bi-monthly research notes

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ENTOMOLOGY

② **Biocidal Effect of Fatty Acids and Soaps on the Balsam Woolly Aphid.** — During a bioassay of todomatuic acid, a juvenile hormone analogue found in certain aphid infested trees (Puritch and Nijholt, Can. J. Bot., in press), we used the unsaturated lipid, oleic acid (C_{18}:1) as a control. To our initial surprise, oleic acid affected the aphid's wool and caused mortality of all stages, including eggs. We then realized that fatty acids and their salts, i.e., soaps, were among the oldest insecticides and had been commonly used for centuries to control garden and orchard pests. Several scientific reports dealing with the use of these compounds as contact insecticides were presented during the 1920's and 30's (Shepherd, H. H. ed. The chemistry and toxicology of insecticides, Burgess Pub. Co. Minn. 1947) but few, if any, appeared since that time. We therefore investigated more fully the effect of these compounds on the balsam woolly aphid and their usefulness as insect control agents.

To further test the effects of oleic acid on various stages of non-dormant balsam woolly aphids [*Adelges piceae* (Ratz.)], 6-year-old *Abies grandis* (Dougl.) Lindl. seedlings were infested with aphids during mid-August according to Carrow and Betts (Can. J. For. Res. 3: 122-139, 1973). In October, eight selected trees were separated into two groups and placed in a growth room at 23 C day/17 C night, 66% day/56% night R.H. and 16 h photoperiod. For each group, the various aphid stages were counted and totalled. Aphids were then sprayed to the drip point on October 24 with a hand sprayer, with either 5% oleic acid emulsified in 0.1% Tween 20 (polyoxyethylene (20) sorbitan monolaurate) or water. A second set of eight infested seedlings was separated into two groups of 4 and, on November 27, sprayed with either 5% oleic acid in 0.1% Tween 20 or 0.1% Tween 20. For both trials, the aphids were counted after treatment and their development was observed for 1 month.

Oleic acid caused total mortality of first-, second- and third-instar aphids, as well as adults in both trials (Table 1). After treatment, the aphid wool lost its hydrophobic properties and collapsed. The aphids, however, remained attached to the stem, appeared bloated and black and showed no signs of activity when probed.

Periodic examination over the following month revealed no signs of life, although control aphids continued normal development. The emulsifier, Tween 20, also caused a certain amount of mortality, especially to the neosistens (Table 1).

Since oleic acid proved effective in killing the aphid, tests were initiated to analyze the effectiveness of various other fatty acids and their potassium (K) soaps. Even carbon-numbered, saturated fatty acids from caproic (C_6) to behenic (C_{22}), along with the unsaturated acids, oleic and linoleic ($C_{18:2}$), were made up as 1% solutions (w/v or v/v) and emulsified with 0.1% Tween 20 and using a Beckmann Polytron Disintegrator. Potassium soaps were made by neutralizing each fatty acid with 1N KOH and adding distilled water to make a 1% solution. Aphid-infested bark was obtained during winter from mature *A. grandis* in the field. The bark was separated into 2-cm diam plugs containing a minimum of 300 aphids and put into separate petri dishes on wet filter paper. One plug was used per treatment and each plug was sprayed to wetness at room temperature with the acid or soap using a hand sprayer. (Further tests are currently underway to ascertain the minimum lethal dose for the aphids.) Excess solution was drained off and plugs kept overnight in a growth room. The filter paper was kept wet to prevent dessication. On the following day, per cent mortality was determined by counting all living and dead aphids within a 0.8 cm^2 area.

Application of the 1% fatty acids caused varying degrees of mortality according to the type of compound used (Fig. 1). The short chain saturated fatty acids from caprylic (C_8) to palmitic (C_{16}) were the most toxic, causing in excess of 95% mortality, while the longer chain saturates, stearic (C_{18}) to behenic (C_{22}), were much less toxic. Oleic and linoleic, the two unsaturated C_{18} compounds, were among the most effective treatments and contrasted with the least effective saturated compound tested, stearic acid, which also has 18 carbons. Neutralization of the fatty acids to make soaps caused little change in their toxicity (Fig. 1), suggesting that the fatty acid component is the major cause of toxicity and not the potassium ion.

