

Effect of imidacloprid foliar treatment and banana leaf age on *Pentalonia nigronervosa* (Hemiptera, Aphididae) survival

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Abstract *Pentalonia nigronervosa*, the banana aphid, is the vector of *Banana bunchy top virus* to banana. This virus is the etiological agent of banana bunchy top disease, a limiting factor in many banana growing regions, including Hawaii, United States. Laboratory bioassays on banana plants were conducted to evaluate the efficacy of imidacloprid (Provado®), to control *P. nigronervosa* by comparing insect mortality at four different insecticide concentrations (120, 60, 24, and 12 ppm) over 1 month, and compared effects on the aphids on new emerging, and old leaf material. We found significant differences between treatments and time after treatment. Aphids survived poorly on newer plant growth, regardless of presence or absence of imidacloprid, or time after treatment. Examining the insecticidal effect on old leaves only, we found that the three highest concentration treatments resulted in high mortality over the 4-week-long period of testing. Our results confirm that imidacloprid foliar application on bananas results in effective control

of aphids and does not become systemic within the plant. From these results, it is likely that management of banana aphid using imidacloprid under field conditions will be effective on old leaves and new leaves that are sprayed, but leaves emerging after sprays will not be completely protected from aphids. Regular scouting for aphids should be implemented in support of imidacloprid applications.

Keywords banana bunchy top virus; pest management; Provado; vector

INTRODUCTION

Pentalonia nigronervosa Coq. (Hemiptera, Aphididae), the banana aphid, is the vector of *Banana bunchy top virus* (BBTV) (Magee 1927; Hu et al. 1996), the causal agent of banana bunchy top disease (BBTD). This disease was first detected in Hawaii, United States in 1989 (Conant 1992), and despite eradication and control efforts, has since spread to all major islands in the state. BBTD is considered to be the most serious problem facing banana growers in Hawaii (National Agricultural Statistics Service 2005). BBTV is the only member of the newly established genus *Babuvirus* (Fauquet et al. 2005) and is transmitted by *P. nigronervosa* in a persistent manner (Hu et al. 1996). Besides being the vector of BBTV, *P. nigronervosa* causes little damage to its host plants, which besides banana include other members of the family Musaceae as well as some plants in the Araceae and Zingiberaceae (Blackman & Eastop 1984).

Until recently, diazinon was the only pesticide widely used by producers in Hawaii to control *P. nigronervosa*. Diazinon is an organophosphate, and its registration for use on banana will soon expire in Hawaii. Diazinon also has limited effectiveness, as it often does not reach aphids located within the leaf whorls (Constantanides & McHugh 2003). The importance of focusing aphid control on young leaf whorls has been challenged recently, as it was shown that the banana aphid occurs more often between

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sheaths of older leaves and the petiole, rather than on young, unfurled leaves (Robson et al. 2006).

Imidacloprid is a neonicotinoid insecticide which interrupts the binding of nicotinic acetylcholine in post-synaptic receptors (Romoser & Stoffolano 1998). In early 2004, a request for emergency exemption for the use of imidacloprid (foliar spray formulation, Provado 1.6F[®], Bayer) against *P. nigronevosa* on banana was approved for the island of Hawaii. In August 2004, the product was approved for use throughout the state and repeated renewals of this emergency registration occurred. In 2007, Provado 1.6F[®] received regular licensing status for distribution and sale in Hawaii (Hawaii Department of Agriculture 2007) has since been renewed, and a permanent label for Provado 1.6F is to be issued in 2007 and is expected to assist in restricting BBTD spread by providing effective suppression of aphid colonies (M. Kawate, University of Hawaii at Manoa pers. comm.).

