

No Detectable Insecticide Resistance in Swallow Bugs (Hemiptera: Cimicidae) Following Long-Term Exposure to Naled (Dibrom 8)

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Abstract

The swallow bug, *Oeciacus vicarius* Horvath, is a hematophagous ectoparasite of the cliff swallow, *Petrochelidon pyrrhonota* Vieillot, and is closely related to bed bugs (*Cimex* spp.). Evolution of insecticide resistance has been documented for bed bugs but not studied in *Oeciacus*. For periods of 17 and 32 yr, two cliff swallow colonies in western Nebraska were treated during the summer breeding season using the organophosphate insecticide Dibrom. Despite continual treatments, *O. vicarius* has been observed frequently within these colonies. We evaluated the efficacy of Dibrom 8 on *O. vicarius* during the 2016 season at two treated colonies and four that had never experienced treatment. Dibrom 8 was found to be effective in 100% of trials, with immobilization within minutes and death within 72 h, for individuals from all colonies. In control treatments (water), individuals collected from treated colonies exhibited greater survival than individuals from untreated colonies, and those from active colonies (bugs fed) had greater survival than those from inactive colonies (bugs unfed). A residual effect was observed in both lab and field trials: 100% mortality occurred in the lab after exposure to filter paper substrates treated both 5 and 10 d earlier, and in the field, nests treated once early in the season had *O. vicarius* counts 43 d later that were <1% of those from untreated nests within the same colony. We hypothesize that the lack of resistance results from the limited potential for resistance allele fixation due to outbreeding and frequent immigration of insecticide-naïve individuals.

Key words: *Oeciacus vicarius*, organophosphate, cholinesterase inhibitor, *Petrochelidon pyrrhonota*

Insecticide resistance has been identified in >500 species of insects worldwide (Corbel and N'Guessan 2013), including many of agricultural and medical significance. Amongst others, mosquitoes, including *Culex*, *Anopheles*, and *Aedes* spp. (Hemingway and Karunaratne 1998, Hemingway et al. 2004, Gordon and Ottea 2012, Liu 2015), house flies, *Musca domestica* L. (Oppenoorth and van Asperen 1960, Walsh et al. 2001), aphids, *Myzus persicae* (Sulzer) (Devonshire et al. 1998), human head lice, *Pediculus humanus capitis* De Geer (Yoon et al. 2014), German cockroaches, *Blattella germanica* (L.) (Cochran 1989, Liu et al. 2000), and bed bugs, *Cimex lectularius* L. (Steelman et al. 2008), have all evolved mechanisms of resistance to multiple classes of insecticides, including organophosphates (OPs). Indeed, by 1969, only 15 yr after initially using OPs in the field, 54 different species were documented to have developed resistance to these insecticides (Brown 1969), while it was reported that only 10 yr were required for genes conferring resistance to OPs to become widespread within populations (Raymond et al. 2001). It may, however, be more

accurate to report insecticide resistance evolution in generations, not years (Georghiou and Taylor 1977).

Resistance to OPs is typically conferred by either 1) metabolic regulation of detoxification proteins, 2) point mutations affecting specific receptors and other target sites, or 3) a combination of these mechanisms (Hemingway et al. 2004, Corbel and N'Guessan 2013, Feyereisen et al. 2015). One widely observed evolutionary response to OPs is the overproduction of carboxylesterase through gene modulation, whereby the abundant esterase enzyme is able to sequester the pesticide for further detoxification inside the insect (Jayawardena et al. 1994, Claudianos et al. 1999, Hemingway et al. 2004).

Cimicid insects, a speciose family of hematophagous ectoparasites of birds and mammals capable of transmitting disease (Usinger 1966, Zorrilla-Vaca et al. 2015), have proven to be valuable models for studying insecticide resistance, with the evolution of multiple modes of resistance reported in two species of human health concern, the common bed bug, *Cimex lectularius*, and the tropical bed bug, *Cimex*

hemipterus (F.) (Busvine 1958, Feroz 1971, Feyereisen 1995, Karunaratne et al. 2007, Adelman et al. 2011, Kilpinen et al. 2011). It is this remarkable ability to resist insecticide treatments that has contributed to the dramatic resurgence of these pests over the past quarter century (Reinhardt and Siva-Jothy 2007). Despite the frequent reports for *C. lectularius* and *C. hemipterus*, insecticide resistance has yet to be investigated in any other member of the Cimicidae.

