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Metasystox-R, Applied in Mauget Injectors, Ineffective in Protecting Individual Ponderosa Pines from Western Pine Beetles

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The effectiveness of registered application rates of the insecticide metasystox-R applied with Mauget tree injectors (INJECT-A-CIDE) was assessed in two strategies: (1) treatment of trees before western pine beetle attack (preventive treatment), and (2) treatment of trees after attack by western pine beetle (remedial treatment) for protection of individual, high-value ponderosa pine. This field test was conducted on the western slope of the Sierra Nevada on the Eldorado National Forest in central California by using three treatments: metasystox-R injected about 14 days before the onset of beetle attack; metasystox-R injected about 7 days after the onset of beetle attack; and an untreated check. Tree injectors were spaced at 15-cm intervals at the root crown around the entire circumference of each

Western pine beetle (*Dendroctonus brevicomis* LeConte) is the most serious insect pest of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) in the western United States.[1] Severe droughts, such as those in California from 1975 to 1977 and from 1988 to 1993, are accompanied by extensive mortality of ponderosa pines and other conifers as a result of attacks by several bark beetle species, including the western pine beetle. In addition to moisture stress, bark beetle-related mortality of high-value trees located in residential and recreational areas can occur as a result of stress associated with overcrowding, soil compaction, or injury due to construction, logging activity, fire, or vandalism. Mortality of such high-value trees could have long-range management effects. Because either the value of these individual trees or the cost of their removal is high, their protection may be justified until they recover from stress. Individual trees can be protected from bark beetles by using effective insecticides.

Carbaryl, lindane, and metasystox-R are the only insecticides currently registered for protection of individual ponderosa pine trees from attack by western pine beetle. The registrations of carbaryl and lindane are primarily the result of small-scale tests.[2,3,4] Subsequent field research demonstrated the effectiveness of several formulations of carbaryl for protecting individual trees from attack by western pine beetle. In an extensive, large-scale field experiment, a 1 percent suspension of carbaryl in the Sevimol formulation appeared as effective as a 2 percent suspension, the concentration currently registered for protecting trees.[5,6] Carbaryl (Sevimol) as a 2 percent suspension was shown to be effective for preventing successful attack by western pine beetle of ponderosa pine for two field seasons.[7] Lindane appears to be effective for 22 months as a 2 percent water emulsion.[3] No studies have been published that demonstrate the efficacy of injected metasystox-R for control of western pine beetle in ponderosa pine.

The production, registration and marketing of carbaryl for forestry has changed often. Rhone-Poulenc purchased the insecticide division of Union Carbide Corporation, the manufacturer of carbaryl. The manufacturer has introduced a new formulation of carbaryl (Sevin SL) and modified the label of Sevimol. Lindane, however, may be unavailable for future use against western pine beetle. Most pest management specialists are hesitant to recommend lindane because of the controversy surrounding its use in forestry.[8] Given the uncertain future availability of any commercial insecticide, alternative insecticides are needed for important uses in forestry, such as control of western pine beetle.

Metasystox-R, applied in the Mauget injectors (INJECT-A-CIDE), is effective for protection of cone crops in coniferous species[9,10,11] and for reducing damage by foliage-feeding insects.[12] This treatment is also registered for protection of western conifers from numerous bark beetle species[13] and is currently marketed for preventive and remedial control

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of western pine beetle in many mountain communities in California.[14] However, because published data do not unequivocally document the efficacy of metasystox-R applied with Mauget injectors for protection of ponderosa pine from attack by western pine beetle, we conducted an efficacy study of metasystox-R applied for both preventive or remedial control strategies.[15]

This note describes a study to assess the effectiveness of the registered application rate of metasystox-R applied with Mauget tree injectors in two strategies: (1) treatment of trees before western pine beetle attack (preventive treatment), and (2) treatment of trees after initial attack by western pine beetle (remedial treatment), on individual, high-value ponderosa pine. Both strategies are currently recommended by arborists and are consistent with the pesticide label for application.[16]

Methods

Research Approach

This field test was conducted on the western slope of the Sierra Nevada between 750 and 1,700 m elevation on the Placerville and Amador Ranger Districts of the Eldorado National Forest in central California by using three treatments: (1) metasystox-R injected into ponderosa pine about 14 days before the onset of beetle attack; (2) metasystox-R injected about 7 days after the onset of beetle attack; and (3) an untreated check. Thirty trees were randomly assigned to each treatment, totaling 90 trees in the experiment.

