Research Article

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Laboratory assays of select candidate insecticides for control of *Dendroctonus ponderosae*

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Abstract

BACKGROUND: The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), is the most destructive bark beetle in western North America. *Dendroctonus ponderosae* can be prevented from successfully colonizing and killing individual trees by ground-based sprays of insecticides applied directly to the tree bole. However, the future availability of several active ingredients, including carbaryl which is most commonly used in the western United States, is uncertain. Two novel insecticides, cyantraniliprole [Cyazypyr™-OD (oil dispersion) and Cyazypyr™-SC (suspension concentrate)] and chlorantraniliprole (Rynaxypyr®), and carbaryl were assayed in both filter paper and topical assays.

RESULTS: Compared with 20 000 mg L^{-1} carbaryl (i.e. the maximum label rate for solutions applied to conifers for protection from bark beetle attack in the western United States), cyantraniliprole OD caused similar rates of mortality in *D. ponderosae* adults at 400-fold weaker concentrations in both bioassays, while cyantraniliprole SC caused similar rates of mortality at 40-fold weaker concentrations. Probit analyses confirmed that *D. ponderosae* is most sensitive to cyantraniliprole OD, while chlorantraniliprole was effective at concentrations similar to carbaryl.

CONCLUSIONS: These results suggest that lower concentrations of carbaryl have merit for field testing than have been previously considered. While cyantraniliprole and chlorantraniliprole have similar modes of action, cyantraniliprole OD appears to have greater promise for protecting individual trees from mortality attributed to *D. ponderosae* attack and should be evaluated in field studies.
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Keywords: carbaryl; chemical control; chlorantraniliprole; cyantraniliprole; *Dendroctonus ponderosae*; mountain pine beetle; Scolytinae

1 INTRODUCTION

About 8% of forests in the United States are classified at risk (defined as >25% of stand density will die in the next 15 years) to insect and disease outbreaks.[1] The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), is ranked most damaging of all agents considered.[1] This species ranges throughout British Columbia and Alberta, Canada, most of the western United States and into northern Mexico, and colonizes several pine species, most notably lodgepole pine, *Pinus contorta* Dougl. ex Loud., ponderosa pine, *P. ponderosa* Dougl. ex Laws., sugar pine, *P. lambertiana* Dougl., whitebark pine, *P. albicaulis* Engelm., and western white pine, *P. monticola* Dougl. ex D. Don.[2] *Dendroctonus ponderosae* typically initiates and concentrates attacks in the lower tree bole, facilitating host colonization through the use of aggregation pheromones.[3] A tree is considered 'mass attacked' when sufficient numbers of beetles are present to overcome host tree defenses. Partial attacks, often referred to as 'strip attacks', may occur if sufficient numbers of beetles are not present, and these trees may survive for many years. In brief, tree death occurs by girdling of the phloem (i.e. layers of cells just inside the bark that transport photosynthate within the tree) by both colonizing adults and developing larvae.[3] In the western United States, >5.5 million ha were impacted by *D. ponderosae* during 2001–2006,[4] while >9 million ha have been impacted in British Columbia, Canada, since 2003.[5]

Attacks by *D. ponderosae* reduce tree growth and hasten decline (as a result of strip attacks), cause tree mortality and subsequent replacement by other tree species and may impact timber and fiber production, water quality and quantity, fish and wildlife populations, recreation, grazing capacity, real estate values, biodiversity, carbon storage, endangered species and cultural resources. Trees located in residential, recreational (e.g. campgrounds) or administrative sites are particularly susceptible to attacks by *D. ponderosae* as a result of increased amounts of stress associated with drought, soil compaction, mechanical

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injury or vandalism.[6] Tree losses in these environments pose potential hazards to public safety. Costs associated with hazard tree removal and litigation can be substantial,[7] and property values may be significantly reduced by mortality of adjacent shade and ornamental trees.[8] The value of these trees, the cost of removal and the loss of aesthetic value often justify protecting individual trees with insecticides, particularly during bark beetle outbreaks.