Because control of the spread of viral pathogens transmitted by insect vectors is usually of greater concern to growers than control of the vector itself, insecticides are often not considered to be the primary method of control for insect-vector viral diseases. This is especially true for non-persistently transmitted viruses, as brief probes (of only seconds in duration) are sufficient for vector inoculation of the pathogen (Perring et al. 1999). However, because acquisition and inoculation times are longer for persistently transmitted viruses, insecticidal control of vectors has been a useful component of control measures for this group of viruses. For example, imidacloprid applications resulted in a reduction in the spread of *Potato leafroll virus* (PLRV) by *Myzus persicae*, also transmitted in a circulative manner (Boiteau & Singh 1999; Mowry & Ophus 2002; Mowry 2005). Imidacloprid has also been shown to assist in the control of other persistently transmitted viruses such as *Beet mild yellowing virus* (BMV) in sugar beets (Dewar et al. 1992), *Barley yellow dwarf virus* (BYDV) in small grains (Gourmet et al. 1994; Gray et al. 1996), and *Bean leaf roll virus* (BLRV), *Faba bean necrotic yellows virus* (FBNYV), and *Soybean dwarf virus* (SbDV) in faba bean and lentil (Makkouk & Kumari 2001). Imidacloprid is a versatile compound, and can be used as a seed treatment, soil application or foliar spray. When this insecticide is used as a seed treatment or applied to the soil, it is mostly metabolised (Nauen et al. 1999). When used as a foliar spray, however, most of the compound remains on the surface as unchanged imidacloprid (Nauen et al. 1998). Some imidacloprid

is taken up into the plant in a translaminar fashion following foliar application, remaining mostly at the application site (Nauen et al. 1999).

Owing to the lack of alternative control strategies to reduce the spread of BBTD in Hawaii and the availability of imidacloprid, we determined its efficacy in controlling the banana aphid under laboratory conditions. Because banana is a fast growing plant and owing to the expected lack of systemic movement of imidacloprid following foliar application, we examined the effect of banana leaf age on the persistence or presence of imidacloprid in plants following foliar application. This aspect is of special interest as new work showed that the banana aphid is found more frequently in the sheaths of old leaves, rather than on new unfurled leaves.

MATERIALS AND METHODS

Insects, plants, and experimental conditions

We established an aphid colony from a single *P. nigronevosa* individual collected from Kahuku, Oahu, Hawaii in June 2004. Aphid colonies were maintained on banana in an air-conditioned greenhouse using Williams variety banana (*Musa* sp. AAA, Cavendish Subgroup) plants that were 30–40 cm in height and planted in 13 cm diameter pots. Plants were tissue cultured following protocols of the International Network for the Improvement of Banana and Plantain (Carlier et al. 2002). We used a mixture of soil-less potting mix (Sunshine Mix, Sun Gro Horticulture Distribution, Inc., Vancouver, Canada), vermiculite and perlite at a ratio of 1:3:1, respectively, as planting medium. Slow release fertiliser (Osmocote 14:14:14 N:P:K, The Scott's Company, Marysville, OH, United States) was added to the medium at time of planting. All plants were maintained in a greenhouse at 28 ± 6°C, (14:10 L: D, c. 70% relative humidity (RH)) and watered manually every other day, avoiding contact of water with leaf surfaces.

Bioassay with *P. nigronevosa*

We treated plants with a foliar spray of imidacloprid (Provado 1.6F[®], Bayer CropScience, United States) at four different concentrations of active ingredient: 120, 60, 24, and 12 ppm. These concentrations are related to the Section 18 (US-EPA) specific exemption labelling for Provado 1.6F[®] in Hawaii, which dictates an application rate of 237 ml per 0.4 ha (8 fluid ounces of product per acre), but does not specify a dilution factor. These dilutions were

selected to correspond to 8 fluid ounces of product per 100, 200, 500, or 1000 US gallons of water (1 US gallon = 3.8 litre), respectively. We added an organosilicone surfactant, Silwet L-77 (Setre Chemical Company, Memphis, TN, United States), to all treatments at a rate of 0.03% v/v. This surfactant was used because it increases both the atomisation and the adhesion of pesticide sprays (Hall et al. 1993). We also had a control spray that consisted of distilled water and the surfactant. We applied the spray treatments using a 1420 ml multi-purpose sprayer, with all surfaces of the plants sprayed to the point of run-off. On the day of treatment, we marked the newest completely unfurled leaf on each plant with a cotton string tied around the petiole.