All sample trees were 28 to 52 cm diameter at breast height (DBH). Trees were selected so they were within 25 m of an access road to facilitate treatment. The spacing between trees, no less than 0.4 km, assured that a sufficient number of beetles were in the vicinity of each tree to rigorously test the efficacy of the treatments.[5,7,17,18] During the course of the experiment (June 1990 to October 1990), one tree was misidentified. We inadvertently selected a Jeffrey pine (*Pinus jeffreyi*) in a plantation composed of a mixture of ponderosa pine and Jeffrey pine. After this tree was dropped from the study, sample size was not equal among treatments.

The preventive treatment was applied June 6 and 7. Insecticide was applied with Mauget tree injectors spaced at 15-cm intervals at the root crown around the entire circumference of the bole of each tree *(fig. 1)*.[19] Each injector contained 3 ml of a 50 percent solution of metasystox-R (S-[2-(ethylsulfinyl)ethyl]O,O-dimethyl). To test the effectiveness of both injected insecticide strategies against western pine beetle, all trees in every treatment, including check trees, were baited with the western pine beetle aggregation pheromone (*exo*-brevicomin, frontalin, and myrcene) (Phero Tech, Inc. WPB Tree Bait) on June 19 or 20, 13 days after the preventive treatment was applied. The pheromone was placed on the tree bole 2 m above the ground.[5,7,17] Untreated check trees were monitored every 2 days. Baits remained on preventive treatment and untreated check trees until 70 percent of the untreated check trees appeared to be successfully attacked, i.e., with boring dust in bark crevices. By June 28 and 29, 21 of the untreated check trees appeared to be successfully attacked. The pheromone baits were then removed from all trees in the preventive treatment and the untreated check to discontinue beetle attacks and treatment challenges.

Trees assigned to the remedial treatment were observed daily to determine successful attack by the western pine beetle. The pheromone baits remained on the trees in the remedial treatment until at least 24 of the trees (80 percent) had been attacked by western pine beetle and had at least 5 pitch tubes visible from the ground. The aggregation pheromone baits were then removed from all trees assigned to this treatment on June 26 and 27, and the remedial metasystox-R treatment was applied exactly as in the preventive treatment *(fig. 1)*. All trees assigned to the remedial treatment demonstrated some level of attack by western pine beetle.

All metasystox-R treatments were applied by a private, licensed pesticide applicator who had extensive experience treating ponderosa pine with the

tree. All trees in every treatment were baited with the western pine beetle aggregation pheromone. The criterion used to determine effectiveness was whether individual trees succumbed to attack by western pine beetle and eventually died. Metasystox-R treatments were considered ineffective if significantly less than 90 percent of the treated trees survived. Residues of metasystox-R were detectable (an average of 0.10 ppm to 0.84 ppm) in the foliage for each collection period (5, 20, and 40 days post treatment) for both the preventive and remedial treatments. Residue levels of metasystox-R in the phloem were not assessed. Both metasystox-R treatments failed to meet the criterion of efficacy (≤10 percent of trees killed). Furthermore, neither metasystox-R treatment resulted in significantly less (or more) mortality than the untreated check treatment. Therefore, the registered use procedures for injections of metasystox-R were not efficacious.

Retrieval Terms: Dendroctonus brevicomis, Pinus ponderosae, *tree protection, insecticide efficacy, Injecticide*

Figure 1—Mauget INJECT-A-CIDE capsules being applied to ponderosa pine on the Placerville Ranger District, Eldorado National Forest, spaced at 15-cm intervals near ground level.

Mauget INJECT-A-CIDE product. Injectors were removed from the trees within 24 hours after injection; none contained significant amounts of metasystox-R. Deployment of WPB Tree Bait, determination of tree mortality, and pesticide residue analyses were accomplished by USDA Forest Service personnel.

Residue Analysis

Residue levels of metasystox-R were determined in foliage to ascertain whether the insecticide was moving within the tree. We unsuccessfully attempted to determine whether metasystox-R was accumulating in the phloem and bark, the target tissue where western pine beetle adults and larvae feed.

Foliage was sampled at 5, 20, and 40 days after treatment for pesticide residue analysis. Foliage was also taken from adjacent ponderosa pine trees on the same day as the treatments were applied to serve as untreated blanks. Six to 10 trees were arbitrarily selected for foliage collection from each treatment strategy. Only trees with a low crown were sampled so that mid-crown foliage samples could be easily taken with a 7-m pole pruner. Foliage was collected from three branches at mid-crown; samples from each tree were pooled.

Samples of phloem and bark tissue were collected 20 days after treatment by punching a 2.5-cm hole through the outer bark and collecting the phloem from two sides of the bole at 1.5 m above ground level. Phloem samples from different trees within the same treatment were combined in order to obtain 5 g of tissue for pesticide residue analysis. Samples of foliage and phloem were kept in a cooler and returned to our laboratory in Berkeley for determination of metasystox-R residue levels.

