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Variation in mortality among populations is higher for pymetrozine, than for imidacloprid and spiromesifen in *Trialeurodes vaporariorum* in greenhouses in Finland

Running title: Variation in insecticide susceptibility of *T. vaporariorum* populations in Finland

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Abstract

BACKGROUND: Insecticide resistance in *Trialeurodes vaporariorum* W. is unknown in the species' northern distribution range where it inhabits mainly commercial greenhouses. Resistance development in whiteflies feeding on year-round crops in greenhouses is possible due to use of chemical treatments to back up biocontrol. We tested the response levels to spiromesifen, pymetrozine and imidacloprid in whiteflies collected from seven greenhouses within a 35 km radius in Western Finland.

RESULTS: All except one (PR) population had LC_{50} values below the recommended concentrations for the tested compounds. However, some populations showed reduced susceptibility to pymetrozine in comparison to reference susceptible population. Resistance ratios to pymetrozine were highly variable (RR 0.5-39.7) even among closely-located greenhouses and higher than those for imidacloprid (RR 1.05-10.5) and spiromesifen (RR 0.8-11.5). LC_{50} values and application frequencies of pymetrozine correlated positively among the sampled populations.

CONCLUSION: High variation in resistance levels to pymetrozine among-populations within natural whitefly dispersal limits reflects variation in the usage of this compound among individual greenhouse crop producers. Thus, resistance management is recommended on individual greenhouse crop producer level, even in a dense production cluster.

Keywords: Finland; imidacloprid; pymetrozine; resistance; spiromesifen; *Trialeurodes vaporariorum*

1 INTRODUCTION

Insecticide resistance among insect populations differs depending on the treatment history and dispersal possibilities of resistant individuals in the area.¹ Crop production clusters with a high density of production units (i.e. fields or greenhouses), in combination with suitable surrounding habitats that promote pest dispersal year round, are likely at a higher risk of pest invasions and often experience difficulties with pest management.² These circumstances can lead to a continuous cycle of pesticide use. On the contrary, more scattered crop producers in climatic zones that are less conducive to pest dispersal between crop production units, e.g. in Northern Europe, may use pesticides rarely and only when biological control agents are not sufficient (Vänninen pers. obs.). Low usage of pesticide with the lack of gene flow among populations could mean that resistance development in pests at the northern limits of their distribution may be less likely. However, with the advance of year-round crop production even in the most remote locations, persisting populations of pests are likely to be exposed to pesticides more frequently, than in seasonal crops, increasing the possibilities for resistance development. Frequent pesticide exposure, in addition to limited dispersal among populations, could lead to variability in the susceptibility of pest populations in the northern latitudes to insecticides.

In this study we focus on the insecticide susceptibility of the greenhouse whitefly (*T. vaporariorum*), a cosmopolitan polyphagous pest feeding on various commercially important plants.³ *T. vaporariorum* has been controlled with insecticides and it is known to have developed resistance to at least 22 insecticide compounds.⁴ For example, resistance to imidacloprid,⁵⁻⁸ pymetrozine⁷ and insect growth regulators (buprofezin,⁹ spiromesifen¹⁰) have been previously reported. Although biological control is the principal method of whitefly management in the greenhouses of Finland, insecticide treatments are needed to back-up biological control of whiteflies in year-round production units during pest outbreaks (Vänninen pers. obs.). Greenhouse crop producers in western Finland observed-reduced efficacy of pymetrozine and imidacloprid after six years of use, but such a decrease was not noticed for spiromesifen. All three insecticides, however, are still used in integrated pest management (IPM) programs of whiteflies. Owing to the selectivity of pymetrozine to whiteflies and not to its biological control agents,¹¹ the greenhouse crop producers are interested in keeping it in their whitefly management programs.

The aim of this study was to measure variability of response to selected insecticides of *T. vaporariorum* populations inhabiting greenhouses with year-round tomato and cucumber crops. Three commonly used insecticides

were assayed against populations from a greenhouse cluster in Ostrobothnia, western Finland, which have varying insecticide application histories. This is the first scientific publication reporting differences in insecticide susceptibility of *T. vaporariorum* in Scandinavia.

