Pheromone-based disruption of *Eucosma sonomana* and *Rhyacionia zozana* (Lepidoptera: Tortricidae) using aerially applied microencapsulated pheromone

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Abstract: Two aerial applications of microencapsulated pheromone were conducted on five 20.2 ha plots to disrupt western pine shoot borer (*Eucosma sonomana* Kearfott) and ponderosa pine tip moth (*Rhyacionia zozana* (Kearfott); Lepidoptera: Tortricidae) orientation to pheromones and oviposition in ponderosa pine plantations in 2002 and 2004. The first application was made at 29.6 g active ingredient (AI)/ha, and the second at 59.3 g AI/ha. Baited sentinel traps were used to assess disruption of orientation by both moth species toward pheromones, and *E. sonomana* infestation levels were tallied from 2001 to 2004. Treatments disrupted orientation by both species for several weeks, with the first lasting 35 days and the second for 75 days. Both applications reduced infestation by *E. sonomana*, but the lower application rate provided greater absolute reduction, perhaps because prior infestation levels were higher in 2002 than in 2004. Infestations in treated plots were reduced by two-thirds in both years, suggesting that while increasing the application rate may prolong disruption, it may not provide greater proportional efficacy in terms of tree protection. The incidence of infestations even in plots with complete disruption suggests that treatments missed some early emerging females or that mated females immigrated into treated plots; thus operational testing should be timed earlier in the season and should comprise much larger plots. In both years, moths emerged earlier than reported previously, indicating that disruption programs should account for warmer climates in timing of applications. The AIs we tested are behaviorally active for 13 other species of *Rhyacionia* and six other species of *Eucosma*, so the approach may have wide application.

Résumé : Deux applications aériennes de phéromones microencapsulées ont été effectuées sur des parcelles de 20,2 ha pour désorienter le perce-pousse occidental du pin (*Eucosma sonomana* Kearfott) et le perce-pousse du pin ponderosa (*Rhyacionia zozana* (Kearfott); Lepidoptera : Tortricidae) et perturber leur oviposition dans des plantations de pin ponderosa en 2002 et 2004. La première application a été faite avec 29,6 g de matière active/ha et la seconde avec 59,3 g de matière active/ha. Des trappes de contrôle appâtées ont été utilisées pour évaluer la perturbation de l’orientation par les phéromones chez les deux espèces de perce-pousses et les niveaux d’infestation par *E. sonomana* ont été déterminés de 2001 à 2004. Les traitements ont désorienté les deux espèces pendant plusieurs semaines, soit 35 jours dans le cas du premier et 75 jours dans le cas du second. Les deux applications ont réduit l’infestation de *E. sonomana* mais le taux d’application le plus faible a provoqué la plus forte réduction absolue, peut-être à cause du taux de l’infestation précédente qui était plus élevé en 2002 qu’en 2004. Dans les parcelles traitées, l’infestation a été réduite des deux tiers les deux années, ce qui indique que si l’augmentation du taux d’application peut prolonger la durée de la perturbation, elle ne se traduit peut-être pas par une efficacité proportionnellement plus grande en terme de protection des arbres. L’incidence des infestations, même dans les parcelles où la perturbation était complète, indique que certaines femelles qui avaient émergé tôt n’ont pas été affectées par les traitements ou que des femelles accouplées ont migré vers les parcelles traitées. Par conséquent, les essais sur le terrain devraient avoir lieu plus tôt dans la saison et porter sur des parcelles beaucoup plus grandes. À chaque année, les perce-pousses ont émergé plus tôt que ce qui avait été rapporté auparavant, indiquant que les programmes de perturbation devraient tenir compte du réchauffement du climat dans le choix du moment des applications. Les substances actives que les auteurs ont testées...
pheromone release system is not long lasting. After maturation, female moths oviposit primarily on terminal shoots, indicating or killing the terminal (Wood et al. 2003). Whether or not the terminal is killed, the lateral shoots often become dominant, causing forking that severely damages tree form. It has been well demonstrated that ovipositing moths select the fastest growing shoots for oviposition (Sower and Mitchell 1987, 1988; Ross 1989; Thier and Marsden 1990; Pruett and Ross 1997). The reduced terminal elongation suffered by infested trees results in an evening of tree heights that masks the effect of infestation, because there are no other obvious external signs of infestation.

