Bioassays of Flonicamid Against the Common Bed Bug (Hemiptera: Cimicidae) Show No Mortality or Feeding Inhibition after Treatment[1]

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Abstract Flonicamid is a systemic pesticide that inhibits the feeding of certain greenhouse pests and certain hemipteran pests of agricultural crops. In a laboratory study directed at the common bed bug (*Cimex lectularius* L.), flonicamid (as Aria®) was evaluated for its ability to either kill or inhibit the feeding of this blood-sucking pest. After flonicamid was applied topically (by spray), as a residual (to bed bug harborages), or fed directly to bed bugs by incorporating the insecticide into the bloodmeal, mortality assessments were made and surviving bed bugs were given the opportunity to feed on untreated blood. At the recommended label rates and at rates that approached the solubility limits of the product in water, it was concluded that this insecticide would be ineffective in a bed bug control program.

Key Words flonicamid, bed bug, *Cimex lectularius*, nymphs, adults

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is a blood-sucking pest of humans that has proven difficult to control (Pinto et al. 2007). A variety of chemical and nonchemical means have been used to control bed bugs (Doggett et al. 2012). Because field populations of bed bugs are considered resistant to pyrethroid insecticides (Romero et al. 2007, Zhu et al. 2010) and documentation of resistance to neonicotinoid pesticides has also been reported (Romero and Anderson 2016), new chemicals are always in demand in an effort to control these pests.

Flonicamid (*N*-cyanomethyl-4-trifluoromethylnicotinamide) is a Group 29 pesticide (Insecticide Resistance Action Committee 2017) that acts as a feeding inhibitor of certain hemipterous insects (Kodandaram et al. 2010). Flonicamid is marketed by the FMC Corporation as Aria® insecticide. Although designed to control certain greenhouse pests, such as whiteflies, aphids, and thrips, the Aria label also includes hemipteran pests, such as plant bugs and stink bugs (Morita et al. 2007, Roditakis et al. 2014, Tariq et al. 2017). However, other formulations containing flonicamid as the active ingredient did not perform well against the brown marmorated stink bug (*Halyomorpha halys* Stål) in apples (Lee et al. 2015), although the results on controlling this particular agricultural pest in peppers was equivocal (Kuhar et al. 2014, Kuhar and Doughty 2016). Still, it was decided to

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examine the activity of flonicamid (as Aria) for the ability to kill bed bugs or inhibit their feeding, which would be considered a form of control because they must ingest and subsequently digest blood to molt and reproduce (Usinger 1966).

Materials and Methods

Insects. A colony of *C. lectularius* was established from bed bugs originally obtained from Harold Harlan (Crownsville, MD). The colony was kept at ambient conditions (25 ± 2°C and 40 ± 15% relative humidity) and fed weekly on expired, human red blood cells and plasma by using an artificial (*in vitro*) feeding system (Feldlaufer et al. 2010). These bed bugs, often referred to as the "Harlan strain," are considered susceptible to pyrethroid insecticides (Moore and Miller 2006). This strain has been maintained by Dr Harlan since originally collected in 1973, in Fort Dix, NJ. For the experiments described below, nymphs and adults of both sexes (in all experiments, approximately 17% were adults and binomial sampling theory yields a standard deviation of 3%) were used and had not been fed for 8 d. First-instar nymphs were excluded (not counted) from all results due to the reported variability of their responses to insecticides (Hinson et al. 2016).

Chemicals and treatments. Aria (50% flonicamid; FMC Corp., Philadelphia, PA) was obtained from a commercial supplier. Distilled water was used for all dilutions and as a control in all experiments. Bed bugs were treated in three ways: (a) topically, by spray; (b) as a residual, where bed bugs were allowed to seek harborage in fan-folded filter paper previously treated with Aria; and (c) by feeding. Although feeding is not a practical method of exposure, under laboratory conditions, it allows for the assessment of innate activity. Additionally, with aphids, flonicamid reportedly had better activity through ingestion than through contact (http://www.iskweb.co.jp/eng/products/pdf/flonicamid.pdf).

For topical treatments, Aria (50% flonicamid) at 16.1 mg was mixed with 50 ml distilled water. This represents the approximate highest label mixture rate (120 g/378.5 L water = 15.85 mg/50 ml) for plant bugs and stink bugs. After mixing, this solution was used to fill a 30-ml spray bottle (Specialty Bottles, Seattle, WA). Mixed stages of bed bugs, confined to glass petri dishes (60 mm × 15 mm) containing a single sheet of filter paper (47-mm diameter), were then sprayed (3 pumps = approximately 4.8 ml) with either the test chemical or distilled water. After spraying, bed bugs were transferred to different petri dishes containing dry (untreated) filter paper. Mortality was assessed at 48-h posttreatment, and surviving bed bugs were given the opportunity to blood feed *in vitro*, by transferring to glass, wide-mouth 240-ml jars (Kerr®, Jarden Home Brands, Daleville, IN), which had a circular piece of filter paper (55-mm diameter, Whatman 1: Whatman International LTD, Maidstone, UK) on the bottom and were fitted with screened tops (Feldlaufer et al. 2014).

