Effectiveness of Cedar Oil Products for Preventing Host Use by *Ips avulsus* (Eichhoff) (Coleoptera: Curculionidae) in a Modified Small-Bolt Assay[1]

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Abstract Insecticide products based on cedar oil are readily available, but evaluations against pine bark beetles (Coleoptera: Curculionidae: Scolytinae) are lacking. In the southeastern U.S., the southern pine beetle, *Dendroctonus frontalis* Zimm, is the major bark beetle pest for which tree protectants are applied. However, *Ips avulsus* (Eichhoff) are more consistently available, are easily trapped and can attain pest status at times, making them an attractive option for the evaluation of certain tree protection products. In this study, we evaluated 2 commercial cedar oil products for their ability to prevent attack by *I. avulsus* on loblolly pine (*Pinus taeda* L.) bolts. The field method we deployed was an extension of a previously developed laboratory method in which small-bolts are treated with the product and provided a challenge by adult beetles. The new method used multiple-funnel traps baited with synthetic attractants to direct *I. avulsus* to test bolts. The method was successful in providing a challenge to treatments with *I. avulsus*, and resulted in neither product being consistently effective for preventing bolt utilization by this beetle. Although not considered effective in the majority of our trials, full-strength (neat) application of cedar oil product reduced bolt utilization by *I. avulsus* during winter trials (November and February), perhaps due to lower attack pressure from beetles. Evaluations at other times of the year (March through May), or that began > 0 days post-application, resulted in the products consistently failing to meet either of our efficacy criteria. Compared with competing insecticide products (e.g., those based on bifenthrin), the cedar oil products appear to be less effective, require more frequent reapplication and be more expensive.

Key Words pesticide evaluation, bioassay, pine bark beetles, tree protection, plant oil

Insecticide products based on cedar oil are readily available and have some desirable properties, including low mammalian toxicity and their categorization by the US-EPA as Registration Exempt 40 CFR 152.25B. Although cedar oil products are commercially available, there is a paucity of published studies that report on the effectiveness of them for insect management, and we are not aware of any evaluations with pine bark beetles (Coleoptera: Curculionidae: Scolytinae).

Southern pine bark beetles are primary pests of nonjuvenile southern yellow pines. There are 5 species commonly associated with the guild: the southern pine beetle (*Dendroctonus frontalis* Zimmermann), the black turpentine beetle (*D. terebrans* [Olivier]), the small southern pine engraver (*Ips avulsus* [Eichhoff]), the southern

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pine engraver (*I. grandicollis* [Eichhoff]) and the six-spined engraver (*I. calligraphus* [Germar]). Of these, the southern pine beetle is historically the most damaging pest; however, it is also the species with the most unstable population dynamics, sometimes declining to levels at which attractive traps cannot detect them (e.g., Billings 2009). *Ips avulsus* is considered more aggressive than *I. grandicollis* (Mason 1970), and we consistently catch greater numbers in our traps than we do of either *I. grandicollis* or *I. calligraphus* (Strom et al., unpubl. data). *Ips avulsus* is common in the Western Gulf region of the U.S. and can attain pest status at times (Thatcher 1960); thus, it is an attractive option for assays when *D. frontalis* is not available.

Strom and Roton (2009) developed a laboratory method using small bolts to assess products for potential utility as tree protectants against bark beetles. The small-bolt method challenges treatments by confining a specified number of adult beetles with each test bolt. In this study we extend the small-bolt methodology by eliminating the need to rear and handle adult bark beetles. Here, we deployed multiple-funnel traps, baited with standard attractants, to funnel adult *I. avulsus* directly onto experimental bolts, thereby promoting a challenge to cedar oil and associated treatments without handling beetles (Fig. 1). This approach resulted in a significant challenge from *I. avulsus* to the cedar oil products, and when insect species and technology allow it (e.g., attractants are available for the pioneering sex), offers an alternative that is less controlled than our laboratory method but logistically easier.

