The Sublethal Effects of a Systemic Insecticide on the Vine Mealybug Parasitoids *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae)

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Imidacloprid is a systemic insecticide used for the control of the vine mealybug *Planococcus ficus*. However, biological control of *P. ficus* is the primary alternate management method recommended for the integrated control of this pest. We therefore aimed to establish the detrimental effects on the development of *Anagyrus.* sp. near *pseudococci* and *Coccidoxenoides perminutus* feeding on imidacloprid-contaminated vine mealybugs as indicated by the subsequent emergence and survival of the F_1 generation. The results imply that *A*. sp. near *pseudococci* and *C. perminutus* were equally susceptible to imidacloprid, based on probit analysis. However, survival was significantly different between the control and insecticide treatment for *C. perminutus* ($\chi^2 = 23.80$; d.f. = 3; $p < 0.001$), but not for *A*. sp. near *pseudococci* ($\chi^2 = 5.07$; d.f. = 3; $p = 0.17$). As this study was laboratory based, the effect of imidacloprid on populations of parasitoids in the field should be assessed further. Treatment recommendations to minimise the impact on parasitoids are discussed briefly.

INTRODUCTION

The vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Sternorrhyncha: Pseudococcidae) is a major pest in vineyards worldwide (Ben-Dov, 1994). One of the primary methods used for its control in vineyards is augmentative releases of parasitic wasps, namely *Coccidoxenoides perminutus* (Timberlake) and *Anagyrus* sp. near *pseudococcidae* (Walton *et al*., 2012). However, the application of synthetic insecticides can hamper biological control efforts, whereby insecticides were shown to have significant acute toxic effects on *C. perminutus* and *A.* sp near *pseudococci*, as opposed to fungicides commonly applied in South African vineyards (Walton & Pringle 1999; Mgocheki & Addison, 2009). Imidacloprid is a systemic insecticide that has soil, foliar and seed uses for the control of sap-sucking pests such as aphids, thrips, whiteflies and mealybugs (Ahmed *et al*., 2001; Widiarta *et al*., 2001; Pringle, 1998). In vineyards it is applied as a soil drench against vine mealybugs at budburst to pea berry size, and then 21 to 45 days after first application if a split application is required (Anonymous, 2007). Imidacloprid belongs to the chloronicotinyl class of insecticides that act on the nervous system, causing a blockage of the post-synaptic acetyl cholinesterase receptors (Buckingham *et al*., 1997; Mukherjee & Gopal, 2000). Soil applied imidacloprid is taken up by roots and is translocated acropetally within the xylem (Sur & Stork, 2003) and provides high persistence in vines (Byrne & Toscano, 2006). The harmful effects of imidacloprid on natural enemies have been documented widely, e.g. for predatory mites in table grape vineyards (James & Price, 2004), for parasitoids and predators of *P. ficus* (Krischik *et al*., 2007; Cloyd & Dickinson, 2006), and for lacewings feeding on flowers in greenhouses (Rogers *et al*., 2007)survival of adult green lacewing, Chrysoperla carnea Stephens (Neuroptera: Chrysopidae

Unlike the non-systemic insecticides (see Mgocheki & Addison, 2009), many systemic insecticides as well as their metabolites are regarded as 'safe' for natural enemies and other beneficial insects, like bees, although mortality may occur depending on the insecticide's persistence (Ozawa *et al*., 1998; Stapel *et al*., 2000). Most bioassays have focused on acute toxicity resulting from parasitoids coming into contact with pesticide residues or sublethal effects resulting in altered host searching or foraging ability, fecundity and

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male:female ratio (Desneux *et al*., 2007). Juvenile stages of parasitoids are subjected to systemic insecticides when they develop in hosts that have fed on treated plants. Such hosts will have fed off plants with weathered pesticide that is not sufficient to cause death in the host or have developed resistance to the systemic pesticide (Stapel *et al*., 2000; Desneux *et al*., 2007).

Imidacloprid SC 350 g/L is considered a medium-risk insecticide for use in vineyards utilising integrated production of wine (IPW) principles (Anonymous, 2000), which seek to limit unnecessary pesticide applications and ensure sustainable wine grape production in South Africa. The inclusion of biological control in an integrated pest management approach against *P. ficus* is highly encouraged, so it therefore is important to identify the risks that imidacloprid presents to vine mealybug parasitoids foraging in treated vineyards. This investigation therefore aimed to establish the detrimental effects on the development of *A*. sp. near *pseudococci* and *C. perminutus* feeding on imidacloprid-contaminated vine mealybugs, as indicated by the subsequent emergence and survival of the F_1 generation. This information is critical for updating the IPW guidelines to ensure that the most efficient and sustainable integrated pest management (IPM) methods are used against *P. ficus*.

