

AN ABSTRACT OF THE THESIS OF

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Title: Effectiveness of Pheromone Mating Disruption for the Ponderosa  
Pine Tip Moth, *Rhyacionia zozana* (Kearfott) (Lepidoptera: Tortricidae),  
and its Influence on the Associated Parasite Complex

Abstract approved:

*Redacted for Privacy*

Gary E. Daterman

*Redacted for Privacy*

Tim D. Schowalter

The importance of pheromones in insect control relies both on their ability to reduce pest populations and on their relatively benign effects on nontarget organisms. This study was conducted to test the effectiveness of a pheromone application for mating disruption of the ponderosa pine tip moth, *Rhyacionia zozana* (Kearfott), and to determine if this treatment had any affect on the abundance or structure of the associated parasite complex.

Chemical analyses, electroantennograms, and field bioassays showed that the most abundant pheromone component for *R. zozana* was E-9-dodecenyl acetate with a lesser amount of E-9-dodecenol also present. Acetate/alcohol ratios averaged 70:30 in gland washes; male moths were most attracted to sticky traps with synthetic baits containing ratios ranging from 70:30 to 95:5.

Sixteen hymenopteran and one dipteran species of parasites were recovered from *R. zozana* larvae and pupae collected in Calif. and

Oreg. Total percentage parasitism was high, averaging 47.2%. The ichneumonid, Glypta zozanae Walley and Barron, was the most abundant parasite, attacking over 30% of the hosts collected. Mastrus aciculatus (Provancher) was second in abundance, accounting for less than 4% parasitism.

Hercon laminated-tape dispensers containing synthetic sex pheromone (a 95:5 mixture of E-9-dodecenyl acetate and E-9-dodecenol) were manually applied on 57 ha of ponderosa pine plantations in southern Oreg. The nominal dosage was 13.5 g of pheromone/ha from 100 releasers per hectare spaced at 10 m. Male moth response to pheromone-baited traps and to virgin females showed nearly total disruption of female sex pheromone communication with males. After the mating-disruption application, larval populations were reduced 83.2% in treated areas. The frequency of damaged terminal shoots was 50% lower in pheromone treated plantations than in check plots.

Total percentage parasitism was not changed due to the disruption treatment. However, in treated areas, abundances of G. zozanae and M. aciculatus, were reduced and increased, respectively, compared to check plantations. A similarity index showed that the parasite community structures of check and treated areas differed more after disruption than before. Accounting for parasitism and reduced mating, surviving host populations in check plantations were over four times greater than in pheromone disrupted areas. The importance of the host's sex pheromone as a potential kairomone, and effects of changes in the host density caused by the mating disruption treatment on the associated parasites are discussed.

Effectiveness of Pheromone Mating Disruption for the Ponderosa Pine  
Tip Moth, Rhyacionia zozana (Kearfott) (Lepidoptera: Tortricidae),  
and its Influence on the Associated Parasite Complex

by

Christine G. Niwa

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*Redacted for Privacy*

Associate Professor of Entomology in charge of major

*Redacted for Privacy*

Associate Professor of Entomology in charge of major

*Redacted for Privacy*

Head of Department of Entomology

*Redacted for Privacy*

Dean of Graduate School

Date thesis is presented October 24, 1988

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"O Freunde, nicht diese Töne!

Sondern laßt uns angenehmere anstimmen und freudenvollere!"

- Friedrich von Schiller

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## CONTRIBUTION OF AUTHORS

This series of studies was conceived by Christine G. Niwa and Gary E. Daterman with the primary objective of evaluating possible effects of a pheromone-based control method on the target pest's natural enemies. In Chapter II, Chemistry and field evaluation of the sex pheromone of ponderosa pine tip moth, Christine G. Niwa designed the majority of the study, performed all laboratory and field work except mass spectrometry, analyzed the data, and wrote the manuscript. Lonnie L. Sower conducted the mass spectrometry analyses. Lonnie L. Sower and Gary E. Daterman participated in planning the study. In Chapter IV, Control of Rhyacionia zozana by mating disruption with synthetic sex pheromone, Christine G. Niwa took the lead in designing the study, performed or supervised all field work, conducted statistical analyses, and prepared the manuscript. Gary E. Daterman, Charles Sartwell, and Lonnie L. Sower participated in the design of the study.

EFFECTIVENESS OF PHEROMONE MATING DISRUPTION FOR THE PONDEROSA PINE  
TIP MOTH, RHYACIONIA ZOZANA (KEARFOTT) (LEPIDOPTERA: TORTRICIDAE),  
AND ITS INFLUENCE ON THE ASSOCIATED PARASITE COMPLEX<sup>1</sup>

I. Introduction

Biorational approaches to pest control have gained favor in recent years, due to the rise in public concern over possible health hazards of pesticides. The use of pheromones for insect control has provided a method that is safe as well as effective for certain pest species. While toxic effects are unlikely, little is known about indirect influences pheromone application may have on associated organisms, particularly natural enemies. For instance, parasites that use pheromones as kairomones for host location, may be either drawn to an area with high pheromone concentrations, or conversely, disrupted by the confusing signal of large amounts of stimulant. Low host densities caused by pheromone treatments could affect parasite species differently, depending on their search behavior.

The ponderosa pine tip moth, Rhyacionia zozana (Kearfott), a pest of young pine plantations, provided an excellent test species because of its consistently low population densities, and relatively high parasitism levels. R. zozana attacks numerous Pinus spp. throughout the western United States. It is univoltine, pupae overwinter attached

<sup>1</sup> This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

to root collars of host trees and adults emerge in the spring. First and second instar larvae feed on needle sheaths within silken tents; third and fourth instars feed gregariously within both terminal and lateral shoots, leaving only the thin outer bark. R. zozana is most destructive in newly planted ponderosa pine (P. ponderosa Lawson) plantations, where repeated attacks can cause growth reductions and stem deformities. Though seedlings are rarely killed by R. zozana, poor growth may eventually lead to mortality because of competition with understory vegetation. Reduced growth and poor stocking can result in lengthened crop rotations, additional site preparation, and costly replanting.

The first objective of this study was to conduct a mating disruption test of R. zozana which would result in significant reductions in mating frequency, population density, and damage levels. The second goal was to determine any influence of the pheromone treatment on the associated parasite complex, including kairomonal effects, changes in parasite abundance, and shifts in the community structure.

In Chapter II, the female sex pheromone of R. zozana is identified using mass spectrometry, gas chromatography, electroantennograms, and field bioassays.

Chapter III, describes the parasites and predators recovered from collections of R. zozana from Calif. and Oreg. Relative densities and parasite biologies are discussed.

Chapter IV describes a mating disruption field test for R. zozana in southern Oreg. The effects of the pheromone application on male

attraction, mating, population density, and host tree damage levels are detailed.

Finally, Chapter V looks at the influence of the mating disruption treatment on the associated parasites. Species abundance, relative distribution, kairomonal response, and host density effects are examined.

II. Chemistry and Field Evaluation of the Sex Pheromone of  
Ponderosa Pine Tip Moth, Rhyacionia zozana  
(Lepidoptera: Tortricidae)

CHRISTINE G. NIWA, LONNE L. SOWER, and GARY E. DATERMAN

Pacific Northwest Research Station  
Forestry Sciences Laboratory  
Corvallis, Oregon 97331

## ABSTRACT

Chemical analyses, electroantennograms, and field bioassays were conducted to determine the sex pheromone of the ponderosa pine tip moth, Rhyacionia zozana (Kearfott). The most abundant pheromone component was E-9-dodecenyl acetate with a lesser amount of E-9-dodecenol also present. While acetate/alcohol ratios averaged 70:30 in gland washes, male moths were most attracted to sticky traps with synthetic baits containing ratios ranging from 70:30 to 95:5. Laboratory and field tests suggest that Z-9-dodecenyl acetate may also be a pheromone component. No Z-9-dodecenol was detected using gas chromatography-mass spectrometry methods.

