Radiographic Detection of *Agathis pumila*, a Parasite of the Larch Casebearer[1,2]

Gene D. Amman and Scott Tunnock[3]

ABSTRACT

Larvae of *Coleophora laricella* (Hübner) while still in their cases were radiographed on Kodak® type M film using an X-ray exposure of 5 kv and 3 ma for 4 minutes. Parasite larvae could not be detected consistently because of density similar to that of the host, hence lack of contrast. However, mature parasite larvae which had left the host's integument to pupate inside the larch-needle case were detected with 100% accuracy. Contents of all cases were identified with up to 97% accuracy. The radiographic method may be useful where a quick check of the casebearer population is needed to determine if the parasite is established and when subsequent preservation of the parasite is desired.

The larch casebearer, *Coleophora laricella* (Hübner), is an important defoliator of larch, *Larix occidentalis* Nutt., and threatens its management in Idaho and Montana. The casebearer was introduced into eastern North America from Europe prior to 1886. It then became established in Michigan, Wisconsin, and Minnesota (Lake States) (Cody et al. 1967) and in Idaho, Montana, and Washington (Denton 1958, 1965). The principal approach to control of this insect has been biological, and the necessary parasites were imported from Europe (Dowden 1962).

After discovery of the casebearer in northern Idaho, the parasite *Agathis pumila* (Ratzeburg) (Hymenoptera: Braconidae) was collected in New England (introduced originally from Europe) and released in Idaho in 1960 (Denton 1965). This parasite has proved quite successful in controlling the casebearer in Lake States forests (Cody et al. 1967), but up to now it has not controlled the casebearer in Idaho.

Examination of the population for the parasite's establishment is a time-consuming job and requires collecting, holding, and in some cases rearing of casebearers until parasites mature and emerge. Also, considerable time is expended in removing casebearers from cases and then dissecting them to search for parasitic larvae.

The purpose of this study was to explore the possibility of using radiography to detect *A. pumila* within its host. Radiography was used by Holling (1958) to detect parasites in prepupae of a sawfly *Neodiprion* sp. Yates (1967) radiographed shoots of loblolly pine to show parasitism of a pine tip moth, *Rhyacionia* sp. Witter[4] detected endoparasites of adult coccinellids, *Aphidecta obliterata* (L.), from radiographs with a high degree of accuracy. Ectoparasites and predators associated with bark beetles also were identified from radiographs of bark (Berryman 1964). In addition, Knight and Albertin (1966) determined the degree of parasitism and identified parasites of several wood borers and weevils found in Michigan trees.

Equipment and Methods.—A portable Picker® X-ray machine mounted in a lead-lined cabinet served as the source of X-rays. The machine has an output of 0–110 kv, 0–10 ma, and variable exposure time. Radiographs were made on Kodak® type M extra-fine-grain high-contrast film in ready packs. Others have used the much faster AA film, but in our tests this proved to be too grainy and lacking in contrast. Radiographs were best when the machine was operated at 5 kv, 3 ma, with a 20-in. target distance and a 4-min exposure.

Casebearers were collected in January 1968 near Sandpoint, Idaho, and taken to Missoula, Mont., where they were held at room temperature until dormancy was broken. The larvae then were fed larch which had foliated in 2 weeks at room temperature. Casebearers from these rearings were sent periodically during March and April to Ogden, Utah, where they were radiographed and then dissected to search for parasites. The final shipments to Ogden were from field collections made in June.

A holder for the larval cases was constructed of ⅛-in. masonite with ¼-in. holes drilled through it. On one side, paper was glued over the holes to form a bottom. One case was placed in each hole. To prevent casebearers from moving about, all were killed by a fumigant prior to placing them in the holder. However, if preservation of parasites was desirable, they could be inactivated by cool temperatures or CO_2. The number of cases radiographed each date varied from 36 to 110, dependent upon the number available.

Radiographs were interpreted with the aid of a 10× hand lens. Contents of the cases were identified as follows: (1) casebearer or parasite, (2) stage of casebearer or parasite, and (3) casebearer parasitized or not.

Results and Discussion.—The number of parasites correctly identified varied from none in the 1st test to 100% in the final test (Table 1). In the 1st group of instars, the parasites were all within the host and were so small that their density did not differ appreciably from that of the host. Hence, the parasite was not detected on the radiograph. Even though density of the larvae increased as they matured, they still were not distinguished consistently from the host's tissue. Over 95% of the errors in identification of case contents were associated with determining whether a casebearer larva was parasitized. About ½ of these errors occurred when nonparasitized casebearers were called parasitized, the other ½ when parasitized casebearers were called nonparasitized.

The principal reason for improved success with the last group was that most parasitic larvae had matured and emerged from the host integument to pupate inside the larch-needle case. This stage permitted use of body-segment shape as a diagnostic character. A longitudinal section of casebearer larva shows squarish segments, usually with indentations near the

[1] Lepidoptera: Coleophoridae.

[2] Mention of trade names does not imply endorsement by USDA Forest Service.

