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Imidacloprid in Melon Guttation Fluid: A Potential Mode of Exposure for Pest and Beneficial Organisms

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ABSTRACT ELISA techniques were used to detect imidacloprid in guttation fluid of young cantaloupe plants in Arizona. Imidacloprid was detected at up to 4.1 $\mu\text{g}/\text{ml}$ (ppm) in a coincidental guttation collection 3 d after a top label rate soil application and at 37 $\mu\text{g}/\text{ml}$ one d after a separate top label rate soil application study. These imidacloprid titers exceed reported median oral toxicities for several insect species by factors of 10 or more. Pesticides in guttation fluid are a relatively unexplored route of exposure for both pest and beneficial insects, and could represent an important risk for both of these groups in guttation-prone environments.

KEY WORDS guttation, imidacloprid, ELISA, melon

Soil-applied plant-systemic insecticides are versatile compounds that have the benefit of focusing exposure to target organisms. However, a recent report on toxic levels of neonicotinoids in the guttation fluid of maize seedlings after seed treatment demonstrates a novel exposure risk for water-seeking honey bees and non-target organisms in general (Girolami et al. 2009).

Guttation is the expulsion of xylem water through leaf hydathodes, most commonly occurring in scenarios of high root pressure and low transpiration capacity (reviewed in Ivanoff 1963). The collection of this fluid on leaf surfaces can be confused with dew condensation, but guttation originates completely from the plant vascular system.

Residues and associated risks of pesticides in guttation fluid are a proposed area of assessment for the International Commission for Plant Bee Relationships Bee Protection Group (Thompson 2010). This paper presents the first data on guttation fluid from cantaloupes that were treated with imidacloprid as a soil application, as well as a contextual overview of imidacloprid ingestion toxicities to various insects.

Materials and Methods

Opportunistic Observation. Two cantaloupe (*Cucumis melo* cultivar ‘Sol Real’) fields were planted on 23 July 2010 for a study on the impact of soil-applied imidacloprid on honey bees (*Apis mellifera* L.). Plantings were at The University of Arizona Maricopa Agricultural Center in Maricopa, AZ. Seeds were planted at 28 cm plant spacing and 101.5 cm row width. Plants were watered via furrow irrigation every 7–10 d. Fields were 0.2 Ha configured in 12 rows of 167 m. One field

was treated with a drip application of Admire Pro (551 g/L, BayerAG, Research Triangle Park, NC) at a top label rate of 767.3 ml/ha on 18 August 2010. Cantaloupe main stems had grown to approximately the sixth node stage at the time of treatment and had begun to bloom. The application was made using an in-line injector (D14MZ2, Dosatron, Clearwater, FL) set at 1:100 vol:vol and attached to drip tubing that drew up the Admire Pro solution at a rate of 2.5 liters/100 m/min. This is double the label application rate on an area-basis, but equivalent on a plant-basis because of a doubling of plant density using our halved row spacing (203 cm is standard for melons). Total water volume used was \approx 13,000 liters, which included prewetting of the ground, treatment injection, line clearing, and watering in. The second field remained untreated (1,200 m separation) but was furrow-irrigated on the same schedule and to a similar soil moisture level as the treated field.

On the morning of 22 August 2010, it was coincidentally observed that melon plants in both the treated and untreated fields had guttation fluid at the leaf margins. Because of the typically low humidity and high temperatures, guttation is rarely observed during summer months in central Arizona. However, an evening monsoon storm event (2 cm rainfall) on 21 August served to increase both soil moisture and humidity in the fields, both are necessary for guttation (Thompson 2010). Guttation samples were collected from five treated and two control plants using a micropipette and stored in centrifuge vials at -80°C until analysis. Guttation droplets had evaporated from leaves by 0800 hours.