Materials and Methods

Two cedar oil products were evaluated in this experiment. Both products were labeled "PCO Choice™", one was distributed by Cedar Oil Industries, San Antonio, TX (COI-PCO), and one manufactured by CedarCide Industries, Spring, TX (CC-PCO). Both products list ingredients as 85% cedar oil and 15% ethyl lactate. A small-bolt method (Strom and Roton 2009) was modified and used to evaluate the products. Although *D. frontalis* would have been preferred, their regional paucity precluded their use for this evaluation. Instead, we used techniques to target *I. avulsus*, a species that is ubiquitous in central Louisiana and one for which effective synthetic attractants and traps exist for their capture (e.g., Strom et al. 2003). This combination provided a simple method for extending our small-bolt assay to *I. avulsus*. The method uses a freshly-cut small loblolly pine bolt (about 10 cm diam by 10 cm long) treated with the test product and then exposed to living beetles (Strom and Roton 2009). For *I. avulsus*, we used multiple-funnel traps, baited with racemic ipsdienol and lanierone (Contech/Phero Tech, Delta, BC, Canada and Synergy Semiochemical Corp., Burnaby, BC, Canada), and fitted with a bucket to hold the bolt and replace the standard collection cup (Fig. 1). Traps were placed in the field, baited, and a bolt secured in each bucket so that trapped beetles were directed into the container and allowed to attack ad libitum.

Products were applied at high concentrations to provide the best opportunity for observing potential effects. Labels for both products state, "Stronger mixtures will enhance results without damaging side effects." Bark beetles are not explicitly listed on the suggested mixing chart of either product; however, examples of insects and associated concentrations that appear on the label are: mosquitoes (1.3% mixture with water), darkling beetles (8 oz [236.5 ml] PCO product per gallon water, 1:16, 5.9%), and palmetto bugs (8.3% mixture with water).

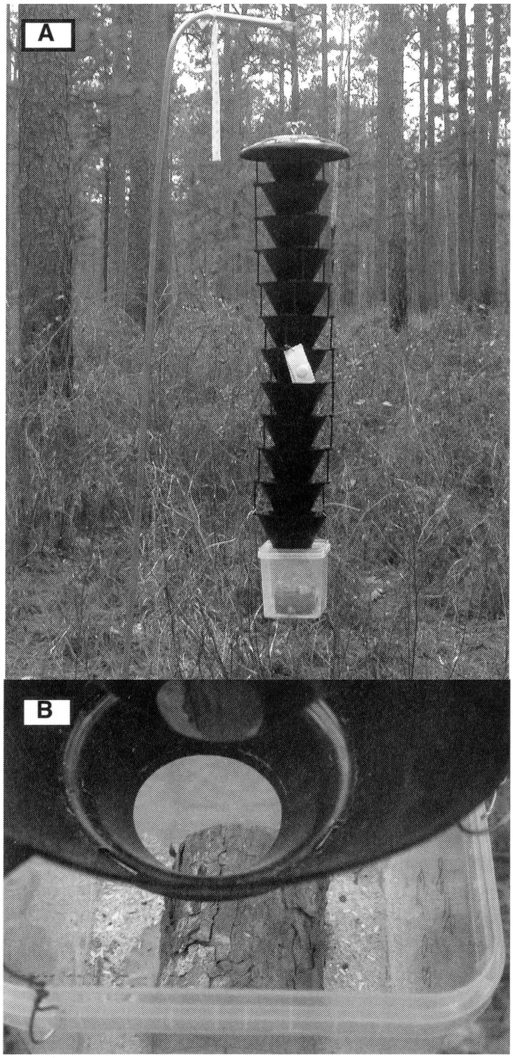

Fig. 1. Modified multiple-funnel trap used to capture bark beetles for evaluating cedar oil products for their potential as tree protectants. Traps were baited with standard attractants for *I. avulsus*, racemic ipsdienol and lanierone (A); attracted beetles fell into the bucket with the small bolt, and thereby provided a challenge to the treatment of interest (B). Bolts remained in traps until beetle activity appeared sufficient for a rigorous test (i.e., untreated bolts were mass attacked), a period that ranged from 3 - 14 days.

