Effect of Buprofezin on Survival of Immature Stages of *Harmonia axyridis*, *Stethorus punctum picipes* (Coleoptera: Coccinellidae), *Orius tristicolor* (Hemiptera: Anthocoridae), and *Geocoris* spp. (Hemiptera: Geocoridae)

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ABSTRACT The effect of buprofezin, a chitin synthesis inhibitor, on development and survival of immature stages of *Harmonia axyridis* (Pallas), *Stethorus punctum picipes* Casey, *Orius tristicolor* (White), *Geocoris pallens* Stål, and *Geocoris punctipes* (Say), was examined in a series of laboratory bioassays. Very few *H. axyridis* larvae (3.1%) treated with buprofezin reached adulthood, although 65% of treated pupae emerged successfully. Buprofezin caused no mortality to eggs of *S. punctum picipes* but 71.1% of treated early instar larvae failed to complete development. Eighty percent of treated late instars and 92.3% of pupae produced viable adults. Early instar nymphs of *O. tristicolor* were unaffected by buprofezin, whereas 47.7 and 85% of *G. punctipes* and *G. pallens* nymphs, respectively, failed to complete development. Treated eggs of *G. pallens* hatched successfully. The use of buprofezin in integrated pest management in Washington state wine grapes is discussed.

KEY WORDS insect growth regulator, buprofezin, development, survival, predators

INSECT GROWTH REGULATORS (IGRs) are valuable chemical tools suitable for, and widely used in, integrated pest management (IPM) programs. They generally combine good efficacy against target pests while conserving predators and parasitoids (Ishaaya 1990). However, some IGRs have been shown to have negative impacts on some beneficial arthropods (Ragusa Di Chiara et al. 1993, Sauphanor et al. 1993, Butaye and Degheele 1995). In particular, IGRs seem to have detrimental impacts on some species of Coccinellidae, including *Rodolia cardinalis* (Mulsant) (Loia and Viggiani 1992), *Chilocorus bipustulatus* (L.) (Peleg 1983), and *Stethorus punctum punctum* (LeConte) (Biddinger and Hull 1995). Buprofezin, a chitin synthesis inhibitor, has been used against pest homopterans (e.g., planthoppers, leafhoppers, whiteflies, scale insects, and mealybugs) in many crops worldwide since the mid-1980s (Nagata 1986, Yarom et al. 1988). The International Organisation for biological and integrated control of noxious animals and plants/West Palaearctic Regional Section joint pesticide testing program on beneficial arthropods concluded buprofezin was harmless (<30% mortality) to 16 of 19 beneficial insect and mite species that were tested (Hassan et al. 1994). The exceptions were a cecid fly *Aphidoletes aphidimyza* (Pondani) and the coccinellids *Coccinella septempunctata* L. and *Semiadalia undecimnotata* Schneider, Smith and Papacek (1990) showed that buprofezin was moderately toxic to larvae of *Cryptolaemus montrouzeri* Mulsant, an important coccinellid used in biological control of mealybugs. Smith (1995) showed that buprofezin caused significant larval mortality and reduced egg production in the scale-feeding coccinellid *Chilocorus circumdatus* Gyllenhal. Field-weathered residues of buprofezin and two other IGRs, pyriproxyfen and triflumuron, were shown by Hattingh and Tate (1995) to prevent egg hatching of *C. montrouzeri* and *Chilocorus nigrita* (F.). Recent studies documented the safety of buprofezin to a lacewing (Liu and Chen 2000) and a parasitic wasp (Hoddle et al. 2001) but reports of incompatibility of buprofezin with coccinellids continue (Magagula and Samways 2000, Grafton-Cardwell 2000, Grafton-Cardwell and Gu 2003).

Buprofezin has recently been registered for use on grapes in Washington state, providing an alternative to broad-spectrum insecticides for the control of leafhoppers and mealybugs (James 2003a). Development of an effective grape IPM program in Washington has been inhibited by widespread early season use of chlorpyrifos, dimethoate, and imidacloprid (James et al. 2002). The availability and use of buprofezin should provide a boost to IPM efforts. However, given the fact that this IGR is not without harmful effects to beneficial insects, I investigated its impact in laboratory

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Table 1. Mean (± SE) percentage of egg hatch or survivorship to adulthood of five beneficial insect species exposed to direct spray and residue of buprofezin (0.0006% [AI]) in laboratory tests

Insect	Developmental stage							
	Egg	*n*	Early instar	*n*	Late instar	*n*	Pupa	*n*
S. punctum picipes	100	35	28.9 (5.2)	46	80.0 (9.9)	50	92.3 (5.3)	45
H. axyridis			0	57	3.1 (0.9)	39	65.0 (7.7)	38
O. tristicolor			100	35				
G. pallens	100	35	15.0 (2.3)	32				
G. punctipes			52.3 (6.7)	42				

bioassays on the immature stages of four species of beneficial insect present in Washington vineyards plus one species available commercially.

