Effects of acetamiprid on *Lipaphis pseudobrassicae* (Hemiptera: Aphididae) resistant and susceptible to the parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae, Aphidiinae)[1]

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ABSTRACT

This study evaluated the relationship between the resistance of *Lipaphis pseudobrassicae* to the parasitoid *Diaeretiella rapae* and the effects of the insecticide acetamiprid on the aphid. Four groups of *L. pseudobrassicae* collected in Uberlândia, MG, were used. The first two were formed by individuals of the same clone (C1), which were resistant (C1R) or susceptible (C1S) to the parasitoid. The third group was formed by resistant individuals to the parasitoid descended from a clone collected in canola (C2R) and the fourth group, from a population collected in a commercial plantation of collard greens (P1). Determination of LD_{50} was done with three replications of 10 aphids, subjected to dose-response assays, with the application of five concentrations of the insecticide acetamiprid (0.01, 0.1, 1, 10 or 100 ng a.i./aphid) and a control treatment consisting of acetone alone. There was no significant difference in the LD_{50} of individuals of the same clone, C1R and C1S (0.06 ng a.i./aphid for both clones). C2R had the highest LD_{50} (0.14 ng a.i./aphid), while P1 had the lowest (0.01 ng a.i./aphid). These results suggest that resistance against the parasitoid is not, therefore, associated with the effects of insecticide on *L. pseudobrassicae*.

Keywords: aphids; biological control; chemical control; insecticide resistance.

INTRODUCTION

The aphid *Lipaphis pseudobrassicae* (Davis) (Hemiptera: Aphididae) is a cosmopolitan pest of Brassica, directly damaging crops by feeding on the leaves, causing deformation and yellowing, and indirectly damaging plants by transmitting more than ten types of plant pathogenic viruses, among them, collard green black ring and the mosaics of cauliflower, turnip and radish (Peña-Martinez, 1992; Blackman & Eastop, 2007). Because it is widely distributed, control of this aphid is extremely important throughout Brassica producing regions in such crops as canola, collard greens, broccoli, and cauliflower (Blackman & Eastop, 2000).

Biological control plays an important role in Integrated Pest Management (IPM) programs, especially considering its low cost and general lack of synthetic chemicals (Parra & Coelho, 2019). The main natural enemy of aphids in Brassica crops is the parasitoid *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae, Aphidiinae) (Starý *et al*., 2007; Akhtar *et al*., 2010; Sampaio *et al.*, 2017); however, the low parasitism rate of *D. rapae* in *L. pseudobrassicae*, both in the field and laboratory, suggests that the population of *L. pseudobrassicae* in Uberlândia-MG is formed mostly of clones resistant to this parasitoid (Oliveira *et al.*, 2013; Sampaio *et al.*, 2017; Ferreira *et al.*, 2018).

The cause of such resistance in *L. pseudobrassicae* is still under investigation; however, association with secondary symbionts is a possibility, since aphids susceptible to the parasitoid have been obtained within

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the offspring of resistant aphids (Ferreira *et al.*, 2018). The association of aphids with these symbionts can result in adaptive advantages, such as increased heat tolerance, the ability to use host plants that, without the symbionts, would not be suitable for aphid feeding, and resistance to parasitoids (Chen *et al*., 2000; Oliver *et al*., 2003; Tsuchida *et al*., 2002; Vorburger *et al*., 2013; Cayetano *et al*., 2014).

Chemical control is commonly used to control agricultural pests, but frequent spraying and the improper use of these products (failure to observe the recommended dose, spraying interval, or rotation of active ingredients) has led to the increased pesticide resistance in populations of many pest insects against a number of insecticides (Chen & Stelinski, 2017). The dose required to eliminate 50% of the population (LD_{50}) of resistant insects is greater than that required for control of susceptible populations, and LD_{50} values are used to monitor the level of resistance in a population or pest (Szendrei *et al.*, 2011; Paramasivam & Selvi, 2017). Moreover, it is not only the lethal effect of an insecticide that determines its effect on an insect's population, but also any sub-lethal effects it may cause, such as decreased fertility and longevity and changes in the development period (Stark & Rangus, 1994; Storch *et al*., 2007; Miao *et al.*, 2016).