Every week for 4 weeks after spraying, five plants were randomly selected from each of the four treatment groups and from the control. These plants were moved to an air-conditioned greenhouse, at $25 \pm 5^\circ\text{C}$. At each week after treatment, apterae adult *P. nigronervosa* were exposed to the treated plants. Apterae were used as this is typically the stage during which colonies expand on bananas and are managed on bananas, before the virus-spreading alatae disperse. Five aphids in each of four clip cages (University of Arizona Center for Insect Science Education Outreach 2001) were placed onto each of the randomly chosen plants. The upper portions of the clip cages were manufactured from 25 mm diameter clear plastic aquarium uplift tubing cut into 2 cm lengths. Organza-type cloth was glued to the top to allow airflow. The lower portion consisted of a plastic washer with an outside diameter of 25 mm and an inner opening of 18 mm in diameter. The whole cage was glued to a metal hairclip 9 cm in length, and craft foam was used on both the top and bottom portions of the cage to provide an escape-proof seal and cushioning on the leaf. We attached two of these cages to either side of the leaf midrib on the basal end of the newest completely unfurled leaf (new growth since treatment), and two on the same location of a lower, older leaf that was present and therefore completely coated with imidacloprid spray at the time of treatment. The clip cages gave aphids feeding access to the underside of the leaf, their observed preference in the laboratory (J. Robson pers. obs.). Because the laboratory colonies of *P. nigronervosa* were also maintained in an air-conditioned greenhouse, we placed the experimental plants and confined aphids in that air-conditioned greenhouse for 3 days to allow aphids to feed and to quantify mortality. Mortality was recorded after 72 h. These bio-assays were conducted for 4 weeks after initial

application of the imidacloprid to generate data on the efficacy of imidacloprid against *P. nigronervosa* over a 1-month period. We repeated the experiment twice. Analysis of mortality data was done with a Mixed Model ANOVA (PROC MIXED) with “experiment” as a random factor with one degree of freedom (SAS Institute 2003). “Experiment” (repetition) was included in the model as a random factor to account for inter-experimental variability. Main (fixed) factors were imidacloprid treatment (concentrations and control), time after treatment, and leaf age. The new and old leaf mortality data were also analysed separately (PROC GLM, SAS Institute 2003), and pairwise comparisons among treatments were made using Tukey’s comparisons.

Persistence of imidacloprid in banana

At the same sampling times described above, we collected tissue samples from the same treated plants to analyse for imidacloprid content. We used a 19 mm diameter punch to take two leaf-disc samples from either side of the leaf midrib on the apical end of the newest, completely unfurled leaf (new growth since treatment), and from a lower, older leaf. Tissue samples were taken from the same leaves that clip cages were attached to. To avoid oxidation, all tissue samples were immediately placed into 2 ml of 75% methanol in 12-well culture plates (Corning, Inc., Corning, NY, United States). Covered plates were placed on a laboratory plate rotator for 60 min at 25°C and leaf discs were subsequently removed from the wells and the methanol extraction transferred to 1.5 ml centrifuge tubes to be held at -80°C until required for ELISA assays. Extraction procedures followed the methods of Byrne et al. (2005). Samples were analysed using the Quantiplate™ kit for Imidacloprid (Envirologix Inc., Portland, ME, United States), following instructions provided by the manufacturer. We sampled plants weekly to determine the persistence of imidacloprid in banana over a 1-month period. This procedure was used to determine relative persistence of imidacloprid on the leaves rather than to provide a direct measure of the amount of product present.

