2000; Juraske et al., 2009, 2011) and thiamethoxam (Karmakar et al., 2006; Karmakar and Kulshrestha, 2009) factors such as soil type and application method can influence efficacy, influencing the way that these products can move throughout the soil (Knoepp et al., 2012), and vary in rate of uptake into the plant (Byrne and Toscano, 2006; Juraske et al., 2009).

In Texas, imidacloprid use on potatoes is widespread; Guenthner et al. (2012) reported its use on over 90% of fields in 2011. High rates of usage were also reported for Kansas and Nebraska where it was applied to 50% of fields in 2011 (Guenthner

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et al., 2012). In California, in 2010, it was used on approximately 42% of planted potato acreage (California Department of Pesticide Regulation, http://www.cdpr.ca.gov/docs/pur/pur10rep/10_pur.htm, retrieved April 2013). In Mexico, there are anecdotal reports of dozens of foliar applications of imidacloprid in a given year. Reported use of thiamethoxam is less than that of imidacloprid, and also less consistent. For example, Guenthner et al. (2012) reported no use in 2011, and its use on a maximum of 30% of fields in Texas in 2010; use of thiamethoxam is similarly inconsistent in Kansas and Nebraska. There are no reports of thiamethoxam use on potatoes in California in 2010, but it was used on over 30,000 acres of tomatoes. Additionally, in California, there was a large increase (22%) of imidacloprid use between 2009 and 2010 (California Department of Pesticide Regulation, http://www.cdpr.ca.gov/docs/pur/pur10rep/10_pur.htm, retrieved April 2013).

To date, there are no published studies of resistance to neonicotinoid pesticides in *B. cockerelli*. However, some elements of imidacloprid susceptibility have been examined. Previous work by Liu and Trumble (2007) demonstrated differences between the concentrations of imidacloprid required to kill 50% of *B. cockerelli* nymphs (LC_{50}) collected in California and Texas. Further, treatment with imidacloprid has also been shown to alter behaviors including probing, cleaning, resting and walking in colonies collected in Texas and maintained in the laboratory for an extended period of time (Butler et al., 2011, 2012). In particular, Butler et al. (2011, 2012) reported a reduction in feeding on imidacloprid treated plants, and similar responses have been documented in Asian citrus psyllid (Boina et al., 2009). Finally, resistance to neonicotinoid pesticides has been examined in the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama). Tiwari et al. (2011a) reported resistance to imidacloprid (RR_{50} of 35) and reduced susceptibility to thiamethoxam (RR_{50} = 15 and 13) in some populations tested in Florida citrus. Notably, both compounds belong to the Insecticide Resistance Action Committee IRAC mode of action group 4-A (http://www.irac-online.org/eClassification/).

In this paper, we examine multiple factors associated with the use of neonicotinoid pesticides for control of *B. cockerelli*. First, we examine potential resistance to imidacloprid in a south Texas population. Second, we examine the previously reported behavioral responses to imidacloprid in the same south Texas population. Finally, we report on two studies associated with application of neonicotinoid pesticides to soil; a lab study examining the influence of irrigation levels on pesticide retention, and a field study examining the efficacy of different application methods. Collectively, these studies will result in more informed decisions about neonicotinoid use, leading to reduced risk of resistance development, more effective control of *B. cockerelli*, and reduced CLP infection.

2. Materials and methods

2.1. Plants and colonies

Laboratory studies were conducted using two colonies of *B. cockerelli*. The 'susceptible' colony (henceforth "Tex06") was established from a lab colony originally collected from tomato and potato fields near Weslaco, Texas and has been maintained in culture for over six years. The field collected colony (Tex12) was collected from tomato near Edinburgh, Texas and was maintained in culture for less than two generations when resistance assays were conducted. Both colonies were maintained on tomatoes (*S. lycopersicum* L. 'Yellow Pear') at conditions of 21–26 °C and 40–60% relative humidity. The lab colony was maintained in greenhouse conditions and at ambient light, the field collected colony was maintained in a rearing room under a 14L:10D light cycle.

All laboratory studies were conducted on greenhouse grown 'Yellow Pear' tomatoes. Plants were initially grown from seed in 10.16 cm pots with UC soil mix (Matkin and Chandler, 1957), fertilized with Miracle Gro® nutrient solution (Scotts Company, Marysville, OH) at label rate, and watered daily. When plants were approximately 12 cm tall, they were transferred to 15 cm diameter (4.9 L) pots. Potatoes (*S. tuberosum*, variety 'Atlantic') for pesticide retention studies were grown from seed pieces in 15 cm diameter pots with UC soil mix, watered *ad libitum* and fertilized with Miracle Gro nutrient solution.