2 MATERIALS AND METHODS

2.1 Insecticides

Three insecticides were selected for the study based on their importance for whitefly management. These products, their modes of action and commercialization history in Finland are described in Table 1. The highest concentration used in the assays correspond to manufacturer' recommended concentration (MRC) for foliar application on tomato and cucumber against whiteflies and other pests. In the bioassays all insecticides were applied as formulated products diluted to required concentrations in distilled water containing 0.1g L^{-1} of non-ionic wetter Agral® (Syngenta). Control treatments contained water and Agral® only.

2.2 Whitefly populations

To evaluate the importance of insecticide treatment history for whitefly resistance development on the individual greenhouse crop producer basis, whitefly populations (Fig. 1, Table 2) were sampled in spring before the start of active whitefly dispersal in the region. Each population was sampled from a different greenhouse crop producer and from multiple locations within a single greenhouse room containing single host plant species. Whiteflies used in spiromesifen assays were collected from five producers in 2011 and those used for pymetrozine and imidacloprid assays were collected from six producers in 2012 (Table 2, Fig. 1). All except one population (BL) had previous, varying treatment histories for all three compounds. Greenhouse crop producers were unwilling to disclose full insecticide treatment histories and only general results representing treatments of the three insecticides which were applied in the last 9 months (winter production) are presented. The frequencies of insecticide applications for PR and ML populations were not provided. The BL population had been controlled without insecticides using only biological control agents during the three preceding years. Equal number of individuals was sampled from each population: 100 adults were aspirated into separate plexi glass cages onto living host plants, and transported to research facilities (MTT, Jokionen), where stocks of whiteflies were reared for bioassays. As a susceptible reference we used a population from Rothamsted Research in Harpenden, Hertfordshire, United Kingdom (Table 2), which had been reared on bean (*Phaseolus vulgaris* L. cv. Canadian Wonder) without insecticides since 1971. Beginning

May, 2012 it was maintained on tomato (*Solanum lycopersicum* L. cv. Encore) for at least three generations before tests since this is the required minimum number of generations for adaptation of *T. vaporariorum* to a novel host.¹² All populations were reared in controlled light (16h light: 8h dark) and temperature (22-24°C) conditions at MTT Agrifood Research, Jokioinen, Finland.

2.3 Test plants

All populations were reared and tested on the same host plant species from which they were initially collected (Table 2). This was done to minimize mortality and any potential lower fitness of the populations due to exposure to novel host plant species¹³ and/or to minimize the impact of plant chemistry on susceptibility of the populations.¹⁴ Tomato (*S. lycopersicum* L. cv. Encore) and cucumber (*Cucumis sativus* L. cv. Eminentia) seedlings for tests were grown at 20-22°C, 16h light: 8h dark photoperiod.

2.4 Bioassays

The bioassays routinely used to determine insect resistance to insecticides were adjusted to make it work reliably and to be able to compare our results with other studies. Adulticides (pymetrozine and imidacloprid), insecticides designed to kill adult insects, were tested using modified^{8, 15, 16} leaf dip bioassays (Fig. 2). Bioassays were performed using 3.7 cm diameter tomato leaf discs. The discs were dipped for 1 min in pesticide concentration, air-dried for 20 min, and then laid abaxial side up on 1% agar in Petri dishes (0.5 cm deep, 3.5 cm in diameter; one disc per Petri dish; Thermo Fisher Scientific Inc.). Five insecticide concentrations were tested (Fig. 3-4) with five replicates per concentration. Twenty-thirty adult whiteflies of mixed gender and age were immobilized on ice for up to 3min in 5 ml plastic pipette tips (serving as collector units of a mouth aspirator) that were covered with sealing film (PARAFILM® M). Immobilized whiteflies from the aspirators were transferred to Petri dishes containing leaf discs treated with insecticides. The insides of perforated (~10 holes, 2mm in diameter) Petri dishes lids (1 cm deep, 3.3 cm in diameter) were fully covered with thin breathable nonwoven milk filter paper (Hygia™) to absorb excess moisture and to prevent the formation of static electricity. We observed that static electricity captured whiteflies onto bare plastic and prevented their contact with the leaf disc unless the filter paper was used. The edges of the Petri dishes were covered with a rubber seal (3.3 cm in diameter) to prevent the leaf disc from drying due to contact with the filter paper. Closed Petri dishes were sealed by wrapping parafilm around the dish's edges to prevent whiteflies from escaping. To imitate natural leaf position (abaxial side down) dishes were inverted and transferred to

