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# RESPONSES TO PLANT EXTRACTS OF NEONATAL CODLING MOTH LARVAE, *CYDIA POMONELLA* (L.), (LEPIDOPTERA:TORTRICIDAE:OLETHREUTINAE)

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## ABSTRACT

A bioassay was designed to test behavioral responses of neonatal codling moth larvae to chloroform and methanol extracts of 25 plant species. Chloroform extractable materials from absinthe wormwood, *Artemisia absinthium* (L.), rabbitbrush, *Chrysothamnus nauseosus* (Pallas), and tansy, *Tanacetum vulgare* (L.) showed promise as possible feeding deterrents to neonatal codling moth larvae.

## INTRODUCTION

In Washington State approximately half the cost of controlling arthropod pests in apples is attributable to the codling moth, *Cydia pomonella* (L.) (Ferro *et al.* 1975). Much of the damage occurs as "stings" made by probing neonatal larvae attempting to penetrate but then not entering the fruit. This "stinging" behavior might be linked to incompletely developed chemoreceptors. Immediately upon eclosion from the egg, larvae may not be able to recognize the fruit as a potential food source. This "non-recognition" phenomenon has been shown by Wiklund (1973) for early instars of *Papilio machaon* (L.) and by Bland (1981) for first instars of acridids. Non-recognition of food by neonates can lead to wandering activities that increase their exposure to abiotic and biotic mortality factors. As a result, in unsprayed apple orchards, death of neonatal codling moth larvae reduces the population by greater proportions than mortalities of any other life stage (Ferro *et al.* 1975, MacLellan 1977). Therefore, new control efforts should be directed to this stage. Disruption of larval feeding behavior by the use of secondary plant compounds may increase wandering and thus mortality.

We surveyed local plants for extracts that might modify the feeding behavior of neonatal codling moth larvae. Extracts that prevented or interrupted feeding activity were considered possible sources for feeding deterrents as defined by Schoonhoven (1982). Twenty-five selected plant species of eastern Washington and northern Idaho were collected in the survey. This study concentrated on neonatal larvae and their feeding behavior rather than on the long-term development of insects fed on artificial diets containing the suspected feeding deterrents.

## MATERIALS AND METHODS

### Plant Collection and Extraction

Test plants were collected during the summer of 1982. Criteria used to select the plants included strong odor, notable lack of herbivore feeding activity, or literature references concerning their repellent properties. An effort was made to include at least one representative from each of a variety of plant families (Table 1).

Plants chosen appeared healthy and free from visible signs of disease. Entire plants were collected including a moist ball of soil around the roots. The roots were wrapped in moist paper towels and covered with a plastic bag for transport. Plant samples were either frozen or extracted within 1 h of collection.

Ten grams of leaves (and flowers, if present) were weighed, wrapped in plastic, and frozen at ca. -16°C to preserve plant components without changes in chemical composition due to enzymatic activity (Draper 1976).

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Frozen or fresh plant material was ground (<4°C) to a slurry in a Sorval Omnimixer® in 30 ml of chloroform and methanol, 2:1 ratio. The slurry was left for 2 h in a covered flask, then filtered through a Büchner funnel. Solvents were transferred to a separatory funnel and the two phases were then collected in vials, flooded with nitrogen, and stored at -16°C until tested.

**Bioassay Design**

"Transparent" variety apples ($\bar{x}$ diam = 5 cm) were collected in late July, 1982, from an abandoned, unsprayed tree near Viola, Idaho in Latah County. Apples were held at 4°C and used within 6 months. A cork borer was used to remove 20 (0.8 cm diam, 0.5 cm thick) plugs from the same apple for each test. Each plug was held by the epidermis with a suction tube and dipped 3 times in liquid Paraplast® tissue embedding medium (melting point 56-57°C) to coat the plug, excluding the epidermis. Each plug was then placed on a filter paper (2.1 cm) to facilitate handling during test procedures. Twenty freshly-prepared plugs were required for each experiment.

