Compatibility of Two Systemic Neonicotinoids, Imidacloprid and Thiamethoxam, With Various Natural Enemies of Agricultural Pests

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ABSTRACT Two systemic neonicotinoids, imidacloprid and thiamethoxam, are widely used for residual control of several insect pests in cotton (*Gossypium* spp.), vegetables, and citrus (*Citrus* spp.). We evaluated their impact on six species of beneficial arthropods, including four parasitoid species—*Aphytis melinus* Debach, *Gonatocerus ashmeadi* Girault, *Eretmocerus eremicus* Rose & Zolnerowich, and *Encarsia formosa* Gahan—and two generalist predators—*Geocoris punctipes* (Say) and *Orius insidiosus* (Say)—in the laboratory by using a systemic uptake bioassay. Exposure to systemically treated leaves of both neonicotinoids had negative effects on adult survival in all four parasitoids, with higher potency against *A. melinus* as indicated by a low LC_{50}. Mortality was also high for *G. ashmeadi*, *E. eremicus*, and *E. formosa* after exposure to both compounds but only after 48 h posttreatment. The two predators *G. punctipes* and *O. insidiosus* were variably susceptible to imidacloprid and thiamethoxam after 96-h exposure. However, toxicity to these predators may be related to their feeding on foliage and not just contact with surface residues. Our laboratory results contradict suggestions of little impact of these systemic neonicotinoids on parasitoids or predators but field studies will be needed to better quantify the levels of such impacts under natural conditions.

KEY WORDS systemic insecticides, beneficial insects, egg parasitoids, predators, laboratory bioassay

The degree of compatibility among various control measures used against agricultural pests is an important consideration in the development of sustainable management programs. For example, a fairly broad selection of insecticides representing different chemical classes, including organophosphates, carbamates, neonicotinoids, and insect growth regulators (IGRs) have proven quite effective in suppressing populations of glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Akey et al. 2001; Bethke et al. 2001; Prabhaker et al. 2006, 2007). Regional control programs that have targeted *H. vitripennis* in citrus (*Citrus* spp.) have proven highly successful in reducing *H. vitripennis* densities in Temecula and Kern counties (Toscano and Gispert 2005). It has been shown that unusually high population densities in citrus can have a direct impact on fruit quality with resultant yield reductions (Hix 2003, 2004). These results confirm that citrus plays an important role in producing large populations of *H. vitripennis* that spill over to other crops and ornamentals and spread a strain of *Xyllella fastidiosa* that causes Pierce's disease in grapes (*Vitis* spp.) (Hewitt et al. 1946). It is therefore essential to address the issue of *H. vitripennis* management in citrus by adopting approaches that will ensure sustainable control.

In California, an integrated pest management (IPM) program of key pests of citrus based on biological control has been successful for many years (Luck 1981, Luck et al. 1986, Grafton-Cardwell and Gu 2003, Morse and Luck 2003, Morse et al. 2007). For example, control of California red scale, *Aonidiella aurantii* (Maskell); yellow scale, *Aonidiella citrina* (Coquillett); purple scale, *Lepidosaphes beckii* (Newman); and cottony cushion scale, *Icerya purchasi* Maskell, is dependent largely on parasitoids and predators such as *Aphytis* spp. and vedalia beetle, *Rodolia cardinalis* (Mulsant), but it is now threatened by the introduction of new pests such as *H. vitripennis* that require renewed use of insecticides to regain control (Grafton-Cardwell and Gu 2003, Morse and Luck 2003, Morse et al. 2007). In particular, the recent registration of newer insecticides for use on citrus is creating uncertainty over the longer term impact they may have on established IPM programs (Grafton-Cardwell and Gu 2003). It is therefore essential to attain greater understanding of the various control options for *H. vitripennis* in citrus and how they can be best integrated with existing, successful IPM programs.