## INTRODUCTION

Ponderosa pine tip moth, Rhyacionia zozana (Kearfott), is distributed throughout the western United States, associated with its main host, Pinus ponderosa Lawson. Larvae feed within elongating shoots, causing growth loss and stem deformities (Stevens 1966). Damage is most harmful in pine plantations where young trees are also injured by other insects and rodents, and where they must compete with surrounding vegetation to survive.

Field screenings of synthetic compounds have shown E-9-dodecenyl acetate (E9-12:Ac) to be a sex attractant for males of this species (Sower et al. 1979, Stevens et al. 1980). This chemical has previously been identified as either a sex pheromone or attractant for several Rhyacionia species (Smith et al. 1974, Roelofs et al. 1979, Hill et al. 1981, Grant et al. 1985). Sower et al. (1979) found that increasing ratios of the Z isomer (Z9-12:Ac) to E9-12:Ac reduced the attraction of R. zozana males to baited traps.

This study was undertaken to further define the pheromone system of R. zozana. Identification of its sex pheromone could enhance monitoring and control efforts for this pest.

## MATERIALS AND METHODS

Specimens used in the laboratory analyses were collected in the field as pupae near Placerville, Calif., and Chiloquin, Oreg. Pupae were put in petri dishes and placed in a photoperiod of 16:8 (L:D) until eclosion.

**Pheromone Extraction.** Terminal abdominal segments of females were extended by placing pressure on the abdomen, then seized with forceps, excised, extracted in double-distilled hexane for 10-20 s, and discarded (methods of Sower et al. 1973). Females were 2-3 d old and extracts were made 1-3 h after the onset of scotophase.

**Chemical Analyses.** Two wall-coated glass capillary columns were used to obtain gas chromatographs (GC) of crude extracts of from 1 to 10 females per sample. A nonpolar column (DB-1, 30 by 0.259 mm ID, J&W Scientific) was used with an initial oven temperature of 55°C for 1.2 min; temperature was then programmed to rise at a rate of 11°C/min to a final value of 240°C. The program for the polar column (Carbowax, 30 m by 0.251 mm ID, J&W Scientific) was oven temperature at 55°C initially for 1 min, then raised at a rate of 7°C/min to 240°C. Carrier gas flow (nitrogen) was 16 ml/min.

Pooled hexane extracts from 290 females were submitted to silica-column fractionation before gas chromatography-mass spectrometry (GC-MS) analysis. The rinse was concentrated to 100  $\mu$ l and purified by eluting it through a 3- by 150-mm column of 325-mesh silicic acid with increasing concentrations (5 ml each of 0, 1, 3, 10, and 30%) of diethyl ether in hexane. The elutant was collected in 25 1-ml fractions.

Each fraction was tested for biological activity using electroantennogram (EAG) techniques generally as described by Roelofs (1984). Excised male antennae were inserted into glass capillary electrodes so that haemolymph was in direct contact with 0.1 M KCl solution at each electrode. Change in electrical potential between the base and tip of the antenna was measured on exposure to a sample using an electroantenna graph amplifier (Murphy Developments). Antennae were exposed to residues from a 50- $\mu$ l aliquot of each fraction placed in the lumen of a 5-ml pipette. The more volatile hexane evaporated quickly and was expelled by repeatedly squeezing the rubber bulb. Additional volatiles, which would include any potential pheromone materials, were then allowed to evaporate into the pipette for at least 1 min. The gas-phase contents of the pipette were then injected into a gentle air stream directed over the antennal preparation. Responses to each fraction, and a hexane residue-only control, were then recorded. This procedure was replicated with three different male antennae. Fractions 14 and 15 elicited electroantennal responses that were much greater (ca. 4X) than responses to solvent residue only. No other EAG active fractions were found.

Fractions 14 and 15 were combined and examined by GC-MS using a Hewlett-Packard 5970B mass-selective detector (Palo Alto, Calif.). The GC columns used were 40-m polar (DB-WAX) or nonpolar (DB-1) capillaries. The program for the polar column was holding at 55°C for 1 min after injection, raising the temperature at 30°C/min to 100°C, and then raising it again at 5°C/min to 240°C. The carrier gas was helium at 12 PSI. Conditions for the DB-1 column were

similar to the polar column, except that the maximum temperature was 300°C.

Five groups of crude gland dips (totaling 270 females) were further analyzed using GC-MS to search for other materials related to the active compound. The abdominal tip of each female was dipped briefly in ca. 20  $\mu$ l of hexane, and 3  $\mu$ l fractions were injected.

Electroantennograms of Synthetic Materials. Responses of seventeen antennae from 20 to 3-d-old males were compared for three single compounds (E9-12:Ac, Z9-12:Ac, and E-9-dodecenol (E9-12:OH)), three blends (E- and Z9-12:Ac at a 95:5 ratio; E9-12:Ac and OH at a 95:5 ratio; and E9-12:Ac, Z9-12:Ac, and E9-12:OH at a 90:5:5 ratio), hexane, and air. These six materials were tested in random order on each antenna. All compounds and blends were tested at concentrations of 100 ng of total component or components in 5  $\mu$ l of hexane.

Field Bioassay Procedures. Synthetic pheromone blends were formulated into polyvinyl chloride controlled-release bait pellets (Daterman 1974). They were all 5 by 3 mm in diameter and contained 0.01% total pheromone components by weight. Each bait pellet was suspended on an insect pin from the interior center of a Pherocon II trap.

E9-12:Ac alone and six pheromone blends were tested: E- and Z9-12:Ac (95:5); E9-12:Ac and OH (95:5, 70:30, 60:40, and 50:50); and E9-12:Ac, Z9-12:Ac, and E9-12:OH (90:5:5). Traps were hung on ponderosa pine branches, from 1.5 to 2 m above the ground and at least 20 m apart. Each set of seven baits was replicated in 14 different locations near Sisters and Chiloquin, Oreg. Traps were in the field continuously from 24 April to 19 June 1985.

Statistical Methods. A two-way analysis of variance (with the 17 antennae used as blocks) and Fisher's Protected Least Squares difference multiple comparison test (Snedecor & Cochran 1980) were used to determine differences in EAG response to the synthetic compounds.

Friedman's two-way analysis of variance and Wilcoxon's signed rank test (Steel & Torrie 1980) were used to analyze the number of males caught in bioassay traps during the entire trapping period.

## RESULTS

Chemical Analyses. Results of the GC analyses of crude gland washes are presented in Table II.1. On both polar and nonpolar columns, there was a large peak with a retention time that coincided with authentic E9-12:Ac (mean = 0.45 ng per female); a smaller amount of material with identical retention times to E9-12:OH (mean = 0.22 ng per female) was also present. The average ratio of acetate/alcohol was 70:30; however, this varied considerably between different batches of hexane dips (range, 39-88% E9-12:Ac).

GC-MS examination of the combined EAG-active fractions 14 and 15 from the purified extract of 290 females showed that ca. 100 ng of a single compound was present. This compound had identical retention times, on both polar and nonpolar columns, to E9-12:Ac. Further, the mass spectrum was identical to that of E9-12:Ac. The 10 biggest ion masses, in descending order of abundance, were: 43, 68, 41, 82, 67, 55, 81, 95, 96, and 166. Subsequent tests by EAG of like quantities (10 ng) of E9-12:Ac and this material gave similar results.

On GC-MS examination, crude extracts of 270 females were found to contain both E9-12:Ac and E9-12:OH. Material peaks had identical retention times and mass spectra to known synthetic materials. This confirmed the presence of E9-12:Ac in the gland extracts. No other materials that had mass spectra similar to 9-12:Ac, the corresponding alcohol, or other known straight-chain 12-16 carbon acetates or alcohols were found except a trace of a material having the same retention time as Z9-12:Ac. Mass-spectra resolution for this trace was

inadequate to either confirm or deny its identity. No evidence of Z9-12:OH was found.