a metal grid shelf (to allow ventilation) in an incubator (16h light: 8h dark photoperiod and 22-24°C temperature). Due to this inversion and to prevent the leaf disc from falling from the agar bed, the lid of the Petri dish was designed to be smaller than the bottom part of the dish, which held the leaf disc. Mortality was scored after 72h for imidacloprid and after 96h for pymetrozine after partially minimizing whitefly activity in the Petri dish by keeping them at +10°C for 10 min. Whiteflies were considered dead if we observed no movement in response to touching them with a brush. The period of insect exposure to adulticides exceeded the period of insect survival without food, since 50% of whiteflies usually die from starvation within 35h in empty Petri dishes¹⁷. Thus, we concluded that majority of alive individuals found on pymetrozine treated leaf discs had fed on the treated leaf discs and thus, showed resistance to this feeding inhibitor, and that the results were not affected by delayed mortality from starvation.

Resistance to the larvicide (spiromesifen), an insecticide designed to kill juvenile insect stages, was tested using a modified method^{18, 19} on leaves of living plants. Leaves of seedlings were dipped in either one of four insecticide concentrations (Fig. 3) (each with three replicate plants). The test leaves had a known number of 2nd instar nymphs that were allowed to develop and hatch into adults. To produce synchronized cohorts of 2nd instar nymphs, whitefly adults were allowed to lay eggs for 24 h, producing progenies of similar age. For that purpose, 15 females and 15 males, immobilized as described above, were released into each mini clip cage (1cm deep, 1cm in diameter) attached to leaves of individually planted tomato seedlings (three clip cages per seedling, one cage per leaf). Clip cages with adults were removed from plants after 24h. The seedlings containing eggs were kept in separate insect-free greenhouses with 16h light: 8h dark photoperiod and 22-24°C. After 12 days from egg-laying on cucumber and 15 days on tomato, the number of 2nd stage nymphs per leaf was counted with the help of a 20x magnifying lens. The mortality of nymphs due to spiromesifen was scored as the abundance of hatched pupal cases after four weeks from nymph counting allowing for a maximum hatching rate.²⁰

2.5 Statistical analysis

All data were corrected for mortality in control treatments using Schneider-Orelli's method²¹ and analyzed with probit regression function in PASW Statistics v. 18²² individually for each compound and population to obtain LC₅₀ values and their 95% confidence limits. Differences in LC₅₀ between insecticides were considered significant if their respective 95% confidence limits did not overlap. Resistance levels were calculated as resistance ratios (RR) by dividing LC₅₀ values of the sampled whitefly populations by the LC₅₀ of the susceptible population. Spearman rank

correlation between population treatment frequency of each insecticide and the corresponding insecticide LC₅₀ values was analyzed using PASW Statistics v. 18.²²

3 RESULTS

Manufacturers' recommended concentrations (MRC) of imidacloprid and spiromesifen (Table 1) on average killed 98% and 94% of tested individuals, respectively, whereas the recommended dose of pymetrozine on average caused 63.5% mortality of tested individuals (Fig. 3-5). Susceptibility among the tested populations was significantly lower and varied more in pymetrozine treatments (RR: 0.46-39.72) than in imidacloprid (RR: 1.05-10.46) and spiromesifen (RR: 0.84-11.47) treatments (Fig. 3-5).

Greenhouse crop producers that had used chemical control applied imidacloprid and spiromesifen 0-3 times each and used pymetrozine 0-2 times, all during the 9 month crop season preceding sampling. The correlation between frequency of applications and LC₅₀ values was not significant for imidacloprid and spiromesifen ($p > 0.05$), whereas it was significant for pymetrozine (Spearman $r = 0.949$, $n = 4$, $p = 0.051$).

Although the resistance ratios were not high in comparison to other studies^{7, 23} (Table 4-3), populations PJ4, PR and PJ 1 were significantly less susceptible to imidacloprid than the reference population, with PJ 4 and PR populations showing the lowest mortality values and highest resistance ratios (Fig. 3). Two of the populations that had been controlled with imidacloprid during the two preceding sampling years (PJ 5 and ML) were susceptible to imidacloprid at the same level as the reference population (Fig. 3). The susceptibility to imidacloprid and pymetrozine of the population that was not exposed to insecticide treatments during the three years before sampling (BL) did not significantly differ from that of the reference population for either insecticide (Fig. 3-4). The maximum resistance ratios to pymetrozine were higher than those to imidacloprid. Populations PR, PJ 5, ML, PJ 1 and PJ 4 (in increasing order) were significantly less susceptible to pymetrozine than the reference population (Fig. 4). Only the population from cucumber (TJ 1) was significantly less susceptible to spiromesifen than the reference population (Fig. 5).