Protection of individual trees from attack by *D. ponderosae* and other bark beetles in the western United States has historically involved applications of liquid formulations of contact insecticides to the tree bole using hydraulic sprayers. For example, benzene hexachloride, fenitrothion and chlorpyrifos were registered for this use, but all three registrations have been canceled or withdrawn. Several pyrethroids (e.g. permethrin and bifenthrin) are registered and effective for protecting individual trees from attacks by bark beetles, but generally provide protection for a period of $\leq$1 year with a single application.[6,9–11] Several researchers have reported that carbaryl is still one of the most effective, economically viable, and ecologically compatible insecticides available for protecting individual trees from bark beetle attack in the western United States,[10] and generally provides protection for a period of 2 years with a single application.[6,10,11] As a result, carbaryl is commonly used to protect trees from attacks by *D. ponderosae*, but its use on trees is continually being challenged, and it is uncertain how long carbaryl will retain registration for this use. This situation emphasizes the need for assuring that effective insecticide treatments are available for protecting individual trees from bark beetle attacks. As these tools are applied in a preventive manner (i.e. in order to prevent tree mortality by protecting trees prior to attack), bark beetles must be killed or incapacitated quickly before successful tree colonization and girdling of the phloem tissue occurs.

The objective of this study was to determine the toxicity to *D. ponderosae* adults of two novel insecticides, cyantraniliprole (Cyazypyr™) and chlorantraniliprole (Rynaxypyr®), which are being considered for future field testing, and carbaryl in both filter paper and topical assays conducted in the laboratory. Chlorantraniliprole recently obtained registration (2006) in the United States for cotton, grapes, tree fruits and certain vegetables as a reduced-risk insecticide (Insecticide Resistance Action Committee, 2009) and controls insect pests through a unique mode of action by activating ryanodine receptors (RyR), which play a critical role in muscle function. Ryanodine receptors act as selective ion channels, modulating the release of calcium. Chlorantraniliprole binds to the RyR, causing uncontrolled release and depletion of internal calcium, thus preventing further muscle contraction and ultimately leading to death. Chlorantraniliprole is of very low toxicity to vertebrates, and results are favorable for a range of tests, including carcinogenicity, mutagenicity, neurotoxicity and reproductive toxicity.[12,13] Cyantraniliprole is a second-generation RyR insecticide with a similar mode of action to chlorantraniliprole. Registration of cyantraniliprole is expected by 2012 in the United States for several agricultural crops. Carbaryl is registered for control of a wide range of insect pests on >100 agricultural crops and non-crop uses. Carbaryl is an acetylcholinesterase inhibitor preventing the cholinesterase enzyme from breaking down acetylcholine, increasing both the level and duration of action of the neurotransmitter acetylcholine, which leads to rapid twitching, paralysis and ultimately death.

2 MATERIALS AND METHODS

2.1 Collection of insects

Live *D. ponderosae* adults were obtained through collections in 16-unit multiple-funnel traps baited with *D. ponderosae* lures consisting of *trans*-verbenol, *exo*-brevicomin, myrcene and terpinolene (Synergy Semiochemicals Corp., Burnaby, BC). A total of 30 traps were deployed in two locations, 20 on the Eldorado National Forest, CA (38.49 °N, 120.15 °W; 2028 m elevation) and ten on the Lake Tahoe Basin Management Unit, CA (38.53 °N, 119.54 °W; 2390 m elevation). Trap locations were selected on the basis of ground surveys indicating that *D. ponderosae* was actively colonizing *P. contorta* in the area. Traps were hung on 3 m metal poles with collection cups 80–100 cm above the ground. Crumpled paper towels were placed in each collection cup to reduce the number of beetles that escaped capture and to decrease damage to and predation of *D. ponderosae* by creating a diverse substrate and refugia (Hayes JL, private communication, 2010). Captures were collected daily (as needed) from 1 July to 5 August 2010 and immediately transported to the laboratory (≈60 and 108 km from collection sites respectively) in wax-coated paper containers (960 mL; Solo Cup Co., Highland Park, IL) with four small circular holes (≈0.8 mm diameter) placed in the top to facilitate ventilation. These containers were placed in coolers containing blue ice (Rubbermaid®, Huntersville, NC) during transport. Upon return to the lab, specimens were identified, sorted [i.e. damaged (loss of any appendages) or weakened (did not immediately walk) individuals were discarded] and stored in wax-coated paper containers (see above) containing a paper towel moistened with distilled water to prevent dehydration (Hayes JL, private communication, 2010). Beetles were stored for up to 48 h in a refrigerator at 5 °C until enough (210 beetles) were accumulated to complete a single replicate. Beetles that were not assayed within 48 h were discarded.

