Purchased by U. S. Department of Agriculture Forest Service, for Official Use

8

Techniques for Radiographing and the Accuracy of the X-Ray Method for Identifying and Estimating Numbers of the Mountain Pine Beetle[1]

GENE D. AMMAN and LYNN A. RASMUSSEN[2]
USDA, Forest Service Intermountain Forest and Range Experiment Station, Ogden, Utah 84401

ABSTRACT

X-ray-exposure curves using 2 kilovoltages (kv) were developed for lodgepole pine slabs of different thickness and moisture content. X-ray exposure at 25 kv for a wood-and-bark slab containing 30% moisture varied, for example, from 4.5 milliampere minutes (mam) for a 1.5-inch-thick slab to 20 mam for a 3-inch-thick slab. The relationship between wood-and-bark thickness and optimum exposure became increasingly curvilinear as moisture content increased. In contrast, X-ray exposure at 45 kv for wood and bark containing 30% moisture varied from 0.4 mam for a 1.5-inch-thick slab to 1.25 mam for a 3-inch-thick slab. At 45 kv the relationship between wood-and-bark thickness and exposure remained approximately linear for all moisture contents.

Identifications and estimates of numbers of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, were obtained from radiographs. Radiographs obtained using an exposure of 25 kv proved to be best for identifying and determining numbers of small and medium-sized larvae. Radiographs obtained using either 25 or 45 kv could be used with about equal accuracy for identifying and estimating numbers of large larvae, pupae, and callow adults. Errors in identification and in estimating numbers of beetles from radiographs obtained using an exposure of 25 kv were less than 10% of the mean and seem acceptable for population studies of bark beetles.

Factors affecting populations of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, have been under investigation in lodgepole pine stands in Idaho, Utah, and Wyoming for several years. However, we are still seeking more accurate methods for obtaining population estimates. Use of X-rays is being considered for this purpose.

Radiography has been used in several studies of bark beetles. Johnson and Molatore (1961) radiographed the Douglas-fir beetle, *D. pseudotsugae* Hopkins, in wood-and-bark slabs of Douglas-fir. They concluded that the X-ray method would be suitable for studying the biology of bark beetles. Berryman and Stark (1962) subsequently used radiography to study the biology of the California five-spined ips, *Ips confusus* (LeConte), in wood-and-bark slabs in the laboratory. DeMars (1963) investigated the use of radiography for obtaining counts of western pine beetles, *D. brevicomis* LeConte, from bark samples of ponderosa pine. He concluded that the X-ray method was superior to the bark dissection method for locating insects but less effective for determining the stage of the insect. DeMars also demonstrated that the radiographic detection method was much less expensive than the dissection method. Fatzinger and Dixon (1965) reported similar findings when radiographing the southern pine beetle, *D. frontalis* Zimmerman, in bark samples from shortleaf pine.

Less exposure is needed and greater detail is obtained when only bark is X-rayed because of the low density of the material. However, when wood-and-bark slabs were radiographed, Berryman and Stark (1962) found that differences in thickness greatly affected quality of the radiograph.

Preliminary radiographs were made of mountain pine beetles in standing lodgepole pine trees. These showed that 2 principal causes of poor radiographic quality were (1) small variations in wood-and-bark thickness, and (2) variations in moisture content of the wood. Therefore, the objectives of this study were (1) to develop exposure curves that would give radiographs of optimum quality, and (2) to determine the accuracy of the X-ray method for estimating numbers and identifying stages of the mountain pine beetle in lodgepole pine slabs.