RESULTS

Bioassay with *P. nigronervosa*

GLM showed significant interactions between main factors and highly significant main effects (Table 1). The treatment \times leaf age ($F_{4, 359} = 33.32$, $P < 0.0001$) interaction is shown in Fig. 1, indicating

consistently high levels of aphid mortality on newer plant material across treatments but decreasing aphid mortality with decreasing treatment concentration on old plant material, which masks the test of main effects, and returns a non-significant main effect for leaf age (Table 1). The significant interaction between week of sampling and leaf age ($F_{3, 359} = 4.99, P < 0.01$), showed that after an initial increase in aphid mortality from week 1 to week 2, mortality rate on older plant material was lower than on young leaf material, across all treatments.

There was also a sampling time by treatment interaction ($F_{3, 359} = 4.26, P < 0.0001$). Main effects included a significant difference in aphid mortality between sampling times ($F_{3, 359} = 4.38, P < 0.01$) and between treatments ($F_{4, 359} = 39.61, P < 0.0001$). A further (reduced) analysis of mortality on new and old leaves separately showed a significant interaction between sampling time and treatment (new: $F_{12, 179} = 4.25, P < 0.001$; old: $F_{12, 179} = 2.12, P < 0.05$). On new leaf material, the difference between sampling times was marginally significant ($F_{3, 179} = 2.74; P = 0.0446$). The treatment effect was significant ($F_{4, 179} = 2.99; P = 0.0203$), but there was no clear pattern in treatment effect, other than the control and lowest concentration treatment being significantly lower at week one (Tukey comparison, $P < 0.050$) (Fig. 2A). On old leaf material, main effects included a significant difference in aphid mortality between sampling times ($F_{3, 179} = 9.40, P < 0.0001$) and between treatments ($F_{4, 179} = 93.53, P < 0.0001$) (Fig. 2B).

Persistence of imidacloprid in banana

ELISA analysis of leaf tissue for imidacloprid content showed that concentrations remained consistently higher in old growth leaves than in new growth leaves (Fig. 3). Initial imidacloprid concentration on

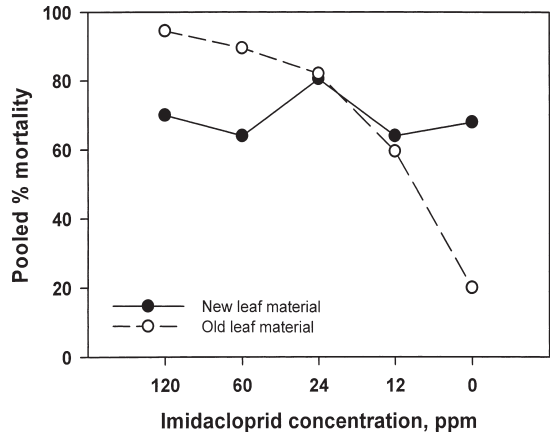


Fig. 1 Mean banana aphid (*Pentalonia nigronervosa*) mortality pooled by leaf age interaction plot for the treatment \times leaf age effect. Overall, mortality decreased as treatment concentration decreased on old leaf plant material, whereas mortality remained consistently high across treatments for new growth material.

young leaves was also in order of only c. 15% of the concentrations on old leaf material, comparable only to the control and lowest concentration treatments on matured leaves (Fig. 3).