Electroantennograms. E9-12:Ac alone, and all three of the compound blends tested, elicited significantly greater EAG responses than did the checks, Z9-12:Ac, or E9-12:OH alone (Table II.2). These results support the chemical analyses, which indicate that E9-12:Ac is a primary pheromone component for this species. The addition of small quantities of Z9-12:Ac, E9-12:OH, or both to E9-12:Ac did not significantly change antennal stimulation.

Field Bioassay. Five of the six pheromone blends of E9-12:Ac, Z9-12:Ac, and E9-12:OH attracted significantly more males than did the 100% E9-12:Ac bait (Table II.3). From the ratios of E9-12:Ac to E9-12:OH tested, a small proportion of alcohol (less than 30%) appears optimal, with attraction dropping off when larger quantities are used. Inclusion of a small amount (5%) of the Z9-12:Ac isomer with E9-12:Ac was more attractive than E9-12:Ac alone, whether or not E9-12:OH was also present in the bait.

## DISCUSSION

Data from chemical analyses, male antennal responses, and field screening establish E9-12:Ac and E9-12:OH as active components of the R. zozana sex pheromone, and suggest that the Z9-12:Ac isomer may also be involved. Inclusion of Z9-12:Ac as a pheromone component needs further clarification; additional field and flight-tunnel studies may help to answer this question.

While acetate/alcohol ratios in abdominal washes averaged 70:30, higher ratios (up to 95:5) were effective in attracting male moths to sticky traps. Similarly, in determining the pheromone of the subtropical pine tip moth (R. subtropica Miller), Roelofs et al. (1979) identified both E9-12:Ac and E9-12:OH in gland washes; however, the presence of as little as 1% alcohol in pheromone traps resulted in a significant decrease in the capture of male R. subtropica.

Recent studies on the pheromone chemistry of Rhyacionia species have identified E9-12:OH as a secondary pheromone component. Gray et al. (1984) found that the addition of about 3% E9-12:OH to E9-12:Ac improved attraction of male R. buoliana to sticky traps. For both R. adana Heinrich and R. busckana Heinrich, lures containing E9-12:Ac and 1-30% of the corresponding alcohol were the most effective in trapping males (Grant et al. 1985). E9-12:OH has also been identified in gland extracts and female effluvium of the Nantucket pine tip moth, R. frustrana (Comstock), but its role in pheromone communication has not yet been determined (Hill et al. 1981).

Several of these Rhyacionia species are sympatric with R. zozana and share similar pheromone components, thus raising the question of

how species isolation is maintained. R. frustrana has been recovered in Calif. (Brown & Eads 1975); however, in addition to E9-12:Ac, this species requires E9,11-12:Ac as an active pheromone component. Though R. buoliana and R. zozana use similar blends of E9-12:Ac and E9-12:OH, R. buoliana mates in June, after most R. zozana have completed their flight. Since R. busckana and the R. zozana share similar distributions and flight periods, species isolation may be sustained by other factors, such as different acetate/alcohol ratios, additional pheromone components, or differences in daily flight times.

Table II.1. Sex pheromone components identified from gas chromatography of hexane washes of female R. zozana abdominal tips

Column	No. of females	E9-12:Ac : E9-12:OH ratio	Total pheromone per female (ng)
Carbowax	10	75:25	0.72
	5	71:29	0.42
	5	74:26	0.77
	4	67:33	0.58
	3	88:12	0.34
	3	83:17	0.71
	1	83:17	0.60
DB-1	8	55:45	1.71
	8	55:45	0.53
	3	78:22	0.50
	2	39:61	1.00
	2	66:34	0.32
	1	77:23	0.52
Average		70:30	0.67

Table II.2. Means and ranges for R. zozana male antennal responses to synthetic pheromone components

Compound(s)	Mean response <sup>2</sup> (mV)	Range
95% E9-12:Ac + 5% Z9-12:Ac	1.9a <sup>3</sup>	1.0-4.4
95% E9-12:Ac + 5% E9-12:OH	1.9a	0.9-3.6
100% E9-12:Ac	1.7ab	0.7-3.6
90% E9-12:Ac + 5% Z9-12:Ac + 5% E9-12:OH	1.5b	0.6-2.9
100% Z9-12:Ac	1.1c	0.3-2.9
100% E9-12:OH	0.7d	0.2-2.0
Air	0.5d	0.1-1.2
Hexane	0.5d	0.1-0.9

<sup>2</sup> n = 17.

<sup>3</sup> Means followed by the same letter are not significantly different (ANOVA  $P < 0.01$ ; Fisher's protected LSD,  $P = 0.05$  [Snedecor & Cochran 1980]).

Table II.3. *R. zozana* males captured by traps baited with various ratios of E- and Z-9-dodecenyl acetate and E-9-dodecenol formulated in polyvinyl chloride with 0.01% active ingredient, 24 April to 19 June 1985

Bait	Mean no. of males per trap <sup>4</sup>
95% E9-12:Ac + 5% E9-12:OH	34.1a <sup>5</sup>
90% E9-12:Ac + 5% Z9-12:Ac + 5% E9-12:OH	26.2a
95% E9-12:Ac + 5% Z9-12:Ac	20.8a
70% E9-12:Ac + 30% E9-12:OH	16.9a
50% E9-12:Ac + 50% E-9-12:OH	4.4b
60% E9-12:Ac + 40% E9-12:OH	2.6bc
100% E9-12:Ac	1.2c

<sup>4</sup> n = 14.

<sup>5</sup> Means followed by the same letter are not significantly different (Friedman's test,  $P < 0.01$ ; Wilcoxon's signed-rank test,  $P = 0.05$  [Steel & Torrie 1980]).

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III. Parasites and Predators Associated with the Ponderosa Pine  
Tip Moth, Rhyacionia zozana (Kearfott) (Lepidoptera: Tortricidae),  
in California and Oregon

CHRISTINE G. NIWA

Pacific Northwest Research Station  
Forestry Sciences Laboratory  
Corvallis, Oregon 97331

## ABSTRACT

Sixteen hymenopteran and one dipteran species of parasites were recovered from Rhyacionia zozana (Kearfott) larvae and pupae collected in Calif. and Oreg. Total percentage parasitism was high, averaging 47.2% in quantitative studies. The ichneumonid wasp, Glypta zozanae Walley & Barron, was the most abundant parasite, attacking over 30% of the hosts collected. Parasitization rates are presented for each parasite species recovered. Two suspected predators, Phyllobaenus binotatus (Chapin) (Coleoptera: Cleridae) and Leptothorax sp. (Hymenoptera: Formicidae), were found in association with R. zozana cocoons. A single Aegilips sp. (Hymenoptera: Figitidae) was recovered from a host cocoon; the relation between this parasite and R. zozana is as yet undetermined. Parasite biologies are discussed.

## INTRODUCTION

Ponderosa pine tip moth, Rhyacionia zozana (Kearfott), is a native pest occurring throughout most of the western United States (Stevens et al. 1980). This moth is univoltine; larvae infest and injure or kill both lateral tips and terminal shoots of young pines (Pinus spp.) from late May through mid-August (Stevens 1966). Pupae overwinter in the soil at the root collars of hosts, and adults emerge the following spring. Shoot destruction by larvae reduces tree growth and thus increases the time a seedling is vulnerable to other insect and vertebrate pests, and may impair the ability of young trees to compete with surrounding vegetation. Early growth loss and poor establishment of young trees will lengthen rotation times and can necessitate new site preparation and replanting.

Although the parasites of other Rhyacionia species have been studied extensively (Yates 1967a, Harman & Kulman 1973), little is known about the parasites of R. zozana. In Placerville, Calif., Stevens (1966) found ca. 10% parasitism of R. zozana larvae and pupae by unidentified ichneumonid wasps. All of these parasites overwintered in cocoons and emerged in early summer to attack the next host generation. An egg parasite, Trichogramma sp., was encountered rarely.

During studies of R. zozana in Calif. and southern Oreg., parasites and other associated insects were reared and identified. This paper reports the species recovered, their relative abundance, and observations on parasite biologies as they relate to this host.