[3] The authors are, respectively, Research Entomologist, USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah 84401; and Entomologist, Division of State and Private Forestry, USDA Forest Service, Northern Region, Missoula, Mont. 59801. Received for publication Oct. 23, 1970.

[4] J. A. Witter. 1967. Bionomics of *Aphidecta obliterata* (L.) (Coleoptera: Coccinellidae), a predator introduced for control of the balsam woolly aphid on Mt. Mitchell, North Carolina. Virginia Polytechnic Institute, M.S. thesis, 91 p.

Table 1.—Accuracy of determining contents of larch casebearer cases from radiographs in 1968.

Date	All contents[a]			*Agathis pumila*		
	Total cases	No. identified correctly	% identified correctly	Total cases	No. identified correctly	% identified correctly
Mar. 13	38	26	68	2	0	0
Mar. 18	58	44	76	8	1	13
Apr. 1	112	69	62	30	5	17
Apr. 4	41	25	61	5	2	40
Apr. 11	96	75	78	16	8	50
Apr. 26	36	31	86	5	3	60
June 18	110	93	85	22[b]	12	55
				4[c]	1	25
June 20	110	107	97	46[b]	46	100
				7[c]	5	71

[a] Larch casebearer and *A. pumila* larvae, pupae, and adults.
[b] Mature parasite larvae that emerged from the host integument.
[c] Immature parasite larvae contained within the host integument.

centers; in contrast, the parasitic larva has rounded segments (Fig. 1).

Knowledge obtained from a sample can often be applied with good results to assessment of subsequent samples. This fact was illustrated by the last 2 samples (June 18 and 20). By using the difference

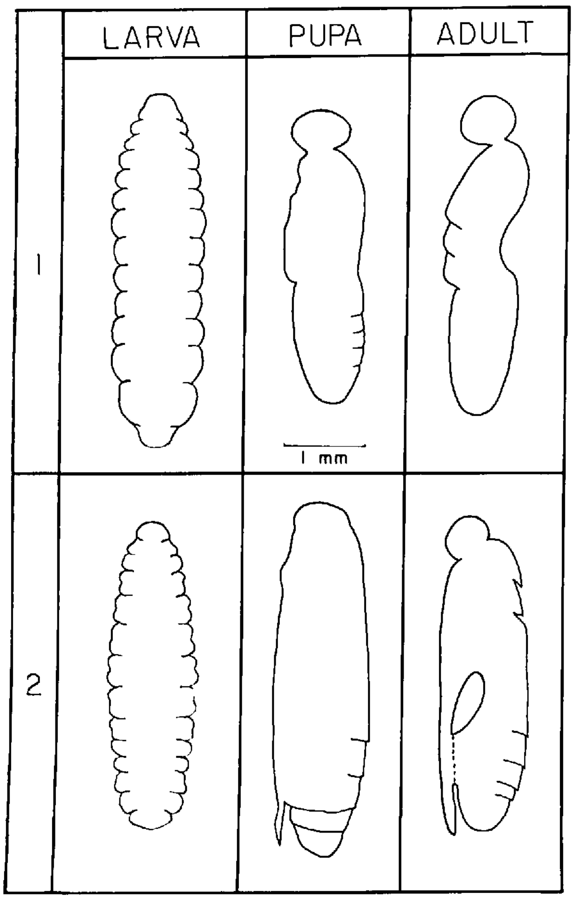

FIG. 1.—Radiographic drawings of larval, pupal, and adult stages of (1) *A. pumila* and (2) the larch casebearer.

noted in shape of body segments when dissecting for parasites from the June 18 sampling, accuracy in identification of mature parasites in the June 20 sample increased from 55 to 100%. Correct identifications of all contents of larch casebearer cases also increased from 68% in the 1st sample (Mar. 13) to 97% in the last one (June 20). Fig. 1 depicts the forms of the larval, pupal, and adult stages of the casebearer and *A. pumila* as they appeared on the radiographs.

The cost of the radiographic detection method in time and expendable materials was calculated. These calculations were based on 12 radiographs (8×10 in.), because this number was the most efficient for the available developing-washing-fixing equipment. The images of 500 cases could be obtained on each radiograph. Total time required for each radiograph is:

Item	Time (avg min/radiograph)
Prepare 500 cases for X-raying	100
X-raying	6
Developing and fixing film	4
Interpreting the radiograph	60
Total	170 min

Preparation of the cases for X-raying requires picking them from larch foliage, placing in containers, and fumigating to kill larvae. Then, the cases are placed in individual holes of masonite holders for radiographing. Preparing cases for radiographing and interpreting radiographs would probably require less time as experience is gained. However, the time required for radiographing, and developing and fixing film would remain constant. Therefore, at this level of experience, 500 cases could be processed in 170 min, or ca. ⅓ min/case. The cost of expendable materials was small. The film cost was 65¢/sheet, and that of the developer and fixer a few cents.

Processing of several thousand cases by the dissection method required an average of 1.6 min/case. However, this average would apply primarily to internal parasites. Once the parasite larvae leave the host's integument (stage when radiographic method is most accurate), processing time would be about the same as that for the radiographic method.

