A Small-Bolt Method for Screening Tree Protectants Against Bark Beetles (Coleoptera: Curculionidae)[1]

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J. Entomol. Sci. 44(4): 297-307 (October 2009)

Abstract A simple, small-bolt method was developed and refined for evaluating and screening treatments being considered as prophylactics against bark beetles (Coleoptera: Curculionidae: Scolytinae). Using this method, 4 insecticide products (3 active ingredients) were evaluated against the southern pine beetle, *Dendroctonus frontalis* Zimmermann, intermittently during a period spanning 1 day to 9 months postapplication. Permethrin (Astro®) showed the best residual effectiveness as determined by the small-bolt assay, followed by bifenthrin (Onyx®) and carbaryl (Ferti-Lome® and Sevin XLR Plus®). Bifenthrin has been reported as effective in field tests with *D. frontalis* and carbaryl as ineffective, lending credence to the small-bolt method. Results with permethrin suggest that a more extensive evaluation may be warranted for this active ingredient. The method as developed provides a useful and efficient tool for identifying preventive treatments that are unlikely to be effective against *D. frontalis*. Its use for screening ineffective products would limit expensive and time-consuming field evaluations to treatments that show significant promise. With additional refinement, the small-bolt assay may provide most of the benefits of more costly testing methods while offering sufficient flexibility for comparing prophylactic treatments that rely on different modes of action.

Key Words pesticide evaluation, bioassay, prophylactic pesticides, pyrethroids, residual efficacy, *Dendroctonus*

Bark beetles in the genus *Dendroctonus* (Coleoptera: Curculionidae: Scolytinae) are major pests of pines, aggressively attacking trees and causing mortality in all types of North and Central American coniferous forests. The southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae: Scolytinae), is the most serious pest of mature yellow pines throughout southeastern North America (Drooz 1985). High-value pines in yards, parks, campgrounds and other aesthetically important areas can be threatened and warrant protective treatments (Cameron 1987, Hayes et al. 1996). Prophylactic pesticide treatments on individual trees can be relatively expensive, but are critical when trees are considered invaluable and imminently threatened by bark beetles. Their use presumes the availability of registered and efficacious products.

Consistently effective bark beetle preventatives are limited to chemical insecticides. Environmental and health concerns and public perceptions are driving the development of alternative, potentially less toxic products, but to date none has proven consistently effective (e.g., Strom et al. 2004, Progar 2005). The potential loss from

[1]Received 21 November 2008; accepted for publication 21 January 2009.

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the marketplace of insecticides currently registered for use against bark beetles makes efficacy evaluation of new synthetic pesticides and biorational alternatives an essential pest management goal.

Traditional efficacy tests of tree protectants are time-consuming and expensive. Typically, one of two methodologies is used: the “hanging bolt” assay (Berisford et al. 1980) which is popular in the southern U.S., and the in situ treatment assay (Shea et al. 1984) which is more frequently used in the western U.S. The hanging-bolt method requires less labor than in situ tests (Berisford et al. 1980) and increases the probability that a rigorous test will be achieved because bolts are transported to active infestations. In situ treatments provide tests more similar to real-world applications, but they are limited to environmental conditions present at the test (e.g., beetle population pressure). Significantly, a product’s efficacy can be directly observed in situ (tree death) and inferred by applying established statistical parameters (Shea et al. 1984). However, beetle populations must be sufficient to generate attack of control trees or the experiment fails (Shea et al. 1984), and, even within established parameters, lower beetle population densities can provide less rigorous tests. Aggressive beetles also can start undesirable infestations near in situ experimental trees (e.g., Strom et al. 2004). Both existing methods demand substantial commitment of resources (labor, travel, active beetle populations, uninfested stands with expendable trees, pesticide application certification and permits). The expense and difficulty of such studies suggest that they should be reserved for products with demonstrated promise; therefore, there is a need for efficient, practical, small-scale screening methods.