A series of 9 trials was conducted to evaluate the effectiveness of the cedar oil products against *I. avulsus*. One trial was excluded due to inadequate colonization of untreated bolts (23.5% of samples were positive), leaving 8 trials for evaluation. The first 2 trials were conducted to estimate the concentrations needed for any activity against *I. avulsus* to be observed. This was necessary because previous information was lacking from either product against any bark beetle. Employing these results, 3 concentrations were selected for further evaluation: full-strength (neat), diluted 5:1 (v/v, water:PCO product; 0.17 [17%], and diluted 10:1 (0.09 [9%]). Three trials were conducted to compare the relative performance of the 2 products, and 3 additional trials to evaluate concentrations and their effectiveness as treatments aged. A vegetable oil treatment (Great Value™ Pure Vegetable Oil, Wal-Mart Stores, Inc., Bentonville, AR) was included in most trials so that an assessment could be made of an oil "control", i.e., one that was unlikely to be insecticidal.

Individual product mixtures were applied liberally with a paintbrush to the outer bark of loblolly pine trees or bolts (see below). Day 0 tests used freshly-cut bolts and began on the same day that treatments were applied. Ends of bolts were always prepared by cutting with a bandsaw, a treatment that greatly reduces attacks on bolt ends (Strom and Roton 2009). Treated bolts were randomly assigned to traps and remained in trap buckets until untreated bolts were mass attacked. Evaluation of treatment durability or longevity required that treatments be aged in situ, so they were applied to the bole of living loblolly pines and allowed to age until their use. For assays, a single freshly-cut bolt was secured horizontally on the floor of the collection bucket at the bottom of each multiple-funnel trap (Fig. 1).

Once it appeared that untreated bolts were mass attacked, 3 - 14 d following installation, all bolts in the trial were collected and refrigerated until their evaluation. The pattern of bolt use by *I. avulsus* (Fig. 2A) did not allow for direct measurement of attack numbers and gallery lengths as we had done with *D. frontalis* (Strom and Roton 2009). Instead, a sampling method was used (Fig. 2B) to provide the proportion (or percentage) of samples that were positive for *I. avulsus* activity. The level of activity of *I. avulsus* in each bolt was estimated by sampling with a 1.27-cm diam round punch that was driven to the sapwood face. The resulting phloem sample was determined to contain beetle activity or not and recorded as such. The number of samples per bolt was dictated by bolt circumference and ranged from 12 - 18. Sample columns (along the length of the bolt) were begun at a randomly selected radial point on the bolt (0 - 360 degrees) and then moved systematically around the circumference with a column of samples being taken about every 4 cm (Fig. 2, right); sampled area represented approx. 6% of the outer bark surface area on average (experiment-wide mean = 15 samples per bolt, mean bolt diam = 9.28 cm, mean bolt length = 10.9 cm, n = 113 bolts). Data were recorded as the proportion of samples in each bolt that was positive for beetle activity.

Analysis of variance (ANOVA) was conducted on arcsin transformed proportions to compare products and concentrations. Residual plots indicated that this transformation improved model performance. Trial was treated as a blocking factor, and tests were conducted for product brand (COI-PCO, CCD-PCO) and concentration (9, 17 and 100%) main effects and their interaction. Otherwise, results were reported in terms of means and standard errors, and product effectiveness determined as described below. Statistical analyses were conducted using the software packages JMP (V. 7.0.2) and SAS (V. 9.2) (SAS Corp., Cary, NC).

Evaluations of product effectiveness for prophylactic application are most appropriately based upon achieving established thresholds of beetle activity and treatment

