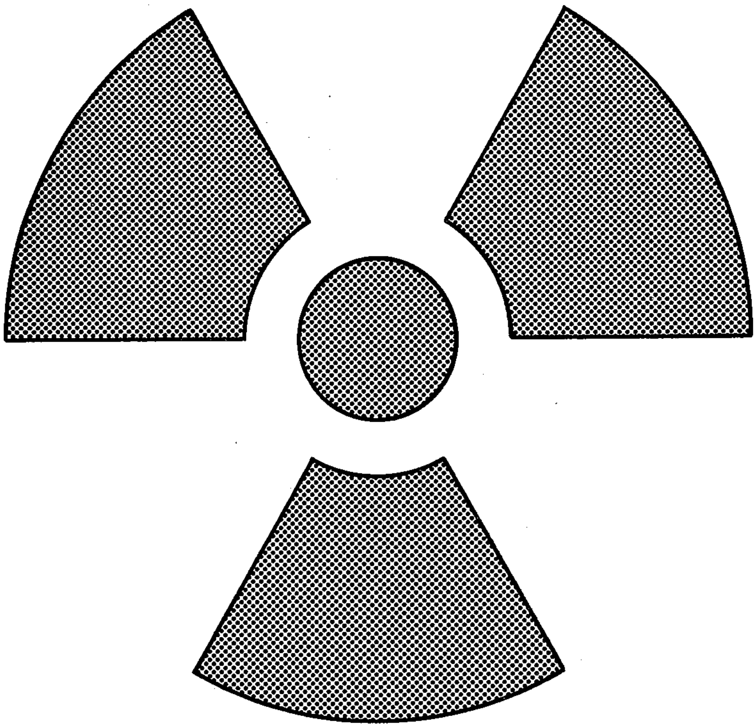

Studies on the Male Sterile Technique for Control of the European Pine Shoot Moth *Rhyacionia buoliana* (Lepidoptera: Olethreutidae)

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STUDIES ON THE STERILE MALE TECHNIQUE FOR CONTROL OF THE EUROPEAN PINE SHOOT MOTH, RHYACIONIA BUOLIANA (LEPIDOPTERA: OLETHREUTIDAE)

S. S. CHAWLA, K. B. TAYLOR AND A. A. BERRYMAN

ABSTRACT

The European pine shoot moth, *Rhyacionia buoliana,* is an introduced pest on ornamental pines in western Washington. The possibility of its spreading into native ponderosa pine stands has worried the timber industry and regulatory agencies.

Studies were undertaken to determine the feasibility of control or eradication of the shoot moth using sterile male releases. Rearing the shoot moth in large numbers from field-collected larvae proved expensive and mortality was high, probably because of bacterial infection. Rearing continuous generations was hampered by lack of success in inducing mating in the laboratory. Male shoot moths were completely sterilized by topical application of 15 μg of tepa without adverse effects on mating ability or longevity. Females required higher doses of tepa to ensure sterility. Males were sterilized by irradiating 11-day-old pupae with 26 K-rads from a $Cobalt^{60}$ source. Longevity and mating ability were not adversely affected at doses up to 35 K-rads. Female fecundity was reduced in direct proportion to the dose applied to the male.

Problems in applying the sterile male method to control or eradicate the European pine shoot moth are discussed.

INTRODUCTION

The European pine shoot moth, *Rhyacionia buoliana* (Schiffermuller), was first recorded in North America in 1914 infesting Scotch pine, *Pinus sylvestris* L., on Long Island (4). It has since spread throughout the North Central United States and the Eastern Canadian Provinces (16). In 1927 it was reported infesting ornamental pines in a nursery near Victoria, British Columbia (15) and in 1959 was found near Seattle, Washington. Since its discovery in western Washington, the shoot moth has become established in many communities in the Puget Sound area but has been largely restricted to ornamental pines in residential areas and nurseries. State and federal quarantines have helped restrict its spread, but spot infestations have occurred in Spokane, Kennewick, College Place, and Walla Walla, Washington and Portland, Salem, Umatilla and Hermiston, Oregon.