Targeted Guttation Study. Because of the surprising findings of the imidacloprid titers in the August guttation samples, a small plot of cantaloupe was planted

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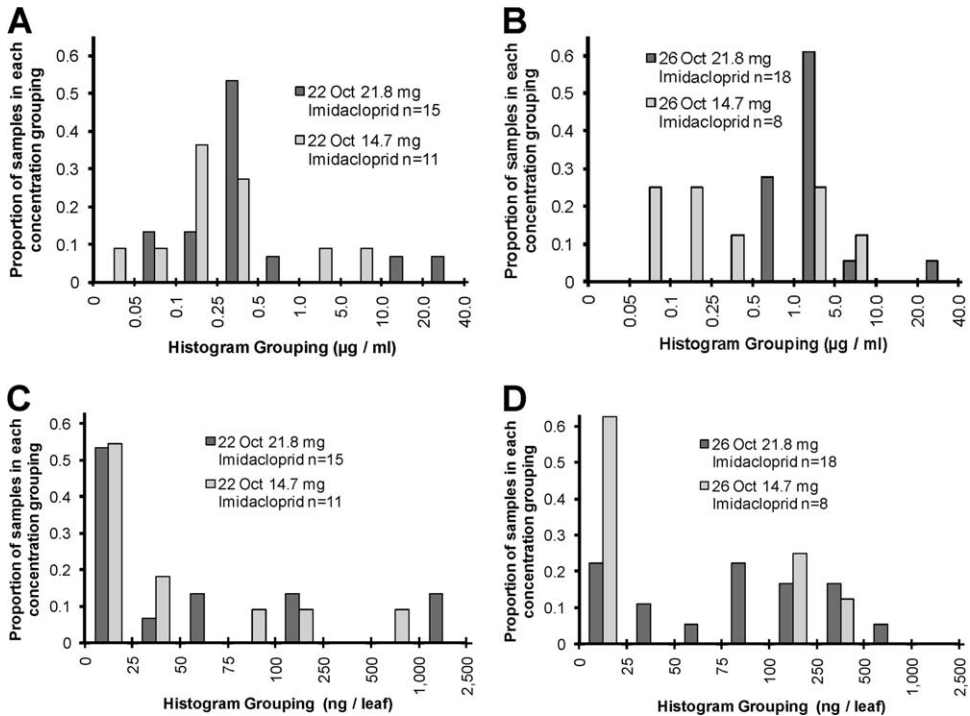


Fig. 1. Histograms of imidacloprid recoveries from melon guttation fluid (A and B) and estimated imidacloprid leaf deposition (C and D) 1 and 5 d after a 21 Oct soil treatment at 21.8 and 14.7 mg imidacloprid per plant.

on 15 September 2010 with the specific goal of monitoring plants for guttation events that might occur under cooler fall conditions. After germination, plants were watered regularly, and an additional fertilizer application (Miracle-Gro 24–8–16, Scotts, Marysville, OH) was made to encourage guttation and promote cantaloupe growth. On 21 October, individual plants were treated with drenches of imidacloprid. Plants were treated individually at either 14.7 or 21.8 mg (AI) imidacloprid in 118 ml water. These application rates correspond to commercially used label rates of 282 and 422 g/ha. Row irrigation was applied immediately after treatment to disperse the application into the root zones of plants.

Plants were observed daily at sunrise for any guttation events. Two events were noted, on 22 October and 26 October. All guttation drops from a single leaf were collected using a micropipette into a 1.5 ml centrifuge vial and volumes were estimated to the nearest 10 µl. For 22 October, 11 and 15 plants were sampled for the 14.7 and 21.8 mg doses, respectively, with five samples from untreated plants. For 26 October, 8 and 18 plants were sampled for the 14.7 and 21.8 mg doses, respectively. Plants sampled on 22 October were not resampled.

Chemical Residue Analysis. Imidacloprid levels were determined using a competitive ELISA kit (EP006, Envirologix, Portland, ME) that has a linear range of 0.2–6.0 ng/ml (ppb). Samples were initially screened at 20-fold dilutions to avoid possible matrix effects (Byrne et al. 2005), yielding a minimum quan-

tification threshold of 4 ng/ml (ppb) imidacloprid. Samples exceeding the linearity range were serially diluted by 20-fold until they fell within the linear standard range. Estimates of imidacloprid quantities deposited on leaves were made by multiplying the collected volume for each sample by the analyzed concentration.

Results

Opportunistic Observation. Imidacloprid was detected in the treated melon fields at mean \pm SEM rate of 2.155 ± 0.547 µg/ml (ppm). The five samples collected from different cantaloupe plants ranged from 1.073 to 4.115 µg/ml. There was no detection of imidacloprid from untreated melons ($n = 2$).