Residue levels in needles were determined by selecting needles (both new and old) from three twigs until a sample of 5 g was obtained.[20] Needles were precut into small pieces and placed in a "titeseal" vial with 20 g of sodium sulfate. Twenty milliliters of ethyl acetate (nanograde) were added, and the needles were pulverized with a Polytron. After the mixture was pulverized, it was centrifuged at 1,500 rpm for 5 minutes. Ten milliliters of activated charcoal were placed in a clean scintillation vial, and the supernatant from the extract was

added. This mixture was shaken on a vortex shaker for 30 sec, and was then filtered with #1 filter paper into a 60-ml round bottom flask. The fluid was evaporated to dryness using a rotovaporator.

The resulting residue was dissolved in 2 ml of acetone (nanograde). This solution was poured into a 125-ml separatory funnel to which 5 ml of 20 percent (w/v) aqueous $MgSO_4$ and 20 ml of freshly prepared 0.5 N aqueous $KMnO_4$ were added. The reaction was allowed to continue for 30 minutes with frequent swirling of the solution. Derivitized metasystox-R (oxydemeton methyl sulfone) was then extracted three times by using 20 ml of chloroform (nanograde). The extract was filtered through sodium sulfate, then evaporated to dryness. The residue was then dissolved in exactly 2 ml of acetone for gas/liquid chromatographic (GLC) analysis.

Samples were analyzed on a Hewlett-Packard 5890 gas/liquid chromatograph using an OV-101 High Resolution capillary column with an NP detector. Injector temperature was 210 °C; column temperature 185 °C; and detector temperature 245 °C. One microliter of the final solution was injected into the GLC. Resulting ng/µl values for metasystox-R sulfone were then converted to ppm.

Similar procedures were used for phloem samples. Phloem discs from more than one tree from the same treatment were combined to obtain 5 g of tissue.

Efficacy Assessment

The criterion used to determine the effectiveness of the insecticide treatment was whether individual trees succumbed to attack by western pine beetle and died.[5,7,17,18] Tree mortality was assessed on October 29 and 30. The period between pheromone removal and mortality assessment was sufficient for tree crowns to "fade," an irreversible symptom of mortality. Presence of western pine beetle galleries and brood was verified in each tree counted as dead or dying. Treatments were considered to have had sufficient western pine beetle pressure if at least 18 of 30 (60 percent) untreated control trees died after western pine beetle attack.[7,17,18] If less than 18 untreated trees were killed after baiting, our criterion of sufficient beetle attack was not met, and any inferences concerning efficacy of either treatment must account for reduced beetle attack pressure.

We considered a treatment ineffective if an average of less than 90 percent of the treated trees survive.[17] To test the null hypothesis that at least 90 percent of the trees survive on average, H_0: $p \geq 0.9$, with a significance level of no more than 5 percent and a power of at least 80 percent to detect a protection rate of only 70 percent, a sample of 30 trees for each treatment was needed. On the basis of our requirements we would reject the null hypothesis by using the one-sample binomial test if more than 6 trees out of 30 died as a result of western pine beetle attack. This rejection would result in a conservative significance level of 2.6 percent when the true protection rate is 90 percent and a power of 84 percent when the true protection rate is only 70 percent.

We examined differences from the untreated control with standard contingency table analysis (testing for equality of proportions between each tree condition class) to determine if the treatment had any effect. Tests were performed at the α=0.05 level.

Results and Discussion

Translocation of Metasystox-R in Ponderosa Pine

Residues of metasystox-R were detectable in the foliage for each collection period (5, 20, and 40 days post treatment) for both the preventive and remedial treatments *(table 1)*. The quantities ranged from an average of 0.10 ppm to 0.84 ppm and indicate that metasystox-R delivered into the xylem with Mauget injectors translocated within ponderosa pine trees under attack by western pine beetles.

We were not able to assess residue levels of metasystox-R in the phloem or bark, the target tissues, for two reasons. First, entire samples from infested trees were difficult to obtain because beetle galleries destroyed the phloem. Second, when phloem samples were processed, we were not able to quantify metasystox-R residues because of a large, interfering peak that eluted precisely where the oxydemeton methyl sulfone eluted. There were indications that the sulfone peak was present, but it was masked by the large interfering peak. Several attempts to clean the samples by using various column substrates failed. The interference peak could not be separated.

In summary, we know metasystox-R is translocated via stem xylem tissue to the foliage in small amounts, but we cannot be certain whether this insecticide then enters stem phloem and is translocated to western pine beetle attack sites.