2.2. Imidacloprid resistance bioassays

While imidacloprid is reportedly effective against both juvenile and adult stages of *B. cockerelli*, nymphs do not move among plants, making them more suited for constant exposure bioassays. In addition, previous studies of susceptibility to imidacloprid have been conducted on nymphs (Liu and Trumble, 2005). Consequently, we used nymphs to conduct bioassays for comparing sensitivity to imidacloprid in the Tex12 and lab colonies. Bioassays were conducted using a protocol similar to that published in Liu and Trumble (2004). Tomato plants were treated with a 100 ml soil drench containing one of four concentrations (24, 48, 96, 192 ml/L) of imidacloprid (Admire Pro, Bayer Crop Science) or a water control. Following pesticide application, plants were maintained in the greenhouse for 6–7 days to allow for uptake and distribution of the pesticide. Following this acquisition period, 20–25 s or third instar *B. cockerelli* nymphs were hand transferred to plants using a fine camel-hair paintbrush. Once nymphs were transferred, plants were examined daily, and the numbers of live nymphs, dead nymphs, and adults (when applicable) were recorded. Following the second round of tests, a leaf sample was collected from the top portion of each plant and stored in an ultra-cold freezer (Forma Scientific, Vernon Hills, IL) and subsequently measured for imidacloprid residual levels.

2.3. Behavioral response to imidacloprid

To determine if *B. cockerelli* from either colony exhibited altered behavioral responses to imidacloprid compared to reports from 2004 (Liu and Trumble, 2004), we conducted behavioral bioassays according to the methods of Liu and Trumble (2004). Assays were conducted on whole intact tomato plants in arenas consisting of a 9 by 12 cm Plexiglass rectangle stage, a 1 by 3 by 6 cm foam square with a 2 hole and a clear glass cover. The elements of the arena were layered so that the foam rested on top of a tomato leaf and was covered by acrylic creating a chamber that both contained a psyllid and provided it access to the abaxial side of the leaf. In conducting the assays, post-teneral adult psyllids were introduced into the arena, and allowed to adjust to the microenvironment for 5 min. Following the 5 min acclimation period, observations began and continued for 15 min during which the behaviors cleaning, jumping, resting, off leaf (abandoning the leaf surface), walking, and behaviors resembling feeding and probing were recorded using the Observer® XT (Noldus Information Technologies, Wageningen, Netherlands) program. Since accessing the phloem is known to take more than an hour (Butler et al., 2012), actual feeding behavior could not be observed and is only an approximate measure. Behavioral assays were conducted using plants treated with imidacloprid as in the resistance bioassays, and on individuals from both the Tex12 and Tex06 colonies. Ten individuals were tested at each imidacloprid concentration from each colony. Behavioral responses to imidacloprid were measured in two ways, duration of behavior and frequency (number of events). Duration was tested using models with the response variables of either duration of behavior or frequency, and the fixed factors colony and dose.

2.4. Greenhouse studies of pesticide retention

Both imidacloprid and thiamethoxam are often soil applied at planting, and both have been shown to exhibit mobility in soil (Cox et al., 1997; Liu et al., 2006; Rouchaud et al., 1996). To determine the potential for leaching of these materials from soil, we conducted a series of greenhouse studies on potatoes treated at planting with either 550 ml of thiamethoxam (Platinum, 141 ml/ha) or imidacloprid (Admire Pro, 756 ml/ha). Following treatment, plants were maintained for 70 days and systematically watered daily at one of three volumes (250, 500 and 1000 ml). A preliminary study indicated that at volumes over 650 ml, water flowed out of pots (Vindiola, unpublished data). One leaf was collected from the top of each plant for quantification of pesticide residue were collected weekly for eight weeks and stored at −80 °C until quantification (as described below). Each treatment by water combination was replicated six times.

2.5. Field studies to compare methods of pesticide application

2.5.1. Field sites

Field studies were conducted at the University of California South Coast Research and Extension Center, Irvine, California (SCREC) (33°41′18″N, 117°43′, 19′W, elevation: 420 ft). Seed sets (variety ‘Atlantic’) were transplanted into a sandy loam type soil. Four replicated plots were established, 3 rows wide (1.5 m centers) by 12.2 m long separated by a buffer row, to give 183 square feet per plot. The potatoes were drip irrigated *ad libitum* (water pH 7.2–7.5).