## MATERIALS AND METHODS

Quantitative study. Collections were made in eight ponderosa pine (Pinus ponderosa Lawson) plantations near Chiloquin, Oreg. The plantations were 4 yr old when the study began in 1984 and trees averaged 78 cm tall.

One larval and two pupal collections (fall, after pupation was completed and spring, before emergence) were conducted during each of two host generations. Larval samples, mostly third and fourth instars, were made by randomly collecting 25 damaged tips per plantation. Shoots were dissected, and the larvae were removed and fed a synthetic diet containing ponderosa pine shoots until pupation. Each pupal sample consisted of 50 randomly selected cocoons, with no more than two cocoons collected per tree.

All insects were reared individually in petri dishes. Host cocoons from rearings of larval and fall pupal collections were chilled for 5 mo at 3°C and then maintained at a photoperiod of 16:8 (L:D) until emergence of either adult moths or parasites. Parasites were identified by specialists at the Systematic Entomology Laboratory USDA-ARS, Beltsville, MD (W.F. Barr (Cleridae), R.W. Carlson (Ichneumonidae), E.E. Grissell (Pteromalidae, Torymidae), P.M. Marsh (Braconidae), A.S. Menke (Figitidae), M.E. Schauff (Eulophidae, Eupelmidae), D.R. Smith (Formicidae), and N.E. Woodley (Tachinidae)); Kings River Conservation District, Fresno, CA (J.A. Halstead (Chalcididae)); and Biosystematics Research Centre, Agriculture Canada, Ottawa, ONT (J.R. Barron (Ichneumonidae)). Unemerged cocoons were dissected for the presence of R. zozana or parasites.

Qualitative Study. Approximately 4,500 larvae and pupae were collected (about 500 pupae from near Placerville, Calif., and the remainder from plantations near Chiloquin, Oreg.) during 1984 to 1987. These specimens were reared as described in the quantitative study, except that the unemerged cocoons were not dissected, and therefore only a qualitative report of species recoveries can be made from these collections.

## RESULTS AND DISCUSSION

Percentage parasitism. Total parasitism averaged 47.2%, ranging from 36.9 to 55.5% in the quantitative collections (Table III.1). This rate is much higher than the average larval and pupal parasitism of 9.7% that Stevens (1966) found in Calif. Parasitism of Rhyacionia buoliana (Denis & Schiffermüller) and Rhyacionia frustrana (Comstock) also varies widely, ranging from a few to over 50% of hosts attacked (e.g. Torgersen & Coppel 1965, Lashomb et al. 1980, Kolk 1982, Staines et al. 1984).

Glypta zozanae Walley & Barron (Ichneumonidae) was the most abundant parasite, attacking over 30% of the hosts in the quantitative collections (Table III.1). Mastrus aciculatus (Provancher) (Ichneumonidae) was second in abundance, though it accounted for less than 4% parasitism. The remainder of the species were encountered infrequently, each parasitizing less than 1% of the R. zozana reared.

Species recoveries and biologies. A total of 16 hymenopteran and one dipteran parasite species were recovered from all of the collections combined (Table III.1). Most species were reared exclusively from Oreg. host material; the exceptions, G. zozanae, M. aciculatus, Itopectis quadricingulata (Provancher), and Tetrastichus turionum (Hartig), occurred in both Calif. and Oreg.; Arotrephes sp. and Eupelmus allynii (French) were reared only from Calif. collections.

Rhyacionia zozana is a new host record for all of the parasites reared except for the ichneumonid Campoplex conocola (Rohwer), which had been reported previously (Krombein et al. 1979). The recovery of

Hockeria tenuicornis (Girault) from R. zozana represents the first host record for this parasite, and marks its first recorded occurrence in Oreg. (Halstead & Niwa 1987). Four of the parasites reared in this study are known to attack other Rhyacionia species (Harman & Kulman 1973, Krombein et al. 1979): I. quadricingulata is a pupal parasite of R. buoliana and Rhyacionia buskana Heinrich in North America; Dibrachys cavus (Walker) is reported as a secondary parasite of R. buoliana in North America and Poland; T. turionum is an introduced, gregarious, larval-pupal or pupal parasite of R. buoliana in North America, and attacks Rhyacionia duplana (Hübner) and R. buoliana (primary and secondary) in Europe; Erynnia tortricis (Coquillett) is a larval-pupal parasite of R. buoliana and Rhyacionia bushnelli (Busck) in North America.

Most R. zozana parasites collected in this study attacked larvae and emerged from host cocoons the following spring (Table III.2). Mastrus aciculatus, I. quadricingulata, Coccygomimus sanguinipes erythropus (Viereck), and Microdontomerus fumipennis Crawford were never recovered from larval collections, and so apparently attacked pupae in the fall. Three parasites, C. conocola, Euderus sp. near cushmani (Crawford), and E. tortricis, attacked larvae and emerged in late summer or early fall the year of attack. These emergence times indicated that these species may also parasitize other hosts. Although most Sinophorus rhyacioniae Sanborne emerged the spring after attack, a few adults were recovered in the preceding summer/fall. All of these individuals came from larval collections that were reared on artificial diet under laboratory conditions, however, which may have affected their emergence time. A few C. sanguinipes erythropus also emerged

early, during the overwintering cold period. Because of their rare occurrence, determination of specific attack periods for Diadegma sp., Arotrephes sp., D. cavus, and E. allynii was not possible.

Sex ratios (F:M) were calculated for four of the parasite species. For nearly 2,000 G. zozanae reared, the sex ratio was 1:1. Slightly more female than male (1.5:1) M. aciculatus were recovered. The sex ratio of the gregarious parasite Bracon sp. was 2:1, with an average of six individuals emerging from each host pupa. An average of 26 T. turionum per host pupa were reared, with a sex ratio of 7:1. In R. buoliana, means of 17-23 T. turionum per host have been recovered, with the same sex ratio found in R. zozana (Juillet 1959, Schaffner 1959, Arthur & Juillet 1961).

In addition to the parasites reared during the study, three other insects were recovered in association with R. zozana cocoons. Two adult clerid beetles, Phyllobaneus binotatus (Chapin), and six female Leptothorax sp. (Hymenoptera: Formicidae) were recovered from fall and spring pupal collections. A single Aegilips sp. (Hymenoptera: Figitidae) was reared from a cocoon collected in the spring.

At least five species of clerids (including three Phyllobaneus spp.) have been recorded as predators of R. bushnelli, R. frustrana, and Rhyacionia rigidana (Fernald) within infested shoots (Miller & Neiswander 1959, Yates 1967b, Eikenbary & Fox 1968). Clerid eggs are usually deposited on needles, and larvae feed within tips, consuming several host larvae and pupae during their development (Wingfield & Warren 1968). The recovery of clerids from cocoons attached to root collars of trees is somewhat difficult to explain because only a single host is available within each cocoon. In the laboratory, Eikenbary &

Fox (1968) found that adult clerids were predaceous on tip moth larvae, pupae, and adults. The clerids collected could have been foraging as adults and used the empty cocoons as refuges.

Several ant species have been observed feeding on pine tip moth larvae and pupae in the field and laboratory (Juillet 1961, Eikenbary & Fox 1968, Jennings 1975). Leptothorax unifasciatus Latr. queens actively remove T. turionum to use the empty R. buoliana pupal niches as shelters (Adlung 1962). Many Leptothorax species nest in preformed cavities, so the specimens collected may have preyed upon host pupae and remained within the cocoons for protection.

Aegilips are exclusively parasites of Hemerobiidae (Neuroptera), but they often are collected from crevices in deeply fissured bark or from old galls that have been inhabited by their hemerobiid hosts (Fergusson 1985). Additional work is necessary to determine if a host-parasite relation exists between R. zozana and Aegilips, or if this recovery was anomalous.

R. zozana is a stable, low-density pest, with only moderate fluctuations in population over time. The high degree of parasitism observed during this study suggests that parasites may be an important factor in maintaining relatively uniform R. zozana densities from year to year.