For residual treatments, harborages constructed of fan-folded filter paper (Whatman 1, 4 cm × 14 cm = 56 cm^2) were treated by pipetting a solution of Aria dissolved in distilled water or of distilled water alone onto the harborage to yield approximately 6×, 60×, 290×, and 590× of Aria. In all cases, dilutions were made so the same volume was pipetted onto each harborage. The highest dose (590×) was selected as the amount that did not result in solubility problems (noticed as solution

turbidity after mixing); other doses were approximate fractions of this highest amount. Amounts were calculated using the highest mixture rate (120 g/378.5 L water) along with an application rate of 94.4 L/acre (for a plant up to 305 mm in height), which equates to approximately 0.74 $\mu g/cm^2$, so 590× represents 437 μg Aria/cm^2. After drying, mixed stages of bed bugs were placed on the treated harborages in mason jars, as described above. Harborages treated with distilled water also received bed bugs after the harborage dried. Mortality was assessed 48 h, and bed bugs in all harborage experiments were given the opportunity to blood feed on untreated blood.

For feeding experiments, Aria (50% flonicamid, 16.0 mg) was dissolved in 0.5 ml distilled water and then mixed with human blood fortified with plasma to a final volume of 50 ml. Bed bugs confined to screened, glass Mason jars (see above) were fed *in vitro* (Feldlaufer et al. 2010) and then given the opportunity to refeed on untreated blood 1 week after the initial feeding. Control groups consisted of bed bugs that were not treated but fed twice (1 week apart) on untreated blood.

In all experiments, dishes or jars containing bed bugs were kept at ambient conditions (25 ± 2°C and 40 ± 15% RH), and mortality was assessed by 48-h posttreatment. Two replicates were conducted in each experiment.

Toxicity. When mortality was assessed, bed bugs were scored as dead, alive, or morbid/moribund. Morbid bed bugs were upright, but did not respond to stimulus by running away, whereas moribund bed bugs remained on their back, with appendages twitching (Feldlaufer et al. 2013). All treatment groups and controls containing bed bugs that were deemed alive were given the opportunity to engorge on untreated blood at various times posttreatment by using an *in vitro* blood-feeding method previously described (Feldlaufer et al. 2010).

Data analysis. Data were assumed to be binomially distributed. In all experiments, standard deviations were calculated and converted to percentages in those instances where neither 0% nor 100% mortality was observed. Other analyses were conducted using the R software (R Core Team 2017) and modeled using "glm" and "glmer" functions, again, assuming the data were binomially distributed (overdispersion was checked and found to be negligible).

Results

In mortality assessments, no morbid or moribund bed bugs were noticed in any group. Therefore, bed bugs were scored as either dead or alive.

In topical (spray) treatments, negligible mortality was observed when mixed stages of bed bugs were sprayed with Aria. Only one bed bug in each of the treatment groups died (1.85% and 2%, respectively) compared to 0% mortality in the control groups (Table 1). After assessment of mortality, the bed bugs were given the opportunity to ingest untreated blood (Table 2). Although only about 43% of surviving bed bugs fed in one of the treatment groups, the other treated group fed at a rate that exceeded 94%. The control groups had feeding rates of about 88% and 98%, respectively. Because only two trials were conducted, the random effect of trials could not be estimated well. It is noted, however, that the one treatment group where approximately 43% of the surviving bed bugs fed statistically separated out

Table 1. Bed bug mortality* after topical treatment (by spray) with either Aria (approximately 1.5 mg; flonicamid) or distilled water (control).**

	Trial#							
	1				2			
Compound	Number Dead	Number Alive	Total	Mortality, % (SD)	Number Dead	Number Alive	Total	Mortality, % (SD)
Aria	1	53	54	1.85 (1.83)	1	49	50	2.00 (1.98)
Control	0	51	51	0.0	0	49	49	0.0

* Mortality was assessed at 48-h posttreatment.
** See Table 2 for feeding after treatment results.

Table 2. Bed bug feeding* on untreated blood after topical treatment with either Aria (approx. 1.5 mg, flonicamid) or distilled water (control).

	Trial#					
	1			2		
Compound	Number Fed	Total	Fed, % (SD)	Number Fed	Total	Fed, % (SD)
Aria	50	53†	94.3 (3.2)	21	49†	42.9 (7.1)
Control	45	51	88.2 (4.5)	48	49	98.0 (2.0)

* After the assessment of mortality, bed bugs deemed alive in Table 1 were given the opportunity to ingest untreated blood at 48-h posttreatment.
† Reflects that one bed bug had died in the previous experiment (Table 1).

from the other three groups (one treatment and two controls). Thus, feeding rates for treated groups were not consistently different from control groups.

In the harborage experiments, Table 3 summarizes the results for mortality and feeding inhibition when mixed stages of bed bugs were placed on harborages treated with varying amounts of Aria. Only the dose corresponding to approximately 590× the recommended dose of Aria exhibited significant mortality, killing over 86% and 84% of bed bugs in the two treatment groups, respectively. By comparison, the doses corresponding to about 290× and 60× times the recommended amount of

Table 3. Bed bug mortality* in groups exposed to harborages treated with various amounts of Aria (flonicamid) or distilled water (control).