2.5.2. Pesticide application

We used two methods of application of imidacloprid and three methods of application of thiamethoxam to determine the relative efficacy of these application methods. Both imidacloprid (Admire Pro®, Bayer Crop Science) and thiamethoxam (Platinum®, Syngenta Crop Protection) were applied as ‘drip treatments’ using the recommended field rate (635.1 ml/ha for imidacloprid, 189.8 ml/ha thiamethoxam) applied through drip tape near the top of soil mounds, as recommended by the product label. Both pesticides were also applied via in furrow drench, with material sprayed into holes immediately following the placement of seed-pieces, prior to closing holes. We also planted seed-pieces treated with a commercial seed treatment containing thiamethoxam (Cruiser-Maxx®, Syngenta Crop Protection). Finally, we examined control plots that were not treated with any neonicotinoid pesticide. Due to the complexities of different watering regimes and application methods, it was not possible to randomize treatments. Consequently, each row was divided into four replicate blocks per treatment. In addition to the experimental neonicotinoid pesticide treatments, all plots were subjected to routine pesticide applications according to standard commercial production practices (Western IPM, 2006).

2.5.3. Sampling for psyllids and collection of samples for pesticide residue analysis

Throughout the season, plants in each experimental plot were sampled for psyllids. During sampling, the numbers of eggs, nymphs and adults on four entire plants were counted in each plot. Concurrent to sampling for psyllids, leaves were collected from the top third of four haphazardly selected plants from across the each plot. Leaf samples were stored in insulated coolers filled with ice during transport to the lab, and were subsequently stored at −80 °C until quantification as described below.

2.5.4. Quantification of pesticide residues

Imidacloprid and thiamethoxam residues in potato leaf tissue were measured by ELISA (QuantiPlate® kit for imidacloprid available from EnviroLogix, 500 Riverside Industrial Parkway, Portland, ME; Thiamethoxam plate kit® available from Beacon Analytical Systems Inc, 82 Industrial Park Rd, Saco, ME). Discs were cut from each leaf using a size four (0.65 cm^2 (methods 2.4, retention experiment) or size five (0.39 cm^2) (all other tests) cork borer, placed in vials containing 100% methanol (1 disc per 200 μl), macerated using a Teflon® pestle and then shaken for 12 h at 25 °C. An aliquot of each extract was dried completely in a TurboVap® LV evaporator (Caliper Life Sciences, Hopkinton, MA, USA) and then reconstituted in either a 0.05% aqueous solution of Triton X-100 (imidacloprid tests) or water (thiamethoxam tests) prior to analysis by ELISA.

2.5.5. Evaluation of zebra chip symptoms

Potatoes infected with CLP results in characteristic patterns within tubers upon frying. To evaluate the efficacy of imidacloprid and thiamethoxam applied using different methods for controlling CLP infection, we fried tuber slices and scored severity of symptoms (symptoms include dark brown striping irregularities) on a scale of 0–3. A score of 0 indicated no symptoms, while a score of 3 indicates significant discoloration and burning. At the end of the growing season, four tubers from four plants were collected from each plot and stored at 4 °C until frying. Two tubers were fried, and two were retained for additional analyses. Tubers were sectioned using a mandolin slicer, and fried in sunflower oil to approximate commercial production of potato chips. Two slices were fried from each tuber.

2.6. Statistical analysis

All analyses were performed using the R statistical language version 2.15 (R Development Core, 2008). LC_{50} and LC_{90} values were calculated by using general linear models (GLM) with probits and the drc (Ritz and Streibig, 2005) and MASS packages (Venables and Smith, 2010). Survivorship analyses were conducted using Cox proportional hazards models implemented with the survival package (Therneau and Grambsch, 2000). Both duration and frequency of behaviors were initially examined using MANOVA with the fixed effects of colony and imidacloprid dose. In analyzing behavior durations, response variables could not be transformed to meet assumptions of normality, and thus values were replaced with ranks. We were also unable to transform frequencies to normality, and therefore analyses likely violate some assumptions of the statistical test. When significant differences were detected in duration, ANOVA was performed and *P*-values were adjusted using Bonferroni's method. Follow-up analyses for frequencies were conducted using GLM fit with either a negative binomial or Poisson probability distribution, chosen based on Akaike information criteria (AIC) values and evaluated with an adjusted *P*-value calculated with Bonferroni's method. Imidacloprid concentrations from ELISA were compared via ANCOVA with the fixed factors: time since application and water level. Due to the limited sensitivity of the ELISA, samples from thiamethoxam needed to be pooled, yielding a single value for each concentration, and preventing statistical analysis. Nymph counts in field plots were examined using linear mixed models and a negative binomial probability distribution. ZC symptoms for fried potato tubers were compared by averaging the scores from two slices of each tuber and then applying a Kruskal–Wallis test.