Results of this study indicate that the radiographic method may be used in monitoring a casebearer population to determine establishment of introduced parasites. However, results are limited to the mature larval stage of the parasite after it leaves the host integument to pupate in the larch-needle case. Advantages of the method are: (1) casebearers from many localities can be collected and radiographed and then the radiographs can be interpreted at a later date; (2) cases containing parasites can be retained for the parasite's rearing, whereas under the dissecting method parasites are killed. The main disadvantage of the radiographic method is that parasites remaining within the host cannot be detected accurately. A possible solution is use of a correction factor based on the number of parasites still contained within the host as determined by dissection of a limited number of casebearers. For example, the June 18 and 20 samples revealed that 13–15% of the parasite population was still within the host. These figures could be used to correct for total parasitism samples which come from areas having similar ecological conditions.

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.

Cody, J. B., F. B. Knight, and S. A. Graham. 1967. The hymenopterous parasites *Agathis pumila* (Braconidae) and *Epilampsis laricinellae* (Eulophidae) on the larch casebearer (Lepidoptera: Coleophoridae) in the northern Lake States. Mich. Entomol. 1: 159–67.

Denton, R. E. 1958. The larch casebearer in Idaho—a new defoliator record for western forests. U.S. Forest Serv., Intermountain Forest Range Exp. Sta. Res. Note 51. 6 p.

1965. Larch casebearer in western larch forests. USDA Forest Serv., Forest Pest Leafl. 96. 6 p.

Dowden, P. B. 1962. Parasites and predators of forest insects liberated in the United States through 1960. USDA Agr. Handb. 226. 70 p.

Holling, C. S. 1958. A radiographic technique to identify healthy, parasitized and diseased sawfly prepupae within cocoons. Can. Entomol. 90: 59–61.

Knight, F. B., and W. Albertin. 1966. Portable X-ray equipment: a useful tool in forest entomological field research. Mich. Acad. Sci. Arts Letters 51: 23–26.

Yates, H. O., III. 1967. Radiographic detection of *Rhyacionia* larvae, pupae, parasites, and predators within pine shoots (Lepidoptera: Olethreutidae). J. Ga. Entomol. Soc. 2: 81–85.

Development and Fecundity of Codling Moths[1] Reared on Artificial Diets or Immature Apples[2,3]

D. O. HATHAWAY, A. E. CLIFT, and B. A. BUTT[4]

Entomology Research Division, Agr. Res. Serv., USDA, Yakima, Washington 98902

ABSTRACT

Laspeyresia pomonella (L.), reared in 30-ml plastic jelly cups on artificial diets developed by Redfern, Howell, International Minerals and Chemical Corporation (IMC), Brinton, and Navon, were compared with moths reared on immature apples. Of the larvae reared on diets developed by Howell and by IMC, 47% developed into adults. Diets developed by Redfern, Brinton, and Navon produced 33%, 30%, and 28% adults, respectively. In comparison, 48% of the larvae reared on immature apples developed into adults. Male moths reared on apples were heavier than those reared on the artificial diets. Only the female moths reared on the IMC diet were comparable in weight to those reared on the immature apples. No significant differences were observed in the numbers of eggs laid, eggs hatched, or adult longevity.

Sterile codling moths, *Laspeyresia pomonella* (L.), have been mass released to test the concept of insect control where the wild population is overflooded with sterile moths in sufficient quantity to prevent fertile matings (Proverbs et al. 1969, Butt et al. 1970). To produce large numbers, many workers have developed artificial diets for rearing the codling moth (Brinton et al. 1969, Howell 1970, International Minerals and Chemical Corporation (IMC), Navon 1968, Redfern 1964, Rock 1967, Sender 1969). Four of these diets plus a commercial diet developed by IMC and immature apples were selected to compare the following: larval development, adult longevity, egg deposition, and egg hatch of the codling moth for 1 generation, and to determine which one, if any, demonstrates the greatest potential in a mass-rearing program.

METHODS AND MATERIALS.—Three of the diets, the modified Redfern diet (Hamilton and Hathaway 1966), the Howell diet, and the IMC multigeneration larval rearing diet (without alfalfa) contain agar, which is used to bind the ingredients together (Table 1). The Brinton diet uses whole wheat flour, paper pulp, and wood chips as a binder. In the Navon diet (personal communication[5]), a binding gel is formed by the reaction between sodium alginate and calcium ions under acid conditions. Immature apples, a natural food of the codling moth, were used as a standard in comparing the artificial diets.

The neonatal larvae used in this experiment were obtained from eggs laid by apple-reared moths held in a 40.6×16.5×11.4-cm waxed paper bread bag, at

[1] Lepidoptera: Olethreutidae.

[2] Mention of a proprietary product does not imply endorsement by the USDA.

[3] Received for publication Sept. 21, 1970.

[4] The authors acknowledge the aid given by G. Tamaki, Research Entomologist, for statistical analysis, and of F. M. Tye and L. J. Morton, insect production workers, in different phases of the testing.

[5] A. Navon, The Volcani Institute of Agricultural Research, Bet-Dagan, Israel.