The objective of this study was to assess a simple, small-bolt assay as a screening tool for insecticides proposed as tree protectants for bark beetles. We evaluated active ingredients previously reported as effective (bifenthrin) or ineffective (carbaryl) in field tests with *D. frontalis* to guide interpretation of the assay’s utility. The small-bolt method uses small, easily manipulated bolts cut from trees prior to treatment, or from trees previously treated and aged over experimental periods. Bolts can be readily used in the laboratory for bioassays with the target bark beetle species, providing a simple, efficient and flexible method for screening products prior to additional testing in the field.

Materials and Methods

A series of experimental tests was conducted from April 2005 to May 2007 to evaluate toxicity and residual effectiveness of various insecticide products against *D. frontalis* in a small-bolt assay. Evaluations included products previously field-tested by other investigators with *D. frontalis* so that results would have a basis for interpretation. Bifenthrin (Onyx®, FMC Corporation, Philadelphia, PA; active ingredient [AI], 23.4%) was previously found to be effective in preliminary hanging-bolt and standing-tree evaluations (Berisford et al. unpubl., Booth unpubl.). Carbaryl products are ineffective against *D. frontalis* (Berisford et al. 1981), so provided a standard by which we could identify product failures in our assay. We employed 2 carbaryl formulations (see below): Ferti-Lome® (Voluntary Products Group, Inc., Bonham, TX, 23.7% AI) and Sevin XLR Plus® (Bayer CropScience LP, Research Triangle Park, NC, 44.1% AI). Astro® (FMC Corporation, AI = permethrin, 36.8%), has a current registration with US-EPA for use against bark beetles. Formulations of permethrin, applied at variable AI rates, have shown activity against other *Dendroctonus* species (Shea et al. 1984, Fettig et al. 2006b); however, effectiveness against *D. frontalis* is uncertain but appears to be less than bifenthrin (Berisford et al. unpubl., Booth unpubl.).

Formulated products were applied using one of two techniques. For evaluations at 1 d posttreatment, insecticides were applied directly to freshly-cut bolts (see below) with a paintbrush and allowed to dry for 24 h before exposing them to beetles. For assessments of residual activity over longer periods, products were applied to standing trees with a hand sprayer (Premium Ace Sprayer, Ace Hardware Corp., Oak Brook, IL) to bark saturation. Small *Pinus taeda* L. trees (approx. 9 cm diam at breast height [dbh]) were sprayed to a height of about 2.5 m of clear bole and left until cutting at the desired time. All insecticide treatments were randomly assigned to trees, and trees were randomly selected for use within each test. Due to practical limitations not all treatments were included in each trial.

Bioassays were conducted in the laboratory within cylindrical polystyrene containers (Pioneer Plastics, Inc., Dixon, KY) measuring 20 by 22 cm (~6900 cm^3) and with screened area of ~210 cm^2 dispersed between top and sides (Fig. 1). Test trees were cut, sectioned into small bolts and exposed to *D. frontalis* as soon as possible thereafter, usually within 2 d. Beetles originated from infestations in Mississippi or Alabama and were handled similarly for all evaluations. Infested trees were cut, sectioned, transported to our laboratory and placed into rearing boxes. Emerging beetles were held in refrigerated containers until they were counted and released with experimental bolts. Holding times varied from < 24 h to 4 d. To reduce handling, beetles were not sexed prior to their use. Assays were run at laboratory room temperature (approx. 25°C) for 48 h, at which time containers and bolts were refrigerated until dissection. Number of attacks and gallery length were determined for each bolt, and the numbers of dead and living beetles found in each container were recorded.