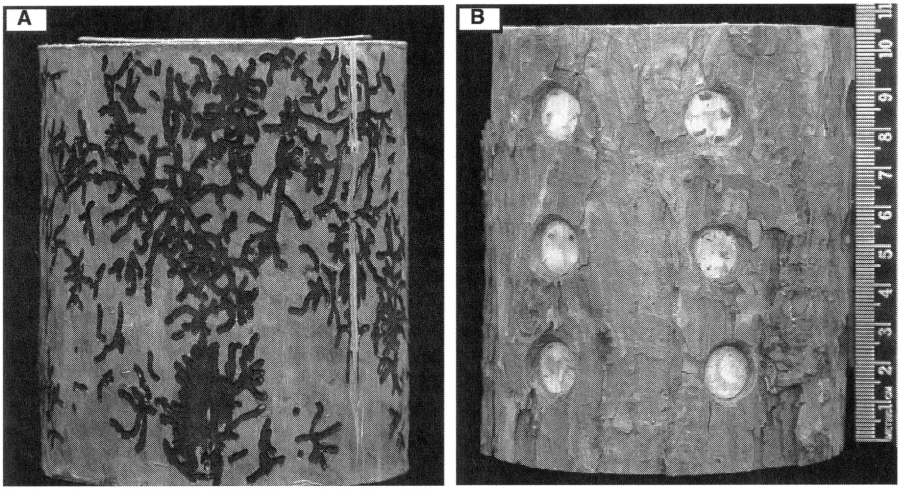

Fig. 2. Due to the extent and pattern of bolt resource use by *Ips avulsus* (A), a sampling method was developed (B) to estimate the extent of the bolt that was colonized. Any presence of *I. avulsus* activity (i.e., beetle, attack or gallery) was considered a positive sample. Scale shows cm.

performance. For example, Shea et al. (1984) suggest for standing trees that efficacy be accepted when the experimental population of untreated trees suffers mortality ≥ 60% and treated trees suffer < 20% mortality. For small-bolt tests with *D. frontalis*, Strom and Roton (2009) suggest that one of two effectiveness criteria be used: an average of fewer than (approximately) 2 attacks per bolt, or a confidence interval for the mean response rate (e.g., average number of attacks) that includes zero. A minimum sample size of 4 or 5 bolts per treatment is recommended for either criterion. Because a sampling procedure was used to estimate bolt utilization by *I. avulsus* in these tests, we used the following guidelines for interpretation: untreated bolts had to average ≥ 50% positive samples (per bolt) for a trial to be considered sufficient or valid for beetle pressure, whereas treatments were only considered effective when the average per bolt percentage of positive samples was ≤ 20%.

Results and Discussion

To estimate the active concentrations of product needed for impacting *I. avulsus*, day 0 tests were accomplished with COI-PCO applied at full-strength ($n = 6$ bolts) and diluted 10:1 with water (9% product; $n = 2$ bolts) (Fig. 3). The full-strength application of COI-PCO reduced bolt utilization from 73.3% (untreated result) to 2.8%. Bolts treated with 9% product had *I. avulsus* activity in 100% of samples, whereas 23.3% of samples from bolts treated with vegetable oil were positive. The full-strength application of COI-PCO during this set of trials was the only time during our series of trials when a cedar oil product reduced attacks sufficiently to be classified as effective by our criteria (Fig. 3). These results suggested that relatively strong concentrations were necessary to impact *I. avulsus* and a 5:1 (17%) dilution was added in subsequent trials.

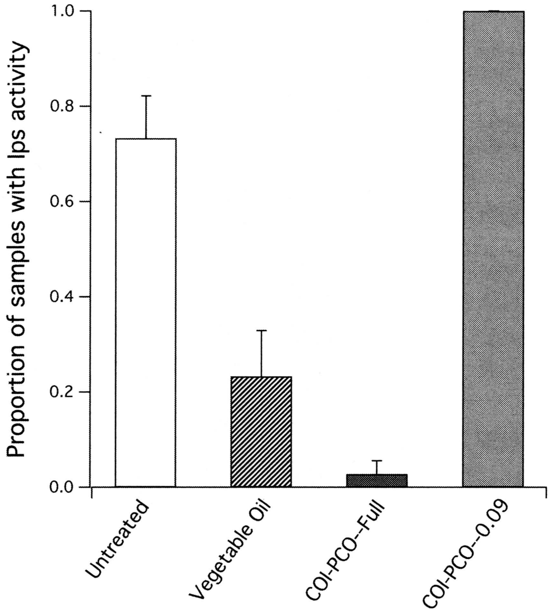

Fig. 3. Mean per bolt proportion of samples that were positive for *I. avulsus* activity in our initial day 0 test with the cedar oil product from Cedar Oil Industries (COI-PCO) applied at full-strength or as a 10:1 dilution with water (0.09). Trials also included untreated bolts and those treated with vegetable oil. Trials were conducted in November 2008 and February 2009. Sample size was 6 bolts for each mean, except for COI-PCO–0.09, which was 2. Error bars depict one SEM.