Targeted Guttation Study. Imidacloprid was detected above the 4 ng/ml threshold at both 1 and 5 d after treatment (Fig. 1A and B) for both application rates. The maximum concentration detected was 37.35 µg/ml. There was a single detection of 72 ng/ml out of six untreated samples, but this is likely because of experimental error (see Discussion). Collected guttation volumes ranged from 10 to 380 µl per leaf and estimated imidacloprid deposition ranged from 3 to 2,267 ng per leaf (Fig. 1C and D).

Discussion

The ELISA technique used here is a useful tool for assessment of imidacloprid presence in guttation fluid.

Table 1. Examples of oral imidacloprid toxicities to selected pest and beneficial insects

Insect	Method	Response	Rate	Citation
<i>Acyrtosiphon pisum</i>	ad libitum Spiked Artificial Diet	LC ₅₀	48 h: 140 ppb 72 h: 30 ppb	Sadeghi et al. 2009
<i>Myzus persicae</i>	ad libitum Spiked Artificial Diet	LC ₅₀	48 h: 73 ppb	Nauen and Elbert 1997
<i>Bombus terrestris</i>	Oral dose	LD ₅₀	24 h: 40 ng/bee 48 h: 20 ng/bee	Marletto et al. 2003
<i>Bombus impatiens</i>	ad libitum Spiked pollen ad libitum Spiked pollen	Reduced foraging rate Reduced worker lifespan, worker oviposition inhibition	30 ng/g pollen 19.2 µg/g pollen	Morandin et al. 2005 Gradish et al. 2010
<i>Apis mellifera</i>	Oral dose Oral dose ad libitum Spiked syrup at feeder	LD ₅₀ Medium term memory impact Foraging impacts	3.7–40.9 ng/bee 12 ng/bee 46 ppb	Schmuck et al. 2001 Decourtye et al. 2004 Ramirez-Romero et al. 2005
<i>Orius laevigatus</i>	ad libitum Spiked water	Fifth instar LC ₅₀ Adult LC ₅₀	50 ppb 1.1 ppm 2.1 ppm	Yang et al. 2008 Delbeke et al. 1997
<i>Podisus maculiventris</i>	ad libitum Spiked water	Fifth instar LC ₅₀ Adult LC ₅₀	4.15 ppm 0.46 ppm	De Cock et al. 1996

The intraplant variability in imidacloprid titers was over 100-fold, but this type of variability has been observed in xylem fluid after soil applied applications to citrus (Castle et al. 2005) and across leaf ages in sugar beets (Westwood et al. 1998).

The competitive ELISA technique used by the commercial imidacloprid test kits has been validated in both water and plant-derived matrices. Because of nonspecific binding of proteins in the original sample, dilutions are necessary to eliminate false positives and inflated imidacloprid titers. Necessary dilutions vary by matrix, but 20-fold dilutions have been found sufficient to avoid interferences in grapevine xylem sap (Byrne et al. 2005a). Byrne et al. (2005b) compared the standard curve of imidacloprid-spiked water with those of 20-fold and 50-fold dilutions of spiked leaf homogenates and found no significant difference. Watanabe et al. (2007) found that 20-fold dilutions were sufficient to eliminate matrix effects for apple and grape juices and a 50-fold dilution was recommended for orange juice. The ELISA technique is extremely robust when compared with HPLC reference methods, with ≥99% correlations between the two in spiked sample pairings across various juice and agricultural homogenates (Watanabe et al. 2004a,b, 2007) and over 94% in more complex leaf homogenates (Xu et al. 2006, Fischer et al. 2009). The xylem-derived cantaloupe guttation fluid has very little protein, and was not found to present any matrix-based deviations when multiple dilutions were applied to the same sample. Similarly, we had no detections of imidacloprid in the 20-fold dilutions of control samples, where matrix-induced titer inflation would be most likely. Our single control detection was not because of matrix effects; the sample was replicate-tested at 20- and 50-fold dilutions and both gave the same adjusted concentration. The imidacloprid detection may be the result of treatment drift from an adjacent treated plant after watering, misapplication, or mishandling of the sample.