	Trial#							
	1				2			
Amount	Number Dead	Number Alive	Total	Mortality, % (SD)	Number Dead	Number Alive	Total	Mortality, % (SD)
6×	0	62†	62	0.0	0	52	52	0.0
60×	1	58	59	1.7 (1.7)	1	51	52	1.9 (1.9)
290×	1	58	59	1.7 (1.7)	1	49	50	2.0 (2.0)
590×	46	7††	53	86.8 (4.7)	50	9†††	59	84.7 (4.7)
Control	1	61	62	1.6 (1.6)	0	41	41	0.0

* Mortality was assessed 48-h posttreatment, and living bed bugs were given the opportunity to feed *in vitro* on untreated blood (Feldlaufer et al. 2010).
† Except where noted (below), all bed bugs in all groups deemed alive fed on blood when offered an untreated blood meal 48-h posttreatment.
†† Only two nymphs from this group fed.
††† Only four nymphs from this group fed.

Table 4. Initial mortality rates in bed bugs one week after feeding on blood containing Aria (50% flonicamid) and refeeding rates of surviving bed bugs fed on untreated blood.

Trial	Mortality, % (SD)	Number Fed	Number Unfed	Total	Refed, % (SD)
Aria (50% flonicamid)†					
1	32.5 (4.3) [38/117]††	26	53	79	32.9 (5.3)
2	32.6 (4.9) [30/92]††	30	32	62	48.4 (6.3)
Controls*					
1	0 [0/101] ††	90	11	101	89.1 (3.1)
2	1.0% (1.0) [1/100] ††	95	4	99	96.0 (2.0)

† Aria (16.1 mg) was dissolved in 0.5 ml distilled water and then mixed with human red blood cells fortified with plasma (Feldlaufer et al. 2010) to a final volume of 50 ml. Mixed stages of bed bugs were allowed to feed on this mixture and then given the opportunity to refeed 1 week (168 h) later on blood without Aria.
†† [dead/total]
* Control groups consisted of bed bugs that did not initially feed on blood that contained Aria.

Aria killed about 2% or less of the bed bugs. At 6× the recommended dose, no mortality was observed. In these latter instances (6×, 60×, and 290×), mortality did not differ significantly from the untreated (distilled water) replicates ($P = 0.53$, likelihood ratio test). As far as the ability to ingest blood at 48-h postexposure, only the highest dosage (590×) inhibited feeding when compared to the other doses and to the control group. Still, nymphs from this highest dose fed, albeit only 2 of 7 nymphs and 4 of 9 nymphs, respectively, actively fed. The fate of these fed nymphs was not followed.

When bed bugs were fed on blood containing Aria, mortality was greater than 32% in both groups of bed bugs; refeeding rates (1-wk posttreatment) of living bed bugs in these groups on untreated blood were also greater than 32% (32.9% and 48.4%, respectively) (Table 4). Mortality and refeeding rates in the two control groups (fed on untreated blood both times) were similar to each other.

Discussion

Flonicamid is a pyridinecarboxamide synthetic insecticide that acts as a feeding inhibitor of certain greenhouse and agricultural pests and occupies an insecticide class by itself (Insecticide Resistance Action Committee 2017). Against pyrethroid-susceptible bed bugs, it did not prove effective in either killing or inhibiting feeding when applied as a topical spray. Our results showed that treatment effects are not predictable, as there was a difference in the feeding rates of bed bugs previously sprayed with Aria. We have no explanation for this disparity in feeding rates in the treated groups previously sprayed with Aria (43% versus 94%), because the numbers of bed bugs in all groups were similar. Still, one could speculate that even the lower feeding of about 43% would not be sufficient in a bed bug control program,

particularly because the compound is an antifeedant. When laboratory harborages were treated with flonicamid (Aria), only the highest dose used (approximately 590× the recommended label dose), exhibited mortality, which was still under 90%. Even at this massive dose, several nymphs survived and ingested blood when given the opportunity.

Although certain insecticides have the potential of being incorporated into liquid baits that bed bugs may ingest, thereby giving effective control (Sierras and Schal 2017), current results with feeding flonicamid (as Aria) were disappointing. Although mortality was greater than 32% in both groups that were fed flonicamid (as Aria), a relatively large percentage of the surviving bed bugs (32.9% and 48.4%, respectively) fed to repletion on untreated blood the following week. Because this active ingredient has an antifeedant effect in certain insects, it was concluded that these feeding rates in surviving bed bugs were unacceptable. It has been my experience that the Harlan strain of bed bugs (pyrethroid sensitive) is more sensitive to all treatments than are pyrethroid-resistant strains of bed bugs. Therefore, tests using Aria with pyrethroid-resistant strains were not conducted, because treatments with Aria did not produce significant mortality nor inhibit feeding in the Harlan strain of bed bugs. In summary, flonicamid (as Aria) does not appear to have any practical use in a bed bug control strategy.

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