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ABSTRACT

Populations of the mountain pine beetle, Dendroctonus ponderosae Hopkins, were sampled by both radiographic and bark-removal methods in standing lodgepole pine, Pinus contorta Douglas. Estimates of live beetle numbers based on the two methods were comparable. However, the radiographic method is not recommended for field sampling because it is more costly and the causes of beetle mortality usually cannot be determined. In addition, beetle populations in areas radiographed were adversely affected, probably because of drying of wood and bark, which resulted in erroneous estimates of brood survival and distorted sex ratios.

INTRODUCTION

Radiography as a tool to sample populations of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, in lodgepole pine, *Pinus contorta* Douglas, was tested in the field. The objective was to determine whether radiography might prove to be a better method of sampling mountain pine beetle populations than the bark-removal method (Carlson and Cole 1965) currently used by this research work unit[1] to obtain life-table data.

Following a cohort of bark beetles through development would be more desirable than obtaining sample estimates from different cohorts. Radiography makes possible such study of cryptic forest insects, and has proved valuable in studying laboratory populations of bark beetles when frequent radiographs could be made (Berryman and Stark 1962a; Johnson and Molatore 1961). However, little field use has been made of radiography in population studies of forest insects.

Knight and Albertin (1966) used radiography in the field to study several insects, including borers, *Oberea schaumii* LeConte and *Saperda concolor* LeConte, in aspen, *Populus tremuloides* Michx., and the white pine weevil, *Pissodes strobi* (Peck), in jack pine, *Pinus banksiana* Lamb. Radiography currently is employed by C. J. DeMars (Entomologist, Pacific Southwest Forest and Range Experiment Station, Berkeley, Calif., personal communication, July 27, 1973) to sample western pine beetle, *Dendroctonus brevicomis* LeConte, populations. The western pine beetle spends much of its developmental time in the outer bark of ponderosa pine, *P. ponderosa* Lawson. Infested bark is removed from trees, taken to the laboratory, and radiographed. Time is saved and accuracy is improved in counting forms on radiographs since the other method entails dissection of thick bark to find insects for direct counting (DeMars 1963). Similar findings were reported for the southern pine beetle, *D. frontalis* Hopkins, when bark samples from short leaf pine, *P. echinata* Miller, were radiographed (Fatzinger and Dixon 1965). However, removal of bark for radiographing still represents a destructive sampling technique and prevents workers from following a cohort through development.

Unlike larvae of the western and southern pine beetles, mountain pine beetle larvae in the thin bark of lodgepole pine feed and pupate primarily in the phloem layer against the sapwood surface. Consequently, removal of bark samples for radiographing would also expose larvae for direct counting. Therefore, any advantage of using the radiographic method in the field would be in the accuracy and speed of sampling beetles *in situ*. Radiographic sampling has been shown to give accurate counts of mountain pine beetles in bark and wood slabs of lodgepole pine in the laboratory (Amman and Rasmussen 1969).

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[1] Population dynamics of primary bark beetles.

METHODS

Twelve beetle-infested lodgepole pines were selected for sampling over a period of time on the Wasatch National Forest south of Evanston, Wyoming. Four of the trees were 20 to 28 cm d.b.h., four trees 30 to 36 cm d.b.h., and four trees 38 cm and larger d.b.h. A chain saw was used to cut two vertical slots into the trunk of each tree for film placement, a technique explored by Berryman and Stark (1962b). This procedure resulted in a "slab" up to 5 cm thick approximately 75 cm above ground level; slots were located at random with respect to cardinal direction (fig. 1). Slots were used because radiographing the entire tree would (1) superimpose images of broods from the near side upon those on the far side, (2) require a much greater exposure time, and (3) result in radiographs of low contrast. The slots were about 20 by 25 cm to accommodate Kodak AA Ready Pack® film of that size, and were open only on one side in the larger trees to slow the rate of tissue drying. In the smaller trees, the trunk was not large enough to keep one side of the slot closed; consequently, slots were open on both sides. However, all slots were sealed with caulking cord to slow the rate of drying between sample dates.