The effectiveness of insecticides against insect pests has been well studied, both empirically and theoretically. More recently, newer and more selective in-

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secticides have been developed that target pest populations while conserving their natural enemies (e.g., Naranjo et al. 2004), but these have not yet been extensively tested for nontarget effects. These compounds have highly specific modes of action to particular taxonomic groups within the Insecta. Greater selectivity has also been achieved by applying certain systemic neonicotinoid insecticides through the soil or irrigation water for uptake and distribution throughout the plant. Systemic exposure to foraging insects is generally thought to impact herbivorous insects feeding on treated plants, but less so to parasitoids and predators searching for prey or hosts (Naranjo and Gibson 1996, Coll and Guershon 2002, Lundgren 2009). Our previous laboratory studies have shown that several foliar insecticides, including IGRs were variably toxic to four adult parasitoids that attack citrus and cotton (*Gossypium* spp.) pests (Prabhaker et al. 2007). Also, there is some concern that predatory insects are exposed to toxic levels of imidacloprid by feeding upon intoxicated herbivorous insects on imidacloprid-treated plants (Grafton-Cardwell and Gu 2003). The degree of compatibility between certain systemic insecticide treatments and particular natural enemy complexes will only be determined by research that investigates such interactions on a case-by-case basis.

Research that focuses on nontarget effects of the new generation of safer and more effective insecticides in combination with increased understanding of predator and parasitoid activities will be important in the control of *H. vitripennis.* The neonicotinoid class of pesticides has a very important role to play in the control of *H. vitripennis* because these materials are relatively safe to humans and the environment and are being substituted for broad-spectrum insecticides such as organophosphates and carbamates. However, the benefit of these putatively selective insecticides for citrus IPM will hinge on their relative toxicity to both pests and natural enemies. Imidacloprid was the first compound from the neonicotinoid class and has systemic activity and both foliar and soil formulations have been developed for use in many agricultural crops. Thiamethoxam is a second generation neonicotinoid with systemic activity and provides good control of many agricultural pests, including aphids (Aphididae), whiteflies (Aleyrodidae), leafhoppers (Cicadellidae), thrips (Thripidae), rice hoppers (Dephacidae), and Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] (Maienfisch et al. 2001). Although both imidacloprid and thiamethoxam have been assumed to be safe for many natural enemies, our preliminary observations under laboratory conditions have shown a limited but detrimental impact of these materials on *Gonatocerus ashmeadi* Girault, suggesting a need for further investigation.

Several species of *Gonatocerus* attack *H. vitripennis* eggs. These parasitoids attack *H. vitripennis* eggs on citrus in large numbers during summer, with peak emergence in July (Prabhaker et al. 2007). Overall parasitism during this period is known to be consistently high (Triapitsyn et al. 1998, Hoddle 2004). *A. melinus* is critical to biological-control of California red scale and biologically-based IPM on citrus (Flint et al. 1991, Haney et al. 1992, Luck et al. 1997, Morse and Luck 2003). *A. melinus* effectively parasitizes several species of armored scales and is widely used for augmentative field releases. Previous studies evaluated the toxicity of *A. melinus* to several insecticides used for control of citrus pests, and some chemicals were found to be compatible with this beneficial insect (Phillips et al. 1983, Bellows et al. 1985, Morse and Bellows 1986, Bellows and Morse 1993). However, these previous studies evaluated the effect of older conventional chemistries applied as foliar sprays but not systemic insecticides introduced recently. Using selective insecticides, parasitoids such as *G. ashmeadi* and *A. melinus* can be protected and conserved as naturally occurring sources of biological control in grape (*Vitis* spp.) vineyards and citrus orchards.

The primary aim of this study was to evaluate the compatibility of two systemic pesticides with two important parasitoids, *G. ashmeadi* and *A. melinus*, in the citrus system. We also used this opportunity to assess the effects of imidacloprid and thiamethoxam, on four additional species of beneficial insects, two parasitoids, *Eretmocerus eremicus* Rose & Zolnerowich and *Encarsia formosa* Gahan that attack the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), as well as two generalist predators, *Geocoris punctipes* (Say) and *Orius insidiosus* (Say), that prey upon a wide range of pests, including whiteflies, in many agricultural systems. Insecticides in general, especially imidacloprid, are important in the management of whiteflies on various agricultural crops, hence the focus on natural enemies of whiteflies. In addition, there is little information on the nontarget effects of these two systemic materials against predators. Both *G. punctipes* and *O. insidiosus* are known to feed on plant tissue (Naranjo and Gibson 1996, Stoner 1970). We hypothesized that exposure of both predators to systemically treated foliage would result in some level of toxicity.