Resistance to insecticides can be due to behavior modification of the insect, changes in cuticle composition reducing penetration, metabolic resistance, or modifications in insecticide target sites (Mbogo *et al*., 1996; Hemingway, 2000; Mathenge *et al*., 2001; French-Constant *et al*., 2004; Dang *et al.*, 2017; Balabanidou *et al.*, 2018). Recently, the fact that secondary symbionts can change enzyme production was recognized (Guidolin *et al.*, 2018), a finding that may be used to create greater susceptibility to insecticides in aphids that present such associations with secondary symbionts (Skaljac *et al*., 2018).

Studies have demonstrated that biological control of the aphid *L. pseudobrassicae* with the parasitoid *D. rapae* is currently not effective due to the aphid's resistance to the parasitoid (Oliveira *et al.*, 2013; Sampaio *et al.*, 2017; Ferreira *et al.*, 2018). A number of chemical control options have been considered, particularly using neonicotinoids such as acetamiprid, since acetamiprid is a systemic insecticide, absorbed by the plant and translocated throughout the plant in the sap in lethal amounts to sap-feeding insects such as the aphids (Tomizawa & Casida, 2003; Faria, 2009). However, it is not known if the resistance of *L. pseudobrassicae* to *D. rapae* changes the effects of the insecticide on aphids. Therefore, this study determined the LD_{50} of the insecticide acetamiprid and its sub-lethal effect on fertility of resistant or susceptible clones of *L. pseudobrassicae* to see if there is such a relationship between resistance of the aphid to the parasitoid and the lethal and sub-lethal effects of the insecticide on *L. pseudobrassicae*.

MATERIAL AND METHODS

Plant production and insect collection and rearing

Collard greens (*Brassica oleracea* var. *acephala* L.), cultivar Manteiga da Georgia, seedlings were produced in the greenhouse for this study, using commercial seeds from TopSeed®. When seedlings had one pair of true leaves, seedlings were transplanted to plastic pots (15 cm height and 13 cm diameter) containing the organic substrate Bioplant®. Plants were used for aphid rearing, and also for experiments when they had six true leaves.

Colonies of *M. persicae* and *L. pseudobrassicae* were maintained in the laboratory. Aphids were reared on 90-mm dia disks of collard green leaves, placed in 100-mm dia Petri plates containing water-agar at 1%. The plates were maintained in a acclimatized chamber (23±2 °C, 70±10 RH% and a 12:12 h L:D photoperiod), and the leaf disks were changed every four days.

Colonies of four groups of *L. pseudobrassicae* collected in Uberlândia were maintained for use in the experiments. The first two groups (C1R and C1S) were created by laboratory rearing and were comprised of individuals of the same clone collected in collard greens plants in the greenhouse (at 18°53'04.7" S and 48°15'36.6" W) at Campus Umuarama of Federal University of Uberlândia (UFU) and then either selected as resistant (C1R) to the parasitoid *D. rapae* or susceptible (C1S). A third group was formed using resistant individuals of another clone (C2R) collected in canola plants at the Água Limpa farm, of UFU. The fourth group (resistance to *D. rapae* unknown) was composed of individuals from a population (P1) collected in a commercial collard green field (19°1'54" S and 48°11'26" W).