2.2 Treatments

Four insecticide treatments and an untreated control were assayed. Five tenfold serial dilutions of each insecticide were prepared in distilled water. Two formulations of cyantraniliprole [Cyazypyr™-OD (oil dispersion) and Cyazypyr™-SC (suspension concentrate); research and demonstration formulations; EI du Pont de Nemours and Company, Stine-Haskell Research Center, Crop Protection Products, Newark, DE] were assayed at 0.5, 5, 50, 500 and 5000 mg AI L^{-1}. Chlorantraniliprole [Rynaxypyr® (Coragen® SC); EI du Pont de Nemours and Company, Wilmington, DE; EPA Reg. No. 352–729] was also assayed at 0.5, 5, 50, 500 and 5000 mg AI L^{-1}. Carbaryl (Sevin® SL; Bayer Environmental Science, Montvale, NJ; EPA Reg. No. 432–1227) was assayed at 20, 200, 2000, 20 000 (maximum labeled rate in the United States) and 200 000 mg AI L^{-1} and included as an internal standard owing to its common use for protecting trees from bark beetle attack in the western United States.

2.3 Filter paper assay

A quantity of 1 mL of each solution (e.g. 20 000 mg L^{-1} carbaryl) was applied with a micropipette to one 9 cm diameter glass microfiber filter disc (Whatman 934-AH, 1.5 µm pore size; Cole-Parmer, Vernon Hills, IL), stored in a 10 cm diameter sterile polystyrene petri dish (Cole-Parmer) and allowed to dry in a fume hood for 2 h (four insecticide treatments × five concentrations + untreated control = 21 dishes per replicate). Prepared petri dishes (i.e. those containing insecticide-treated and dried glass microfiber filter discs) were stored for later use in airtight plastic bags at 5 °C for $\leq$7 days, after which time they were discarded.

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The experiment was replicated 8 times ($N = 168$ dishes). Ten *D. ponderosae* were randomly selected from recently captured individuals (i.e. stored up to 48 h) and placed on each treated filter paper in the bottom of the petri dish ($N = 1680$ beetles). Small holes (≈0.8 mm diameter) were drilled into the tops of each petri dish to facilitate ventilation. Petri dishes were stored in a large fume hood (Model No. 4 863 000; Labconco Corp., Kansas City, MO) with airflow of 4.1 m^3 min^{-1} under static abiotic conditions (≈20 °C, RH = 45%, 14.5 h light), and the number of dead and moribund individuals (i.e. defined as those that could no longer right themselves and walk) was recorded within each dish at 6, 12, 24, 48, 96, 120, 144 and 168 h. Dead individuals were immediately removed from petri dishes, and the gender was later determined through examination of the seventh abdominal tergite[14] for the presence of an angular margin (serving as a stridulating organ in males) with a compound microscope.

2.4 Topical assays

Ten *D. ponderosae* were randomly selected from recently captured individuals (i.e. stored for up to 48 h) and treated topically with 0.5 µL of each insecticide solution to the ventral surface of the mesothorax of each *D. ponderosae* using a micropipette. Treated *D. ponderosae* were then transferred into petri dishes (four insecticide treatments × five concentrations + untreated control = 21 dishes per replicate; ten *D. ponderosae* per dish) lined with untreated 9 cm diameter glass microfiber filter discs (Whatman 934-AH). Micropipette tips were discarded after application of each solution. Petri dishes were then stored in a large fume hood under static conditions (Section 2.3), and the number of dead and moribund individuals was recorded within each dish at 6, 12, 24, 48, 96, 120, 144 and 168 h. The experiment was replicated 8 times ($N = 1680$ beetles). As above, dead individuals were immediately removed from petri dishes and the gender was determined through examination of the seventh abdominal tergite.[14]

2.5 Statistical analysis

2.5.1 Survival curves

The life-table method[15] was used to estimate the survival probability of *D. ponderosae* subjected to different doses of each insecticide, and to compare the survival curves from filter paper and topical assays. The non-parametric Mantel log-rank test with the Dunnet adjustment was used to compare treatment doses with 20 000 mg L^{-1} carbaryl and the untreated control. The SAS (SAS v.9.2; SAS Institute, Cary, NC) Lifetest procedure was used to estimate the survival probabilities and confidence intervals, and to test the multiple comparisons.