Materials and Methods

Insects. The main source of *Gonatocerus* spp. for toxicological tests was from field collections as described by Prabhaker et al. (2007). Insects were collected by two methods. Collections of leaves from citrus (orange, *Citrus sinensis* L. 'Valencia' and lemon, *Citrus limon* Burm. f. 'Lupe') and willow (*Salix goodingii* Ball.) trees infested with egg masses of *Homalodisca* spp. located in the vicinity of fields 5 and 7, respectively, at Agricultural Operations at the University of California, Riverside, CA, were made from July to September 2004. Egg-infested leaves were transferred to the laboratory in plastic bags and held to allow emergence of the pest and/or parasitoid. Egg masses on both citrus and willow leaves collected in this region are a mixture of two species of sharpshooter eggs, *H. vitripennis* and *Homalodisca lacerta* (Fowler), and they were not identified for this study because eggs of both species are parasitized by *Gonatocerus* spp. (Al-Wahaibi 2004). The sections of leaves con-

taining the sharpshooter egg masses were excised and placed on agar beds (1.5%) in petri dishes (60 mm) to allow emergence of parasitoids. Freshly emerged insects in petri dishes were subsequently transferred to screened cages with citrus plants and maintained for 3–4 d before running toxicity tests. Honey drops were placed on the sides of the cage and on citrus leaves to provide a food source. To minimize control mortality, only flying or actively feeding parasitoids were selected for each bioassay. Several field collections also were made during summer in citrus and willow trees in Riverside to obtain additional *Gonatocerus* spp. by using sweep net and bucket sampling devices (Castle et al. 2005). The majority of the *Gonatocerus* spp. that were collected by both methods was *G. ashmeadi*, with <1% of a second species, *Gonatocerus novifasciatus* Girault present on any individual date (Prabhaker et al. 2007). Toxicological tests on *Gonotocerus* spp. were conducted on the same day of collection. Citrus and willow in this area in Riverside had no exposure to insecticides over the past 15 yr (except for herbicides used on weeds around the citrus).

Insectary-reared *A. melinus* were provided by a commercial insectary, Foothill Agricultural Research, Inc., Corona, CA. Insects were shipped as 2–3-d-old emerged adults in containers with honey. Insects were used in toxicological tests on the day of delivery. Details on the background and maintenance of this species at the insectary are reported in Prabhaker et al. (2007). In an effort to maintain genetic variability, the commercial insectary collects *A. melinus* from the citrus groves around their location each fall and rears them for at least three generations before mixing with the previous year's colony. Citrus in this area of southern California receives relatively little pesticide use in contrast to citrus in the San Joaquin Valley (Morse et al. 2007). Therefore, the tested *A. melinus* represents a relatively "insecticide-susceptible" strain of this parasitoid.

Both species of whitefly parasitoids, *E. eremicus* and *E. formosa*, were supplied as pupae protected in their host whitefly pupae by Syngenta Bioline Inc., Oxnard, CA (see Prabhaker et al. 2007). These parasitized whitefly nymphs were obtained loose in a bottle mixed with bran flakes. Insects were in culture at the source insectary for ≈5 yr before use in this study. No details of previous exposure of these parasitoids to insecticides were available. Insects that emerged within 2–3 d after shipment were used in the tests. Because these insects were reared in an insectary, results obtained for both whitefly parasitoids and *A. melinus* might not be representative of the responses as those of field populations of these parasitoids that may have been exposed to insecticides for a substantial period.