Parasitoids (*D. rapae*) were reared from *M. persicae*, *B. brassicae*, and *L. pseudobrassicae* colonies that contained parasitized aphids (mummies), collected from commercial area of collard greens production in Uberlândia-MG in field and in the greenhouse at Campus Umuarama of UFU. Each mummy was placed in a 2-mL Eppendorf tube and held in the laboratory (at 23±2 °C, RH = 70±10% and a 12:12 h LD photoperiod) until adult parasitoids emergence. Subsequently, adults were fed with droplets of honey diluted in water (honey 50%). The parasitoids were maintained in pairs in the same type of tubes and, after mating, females were used to produce more parasitoids. Parasitoid rearing was done using 2nd instar nymphs of *M. persicae* as hosts. First instar nymphs were obtained from adults that were placed in Petri plates

containing leaf disks of collard greens for 24 hours, after which the adult aphids were removed and the first instar nymphs left in the dish. Twenty-four hours later, these nymphs were in the 2nd instar. One mated female of *D. rapae* with no previous oviposition experience was released in each Petri plate, each containing forty 2nd instar nymphs of *M. persicae* and left for two hours for oviposition. Subsequently, the female parasitoid was removed and the aphid nymphs were held in an acclimatized chamber (22 ºC and a 12:12 h L:D photoperiod) until mummies were formed, about 10 to 15 days later and the parasitoids were used to select resistant clones.

Selection of clone C1R and C1S of *L. pseudobrassicae* collected in collard green

Colonies of *L. pseudobrassicae* were collected in collard greens plants in the greenhouse (at 18°53'04.7" S and 48°15'36.6" W). Leaves containing aphids were taken to the laboratory and eight individual aphids were placed in individual Petri plates containing collard green leaf disks overlaying the water-agar (1%) to form one colony of each clone (C1, Cx1, Cx2, Cx3, Cx4, Cx5, Cx6, Cx7). Aphids reproduce by thelytokous parthenogenesis, and thus each colony arising from a single individual is formed by genetically identical individuals (Blackman & Eastop, 2000). After population growth of the colonies, adults of each clone were maintained in distinct Petri plates, containing the collard green disks over water-agar (1%), and removed after 24 hours, to form a group of only 1st instar nymphs. These *L. pseudobrassicae* nymphs reached the 4th instar in 72 hours and were used to assess the level of resistant to *D. rapae* in each of the eight clones started from aphids collected from collard greens (see method details below).

Approximately twelve adult aphids of each of the eight clones were placed in separate Petri dishes with a collard green leaf disk. Adults were removed 24 hours later, and the 1st instar nymphs held for a further 72 hours until they reached the 4th instar. Twenty-two to 35 4th instar nymphs of each clone were then held individually in Petri dishes and subjected to a single oviposition by *D. rapae*. Oviposition was observed under the stereoscopic microscope, and each aphid nymph received one oviposition in the abdomen; if parasitism occurred on the legs, head or siphunculus, the nymphs were discarded. Parasitized aphids were then maintained in individual Petri plates in an acclimatized chamber. Among the clones evaluated, two had only individuals susceptible to the parasitoid and were discarded and six were selected as resistant (all aphids reached adulthood and did not mummify). The clone C1 was read in the laboratory specifically to select both resistant and susceptible individuals from the same clone.

To obtain susceptible individuals within a resistant clone, fourth instar nymphs of the clone C1 were subjected to a single oviposition of *D. rapae* (as previously described above). Each female parasitoid was allowed to parasitize a maximum of ten nymphs until 65 aphids were parasitized. Nevertheless, it is important to mention that the mummification proves the susceptibility of the aphid, but the survival does not necessarily prove resistance, thus the parasitism of a great number of individuals is necessarily to prove the resistance of the clone. Daily observations were made for 10 days, evaluating the number of aphids reaching adulthood and remaining alive (resistant) and those that were soon after molting to adults transformed into mummies (susceptible) (Ferreira *et al.*, 2018). Fourth instar nymphs were used because even susceptible aphids that are successfully parasitized by *D. rapae* at this developmental stage can reach adulthood and reproduce before dying and becoming mummies (Ferreira *et al.* 2018).