Pheromone-based disruption of moths toward potential mates or baited sentinel traps, often referred to as mating disruption (see References), is a well-developed pest control strategy that is especially appropriate for crops that can be managed on an area-wide basis (Cardé and Minks 1995), such as intensively managed pine plantations. We use the term “pheromone disruption” in this report, because our test methods allow conclusions about orientation toward pheromone-baited traps, but do not directly assess disruption of mating. Successful mating disruption may be inferred by population reductions, but cannot be explicitly proven without use of virgin female moths in traps, as well as proof that other mechanisms did not contribute to the population reductions. The pheromone blends for *E. sonomana* and various *Rhyacionia* species have been well documented (Sartwell et al. 1980; Skellen et al. 1997; El-Sayed 2005), and several pheromone release systems have been tested for *E. sonomana* disruption including attract-and-kill, lure-tapes, hollow fibers, and laminated flakes (Overhulser et al. 1980; Sartwell et al. 1980b, 1983; Sower et al. 1982; Sower and Mitchell 1994; Daterman et al. 2001). Niwa et al. (1991) demonstrated successful control of *R. zozana* using pheromone disruption techniques. Evidence has been presented indicating that previous *Rhyacionia frustrana* (Comstock) infestations predispose trees to heavier subsequent attacks (Coody et al. 2000), so protection from *R. zozana* attack may similarly confer protection for more than 1 year. Economic analyses have shown that pheromone-based treatments for the control of *E. sonomana* were cost effective (Williams et al. 1989), but some of the available products are excessively labor intensive because they require manual placement of pheromone point sources on the trees from ground level. Many western pine plantations are in very steep terrain, and road access at the beginning of moth flight (late March or early April) is problematic because it occurs just as the snowpack is melting. In addition, we felt that an aerially applied product might prove more effective because it would leave pheromone sources both on the forest floor, where *E. sonomana* overwinters, and on the trees, where mating takes place. For these reasons, we initiated a series of studies aimed at testing a new formulation of sprayable, microencapsulated (MEC)
pheromone (3M of Canada, London, Ontario, Canada). Our objective was to assess the effect of the aerially applied MEC on both pheromone disruption (E. sonomana and R. zozana) and tree infestation levels (E. sonomana only) in a northern California ponderosa pine plantation, using large plots to minimize the effect of immigration of gravid female moths from outside the treated plots.

Materials and methods

Pheromone formulation and application

The MEC formulation consisted of 20% 80:20 Z:E-9-dodecenyl acetate (active ingredient (AI) purchased from Bedoukian Research, Danbury, Connecticut) in a water suspension with 0.00125% (v/v) colorfast red dye (Becker Underwood, Inc., Ames, Iowa) added as a tracer and 0.00125% (v/v) Nu-Film-17 (Miller Chemical Corp., Hanover, Pennsylvania) added as a sticker. The formulation, which consists of 25 μm microcapsules containing the AI, was diluted in distilled water to achieve an application rate of 29.6 g AI/ha for 2002 treatments and 59.3 g AI/ha for 2004 treatments. Other than this difference in dilution rate of the MEC, there was no difference between the formulations for the two applications. Application was made at the rate of 18.7 L of formulated pheromone in water per hectare using a Bell 47-G3B2A turbine helicopter equipped with a 12 m boom fitted with 18 T-Jet D-4 nozzles oriented at 90° downward from the direction of flight. The target average volume mass diameter was 400 μm, and the airspeed during application was 72.5 km/h.

Study location

Plots were located in Siskiyou and Modoc Counties at elevations of 1200–1500 m (exact locations of plots and plantation stand characteristics shown in Table 1). Plot names are derived from the original plantation names, which are based on the names given to large wildfires that created the conditions for large, even-aged pine monocultures. Stands consisted of P. ponderosa seedlings aged 10–13 years at the time of the study installation in 2001.

Experimental design

The study was conducted on ten 20.2 ha plots with a minimum of 1 km between plots to avoid cross-contamination of treatments. We selected paired plots at each of five localities, with plots matched by apparent infestation levels, elevation, stand age, and stand structure (Table 1). Plots to be treated with the MEC were randomly selected from within each pair at each location. Applications were scheduled for 2002 and 2004, because previous studies (Sower and Overhulser 1986) had shown that a single application could suppress moth populations for 2 years. The plots treated in 2002 were used as treatment plots again in 2004, and the control plots were the same in both 2002 and 2004. A core sampling plot 0.405 ha in size was located at the center of each 20.2 ha plot. Every third tree (50 trees/plot) in each core plot was assessed at the end of the season for E. sonomana infestation. Infestation levels were thus measured before and after each pheromone application. Immediately following treatments in 2002 and 2004, five Pherocon Wing traps (IPM Technologies, Bend, Oregon) were suspended in each core plot at approximately 2 m in height from tree branches to serve as sentinel traps for assessment of efficacy of pheromone disruption. These traps were baited with rubber septa loaded with 0.05 mg 80:20 Z:E-9-dodecenyl acetate (IPM Technologies, releasing ca. 1.0 μg/day of the pheromone (Vrock et al. 1988). Separate moth collections were made using the same baits with Unitraps® (Phero Tech, Inc., Delta, British Columbia, Canada) to collect voucher specimens for deposit at the Essig Museum of Entomology, University of California, Berkeley, California, USA.