2.5.2 Lethal doses

To compare dose assays, a logistic regression model with the probit link from the family of the generalized linear model (GLM)[16,17] was fitted to the data (number of dead *D. ponderosae* from ten initial *D. ponderosae* for each replicate), assuming a binomial distribution. The statistical model for the proportion of dead beetles for each time period (0–6, 0–12, 0–24, 0–48 and 0–72 h; because of the high mortality rates after 72 h, longer time periods were not included in this analysis) is as follows:

$$P_{ij} = \Phi[a_{ij} + b_{ij} \times \log(\text{dose})]$$

where P_{ij} is the probability of mortality at the end of a given period j for treatment i for a given dose, Φ is the cumulative standard normal distribution function, a_{ij} and b_{ij} are the intercept and slope for the respective treatment i and log(dose) is the logarithm of the dose (the log function of the dose improved the fitting). For each time period j and treatment i, LC_{50} was calculated as

$$LC_{50} = e^{-\frac{a_{ij}}{b_{ij}}}$$

The SAS NLMIXED procedure was used to estimate the coefficients and compare the LC_{50} values among treatments at a given time period. The Bonferroni approach was used for pairwise comparison tests to attain an experiment-wise error rate equal to 0.05.

3 RESULTS AND DISCUSSION

3.1 Survival probability

In the field, the effectiveness of carbaryl for protecting individual trees from *D. ponderosae* attack has been well established for some time.[6,10,11,18–22] For example, Shea and McGregor[22] evaluated 0.5, 1.0 and 2.0% carbaryl and found that all concentrations and formulations were effective for protecting *P. contorta* from *D. ponderosae* attack for 1 year. In the filter paper assay, no significant differences were found in survival probability between 20 000 mg L^{-1} carbaryl (i.e. the maximum label rate typically used in commercial applications, hereafter referred to as the internal standard) and other concentrations of carbaryl until the concentration was reduced 1000× (20 mg L^{-1}) (Table 1). The survival of *D. ponderosae* exposed to 20 mg L^{-1} of carbaryl was >70% at 24 h (Fig. 1) and was not significantly different from the untreated control (Table 1). In the topical assay there was no significant difference in survival probability between the internal standard and 200 000 mg L^{-1} of carbaryl, but there was significantly higher survival probability with carbaryl concentrations of ≤2000 mg L^{-1} compared with the internal standard (Table 1). Survival probabilities of *D. ponderosae* exposed to ≤20 mg L^{-1} of carbaryl in topical assays was >85% at 24 h (Fig. 2). When compared with the untreated control, *D. ponderosae* exposed to carbaryl residues of ≥2000 mg L^{-1} in the topical assay had significantly lower survival probability (Table 1). While significant data exist on the effectiveness of carbaryl for protecting individual trees from attack by *D. ponderosae*, these are the first data detailing its toxicity to *D. ponderosae* in laboratory assays. Results from the filter paper assays suggest that lower concentrations than the internal standard (100× lower, 200 mg L^{-1}) could be considered for field testing, but this is not supported by the topical assays (where lower concentrations exhibited significantly higher survival probabilities).

Cyantraniliprole is believed to hold great promise for insect control, based on its properties of improved plant mobility, significant activity on Lepidopteran pests and an increased spectrum of activity that is known to include members of Hemiptera.[23] However, no data have hitherto been published on its effectiveness for controlling forest Coleoptera, probably because the chemistry was only recently developed. Similar trends were observed for cyantraniliprole OD (as compared with the internal standard) in both filter paper and topical assays (Table 1). Cyantraniliprole OD had significantly higher survival probability than the internal standard at concentrations of ≤50 mg L^{-1} (Table 1). Survival rates of *D. ponderosae* exposed to ≤5 mg L^{-1} in filter paper assays were >60% at 24 h (Fig. 1). No significant difference was observed between the internal standard and ≥500 mg L^{-1} of cyantraniliprole OD. In the filter paper assay,

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Table 1. The χ^2 values for pairwise comparisons, using the log-rank test, of survival probabilities of *Dendroctonus ponderosae*, comparing treatments with 20 000 mg L^{-1} carbaryl (internal standard; maximum label rate typically used in commercial applications) and the untreated control, Placerville, California[a]