4 DISCUSSION

After 4-13 years of use in Finland imidacloprid and spiromesifen were still effective in all populations (LC₅₀<MRC), whereas pymetrozine had reduced efficacy and was marginally effective in one population

($LC_{50} > MRC$) (Fig. 3-5). Furthermore, recommended concentrations of imidacloprid and spiromesifen induced up to 90% mortality in majority of studied populations (Fig. 3 and 5). The high susceptibility of Finnish whitefly populations suggests that compared to other regions resistance development in the northern Europe has been relatively slow as resistance to neonicotinoids has been recorded in other whitefly populations (in Israel and Arizona, USA) to occur after only 2-3 years of use.²⁴ However, there was variability among responses of populations to the tested compounds (Fig 3-5), which might reflect different treatment frequencies in the region.

The susceptibility of whiteflies to pymetrozine compared to imidacloprid was clearly reduced although both compounds have been used in greenhouses for a similar period of time (Table 1). Although cross resistance of pymetrozine and imidacloprid have been reported in other studies,^{7, 8, 25} only one population in our study (PR) showed low susceptibility to both compounds. However, it is not clear whether this case represents cross resistance between pymetrozine and imidacloprid or several non overlapping modes of resistance for these compounds.^{7, 7}

Over the years, the use of pymetrozine has been more prevalent in the study area due to its better compatibility with biological pest control.¹¹ Indeed there was higher variation among populations in the LC_{50} values for pymetrozine (Fig. 4) which could be explained either by limited dispersal among whiteflies or selection by insecticides. Since the BL population, which was the most susceptible to pymetrozine (Fig. 4), was located within the whiteflies' potential distribution radius of 7-20 km^{26, 27} from other less susceptible populations (Fig. 1), it could be that the spread of resistant individuals has been restricted in the sampled area. Limited dispersal might be also supported by observations of greenhouse crop producers of different *T. vaporariorum* susceptibility levels even among greenhouses managed by the same producer or in our data among the different populations in the same village (PJ 1, 4 and 5, Fig. 3-5). However, in another study (Ovcarenko et al., unpubl.) analysis of these populations using microsatellite markers indicated that PJ 1, 4 and 5 populations represent a single genetic population. Thus, the observed variation in susceptibility to pymetrozine described here might be due to behavioural plasticity of local populations that share the same genetic background and/or due to differential selection pressure on the genes responsible for resistance development.^{8, 28} Although our sample size is small, we found a positive correlation between insecticide usage history and LC_{50} values for pymetrozine suggesting that variable use of insecticides might explain the higher variation in pymetrozine susceptibility. We do not suggest that two applications are enough to cause the higher tolerance as correlative data do not provide evidence for cause –effect and is just reflecting the trend of insecticide application likelihood in the past. This is also supported by the absence of exposure to

insecticides of the most susceptible population to all three compounds for at least three years. The observed results call for a need to monitor whitefly resistance at the level of individual greenhouse crop producers, even in a dense production cluster.

Only the population from cucumber had decreased susceptibility to spiromesifen (Fig. 5) either due to initial resistance development or to the host plant effect.^{19, 14} Insects feeding on cucumber for a few generations are known to have relatively high levels of detoxification enzymes and therefore low susceptibility to insecticides.^{29, 30} However we can only speculate if this is the reason for the observed lower susceptibility of the TJ 1 population to spiromesifen, because only one population from cucumber was tested.

The high susceptibility levels to imidacloprid and spiromesifen might be explained by the loss of resistant individuals during the 3 and 5 generations (respectively) when whiteflies were kept in rearing before our bioassays (Table 1). It is known that, due to fitness costs, resistance can decrease when insects are grown for several generations without insecticides.^{8-10, 31, 32} However, the length of rearing period in the laboratory before bioassays is usually not reported in publications,^{8-10, 31} and the rate of decrease in whitefly resistance is only known for imidacloprid. A decrease in *T. vaporariorum* susceptibility to imidacloprid was observed after three generations spent in rearing without selection pressure, whereas for *B. tabaci* only after 10 generations.^{6, 33} Thus, it is unlikely that imidacloprid resistance in our populations disappeared completely during the three generations of rearing before bioassays. Furthermore, the same generation of whiteflies was tested using imidacloprid and pymetrozine and variation in susceptibility to pymetrozine among populations suggests that three generations is not enough to abolish resistance at least to this compound. Therefore, the observed variation among populations in susceptibility to the tested compounds most likely reflects the local insecticide application history rather than a reduction in susceptibility levels prior to testing.