The two predators, *G. punctipes* and *O. insidiosus* were obtained from the USDA–ARS center in Maricopa, AZ. The colonies of both predators originated from cotton and alfalfa, *Medicago sativa* L., fields at Maricopa, AZ, in 1997. Predator larvae were reared on a green bean, *Phaseolis vulgaris* L., diet and pink bollworm, *Pectinophora gossypiella* (Saunders), eggs and held in climate-controlled chambers at 27°C with a photoperiod of 14:10 (L:D) h (Naranjo 2007). Cultures of both predators were augmented with field collections (mainly from alfalfa) annually. Adults of both species were used in toxicity tests. Neither predator was exposed to insecticides while in laboratory culture.

Insecticides. Two systemic neonicotinoid insecticides of formulated grade were provided by the respective manufacturers: 1) imidacloprid (Admire 2F, 0.24 kg [AI]/liter, Bayer Ag, Kansas City, MO and 2) thiamethoxam (Platinum 2 SC, 0.24 kg [AI]/liter, Syngenta [formerly Novartis], Greensboro, NC). Stock and serial dilutions for the formulated compounds were made with water on the day of tests for use in systemic bioassays. At least five concentrations of each insecticide were used to obtain mortality that ranged from 5 to 95%.

Bioassay for Systemic Insecticides. A simple uptake bioassay was developed that used detached Valencia orange terminals with two leaves to take up each systemic compound through the stem (Prabhaker et al. 2006). The leaves at the end of terminals were young, bright green (new flush, but fully expanded) to which test insects were exposed to evaluate the systemic efficacy of imidacloprid and thiamethoxam. Appropriate concentrations of each insecticide were prepared on the day of plant exposure, and 9.5-ml aliquots of each dilution was placed in a 11.4-cm (4.5-in.) floral aquapik (Floral Supply.com, SYND57-97). Excised stems were placed in serial dilutions of imidacloprid and thiamethoxam in aquapiks for 24 h. Treated leaves were then transferred to a duplicate set of aquapiks containing water only just before bioassay insects were introduced. The control leaves were placed in water alone. Adults of each species of natural enemies were exposed to the center of the abaxial side of treated leaves in small, circular clip cages (3.8 cm in diameter, 11.34-cm^2 surface area, 1.3 cm in height off the leaf) that were screened on the upper surface to allow air flow. Typically leaves were 8.71 cm in length and 3.90 cm in maximum width (mean surface area of 20 leaves was 30.75 ± 1.7 cm^2), and the maximum length of a leaf stem was 3.34 ± 0.16 cm in length (mean surface area of 20 stems was 3.10 ± 0.16 cm^2). For exposure, 10 parasitoids and five predators of each species were enclosed per clip cage on the treated leaves with the exception of *A. melinus* (20 per clip cage). A minimum of five concentrations plus untreated controls were included in each test. The number of replicate clip cages at each concentration varied from six to 10 per test, depending on the species availability on the day of the test for each insecticide. Each test was repeated at least five times on different days. For both parasitoids and predators, honey was smeared on the clip cage lid as a food source, i.e., it was not in contact with the leaf. Mortality was checked after 24 and 48 h for parasitoids and after 96 h for predators. All tests were conducted and maintained at 27 ± 1°C, 22–24% RH, and a photoperiod of 12:12 (L:D) h. Relative humidity inside the clip cage was 26–28%.

Table 1. Toxicity of imidacloprid to six species of adult natural enemies by using a systemic uptake bioassay

Insect species	Exposure time (h)	*n*	Slope ± SE	LC_{50} (mg [AI]/ml) (95% FL)[a]	χ^2 (df)	g
Parasitoids						
A. melinus	24	5,408	1.2 ± 0.04	0.246 (0.089–0.465)a	11.68 (4)	0.17
G. ashmeadi	48	2,038	1.2 ± 0.06	2.63 (1.56–4.16)c	8.69 (5)	0.13
E. eremicus	48	3,703	1.7 ± 0.05	1.93 (1.33–2.67)bc	6.11 (4)	0.05
E. formosa	48	1,477	1.5 ± 0.13	0.980 (0.467–1.53)b	12.93 (4)	0.19
Predators						
G. punctipes	96	799	1.4 ± 0.07	5.18 (2.33–10.02)c	4.96 (4)	0.08
O. insidiosus	96	1,065	1.9 ± 0.12	2.78 (1.42–4.26)bc	10.47 (4)	0.11

[a] LC_{50} values across the six tested beneficial species followed by the same letter are not significantly different based on overlap of 95% FL.