The 2 brands of cedar oil product, COI-PCO and CC-PCO, were compared in 3 trials (Fig. 4). There was no significant difference between brands in day 0 tests, either averaged over concentrations or separately at any of the 3 concentrations evaluated, full-strength, 5:1 dilution with water (17% product) and 10:1 dilution with water (9% product) (effect of product brand, $P = 0.20$; effect of the interaction between product brand and concentration, $P > 0.20$). Averaging over products (combining the 2 brands), when each concentration was compared with the untreated control, only the full-strength application impacted bolt utilization by *I. avulsus* at day 0 ($P < 0.001$), and even this treatment suffered bolt utilization too extensive to be classified as effective by either of our criteria. Both brands of cedar oil applied at full-strength and evaluated at day 0 received > 20% bolt utilization by *I. avulsus* (Fig. 4), and none had confidence intervals that included zero. The lowest utilization rate was observed with CC-PCO full-strength, which showed an average of 27% positive samples and provided a confidence interval of 0.06 - 0.49 (6 - 49% bolt utilization).

Treatment durability (longevity) and effective concentration are related (concentrations wane over time) and were evaluated together over the course of 3 additional trials (Fig. 5). At day 0, full-strength COI-PCO was ineffective by our criteria, but observed *I. avulsus* activity (mean = 43% of samples positive) was lower than for bolts

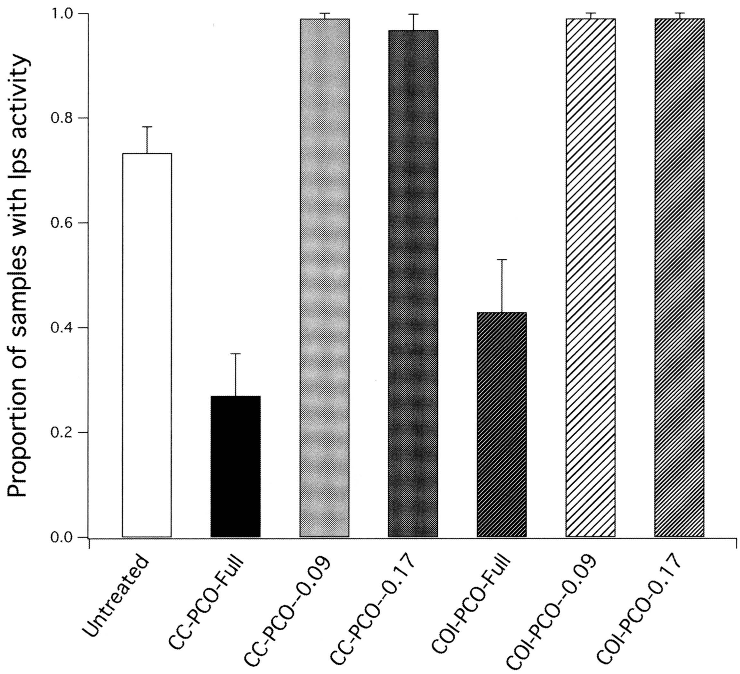

Fig. 4. Comparison of two cedar oil PCO products, one from CedarCide Industries (CC-PCO) and one from Cedar Oil Industries (COI-PCO), for their impact on bolt utlization by *I. avulsus* when applied at three concentrations (Full [neat], 9% (0.09) and 17% (0.17). Bars indicate the mean proportion of samples within each bolt that were positive for *I. avulsus* activity. Error bars show one SEM. Sample sizes were 5 or 6 bolts per treatment, except for Untreated, which was 11. Trials conducted during March and April 2009.

treated with 17% product (mean = 99%) or untreated (mean = 92%). By day 30, however, treatments had become more similar, with even the full-strength application becoming roughly equivalent to the other treatments, and the vegetable oil appearing best. No treatment at any test period in this evaluation was considered effective, and treatments appeared to be indistinguishable after day 30; treatment failures continued for all concentrations over the next 2 periods evaluated, 46 and 67 d post application (Fig. 5).