MATERIALS AND METHODS

Using Confidor 350SC, or 350 g/l pure active ingredient (Bayer Crop Science, Paarl, South Africa), a stock solution of the highest dose (12 ml imidacloprid/1 000 ml water for the pot experiment, = four times field recommended rate) was prepared and serial dilutions were made with distilled water to give double, field, ½ and ¼ recommended rates. Potted vines were established in the laboratory under ambient conditions in the dormant stage. The experiments were initiated just before bud break. Vines were pre-watered at least one hour before application of the insecticide to ensure adequate wetting. Then 166 ml of imidacloprid was applied as a soil drench around the base of each of five potted vines for all application rates. A blank treatment with no imidacloprid (water control) was included as a sixth treatment and the experiment was replicated five times. The pesticide was allowed to translocate for 48 hours and then 150 ml of clean water was applied to each vine to wash the imidacloprid into the soil. Thereafter the vines were irrigated with the same amount of water every three days until 21 days after treatment. Vines were infested with 100 1st and 2nd instar *P. ficus* (for *C. perminutus* bioassays) and 100 3rd instar to pre-ovipositing female *P. ficus* (for *A*. sp. near *pseudococci* bioassays). *Anagyrus* sp. was field collected from *P. ficus* and incubated in the laboratory at room temperature until emergence. Colonies were maintained in the laboratory on 3rd instar to adult vine mealybugs feeding on butternut squash (Islam & Copland, 1997). *Coccidoxenoides perminutus* were sourced from DuRoi IPM (Letsitele, South Africa) as mature pupae. Newly emerged individuals were allowed to feed for 24 h on a honey water solution (50:50), after which they were used in the experiments. The vines were covered in clear muslin cloth and mealybugs were allowed to feed for two days. Parasitoids (n = 20) were then released onto the vines with mealybugs for 24 hours, after which they were removed. Mealybugs were allowed to feed on the vines for a further 48 hours, after which they were kept in vials at 26 ± 0.5°C, 65 ± 5% RH and a 12:12 (L:D) photoperiod. They were inspected daily between 12:00 and 15:00 hours for any emerged parasitoids. When no more parasitoids emerged, the percentage of emerged parasitoids was calculated.

The emerged parasitoids were allowed to reproduce on mealybugs feeding from the same treated vines (but with weathered pesticide) and their offspring (F_1 generation) were examined for longevity over 21 days (*A*. sp. near *pseudococci*) and seven days (*C. perminutus*), based on adult survival rates (Ceballo & Walter, 2005; Suma *et al*. 2012). *A*. sp. near *pseudococci* females were mated, while the parthenogenetic *C. perminutus* were not.

Data analysis

The bioassay data for each parasitoid species were pooled for each treatment replicate to obtain homogeneity before Probit analysis (Polo-PC LeOra Software, 1987) after correction for control mortality using Abbott's formula (Abbott, 1925). Repeated measures ANOVA, followed by Tukey's HSD test, were performed to compare differences in emergence rate (or mortality as shown by the percentage of un-emerged parasitoids) of the two parasitoid species. Longevity of the F_1 generation females was analysed using the Kaplan-Meier (product limit) survival analysis in Statistica 12 (StatSoft Inc., 2012). .

RESULTS

Probit regression showed that fiducial limits for the two parasitoids overlapped (Table 1) and therefore mortality did not differ significantly between the two parasitoid species (Robertson *et al*., 2007). As expected, both *A*. sp. near *pseudococci* and *C. perminutus* failed to emerge at high doses of imidacloprid. The probit regression line intercepts and slopes (Table 1) for both *A*. sp. near *pseudococci* and *C. perminutus* did not differ significantly, and therefore the hypothesis of equality of regression lines was accepted ($\chi^2_{df=2}$ = 5.78; p = 0.055), as well as that of parallelism ($\chi^2_{df=1}$ = 0.19; p = 0.664). This implies that *A*. sp. near *pseudococci* and *C. perminutus* are similar in their susceptibility to

TABLE 1. Probit parameters of dose responses of *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* parasitising the vine mealybug, *Planococcus ficus,* feeding on imidacloprid SC (350 g/L) treated vines.

Parasitoid	LC_{50} (ml/L)	95% fiducial limits	LC_{90} (ml/L)	95% fiducial limits	Intercept* (± std. err.)	Slope* (± std. err.)
A. sp. near *pseudococci*	1.1198	0.57 to 1.67	11.3572	7.21 to 25.76	4.7961 (0.8578)	1.2272 (0.1449)
C. perminutus	1.7608	0.49 to 3.33	23.1282	8.95 to 891.5		

*Common intercept and slope.

imidacloprid (Fig. 1).