Statistical analysis

We used the generalized linear model, a logit–normal model (McCulloch and Searle 2001), for the probability of infested trees in treated years regressed on treatment (control vs. sprayed), year of treatment application (2002 and 2004), and

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Table 1. Site and stand characteristics of plots in Lassen County, California.

<table>
<thead>
<tr>
<th>Plot name</th>
<th>Plot type</th>
<th>North UTM</th>
<th>East UTM</th>
<th>Elevation (m)</th>
<th>Mean tree DBH (cm)</th>
<th>Tree height (m)</th>
<th>Year planted</th>
<th>Mean % infestation, 2001</th>
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<tr>
<td>Pondsos1</td>
<td>C</td>
<td>121.620</td>
<td>41.194</td>
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<td>3.39</td>
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<td>41.195</td>
<td>1250</td>
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<td>1993</td>
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</tbody>
</table>

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3Mention of a product does not constitute endorsement or recommendation for its use.
Fig. 1. (a) Mean number of *Eucosma sonomana* moths per trap, 2002; (b) mean number of *Rhyacionia zozana* moths per trap, 2002; (c) mean number of *Eucosma sonomana* moths per trap, 2004; (d) mean number of *Rhyacionia zozana* moths per trap, 2004; for Modoc and Siskiyou Counties, California.

The logit model is as follows:

\[
\text{logit } p_{ijk} = \log \left( \frac{p_{ijk}}{1 - p_{ijk}} \right) = \text{Intercept} + \text{Treat}_i + \text{Year}_j + \text{Slope}_j \times \text{PP}_{ijk} + \epsilon_k
\]

or, equivalently, solving for \( p_{ijk} \),

\[
p_{ijk} = \frac{e^{\text{Intercept} + \text{Treat}_i + \text{Year}_j + \text{Slope}_j \times \text{PP}_{ijk} + \epsilon_k}}{1 + e^{\text{Intercept} + \text{Treat}_i + \text{Year}_j + \text{Slope}_j \times \text{PP}_{ijk} + \epsilon_k}}
\]

where \( i = 1, \text{ and } 2, \) for treatment: 1, control; 2, treated; \( j = 1, \text{ and } 2, \) for year: 1, 2002; 2, 2004; \( k = 1, 2, 3, 4, \text{ and } 5, \) for site; \( p_{ijk} \) is probability (tree infested in site \( k \) for treatment \( i \) in year \( j \)). We assume that the \( x_{ijk} \) is the number of trees infested for a given site \( k \) has the binomial distribution \( B (p_{ijk}, n = 50) \), \( n = 50 \) is the total number of trees per treatment level and site. The explanatory variables are defined as follows: Treat\( i \) is effect of treatment \( i \), Year\( j \) is effect of year \( j \), Slope\( j \) is the coefficient of the percentage of infested trees in prior untreated year slope for year \( j \), PP\( _{ijk} \) is percentage of infested trees in the prior untreated year for year \( j \) for site \( k \) with treatment level \( i \) in year \( j \), and \( \epsilon_k \) is site \( k \) effect, assumed to be a random effect. Intercept is the overall mean. We used SAS (version 9.1.2) GENMOD procedure to estimate the effect parameters and make comparisons (SAS Institute Inc. 2004). The Wald \( \chi^2 \) test was used to test the difference between the slopes in 2002 and 2004.

| Parameter | Estimate | SE  | \( P >|Z| \) |
|-----------|----------|-----|-------------|
| Intercept | 4.46     | 0.67| <0.0001     |
| TreatControl | 1.40 | 0.23| <0.0001     |
| TreatTreated  | 0.00 | 0.00|             |
| Year 2002   | 2.28 | 0.37| <0.0001     |
| Year 2004   | 0.00 | 0.00|             |
| Slope2002   | 1.96 | 0.28| <0.0001     |
| Slope2004   | 3.91 | 0.73| <0.0001     |

Note: SE, standard error.
Results

Pheromone disruption

In 2002, *E. sonomana* and *R. zozana* emergence was well underway by the time of the first collection of trapped moths about 2 weeks following application of treatments (Figs. 1a and 1b). The treatments resulted in very good pheromone disruption of *E. sonomana* and excellent disruption of *R. zozana* until 29 April, at which point the efficacy of the treatments fell off steeply for *R. zozana*, and *E. sonomana* flight tapered off to zero in untreated stands. Several individuals of *E. sonomana* were counted in treated plots, mostly from the 29 April collection, which motivated the increase in pheromone application rate for 2004. There is indirect evidence that *E. sonomana* moths are capable of immigrating into the interior of plots this size (J.N. Webster, unpublished data), so we cannot say for certain whether they originated from inside or immigrated from outside the plots. Since it was not possible to slow the microcapsule release rate per se, we doubled the concentration of microcapsules in the tank mix for 2004 treatments to achieve higher pheromone levels over time following application.