A 9 cm plastic petri dish served as the test arena (Fig. 1). A 1.2 cm hole was drilled in the center of the dish and covered with nylon screen (100 $\mu$m mesh) to prevent escape by the larvae. A section of clear plastic tubing (I.D. 2.1 cm, 1.2 cm tall), with four 0.3 cm holes drilled in the base at 90° intervals, was secured over the center hole. A polyethylene tube (O.D. 1.4 cm, I.D. 1.0 cm) for connecting a vacuum line was glued in place over the 1.2 cm hole on the bottom of the dish. Four 0.3 cm holes were drilled at 90° intervals in the center of the side wall of the dish and covered with nylon screen. A thin layer of vacuum grease was applied to the top lip of the petri dish and the lid was secured with 3 rubber bands. Five arenas were placed in a series and vacuum was applied to create an air flow of 24 cc/sec/arena. The air flow permitted test larvae to find the apple plugs by following the odor gradients and also prevented a buildup of odors. Dense smoke demonstrated that the air flow pattern was uniform.

**Bioassay Procedure**

Three milliliters of chloroform extract were transferred to a pre-weighed round-bottom flask and flash-evaporated to dryness under vacuum at room temperature. The flask and residue were re-weighed and enough 1% Triton-X:water was added to make a 1% (w/v) solution of plant extract. A similar procedure was followed for methanol extracts, but 1% methanol:water was added to the dry residue to obtain a 1% (w/v) solution of plant extract.

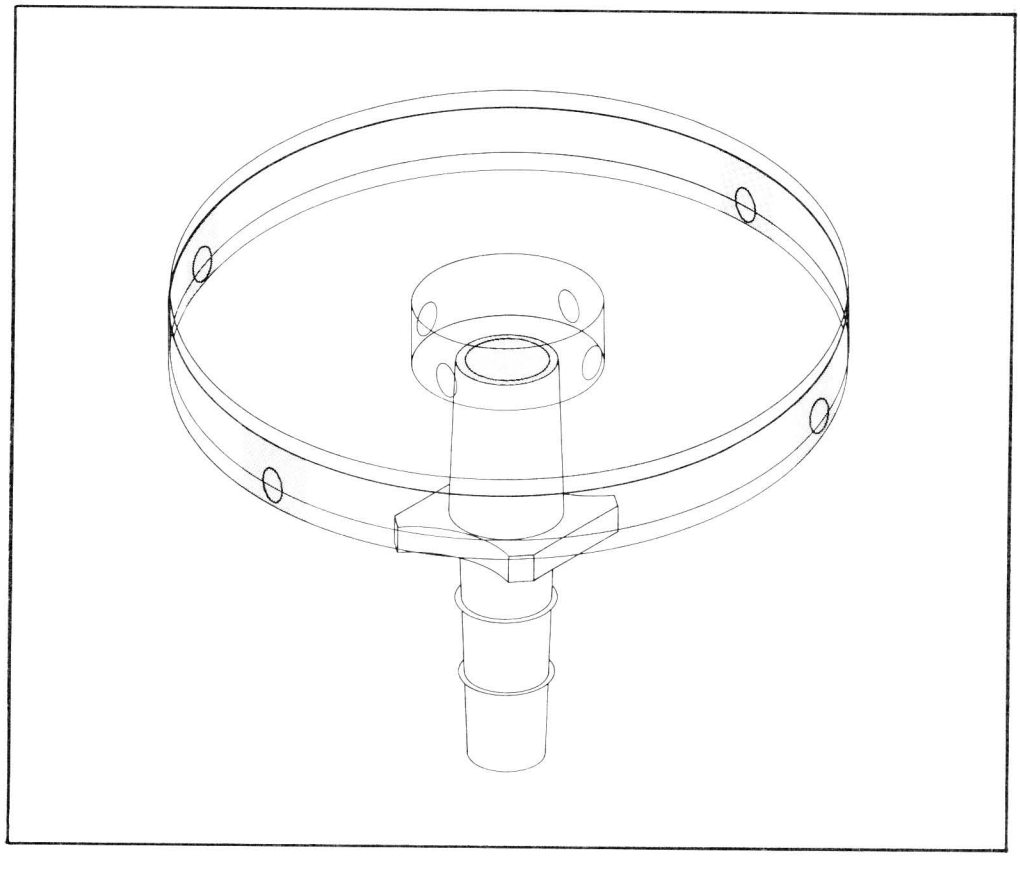

Fig. 1 Modified petri dish used for test arena bioassay.