The swallow bug, *Oeciacus vicarius* Horvath, a close relative of both *C. lectularius* and *C. hemipterus* (Balvin et al. 2015), is a specialized hematophagous ectoparasite of the cliff swallow, *Petrochelidon pyrrhonota* Vieillot. *Oeciacus vicarius* live exclusively in the crevices of the cliff swallows' nesting substrate, and crawl into the nest to feed on nestling and adult birds. These insects synchronize their reproduction with the spring arrival of their host at the breeding colonies (Loye and Hopla 1983), and then survive harsh climates by overwintering in a state of diapause (Brown et al. 2009, 2010). *Oeciacus vicarius* undergo five nymphal instars prior to molting into adults (Usinger 1966), with the process from egg laying to adult appearance taking ~60 d (Loye 1985). As such, the number of generations per season is unlikely to exceed two. After feeding, adult females lay on average 16 eggs (range 7–27), which hatch within 3–5 d (Loye 1985). *Oeciacus vicarius* has been found to vector Buggy Creek virus (Togaviridae), an alphavirus not known to negatively affect their primary host, but found to cause extensive mortality in nestling house sparrows, *Passer domesticus* (O'Brien and Brown 2012). The recent host-species expansion of *O. vicarius* onto alternative hosts such as cave swallows, *Petrochelidon fulva* (Weaver and Brown 2005), barn swallows, *Hirundo rustica* (Kopachena et al. 2007), and house sparrows (O'Brien et al. 2011), creates the potential for range expansion of the ectoparasite and the infestation and potential spread of Buggy Creek virus to additional species outside of the current range. Thus, evaluating the ability of *O. vicarius* to develop insecticide resistance could be important in determining how best to control its infestations.

This study takes advantage of a long-term field experiment in western Nebraska to examine to what degree *O. vicarius* are able to resist the OP insecticide Dibrom (AMVAC Chemical Corporation, Los Angeles, CA) after repeated annual exposure at two active host nesting sites. Despite season-wide (May through July) treatments for periods of 17 and 32 yr, *O. vicarius* are commonly observed at these sites; thus, the potential for insecticide resistance to Dibrom was questioned. Dibrom is a cholinesterase inhibitor (Raouf et al. 2006) for which some resistance has been identified within populations of the mosquito, *Culex nigripalpus* Theobald (Diptera: Culicidae; Dongyoung and Smartt 2016) and onion maggot, *Delia antiqua* (Diptera: Anthomyiidae) (Carroll et al. 1983). Dibrom breaks down into dichlorvos, another OP insecticide, and is known to be moderately toxic to birds and fish upon high exposure (Cox 2002). It is most often used for aerial mosquito spraying, including recently in attempts to control Zika virus transmission (CDC 2016).

Given the morphological, physiological, and evolutionary similarity of *O. vicarius* to *C. lectularius* (Usinger 1966, Balvin et al. 2015), the potential for its range expansion to alternative hosts, and the ability for Dibrom resistance to manifest within other insects (Sullivan et al. 1960, Cheng 1988, Marcombe et al. 2009), we examined whether some level of resistance to this fumigant could develop in *O. vicarius* with long-term exposure under field conditions.

Materials and Methods

A long-term field experiment was conducted in western Nebraska in which the insecticide, Dibrom 8 (also known as naled), was applied

to two *P. pyrrhonota* colony sites for periods of 17 and 32 yr, respectively, to control numbers of *O. vicarius* in and on the birds' nests for studies of behavioral ecology (Brown and Brown 1996, 2004). At each site, a dilute solution of Dibrom was lightly misted onto the outsides of the nests, the cracks in between nests, and surrounding nesting substrate (concrete wall) at intervals of ~10 d throughout each summer (mid-May to mid-July). Both colony sites were occupied by *P. pyrrhonota* and fumigated each year of the study. While Dibrom is thought to function mostly as a contact insecticide, we use the term "fumigation" to refer generally to its application to *P. pyrrhonota* colony sites (Brown and Brown 1996). Field application of Dibrom to cliff swallow colony sites has not resulted in any observable negative effects on the birds, with individuals from sprayed nests often exhibiting better body condition and higher survival than those from unsprayed nests (Chapman and George 1991, Brown and Brown 1996). The dosage used (1 part Dibrom:170 parts water) followed that in a 1970's study of cliff swallows in which bugs were removed from nests (Chapman and George 1991).