Dispersal of the greenhouse whitefly in Finland is currently being analyzed with microsatellite markers, and preliminary results indicate relatively persistent populations in the same greenhouses over two consecutive years (Ovcarenko et al. unpubl). *T. vaporariorum* persistence highlights the need for diligent use of pest management strategies to avoid increased insecticide use, which could lead to resistance development. Microsatellite data also suggest that different greenhouse crop producers within a village (PJ1-5) have come to share a relatively homogeneous whitefly population over time. When combining this evidence for homogeneity, challenges associated

with inter-greenhouse movement by whitefly in Finland with variation in mortality from tested compounds described in this paper it appears that insecticide use patterns continue to be a leading factor contributing to the creation of variability in response to insecticides.

Table 1. Physical and chemical qualities of the compounds used in the study.

Compound	Product	Manufacturer	Chemical group	Mode of action
Imidacloprid ^a	Confidor WG-70	Bayer CropScience	neonicotinoid	inhibits acetylcholine receptors in the central nervous system, causing paralysis followed by death
Pymetrozine ^b	Plenum WG-50	Syngenta	pyridine-azomethine	neuroactive insecticide that inhibits the feeding system by preventing insects from inserting their stylus into the plant tissue leading to death from starvation
Spiromesifen ^c	Oberon SC-240	Bayer CropScience	tetronic acid derivative	inhibits lipid synthesis, interferes with development of the egg and immature stages and reduces adult female fecundity

^a Available for use in Finland since 2000.

^b Plenum WP-25₇ formulation was available for use in Finland from 2000 until 2007. Thereafter, Plenum WG-50 was available from 2007 until 2013.

^c Available for use in Finland since 2009.

Table 2. Abbreviations of *T. vaporariorum* population names, their original host plants and cultivars, date of sampling and testing, analyses conducted and number of whiteflies tested (N). Each population was sampled from different crop production company.

Population	Original host	Cultivar	Sampling / Testing dates	Compounds tested	N
REF	Bean	Canadian Wonder	5.2012 / 9.2012	Imidacloprid	1387
			5.2012 / 8.2012	Pymetrozine	964
			5.2012 / 9.2012	Spiromesifen	891
BL	Tomato	Encore	5.2012 / 9.2012	Imidacloprid	1545
			5.2012 / 8.2012	Pymetrozine	964
PJ 1	Tomato	Encore	6.2011 / 11.2011	Spiromesifen	2056
			5.2012 / 9.2012	Imidacloprid	1526
			5.2012 / 8.2012	Pymetrozine	945
PJ 4	Tomato	Dometica	6.2011 / 11.2011	Spiromesifen	1223
			5.2012 / 9.2012	Imidacloprid	2041
			5.2012 / 8.2012	Pymetrozine	914
PJ 5	Tomato	Dometica	6.2011 / 11.2011	Spiromesifen	1676
			5.2012 / 9.2012	Imidacloprid	2036
			5.2012 / 8.2012	Pymetrozine	926
TJ 1	Cucumber	Logica	6.2011 / 11.2011	Spiromesifen	1625
PR	Tomato	Careza	6.2011 / 11.2011	Spiromesifen	1126
			5.2012 / 9.2012	Imidacloprid	924
			5.2012 / 8.2012	Pymetrozine	907
ML	Tomato	DRW	5.2012 / 9.2012	Imidacloprid	1411
			5.2012 / 8.2012	Pymetrozine	901

Table 3. Variation among *T. vaporariorum* populations' susceptibility to insecticides (used in present study) reported in the literature. Susceptible population from bean, UK corresponds to our reference strain obtained from N. Karatolos in 2012.