		Filter paper (χ^2)		Topical (χ^2)	
Insecticide	Conc. (mg L^{-1})	Carbaryl (20 000 mg L^{-1})	Untreated control	Carbaryl (20 000 mg L^{-1})	Untreated control
Control	–	137.1*	–	84.8*	–
Carbaryl	20	106.2*	2.2	101.8*	0.5
	200	2.6	105.2*	90.7*	0.02
	2000	0.1	131.4*	34.4*	11.8*
	20 000	–	138.7*	–	84.8*
	200 000	2.5	104.7*	1.2	104.4*
Cyantraniliprole OD	0.5	166.8*	1.0	126.5*	3.7
	5	84.6*	6.7	97.0*	0.4
	50	29.1*	41.6*	98.3*	0.4
	500	4.7	94.1*	7.1	43.3*
	5000	2.0	108.4*	0.3	94.7*
Cyantraniliprole SC	0.5	136.8*	0.02	97.4*	0.4
	5	70.5*	11.4*	81.7*	0.03
	50	15.5*	62.0*	84.0*	0.02
	500	13.7*	65.6*	32.0*	13.0*
	5000	5.7	89.3*	0.5	72.9*
Chlorantraniliprole	0.5	134.7*	0.2	93.3*	0.2
	5	146.8*	0.1	99.2*	0.5
	50	63.3*	14.6*	108.5*	1.2
	500	32.2*	37.1*	84.4*	0.01
	5000	22.3*	49.8*	3.3	54.7*

[a]* Significant difference at an experiment-wise error rate of $\alpha = 0.05$.

concentrations of $\geq$50 mg L^{-1} of cyantraniliprole OD had lower survival probability than the untreated control, while in the topical assay the 50 mg L^{-1} concentration was not significantly different from the untreated control (Table 1). In the topical assay, *D. ponderosae* exposed to concentrations of $\leq$50 mg L^{-1} had survival rates of >85% at 24 h (Fig. 2). In both assays, cyantraniliprole SC had significantly higher survival probability than the internal standard in all concentrations but 5000 mg L^{-1} (Table 1, Figs 1 and 2). When compared with the untreated control, cyantraniliprole SC had lower survival probability at concentrations of $\geq$5 mg L^{-1} in the filter paper assay, but only at concentrations of $\geq$500 mg L^{-1} in the topical bioassay. In the topical assay, *D. ponderosae* exposed to concentrations of $\leq$50 mg L^{-1} had >85% survival at 24 h (Fig. 2).

While the toxicity of chlorantraniliprole to Lepidoptera is becoming well documented,[24,25] few data are published on its effect on Coleoptera. A recent study[26] reported that surface drenches of chlorantraniliprole significantly reduced the number of black vine weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae), larvae in *Sedum* plants by 92% compared with the untreated control, but was ineffective for controlling adults. In the present study, chlorantraniliprole had significantly higher survival probability than the internal standard at all concentrations evaluated in the filter paper assay, and in all but the highest concentration (5000 mg L^{-1}) in the topical assay. When compared with the untreated control, chlorantraniliprole had significantly lower survival probability at concentrations of $\geq$50 mg L^{-1} in the filter paper assay, but only the highest concentration was significantly different from the untreated control in the topical assay (Table 1). Survival rates of *D. ponderosae* exposed to $\leq$500 mg L^{-1} in topical assays were >80% after 24 h (Fig. 2). These results suggest that, while chlorantraniliprole and cyantraniliprole have similar modes of action, the latter holds greater promise for controlling *D. ponderosae* adults and for protecting individual trees from *D. ponderosae* attack.

3.2 Comparisons of LC_{50}

In the filter paper assay, mean LC_{50} values ranged from 3.0 to 132.9 mg L^{-1} for cyantraniliprole SC (24 h) and carbaryl (12 h) respectively (Table 2). In the topical assays, mean LC_{50} values ranged from 141.5 to 6298.9 mg L^{-1} for cyantraniliprole OD (24 h) and chlorantraniliprole (12 h) respectively (Table 2). Cyantraniliprole OD and cyantraniliprole SC had the lowest LC_{50} estimates, and only differed from one another in the 12 h topical assay analysis, in which the oil dispersed (OD) formulation was more toxic to *D. ponderosae*. The oil dispersed formulation may have allowed better adherence of cyantraniliprole to the beetle's venter and/or enhanced penetration of the active ingredient through the insect cuticle, thereby increasing toxicity in the topical (12 h) assay. On the other hand, LC_{50} values for carbaryl and chlorantraniliprole were not significantly different, but carbaryl had significantly higher LC_{50} values than cyantraniliprole OD in all analyses (Table 2). Chlorantraniliprole had the highest LC_{50} estimates of all treatments (Table 2).