Some speculation has taken place in the past few years on the potential impact of *R. buoliana* on the ponderosa pine (*Pinus ponderosa* Lawson) industry of the Pacific Northwest. Miller and Heikkenen (17) rated ponderosa pine as medium in its susceptibility to shoot moth attack and this insect is frequently found infesting ornamental ponderosa pine in the Seattle area. However, only two specimens have been identified on native pines growing in natural stands in the Pacific Northwest, and one of these identifications was on ponderosa pine near Summerland, British Columbia. Nevertheless, it is surprising that the shoot moth has not become generally established in the ponderosa pine forests of eastern Washington, particularly during the spot infestation at Spokane, which is in the heart of the northeastern ponderosa pine country.

The economic analysis of Flora (9) was based on the assumption that the shoot moth could survive in parts of interior Washington and Oregon. He showed that the insect posed a potential threat to pine stands, particularly those growing on poor or medium sites (13). This precipitated action by the lumber industry and the U.S. Forest Service (1).

The European pine shoot moth appears amenable to control or eradication by the sterile male method (5, 14) that proved so successful against the screw worm fly (*Cochliomyia hominivorax* Coquerel) (2). The distribution of the shoot moth in the Pacific Northwest is largely restricted to residential areas and nurseries west of the Cascade Range and north of the Columbia River. Furthermore, the female moth mates only once (18), and this reduces the problems encountered should the sterilizing treatment affect sperm function (3).

The study reported here was a preliminary examination of the potential of sterilizing techniques for controlling or eradicating the European pine shoot moth from western Washington. The sterilizing methods tested were gamma-irradiation, and chemosterilant tepa (tris (1-aziridinyl) phosphine oxide). These methods were chosen because of their success in sterilizing the codling moth, *Carpocapsa pomonella* (L.), another Olethreutid moth (10, 11).

LABORATORY REARING

Methods

Terminal shoots of mugo, Scotch, and red pines were collected near Seattle and transported in insect-proof containers to the quarantine laboratory at Pullman. Shoot moth larvae dissected from buds were then reared on an artificial diet (Bo) developed by Chawla and Harwood (6). Larvae were reared individually in hard plastic vials at a constant 25C and a 15-hr photoperiod. Pupae were removed from the diet and held in petri dishes, at 25C and 15-hr day length, until the adult moths emerged.

The European pine shoot moth mates under specific light and temperature conditions (7, 18) and requires the development of special techniques before mating can be achieved in the laboratory. A mating cage was used which was essentially similar to that described by Daterman (7). A stream of air drew sex attractant from females positioned on toothpick perches at one end of the mating chamber towards males which usually congregated at the lighted end of the chamber. Females were confined to the perches by clipping their wings and dusting the petri dish with Teflon powder. Release of sex attractant and male flight were stimulated by reducing light intensity. Lights were controlled by a time clock and a motorized variable rheostat, which allowed simulation of a dusk period. A 16-hour day length and 60-minute dusk period were used. Temperature and humidity were maintained at a constant 20C and 55% relative humidity.

At the end of each mating test, shoot moth pairs joined in copulation were removed from the mating cages and left together overnight. Next morning, the males were marked with a felt tipped pen and released in the mating cage. Females were placed in a clear plastic bag (6 x 3 x 15 inches) which, upon inflation, served as an egg laying chamber. These chambers were sealed with rubber bands and put on shelves in the mating room. Most ovipositing occurred within 5 or 6 days of mating and eggs hatched about 10 days after oviposition. On the eighth day, the eggs were shaken from the plastic bags, counted, and placed in an incubator at 25C. The females were then put in petri dishes in the mating room until they died. At this time, any additional eggs laid were counted and removed. Larvae hatching from the eggs were reared on artificial diets.