Table III.1. Percentage of parasitism in quantitative and qualitative collections of R. zozana larvae and pupae from Calif. and Oreg., 1984-1987

Species	Quantitative collections												Qualitative collections (ca. 4,500) No.		
	'84 larval (271) <sup>6</sup>		'84 fall pupal (431)		'85 spring pupal (382)		'85 larval (458)		'85 fall pupal (472)		'86 spring pupal (382)			Total (2396)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		No.	%
Hymenoptera															
Ichneumonidae															
<u>Glypta zozanae</u>	71	26.2	155	36.0	135	35.3	156	34.1	140	29.7	94	24.6	751	31.3	1180
<u>Mastrus aciculatus</u> <sup>7</sup>	0	0.0	15	3.5	12	3.1	0	0.0	31	6.6	28	7.3	86	3.6	174
<u>Itoplectis quadricingulata</u>	0	0.0	0	0.0	1	0.3	0	0.0	0	0.0	1	0.3	2	0.1	11
<u>Sinophorus rhyacioniae</u>	1	0.4	0	0.0	5	1.3	9	2.0	2	0.4	3	0.8	20	0.8	15
<u>Coccygomimus sanguinipes erythropus</u>	0	0.0	3	0.7	1	0.3	0	0.0	1	0.2	2	0.5	7	0.3	3
<u>Campoplex conocola</u>	0	0.0	0	0.0	0	0.0	7	1.5	0	0.0	0	0.0	7	0.3	5
<u>Diadegma</u> sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1
<u>Gelis</u> sp.	3	1.1	1	0.2	3	0.8	0	0.0	0	0.0	1	0.3	8	0.3	0
<u>Arotrepes</u> sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1
Braconidae															
<u>Bracon</u> sp. poss. <u>variabilis</u>	1	0.4	2	0.5	5	1.3	1	0.2	3	0.6	6	1.6	18	0.8	22
Chalcididae															
<u>Hockeria tenuicornis</u>	1	0.4	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	2	0.1	3
Torymidae															
<u>Microdontomerus fumipennis</u>	0	0.0	0	0.0	1	0.3	0	0.0	0	0.0	1	0.3	2	0.1	4
Pteromalidae															
<u>Dibrachys cavus</u> <sup>8</sup>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.3	1	<0.1	1
Eulophidae															
<u>Tetrastichus turionum</u> <sup>8</sup>	3	1.1	5	1.2	1	0.3	0	0.0	3	0.6	4	1.1	16	0.7	27
<u>Euderus</u> sp. near <u>cushmani</u> <sup>8</sup>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4
Eupelmidae															
<u>Eupelmus allynii</u> <sup>8</sup>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2
Diptera															
Tachinidae															
<u>Erynnia tortricis</u>	4	1.5	5	1.2	5	1.3	2	0.4	1	0.2	1	0.3	18	0.8	0
Unidentified	16	5.9	11	2.6	26	6.8	79	17.3	33	7.0	27	7.1	192	8.0	---
Total	100	36.9	197	45.7	195	51.1	254	55.5	215	45.6	169	44.2	1130	47.2	1453

<sup>6</sup> Number of host larvae, cocoons, or both in collection.

<sup>7</sup> Secondary parasite in other hosts, a primary parasite of R. zozana.

<sup>8</sup> Secondary parasite in other hosts, status unknown for R. zozana.

Table III.2. Attack and emergence periods of R. zozana parasites

Species	Collections reared from	Stage attacked	Emergence period
Hymenoptera			
Ichneumonidae			
<u>G. zozanae</u>	all <sup>9</sup>	larvae	spring
<u>M. aciculatus</u>	fall & spring	pupal pupae	spring
<u>I. quadricingulata</u>	fall & spring	pupal pupae	spring
<u>S. rhyacioniae</u>	all	larvae	mostly spring
<u>C. sanguinipes</u>	fall & spring	pupal pupae	mostly spring
<u>C. conocola</u>	larval	larvae	late summer/fall
<u>Diadegma</u> sp.	fall	pupal ---	spring
<u>Gelis</u> sp.	all	larvae	spring
<u>Arotrephes</u> sp.	spring	pupal ---	spring
Braconidae			
<u>Bracon</u> sp.	all	larvae	spring
Chalcididae			
<u>H. tenuicornis</u>	larval & fall	pupal larvae	spring
Torymidae			
<u>M. fumipennis</u>	fall & spring	pupal pupae	spring
Pteromalidae			
<u>D. cavus</u>	fall & spring	pupal ---	spring
Eulophidae			
<u>T. turionum</u>	all	larvae	spring
<u>Euderus</u> sp.	larval	larvae	late summer/fall
Eupelmidae			
<u>E. allynii</u>	spring	pupal ---	spring
Diptera			
Tachinidae			
<u>E. tortricis</u>	all	larvae	late summer/fall

<sup>9</sup> Larval, fall pupal, and spring pupal.

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IV. Control of Rhyacionia zozana (Lepidoptera: Tortricidae)  
by Mating Disruption with Synthetic Sex Pheromone

CHRISTINE G. NIWA, GARY E. DATERMAN,  
CHARLES SARTWELL, and LONNE L. SOWER

Pacific Northwest Research Station  
Forestry Sciences Laboratory  
Corvallis, Oregon 97331

## ABSTRACT

Hercon laminated-tape dispensers containing synthetic sex pheromone (a 95:5 mixture of E-9-dodecenyl acetate and E-9-dodecenol) of the ponderosa pine tip moth, Rhyacionia zozana (Kearfott), were manually applied on 57 ha of ponderosa pine plantations in southern Oreg. The nominal dosage was 13.5 g of active ingredient (AI)/ha from 100 releasers per hectare spaced at 10 m. Male moth response to pheromone-baited traps and to virgin females in mating tables showed nearly total disruption of female sex pheromone communication with males. After the mating-disruption application, larval populations were reduced 83.2% in treated areas. The frequency of damaged terminal shoots was 50% lower in pheromone-treated plantations than in check plots.

## INTRODUCTION

Ponderosa pine tip moth, Rhyacionia zozana (Kearfott), is a native pest of young pines. Occurring throughout much of the western United States (Stevens et al. 1980), it infests shoots of several Pinus species with ponderosa pine (P. ponderosa Lawson) as its principal host. Trees up to 1.3 m tall are most frequently attacked. Repeated attacks result in severe stem deformities, growth loss, and increased susceptibility to secondary pests (Stevens 1966). Terminal growth loss during a seedling's first few years is critical because it may permit brush to gain a competitive edge, which can result in tree mortality. Poor seedling growth and survival lengthen crop rotations and necessitate additional site preparation and replanting.

Chemical analysis, electroantennograms, and field trapping have shown that E-9-dodecenyl acetate (E9-12:Ac) and E-9-dodecenol (E9-12:OH) are active components of the female sex pheromone (Sower et al. 1979, Niwa et al. 1987). These compounds caught the most male moths at a 95:5 (acetate:alcohol) ratio. Identification of these pheromone components has made possible the testing of mating disruption as a control technique for R. zozana. Pheromone disruption tests of other Rhyacionia species have reduced male attraction to female-baited traps, but have not demonstrated subsequent reductions in damage (Daterman et al. 1975, Berisford & Hedden 1978).

This report presents results of a mating disruption test with synthetic pheromone formulated in commercial dispensers. Treatment efficacy was assessed by response of males to attractant-baited traps,

mating of virgin females, and reduction of damage and population density.

## MATERIALS AND METHODS

The study was conducted near Cave Mountain, about 8 km northeast of Chiloquin, Klamath Co., Oreg. The eight test plantations ranged in size from 7-28 ha. Trees were 6 yrs old and generally 0.5-1.5 m tall when treated.