3.3 Differences due to gender

In *D. ponderosae*, host colonization is initiated by females and mediated through aggregation pheromones[27–29] and host

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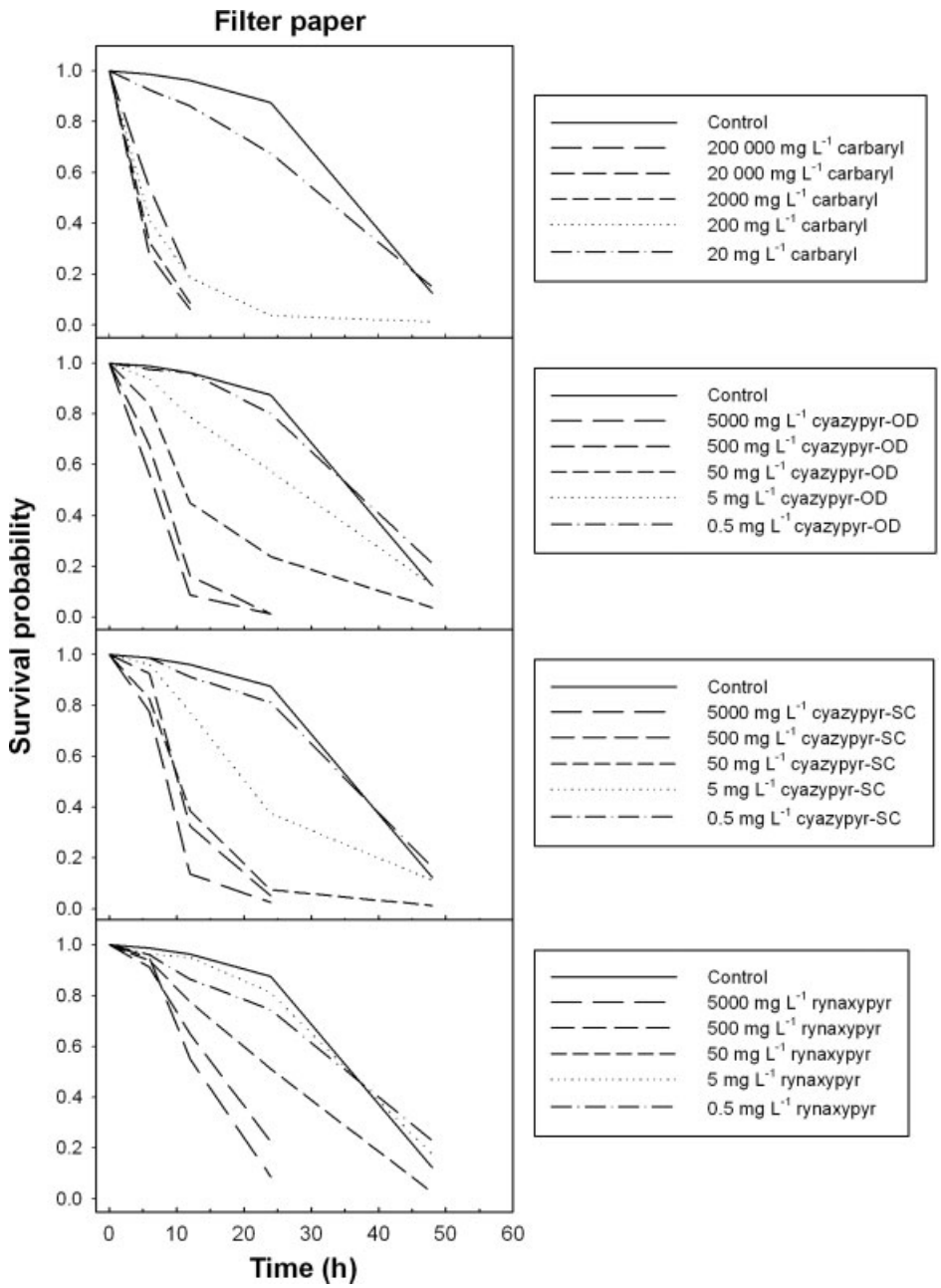

Figure 1. Survival probability curves for *Dendoctronus ponderosae* for carbaryl, cyantraniliprole (Cyazypyr) and chlorantraniliprole (Rynaxypyr) in the filter paper assay, Placerville, California.

kairomones. In this regard, it is obvious that, when evaluating tools for protecting trees from *D. ponderosae* attack, gender effects may be important. The results in the present analyses with respect to gender were inconsistent. In the filter paper assay there were significant differences in LC_{50} estimates owing to gender for cyantraniliprole OD and chlorantraniliprole, with males being more sensitive than females (lower LC_{50} estimates) in both cases (Table 3). However, in the topical assay, no gender effects were observed (Table 3).