Efficacy of Metasystox-R against Western Pine Beetle

Although we barely failed to achieve the predetermined and arbitrary level of mortality in the untreated check trees, this does not seriously alter our conclusions. The results show that the treatments were rigorously tested: 17 of 29 (58.6 ± 17.9 percent [$\bar{X}$ ± 95 percent CI]) untreated trees were killed by western pine beetle *(table 2),* a percentage that is not significantly different from 60 percent. Of the 12 untreated trees that survived, 7 had numerous pitch tubes with boring dust in bark crevices and had, in our opinion, experienced lethal levels of attack, but were simply slow to exhibit signs of fading *(table 2).*

Table 1—*Residues of oxydemeton methyl sulfone (ppm) in needles of ponderosa pine resulting from treatment with Mauget injectors, June 1990.*[1,2]

	Days after treatment					
	5		20		40	
Treatment	n	ppm	n	ppm	n	ppm
Preventive	8	0.62 (1.3)	9	0.10 (.28)	8	0.11 (.14)
Remedial	10	0.72 (.89)	6	0.84 (1.4)	8	0.45 (.77)

[1]Mean (Standard deviation)

[2]Only one of nine foliage samples from untreated trees contained oxydemeton methyl sulfone residues, but this one sample was below 0.01 ppm.

Table 2—*Condition of ponderosa pine trees 4 months after treatment with metasystox-R in Mauget injectors either 13 days before baiting (preventive treatment) or 7 days after baiting (remedial treatment) with western pine beetle aggregation pheromone (WPB Tree Bait).*

	Number of trees in various conditions[1]					
Treatment	n	1	2	3	4	3 and 4
Preventive	30	4	6	2	18	20
Remedial	30	5	7	2	16	18
Untreated	29	5	7	0	17	17

[1] Condition 1: Less than 10 "white" pitch tubes, no boring dust, foliage still green. Condition 2: Numerous pitch tubes (≥10) with boring dust present in bark crevices, but no fading of foliage. Condition 3: Numerous pitch tubes (≥50), boring dust abundant, and foliage beginning to show signs of fading. These trees were, in our opinion, dying. Condition 4: Numerous pitch tubes, abundant boring dust, *and* foliage faded to yellow, red, or brown color. These trees were dead.

Both metasystox-R treatments failed to meet the criterion of efficacy (six or less trees killed) set forth in the experimental design. Therefore, neither treatment was considered effective in protecting ponderosa pine from lethal attack by western pine beetle when trees were challenged with mass attack. Furthermore, neither metasystox-R treatment resulted in significantly less (or more) mortality than the untreated check treatment (Chi-square = 0.453 with 2 df). Thus, the ratio of live to dead trees did not vary among treatments more than by chance alone.

Conclusion

The results of this and similar experiments,[5,7,17,18,21] combined with information on application and handling, safety, environmental considerations, and cost information, can be used by land managers, pest control specialists, and private property owners to make informed decisions regarding individual tree protection. Management scenarios that may require protection of individual ponderosa pine from lethal attack by western pine beetle could include situations involving developed recreation sites, shade trees, or individual trees that possess some unique characteristic (specimen tree or seed production). Trees recovering from injury because of construction, logging activity, or fire may also require protection for a given amount of time. If duration of protection needed is short, e.g., part of one summer season, previous studies show that a registered insecticide, such as carbaryl, applied in a spray to the bole of the tree to a height of 10 m or into the crown, provides a high probability of protection.[5,7] However, if maximum time of protection were an important consideration, then more than one application of the insecticide may be justified.[5,7] Whatever the protection requirements, the registered use procedures were not efficacious, and this insecticide applied in Mauget injectors for either preventive or remedial control of western pine beetle to protect high-value ponderosa pine is not recommended based on the results of this study. Therefore, these treatments will not provide forest pest control specialists, land managers, or private citizens with an effective alternative to insecticides such as lindane and carbaryl applied to the bole of trees as toxic sprays for protection of high-value ponderosa pine from attack of western pine beetle.

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[13] Metasystox-R (S-[2-(Ethylsulfinyl)ethyl]O,O-dimethyl phosphorothiate), applied as a 50 percent solution with the Mauget INJECT-A-CIDE device, is registered for internal treatment by micro injection for systemic suppression of certain insects on ornamental trees. INJECT-A-CIDE applications for pines (except piñon) include engraver beetle (five-spined and Monterey), flathead borer, mountain pine beetle, red turpentine beetle, black turpentine beetle, and western pine beetle.

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[16] This note neither recommends the pesticide uses reported nor implies that they have been registered by the appropriate governmental agencies.

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