TABLE 1
Effect of topically applied oleic acid on balsam woolly aphid

	October Treatment: No. of live aphids before Spray Oct 24				No. of live aphids after Spray Nov 29			
	Instar I	Instar II & II	Adults	Eggs	Instar I	Instar II & II	Adults	Eggs
5% oleic acid	42	10	0	0	0	0	0	0
Control	11	28	4	13	41	26	4	0
	November Treatment: Before Spray Nov 27				After Spray Dec 18			
5% oleic acid	53	21	1	0	0	0	0	0
.1% Tween 20	18	17	0	0	1	17	1	6

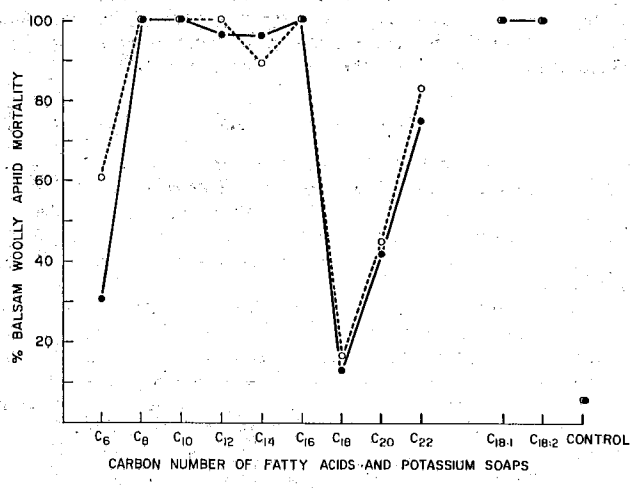

Figure 1. Effect of 1% fatty acids (—) and 1% potassium soaps (- - -) on mortality of the balsam woolly aphid (*Adelges piceae* (Ratz.)).

Fatty acids and their soaps are effective insecticides for the balsam woolly aphid. These compounds are natural constituents of plants and animals, are relatively cheap, biodegradable and low in phytotoxicity. The unsaturated fatty acids, oleic and linoleic, are also readily oxidized and are low in toxicity to fish. Rogers (Pulp Pap. Mag. Can. 74: T303-T308, 1973 and personal comm.) found that oleic acid was about eight times, and linoleic about four times, less toxic to coho salmon than the resin acid, abietic, which has an incipient lethal level of 2.2 ppm. These compounds have also been shown at this laboratory to alter development of certain insects and to cause instant paralysis and death of other important forest pests, including the spruce budworm [*Choristoneura fumiferana* (Clem.)] (Puritch, unpublished results). The soaps and fatty acids thus hold promise as insecticides and agents to prevent insect development and may provide an alternative to the more toxic petro-chemicals. — George S. Puritch and M. Talmon de l'Armee, Pacific Forest Research Centre, Victoria, B.C.

FOREST PRODUCTS

Diazo Naphthalene-disulfonic Acid and Fungal Membrane Permeability. — Paradee and Watanabe (J. Bacteriol. 96:1049-1054, 1968) devised a reagent, diazo-7-amino-1,3-naphthalene-disulfonic acid (diazo-NDS) that inactivates enzymes and does not penetrate bacterial cytoplasmic membranes. This reagent is useful for determining on which side of the cell membrane a particular enzyme is located.

The site of cellulase (EC. 3.2.1.4), the main enzyme associated with wood rot, has not been definitely established for fungal species. An investigation was therefore begun and diazo-NDS used in the first tests.

Cell-free cellulase of *Myrothecium verrucaria* was shown to be inactivated by diazo-NDS. To determine this, freeze-dried culture supernatant obtained from growth of *M. verrucaria* on glucose in shake culture (Hulme and Stranks, J. Gen. Microbiol. 69: 145-155, 1971) was treated following the procedure of Day and Ingram (Can. J. Microbiol. 17: 1025-1028, 1971). After exposure for 1 hour at room temperature the cellulase preparation was 80% inactivated.

Bound cellulase, which is that remaining with the mycelium after washing, was then treated with the diazo-NDS reagent using the same procedure. After treatment the reagent was washed away, the mycelium resuspended in buffer (0.1M tris (hydroxymethyl) aminomethane HCl, pH 7.1), the whole disintegrated at 20 kHz, the debris centrifuged off, and the clear supernatant recovered. The cellulase activity of the supernatant was determined and then compared with that for an untreated control. Complete inactivation of the bound cellulase for 3-day-old and older culture material was observed (Table 1). If the membrane of this organism did not leak diazo-NDS under the test conditions, the bound cellulase could then be considered to be outside the membrane. To check for possible leakage, reaction of this reagent with fumarase, an

TABLE 1
Inactivation of cell-bound cellulase of *M. verrucaria* by diazo-nds

Growth Period (days)	Cellulase (units */10 mg oven dry mycelium) Control	Treated	Cellulase Inactivation (%)
2	0.32	0.03	90.6
3	0.66	0.00	100.0
4	0.12	0.00	100.0
7	0.04	0.00	100.0

*(Stranks, Can. J. Microbiol. 19:1523-1526, 1973).