Table 1
Measures of lethal imidacloprid doses (mg a.i.). $RR_{50} = 3.4$, $RR_{90} = 6.4$.

	LC_{50}	SE	CI	LC_{90}	SE	CI	Slope	SE
Tex06	21.7	0.05	18.7–21.7	130.2	0.08	83.5–98	2.23	0.10
Tex12	74.8	0.06	66.6–84.0	839.7	0.14	558.8–1262.1	1.45	0.16

3. Results

3.1. Imidacloprid resistance bioassays

Tex12 was 3.4 times less susceptible to imidacloprid than the lab colony, exhibiting LC_{50} and LC_{90} values with non-overlapping confidence intervals (Table 1). Survival analysis revealed significant effects of both dose ($X^2 = 375.6$, d.f. = 4, $P < 0.001$), colony ($X^2 = 4.19$, d.f. = 1, $P < 0.05$), and the interaction of colony and dose ($X^2 = 72.16$, d.f. = 4, $P < 0.001$). Results from ELISA of samples collected following the death of all nymphs revealed an average of 131.7 (±71.2), 176.2 (±75.6), 458.7 (±267.0) and 2643.5 (±71.2) ng imidacloprid leaf disc^{-1} in plants treated with 24, 48, 96 and 192 ml L^{-1}, respectively. Imidacloprid was not detected in untreated control plants.

3.2. Behavioral response to imidacloprid

When behaviors were measured as a proportion of the observation period, there was a significant effect of colony (MANOVA: $F_{6,76} = 3.7$, $P < 0.01$), but there was no significant effect of dose (MANOVA: $F_{24,316} = 1.27$, $P = 0.17$) nor was there a significant colony by dose interaction (MANOVA: $F_{18,234} = 1.2$, $P < 0.23$). When behaviors were examined using individual ANOVAs (Supplemental 1) and adjusted P-values, only the feeding behavior differed significantly between colonies ($F_{1,88} = 13.9$, $P < 0.0001$). A similar pattern was found when the frequencies of behaviors were tested. Specifically, there was a significant colony effect (MANOVA: $F_{7,78} = 4.1$, $P < 0.01$), but there was no significant effect of dose (MANOVA: $F_{28,324} = 1.2$, $P = 0.16$) nor was there a significant interaction (MANOVA: $F_{21,231} = 1.4$, $P = 0.14$). Again, follow up revealed a significant difference between colonies with respect to feeding ($Z_{1,88} = -4.36$, $P < 0.001$), probing ($Z_{1,88} = -2.89$, $P < 0.001$), and cleaning ($Z_{1,88} = -3.19$, $P < 0.001$) (Supplementary 2).

3.3. Greenhouse studies of pesticide retention

The greenhouse studies of pesticide retention demonstrated a significant effect of both watering amount (volume) (ANCOVA: $F_{2,153} = 22.7$, $P < 0.001$) and elapsed time since pesticide application ($F_{7,146} = 9.9$, $P < 0.001$) on imidacloprid levels in plant tissue, in addition to a significant time by water interaction ($F_{7,146} = 4.1$, $P < 0.001$) (Fig. 1a). More specifically, the lower watering rates were not significantly different, but the 500 ml watering rate differed from 1000 ml, and the 1000 ml rate differed from 250 ml. A similar overall trend was observed with thiamethoxam (Fig. 1b); however, the need to pool samples for ELISA prevented us from performing statistical comparisons.

3.4. Field studies to compare methods of pesticide application

To evaluate the relative efficacy of thiamethoxam and imidacloprid applied in the field using different methods, we compared the number of nymphs sampled at three time points (Fig. 2) using a general linear model with a negative binomial probability distribution. Overall, there was a marginal, but non-significant effect of pesticide treatment ($X^2 = 10.3$, d.f. = 5, $P = 0.07$), but both the effect