For clone C1, only one specimen was transformed into mummy and the other 64 were considered to be resistant. Thus, the clone C1 was reared as two groups, one of individuals resistant to *D. rapae* (C1R) and the other of susceptible individuals (C1S). Twenty 2nd instar nymphs of C1S and twenty of C1R were subjected to a single oviposition of *D. rapae* to confirm their susceptibility or resistance. Daily observations were made for 15 days and the number of aphids that reached adulthood and reproduced (resistant) and those that were mummified (susceptible) was recorded. None of the twenty C1R nymphs parasitized by *D. rapae* mummified, confirming that 100% of the individuals were resistant. In contrast, 19 out of the 20 C1S parasitized nymphs were mummified, confirming the susceptibility of C1S.

Selection of clone C2R of *L. pseudobrassicae* collected in canola

Colonies of *L. pseudobrassicae* were collected in canola plants in the Capim Branco farm of UFU (18º 52' 50.63" S and 48º 20' 32,07" W). Leaves containing aphids were taken to the laboratory and two individual aphids (clones C2 and C3) were placed in individual Petri plates containing collard green leaf disks overlaying the water-agar (1%) to form one colony of each clone. After population growth of the colonies, 4th instar nymphs of *L. pseudobrassicae* were used to assess the level of resistant to *D. rapae* (as described above) in each of the two clones started from aphids collected from canola fields.

To assess the level of resistance to parasitism of the two canola field-origin aphid colonies, ten 4th instar nymphs from each a *L. pseudobrassicae* clone (C2, C3) were placed in batches (of 10 aphids) in Petri plates containing a collard leaf disk, where one *D. rapae* mated

female, no older than 48 hours and with no previous oviposition experience was released. Oviposition was observed under the stereoscopic microscope (as previously described above). Parasitized aphids were removed from the plate immediately after been parasitized and then maintained in individual Petri plates in an acclimatized chamber. Each parasitoid female was allowed to parasitize from six to 10 aphids of the aphids in a given dish (10 aphids), until 23 aphids were parasitized per clone (using three parasitoid females per clone). Daily observations were made for 15 days to evaluate the number of aphids that did not mummify (resistant) and the number of those that became mummies (susceptible).

For clone C2, none of the 23 parasitized aphids transformed into a mummy, and therefore we considered clone C2 to be resistant to the parasitoid. Similarly, for clone C3, only one specimen was transformed into mummy and the other 22 were considered to be resistant. Thus, clone C2 was also chosen for the experiment as a resistant clone.

Twenty 2nd instar nymphs of C2 clone were subjected to a single oviposition of *D. rapae* to confirm their resistance. Daily observations were made for 15 days to confirm the formation of mummies. Since all 20 aphids reached adulthood and did not mummify, resistance of clone C2 to *D. rapae* was confirmed and it was designated as C2R.

Experimental protocol

The lethal and sub-lethal effects of acetamiprid on *L. pseudobrassicae* clones that were either resistant or susceptible to the parasitoid *D. rapae* were evaluated with dose-response assays, using LD_{50} values as the measure of mortality and the sub-lethal effects of acetamiprid on aphid fecundity. The doses to be applied per aphid were determined in preliminary tests. Insecticide solutions were prepared from the technical product, using acetone as the solvent. Five concentrations (0.01, 0.1, 1, 10 or 100 ng a.i./aphid) of acetamiprid and a control treatment with just acetone were used. A micro syringe coupled to an automatic dispenser was used to apply a volume of 0.20 µL on each aphid. This volume was based on the average body mass of an adult aphid (0.4809 mg), obtained from the evaluation of the mass of 350 adult aphids, weighed in 10 groups of 35 specimens, using an analytical scale.

Three replications were done for each insecticide dose and aphid group, each replicate being set up in a 100-mm dia Petri plate containing one collard green disk over water-agar (1%). In each dish (replicate) we placed ten 24-hour old *L. pseudobrassicae* adults. The plates were incubated in an acclimatized chamber at 25±2°C, RH70% and a 12:12 h L:D photoperiod immediately after the appropriate insecticide dose had been applied to each aphid. Evaluations of aphid survival were done at 24, 48 and 72 hours after the application, determining the number of dead specimens as well as the number of aphid nymphs produced by each chemically treated mother aphid.