Oeciacus vicarius Collection Sites

For the experimental trials, *O. vicarius* were collected from six colony sites. Whitetail (41° 13.301' N, 101° 37.145' W), a concrete road culvert in Keith County, NE, was fumigated in its entirety each year from 1985 through 2015, with approximately half of the site also fumigated in 1984. Samples were collected, with forceps, from the outsides of the swallows' nests on 17 May 2016 prior to any fumigation in 2016. Junkyard (41° 15.380' N, 101° 37.043' W), a concrete culvert underneath railroad tracks in Keith County, was fumigated in its entirety each year from 1998 through 2014, with most of the site also fumigated in 2015. Samples were collected on 19 May 2016 prior to any fumigation in 2016. Whitetail and Junkyard were termed "fumigated" sites. Both were active (i.e., birds present in nests) in 2016 at the time bugs were collected.

Samples were collected from two colony sites in Morrill County, NE, on 18 May 2016: Haybale (41° 37.508' N, 102° 56.960' W), a road culvert, and Wild Rose South (41° 30.300' N, 102° 37.467' W), a railroad culvert. Both were inactive sites (no birds present) that had never been fumigated. At inactive sites, *O. vicarius* cluster at the entrance of the gourd-shaped nests (Moore and Brown 2014), and were collected by chipping off portions of the nest entrances into plastic bags. Finally, *O. vicarius* were collected from the outsides of nests from two never-fumigated sites that were active (i.e., birds present in nests): CaddiShack, a railroad culvert in Keith County (41° 15.794' N, 101° 40.955' W), and Wild Rose, a road culvert in Morrill County (41° 32.742' N, 102° 42.784' W), on 20–22 May 2016. At the four active sites where *P. pyrrhonota* were present, most *O. vicarius* had recently fed (blood visible in abdomen), whereas the two inactive sites had few, if any, *O. vicarius* that were fed, and most of those had not fed for a minimum of ~10 mo.

Insecticide Trials

For each of the six colony sites, five different insecticide trials were performed. Each trial used ~20 randomly selected adult *O. vicarius* placed into 100-by-15-mm VWR petri dishes lined with two sheets of Whatman 9 cm #1 filter paper. The treatments were 1) filter paper that was soaked in Dibrom and blotted but still remained moist when *O. vicarius* were added, 2) filter paper that was soaked in Dibrom but had dried when *O. vicarius* were added, 3) filter paper that was both soaked (and remained moist) and lightly misted with Dibrom from a ~30-cm distance (using the same sprayer used in the field) immediately after *O. vicarius* were added, and 4) filter paper

without insecticide that was lightly misted with Dibrom after *O. vicarius* were added. The control treatment (5) contained filter paper that was dipped into tap water and remained damp in the petri dish when *O. vicarius* were added. Experiments were performed at room temperature ($\sim 25^{\circ}\text{C}$) for the duration of the experiments. Dibrom concentrations followed those of field applications across the 17- and 32-yr treatments (1 part Dibrom:170 parts water), and that of Chapman and George (1991).

Residual effects of the fumigant were assessed by taking *O. vicarius* collected from Wild Rose only and placing them into petri dishes in which the (by then, dry) filter paper had been treated with Dibrom 10 and 5 d earlier, respectively. Controls for this experiment used filter paper that had been exposed only to water at the same time in the past. Residual effects of Dibrom in the field were assessed in 2013 at a colony site, CR 1 in Keith County ($41^{\circ} 12.54' \text{ N}$, $101^{\circ} 38.093' \text{ W}$), which had been fumigated in its entirety in 2011 and 2012. One wall of this road culvert was fumigated once on 15 May 2013; the other wall was not treated at all in 2013. Both walls had equivalent numbers (~ 75) of active cliff swallows' nests. *Oeciacus vicarius* were counted (Brown and Brown 2004) on the outsides of all nests in the colony at 8-d intervals from 3 June 2013 to 4 July 2013, by which time most of the nestling cliff swallows at the site had fledged.

Oeciacus vicarius in all treatments plus the controls were assessed for mobility after 15 min of exposure to Dibrom and again after 24 and 72 h. Statistical analyses employed chi-square contingency tables or analysis of covariance (ANCOVA).