In 2004 (Figs. 1c and 1d), *E. sonomana* flight was well underway by the first collection on 8 April, but *R. zozana* flight had not yet begun. Pheromone disruption for both moth species was nearly absolute in 2004 through the entire flight periods of both species, with only nine individuals of *R. zozana* trapped and one of *E. sonomana* trapped in treated plots during their entire flight periods.

Infestation levels

All effects in the model (year, treatment, and prior infestation level) were significant (Table 2) ($\alpha = 0.5$). The estimated control level was greater than the estimated treatment level (Table 2), moreover they were significantly different (Table 3) ($P < 0.0001$). Similarly, the year effect in 2002 was significantly different from that in 2004 (Tables 2 and 3) ($P < 0.0001$). Mean infestation levels in 2002 were 54.8% in controls versus 18.8% in treated stands; in 2004 they were 20.4% in controls and 6.8% in treated stands (derived from data shown in Fig. 2). The slopes for both 2002 and 2004 were significantly different from zero (Table 2), indicating that infestation levels in the year prior to treatment (i.e., baseline infestation levels) had a significant effect on infestation rates the year of the treatment. In addition, the slope in 2002 was significantly shallower than the slope in 2004 (Tables 2 and 3), suggesting that baseline (prior year) infestation levels affected current-year infestation levels differently in 2004 than in 2002.

The effect of baseline infestation in the model is confirmed by plotting percentage of infestation as a function of prior infestation (Fig. 3). First of all, the positive slopes in this graph confirm the assumption that prior infestation levels are positively related with prior infestation levels. However, the rate of increase of infestation levels was different in the two years, with significantly greater increase (as a function of prior infestation levels) in 2002 than in 2004. That is, the slopes for 2004 increase much more sharply at higher prior infestation levels than do the slopes for 2002, regardless of treatment. This graphic portrayal also clarifies that (i) regardless of the treatment applied (control or sprayed), infestations were significantly higher in 2002 than 2004; (ii) regardless of application year (2002 or 2004), the positive slopes indicate that the higher level of infestation in the untreated prior year explains the greater level of infestation in the treated year; and (iii) for both the treated years, the controls have a steeper slope than treated plots, indicating a lower rate of infestation response for the treated sites.

Discussion

In 2002, we were unable to apply the pheromone before the *E. sonomana* and *R. zozana* flights began. In 2004, we made the application before the *R. zozana* flight began but we again missed the beginning of the *E. sonomana* flight. Although we had deployed monitoring traps to time our applications, moth flight had already begun before the roads were passable in the early spring. Timing of applications was assumed to be a tradeoff between protection from early-emerging insects and maintaining sufficient protection over the entire flight period, because we were not sure that the formulations would last much longer than the insect flight period. A longer lasting formulation such as that applied in 2004, however, may provide sufficient protection even with an earlier application. Operational tests could undoubtedly be conducted before moth flight begins in the spring, but moth flight cannot be reliably quantified without the use of monitoring traps, which depend upon passable roads for their deployment and maintenance. Thus, although it should be feasible to achieve successful disruption and crop protection with this pheromone formulation, it may be difficult to demonstrate that success in an experimental setting in a steep, remote site where access is difficult until roads are dry. We recommend that for operational treatments, land managers apply such pheromone formulations 2–4 weeks earlier than we did, or as soon as possible in late winter, given uncertain weather conditions. Applying treatments earlier will help ensure disruption of moths even with fluctuating climate and consequent unpredictable moth emergence dates.
Fig. 2. Mean proportion of terminals infested per plot for year 2001, 2002, 2003, and 2004 (arrows indicate approximate timing of pheromone applications; closed green circles indicate plots scheduled for treatment in 2002 and 2004 and open red circles indicate control plots); for Modoc and Siskiyou Counties, California.