Data analysis

The experimental design was completely randomized, with three replications, and the analyses were done with the software R (2016). Data were submitted to Shapiro and Fligner tests to confirm the assumptions of normality of error distribution and homogeneity of variances, respectively. Mortality data were analyzed using the package DRC (Ritz & Streibig, 2005) using the Chi-square test. We determined the values of acute toxicity (LD_{50}) and the 95% CI of aphid mortality at 48 hours after insecticide application. LD_{50} values were expressed in nanograms of active ingredient per aphid (ng a.i./aphid) and in nanograms of active ingredient per milligram of aphid (ng a.i. mg^{-1}aphid). The data on *L. pseudobrassicae* fertility (number of nymphs produced after 72 hours) were analyzed using the General Linear Model and adjusted to the Poisson distribution, with a "log" link function, in which the aphid groups, dilutions and its interaction were considered as fixed factors.

RESULTS

The greatest mortality from acetamiprid occurred in clone C1S and population P1 at 0.01 and 0.1 ng a.i./aphid, with values of 53.3% and 53.3%, and 76.6% and 66.6% dead aphids (out of 30 aphids per treatment), respectively. Mortality of 93,3%, 86,6%, and 90% of the specimens in the clones C1R, C1S and population P1, respectively, was observed at the concentration 1 ng a.i./aphid of acetamiprid after 72 hours of exposition. Mortality of the clone C2R was lower than that of the other groups at the concentrations of 0.01, 0.1 and 1 ng a.i./aphid. The insecticide acetamiprid caused 100% mortality at the two greatest concentrations, 10 and 100 ng a.i./aphid, while the control treatment caused no mortality (Table 1).

The clone C2R had the highest LD_{50} value. The population P1 had the smallest LD_{50} value and, therefore, was considered the most susceptible to the insecticide acetamiprid. The LD_{50} values of the C1R (resistant) and C1S (susceptible) clones were not statistically different (Table 2), indicating that resistance to the parasitoid did not change the effect of the insecticide acetamiprid on *L. pseudobrassicae*. Moreover, the LD_{50} values of the two resistant clones (C1R and C2R) were different (Table 2). The clone C2R had a greater LD_{50} than that of clone C1R, indicating that the mortality due the insecticide acetamiprid is related to the clone, not to resistance to the parasitoid (Table 2).

Fertility of aphids was affected by pesticide applications but not by their degree of resistance to the

parasitoid. Significant differences were observed for the number of nymphs produced by each clone 72 hours after applying the insecticide acetamiprid ($W = 1.057e^{-07}$). Low pesticide doses (0.01 and 0.1 ng a.i./aphid) did not affect nymphal production for any of the aphid treatment groups, including the control (Figure 1). Reduction in nymphal production started at 1 ng a.i./aphid for all aphid groups. At 10 ng a.i./aphid, C1R, C1S and P1 ceased reproduction and no aphid group reproduced at the greatest dose evaluated, 100 ng a.i./aphid. The clone C2R had the greatest fertility, in the control as well as at the doses 0.01, 0.1, 1 and 10 ng a.i./aphid (Figure 1). The production of nymphs in clones C1R and C1S and the population P1 did not differ among themselves or with the control at 0.01, 1 and 10 ng a.i./aphid (Figure 1). In contrast, at 0.1 ng a.i./aphid, C1S produced more nymphs than C1R and P1. The greatest reproduction of clone C2R for all the doses of acetamiprid for which reproduction was observed,

Table 1: Percentage of mortality of four groups of *Lipaphis pseudobrassicae* (30 aphids of each group per dose) resistant and susceptible to the parasitoid *Diaeretiella rapae*, after topical application of five doses of the insecticide acetamiprid in nanograms of active ingredient per aphid (ng a.i./aphid) and in the control (acetone)