Results

At the beginning of this study, larval survival to pupation on artificial diets exceeded 70% (6). However, in the last 2 years, survival in the quarantine laboratory has been declining. Pupal survival was also fairly high initially, but a marked increase in mortality occurred over the study period (table 1). Clinical examination of dead larvae and pupae revealed the presence of *Bacillus cerus* Frankland and Frankland in 1968 and *B. cerus* and *Streptococcus faecalis* Andrewes and Harder in 1969.

High mortality also occurred in field-collected pupae (table 1), although no evidence of microbial infection was found in these pupae. Attempts to increase pupal survival

TABLE 1. Survival of laboratory-reared and field-collected R. buoliana **pupae from 1965 to 1969.**

Year	Pupae reared	Adults emerged	Survival (%)
1956-66[a]	800	600	75.00
1966-67[a]	3476	1738	50.00
1967-68[a]	3313	1053	31.78
1968-69[a]	2002	731	36.51
1967[b]	210	94	44.76
1968[b]	642	237[c]	36.92

[a] Laboratory rearings during September to May each year.
[b] Pupae dissected from shoots collected in the field in June.
[c] 29 parasites (**Exerstes comstockii** (Cresson) and **Itoplectis quadricingulata** (Provergs)) emerged from these pupae.

by increasing and decreasing humidity and temperature, by leaving pupae in their silk tube, and by allowing pupae to remain in the rearing tubes were all unsuccessful.

The cost of rearing adult shoot moths from field-collected larvae was calculated, assuming larval and pupal survival can be increased by controlling bacteria (table 2).

Mating the European pine shoot moth in the laboratory was a major problem. Only 25% of all females used during this study mated successfully during an average dusk period. Many variables were introduced in attempts to improve mating success. Increasing or decreasing the dusk period and the use of fluctuating daily temperatures had no effect (20). However, mating success was improved by increasing the number of males in the mating chamber.

The best results were obtained with 3-4 females and 15-20 males per cage (20). Preliminary tests also indicated that the probability of a female's mating dropped sharply with female age (fig. 1). Therefore, in subsequent tests, newly emerged females were introduced every 1-2 days. Male mating propensity dropped much more slowly with age (fig. 1) and therefore males were left in the cages until death. Males that had mated successfully were re-

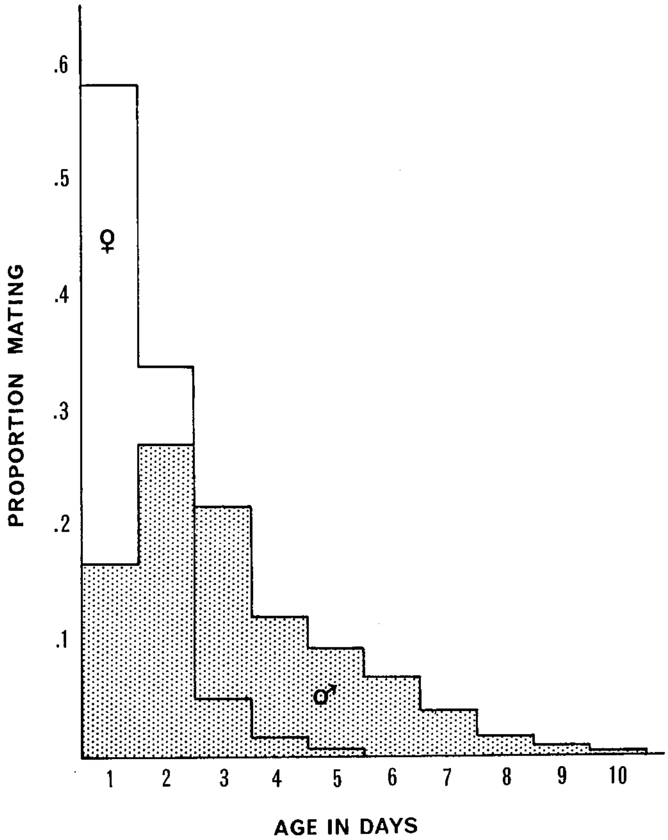
TABLE 2. Cost analysis of rearing R. buoliana **adults from field-collected pine shoots.**