4 CONCLUSION

Recently, the US Environmental Protection Agency received voluntary cancellation requests for several uses of carbaryl (USA Federal Register, 20 August 2008), including backpack sprayer applications and liquid formulations to residential lawns. These actions have heightened concerns that the availability of carbaryl for protecting individual trees from bark beetle attack may be limited in the future. To the authors' knowledge, these are the first data evaluating the toxicity of carbaryl, cyantraniliprole and chlorantraniliprole to *D. ponderosae* in laboratory assays. While significant data exist on the effectiveness of carbaryl for protecting individual trees from attack by *D. ponderosae*, results from the filter paper assay suggest that lower concentrations have merit for field testing than have been previously considered, but this is not supported by the topical assay. Neither assay method mimics field conditions, but the authors feel that the filter paper assay more closely approximates conditions under which

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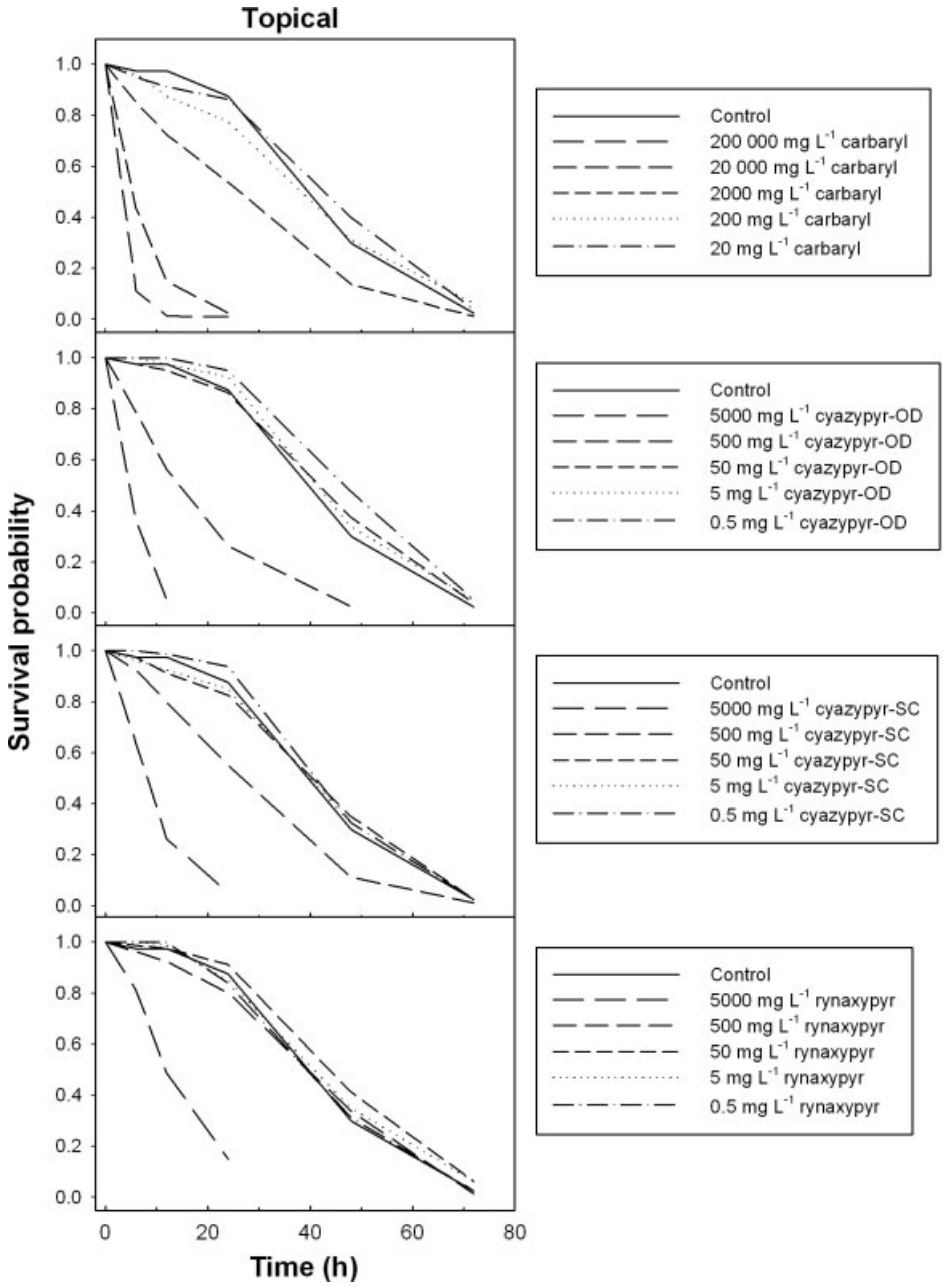