Concentration (ng a.i./aphid)	Clones and population	Mortality (%)			
		Exposition (hours)[1]			Total
		24	48	72	
0.01	C1R	0.0	10.0	16.6	26.6
	C1S	3.3	13.3	36.6	53.3
	C2R	0.0	3.3	13.0	16.6
	P1	40.0	3.3	10.0	53.3
0.1	C1R	10.0	10.0	6.6	26.6
	C1S	10.0	23.3	43.3	76.6
	C2R	3.3	6.6	3.3	13.3
	P1	60.0	3.3	3.3	66.6
1	C1R	20.0	46.6	26.6	93.3
	C1S	20.0	50.0	16.6	86.6
	C2R	16.6	26.6	10.0	53.3
	P1	70.0	16.6	3.3	90.0
10	C1R	90.0	10.0	-	100.0
	C1S	66.6	26.6	6.6	100.0
	C2R	76.6	23.3	0.0	100.0
	P1	100.0	-	-	100.0
100	C1R	100.0	-	-	100.0
	C1S	100.0	-	-	100.0
	C2R	63.3	36.6	-	100.0
	P1	100.0	-	-	100.0
Control	C1R	0.0	0.0	0.0	0.0
	C1S	0.0	0.0	0.0	0.0
	C2R	0.0	0.0	0.0	0.0
	P1	0.0	0.0	0.0	0.0

[1] Hours after exposition to the product; C1R: Clone one, resistant; C1S: Clone one, susceptible; C2R: Clone two, resistant; P1: Population collected in a commercial area of collard greens. *average body weight of one aphid: 0.4809 mg (n = 350).

Table 2: Acute toxicity (LD_{50} – 48 hours) of acetamiprid in four groups of *Lipaphis pseudobrassicae* resistant and susceptible to the parasitoid *Diaeretiella rapae*

Clone	LD_{50} [a]	CI95%[b]	LD_{50} [c*]	CI95%[d*]	χ^2 [e]	*Df* [f]
C1R	0.06	0.01 – 0.10	0.12	0.02 – 0.21	12.924	10
C1S	0.06	0.03 – 0.10	0.12	0.06 – 0.21	15.194	13
C2R	0.14	0.07 – 0.20	0.29	0.14 – 0.41	12.294	10
P1	0.01	0.00 – 0.02	0.02	0.00 – 0.04	8.2731	10

[a] LD_{50} in nanograms of active ingredient per aphid (ng a.i./aphid); [b] Confidence interval of 95% in ng a.i./ aphid; [c] LD_{50} in nanograms of active ingredient per milligram of aphid (ng i.a. mg^{-1} aphid); [d] Confidence interval of 95% in ng i.a. mg^{-1} of aphid; [e] Chi-square of the model; [f]Degrees of freedom; *average body mass of an aphid: 0.4809 mg (n = 350); C1R: Clone one, resistant; C1S: Clone one, susceptible; C2R: Clone two, resistant; P1: Population collect in a commercial area of collard greens.

indicates that this is a characteristic of the clone and is not related to resistance to the parasitoid or to a smaller sub-lethal effect of the insecticide on this clone (Figure 1). In general, a reduction in production of nymphs was most related to increased aphid death, especially 24 and 48 hours after exposure to acetamiprid (Table 1, Figure 1).

DISCUSSSION

Few studies have been done determining the LD_{50} of insecticides to aphids, and those that have were done mostly with plant extracts and essential oils. There are more studies determining LC_{50} (median lethal concentration) values, probably due to the greater difficulty of the topical application on aphids for the determination of LD_{50}, especially due to their small size (Konno & Omoto, 2006; Silva *et al*., 2009; Breda *et al*., 2011; Tran *et al*., 2016).