Results

All *O. vicarius* (100%) from all sites in insecticide trials (1–4) were immobilized within 15 min of being placed into the petri dishes. Once immobilized, some twitched their appendages and contracted their abdomens for up to 24 h after Dibrom exposure. All *O. vicarius* in insecticide trials were dead within 72 h, with no discernible body movements. In contrast, water-control trials (from all sites combined) showed 61 of 120 bugs still mobile after 24 h; the difference between insecticide trials and controls at 24 h was highly significant ($\chi^2_1 = 81.8$, $P < 0.0001$). The 5-d and 10-d residual insecticide trials yielded 20 of 20 and 18 of 20 bugs immobile at 12 h, respectively, with 100% of each dead by 24 h. In contrast, the 5-d and 10-d residual water-control trials yielded only 1 of 20 and 1 of 19 bugs immobile at 12 h, respectively, and 1 of 18 and 2 of 16 immobile at 24 h.

In the field residual experiment, on 3 June, 16 June, 26 June, and 4 July, the wall that was sprayed once on 15 May yielded a mean (\pm SE) 0.09 (± 0.03), 0.05 (± 0.03), 0.15 (± 0.04), and 0.24 (± 0.05) *O. vicarius* per nest, respectively, compared with 7.53 (± 1.31), 56.13 (± 8.09), 150.49 (± 17.42), and 27.48 (± 6.10) *O. vicarius* per nest for the wall not sprayed. *Oeciacus vicarius* counts were significantly affected by both fumigation status ($F_{1,522} = 132.57$, $P < 0.0001$, ANCOVA) and date ($F_{1,522} = 15.36$, $P = 0.0001$).

The 24-h survival of *O. vicarius* in the water-control trials varied with the fumigation status of the site from which they were collected and whether the site did or did not have *P. pyrrhonota* hosts present (Table 1). For fumigated active sites, 90% of *O. vicarius* in the water-controls were still mobile after 24 h, compared with only 48.7% of *O. vicarius* from nonfumigated active sites; the difference was highly significant ($\chi^2_1 = 15.9$, $P < 0.0001$). For inactive nonfumigated sites, only 14.6% of *O. vicarius* in water-controls were

Table 1. Ratio of mobile to immobile *O. vicarius* after 24 h of exposure to water-control treatment.

Site	Fumigated		Nonfumigated			
	Active sites		Active sites		Inactive sites	
	Whitetail	Junkyard	Wild Rose	Caddishack	Haybale	Wild Rose South
Ratio	19:1	17:3	4:16	15:4	6:15	0:20
Total	36:4		19:20		6:35	

mobile after 24 h, compared with the 48.7% of *O. vicarius* in active nonfumigated sites; the difference was significant ($\chi^2_1 = 10.8$, $P = 0.001$).

Discussion

These results show that regardless of application method within our treatments (e.g., filter paper soaked in insecticide versus direct spraying of insecticide onto the live specimens), Dibrom was equally effective at immobilizing and killing *O. vicarius*. Furthermore, the test of the residual effect of this insecticide on filter paper substrates showed that it remains highly effective for up to 10 d after application. The field test (on nests) showed an even greater residual effect of the fumigant: for example, on 26 June, 43 d after spraying the nests only once, the fumigated nests had a mean *O. vicarius* count only 0.097% that of nests that had not been treated that year.

With our experimental trials showing all *O. vicarius* from fumigated sites being killed, we thus found no evidence that the use of this OP insecticide at colony sites for prolonged periods (17–32 yr) has resulted in the evolution of any detectable insecticide resistance. This is contrary to what might be expected, given that resistance to OPs can arise rapidly (Brown 1969); however, it should be noted that while the number of years of continued treatment exceeds the time previously reported for the evolution of resistance to OPs (Brown 1969), the number of generations may still be below that expected for resistance to evolve (e.g., see Raymond et al. 2001). A lack of resistance may therefore reflect the limited number of generations per year, few offspring per generation, and continual immigration of individuals lacking the mutation into each treated population, diluting the frequency of any resistant alleles that may arise.