Materials and Methods

The effect of buprofezin on development and survival of immature stages of generalist coccinellid *Harmonia axyridis* (Pallas), mite-feeding coccinellid *S. punctum picipes,* anthocorid *Orius tristicolor* (White), and the geocorids *G. pallens* and *Geocoris punctipes* (Say), was examined in a series of laboratory bioassays conducted during June through November 2002. All insects except *G. punctipes* were obtained from an unsprayed hop yard at Washington State University's Irrigated Agriculture and Extension Center at Prosser in south central Washington. *G. punctipes* was obtained from Entomos LLC, Gainesville, FL. Developmental stages tested for each species (identified by daily observations of cohort development) were as follows: for *S. punctum picipes,* eggs, early instars (1–2), late instars (3–4), and pupae; for *H. axyridis,* early instars (1–2), late instars (3–4), and pupae; for *O. tristicolor,* early instars (1–2); for *G. pallens,* eggs and early instars (1–2); and for *G. punctipes,* early instars (1–2). Insects were directly sprayed with the maximum recommended dosage of buprofezin (Applaud 70 WP Insect Growth Regulator, Nichino America Inc., Wilmington, DE) for grape leafhoppers (0.54 kg [AI]/ha, 0.0006% [AI]), assuming a dilution factor of 935 liters of water per hectare. Bioassays were conducted using a Potter Precision Spray Tower (Burkard, Rickmansworth, UK) (Potter 1952). The tower spraying pressure was 50 kPa and 2 ml of liquid was used giving a deposit of 1.6–1.8 mg of liquid per square centimeter. All insects were treated within 24 h of collection or receipt from Entomos LLC. Small plastic cups (44 mm in diameter) with muslin lids served as the basic bioassay setup. Eggs of *S. punctum picipes,* and *G. pallens* were excised from hop leaves by cutting a small leaf platform for individual eggs. Up to five eggs were placed in single cups containing a grape leaf disc (same diameter as the cup) resting on moist cotton wool. Larvae and nymphs of the five insect species were transferred, by using a fine, soft brush, from hop leaves to plastic cups containing moist cotton wool and grape leaf discs as the substrate. Pupae were excised from leaves on platforms. A single larva, nymph, or pupa was placed in each cup to avoid problems with cannibalism and to allow the fate of individuals to be monitored. All insects were sprayed in their holding cups and held after treatment in a constant growth chamber maintained at 28 ± 1°C under constant illumination. Observations on insects were made daily. Eggs were recorded as hatched or dead after 5–10 d. Ecdysis and mortality were recorded for larvae and nymphs until adulthood was attained. Adult emergence and postemergence survival (48 h) were recorded for pupae. A minimum of 10 individuals constituted a replicate for each tested insect stage with three to five replicates performed. Control (sprayed with water) replicates were also conducted for each insect stage tested. Untreated eggs and motiles of twospotted spider mite, *Tetranychus urticae* Koch, were provided daily as prey for *S. punctum picipes* larvae and nymphs of *O. tristicolor* and *Geocoris* spp. Untreated aphids [primarily pea aphid, *Acyrthosiphon pisum* (Harris)] were provided daily as food for *H. axyridis* and as supplemental food for *O. tristicolor* and *Geocoris* spp. Data for all insects were corrected for control mortality (Abbott 1925), and tests were discarded if mortality exceeded 10%.

Results

S. punctum picipes. Buprofezin caused no mortality to eggs. However, it prevented ecdysis in 71.1% of early instars. Twenty percent of treated late instars failed to pupate, died in the pupal stage, during adult emergence, or in the 48 h after emergence. Only 7.7% of treated pupae failed to emerge (Table 1). All emerging adults survived for at least 48 h. All larvae failing to complete development exhibited similar symptoms: flattened appearance and ecdysis or partial ecdysis with larvae "trapped" in the old cuticle.

H. axyridis. No early instars and very few late instar larvae (3.1%) completed development after exposure to buprofezin (Table 1). Treated pupae had a higher survival rate with 65% of individuals emerging and surviving for at least 48 h. The remainder emerged but failed to harden their cuticles, leaked fluid, and died within hours of emergence. Affected larvae characteristically became "plump" and unable to ecdyse. In some instances, partial ecdysis occurred. Treated late instars usually died in the prepupal stage, unable to ecdyse to pupae.

O. tristicolor. Nymphs of this species suffered no mortality with all treated individuals reaching adulthood and surviving the 48 h postemergence period (Table 1).

***Geocoris* spp.** All eggs of *G. pallens* sprayed with buprofezin hatched. Buprofezin interfered with ecdysis in nymphal geocorids, resulting in 52.3% survival to adulthood in *G. punctipes* and only 15% survival in *G. pallens* (Table 1). Interference occurred primarily to the molt after treatment; if this molt was successful, adulthood was generally achieved.

Discussion

The results from this laboratory study indicate the effect of buprofezin on five predatory insects varied according to species. The bioassay methodology used in this study guaranteed exposure of the test insects to a direct spray and residual concentration of buprofezin equivalent to the maximum dosage likely to be encountered in Washington vineyards. Pesticide applications in the field are subject to numerous variables, including weather, spray equipment calibration, coverage, insect behavior, and human error, most of which serve to reduce the chances of individual insects receiving the maximum dose. Thus, the pesticide effects on insects observed in studies such as this represent an optimal situation. If no direct lethal effects are observed in the laboratory, then it is unlikely that they will occur in the field.