Comparing the LD_{50} of *L. pseudobrassicae* with that of other insects with greater body mass makes it clear that the reduced size of the aphid results in a low acetamiprid LD_{50}, varying from 0.01 to 0.14 ng a.i./aphid. For instance, studies done with a topical application of imidacloprid, another neonicotinoid, on *Apis melifera* (L.) bees, presented LD_{50} of 49 to 102 ng /bee (Nauen *et al*., 2001; Schmuck *et al*., 2003). The toxic effect of neonicotinoids on aphids is known to be greater than that of insecticides of other chemical groups, Gaber *et al*. (2015) demonstrated the great efficacy of the commercial product Mospilan, manufactured with acetamiprid, in the control of the aphid *A. gossypii* on cotton. Those authors observed a reduction of 85% of the aphid population 21 days after the treatment. According to Konno & Omoto (2006), for *Aphis gossypii* Glover, the lethal concentration of imidacloprid was half that of carbosulfan (a carbamates) and only about 2% of endosulfan (a cyclodiene chlorates). Similarly, Skaljac *et al*. (2018) found values for the lethal concentration of imidacloprid for *Acyrthosiphum pisum* (Harris) to be only about 4% of cyantraniliprole, 0.1% of spirotetramat, 0.03% of methomyl and 0.006% of chlorpyriphos-methyl.

We found no relation between resistance of *L. pseudobrassicae* to the parasitoid *D. rapae* and the effects of the insecticide acetamiprid, be it through its lethal effect, evaluating the LD_{50}, or as sub-lethal effects, through the evaluation of *L. pseudobrassicae* fertility. Aphids resistant to the parasitoid have a clear adaptive advantage in the presence of the parasitoids (Oliveira *et al*., 2013); however, in the absence of the parasitoid there can be adaptive advantages (Cayetano *et al*., 2014) or costs (Vorburger *et al*., 2013; Skaljac *et al*., 2018) to being resistant to the parasitoid. For instance, *Aphis fabae* Theobald infected by *Hamiltonella defensa*, was resistant to the parasitoid *Lysiphlebus fabarum* (Marshall) and its life expectation and reproduction were greater than that of individuals that were not resistant to the parasitoid (Cayetano *et al*., 2014). In contrast, Skaljac *et al*. (2018), found a reduction in reproduction and size of aphids that were resistant to the parasitoid *Aphidius ervi* (Haliday) through symbiosis with *Serratia symbiotica*, although clones of *A.pisum* that were resistant to the parasitoid were more susceptible to the insecticides imidacloprid, chlorpyriphos-methyl, methomyl, cyantraniliprole, and spirotetramat, in contrast to the present study where no adaptive advantage or disadvantage was observed in the aphids resistant to *D. rapae* in relation to the effects of the insecticide.

The evaluation of nymphal production in our control, with no insecticide application, demonstrated that the clone C2R, resistant to the parasitoid, had the greatest fertility. However, this is a clone effect, not an effect from the resistance to the parasitoid, since resistant (C1R) and