Metal staples were placed in the corners of 15.2-cm^2 areas to be radiographed. Staples were visible on the radiographs and delinated areas from which population counts would be made. During development of the beetle, radiographs were obtained October 14, 1969, June 2, 1970, and June 30, 1970. Plastic screen cages, 15.2 cm^2, to which test tubes had been attached, were stapled over the area delineated by the staples after the last sample. Emerging insects were collected weekly from the test tubes, and numbers, sexes, and sizes were recorded.

Figure 1.--A chain saw was used to cut vertical slots in trees for film placement.

For purposes of comparison, two 15.2-cm^2 areas of bark within 31 cm of breast height were removed from the trees each time populations were radiographed. All insects, mountain pine beetle egg galleries, and gallery starts in the samples were counted. Plastic screen cages also were stapled on the trees in this area to collect insects emerging from the bark.

Moisture content of the trees was determined by means of an electrical resistance meter. Radiographic expose time was adjusted according to moisture content. Exposure curves for different slab thickness-moisture content combinations (fig. 2) were presented by Amman and Rasmussen (1969).

A portable Picker® X-ray machine with up to 110-kV output was used. All radiographs were taken at 25 kV, 5 mA, and at a 51-cm film distance. The 25 kV level gave better contrast than higher kilovoltage levels in laboratory tests (Amman and Rasmussen 1969). A cord that allowed the operator to be at least 12.5 m from the tube head during operation was used to protect him from possible radiation exposure. All legs of the quadruped stand telescoped for rapid height adjustment; wheels, mounted on the rear set, facilitated movement of the head from tree to tree. A 1,500-watt gasoline-powered alternator provided electricity for the unit. All equipment was easily moved by two men.

Because of trunk curvature, thickness of the area radiographed varied from about 5 cm in the center to about 2 cm on the outer edge. To avoid overexposure of sample portions thinner than 5 cm, an aluminum foil filter, that thickened laterally from the center, was placed over the sample area. The number of sheets of foil ranged from two near the center to six at the outer edge of the sample.