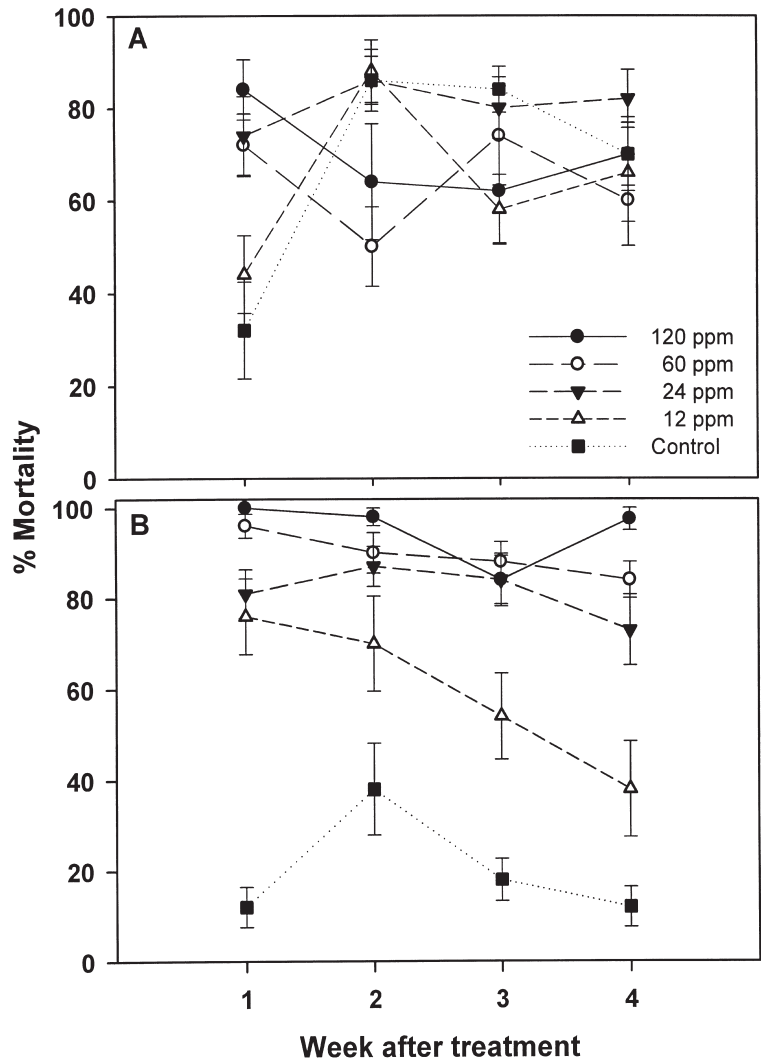
DISCUSSION

Management options to reduce *P. nigronervosa* populations on banana in Hawaii are limited. The objective of this work was to study the potential of imidacloprid as a management option for this pest, considering aspects of aphid and banana biology, their interactions with this compound, and potential impacts on efficacy of the insecticide. We initially hypothesised that foliar application of imidacloprid

Table 1 Mixed model ANOVA summary for main effects and interactions for banana aphid (*Pentalonia nigronervosa*) mortality on old and new leaves, treated with four imidacloprid concentrations and a control, over a 4-week period following treatments (experiment was included as a random factor with 1 d.f. and error d.f. = 359).

| Source of variation | d.f. | F | P |
|---|------|-------|---------|
| Week after treatment | 3 | 4.38 | 0.0048 |
| Treatment | 4 | 39.61 | <0.0001 |
| Leaf age | 1 | 0.01 | 0.9313 |
| Week \times treatment | 12 | 4.26 | <0.0001 |
| Week \times leaf age | 3 | 4.99 | 0.0021 |
| Treatment \times leaf age | 4 | 33.32 | <0.0001 |
| Week \times treatment \times leaf age | 12 | 2.14 | 0.0139 |

Fig. 2 Mean mortality of banana aphids (*Pentalonia nigronervosa*) on **A**, new leaf material and **B**, old leaf material over a 4-week period following imidacloprid application. Tukey pairwise comparisons showed that the three most concentrated treatments on old leaf material induced consistently higher mortality than the lower concentration ($P < 0.050$) and statistically similar ($P > 0.050$) mortality within those three treatments, for *P. nigronervosa* over the 4-week period of testing.



would retain efficacy for the full 4-week duration after treatment and that it would not be systemically translocated through the plant into newly produced leaf material. Our results demonstrated complex interactions among factors and interesting results regarding aphid mortality on leaves of different ages, particularly overall mortality on young leaf material essentially devoid of imidacloprid even on treated plants. We discuss conclusions drawn regarding tissue-specific persistence of imidacloprid in banana and its impact on aphid survival below.