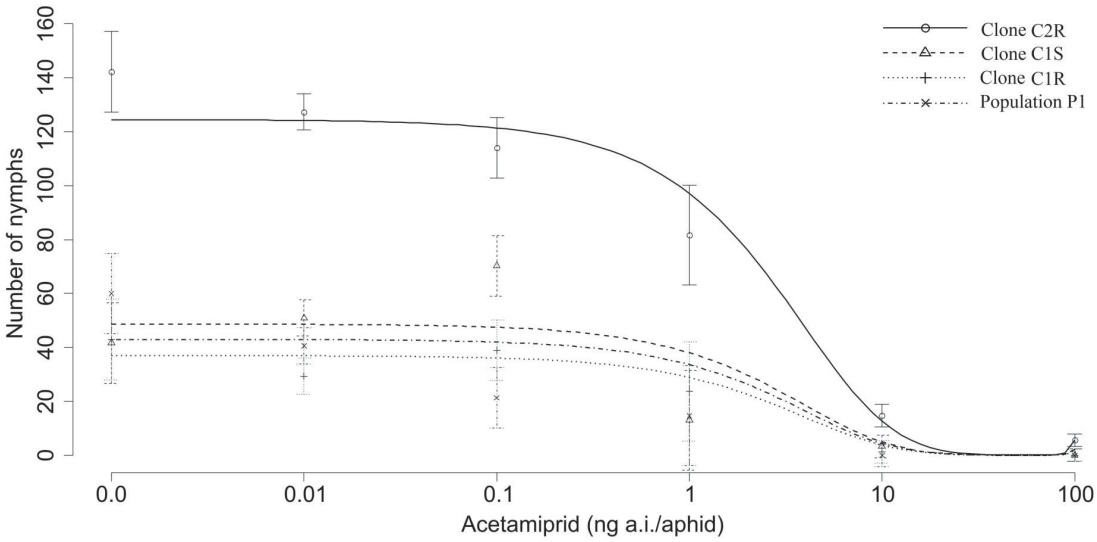

Figure1: Number of nymphs produced by four groups of *Lipaphis pseudobrassicae* for 72 hours after the application of five doses of the insecticide acetamiprid, in nanograms of active ingredient per aphid (ng a.i./aphid) and in the control (acetone).

susceptible (C1S) specimens of the same clone had the same fertility. Similarly, the fertility of susceptible clone C1S was similar to that of population P1.

Another adaptive aspect to be considered is the negative effect of resistance to insecticide, since there tend to be adaptive costs linked to such resistance. In this study, the clone C2R, resistant to the parasitoid, also had greater LD_{50} (0.29 µg a.i./mg aphid) and also greater nymphal production than the other clones, with no adaptive cost, at least in terms of fertility. The superior reproduction of clone C2R compared other treatments was observed both in the absence (control) and in the presence of selection pressure (insecticide treatments), indicating that this is an advantageous characteristic of the clone, which, by having the smallest mortality, had the greatest population increase, even when exposed to acetamiprid. According to Belinato & Martins (2016), in the absence of insecticides, susceptible individuals can have reproductive advantages, and therefore levels of resistance in the population tend to decrease. Studies related to the adaptive cost associated to resistance have found contrasting results. Hollingsworth *et al*. (1997) observed that a line of *A. gossypii* resistant to the insecticide methomyl, had greater fertility than a susceptible line. Similarly, Eggers-Schumacher (1983) reported that a resistant line of *M. persicae* had a reproductive advantage in relation to the susceptible line in the absence of selection pressure. In contrast, Konno & Omoto (2006) observed that the line of *A. gossypii* resistant to the insecticide carbosulfan was at a reproductive disadvantage in relation to the susceptible line in the absence of selection pressure, similar to Stone *et al*. (2000), who reported that lines of *Schizaphis graminum* (Rondani) resistant to five organophosphate insecticides had a reproductive disadvantage compared to susceptible ones.

Adaptive costs of resistance can be related to other characteristics besides those associated with aphid reproduction, such as mobility or response to alarm pheromones. Foster *et al*. (2010) observed that *M. persicae* resistant to insecticides had lower mobility, reducing its ability to flee from attack by *D. rapae* compared to susceptible aphids, leading to higher parasitism. The present study, however, did not consider behavioral aspects in the evaluation of the consequences of resistance to acetamiprid.

The parasitoid *D. rapae* is the most important natural enemy for aphid control in Brassica crops (Sampaio *et al.*, 2017). However, a high proportion of resistant individuals in the population of *L. pseudobrassicae* in Uberlândia makes biological control from this parasitoid a less effective tool (Oliveira *et al.*, 2013; Sampaio *et al.*, 2017; Ferreira *et al.*, 2018).

CONCLUSION

The use of the parasitoid *D. rapae* for the control of the aphid *L. pseudobrassicae* in Brassica crops in Uberlândia-MG is ineffective since most individuals are resistant to the parasitoid. Thus, the confirmation that there is no relation between resistance to the parasitoid and the effects of the insecticide acetamiprid makes the use of chemical control with neonicotinoids a possible option for the control of *L. pseudobrassicae* in Brassica.

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