A total of 136 bolts was evaluated from 1 - 275 d (0 - 9 months) posttreatment. The sample size for each product was: Astro, 29 bolts; Ferti-Lome, 21 bolts; Onyx, 33 bolts; Sevin XLR Plus, 14 bolts and untreated, 39 bolts. The sample size for each period evaluated was: 0 month (4 - 19 bolts per treatment), 2 months (5 - 11), 4 months (10 - 13) and 9 months (2). Standard treatments were determined by the maximum

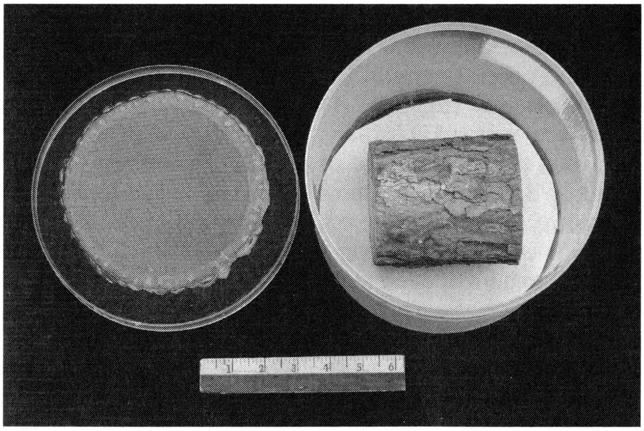

Fig. 1. Assay chamber and lid used for evaluating prophylactic performance of insecticides with *D. frontalis*. Ruler (6.25 inches = 15.9 cm) is included to show scale.

bark beetle rate provided by their labels, excepting the Ferti-Lome carbaryl product, which was mixed to achieve a 2% AI concentration to match the recommended rate of carbaryl for bark beetles (Haverty et al. 1985, Sevin XLR Plus label). This provided a bifenthrin product at 0.06% AI (0.95 L Onyx / 378.5 L water) and permethrin at 0.5% AI (5.1 L Astro / 378.5 L water; see labels, FMC Corporation). All bolts were *P. taeda* and measured ($\bar{X} \pm 1$ SEM): 9.25 ± 0.08 cm diam by 11.1 ± 0.05 cm long (321.6 ± 3.2 cm^2 bark surface area; n = 136). Bark surface area of untreated bolts was not a significant explanatory factor for number of attacks ($r^2 = 0.01$, $P > 0.50$) or gallery length ($r^2 = 0.03$, $P > 0.32$; linear regression), so size variation among bolts was not considered further.

Every trial included untreated bolts to assure beetle quality. For all evaluations we used typical precautions for selecting vigorous beetles (i.e., able to walk up the sides of the collection container; Hayes et al. 1994). Despite this there were 2 tests (of 28) where the mean number of attacks on untreated bolts was < 5; these 2 tests were discarded. As expected (e.g., Smith 1982, Berisford et al. 1980), ends of bolts provided a preferred surface for *D. frontalis* to initiate galleries. This was undesirable, especially for assessments of effective treatment duration. To combat this we cut bolts using a bandsaw so that ends were nearly smooth. This did not always prevent attacks but it reduced them in all cases to < 4 and usually to 0 or 1. Attacks initiated on bolt ends, and their consequent galleries, were excluded.

Exposure of treated bolts to beetles was dependent upon availability of trees infested with *D. frontalis*. This caused the number of days to vary somewhat, so we coded time periods in months using: 0 - 1 day post treatment, 0 month; 28 - 32 days, 1 month; 63 - 70 days, 2 months; 106 - 128 days, 4 months; and 275 days, 9 months. This also caused variation in the number of individual beetles released into containers. Forty to 104 unsexed beetles (source and handling as above) were added to each container (Fig. 2; $\bar{X} = 73.8 \pm 0.97$) with the total being consistent within each trial. The influence of beetle number on attack number of untreated bolts was evaluated using linear regression (Fig. 2).