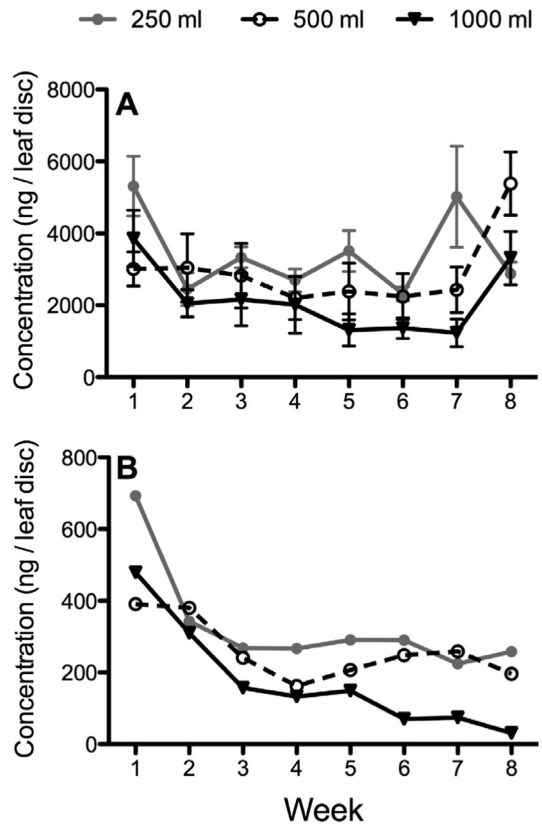

Fig. 1. Mean concentration and (SEM) of imidacloprid (A) and pooled concentration of thiamethoxam (B) over time for plants consistently watered daily with volumes of 250, 500 or 1000 ml.

of sample date ($X^2 = 14.5$, d.f. = 1, $P < 0.001$) and the sample date by treatment interaction were significant ($X^2 = 22.1$, d.f. = 5, $P < 0.001$). Within time points, we found that only the seed treated thiamethoxam reduced the number of nymphs in the first time point (Fig. 2), while both imidacloprid applications reduced the number of nymphs relative to the control in the second and third samples, as did thiamethoxam applied via the open hole soil drench.

ELISA revealed a significant effect of treatment method for imidacloprid (Fig. 3) (GLM: $T = 7.53$, d.f. = 1, 57, $P < 0.001$) and also a significant treatment by sample time interaction (GLM: $T = -4.0$, d.f. = 3, 55, $P < 0.001$), but the sampling times did not differ significantly (GLM: $T = -8.4$, d.f. = 1, 57, $P = 0.6$). Analysis of thiamethoxam levels at the first sample point revealed an overall significant difference among application methods ($F = 26.9$, d.f. = 2, 37, $P < 0.0001$), which was due to a reduced level in the seed treated plants relative to those treated via drip (Fig. 4). Sample points two and three for thiamethoxam were below the detection levels of the ELISA.

3.5. Evaluation of zebra chip symptoms

There was a significant difference in zebra chip severity among pesticide application methods (Kruskal–Wallis Test: $X_2 = 15.157$, d.f. = 5, $P < 0.001$) (Fig. 5). However, this effect was due exclusively to the thiamethoxam drip treatment in which all tubers exhibited severe zebra chip symptoms. When the drip applied thiamethoxam treatment was removed from the analysis, there was no significant difference among treatments (Kruskal–Wallis Test: $X^2 = 2.8$, d.f. = 4, $P = 0.6$).

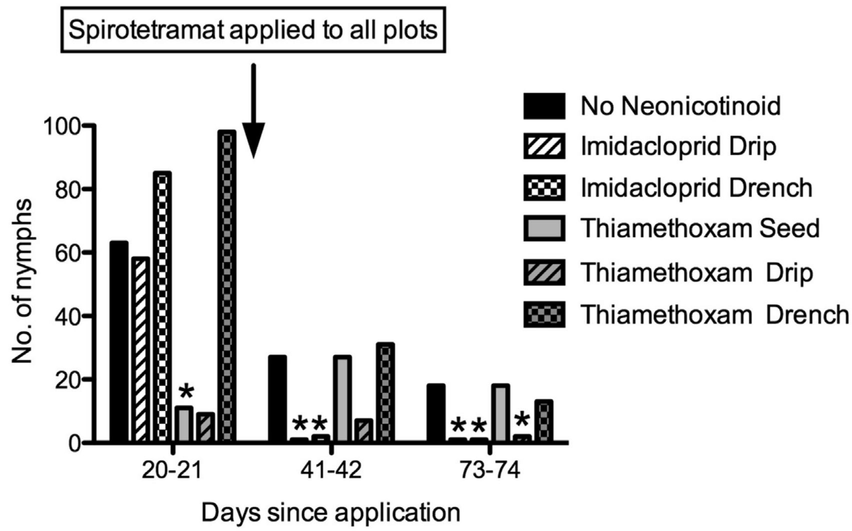

Fig. 2. Number of nymphs collected in plots treated with imidacloprid (white bars and patterns) and thiamethoxam (gray bars and patterns) applied using drip tape, open hole soil drench at planting, or via seed treatment containing thiamethoxam. Asterisks indicate significant differences from untreated control plots within a given sampling period at $P < 0.05$. Drip tape applications occurred one day later than soil drench.