Statistical Analysis. The LC_{50} values expressed as micrograms (active ingredient per milliliter, 95% fiducial limits [FL]), slopes of the regression lines, and the g factor were estimated by probit analysis by using POLO (Russell et al. 1977, LeOra 1987). The POLO probit analysis model generates a g factor to indicate the level of fit for analyzed data. With almost all good sets of data, g will be substantially smaller than 1.0 and seldom >0.4 (Russell et al. 1977). Any data with a g factor >0.5 was discarded. Differences in LC_{50} values at 24- and 48-h exposures among different species to a particular insecticide were considered significant if there was no overlap of 95% FL.

Results

Systemic Insecticide Toxicity Across the Four Parasitoid Species. A significant species and insecticide interaction was detected in systemic toxicity tests with *A. melinus*, *G. ashmeadi*, *E. eremicus*, and *E. formosa* ($F = 2.48$; df = 24, 672; $P = 0.0001$). Contrary to expectations, both imidacloprid and thiamethoxam were toxic to all four parasitoid species.

This was especially true for *A. melinus* for which exposure to imidacloprid through systemically treated foliage resulted in an LC_{50} of 0.246 μg (AI)/ml (Table 1). Among the four parasitoids tested, *G. ashmeadi* was the least sensitive to imidacloprid (2.26 μg [AI]/ml followed by a similar response by *E. eremicus* (1.93 μg [AI]/ml) (Table 1). *E. formosa* was two-fold more susceptible than *G. ashmeadi* or *E. eremicus* to imidacloprid.

Exposure to residues of thiamethoxam taken up into the leaf systemically caused 2× greater mortality of *A. melinus* compared with imidacloprid 24 h after treatment (Table 2). Thiamethoxam also caused higher mortality 48 h after treatment to *E. formosa* (0.397 μg [AI]/ml) than imidacloprid, but there was no significant difference (Table 2). By 48 h posttreatment, both parasitoid species, *G. ashmeadi* and *E. eremicus*, responded similarly to thiamethoxam (LC_{50} values of 1.35 and 1.01 μg [AI]/ml, respectively) (Table 2). Compared with *A. melinus*, both *G. ashmeadi* and *E. eremicus* were 13- and 10-fold less sensitive to thiamethoxam, respectively (Table 2). Imidacloprid, however, was less toxic to *G. ashmeadi* and *E. eremicus* adults compared with thiamethoxam based on lower LC_{50} values for the latter, although the difference was not significant based on overlapping FL (Tables 1 and 2). For *E. formosa*, toxicity of thiamethoxam was threefold greater than that of imidacloprid, indicating that this insecticide was more toxic among the two insecticides when applied systemically, but the difference was not significant. Results also showed that thiamethoxam residues were 4× more toxic to *A. melinus* than *E. formosa* adults (Table 2). All treated adult parasitoids died within 4 d posttreatment.

Systemic Insecticides Toxicity to *G. punctipes* and *O. insidiosus*. Systemic applications of both imidacloprid and thiamethoxam showed direct toxicity to the two predators. Both compounds were numerically more toxic to *O. insidiosus* than *G. punctipes;* however, there were no significant differences based on overlap of FLs (Tables 1 and 2). Survival of both predators confined on treated leaves was lower compared with those on untreated leaves after 96 h posttreatment. Most of the test insects were alive for at least 7 d on untreated control leaves suggesting that toxicity was related to insecticide exposure. Survival of the adult predators was higher on the systemically treated

Table 2. Toxicity of thiamethoxam to six species of adult natural enemies using a systemic uptake bioassay