Insecticide ^{REF}	Population	Host	Application method	LC ₅₀ (mg L ⁻¹ a.i.)	RR	Country
Imidacloprid ¹	Sampled	strawberry	foliar	298 ^a	-	California
	Sampled	strawberry	systemic	87.4 ^b	-	California
Imidacloprid ²	Susceptible	bean	foliar	8.12	1	UK
	Sampled	rose, tomato, cucumber	foliar	60.2-193	7.41-23.8	UK, Spain, Italy
Imidacloprid ³	Susceptible	cucumber	foliar	94.4		Greece
	Sampled	cucumber	foliar	138-417.5	1.5-4.4	Greece
Imidacloprid ⁴	Sampled	cucumber	foliar	211	-	China
	Sampled	cotton, pumpkin, vegetable marrow	foliar	52.5-65.3	-	China
Imidacloprid ⁵	Susceptible	bean	systemic	5.3	1	UK
	Sampled ^c	tobacco	systemic	3	0.57	UK
	Sampled	fuchsia	systemic	4.9-8.6	0.93-1.62	UK
Imidacloprid ⁶	Susceptible	bean	systemic	8.12	1.00	UK
	Sampled	bean	systemic	3.96-29.7	0.49-3.66	UK, Netherlands
Imidacloprid ⁷	Susceptible	bean	foliar	17.2	1	UK
	Sampled	tomato, vegetables, aubergine, ornamentals	foliar	44.9-374	2.61-21.8	UK, Spain, Turkey, China, Germany
Pymetrozine ⁷	Susceptible	bean	foliar	38.8	1	UK
	Sampled	tomato, vegetables, aubergine, ornamentals	foliar	160-792	4.13-20.4	UK, Spain, Turkey, China, Germany
Spiromesifen ⁸	Susceptible	bean	foliar	0.61	1	UK
	Sampled	ornamentals and vegetables	foliar	2.75 - 15.7	4.49-25.7	UK, Turkey, Germany

^a µg a.i. mL⁻¹

^b mg a.i. plant⁻¹

^c No previous imidacloprid exposure in the field

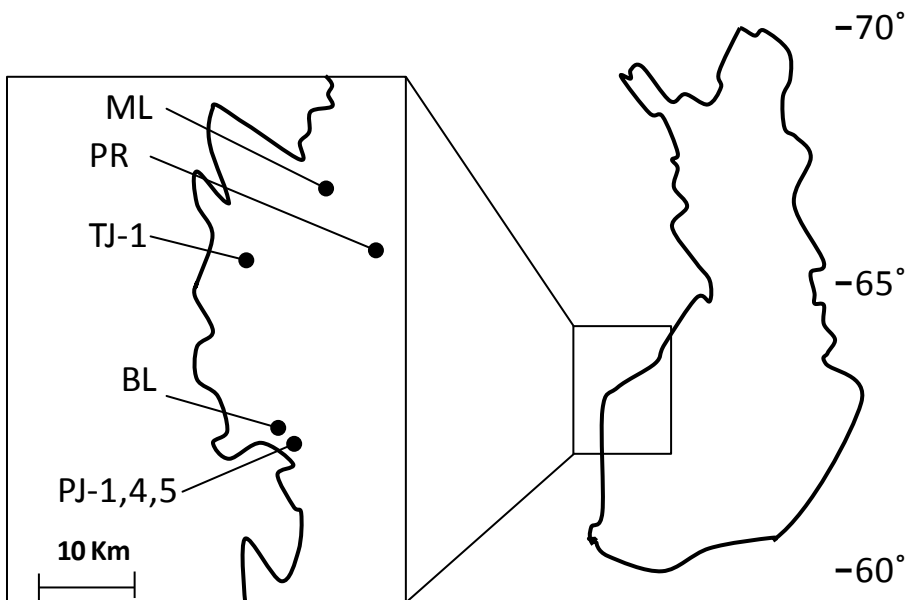


Fig. 1 Map of sampling locations in Finland. Letters refer to populations described in Table 2.