4. Discussion

4.1. Imidacloprid resistance

In imidacloprid susceptibility assays, field collected populations were over three times less susceptible than those colonies that were maintained in the laboratory and unexposed to imidacloprid. This reduced susceptibility is in keeping with Texas growers' reports of limited efficacy (Prager, pers. communication). It is, however, difficult to assess the true impact of this level of resistance because imidacloprid is used in rotations that can involve near weekly applications of a series of different insecticides (Guenthner et al., 2012). Additionally, losses arising from *B. cockerelli* in potato are mostly from 'zebra chip disease' rather than from direct insect feeding (Butler and Trumble, 2012a,b). Therefore, it is difficult to evaluate efficacy of individual insecticides in economic terms since populations may be reduced without reducing ZC rates in the field. In comparison to the rates we report here, a previous study of the same lab colony (Liu and Trumble, 2007) resulted in an LC_{50} value of 20.318 mg a.i.$^{-1}$, nearly identical to what we found in this study despite over 5 years elapsed time (approx. 50 generations). Liu and Trumble (2007) also reported LC_{50} values of 26.189 mg a.i.$^{-1}$ for a California population of psyllids. These results suggest that psyllids in California are more susceptible to imidacloprid than Tex12, and that the baseline LC_{50} is close to 20 mg a.i.$^{-1}$. The resistance ratios we detected can be contrasted with findings for Asian citrus psyllid in Florida where LC_{50} resistance ratios for imidacloprid ranged from 7.5 to over 35 (Tiwari et al., 2011a). Other reported instances of imidacloprid resistance include green peach aphids (*Myzus persicae* (Sulz) (Choi et al., 2001) and tobacco whiteflies (*Bemisia tabaci*) (Nauen et al., 1998). Resistance has been linked to increased levels of cytochrome P450-monooxygenase activity in brown plant hoppers (*Nilaparvata lugens*) (Zewen et al., 2003). Finally, it is worth noting that imidacloprid has long been used for control of Colorado potato beetle (*Leptinotarsa decemlineata*) (Say), for which baseline susceptibility and potential resistance to imidacloprid with LC_{50} values of up to 29 times higher in tolerant populations have been reported (Olson et al., 2004, 2000). It is therefore likely that populations of *B. cockerelli* were exposed to high levels of imidacloprid prior to its specific use for *B. cockerelli* control, and some resistance (or decreased susceptibility) may already have been developing.

4.2. Behavioral responses

Multiple studies have demonstrated the influence of imidacloprid on psyllid behavior (Boina et al., 2009; Butler et al., 2011;

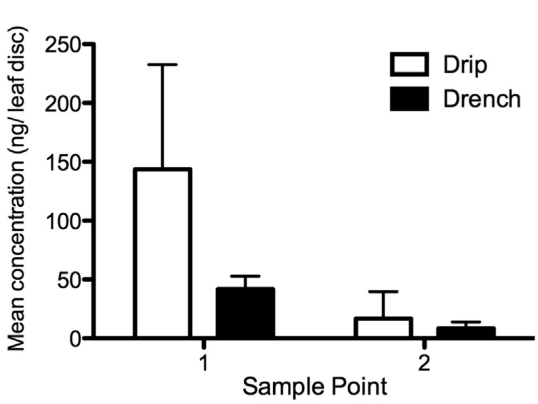

Fig. 3. Mean imidacloprid concentration in leaves (ng/leaf disc) applied via drip tape or open hole soil drench.

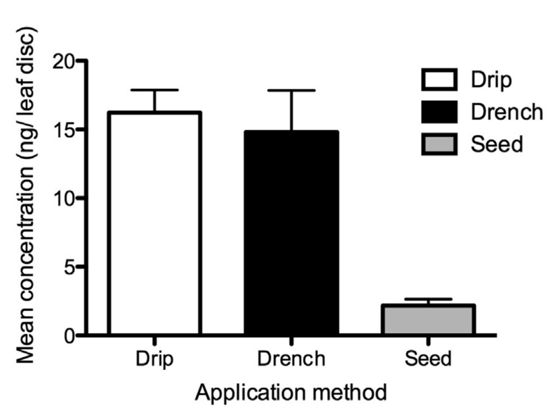

Fig. 4. Mean thiamethoxam concentration in leaves (ng/leaf disc) when applied via drip tape, open hole soil drench or as seed treatment.