Fig. 3. Mean proportion of terminals infested in treated year plotted as function of infestation level in the year prior to treatment (red indicates year 2002 and green indicates year 2004; open squares and circles indicate controls, and filled squares and circles indicate treated plots). (Observed \( \hat{p}_{ijk} \) and \( p_{ijk} \) is estimated percentage of infested trees in treated year versus percentage of infested trees in prior untreated year for the logit model.)
Although the absolute reduction of infestation was greater in 2002 than in 2004 (Fig. 3), it appears from the data from sentinel traps that the 2004 treatment disrupted mating more effectively (Figs. 1a and 1c). The greater reduction in infestation in 2002 is probably a result of increased competition for resources in that year, since baseline moth populations were higher that year. The steeper slopes of the responses in 2004 versus 2002 (Fig. 3) support this explanation.

Regardless of existing moth populations, however, the treatments provided significant pheromone disruption in both years, especially for R. zozana in 2004, and clearly reduced infestations of E. sonomana in both years. The assessments of moth infestation were directed solely at E. sonomana, because we did not have the resources to assess R. zozana infestations as well. However, we plan to take 5-year height and diameter growth measurements, and those will reflect the cumulative effect of excluding both moth species for 2 of the 5 years. Previous cost–benefit analyses (Williams et al. 1989) supported the use of pheromone disruption in terms of increased volume at harvest, and our results provide yet another means of application that is especially promising, because it offers another, simpler method of aerial application, which will allow applications to take place even when roads are impassable in late winter or early spring, when moths are emerging. Other aerially applied formulations, such as laminated flakes, fibers, and pellets, may offer similar advantages. In areas where access by road is not problematic and where the terrain is accessible by field crews on foot, hand-applied pheromone disruption techniques, such as lure-tapes, puffers, and attract-and-kill (Daterman et al. 2001) may also be cost effective.

Pine plantations are routinely thinned to reduce competition among trees for water and sunlight, and E. sonomana infestation levels typically increase the year following a thinning (J.N. Webster, personal observation). The probable mechanism for this phenomenon is simply a reduction (normally by about half) in the available resource for oviposition, resulting in a concentration of oviposition on fewer available terminals. Although we know of no documentation for this explanation for E. sonomana, this mechanism is known to be important in cone and seed insect population dynamics (Daniel et al. 1979, p. 145). Thus, it could be beneficial to time the application of pheromone disruptions in tandem with stand thinning, to avoid concentrating the oviposition of moths on a smaller number of trees.

Sower and Overhulser (1986) reported that recovery of E. sonomana populations usually begins within 1–2 years following treatment, and our results concur (Fig. 2). It is thus important to treat every two years or so until trees are large enough to tolerate infestation without severe growth reductions. Large-scale pheromone applications have never been attempted (Wood et al. 2003), but area-wide coordinated treatments should be given serious consideration, because treatments on a large scale would minimize edge effects and delay the recovery of moth populations, thus reducing both immigrating gravid females during the year of treatment and reinvasion of moths in years following the treatment. Such treatments should be doubly effective if timed to coincide with thinnings. The active ingredients in the pheromone blend that we evaluated have also shown behavioral activity for 13 other species of Rhyacionia and 6 other species of Eucosma (Grant et al. 1985, 2002; Skillen et al. 1997; http://www.pherobase.com), so this approach may have a broad application for control of other damaging pest species of pine plantations and Christmas tree farms in the Pacific Northwest and in the Southeastern United States.

Acknowledgements

We thank G.E. Daterman (retired from USDA Forest Service, Corvallis, Oregon) and D. Czokajo (IPM Technologies, Bend, Oregon) for valuable advice. S. Henson (Roseburg Resources, Weed, California), B. Rynearson (Beatty and Associates, McArthur, California) and M. Gray (Sierra Pacific Industries, Anderson, California) provided access to study plots on private lands and assisted in implementation of the study. G.G. Grant (Environment Canada, Ottawa, Ontario) generously provided guidance in study design and implementation. C.L. Peatman and G. Oliver (3M of Canada) provided microencapsulated E.Z-9-dodecencyl acetate. G.O. Fiddler (USDA Forest Service, Redding, California), G. Ferrell (USDA Forest Service, retired), C. Shestak (USDA Forest Service, Redding, California), Gaylord Briggs, and Joseph Turner (Total Forestry, Anderson, California) provided valuable assistance in plot installation and assessment of infestation rates. J. Powell and P. Oboyski (University of California, Berkeley) confirmed the identity of the specimens. John Mateski (Western Helicopter) conducted an impeccable application of the MEC.

References


Hessburg, P.F., Smith, B.G., Salter, R.B., Ottman, R.D., and Alvarado, E. 2000. Recent changes (1930s–1990s) in spatial pat-