Effect of leaf age

The interaction between treatment and leaf type shows that mortality depends not only on treatment

(i.e., concentration of imidacloprid), but also on the age of leaf growth the aphids were placed on. We had little reason to believe that imidacloprid would translocate to new plant growth, as it has a translaminar effect after foliar application. That control mortality on young leaves was not lower than on young leaf material from treated plants was particularly interesting, showing that the aphids did not survive exceptionally well on newly developed leaves. The high control mortality also suggests that imidacloprid metabolites, which may have higher insect toxicity and systemic activity than imidacloprid (Nauen 1999), did not translocate into the new leaves, and did not increase aphid mortality. Other work has shown that imidacloprid has differing

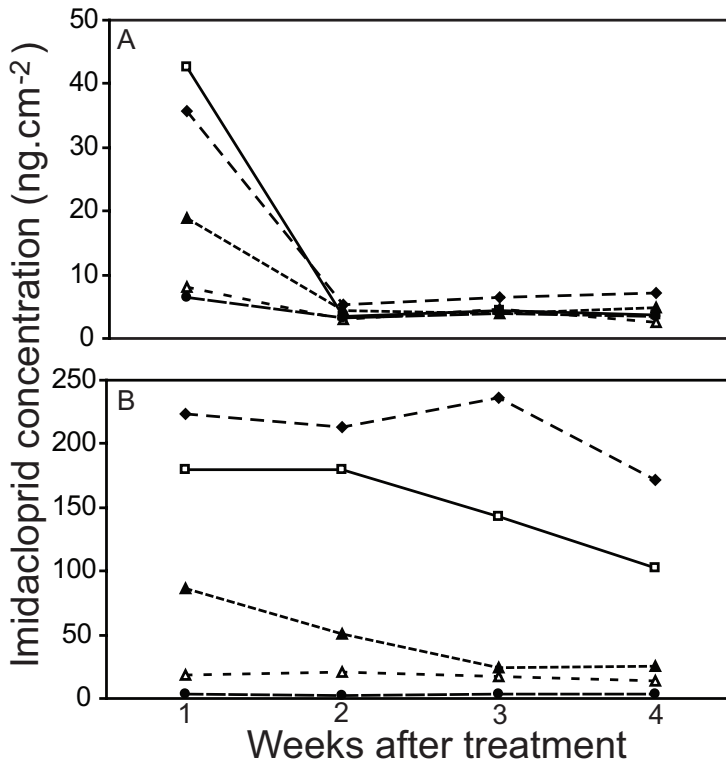


Fig. 3 Concentration of imidacloprid measured by ELISA on **A**, new leaf and **B**, old leaf growth on banana plants in the laboratory. (Filled diamonds, treatment spray of 120 ppm; open squares, 60 ppm; filled triangles, 24 ppm; open triangles, 12 ppm; filled circles, control.) Note differences in scale for A versus B.

degrees of translaminar activity depending on the type of plant tested, and also moves only locally within a treated leaf (Buchholz & Nauen 2001). The mobility of imidacloprid metabolites within banana plants would be worth investigating further. Actual levels of systemic movement of imidacloprid in hops were 1% or less after foliar application for example (Weichel & Nauen 2003). As would be expected, aphid mortality decreased as treatment concentrations decreased on old leaf material, but aphid survival is consistently low on new leaf material, regardless of the imidacloprid concentration of the treatment (see Fig. 1). Indeed, there is little difference in mortality on new growth across treatments, including the control. These results corroborate our field data (Robson et al. 2006), where we found aphids significantly more likely to be located under sheaths of older leaves than on the newest growth.