Insect species	Exposure time (h)	*n*	Slope ± SE	LC_{50} (mg [AI]/ml) (95% FL)[a]	χ^2 (df)	g
Parasitoids						
A. melinus	24	5,435	1.2 ± 0.03	0.105 (0.054–0.181)a	7.37 (4)	0.06
G. ashmeadi	48	2,032	1.0 ± 0.03	1.44 (0.737–2.41)cd	13.26 (6)	0.07
E. eremicus	48	1,759	1.4 ± 0.07	1.01 (0.578–1.51)bc	10.69 (4)	0.07
E. formosa	48	1,690	1.0 ± 0.03	0.397 (0.221–0.664)b	4.30 (4)	0.02
Predators						
G. punctipes	96	722	1.8 ± 0.09	2.17 (1.57–3.79)d	5.36 (4)	0.12
O. insidiosus	96	909	1.6 ± 0.14	1.67 (0.752–2.65)cd	7.32 (4)	0.16

[a] LC_{50}'s followed by the same letter are not significantly different based on overlap of 95% fiducial limits (FL).

leaves compared with that of the four adult parasitoids based on LC_{50} values recorded at 96 h (predators) and 48 h (parasitoids) (Tables 1 and 2). No significant differences were observed in toxicity to imidacloprid between *O. insidiosus* and the parasitoids *G. ashmeadi and E. eremicus;* however, the values are based on mortality counts recorded at different exposure times. Observations also showed that after 5 d, 98% of predators on treated leaves of higher doses were dead compared with the insects on lower doses or control leaves. Lower mortality was observed on the leaves treated with lowest dose. After 10 d, mortality of adults on the lower doses was 100%.

In all experiments with three of the species of parasitoids—*G. ashmeadi*, *E. eremicus*, and *E. Formosa*—and the two predators *G. punctipes* and *O. insidiosus*, control mortality was always <10%. However, with *A. melinus* data sets, control mortality was higher than 10% in a few cases. These bioassays were excluded from the analysis and the tests redone. The g factor for all data sets was ≤0.40 (Table 1). The size of the parasitoids varied, with *A. melinus* (range, 0.5–0.8 mm; mean length ± SD, 0.6 ± 0.10 mm; $n = 20$) and *E. formosa* (range, 0.6–1.0 mm; mean length ± SD, 0.7 ± 0.13 mm; $n = 40$) being the smallest parasitoids tested, followed by *E. eremicus* (range, 0.8–1.1 mm; mean length ± SD, 0.95 ± 0.12 mm; $n = 40$) and then the relatively larger *G. ashmeadi* (range, 1.1–1.8 mm; mean length ± SD, 1.5 ± 0.22 mm; $n = 40$) (Prabhaker et al. 2007). Size differences also were measured for both *G. punctipes*, which was larger (range, 4.2–5.1 mm; mean length ± SD, 4.8 ± 0.21 mm; $n = 10$) than *O. insidiosus* (range, 2.0–2.8 mm; mean length ± SD, 2.5 ± 0.15 mm; $n = 10$).

Discussion

Systemically applied neonicotinoids such as imidacloprid and thiamethoxam are generally assumed to be safe to natural enemies unless they 1) feed on plant tissue or excretions or 2) are exposed to the pesticide via food chain toxicity. Obviously, predators can be exposed to systemic pesticides when they feed on prey that has fed on systemically treated plants. In this study, however, bioassayed predators were provided honey as a food source and this honey was not in contact with the leaf. Adult parasitoids could come in contact with systemic pesticides in a similar fashion if they host-fed on hosts that had taken up the systemic pesticide but again, this opportunity was excluded in our bioassays. We know of no reports that suggest any of the four adult parasitoids we tested will feed on leaves and the clip-on cages used in our bioassays did not seem to contain any plant surface exudates such as might be found associated with flowers or extrafloral nectaries. How then were these parasitoids acquiring a toxic dose of these systemic pesticides?