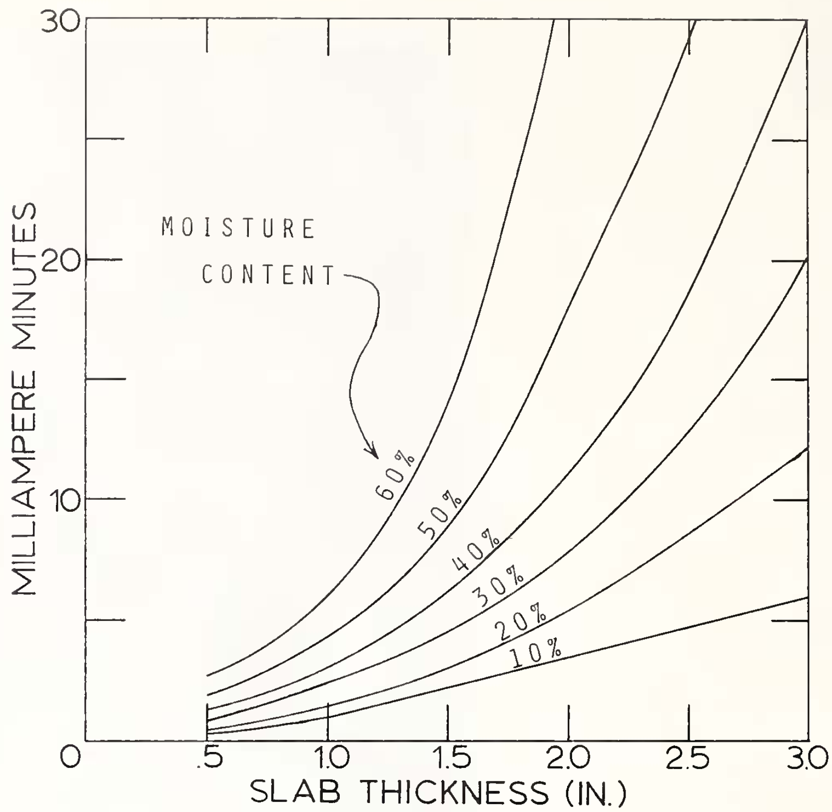

Figure 2.--X-ray exposure curves for wood and bark slabs (lodgepole pine) of different thickness and moisture content when the X-ray unit is operating at 25kV, 5mA, using a 51-cm (1 inch = 2.54 cm) target film distance (from Amman and Rasmussen, J. Econ. Entomol. 62:631-634, 1969).

Radiographs were processed and then interpreted independently by two observers. A light table, on which all light except for a 15.2-cm^2 area had been blocked out, was used for radiograph interpretation. Insects and their identification were recorded on mattex acetate placed over the radiograph. Counts of insects and stages were made from the overlays.

Data were analyzed by means of analysis of variance. The comparisons made between radiographic and bark-removal sampling were based on averages of estimates obtained by both observers.

RESULTS AND DISCUSSION

An evaluation of the radiographic method of sampling mountain pine beetle populations was based on both statistical and operational considerations.

Population estimates.--Assuming negligible location effects, no significant difference at the 0.05 level of probability was revealed among population estimates obtained by using the bark-removal method and estimates made from radiographs for any of the three sample dates (table 1). One source of difference was noted between estimates obtained by the two methods for the first sample; eggs could be counted in bark samples, but not on radiographs. As observed previously (Amman and Rasmussen 1969), radiographing mountain pine beetles through wood precluded detection of the slight difference in density of eggs. Eggs of the western pine beetle have been seen on radiographs when only bark was radiographed (C. J. DeMars, personal communication July 27, 1973). The authors detected no significant difference between methods for estimates of centimeters of egg gallery nor of the number of gallery starts. Therefore, estimates from radiographs appear to be comparable to those obtained by bark-removal sampling.

Comparisons of estimates made from radiographs by the two observers revealed no significant difference for brood populations and centimeters of egg gallery (table 2). However, the observers gave significantly different estimates for the numbers of gallery starts ($P < 0.01$). With the exception of gallery starts, observer differences are small and adequately trained observers can be expected to give comparable estimates from radiographs.

Table 1.--*Comparison of estimates from bark-removal and radiographic sampling.*

	Bark-removal sampling	Radiographic sampling[1]	Significance
	--------*Average/30.4 cm²*--------		
Brood number			
Observation 1	215.5	177.5	$P > 0.10$
Observation 2	83.2	78.3	$P > 0.10$
Observation 3	48.2	40.6	$P > 0.10$
Gallery cm	261.1	238.3	$P > 0.10$
Gallery starts	10.0	8.9	$P > 0.10$

[1] Estimates made by two observers were averaged and the average was compared with the estimates obtained by bark-removal sampling.

Table 2.--*Comparison of observer estimates made independently from the same sets of radiographs.*

	Observer 1	Observer 2	Significance
	-------*Average/30.4 cm²*-------		
Brood Number			
Observation 1	171.5	183.5	$P > 0.05$
Observation 2	82.9	73.6	$P > 0.10$
Observation 3	45.2	36.0	$P > 0.10$
Gallery cm	234.4	242.1	$P > 0.10$
Gallery starts	6.3	11.5	$P < 0.01$

Time.--An analysis of time required to accomplish sampling by the two methods demonstrated that the bark-removal method is much faster than the radiographic method. Time required for the first sample averaged 38.1 min per sample for the radiographic method compared to only 16.3 min per sample for the bark-removal method (table 3). Time required to accomplish the radiographic sample decreased during the second and third samplings because no new slots had to be cut in the trees. In addition, fewer insects in the last two samples speeded interpretation of radiographs. Also, a slight saving occurred in exposure time, which changed from an average of 1.4 min per sample for the first sample date to 0.78 min per sample for the last two sample dates because of decreasing moisture in the wood and bark. Ratios of time (bark removal method: radiographic method) ranged from 1:2.35 min per sample for the first sampling date to 1:1.66 min per sample for the third sampling date (table 3).