Oeciacus vicarius disperse among colony sites by clinging to the feet of transient cliff swallows (Brown and Brown 2004, 2005; Moore and Brown 2014). During the breeding season, immigration can be frequent. For example, Brown and Brown (1996) reported observing >13,000 adult *O. vicarius* infesting 115 nests over a 4-wk period at a site previously unoccupied by cliff swallows. Given that this was a newly colonized site, *O. vicarius* observed were assumed to represent immigrants. Assuming these immigrants then randomly mated with residents in fumigated colonies that might have had a resistance allele, immigration of unexposed individuals could prevent selection and long-term establishment of any resistance mutations (Tabashnik and Croft 1982). Furthermore, across colonies and within single nests, genetic diversity is high (Moore 2016), and, unlike in other cimicids (Booth et al. 2012, 2015; Saenz et al. 2012; Fountain et al. 2014), inbreeding in this species is likely insignificant. The establishment of resistance alleles, assuming they arise, is therefore likely to be inhibited by the frequent immigration of nonresistant individuals coupled with outbreeding. As such, due to

these biological and ecological factors, the pressure exerted from insecticide treatments may have simply been insufficient to result in selection for, and a subsequent increase in, resistance genes at these sites.

Although we found no detectable insecticide resistance at fumigated colonies, differences were observed in the survival of *O. vicarius* unexposed to Dibrom (i.e., control treatments). This varied with both fumigation history and bird occupancy status of the colonies from which they came. *Oeciacus vicarius* from the long-term fumigation sites had much higher survival than ones from never-fumigated sites, after controlling for occupancy status. This suggests that *O. vicarius* present in fumigated sites—likely ones recently immigrating into that site since its last treatment—may have higher fitness than *O. vicarius* collected at unmanipulated sites. Why this may be is unclear; however, it is possible that immigrating individuals were those that had survived the winter diapause, and thus on average may have had higher fitness than individuals that chose to remain at their natal colony site. Additionally, a disperser may enter a colony with abundant resources due to a lack of competition from individuals in an established population, thus experiencing a release from competition; the increased opportunity to feed then elevates individual fitness. We also found relatively low survival of *O. vicarius* from inactive sites, compared with active ones, once controlling for fumigation status. This may reflect in part the feeding status of *O. vicarius*: those at inactive sites had not fed since at least the previous summer, whereas all at active sites had taken a recent bloodmeal. With individuals from inactive sites largely quiescent, experimental manipulation (i.e., collecting them) likely raised their metabolic rate, depleting the limited stored energy and resulting in the observed elevated mortality in relation to individuals at active sites.

Within cimicid insects, resistance to OPs has been widely reported in both *C. lectularius* and *C. hemipterus* (Feroz 1971, Karunaratne et al. 2007, Tawatsin et al. 2011); however, studies of resistance in other cimicid species are lacking. Our work shows that the OP Dibrom can remain an effective control agent on *O. vicarius*, even with prolonged exposure (32 yr at certain sites) and validates its use in long-term parasite removal studies (Brown and Brown 2015). In light of the recent host-species expansion of *O. vicarius* onto other species that reside within cliff swallow colonies, and thus the potential for a geographic range expansion via these alternative hosts, our findings suggest that Dibrom might be effective in other situations where unwanted infestation may occur.

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No Detectable Insecticide Resistance in Swallow Bugs (Hemiptera: Cimicidae) Following Long-Term Exposure to Naled (Dibrom 8). J Med Entomol. Evolution of insecticide resistance has been documented for bed bugs but not studied in *Oeciacus*. For periods of 17 and 32 yr, two cliff swallow colonies in western Nebraska were treated during the summer breeding season using the organophosphate insecticide Dibrom. A residual effect was observed in both lab and field trials: 100% mortality occurred in the lab after exposure to filter paper substrates treated both 5 and 10 d earlier, and in the field, nests treated once early in the season had *O. vicarius* counts 43 d later that were <1% of those from untreated nests within the same colony. No Detectable Insecticide Resistance in Swallow Bugs (Hemiptera: Cimicidae) Following Long-Term Exposure to Naled (Dibrom 8). Abstract The swallow bug, *Oeciacus vicarius* Horvath, is a hematophagous ectoparasite of the cliff swallow, *Petrochelidon pyrrhonota* Vieillot, and is closely related to bed bugs (*Cimex* spp.). Expand. 5. No Detectable Insecticide Resistance in Swallow Bugs (Hemiptera: Cimicidae) Following Long-Term Exposure to Naled (Dibrom 8). Journal of Medical Entomology 54.4 (2017): 994-998. Print. Sorger, D. et al. Molecular Markers Reveal Infestation Dynamics of the Bed Bug (Hemiptera: Cimicidae) Within Apartment Buildings. Vol. 49, Journal of medical entomology, 2012, pp. 535-546. Singham, G., E. Vargo, W. Booth, A. Othman, and C.-Y. Lee.