Neither of the cedar oil products showed sufficient activity against *I. avulsus* to warrant recommendation as tree protectants. The best performance we observed by any treatment, and the only set of trials in which effectiveness was achieved, was full-strength COI-PCO at day 0 in trials run in November 2008 and February 2009 (Fig. 3). In trials conducted during nonwinter months (March through May 2009), not even the full-strength, day 0 applications were effective (Figs. 4 and 5). It appears that beetle pressure on treatments may have been lower during the initial winter trials (73% of samples from untreated bolts were positive for *I. avulsus*) than during later trials (92%

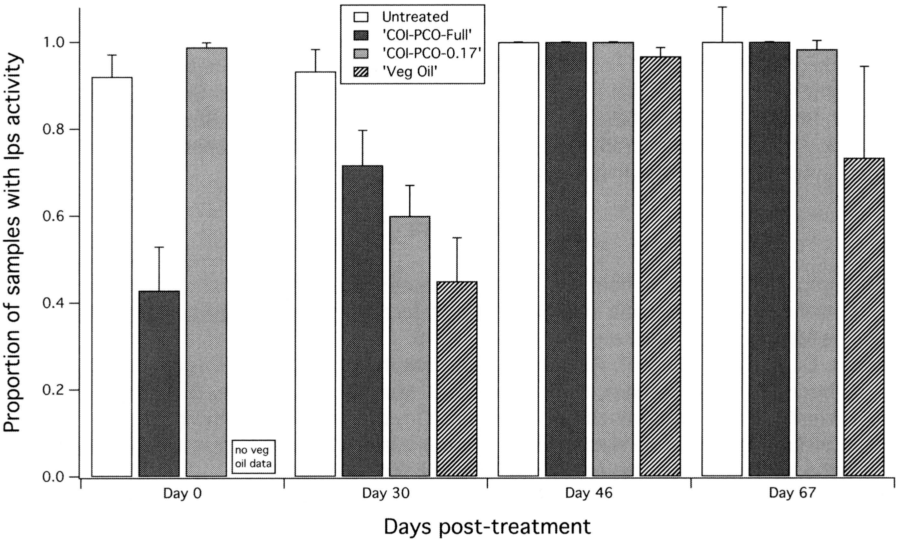

Fig. 5. Mean per bolt *I. avulsus* utilization (proportion of positive samples) of loblolly pine bolts treated with COI-PCO (Cedar Oil Industries) at two concentrations (Full or 0.17 in water) and 0 - 67 days after treatment. Treatments were applied to standing trees and aged until cutting for evaluation. Error bars show one SEM. Day 0 sample sizes from Fig. 4, with the remaining treatments being 4 bolts each. Trials conducted from March to May 2009.

of samples from untreated bolts were positive), which may have improved product performance. However, even under this "best-case scenario," product applications would apparently have to be repeated at least every month.

The retail cost of 3.8 L of PCO choice cedar oil product was US $200.00 to US $250.00 in December 2009 (www.pestigator.com, www.cedarcidestore.com). Assuming 7.6 L as a minimum volume necessary to cover a tree bole to about 12 m for adequate protection, the minimum per tree application cost for product alone would be US $400.00 to US $500.00. As mentioned, application would need to be repeated at least every month, and perhaps more frequently. For comparison, Onyx® (FMC Corporation, active ingredient [AI] bifenthrin, 23.4%) applied at the label rate of 0.06% active ingredient is considered effective for season-long tree protection against pine bark beetles in the western U.S. (Fettig et al. 2006) and has been demonstrated effective against the southern pine beetle for 4 months or more in 2 different tests (Berisford et al., unpubl. data, Strom and Roton 2009). If one again assumes a 7.6 L application at the label rate, and the December 2009 retail cost of US $129.95 / 0.95 L for Onyx, the comparative product cost would be about US $2.60 per tree.

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