Plots were paired on the basis of proximity and infestation densities before treatment. One of each of four pairs of plots was randomly selected and used as an untreated check; the other was treated with synthetic pheromone. Pheromone was manually applied in Hercon laminated-tape dispensers (Hercon Division, Health-Chem Corp., New York) at a nominal dosage of 13.5 g/ha of active ingredient (AI). Releasers were spaced 10 m apart. Treatments were applied 19-27 March 1986 by tying dispensers to tree branches about 1 m above the ground. Where plantations were bounded by natural pine stands, a 40- to 80-m-wide buffer strip was also treated but with only half the releaser density and dosage. Buffers were applied to extend the effective disruption area, in an effort to minimize the amount of immigration of mated females from nearby untreated areas. A total of 57 ha of plantations and about 40 ha of buffer zone were treated.

The pheromone applied was a 95:5 mixture of E9-12:Ac and E9-12:OH. Chemicals were purchased from Orsynex, Inc., Columbus, Ohio, and contained less than 2% total impurities.

**Application Assessment.** A set of releasers placed in the study area on 19 March was used to measure residual pheromone throughout the test period. Four strips were collected every 2 wk until 8 July. Gas

chromatography was used to estimate the average amount of pheromone remaining in the four strips.

**Assessment of Disruption of Orientation and Mating.** In each plot, sets of five Pherocon II sticky traps were spaced at least 30 m apart in a line to evaluate male response to the disruption treatment. Traps contained polyvinyl chloride baits (3- x 5-mm, with 0.1% pheromone by weight) made according to Daterman (1974). Trap baits had the same pheromone blend as the treatment releasers.

Virgin females, 2-3 days old, were placed in mating tables constructed from half-gallon cartons to assess mating frequency in treated and check plantations. Windows were cut in the cartons and covered with fine mesh screen to enhance pheromone dispersal. To prevent escape of the female moth, one wing was clipped and talcum powder was sprinkled along the inner rim of the carton to make the sides of the container slippery. Tables were attached to bamboo poles and placed at about the same height as nearby terminal shoots; each table contained a ponderosa pine shoot and two moths. Tables were left in the field overnight, and females were dissected the following day to determine the presence of spermatophores in the bursa copulatrix. Mating tables were placed in a different pair of plots on each of three nights. The number of females in each plot varied between 10 and 20, depending on their availability; in total, 42 females per treatment were used.

**Damage and Population Density Evaluations.** Pre- and posttreatment damage was recorded after tree growth had terminated in the falls of 1985 and 1986. Data from 15 subplots (6 m in diameter and spaced ca. 50 m apart) were recorded in each plantation. All trees within a

subplot were measured, with the same trees used both sample years. The following factors were assessed: current year's height growth, number of infested shoots, and condition (attacked or nonattacked) of the terminal shoot.

Because larvae often feed gregariously, a collection of 25 infested terminal and lateral tips (from 25 trees selected at random) was made in each plantation in 1985 and 1986 to determine the average number of larvae feeding within attacked shoots. Shoots were collected when larvae were mostly third and fourth instars, tips were dissected, and the number of larvae per shoot was determined.

Data Analysis. Analysis of the mating-table data was by a  $\chi^2$  test of the number of mated and unmated females in check and treatment plots.

Because impacts to both tree growth and form result primarily from damage to terminal shoots, only injuries to terminals were considered in assessing damage. The percentage of attacked terminals in check vs. pheromone-treated plantations and the current year's height growth in trees with infested vs. trees with uninfested terminals were compared using paired Student's t-tests.

Population reduction was determined by calculating posttreatment larval density (mean number of larvae per infested shoot X mean number of infested shoots per tree) as a percentage of pretreatment densities. Population changes in treated and check plantations were subjected to a paired Student's t-test. Abbott's formula (1925) was used to express percentage control of populations from the mating-disruption treatment.

The arcsin squareroot transformation was used in the statistical analysis of all percentages; untransformed percentage figures are used in the presentation of data here.

## RESULTS AND DISCUSSION

Application Evaluation. After 14 weeks of exposure in the field, 3.8% of the original pheromone remained in the dispensers. Thus, the calculated seasonal dosage was 13.0 g/ha. Pheromone release decreased substantially after 2 wk of field exposure; release rate averaged 5.2 mg/(h ha) during the period of peak male moth flight (3 April-28 May) (Figure IV.1).

During the initial 2 wk in the field, the amount of E9-12:OH in the tapes decreased faster than E9-12:Ac. From 2 to 10 wk after treatment (quantities of E9-12:OH were too low to measure after this time) release rates for both compounds were similar. Butler & McDonough (1981) and McDonough & Butler (1983) determined that the half-lives of E9-12:Ac and E9-12:OH in rubber septa are 38.4 and 16.6 days, respectively. Although laminated tape dispensers and rubber septa probably differ in their rates of pheromone release, more rapid evaporation of alcohols than corresponding acetates may occur in many types of dispensers.

Male Disorientation. The disruption treatment was almost totally effective in preventing male moths from locating synthetically baited traps. A single male was recovered in the 20 traps placed in pheromone-treated plantations, but mean trap catch for the season ranged from 7.0-57.6 males per trap in the untreated areas.

Disorientation of males was 100% effective in the mating-table experiment. Only one of the virgin females placed in mating tables was not recovered. All but three of the 42 females placed in untreated plantations had mated overnight, but in pheromone-treated areas, none

of the 41 females recovered was found to have mated. Consequently, the  $\chi^2$  analysis showed a highly significant ( $P < 0.01$ ) difference in the ability of males to locate and mate with females in pheromone-treated and check plantations.

**Damage and Population Reduction.** Mating disruption was successful in reducing growth impacts of larvae feeding on young pines. In the year preceding pheromone application, no significant difference was found between the percentage of damaged terminals in plantations to be treated and those designated as checks (mean = 20.1% and 17.6%, respectively). After mating disruption, the incidence of injured terminals was 50% lower in treated plantations than in check plots ( $P < 0.01$ , mean = 4.9% and 11.0%, respectively). The average yearly growth of trees with infested terminals was almost 5 cm less than that of undamaged trees ( $P < 0.05$ , mean = 25.1 cm and 29.7 cm, respectively).

Subsequent larval populations were greatly reduced in pheromone-treated areas (Table IV.1). In 1986, the average number of larvae per tree in check plantations increased to 137.3% of that in 1985, and posttreatment larval densities in treated plots averaged only 27.8% of pretreatment densities. The difference in average larval-population reduction between check and treated plantations is highly significant ( $P < 0.01$ ). By Abbott's formula, the percentage control of R. zozana population density was 83.2% with the mating-disruption application.

The damage survey and larval counts enabled us to estimate the density of adults during the disruption test at approximately 3,000-7,000 R. zozana per ha. This estimate should be considered the maximum adult density possible, as there may be high mortality during

the late larval and pupal stages. Many Rhyacionia species occur as low-density pests, making them good candidates for control using mating disruption. Our ability to demonstrate a significant amount of damage reduction is probably due to the relatively large plots treated, which limited the effect of invasion by females mated outside of treated areas.

Table IV.1. Effect of a mating-disruption treatment on R. zozana larval populations in ponderosa pine plantations, Chiloquin, Oreg., 1986

Treatment	No. larvae per tree		Post/pre <sup>10</sup>	Control
	1985	1986	(%)	(%)
Check	2.36	3.29	137.3	----
Mating disruption	3.03	0.71	27.8	83.2

<sup>10</sup> Means are significantly different, (Student's t-test,  $P < 0.01$ ).