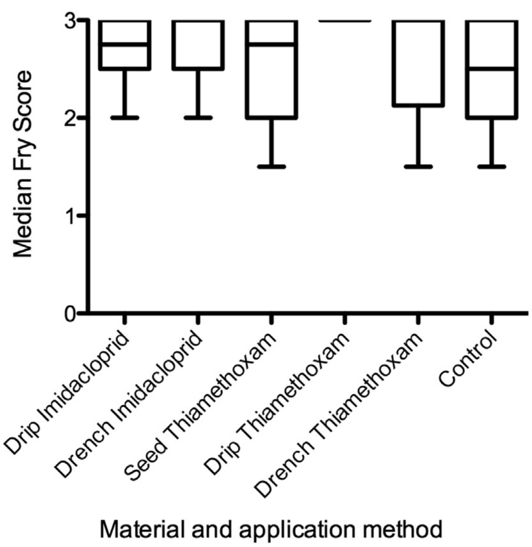

Fig. 5. Median fry score (average score of 2 chips per tuber) from tubers collected from plants in plots treated with either imidacloprid or thiamethoxam applied via different methods. Whiskers represent maximum and minimum values.

Serikawa et al., 2012). In particular, they all suggested that imidacloprid leads to a change in feeding and probing behavior, a finding that is also supported by findings from electrical penetration graph studies (Butler et al., 2012). In addition, Liu and Trumble (2007) found psyllids to be more unsettled (increased walking for instance) on plants treated with imidacloprid. Butler et al. (2011) found the walking effect was variable over time, but also detected differences in time spent off-leaflet, resting and cleaning. We found no dose effects for any behavior, suggesting that the behavioral effects of imidacloprid may mostly have been lost in both Tex06 and Tex12. However, we did find that Tex06 and Tex12 differed with respect to feeding and probing associated behaviors and also behaviors that reflect settling onto the leaf such as walking and resting. Critically, these differences result from more probing, and less 'settling' behaviors than in the lab colony. In Asian citrus psyllid, imidacloprid has been shown to elicit a concentration-dependent reduction in honeydew production, which has been interpreted as a reduction in feeding (Boina et al., 2009), but this has not been examined in the context of resistance.

As in previous studies, there was some inconsistency between duration of a behavior and frequency of a behavior. It is not clear exactly what causes these discrepancies, but they may be derived from the rarity of some behaviors and also the variation that is exhibited with respect to some behaviors. Regardless, it would appear that behavioral responses to imidacloprid may be lost over time, and that these differences may by partly associated with the reduced susceptibility in the Tex12 population.

4.3. Greenhouse studies of pesticide retention

In our systemic studies of pesticide retention, we found two critical patterns. First, it is apparent that imidacloprid can be found in potato leaves longer than thiamethoxam. Second, there is a statistically significant effect of watering level on imidacloprid levels, with higher water regimes resulting in lower residual levels in the tissue. Although we were unable to test this statistically for thiamethoxam, a similar effect of high water leading to decreased residues is visually apparent in Fig. 1b. Moreover, it is documented that thiamethoxam can leach through soil at moisture levels approximating 65 mm of rainfall (Gupta et al., 2008). Conversely, multiple studies indicate that imidacloprid does not substantially leach from soil, and remains primarily in the root zone (Leib and Jarrett, 2003). While they did not measure levels of imidacloprid directly, Olson et al. (2004) used mortality and development of Colorado potato beetle as a proxy of concentration to suggest a spike in levels of imidacloprid about 12 weeks post planting. Since this was also based on an in-furrow application at maximum rate, it is quite comparable to our results that also showed a spike in concentration at about 6 weeks, followed by a reduction in levels after about 12 weeks. Contrary to imidacloprid, thiamethoxam is known to have substantial mobility in soil (Ghidiu et al., 2012), and this would agree with the results of high water rates used in these assays.