DESCRIPTION AND HABITS OF THE MOUNTAIN PINE BEETLE.—The adult mountain pine beetle is about ⅕ in. long and is dark brown. The female starts construction of a vertical egg gallery in the living bark of lodgepole pine in late summer and is soon joined by the male. Eggs are laid singly in niches alternately arranged along the sides of the gallery as the gallery is being constructed. When the larvae hatch, they make short feeding galleries, usually at right angles to the egg gallery. The beetle overwinters in the larval stage and completes development in the spring. A pupal chamber is then formed within the bark against the sapwood, where the larva pupates in late spring to early summer and soon becomes a callow adult. After a short period of maturation feeding the callow adult turns dark brown and emerges in mid-to late summer to infest a living tree. The entire life cycle requires 1 year in lodgepole pine in the Intermountain area.

EQUIPMENT AND METHODS.—A portable Picker X-ray machine[3] was used. The machine has an output of 0–110 kv, 0–10 ma, and variable exposure time. The X-ray head was mounted in a lead-lined steel cabinet in the laboratory. Kodak AA Industrial ready pack film was used.

Radiographs were obtained by operating the X-ray machine at (1) 25 kv, 5 ma and (2) 45 kv, 5 ma, using an aluminum filter 2 mm thick over the X-ray window.

Stacey and Mothershead (1965) reported that low energy rays caused secondary radiation when interacting with the specimen being radiographed. They noted that these rays did not contribute to the image, but reduced image contrast and therefore were detrimental to optimum radiographic quality. They used thin lead filters in front and behind the film to remove secondary radiation, whereas a filter over the X-ray window to remove the long rays which cause secondary radiation was used in these tests.

However, in our study there was little difference between radiographs taken at 45 kv with filters and

[1] Accepted for publication Dec. 30, 1968.
[2] Research Entomologist and Biological Technician, respectively.
[3] Use of trade names is for identification only and does not imply product endorsement by the USDA Forest Service.

those without filters. As a result, placing a filter over the window of the X-ray machine is not recommended when radiographing mountain pine beetles in slabs less than 3 in. thick. Furthermore, Stacey and Mothershead (1965) were radiographing power poles, which were much thicker than the slabs used in our study.

An electrical resistance meter was used to determine moisture content of wood. This meter was calibrated to measure moisture content of lodgepole pine by correlating meter readings with actual moisture content determined by the ovendry method. Meter readings were obtained from 20 lodgepole pine slabs. Small pieces of wood were then removed from the slabs and placed in glass vials, which were sealed with rubber stoppers and weighed. The wood then was removed from the vials, dried to a constant weight, returned to the vial, and reweighed. The percent moisture of the original sample was then calculated. Meter readings were highly correlated with actual moisture contents ($r=0.94$), which varied from 12 to 63%.

Exposure curves were developed for step wedges having moisture contents ranging from 10 to 60%. The wedges varied in thickness from ½ to 3 inches in ½-in. steps when a ⅛-inch-thick piece of bark was placed on each step. Each piece of bark contained a larva and an adult of the mountain pine beetle.

To test the accuracy of the X-ray method for estimating populations of the mountain pine beetle, infested bark-and-wood slabs varying from 1½ to 2 in. thick were radiographed. These slabs contained beetles grouped as follows: (1) small larvae; (2) medium-sized larvae; and (3) large larvae, pupae, and callow adults. Ten slabs were used for each grouping.

Metal staples or nails, which were visible on the radiograph, were placed in the corners of a 4×9-in. area on each slab to delineate the area where beetles were to be identified and counted. All insect inclusions were drawn and identified on a mattex acetate overlay, a technique used by Fatzinger and Dixon (1965). Characteristics used in the separation of radiographic images of insect stages have been covered adequately by Berryman (1964) and Fatzinger and Dixon (1965). Attack density of parent adults, which is determined by the number of entrance holes, and inches of egg gallery constructed by parent adults also were measured from the radiographs. The radiographs were read on a light table; all light was excluded except from a 4×9-in. area. Each radiograph was read by 2 observers working independently.