The cumulative proportion surviving, i.e. the cumulative proportion of the F_1 generation of *A*. sp. near *pseudococci* and *C. perminutus* surviving up to 21 and seven days respectively, is shown in Fig. 2 (*C. perminutus*) and Fig. 3 (*A*. sp. near *pseudococci*). The survival function of *A*. sp. near *pseudococci* drops off sharply in the first eight days, and thereafter declines steadily until 17 days. Survival was significantly different between the control and insecticide treatment for *C. perminutus* ($\chi^2 = 23.80$; d.f. = 3; $p < 0.001$), but not for *A*. sp. near *pseudococci* ($\chi^2 = 5.06$; d.f. = 3; $p = 0.168$). Survival could not be compared directly between the two parasitoid species because of natural differences in lifespan.

DISCUSSION

The success of a biocontrol agent relies not only on the timing and number of natural enemies released, but also on their quality. The residual impact of systemic insecticides, like imidacloprid, has been investigated on the emergence of natural enemies (e.g. Preetha *et al*., 2010). However, the longevity, fecundity and general searching ability of natural enemies, in particular parasitoids, has not been addressed adequately.

The cumulative negative impacts of neonicotinoids indicate that even the lowest concentrations have toxic effects if sustained over a long period (Tennekes & Sanchez-Bayo, 2011), which is especially relevant for species with a long lifespan or those whose larval or nymphal stage occurs in contaminated hosts. This investigation has demonstrated that, when parasitoid larvae develop in imidacloprid-contaminated

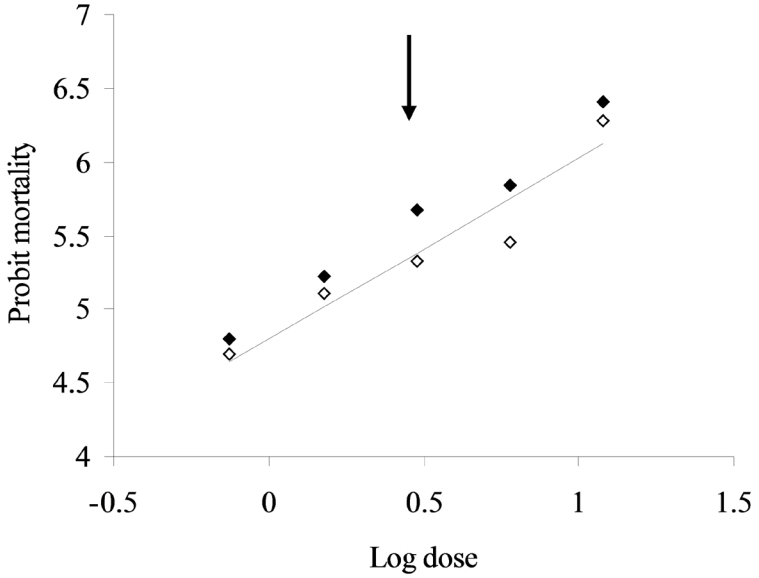

FIGURE 1

Probit mortality (inability to emerge) of *Anagyrus* sp. near *pseudococci* (♦) and *Coccidoxenoides perminutus* (◊) to various doses of imidacloprid SC (350 g/L), using *Planococcus ficus* feeding on treated vines. Arrow indicates field dose rate (3 ml/L).

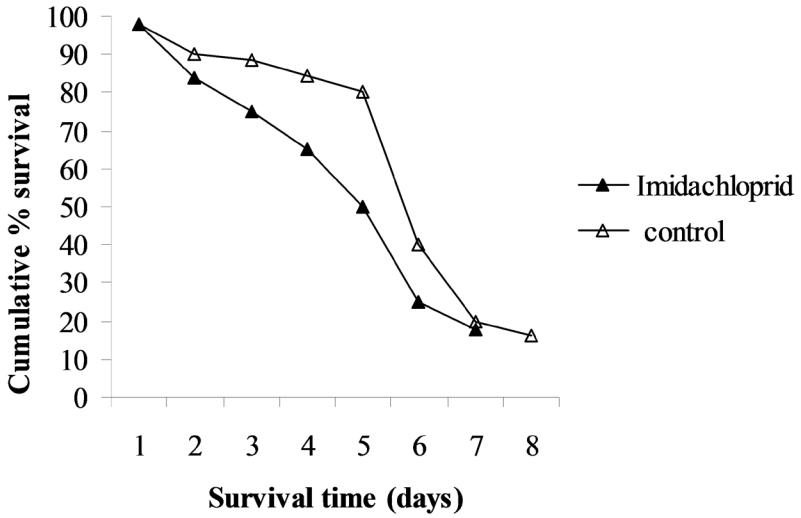

FIGURE 2

Survival function of *Coccidoxenoides permunitus* F_1 generation females that emerged from imidacloprid-contaminated *Planococcus ficus* individuals.