4.4. Imidacloprid levels and resistance

Integrating results from resistance bioassays and application experiments leads to some important findings. First, we found that in susceptible colonies (Tex06) of *B. cockerelli*, a dose of 21.7 mg a.i. is required to kill 50% of individuals, and a dose of 130.2 mg a.i. is necessary to kill 90%. The latter equates to approximately 60% of the maximum rate tested, which is approximately equivalent to the maximum field rate. The residual amount of imidacloprid for those plants, tested at three weeks is approximately 2.6 mg/L of imidacloprid, 60% of which would be 1.59 mg/L. When the imidacloprid resistant population is considered, a rate of 74.8 mg a.i. is necessary to kill 50% of individuals and 839.7 mg a.i. to kill 90%. Thus, this LC_{90} value is over 4 times the maximum rate, and even at the low end of the confidence interval is above the maximum rate. The LC_{50} value equates to 39% of the maximum rate, or nearly 1.1 mg/L. These values can be compared to those from the watering and residue experiments which were conducted on potatoes planted and insecticide treated under identical conditions to those in the resistance bioassays. Such a comparison reveals that at 21 days, a low watering (250 ml) rate resulted in 3.3 mg/L of imidacloprid, versus 2.2 mg/L for the highest watering volume (1000 ml). In comparing this to those levels observed in the field at 3 weeks, we detected 114.2 PPB of imidacloprid when applied via drip tape versus only 0.16 mg/L in drench applications.

A critical consideration in the use of systemic insecticides, including the neonicotinoid compounds examined here, is that the target pest must ingest them. When attempting to control a vector, this has important implications. First, it means that in acquiring the toxin, the pest may also be transmitting the pathogen that is the true cause of damage. This is critical in *B. cockerelli* because the pathogen can be transmitted in a matter of hours (Buchman et al., 2011; Butler et al., 2012), which is a shorter period of time than that required for the insect to be killed. Second, an important implication is the behavior-modifying effect of imidacloprid, which is known to reduce feeding, and thus prevent CLP transmission. And third, it means that a near-zero tolerance approach in which mortality approximates 100% must be adopted, since surviving psyllids would be able to transmit the disease among plants. Such low tolerance would mean that desired mortality would be in the range of LC_{90} values. As demonstrated, these values are not achievable in Tex12, but they are likely still possible in California. These factors may explain our finding of zebra chip symptoms in the field despite the relatively low psyllid pressure and significant effects of some neonicotinoid treatments. Since mortality was not 100%, enough psyllids may have survived to transmit the bacteria and lead to infection in plants. Critically, this highlights the difficulty of managing a disease vector where even low numbers of individuals can lead to loss.

Overall, these findings integrate to raise some important concerns for the use of imidacloprid. First, they suggest that in almost all contexts, resistant colonies will not be exposed to high enough levels of material to result in control. This is especially the case if one considers the context of disease management where almost any survival and feeding is unacceptable. Second, these findings suggest that both method of application and post-application cultural practices (watering) will influence the efficacy of imidacloprid.

An important consideration in this work is that all field samples for ELISA were taken from the top third of plants. It has been previously demonstrated that in 'Atlantic' variety potatoes, there is uneven distribution of imidacloprid and its metabolites within foliage at different vertical positions (Olson et al., 2004). Both Oslon et al. (2004) in studies on potato and Westwood et al. (1998) in studies on sugar beet, demonstrated that younger tissues near the tops of plants have lower concentrations of imidacloprid. Importantly, while we measured the least toxic segment of plants, thereby underestimating the total amount of imidacloprid in plants, potato psyllids are known to prefer the upper third of potato plants (Butler and Trumble, 2012a,b; Martini et al., 2012). Thus, our measurements at the sites where psyllids are typically aggregating and feeding are most representative of what the insects would encounter, and be exposed to, in the field. A second important consideration is that all laboratory studies were conducted on psyllids from colonies infected with CLP, and while the effect of infection on insecticide susceptibility has not been examined in *B. cockerelli,* there is limited evidence for an increased susceptibility of Asian citrus psyllids when they are infected with the pathogen, *Candidatus* Liberibacter asiaticus (Tiwari et al., 2011b). Finally, we note that while greenhouse studies were all conducted in equivalent pots of prepared soil mix, field studies were obviously not. Since it is known that soil conditions can influence the behavior of neonicotinoid pesticides, especially with respect to uptake (Byrne et al., 2012), some care must be taken in extrapolating results to other soil types.

5. Conclusions

Collectively, these results suggest that 1) imidacloprid is unlikely to have efficacy for controlling potato psyllids in south Texas; 2) that its use needs to be carefully considered in other locations with special attention paid to potential resistance; and 3) that thiamethoxam may be a useful alternative in some scenarios. We also note the substantial effects that irrigation level, application method, and timing of application have on the utility of both thiamethoxam and imidacloprid, and that these need to be carefully considered to maximize efficacy and manage resistance.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cropro.2013.08.001.

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