Our primary goal was to develop a screening technique for prophylactic products; however, we were also interested in evaluating our assay method and identifying factors that may limit its utility. Toward this end we included evaluations of noncontact exposure (effects of fumes) to bifenthrin, permethrin and carbaryl and the effects of low-dosage (sublabel) applications of bifenthrin and carbaryl. Although containers were screened to provide air exchange (Fig. 1), effects of fumigation were uncertain. To test this, bolts were encased in screen after application of insecticides (2 tests) or by placing filter paper, with an aliquot of insecticide formulation estimated to be equal to that applied to bolts, into the bolt assay container without any host material (3 tests). In both assays, beetles were protected from direct contact with insecticides while exposed to whatever fumes were in the assay containers. Following exposure to fumes for 24 h, the numbers of dead and living beetles were recorded (all tests) and surviving beetles were then released with untreated bolts (3 tests). After 48 h of exposure to untreated bolts, attacks and gallery length were recorded as above.

The effect of sublethal treatment exposure on gallery construction was evaluated by graphing attacks and consequent gallery length for each treatment (Fig. 3). In addition, low-dosage treatment rates were applied and evaluated 1 day after treatment (Fig. 4) using bifenthrin (6 rates: 5, 12.5, 20, 25, 35 and 50% of label for bark beetles) and carbaryl (Ferti-Lome, 7.3% of carbaryl label for bark beetles). If our assay method performed as expected, lower concentrations of AI would facilitate a greater number of attacks.

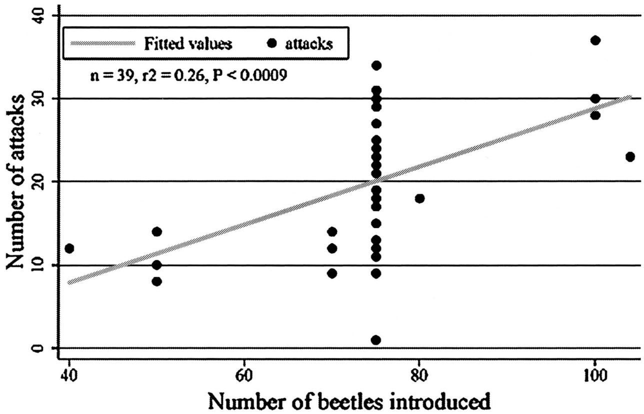

Fig. 2. Relationship between number of *D. frontalis* adults released into a container with untreated bolts and the number of resulting attacks after 48 h. It appears that 25 attacks is a reasonable goal for bolts of this size (ca. 9.25 by 11.1 cm), requiring that about 75 unsexed beetles be released for ~99% confidence that the necessary number of females will be present. Line shows the best-fit linear regression.

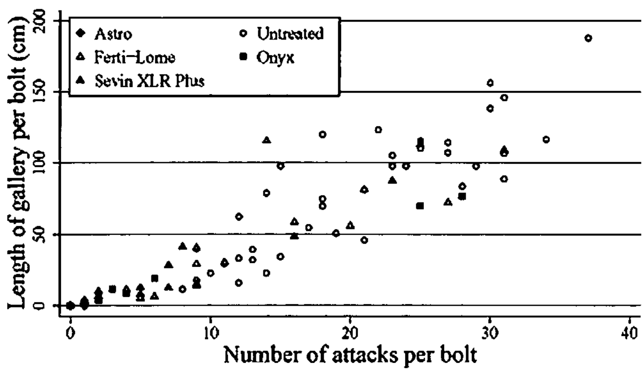

Fig. 3. Relationship between number of *D. frontalis* attacks and gallery length by treatment. No obvious sublethal effects were observed, although differences in the attack numbers between untreated and treated bolts may make some effects difficult to elucidate.