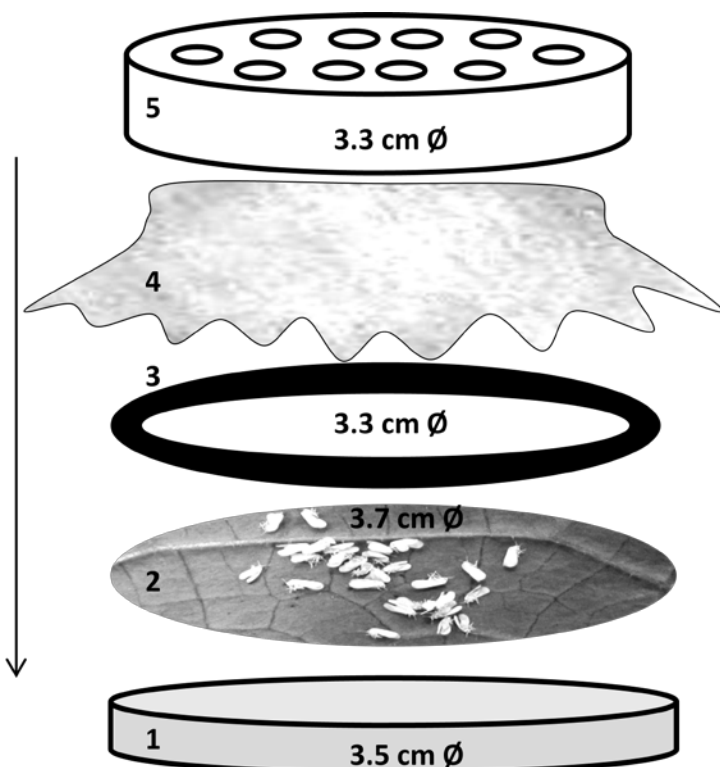


Fig. 2 Schematic of Petri dishes used for the adulticide bioassays: 1 - petri dish (0.5 cm deep, 3.5 cm in diameter) containing 1% agar, 2 - leaf disc treated with insecticides covered with 30 immobilized adult whiteflies, 3 - rubber seal (3.3 cm in diameter), 4 - thin breathable nonwoven milk filter paper (Hygia™), 5 - perforated (~10 holes, 2mm in diameter) and Petri dish lid (1 cm deep, 3.3 cm in diameter). After construction, the Petri dishes were inverted to imitate the natural leaf position (see text).

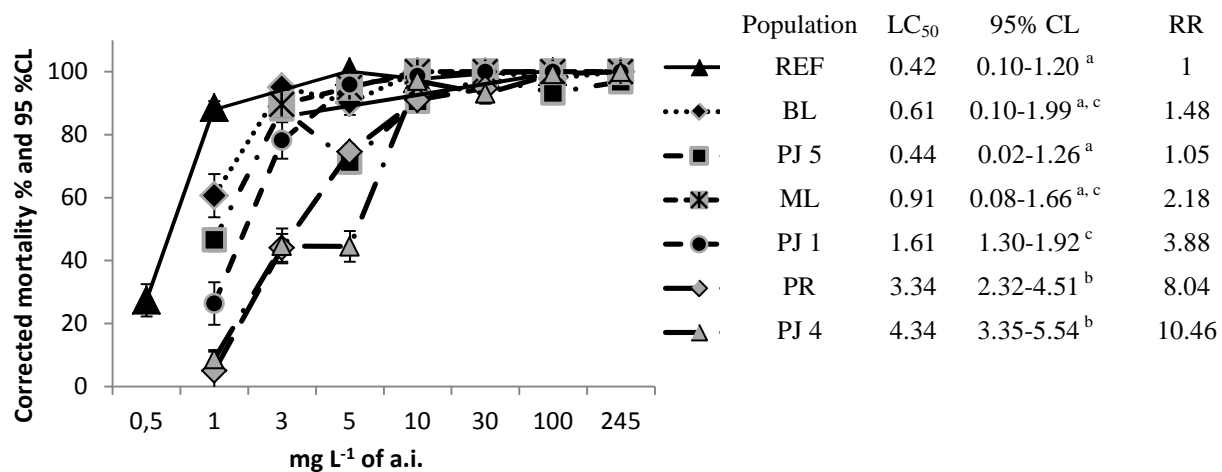


Fig. 3 Variation in Shneider-Orelli's corrected mortality of adult whitefly populations after imidacloprid treatment. Bars show 95 % confidence limits. Response of each population is described by number of whiteflies tested (N), LC₅₀ (mg L⁻¹ of a.i.) and their 95% confidence limits (CL), as well as resistance ratios (RR) relative to REF population. Shared letters indicate overlapping 95% CL and no significant difference between LC₅₀.