In addition to imidacloprid, the ELISA test kit used here is mildly cross-reactive to the insecticidal olefin

and hydroxy forms of imidacloprid as well as the relatively inactive urea, guanidine (desnitro) and N-nitroso metabolites (Lagalante and Greenbacker 2007). Plant metabolism of imidacloprid is variable, but the insecticidal components represent a significant proportion of the metabolite profile (Laurent and Rathahao 2003, Sur and Stork 2003). The concentrations reported here may be an overestimate of the imidacloprid parent compound, but may actually be an informative test as it reports the contributions of multiple toxic imidacloprid metabolites. Such a total report, in conjunction with time-series behavioral studies can give a perspective on the contribution of active and inactive metabolites (Byrne et al. 2005).

The imidacloprid titers found in melon guttation (maximum of 37 µg/ml) are in line with those recently reported in corn seedling guttation. In field and greenhouse studies with corn germinated from imidacloprid-coated seeds, imidacloprid titers in seedling guttation ranged from 17 to 346 µg/ml at application rates of 0.5–1.25 mg/seed (Gaucho, Bayer CropScience, Research Triangle Park, NC) (Girolami et al. 2010, Tapparo et al. 2011).

Imidacloprid has both ingestion and contact activity, and guttation drops could serve as a water source for both pest and nontarget organisms. Most published bioassays report on direct topical application results or mixed exposure modes (leaf dip or field residual assays) that are not easily compared with these guttation data. However, there are some pest and nontarget examples of high-resolution ingestion data derived from methods that assess per-insect dosing or responses to known pesticide concentrations (Table 1). Oral LC₅₀ rates for the pea aphid *Acyrtosiphon pisum* (Harris) and the green peach aphid *Myzus persicae* (Sulzer) were <200 ppb (ng/ml) imidacloprid, and the bumblebee *Bombus terrestris* (L.) had a 24 h oral LD₅₀ rate of 40 ng/bee (Nauen and Elbert 1997, Marletto et al. 2003, Sadeghi et al. 2009). Similar per-insect LC₅₀ rates were seen in the honey bee *Apis mellifera* (L.) (Schmuck et al. 2001). For context, the *A. mellifera* honey stomach (also used for water-carrying)

has a volume of around 50 μl ; 10 μl of 10 ppm ($\mu\text{g}/\text{ml}$) imidacloprid represents a 100 ng load of active ingredient. Sublethal individual and hive-level effects have been assessed using several methods. Medium-term memory was impacted at doses of 12 ng/bee (Decourtye et al. 2004). Colony-level foraging impacts from exposure to treated sugar syrup were not noted at five ppb (Faucon et al. 2005) but have been seen at higher rates (Ramirez-Romero et al. 2005, Yang et al. 2008) (Table 1). Guttation fluid is generally low in sugar content, and thus not highly attractive for foraging honey bees. However, water collecting in honey bees is intensive in arid regions and it is unclear to what degree ephemeral and rare sources such as guttation would be used over more permanent water supplies.

The reviewed data of imidacloprid oral toxicities suggest that imidacloprid concentrations below 20 ppm ($\mu\text{g}/\text{ml}$) are toxic to a broad range of insects. For the most sensitive species, the guttation concentrations recorded here exceed median lethal concentrations by a factor of 10 or more.

From an environmental fate perspective, guttation repartitions soil and systemic pesticide residues to external surfaces of leaves. For melons and many other plants, this is primarily at the margins of leaves and may provide an additional measure of protection as the guttation drops evaporate and leave residue deposits on the leaf surface. Margin-feeding insects could encounter significantly higher concentrations of pesticide in plants that have experienced guttation relative to the baseline "systemic" titer, and surface contact becomes a viable mode of exposure. Conversely, guttation drops can solubilize surface deposits and repartition material into the leaf vascular system (Curtis 1943, 1944).

Our results affirm recent reports (Girolami et al. 2009, Thompson 2010, Tapparo et al. 2011) that viewed guttation fluid containing imidacloprid as a risk factor to honey bees and other sensitive beneficial insects. The occurrence of guttation in arid environments such as Arizona is probably rare, but the fact that guttation fluid was observed and collected unexpectedly in August, and again in October as part of a planned study, suggests a need for future attention. Crops routinely treated with imidacloprid are grown under environmental conditions that are more conducive to guttation. The potential and realized impacts that imidacloprid-contaminated guttation fluids have on pest and beneficial insects as well as other fauna in a crop remain to be determined.

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