Source of cost	Quantity	Man-hrs.	Cost ($)
Collection and transportation	32,789 shoots	485	928.90
Dissection of larvae	32,789 shoots	1469	2387.10
Diet materials and preparation	109 quarts	109	339.00
Supervision of rearings and removal of pupae	10,889 vials	300	900.00
TOTALS		2963	4555.00

Initial number of larvae in rearing	= 21,779
Final number of pupae reared[a]	= 15,245
Cost per pupa	= $0.30
Final number of adults reared[a]	= 10,672
Cost per adult	= $0.43

[a] Assuming 70% survival of larvae and pupae.

introduced because of their multiple-mating tendencies. Over all tests, 13% of the males mated twice and 2% three times. Mating success of females emerging from field-collected pupae was no better than that of females emerging from laboratory-reared pupae.



1. Effect of age on the mating propensity of European pine shoot moth males and females.

Methods

Emerging adult moths were anesthetized with carbon dioxide, the wings of males were marked with various colored felt-tipped pens, and females had one wing clipped.

Dilutions of 5, 10, 15 and 20 μg of tepa [tris (1-aziridinyl) phosphine oxide] per μliter of alcohol were used in the topical application tests. Applications were made with a 50-μliter syringe mounted on a micrometer. One μliter of tepa of desired concentration was applied to the ventral surface of the abdomen of anesthetized moths. After treatment, male moths were released in the mating cage and females placed on toothpick perches.

Limited tests were made with tepa applied to adult moths as an aerosol using an applicator patterned after Hathaway *et al.* (12). Moths were subjected for 10 min to an aerosol produced from a 5% tepa-water solution.

Results

Treatment with tepa had little effect on the mating ability or longevity of *R. buoliana* males or females (table 3). There was an indication that low topical doses increased the mating ability and longevity of males, although the female did not show this tendency.

Fecundity was extremely variable (range: 0-256 eggs/female) in both treated and untreated females. Although egg production was slightly higher than the control in both treated females and normal females mated with treated males, these differences were not significant at the 5% level (table 4). Male sterility was affected at lower doses than female sterility. Matings were obtained with tepa-treated males up to 7 days old and there was no indication that sterility was not permanent. Preliminary observations indicate that aerosol application of tepa can be used to sterilize the European pine shoot moth (table 4).

TABLE 3. Effect of tepa on the mating ability and longevity of R. buoliana.

	Males				Females			
Dose	N	% Mated	N	longevity (days)	N	% Mated	N	longevity (days)
5 g topical	46	30.4*	32	10.7*	45	26.7	12	13.9
10 g topical	69	17.4	29	9.2	58	17.2	11	15.9
15 g topical	58	17.2	34	8.9	60	20.0	12	13.7
20 g topical	......	....	......	....	20	25.0	5	13.8
5% aerosol	14	14.3	7	8.1	11	45.5	5	10.8
Control	612	21.6	179	8.2	588	25.7	92	16.8

* Significant difference from the control at 5% level.

TABLE 4. Effect of tepa on fecundity and fertility of normal and treated R. buoliana females mated with treated and normal males, respectively.

Treatment	Treated female x normal male			Normal female x treated male		
	N	Eggs/female	Hatch (%)	N	Eggs/female	Hatch (%)
5 μg topical	12	82.0	51.5	17	75.2	33.6
10 μg topical	11	75.4	16.0	11	75.4	0.8
15 μg topical	12	76.1	9.3	11	57.4	0
20 μg topical	5	57.8	3.1	......	....	....
5% aerosol	5	71.4	41.2	2	82.0	3.7
Control	Normal female x normal male			94	71.6	71.0

STERILIZING R. BUOLIANA WITH GAMMA-RADIATION

Methods

Pupae of known age were sexed, placed in gelatin capsules, and then irradiated at different doses of gamma-radiation with a Cobalt[60] source. The pupae were then put in petri dishes until eclosion. Emerging males were marked and females had their wings clipped before they were placed in mating cages.