The bark between the metal staples was removed from the slabs, and the location as well as the identity of insects were compared with those drawn on the mattex acetate overlays. These comparisons were: (1) stage of mountain pine beetle identified correctly, (2) stage of beetle identified incorrectly, (3) beetle counted where it did not exist, (4) beetle omitted that did exist, (5) beetle incorrectly identified as being other species of insect, and (6) other species of insects (*Ips, Pityogenes, Enoclerus, Thanasimus, Medetera,* and *Coeloides*) incorrectly identified as pine beetles.

RESULTS AND DISCUSSION.—*Exposure Curves.*—Exposure time at 25 kv was 1.0 milliampere minute (mam) for a 1-inch-thick block containing 10% moisture. The exposure had to be increased to 6 mam for a block 3 in. thick containing 10% moisture and for a block 1 in. thick containing 60% moisture (Fig. 1). Little

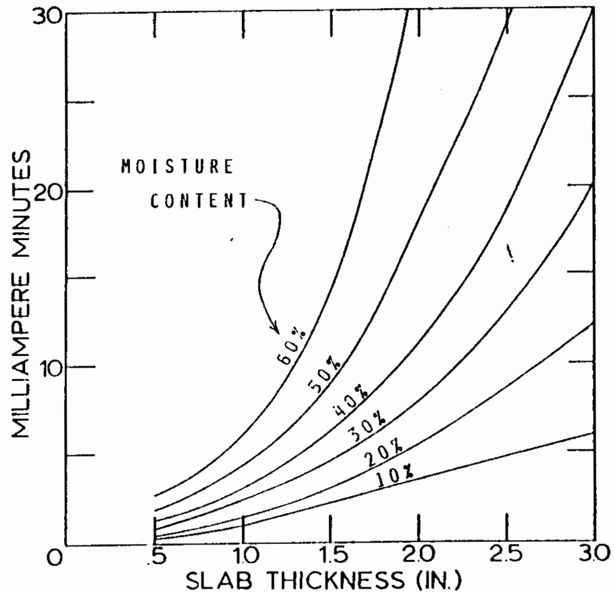

FIG. 1.—X-ray exposure curves for wood-and-bark slabs of different thickness and moisture content when the X-ray unit is operating at 25 kv, 5 ma, using a 20-in. target-to-film distance.

difference could be detected generally in radiograph quality within ±5% moisture content. The relationship between thickness of wood and bark and exposure was almost a straight line when the wood contained 10% moisture but became curvilinear as moisture increased. The curvilinearity became more pronounced at the higher moisture contents.

At 45 kv, exposure time was greatly reduced. For example, a 2-inch-thick piece of wood and bark containing 60% moisture required an exposure of 30 mam at 25 kv but only 1.5 mam at 45 kv (Fig. 2). Exposure curves at 45 kv were almost straight lines regardless of moisture content because of the greater proportion of short high-energy rays which are more penetrating than long rays.

The curvature of the surface of the slab resulted in an overexposure of the thinner outer edges, a prob-

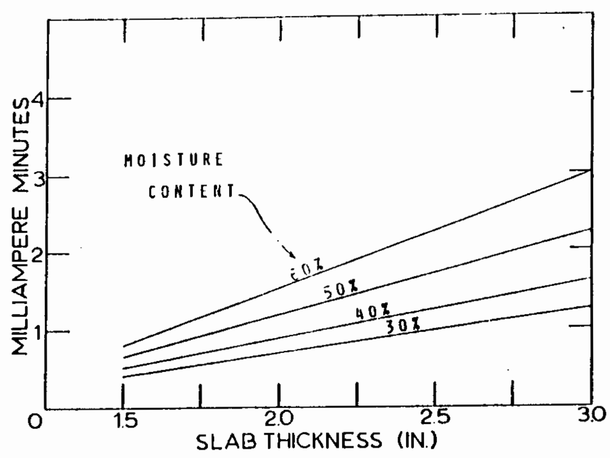

FIG. 2.—X-ray exposure curves for wood-and-bark slabs of different thickness and moisture content when the X-ray unit is operating (without filter) at 45 kv, 5 ma, using a 20-in. target-to-film distance.

lem encountered also by Berryman and Stark (1962). Exposure was adjusted for the thickest portion of the slab because it was easier to identify insect inclusions on slightly overexposed than on underexposed areas. Our problem of overexposure probably was not so serious as that of Berryman and Stark, because inclusions were identified only in the central 4×9-in. area of each slab. Within this area, differences in thickness of the slab were generally small.