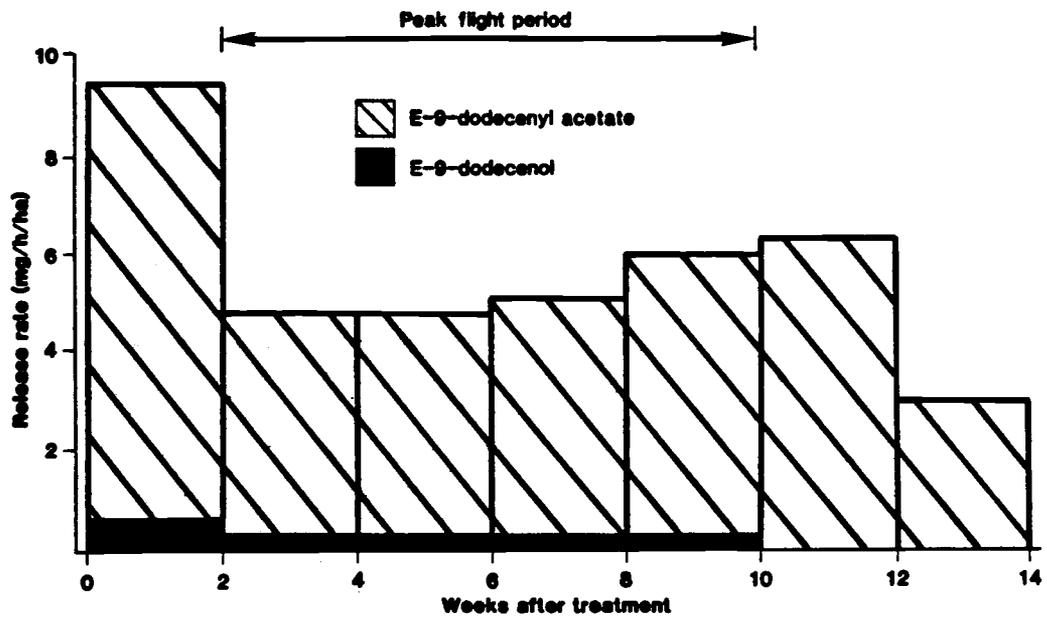


Figure. IV.1. Pheromone release rates from Hercon laminated-tape releasers and peak flight period of R. zozana

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V. Pheromone Mating Disruption of Rhyacionia zozana (Lepidoptera:  
Tortricidae): Influence on the Associated Parasite Complex

CHRISTINE G. NIWA and GARY E. DATERMAN

Pacific Northwest Research Station

Forestry Sciences Laboratory

Corvallis, Oregon 97331

## ABSTRACT

Larval and pupal parasites were collected before and after a mating disruption-test of the ponderosa pine tip moth, Rhyacionia zozana (Kearfott), to determine if the pheromone treatment affected either the species distribution or abundance of the parasite complex. Total percentage parasitism was not changed due to the disruption treatment. In treated areas, however, abundance of the most numerous tip moth parasite, Glypta zozanae Walley & Barron, was reduced significantly below check plantation levels. In contrast, abundance of the pupal parasite Mastrus aciculatus (Provancher), was higher in pheromone-disrupted areas. No changes in the sex ratios of these species were attributable to the pheromone treatment. Parasite community structures were compared using a similarity index. The degree of likeness between treated and check plantations decreased after disruption, probably due to the changes in parasitism rates of G. zozanae and M. aciculatus. Accounting for parasitism and reduced mating, surviving host populations in check plantations were over four times greater than in pheromone-disrupted areas. The importance of the host's sex pheromone as a potential kairomone, and effects of changes in the host density caused by the mating-disruption treatment on the associated parasite complex are discussed.

## INTRODUCTION

Environmental safety is one of the major advantages for the use of pheromones in pest control. Because the low toxicity of pheromones has been adequately demonstrated (Beroza et al. 1975, Kydonieus & Beroza 1982), few studies have investigated the effects pheromone applications may have on the behavior of associated organisms, in particular, that of natural enemies. The attraction of parasites to pheromones of hosts in several orders has been well documented: Lepidoptera (Lewis et al. 1982, Noldus & van Lenteren 1985); Coleoptera (Rice 1969, Kennedy 1984); Hemiptera (Mitchell & Mau 1971); and Homoptera (Sternlicht 1973). In addition, laboratory, greenhouse, and field studies have demonstrated the kairomonal activity of host pheromone gland extracts and synthetic sex pheromone blends in stimulating increased egg parasitism of the corn earworm, Heliothis zea (Boddie) (Lewis et al. 1982), fall armyworm, Spodoptera frugiperda (J.E. Smith) (Nordlund et al. 1983), S. littoralis (Boisd.), and H. armigera (Hübner) (Zaki 1985). Oviposition by Brachymeria intermedia (Nees), a pupal parasite of the gypsy moth, Lymantria dispar (L.), was increased slightly when caged with hosts in the presence of disparlure (Cameron & Rhoads 1973).

Pheromone application in mating-disruption tests, however, has not shown similar results. Disruption treatments with synthetic sex pheromone had no effects on egg parasitism of either the Douglas-fir tussock moth, Orgyia pseudotsugata (McDunnough) (Sower & Torgersen 1979) or L. dispar (Brown & Cameron 1979). Larval parasitism of the tobacco budworm, H. virescens (Fabricius), was similar in plots treated

with a combination of pheromone and insecticides, and in plots receiving only insecticidal treatments (Tingle & Mitchell 1982). Mitchell et al. (1984) found no effects on the level of parasitization of S. frugiperda by the two principal larval parasites in pheromone-permeated fields.

Aside from kairomonal effects, pheromone-disruption treatments may also influence parasite effectiveness indirectly, by reducing host densities. Schroder (1974) found that lower host densities supported increased rates of super- and multi-parasitism by internal larval parasites of the European pine shoot moth, Rhyacionia buoliana Denis & Schiffermüller.

In a recent mating-disruption test of the ponderosa pine tip moth, Rhyacionia zozana (Kearfott), significant reductions in mating, population density, and damage were achieved (Niwa et al. 1988). In the present study, our objectives were to detect effects of the pheromone treatment on the abundance or relative species distribution of the associated parasite complex. Some of the possible changes we anticipated might occur were: increased parasites in treated areas due to kairomonal responses to the host's pheromone; decreased parasitism due to dispersal away from scarce host densities in treated plantations; increased percentage parasitism by an unaffected parasite complex attacking lower-density host population in disrupted areas; or changes in the natural enemy community, due to differential responses of parasite species to decreased host densities.

## MATERIALS AND METHODS

Four plantations near Chiloquin, Klamath Co., Oreg., were treated with 13.5 g/ha of E-9-dodecenyl acetate and E-9-dodecenol (95:5 blend) between 19-27 March 1986 to disrupt mating of R. zozana (Niwa et al. 1988). Four untreated check plantations were paired with treatment plantations based on proximity and infestation densities before treatment.

Rhyacionia zozana were collected and reared for parasites the generation before and the generation after pheromone application in all eight plantations (Niwa 1989). One larval and two pupal collections (one in the fall, after pupation was completed, and one in the spring, before emergence) were conducted each year. Larval samples, mostly 3rd and 4th instars, were made by rearing larvae from 25 damaged tips per plantation. Each pupal sample consisted of 50 cocoons. Both larval and pupal samples were randomly collected along transects through plantations. Parasites were identified by specialists at the Systematic Entomology Laboratory USDA-ARS, Beltsville, MD; Kings River Conservation District, Fresno, CA; and the Biosystematics Research Institute, Agriculture Canada, Ottawa, ONT.

Five Pherocon II sticky traps were placed in each plantation in an attempt to determine kairomonal attraction of parasites to the pheromone. Traps were baited with polyvinyl chloride pellets containing 0.1% of the same pheromone blend as the treatment releasers.

Final host population densities were calculated for the generation following treatment, taking into account both larval density after treatment application and the amount of mortality caused by

parasitism. The mean number of R. zozana larvae per tree was determined by multiplying the mean number of larvae per infested shoot (from the larval collection) by the mean number of infested shoots per tree (from a damage survey (Niwa et al. 1988)). Finally, the product of the mean number of larvae per tree and [1 - the proportion of total parasitism] gives the average number of R. zozana per tree surviving to the next generation.