Competition was studied by releasing similar numbers of normal and sterilized males in the mating chambers. Treated and untreated males were dusted with different colored fluorescent pigment which enabled their identification under ultra violet light.

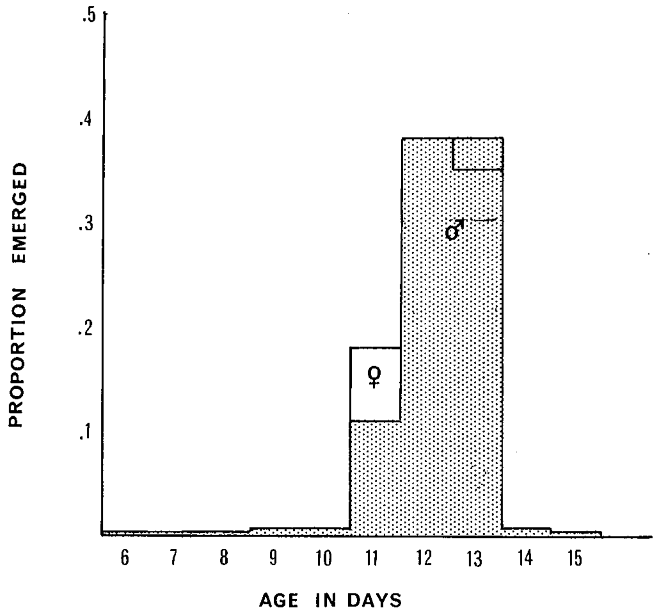
Semi-field tests on the competitive ability of irradiated males were made in a cage 10 ft x 10 ft x 6½ ft high. These tests were performed in the Seattle area in June, 1967. A single potted pine was placed in the center of the cage, and females were confined, by clipping their wings, to toothpick perches placed within the foliage. Approximately equal numbers of treated and untreated males were marked and released into the cage.

Results

Irradiation of different aged pupae showed that the older pupae were more resistant to the effects of gamma-radiation (table 5). The rate of pupal development at a constant temperature of 25C is shown in figure 2. Peak adult eclosion occurred on the 12th and 13th days. In all subsequent studies, 11-day-old pupae were irradiated.

TABLE 5. Survival of R. buoliana pupae subjected to various doses of gamma-irradiation at different ages (corrected by Abbott's Formula).

Dose (K-rads)	Per Cent Survival					
	7 days old		9 days old		11 days old	
	Males	Females	Males	Females	Males	Females
35	7.7	17.3	68.9	52.0	100.0	68.7
52	0.0	5.2	16.2	24.1	86.1	83.8
70	6.9	0.0	8.4	10.1	19.1	72.3
87	0.0	0.0	0.0	0.0	14.8	49.6
105	0.0	0.0	0.0	0.0	5.5	14.5



2. Emergence time of adult European pine shoot moth from pupae reared at constant 25C.

Longevity of *R. buoliana* adults was not significantly affected when pupae were treated with 35 K-rads (table 6); in fact, longevity was increased slightly at this dosage. Higher dosages had an increasing deleterious effect on adult longevity.

European pine shoot moth males were sterilized completely by gamma-radiation dosages above 26 K-rads (table 7). However, fecundity of females mated with treated

TABLE 6. Effect of gamma-irradiation on longevity of R. buoliana adults treated as 11-day-old pupae.