Analysis of covariance, with the number of beetles introduced serving as the covariate, was applied to square-root transformed numbers of attacks, and to total gallery length, for treated bolts at measurement periods 2 and 4 months. The covariate was not significant, and these results are not reported. Instead, two-way analysis of variance (ANOVA), with factors trial and treatment, was conducted on square-root transformed attack numbers and on total gallery length. Analyses were done separately at months 2 and 4. Following each ANOVA, Tukey's HSD was used to separate treatment

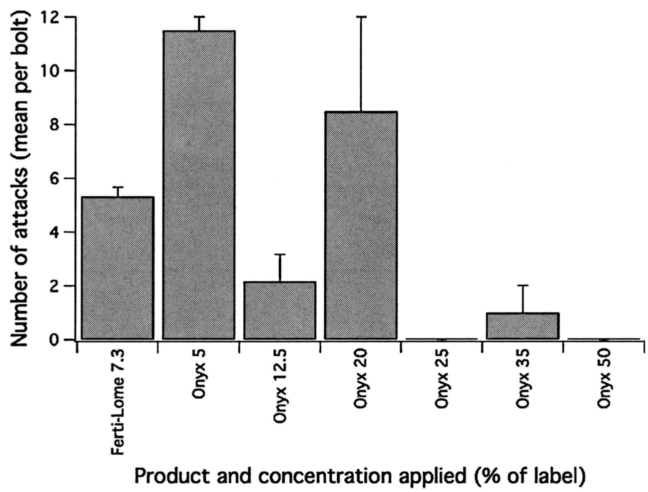

Fig. 4. Mean number of *D. frontalis* attacks when released on bolts treated with low dosage (sublabel) concentrations of a carbaryl insecticide formulation (Ferti-Lome®) and a bifenthrin insecticide formulation (Onyx®). Error bars indicate one SEM. Concentrations are given as percentages of maximum label rate for scolytids. Sample sizes were: Ferti-Lome 7.3%, n = 6; Onyx 5, 20, 25, 35 and 50%, n = 2 each; 12.5%, n = 12.

means if the effect was significant. Data from month 0 or month 9 were not subjected to ANOVA. At month 0, the response variable was 0 for all but the Sevin XLR Plus treatments, and at month 9 there were only 2 replicates of each treatment. As mentioned, data with carbaryl were generated with 2 different products: Ferti-Lome and Sevin XLR Plus. Both products were mixed to standard 2% AI concentrations, but bolts treated with Ferti-Lome received fewer attacks 1 day after treatment than did those treated with Sevin XLR Plus (Fig. 5). For this reason, the products were separated for evaluations. Data from untreated bolts were not included in these analyses because demonstrating differences between the insecticide treatments and the untreated check was not of interest. Adjusting for trial effects made little difference to treatment means, and so the overall mean for the untreated bolts was presented together with treatment means and standard errors for attack numbers (Fig. 5); gallery lengths mirrored attack numbers so are not shown. This facilitated graphical comparisons with the untreated check treatment and with a threshold value proposed for use in screening products. Confidence intervals were also constructed for square-root transformed attack numbers to provide an alternative approach for evaluating product failure and durability (Fig. 6). All statistical analyses were conducted using the software package JMP (V. 7.0.2, SAS Corp., Cary, NC) and significance was determined using a level of 0.05.

Results

The number of *D. frontalis* released into a container had a significant effect on the number of resulting attacks on untreated bolts (Fig. 2; linear regression, n = 39, r^2 =

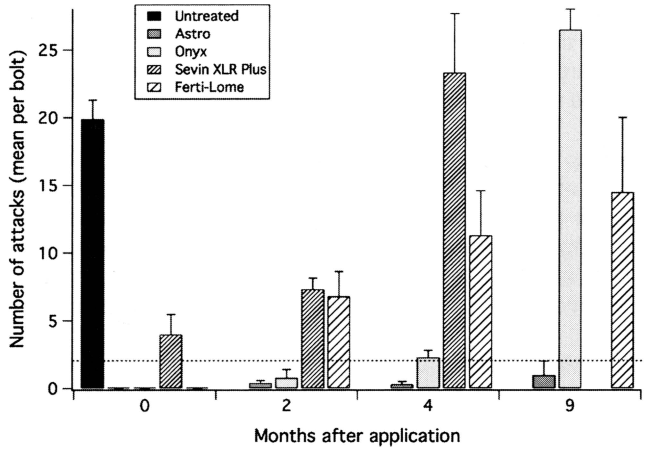

Fig. 5. Mean number of attacks resulting from *D. frontalis* adults being released into containers with small bolts of *P. taeda* coated with insecticide formulations. Insecticides were applied to standing trees and aged in-place until being cut for testing as small-bolts. In each test, bolts were exposed to *D. frontalis* adults for 48 h. Error bars indicate one SEM. Line at 2 attacks indicates our approximate suggestion for choosing products that may deserve additional testing. Gallery lengths mirrored these results so are not displayed.