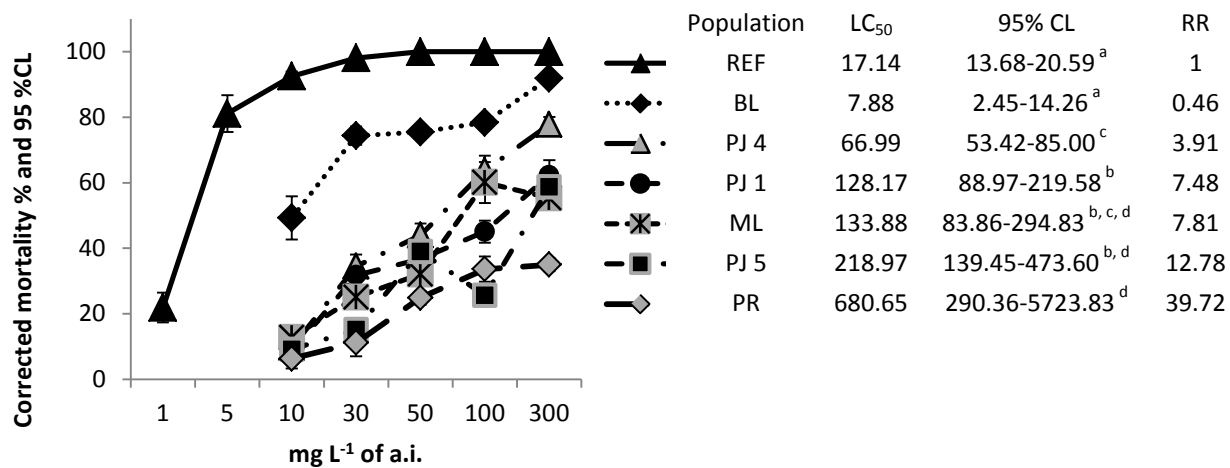


Fig. 4 Variation in Shneider-Orelli's corrected mortality of adult whitefly populations after pymetrozine treatment.

Bars show 95 % confidence limits. Response of each population is described by number of whiteflies tested (N),

LC₅₀ (mg L⁻¹ of a.i.) and their 95% confidence limits (CL), as well as resistance ratios (RR) relative to REF

population. Shared letters indicate overlapping 95% CL and no significant difference between LC₅₀.

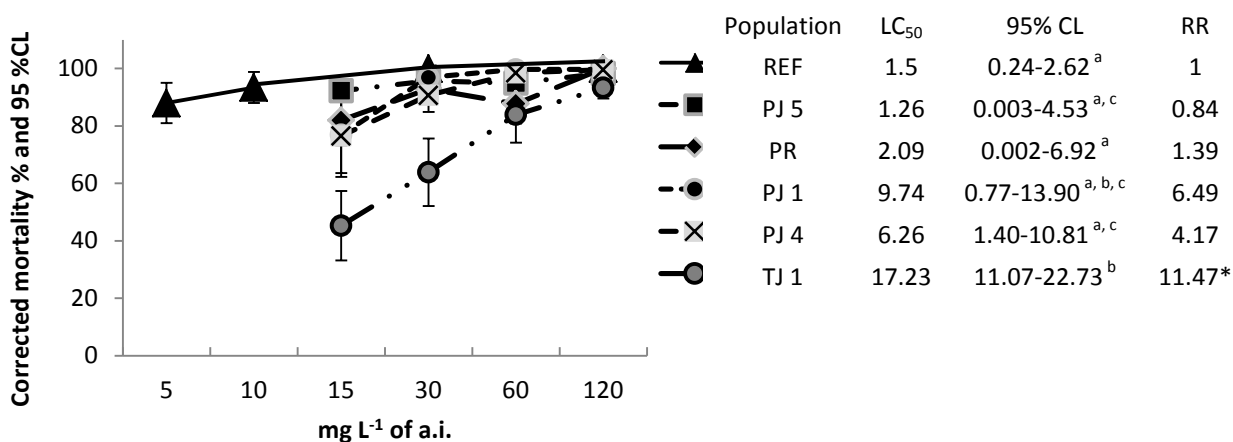


Fig. 5 Variation in Shneider-Orelli's corrected mortality of 2nd instar whitefly nymphs on spiromesifen treated leaves. Bars indicate 95 % confidence limits. Response of each population is described by number of whiteflies tested (N), LC₅₀ (mg L⁻¹ of a.i.) and their 95% confidence limits (CL), as well as resistance ratios (RR) relative to REF population. Shared letters indicate overlapping 95% CL and no significant difference between LC₅₀. * RR of TJ 1 was obtained from comparison with LC₅₀ of REF population from tomato and could be lower when compared to LC₅₀ of REF population from cucumber, which was not available for this study.

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