Sex	Longevity in days					
	Control	35 K-rads	52 K-rads	70 K-rads	87 K-rads	105 K-rads
Male	10.3	10.9	8.8	....	7.7	7.0
Female	15.4	18.7	12.7	13.0	8.8	7.3

males was reduced in direct proportion to the gamma-ray dosage. Preliminary analysis of competitive mating ability of sterilized males under laboratory and semi-field conditions indicated that gamma-irradiation in the 26-35 K-rad range had little affect on mating ability (table 8). The expected probability of mating with a sterile male, based on release proportions of sterile to normal males, was not significantly different (at the 1% level) from the observed probability of mating.

TABLE 7. Fecundity and fertility of normal R. buoliana **females mated to males sterilized by various doses of gamma-radiation.**

Dose (K-rads)	N	Eggs/female	Hatch (%)
20	7	40.0	54.6
24	6	28.7	1.2
26	10	22.3	0
30+	14	21.4	0
Control	94	71.6	71.0

TABLE 8. Effect of gamma-ray induced sterility on the mating competitiveness of R. buoliana **males in cages in the laboratory and field.**

Female	Male release			Female matings			
release	Sterile	Fertile	$P^e = \frac{S}{S+F}$	Success (%)	Sterile	Fertile	$P^e = \frac{S}{S=F}$
206[a]	127[c]	111	0.5336	9.22	10	9	0.5263
115[b]	57[d]	55	0.5089	9.96	4	4	0.500

[a] 5 tests conducted in the laboratory mating cages.
[b] 1 test conducted in field cage
[c] Sterilized with 26 K-rads.
[d] Sterilized 35 K-rads.
[e] Probability of mating with a sterile male.

DISCUSSION

The results of this investigation indicate that the European pine shoot moth can be sterilized effectively by the chemosterilant tepa (15 μg topical) and gamma irradiation (26-35 K-rads) at doses that do not seriously affect the mating behavior, aggressivenes, or longevity of the male moths. However, the fecundity of females mated to males sterilized by gamma radiation was reduced, indicating that irradiation may render the sperm nonfunctional. This conclusion is supported by previous work (19). Further studies are needed to determine if these females will mate a second time.

Recent knowledge of the effects of gamma irradiation on the fertility of the sugarcane borer, *Diatraea saccharalis* (F.), suggests that doses resulting in partial sterility may be more effective through their inherited effects on subsequent generations (21). If this hypothesis proves true and applies to other lepidoptera, including *R. buoliana,* then the practicality of their control or eradication will be increased. Although logistics problems will undoubtably be met in sterilizing large numbers of moths for release into the field, the more serious and immediate problems seem to be in mass propagation and release.

In the present study, shoot moth larvae were reared mainly from field-collected material. The cost of collecting and dissecting infested shoots, preparing diets, and tending the rearings is a major consideration. Even with the optimistic assumption that larval and pupal survival can be maintained near that achieved at the beginning of this program, the cost of 43 cents per adult reared seems prohibitive to a large scale release program.

The alternative approach of mass propagation by laboratory mating seems equally bleak. In our study, an average of 25% of all females mated in the single night they were exposed to males, although Daterman (8) has achieved much better results with a modified cage design. However, physical and economic problems of building and supervising complex mating cages, and biological problems that may arise as a result of inbreeding are still formidable obstacles to mass propagation.

Apart from the feasibility of mass propagation and sterilization of *R. buoliana* males, serious problems can be foreseen in the release of vast numbers of insects in a densely populated area, such as Seattle and its vicinity. Furthermore, there is little information on the density and distribution of the host pines within residential areas, although methods have been tested for estimating shoot moth population densities (Carolin, personal communication). Information of this type is essential for determining release numbers and strategies.

It is apparent that more research is needed on this introduced pest, particularly on laboratory propagation. Even more important is the population ecology of the moth in western Washington, especially its capacity to invade and survive in the ponderosa pine forests of the Pacific Northwest. The latter question is important in determining the potential threat of the European pine shoot moth to the economy of the pine industry.

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