We hypothesize that the systemic pesticides are being taken up into bioassay leaves and are then "leaking" out of leaves as liquids or volatiles that perhaps condense on the leaf surface or even coagulate near the site of release. Hydathodes are water pores situated at leaf margins and at vein endings in the leaf and are the structures through which water is released from leaves (Thompson 2010). The mechanism of water release from plants is known as guttation and is most conspicuously associated with leaf margins where small droplets of water are sometimes visible under conducive conditions. For example, guttation is most prevalent when soil moisture is high and transpiration low and is often observed on cool mornings followed by warm days that promote higher soil temperatures and more active water absorption (Thompson 2010). The visible presence of guttation droplets often is more apparent on certain plants with waxy leaves and on most cereals and grasses. However, on other plants such as tobacco (*Nicotiana* spp.), potato (*Solanum* spp.), lettuce (*Lactuca sativa* L.), and beans, much of the leaf surface exudes the liquid, which may result in its spread over the entire surface, at times even pooling at the base or within convolutions of the leaf (Thompson 2010).

Recently it has been demonstrated that neonicotinoid insecticides applied as a seed coat were being translocated into the seedling plants and occurring in guttation droplets at cotyledon and leaf margins, thereby representing a potential source of intoxication for foraging honey bees, *Apis mellifera* L. (Girolami et al. 2009). In our study, the mode of exposure would probably not be guttation droplets on leaf margins because clip cages containing test insects were always attached well onto the leaf blades away from the margins. We are intrigued by the possibility that exudation of water containing imidacloprid that has been taken up by excised leaves is being released through hydathodes spread across the leaf surface and creating a residue containing imidacloprid. One other study examined the impact of systemically applied imidacloprid on *H. vitripennis* eggs inserted beneath the epidermis of citrus leaves (Byrne and Toscano 2007). The degree to which *G. ashmeadi* was killed by exposure to pesticide residues in the leaf as they emerged out of the sharpshooter egg was evaluated. However, the result we are describing is clearly a leaf surface phenomenon and not a subsurface occurrence where it is expected that a systemic compound such as imidacloprid will be present because it is translocated acropetaly in the xylem. Obviously, the mechanism of exposure needs to be examined in greater detail and include field-treated trees to determine whether this phenomenon also occurs in citrus orchards. Although there has traditionally been little expectation that imidacloprid residues may be present on leaf surfaces, the potential risk of exposure to translocated neonicotinoid insecticides contained in guttation water that was described for honey bees (Girolami et al. 2009, Thompson 2010) also may extend to other beneficial insects that are resident upon plants.

Laboratory bioassays based on systemic delivery of the compounds also demonstrated that imidacloprid and thiamethoxam were toxic to *G. punctipes* and *O. insidiosus.* In the field, predators forage on the plant surface and also may feed on plants that harbor the prey. Plant feeding behavior of certain predators can

expose them to systemic insecticides (Ridgway et al. 1967, Morrison et al. 1979, Hough-Goldstein and Whalen 1993). *G. punctipes* is a generalist predator that is best known for feeding upon numerous kinds of insect and mite pests of ornamental and agricultural crops, and both nymphs and adults are effective predators in cotton (Orphanides et al. 1971, Naranjo and Gibson 1996, Naranjo and Hagler 1998). However, food choices of *G. punctipes* also include feeding on cotton and other plant species regardless of prey availability (Naranjo and Gibson 1996, Tillman and Mullinix 2004). In the field, possible routes of insecticide exposure to the two predators include ingestion of either insecticide on contaminated plant tissue, through residual contact by moving on treated leaves, or by feeding on contaminated prey. Results in the laboratory indicate exposure of systemic insecticides to the predators either through contact of insecticide residues on the surface of treated-leaves or through leaf-feeding within the clip cage. Observations (data not shown) showed that there was damage in the form of punctures to treated leaves by *G. punctipes*, suggesting that *G. punctipes* was feeding on the foliage. Feeding damage by the much smaller *O. insidiosus* was not obvious in this laboratory study, although they are well known to be facultatively phytophagous predators (Armer et al. 1998, Lundgren et al. 2009). The higher toxicity of imidacloprid and thiamethoxam to *O. insidiosus* than *G. punctipes* may be related to the possibility that feeding of contaminated plant tissue did occur but without leaving any trace, and if so that *O. insidiosus* was more susceptible to each compound, perhaps due to its smaller size. Whether the responses of these two predators on treated plants in the field would be similar to the laboratory findings needs to be studied further.