Figure 2. Survival probability curves for *Dendroctonus ponderosae* for carbaryl, cyantraniliprole (Cyazypyr) and chlorantraniliprole (Rynaxypyr) in the topical assay, Placerville, California.

D. ponderosae encounters toxicants during host colonization, and therefore that lower concentrations of carbaryl (e.g. 2000 mg L^{-1}) should be evaluated in the field. The experimental formulations of cyantraniliprole and chlorantraniliprole evaluated here all proved lethal to *D. ponderosae* in both filter paper and topical assays at concentrations within the present test range, including concentrations as low as 5 mg L^{-1} in the filter paper assay. While cyantraniliprole and chlorantraniliprole have similar modes of action, cyantraniliprole OD appears to have greater promise for protecting individual trees from mortality attributed to *D. ponderosae* attack and should be evaluated in the field as an alternative to the insecticides that are currently registered. Results from the filter paper assay suggest that concentrations of 50–500 mg L^{-1} are expected to cause significant (Fig. 1) mortality of *D. ponderosae* adults within 24 h. This is important, as beetles must be killed or incapacitated quickly before successful host colonization occurs.

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Table 2. Comparisons of LC_{50} concentrations for *Dendroctonus ponderosae* at 12 h and 24 h after application in filter paper and topical assays, Placerville, California

Application	Insecticide	LC_{50} (95% CL) (mg L^{-1})[ab] 12 h	24 h
Filter paper	Carbaryl	132.9 (57.2–208.7) a	19.1 (12.1–26.2) a
	Cyantraniliprole OD	46.1 (26.1–66.2) b	6.2 (3.5–8.9) b
	Cyantraniliprole SC	56.8 (26.5–87.1) ab	3.0 (1.5–4.4) b
	Chlorantraniliprole	–	34.9 (15.1–54.7) a
Topical	Carbaryl	2529.1 (1433.8–3624.5) a	628.7 (326.5–950.8) ac
	Cyantraniliprole OD	521.8 (349.4–694.2) b	141.5 (87.2–195.8) b
	Cyantraniliprole SC	1851.4 (672.3–3030.4) a	270.6 (133.5–407.6) ab
	Chlorantraniliprole	6298.9 (2128.9–10 468.9) a	1384.4 (73.3–2695.5) c

[a] Values within the same column and application type followed by the same letter are not significantly different at Bonferroni's adjusted alpha = 0.05/4 = 0.0125 to attain an experiment-wise error rate of $\alpha = 0.05$.
[b] – Estimate not significant at the $\alpha = 0.05$ level.

Table 3. Comparisons of LC_{50} estimates at 24 h post-treatment for female and male *Dendroctonus ponderosae* in filter paper and topical assays, Placerville, California

	Insecticide	LC_{50} (95% CL) (mg L^{-1})[a] Female	Male
Filter paper	Carbaryl	17.6 (9.9–25.2) a	22.9 (10.5–35.2) a
	Cyantraniliprole OD	10.1 (5.8–14.4) a	1.7 (0–3.4) b
	Cyantraniliprole SC	2.8 (1.2–4.3) a	3.4 (0.97–5.8) a
	Chlorantraniliprole	72.7 (30.8–114.6) a	11.0 (0–22.4) b
Topical	Carbaryl	682.2 (290.8–1073.6) a	766.1 (136.4–1395.9) a
	Cyantraniliprole OD	164.8 (98.2–231.4) a	102.0 (36.8–167.2) a
	Cyantraniliprole SC	257.8 (91.5–424.0) a	301.5 (79.8–523.1) a
	Chlorantraniliprole	1112.2 (0.35–2224.1) a	1751 (0–4588.7) a

[a] The LC_{50} estimates followed by the same letter are not significantly different at $\alpha = 0.05$ for comparing female and male LC_{50} values exposed to the same insecticide.

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