Estimates of Beetle Numbers.—An analysis of variance was used to analyze the differences between actual and estimated numbers of mountain pine beetles. This analysis revealed significant differences between estimates from radiographs taken at the 2 kv levels and also among the 3 insect stages ($P<0.005$). However, the interactions and the differences between observers were not significant ($P>0.05$).

Estimates of beetles from radiographs taken at 25 kv, which departed from the actual count by an average of 3.3 beetles, were more accurate than those made from radiographs taken at 45 kv, which deviated an average of 6.8 beetles (Table 1). Better contrast and clarity accounted for the difference.

Average differences between actual and estimated numbers of beetles were greatest in the small larval stage and least in the large larval, pupal, and callow adult stages. Differences between stages were significant at the 0.05 level of probability. As the insects increased in size, accuracy of estimates increased and differences between kilovolt levels decreased. The differences observed between kilovolt levels in estimating large larval, pupal, and callow adult populations were not significant.

The estimates were compared with known numbers of insects using the "t-test" (Table 2). These comparisons showed only 3 significant differences for the 12 tests: small larvae from radiographs taken at 25 kv differed at the 0.01 level of probability, and medium larvae from radiographs taken at 25 kv and at 45 kv differed at the 0.05 level of probability. However, these differences were associated with only 1 observer and probably may be eliminated when that observer gains additional experience.

An arc sine transformation of data was used because of the wide range of proportions of insects correctly identified in this study. The analysis of variance revealed highly significant differences between the proportions of insects detected at the 2 kv levels and the kilovolt-insect stage interaction ($P<0.005$). At 25 kv, 91 and 95% of the small and medium larvae, respectively, were identified correctly compared with only 84 and 83% at 45 kv. These differences were significant at the 0.05 level of probability. However, a significant difference could not be detected between correct identifications of large larvae, pupae, and callow adults at 25 and 45 kv (92 and 93% correct, respectively) ($P>0.05$).

Table 1.—Average differences between actual and estimated numbers of mountain pine beetles from radiographs obtained at 25 kv and 45 kv.

Insect stages	25 kv Actual	25 kv Difference	45 kv Actual	45 kv Difference
Small larvae	72	5.3	49	10.5
Medium larvae	44	3.0	58	7.3
Large larvae, pupae, callow adults	58	1.5	77	2.1

Table 2.—T-test comparisons of known numbers of the mountain pine beetle with estimates from radiographs taken at 25 kv and 45 kv.

Observer	Insect stage	Actual population	Estimated population	% of actual	Probability
		25 kilovolts			
1	Small larvae	717	695	97	>0.2
2	"	717	767	107	< .01
1	Medium larvae	442	444	100	> .5
2	"	442	486	110	< .05
1	Large larvae, pupae, callow adults	576	573	99	> .5
2	"	576	569	99	> .5
		45 kilovolts			
1	Small larvae	485	445	92	> .5
2	"	485	507	105	> .5
1	Medium larvae	579	555	96	> .2
2	"	579	505	87	< .05
1	Large larvae, pupae, callow adults	772	771	100	> .5
2	"	772	753	98	> .1

Medium-sized larvae were identified from radiographs taken at 25 kv with slightly greater accuracy than the other stages. Medium-sized larvae could be detected and differentiated from other bark inclusions more easily than small larvae. In addition, there was no chance for error in identification of stage such as existed when large larvae, pupae, and callow adults were present. Movement by large larvae and callow adults, which caused blurred images on the radiographs, further complicated the problem of identification.