The current study suggests that compounds assumed to be unavailable to nonfoliage feeding natural enemies such as parasitoids can cause high rates of mortality. This is especially true for *A. melinus* in which mortality on systemically treated leaves with either imidacloprid or thiamethoxam was extremely high. *G. ashmeadii* also was negatively affected when caged on the plant surface, as were the two whitefly parasitoids *E. eremicus* and *E. formosa.* These results are unexpected because systemic insecticides, including neonicotinoids, are conventionally thought to be nonlethal to beneficial insects. It is also assumed that systemic insecticides applied in soil are less harmful to chewing predators and parasitoids than foliar sprays because direct contact is less likely (Ruberson et al. 1998, Torres et al. 2002). The deleterious effects of topical applications of foliar insecticides are expected and are well known from numerous reports, including a recent study on the same four species of parasitoids when exposed to foliar insecticides (Prabhaker et al. 2007). Little data are available relative to the effects of systemic neonicotinoids on beneficial insects through exposure on the leaf surface. Further research is needed to pin down the specifics of this mode of action.

In contrast to our study, data published on the direct nontarget effects of imidacloprid and thiamethoxam on other natural enemies showed that these insecticides are reasonably safe (Franz et al. 1980, Hassan et al. 1987, Jansen 2000, Gautam and Tesfaye 2002). Thiamethoxam was moderately toxic to parasitized whitefly pupae (Ogata 1999) and cotton aphid mummies (Torres et al. 2003), but imidacloprid did not have any effect on emergence of *Anagrus takeyanus* Gordh, an egg parasitoid of the azalea lace bug, *Stephanitis pyrioides* (Scott) (Baldson et al. 1993). Imidacloprid and thiamethoxam are effective in suppressing pests such as glassy-winged sharpshooter, California red scale, and whiteflies (Palumbo et al. 2001; Stone-Smith et al. 2005; Grafton-Cardwell et al. 2006, 2008), but our findings of a negative impact of these compounds on *G. ashmeadi* under laboratory conditions that include confinement to a treated leaf may not be representative of nonsurvival in the field.

Specific studies on the nontarget effects of systemic applications of imidacloprid on predators vary throughout the literature. For example, certain studies reported low toxicity to some predatory beetles and bugs, spiders, and lacewing *Chrysoperla carnea* (Stephens) (Hough-Goldstein and Whalen 1993, Kunkel et al. 1999, Elzen 2001, Gautam and Tesfaye 2002, Varghese and Beevi 2004). Other studies have shown imidacloprid to be highly toxic to other predator species (Mizell and Sconyers 1992, Stark et al. 1995, Delbeke et al. 1997, James and Coyle 2001, Huerta et al. 2003). Another study showed that imidacloprid and thiamethoxam, either through direct or residual contact, affected the survival and the predatory behavior of *Podisus nigrispinus* (Dallas) (Torres et al. 2002). The predator *Orius tristicolor* (White) was susceptible when confined on imidacloprid-treated corn seedlings (Sclar et al. 1998). These studies have shown that predator mortality can result from contact with systemic insecticides, from the consumption of insecticide-contaminated leaf tissue, or both.

In general, the use of selective insecticides that allow the survival of beneficial insects is important for the success of IPM programs. Selective insecticides integrated with biological control can minimize adverse effects to natural enemies (Johnson and Tabashnik 1999). It is therefore important to incorporate insecticides that are selective, with least disruption to natural enemy–prey relationships. Our laboratory results have established the risks of imidacloprid and thiamethoxam to some natural enemy species. However, the determination of risk, which includes evaluation of multiple routes of exposure from these compounds, will require further laboratory and field testing and as such, our conclusions need to be interpreted with caution until tests are conducted under field conditions under more realistic conditions. The two systemic pesticides may have varying nontarget effects on the parasitoid and predator populations under field conditions that may be related to other factors and not just insecticides.

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