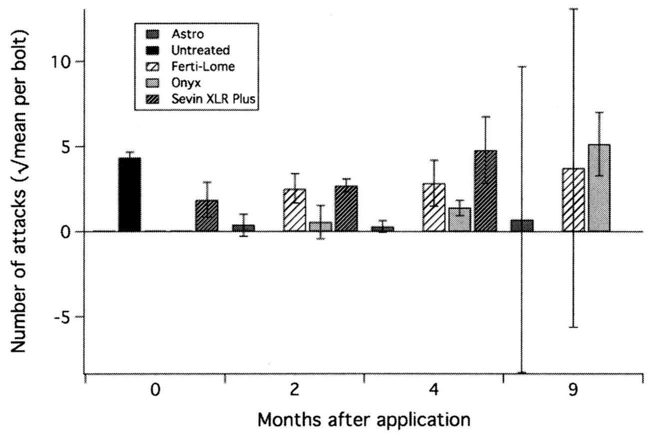

Fig. 6. Mean number of D. frontalis attacks (as in Fig. 5 except that data here are transformed by square root) and 95% confidence intervals observed on small-bolts as treatments aged. Treatments with confidence intervals that encompass zero (or other small number) may be identified as effective enough to warrant further investigation via this screening technique when sufficient replication exists. Note that the Ferti-Lome treatment at 9 months posttreatment resulted in 9 and 20 attacks, but the 95% confidence interval still encompasses zero due to insufficient replication (n = 2).

0.26, $P < 0.0009$). Results with untreated bolts also provided information about the number of attacks that bolts of this size may accommodate. We estimate that 25 attacks is a good target, requiring at least 25 females and suggesting that about 75 unsexed beetles be released so that this may be achieved with slightly more than 99% confidence.

The relationship between attack number and total gallery length was plotted to observe potential effects from sublethal insecticide exposures on gallery formation (Fig. 3). A simple positive relationship was evident with points clustered more tightly near the origin due to the effectiveness of the insecticide treatments on reducing number of attacks. This result was expected with the contact poisons used in this study, but may change if treatments with different modes of action were to be evaluated.

Insecticides applied at their label rates were mostly effective at eliminating *D. frontalis* attacks on small bolts 1 day after application (= 0 month; Fig. 5). Bifenthrin applied at the maximum label rate for scolytids (0.06% AI), was effective when first applied; both attacks (Fig. 5) and gallery length were 0. It began to lose effectiveness slightly at 2 months, and between 4 and 9 months became ineffective. As expected, insecticide formulations containing 2% carbaryl were the least effective for preventing attacks by *D. frontalis*. Sevin XLR Plus was the poorest performer at months 0, 2 and 4, which further suggests our evaluation has merit for identifying ineffective products. For reasons that are unclear, Ferti-Lome was more effective than Sevin XLR Plus at the 0 month evaluation; however, it lost effectiveness and was similar to Sevin XLR Plus at 2 and 4 months. Permethrin was surprisingly effective in this test and appears to deserve consideration for additional testing. It was as effective as bifenthrin at 2 and 4 months, and better thereafter.