Table 3.--*Time analysis of radiographic and bark-removal methods to sample mountain pine beetle populations.*

	Observation		
	1	2	3
	- - - *Man-minutes* - - -		
RADIOGRAPHIC METHOD			
Cutting slots in trees	60	--	--
Setting up and taking down equipment	60	60	60
Determining moisture content of trees	60	60	60
Radiographing and moving equipment from tree to tree	300	285	285
Sealing saw slots in trees	60	60	60
Developing and fixing film	60	60	60
Interpreting radiographs	257	127	80
Summarizing counts from overlays	60	48	44
Totals	917	700	649
Average/sample	38.2	29.2	27.0
BARK-REMOVAL METHOD			
Removing bark and counting insects	360		
Coating exposed wood with wax	30		
Total	390		
Average/sample	16.3		
Ratios (bark-removal:radiographic)	1:2.35	1:1.79	1:1.66

One might expect the saving of time experienced with radiographic sampling when insect numbers were fewer to carry over to bark-removal sampling as well. However, this saving did not occur because more time was required to remove the drier and tighter bark in the latter samples.

Cost.--The initial cost of equipment needed to sample populations by the two methods was not considered; only the cost of expendable items used during the sampling process and sampling time were evaluated. The radiographic method cost a total of about $46, or 64 cents per sample, more for materials than the bark-removal method. Costs were film $32, developer $7, and fixer $7.

Total costs for time (based on GS-5 level) and materials follow: bark-removal method, $1.01 per sample; radiographic method, first date, $3 per sample, second date, $2.44 per sample, and third date, $2.31 per sample.

Assessment of mortality factors.--A major disadvantage of the radiographic method was the inability of the observers to assess mortality from the radiographs. Some predators could be seen and counted on the radiographs. The only one of consequence was *Medetera aldrichii* Wheeler (Diptera: Dolichopodidae). Dead beetles that had dried completely were not often detected; those that were, usually could not be assigned to specific mortality causes. In addition, larvae that had recently died, but still contained much moisture, could not be distinguished from living larvae. The bark-removal sampling technique is definitely superior in this respect because the cause of death of most larvae can be determined. Consequently, the bark-removal sampling method will continue to be the choice for life-table sampling of mountain pine beetles where assessment of mortality factors is of primary concern.

Brood survival.--Although estimates of brood by the bark-removal and radiographic methods were comparable, the number of brood that completed development and emerged was only 4.0 adults per 30.4 cm^2 for the radiographed areas compared to 27.8 per 30.4 cm^2 for nonradiographed areas. In addition, the sex ratio was more in favor of females in radiographed areas than in nonradiographed areas--1:2.50 compared to 1:1.67 (males to females). A greater rate of drying in radiographed areas is suspected of causing these differences (unpublished data on file at Intermountain Forest and Range Experiment Station, Ogden, Utah).

Bluestain fungi introduced by the parent beetles interfere with movement of water in the infested tree (Nelson 1934). However, after the initial sample was taken, bark and wood in the area sampled by the bark-removal method appeared to contain more moisture than that sampled by the radiographic method. The vertical slots made by the saw to accommodate the film may have prevented moisture from moving horizontally from inner sapwood to outer sapwood and bark being radiographed. In addition, evaporation took place from the cut surfaces.