Detection and identification of small and medium larvae from radiographs taken at 45 kv were difficult because of lack of contrast in the radiographs. The differences in correct identifications between these 2 larval stages were not significant ($P>0.05$). Correct identifications of large larvae, pupae, and callow adults were significantly more accurate than those of small and medium larvae.

Estimates of Attack Density and Egg Galleries.—Estimates of attack density from radiographs at 25 and 45 kv did not differ from actual numbers ($P>0.1$ to >0.5) (Table 3). Inches of egg gallery could be determined accurately from radiographs taken during the small and medium larval stages at 25 kv and during the small larval stage at 45 kv ($P>0.05$ to >0.4). However, during the large larval, pupal, and callow adult stages it was almost impossible to make an accurate estimate of egg gallery length because of extensive mining.

CONCLUSIONS AND RECOMMENDATIONS.—(1) X-ray exposure should be adjusted for small differences in thickness and moisture content of wood and bark to obtain the most readable radiographs. (2) 25 kv should be used to obtain radiographs from which counts of small and medium-sized larvae will be made. Either 25 or 45 kv can be used to prepare radiographs from which counts will be made of large larvae, pupae, and

Table 3.—T-test comparisons of actual average attack density and inches of egg gallery of the mountain pine beetle with estimates made from radiographs taken at 25 kv and 45 kv.

Insect stage	Attack density				Gallery in.			
	Actual	Estimate	Difference range	Prob-ability	Actual	Estimate	Difference range	Prob-ability
				25 kilovolts				
Small larvae				[a]	22.0	20.9	−11–+ 6	0.4
Medium larvae	2.3	2.5	−2–+3	0.5	15.7	18.2	0–+18	.05
Large larvae, pupae, callow adults	3.4	2.6	−4–+3	.5	29.5	22.5	−18–+13	.05
				45 kilovolts				
Small larvae	0.7	1.0	0–+2	.1	11.7	12.9	− 6–+10	.3
Medium larvae	3.6	3.5	−2–+5	.5	34.5	25.7	−36–+31	.02
Large larvae, pupae, callow adults	2.3	2.0	−2–+1	.2	33.8	20.0	−38–+ 2	.01

[a] Not recorded.

callow adults. (3) Errors in estimating and identifying stages of the beetle from radiographs taken at 25 kv were less than 10% of the mean and are considered acceptable for population studies of bark beetles. (4) Estimates of egg gallery length should be taken from radiographs only when the larvae are small. (5) Attack density of parent adults can be determined from radiographs taken during any stage of beetle development. (6) The exposure curves presented here should be used with caution when radiographing insects in bark-and-wood slabs from trees other than lodgepole pine because of differences in density of wood and in proportions of bark to wood.

REFERENCES CITED

Berryman, A. A. 1964. Identification of insect inclusions in X-rays of ponderosa pine bark infested by western pine beetle, *Dendroctonus brevicomis* LeConte. Can. Entomol. 96: 883–8.

Berryman, A. A., and R. W. Stark. 1962. Studies on the effects of temperature on the development of *Ips confusus* using radiographic techniques. Ecology 43: 722–6.

DeMars, C. J., Jr. 1963. A comparison of radiograph analysis and bark dissection in estimating numbers of western pine beetle. Can. Entomol. 95: 1112–6.

Fatzinger, C. W., and J. C. Dixon. 1965. Use of X-rays to detect southern pine beetles in shortleaf pine bark. J. Forest. 63: 451–5.

Johnson, N. E., and H. D. Molatore. 1961. X-ray detection of Douglas-fir beetles reared in slabs. Can. Entomol. 93: 928–31.

Stacey, S. S., and J. S. Mothershead. 1965. Applicability of radiography to inspection of wood products. Second Symp. Nondestructive Testing of Wood Proc.: 307–31.

Reprinted from the
JOURNAL OF ECONOMIC ENTOMOLOGY
Volume 62, Number 3, pp. 631-634, June 1969