Effects of low-dosage applications of carbaryl and bifenthrin also suggest that our assay can identify treatments that are unlikely to be effective. Small-bolts treated with low-dosage Ferti-Lome (7.5% of the bark beetle label for carbaryl) received more attacks (Fig. 4) than did those that received the label rate (Fig. 5, 0 month). The number of attacks on bolts treated with bifenthrin generally was higher with lower treatment concentrations (Fig. 4). However, the rates of bifenthrin necessary for failure were low, suggesting that this insecticide is highly toxic to *D. frontalis* adults. High levels of 0 month mortality with bifenthrin are not surprising in our assay given that beetles are unable to escape containers and pyrethroid insecticides are effective toxicants when freshly applied (Elliott et al. 1978). To our knowledge, a range of bifenthrin concentrations has not been evaluated with *D. frontalis* so soon after treatment, so the consistency of this result with field applications is unknown.

Fumigation effects from bifenthrin, permethrin and carbaryl products were negligible. In our first evaluation we observed only 1 dead *D. frontalis* adult, and it was exposed to fumes from an untreated bolt. Another 4 *D. frontalis* were considered moribund: 2 exposed to bifenthrin, 1 exposed to permethrin and 1 exposed to an untreated bolt. Surviving beetles were combined from the 2 replicate fume exposures (by treatment) and placed onto untreated bolts to evaluate their ability to attack following exposure. Beetles exposed to bifenthrin fumes for the previous 24 h ($n = 92$) produced 22 attacks with 48.3 cm of gallery. Beetles similarly exposed to permethrin ($n = 85$) produced 21 attacks with 42.9 cm of gallery, and those exposed to an untreated bolt ($n = 96$) produced 23 attacks with 49.5 cm of gallery.

Our second, third and fourth evaluations of fume effects used filter paper as the medium to which insecticides were applied (rather than a bolt) and additionally included Sevin XLR Plus. In the second test we observed the following mortality of *D. frontalis* adults after 24 h exposure to fumes: Onyx, 5/92; Astro, 3/98; Sevin XLR Plus, 2/99; tap water, 8/100. Surviving beetles were again combined within treatments and allowed to attack untreated bolts, resulting in: Onyx exposed, 26 attacks with 71.9 cm of gallery; Astro exposed, 30 attacks with 86.9 cm of gallery; Sevin XLR Plus exposed, 29 attacks with 90.2 cm of gallery; and water exposed, 32 attacks with 91.4 cm of gallery. The third test only evaluated exposure mortality after 24 h and resulted in: Onyx exposed, 6/76; Astro, 5/72; Sevin XLR Plus, 4/78; water, 1/79. The fourth test similarly produced: Onyx exposed, 7/93; Astro, 3/97; Sevin XLR Plus, 8/96; water, 10/100. Combining across filter paper replicates gave 24 h mortality rates of: 6.9% for Onyx, 4.1% for Astro, 5.1% for Sevin XLR Plus and 6.8% for water. Apparently the toxicity observed in our tests was not due to fumigation.

Discussion

By evaluating products previously field-tested for efficacy against *D. frontalis* adults, we were able to assess a small-bolt method for its ability to effectively screen insecticide products. Evaluations 1 day after application (0 month) appeared to provide a reasonable estimate of initial insecticide toxicity. Regardless of evaluation method, tests so soon after application are better suited for identifying product failures than estimating actual field effectiveness, which requires knowledge of a treatment's effective duration. When insecticide products were aged on tree boles, the small-bolt assay produced results with *D. frontalis* that were similar to those obtained by previous investigators in field tests. Carbaryl products were ineffective and bifenthrin was effective, especially over short durations (< 9 months). There are not sufficient field data with permethrin and *D. frontalis* for adequate comparison with our results, but this AI performed better than we expected. Because it is 1 of 3 contact insecticides labeled for tree protection against bark beetles, and 1 of only 2 with potentially good activity against *D. frontalis*, it warrants additional study.