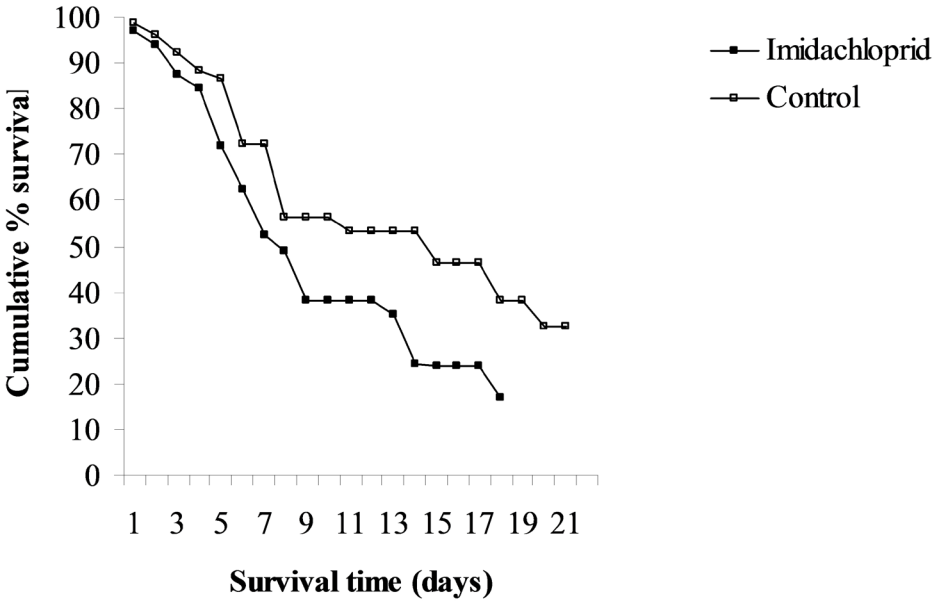

FIGURE 3
Survival function of *Anagyrus* sp. near *pseudococcus* F_1 generation that emerged from imidacloprid-contaminated *Planococcus ficus* individuals.

vine mealybugs, their development and longevity are indeed affected negatively, with failure to emerge at higher doses. At low doses (¼ and ½ and full recommended field rate), some parasitoids managed to emerge, but the longevity of the F_1 generation was significantly reduced for *C. perminutus*. Stapel *et al*. (2000) also reported reduced longevity of the parasitoid *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) after feeding on extra floral nectar from cotton treated with soil-applied imidacloprid. Imidacloprid significantly reduced the survival of *C. perminutus*, while the survival of the F_1 generation of *A*. sp. near *pseudococci* was affected less. *Coccidoxenoides perminutus* is comparably smaller than *A*. sp near *pseudococci*, rendering it more susceptible to pre-imaginal sublethal effects of imidacloprid (Sohrabi *et al*., 2012).

Soil-applied imidacloprid is persistent and can continue to kill pests for more than 30 days (Widiarta *et al*., 2001). Therefore, soil-applied imidacloprid is particularly detrimental to *C. perminutus*, as this parasitoid species should be released early in the growing season, when temperatures are cooler, to effectively discourage build-up of high mealybug populations (Walton & Pringle, 2005), while *A*. sp. near *pseudococci* is ideally adapted for hotter temperatures later in the season (Wohlfarter & Addison, 2014). Alternate products are available that are not detrimental to parasitoids, in particular *A*. sp. near *pseudococci* parasitising *P. ficus*, including insecticidal soaps (e.g. Prev-Am®) and tetramic acid insecticides (Mansour *et al*., 2011).

CONCLUSIONS

This study has indicated that *C. perminutus* and *A*. sp. near *pseudococci* are equally susceptible to imidacloprid systemic residues, as shown by the emergence rate and/or mortality of the *F1* generation. However, the progeny of imidacloprid-contaminated *A*. sp. near *pseudococci* and *C. perminutus* could still survive long enough to have an impact on mealybugs. This investigation did not establish the impact of this insecticide on important physiological activities like oviposition, searching ability and host recognition (Ruberson *et al*., 1998). It should also be noted that we did not assess whether there were any differences in mealybug acceptance of imidacloprid vines treated at different concentrations, as this was beyond the scope of this trial. Split applications could be substituted by one imidacloprid treatment at budburst to pea berry size, followed by the release of parasitoids about 45 days after treatment. Since this investigation was laboratory based, it is expected that imidacloprid could be more harmful under field conditions, as it works synergistically with other agrochemicals (e.g. Koppenhöfer *et al*., 2000; Van Dijk *et al*., 2013; Rondeau *et al*., 2014). This therefore should be investigated in a field-based trial.

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