The above effect is further corroborated by the significant interaction between time after treatment and leaf type. Aphids placed on old growth leaves showed a general pattern of decreasing mortality over the 4 weeks of sampling (which correspond to decreasing imidacloprid levels), whereas aphids placed on new growth had relatively high mortality

over the entire duration of the experiment, ranging from 61% to 75% (Fig. 2). Mortality on both old and new leaves was greater in the first 2 weeks of evaluation. Over time, pooled mortality decreased on old leaf material, likely reflecting breakdown of the imidacloprid. Mortality remained higher on the new growth material, indicating that new growth material is a less optimal substrate for *P. nigronevosa* survival.

Imidacloprid concentration

Because of the consistent high mortality on new leaves, we separated new and old growth mortality data in a further analysis to exclude the effect this interaction had on interpreting the main effect, concentration of imidacloprid. Mortality of aphids on young leaves was initially lowest on the control and lowest imidacloprid application (Fig. 3A, week 1), but then increased to a point where control mortality was statistically indistinguishable from treatment mortality (Fig. 1A, weeks 2–4).

Over time, the lowest concentration treatment (12 ppm imidacloprid) inflicted less mortality than the other treatment concentrations on old leaves, following relatively high mortality in week one on

old leaf material (Fig. 2B). The three higher concentration treatments provided relatively consistent aphid mortality over the 4-week period (Fig. 2B).

Banana is a relatively fast growing plant, with each plant producing a new leaf every 7–10 days (Gowen 1995). Concern exists regarding pesticide application in this respect, particularly whether new plant material produced after pesticide application would have the same level of protection against banana aphid as those leaves that had contact with the insecticide at time of treatment. Imidacloprid level in new growth leaves was virtually the same as the control after week one—yet aphid mortality was high, confirming that new growth leaves are not suitable for aphid survival. It will be important to evaluate the efficacy of imidacloprid in reducing transmission of BBTV by *P. nigronervosa* with access to whole plants under field conditions.

Imidacloprid residues on new growth leaves declines very quickly after application compared to imidacloprid content of old growth leaves (Fig. 3). Small amounts of imidacloprid were detected using ELISA on new growth leaves 1 week after treatment (probably because these leaves received some insecticide at application), after which levels dropped to almost zero. In contrast, old growth leaves showed much higher initial levels of imidacloprid, especially with the three most concentrated treatments, and these levels dropped off comparatively slowly. This confirms that imidacloprid is not translocated in the plant from old to new leaves following foliar application. It also further supports our findings that new growth leaves support aphids poorly.

The newest plant growth on our laboratory banana plants was consistently a paler green colour than previously established growth. As with many plants, leaf greenness is correlated with nitrogen concentration in banana leaves (Gowen 1995). Nitrogen is redistributed from older leaves to younger ones in banana (van der Vorm & van Diest 1982), but the exact rate at which this occurs has not been evaluated. Aphid primary symbionts do not fix atmospheric nitrogen, leaving aphids to rely solely on dietary nitrogen (Dixon 1998). Because we placed aphids onto new plant growth immediately following its emergence, we probably evaluated aphid mortality in this location before amounts of nitrogen adequate for aphid survival had a chance to be redistributed.

This study was not a typical toxicology study seeking to quantify and compare lethal doses of a compound, but a study investigating the relative aphid mortality on banana leaves of various ages

after imidacloprid treatments, and persistence of the product over time and on young and old leaves. Our results show clearly that imidacloprid (formulated as Provado 1.6F® and with foliar application under greenhouse conditions) can cause high aphid mortality over at least 4 weeks after a single application on banana, and that it is only effective locally. We have confirmed that new leaves will not be protected by imidacloprid, and although aphid survival was poor on young leaves, survivors may still pose a virus transmission risk upon those leaves. In addition to applying imidacloprid, growers should persist with pest scouting (Robson et al. 2006) to ensure that they detect infestations of banana aphid before they burgeon into unmanageably large populations, as they are capable of doing under optimal conditions, with populations doubling about every 5 days (Robson et al. 2007).

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