Insecticides are important tools for managing resources threatened by bark beetles. Most important are treatments that prevent attack of standing trees. Experiments to evaluate treatments in the field are expensive and require significant investments in space, money, time and other resources. This is magnified for in situ experiments, which also may fail to produce useful results due to a lack of beetle attack pressure (Shea et al. 1984, Fettig et al. 2006a, b) and, at higher pest pressures, may lead to undesirable infestations near experimental trees (Strom et al. 2004). The hanging-bolt technique, pioneered by Berisford et al. (1980), reduces some of the costs of in situ experiments and increases control over assessing the temporal durability of insecticide treatments. This approach also increases the probability of a successful experiment (i.e., one in which treatments receive verifiable challenge from the target species) and eliminates the importance of unwanted tree attacks. However, significant resources are still required, as active infestations of sufficient size must be located, trees > 15 cm dbh sprayed and treated bolts 1.5 m long transported and handled. Similar to the small-bolt method, the hanging-bolt method requires indirect assessment of treatment success from attack information (i.e., tree mortality is not observed).

Pesticides have been screened for efficacy against *D. frontalis* by direct application to individual beetles in the laboratory (Hastings and Jones 1976). We did not use this approach primarily for two reasons. First, it ignores environmental effects on insecticide formulations and therefore cannot directly assess residual activity and, second, it is incompatible with products that work via mechanisms other than contact toxicity (e.g., ingestibles or deterrents). Most new products being proposed as tree protectants against bark beetles are not contact insecticides, so this approach does not provide the flexibility necessary for their evaluation.

We believe that the small-bolt assay provides many of the advantages of the hanging-bolt method with less work and cost. Because small trees are used, and pesticides are only applied to a small portion of the lower bole, environmental and worker exposures to pesticides should be reduced. Our primary goal was to provide a screening technique to eliminate ineffective products prior to field-testing. It appears that evaluations of products 1 day after application can identify products that are unlikely to be effective against bark beetles. Exposing bolts of this size (about 11 cm long by 9 cm diam) to about 75 beetles provided a useful test and identified a threshold failure level of about 2 attacks per bolt. This level would have screened out Sevin XLR Plus at 1 day posttreatment. The Ferti-Lome product would have failed between 1 d and 2 months, whereas bifenthrin would have failed at 4 or more months postapplication (though only slightly at 4 months; Fig. 5). Alternatively, or in conjunction with the above, confidence intervals could be used. We suggest using 4 - 5 replicate bolts for either screening rule, as fewer replicates will produce large standard errors and wide confidence intervals. For example, bolts with the Ferti-Lome treatment at 9 months had either 9 or 20 attacks but produced a confidence interval that encompassed 0 (Fig. 6). Whether 0 or a small number (e.g., 1 or 2) should be chosen as the critical value for confidence intervals will depend on experimental objectives and should become clearer as additional data are generated using this method.

Whether this technique can be refined to provide an adequate substitute for in situ or hanging-bolt tests remains to be determined. The residual effectiveness of carbaryl and bifenthrin products over the 9 month duration of our testing was similar to that reported from field tests conducted by previous investigators. This suggests that with further development and testing, the small-bolt assay may be useful for predicting field performance of insecticides deployed against *D. frontalis*. The method provides more control over beetle pressure with less resource use than either method currently used, whereas also providing the flexibility necessary for directly comparing products with different modes of action. The details included in this study allow subsequent investigations to focus more on questions of treatments and less on methods. Consequently, experimental designs that are complete and simple to analyze (e.g., randomized complete blocks with all treatments included in each trial) can be efficiently used. Over time, results garnered from this simple assay will provide the information necessary to evaluate and refine our proposed rules for efficacy and determine the utility of this assay for inferring results to real-world applications.

Acknowledgments

The authors thank B. Parpart, J. Parpart, C. Reed, E. Vallery, S. Walters and C. Young for technical assistance, the Winn Ranger District, Kisatchie National Forest (LA) for providing suitable stands for this work, C. Brownie for statistical review and advice, and C. Brownie, A. Mangini, J. Meeker, K. Reed and two anonymous reviewers for their reviews of earlier versions of this manuscript.

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