To measure the degree of likeness between the parasite communities of each pair of pheromone-treated and check plantations, a similarity index devised by Stander (1970) was used:

$$\sum_{i=1}^s p_{1i} p_{2i}$$

Similarity =  $\frac{\sum_{i=1}^s p_{1i} p_{2i}}{\sqrt{\sum_{i=1}^s p_{1i}^2} \sqrt{\sum_{i=1}^s p_{2i}^2}}$

$$\sqrt{\sum_{i=1}^s p_{1i}^2} \quad \sqrt{\sum_{i=1}^s p_{2i}^2}$$

$p_{1i}$  = Proportion of the  $i$ th species in the first collection.

$p_{2i}$  = Proportion of the  $i$ th species in the second collection.

$s$  = Number of species in sample.

Values for this index range from 0-1, which, compared to an index with a variable upper limit, facilitates comparisons between pairs. A one-tailed, paired t-test was conducted to determine if the similarity in parasite community structure between treatment and check areas was reduced by mating disruption with pheromone.

Two-tailed, paired t-tests were used to detect differences between check and disruption plantations before pheromone treatment (1985), and after (1986). The variables analyzed were: percentage parasitism by all species combined; percentage parasitism by each of the two most abundant parasite species (Niwa 1989), Glypta zozanae Walley & Barron and Mastrus aciculatus (Provancher); sex ratios (F:M) of G. zozanae and M. aciculatus; and the average number of R. zozana per tree surviving to the next generation.

## RESULTS

None of the pretreatment t-tests showed differences ( $P > 0.05$ ) in any of these variables between plantations designated as checks and those to receive pheromone applications.

After mating disruption, no real difference was found in the total percentage of parasitism between check and pheromone-disrupted plantations (Table V.1). The mean percentage of parasitism by all species combined was 34.8% in check plantations compared to 28.2% in treated sites.

During the study, G. zozanae accounted for 66.5% and M. aciculatus for 7.6% of the parasites collected; the next most abundant species made up less than 2% of the total number of parasites reared (Niwa 1989). The generation after disruption treatment, attack by G. zozanae was higher in check than in pheromone-treated plantations (Table V.1). Conversely, fewer M. aciculatus occurred in untreated plantations than in disrupted areas (Table V.1).

Sex ratios (F:M) of G. zozanae and M. aciculatus were not different in check and disrupted plantations ( $P > 0.05$ ; mean check = 1.7, treated = 2.0;  $P > 0.05$ ; mean check = 2.8, treated = 2.1, respectively).

Before treatment, the degree of likeness in parasite complexes between check and disruption areas was very high in all four replicates, as indicated by their large similarity indices (Table V.2). After the pheromone application, similarity between parasite community structures was reduced in all treated and check plantation pairs (Table V.2). These changes probably reflect the

fluctuations in relative abundance of G. zozanae and M. aciculatus because all other species were sporadic and accounted for a lower proportion of the total parasitism.

Because of their poor condition, identification of parasites caught in sticky traps was limited to genus. Three specimens from two genera of minor R. zozana parasites were recovered. One Microdontomerus sp. and two Tetrastichus spp. were caught in traps from check plantations, and no parasites were caught in traps from treated sites.

Calculations of final host density of the 1986 generation, after accounting for both disruption of mating and parasitism, was significantly higher in check than in pheromone-treated plantations ( $P < 0.05$ ; mean check = 2.1 R. zozana per tree, treated = 0.5 R. zozana per tree).

## DISCUSSION

Our evaluation shows that although the total percentage of parasitism was not changed, the relative abundance of certain parasite species was altered after mating disruption of the ponderosa pine tip moth.

Egg parasites often use female sex pheromones as kairomones to locate areas likely to contain hosts; however, these airborne pheromones are not present when larval and pupal parasites are searching for hosts and so are most likely not used in this manner. The very low capture of tip moth parasites in baited sticky traps in both treated and check plantations may indicate that neither attraction of parasites to the pheromone nor disruption of the parasites' ability to find hosts using kairomonal cues are factors in the change in relative abundance of certain parasite species in pheromone-treated areas. Parasites may be attracted but not caught, however, because the efficiency of these traps in capturing parasites has not been evaluated.

Host density is rarely considered when evaluating the effects of pheromone release on the associated parasites. Hosts were always provided in abundance in laboratory, greenhouse, and field tests where the presence of pheromone was shown to increase egg parasitism (Lewis et al. 1982, Nordlund et al. 1983, Zaki 1985.) In large-scale field tests that found mating disruption had no effects on parasite abundance (Brown & Cameron 1979, Sower & Torgersen 1979, Tingle & Mitchell 1982), hosts were relatively high-density pests (with the possible exception of H. virescens), with tens to hundreds of thousands of insects per

ha. Ponderosa pine tip moth densities, in contrast, typically range from about 3,000 to 7,000 larvae per ha. When populations of a low-density pest are reduced dramatically, as in the present experiment, the remaining hosts are so sparsely distributed that parasites may be less efficient in locating them. As a result, parasite species with relatively poor searching capabilities may be at a disadvantage, and parasites with alternate hosts may search elsewhere when tip moths are difficult to find.

The two most abundant parasite species responded very differently following pheromone treatment: although parasitism by G. zozanae was lower in treated than untreated plantations, M. aciculatus parasitized more hosts in treated versus untreated areas. These two species have different host-finding strategies, G. zozanae is a larval parasite, attacking early instar hosts feeding in silken tents spun on needle sheaths; M. aciculatus attacks pupae that are attached to root collars of trees. Glypta zozanae has a relatively large environment in which to search for hosts (the whole tree crown). This large area could make finding sparse hosts less efficient and, in turn, might induce dispersal to search other areas. This dispersal may account for the reduced abundance of hosts parasitized by G. zozanae in pheromone-treated plantations. Mastrus aciculatus, on the other hand, has a limited field of search (root collars) and appears to be naturally more efficient at finding sparsely distributed pupae; thus, dispersal from areas of lower host densities might not occur as readily. Because of the lack of any toxic or other direct detrimental effect caused by the disruption treatment, the surviving and relatively unchanged numbers of

M. aciculatus could then attack a greater proportion of the hosts remaining in treated areas.

Taking into account both parasitism rates and host densities after mating disruption, tip moth populations at the end of the generation were more than four times higher in check than in pheromone treated plots. Although shifts in relative abundance of some parasite species must be considered, pheromone disruption is apparently an effective technique for control of the ponderosa pine tip moth.

Table V.1. Percentage parasitism of R. zozana by all species combined, G. zozanae alone, and M. aciculatus alone, Chiloquin, Oreg., 1986

Replicate treatment-check pair	<u>Percentage parasitism</u>					
	Total parasitism		<u>G. zozanae</u> only		<u>M. aciculatus</u> only	
	<u>Check</u>	<u>Treated</u>	<u>Check</u>	<u>Treated</u>	<u>Check</u>	<u>Treated</u>
I	32.1	28.0	18.4	9.3	5.6	9.3
II	39.7	33.1	13.1	9.3	6.5	8.5
III	29.8	27.3	17.3	8.4	4.2	8.4
IV	37.3	24.4	18.7	6.1	7.3	10.6
Mean	34.8	28.2	16.9	8.3 <sup>11</sup>	5.9	9.2 <sup>12</sup>

<sup>11</sup> Treated mean is significantly lower than that of checks  
(2-sided, paired t-test,  $P < 0.05$ ).

<sup>12</sup> Treated mean is significantly higher than that of checks  
(2-sided, paired t-test,  $P < 0.01$ ).

Table V.2. Similarity indices of R. zozana parasite community structure between pairs of check and pheromone-treated plantations, Chiloquin, Oreg., 1985 & 1986

	Replicate treatment-check pair				Mean
	I	II	III	IV	
Before treatment	.9966	.9797	.9969	.9940	.9918 <sup>13</sup>
After treatment	.9201	.9505	.8448	.7789	.8736 <sup>13</sup>
Amount of change	-.0765	-.0292	-.1521	-.2151	-.1182

<sup>13</sup> Mean similarity significantly lower after treatment (1-sided, paired t-test,  $P < 0.05$ ).

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