The amount of radiation received by the developing brood should not have caused the differences noted; no such abnormality was observed during laboratory investigations. In addition, the greatest total exposure to which any group of beetles was subjected for the three sample dates was about 800 *R*. This exposure was far below that generally found to sterilize insects or to interfere with insect behavior. For example, Davich and Lindquist (1962) found that at an exposure of 2,500 *R*, behavior and egg laying were about normal in the boll weevil (*Anthonomus grandis* Boheman).

Average size of mountain pine beetle brood adults did not appear to differ significantly between radiographed and nonradiographed areas: males in radiographed areas averaged 4.64 mm in length compared to 4.75 mm for males from nonradiographed areas; females in radiographed areas averaged 5.07 mm in length compared to 5.11 mm for nonradiographed areas (table 4).

Radiographic quality.--A problem that affected radiographic quality was unequal moisture content of the bark and wood within some of the radiographed areas. Where moisture was uniform, high quality radiographs were obtained (fig. 3); however, where moisture was uneven, radiographs were of unequal density and difficult to interpret (fig. 4). Streaks of high moisture content occurred where bluestain fungi were absent; absence was the result of beetle attacks that left some areas of bark lightly infested and, consequently, uninfected by bluestain fungi. Uneven radiographic exposure occurred when the probe for the electrical resistance meter was unknowingly placed in a moisture streak. This error resulted in overexposure of the radiograph for the areas surrounding the moisture streak. A less serious error occurred when the moisture probe was placed in fungus-infected wood near a moisture streak, which resulted in underexposure of the moisture streak. Usually, this area contained few larvae from nearby galleries; consequently, this error would not have affected the total count as much as that of the first type. Where a moisture streak is indicated on the first radiograph, possible solutions are either to take two radiographs of the area (one adjusted to the moisture streak, the other to the area surrounding the streak) or to develop a filter to reduce exposure of the area surrounding the streak. Poor radiographs necessitated a return trip to the field to retake radiographs at a more optimum exposure. A portable daylight developer unit would provide on-the-spot development; poor radiographs could be retaken immediately.

Table 4.--*Number, size, and sex ratio of beetles emerging from areas on the same trees where radiographic and bark-removal sampling were used.*

	Number[1]	Average length	Standard deviation	Number/ 30.4 cm^2
		- - - - - *mm* - - - - -		
		RADIOGRAPHIC METHOD		
Male	6	4.64	0.62	1.0
Female	15	5.07	.53	2.5
Unidentified[2]	3	--	--	.5
Totals	24			4.0
		BARK-REMOVAL METHOD		
Male	54	4.75	.49	9.0
Female	90	5.11	.51	15.0
Unidentified[2]	23	--	--	3.8
Totals	167			27.8

[1] Sex ratios (males to females) are 1:2.50 in radiographed areas, and 1:1.67 in nonradiographed areas.

[2] Beetles could not be sexed because of broken abdomens.

Figure 3.--Radiographs of good quality can be produced where moisture content of wood and bark is uniform and a filter is used to prevent overexposure of thin areas.

Figure 4.--Radiographs of poor quality result when moisture content of wood and bark is not uniform. Light streak represents area of high moisture content, the result of unequal distribution of bluestain fungi.

The radiographic method does not appear to offer any clear-cut advantage over the bark-removal method of sampling field populations of mountain pine beetles at this time. Although estimates of live beetle numbers obtained by the two methods were comparable, disadvantages of the radiographic method should be taken into account. These are (1) costs of both time and material; (2) difficulty in detecting dead beetles on radiographs and in assessing specific causes of mortality; and (3) lower emergence of brood adults, but higher female survival rates in radiographed areas.

Although these disadvantages of the radiographic method exist when sampling field populations of mountain pine beetles, radiography could be a useful technique for studying some aspects of bark beetle biology and behavior. In addition, radiography will continue to be an important means for studying laboratory populations of bark beetles.

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- Logan, Utah (in cooperation with Utah State University)
- Missoula, Montana (in cooperation with University of Montana)
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