H. axyridis, a generalist coccinellid feeding on prey as diverse as aphids (Hukusima and Kamei 1970), weevil eggs (Kalaskar and Evans 2001), and mites (Lucas et al. 1997), was the species most affected in this study. Exposure of larvae to buprofezin at any age caused almost 100% mortality. Pupae were less affected. Eggs were not tested but given its severe effect on larvae, buprofezin is likely to have a substantial impact on vineyard populations of *H. axyridis.* Single tests on small groups of *Hippodamia convergens* Guérin-Méneville larvae ($n = 15$) and pupae ($n = 10$) showed $\approx$50% survival to adulthood in both cases (D.G.J., unpublished data). This species, along with *Coccinella transversogutatta* Brown, is also common in Washington vineyards and both should be examined in more detail for buprofezin susceptibility. Although this seems to be the first report on the adverse impact of buprofezin on *H. axyridis*, similar observations have been made concerning this IGR and immature development of other coccinellids. For example, Magagula and Samways (2000) found buprofezin to be highly detrimental to larvae of the scale insect-feeding coccinellid *C. nigritus* (=*nigrita*). Similar results were found by Smith and Papacek (1990) for *C. montrouzeri* and by Smith (1995) for *Chilocorus circumdatus* Gyllenhal. Buprofezin is not toxic to adult coccinellids (Smith and Papacek 1990, Lo and Blank 1992) but seems to have a sterilizing effect on some species. The viability of eggs laid by *C. nigrita* exposed for six days to three-week old residues of buprofezin was significantly reduced (Hattingh and Tate 1995). The use of pyriproxyfen and buprofezin in South African and Californian citrus orchards for control of California red scale, *Aonidiella aurantii* (Maskell), in the early and late 1990s, respectively, caused major outbreaks of cottony cushion scale, *Icerya purchasi* Maskell (Hattingh and Tate 1995, Grafton-Cardwell 2000; Grafton-Cardwell and Gu 2003). Detrimental effects of these two IGRs on larval development and viable egg production in vedalia, *Rodolia cardinalis* (Mulsant), are considered to be the prime cause.

S. punctum picipes is an important spider mite predator on several crops in Washington, including grapes. Antonelli et al. (1996) and James (2003b) reported on the susceptibility of *S. punctum picipes* to several synthetic pyrethroid and neonicotinoid insecticides and its relative tolerance to some organophosphate compounds. This is the first study to evaluate the impact of an IGR on *S. punctum picipes,* although Biddinger and Hull (1995) tested the effects of several IGRs (not buprofezin) on the congeneric *S. punctum punctum.* They found teflubenzuron, another chitin synthesis inhibitor, to have no detrimental effect on eggs or larvae, although individuals exposed as larvae suffered high mortality as pupae. Buprofezin was not ovicidal to *S. punctum picipes* but caused high mortality to larvae treated as first or second instars. Beetles treated as late instars or pupae were fairly tolerant of buprofezin. These results indicate *S. punctum picipes* in vineyards might be less affected by buprofezin than *H. axyridis.* However, we did not examine the possibility that exposure of adults to buprofezin may affect the viability of eggs produced because it does in some other coccinellids (Hattingh and Tate 1995). It is also possible that consumption of spider mites contaminated with buprofezin might increase the effect on larvae of *S. punctum picipes.*

No impact of buprofezin was observed on nymphal development of *O. tristicolor.* Hassan et al. (1994) reported buprofezin was harmless to the European pirate bug, *Anthocoris nemoralis* (F.). Conversely, the other heteropterans evaluated in this study, two species of bigeyed bugs, seemed to be affected by buprofezin with nymphal mortality of up to 85% (*G. punctipes*), but there was no effect on eggs of *G. pallens.* No serious impact of buprofezin on the survival and viability of other noncoccinellid beneficial arthropods has been reported (Hassan et al. 1994). Several studies have shown no or minimal impact of buprofezin on hymenopteran parasitoids, particularly Aphelinidae (Garrido et al. 1985, Smith and Papacek 1990, Gerling and Sinai 1994, Hoddle et al. 2001). A demographic approach to measurement of toxicological effects on natural enemies is currently advocated (Stark and Banks 2003) and would determine more accurately the sublethal effects of buprofezin (e.g., reduced viability of eggs and prolonged developmental) on beneficial arthropod populations. However, in the context of grape pest management in Washington state, it is clear that buprofezin is an insecticide more compatible with biological control than alternative broad-spectrum insecticides such as chlorpyrifos (James 2001). Although some deleterious impacts on beneficial arthropod populations can be expected with buprofezin, they will likely not be catastrophic. None of Washington's grape pests have coccinellid beetles as key controlling agents, in the way that vedalias are primarily responsible for control of cottony cushion scale in citrus (Grafton-Cardwell 2000). Coccinellids play some role in the biological control of mealybugs, leafhoppers, and mites in Washington grapes, but parasitic hymenoptera and predatory mites are probably more important (Cone et al. 1990, James et al. 2002).

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