The epidermis of the apple plugs was immersed in weighed test solutions, and excess fluid was allowed to drain back into the reservoir for several seconds. The amount of 1% solution adhering to each plug was determined by re-weighing the test solution reservoir. Plugs to be used as controls were immersed in 1% Triton-X:water mixture (for chloroform extraction tests) or a 1% methanol:water mixture (for methanol extraction tests). In each arena a plug was placed at each 90° interval with test and control plugs alternating, for a total of 4 plugs.

### Test Animals

The codling moths used for this research were collected as larvae from unsprayed apple trees near Viola and reared through two generations on an agar-wheat germ diet (Howell and Clift 1972). All experiments were conducted in a controlled environment with long day (16 h light:8 h dark) illumination from overhead fluorescent lighting, a temperature of 29°C ± 1°, and RH of 55-60%. For each test, 10 larvae (<24 h old) were transferred to each of 5 arenas. After 24 h the location of each larva was recorded. Results were analyzed by one-way analysis of variance (Fisher's LSD $P<0.001$). Mortality and feeding behavior were noted.

### Feeding Stations

Initially, experiments were conducted to determine the optimal number of feeding stations necessary to ensure that neonates would find the apple plugs with minimal wandering. This was done by varying the number (1 to 4) of untreated apple plugs in each arena. Four apple plugs resulted in establishment of 90% of the larvae. Therefore, all tests were conducted using 4 stations. In addition, results obtained while using 4 untreated plugs/arena showed an even distribution of larvae on each of the stations with no significant ($P<0.05$) feeding preference for any 1 station. There was also no observed hesitation by the larvae to feed on any of the feeding stations treated with either control solvent, 1% Triton-X:water or 1% methanol:water.

Tests were made of chloroform and methanol extracts of 25 plants (Table 1). Extracts that reduced feeding on treated stations to ≤20% were re-tested (Table 2). Methanol extracts were generally ineffective, and only the alcohol extract of bittersweet, *Solanum dulcamara*, was re-tested. The most promising materials, chloroform extracts from absinthe wormwood, *Artemisia absinthium* (L.), rabbitbrush, *Chrysothamnus nauseosus* (Pallus), and tansy, *Tanacetum vulgare* (L.), were then used in a third series of tests (Table 3).

**TABLE 1.** Plants from the Palouse area of Washington and Idaho collected and extracted to test for compounds modifying behavior of neonatal codling moth larvae.[a]

| Family | Scientific and common name[b] | Date collected |
|---|---|---|
| Pinaceae | Abies grandis (Douglas) Grand Fir | VI-23-82 |
| | Pinus monticola (Douglas) Western White Pine | VI-23-82 |
| | Pseudotsuga menziesii (Mirbel) Douglas fir | VI-23-82 |
| Liliaceae | Allium sativum (L.) Garlic | VIII-19-82 |
| | Veratrum californicum (Durand) False Hellebore | VI-23-82 |
| Aristolochiaceae | Asarum caudatum (Lindley) Wild Ginger | VI-23-82 |
| Geraniaceae | Geranium viscossissimum (Rydberg) Sticky Geranium | VII-12-82 |

| | | |
|---|---|---|
| Leguminosae | Lupinus argenteus (Pursh)<br>Silky lupine | VI-10-82 |
| Labiatae | Nepeta cataria (L.)<br>Catnip | VI-24-82 |
| Cruciferae | Tropaeolum majus (L.)<br>Nasturtium | IX-19-82 |
| Convolvulaceae | Convolvulus arvensis (L.)<br>Field Bindweed | VI-25-82 |
| Solanaceae | Solanum dulcamara (L.)<br>Bittersweet | IX-13-82 |
| | Capsicum annuum (L.)<br>Pepper | X-14-82 |
| Umbelliferae | Conium maculatum (L.)<br>Poison Hemlock | VI-16-82 |
| Asteraceae | Achillea millefolium (L.)<br>Yarrow | VI-25-82 |
| | Anthemis cotula (L.)<br>Mayweed | IX-17-82 |
| | Artemisia absinthium (L.)<br>Absinthe Wormwood | VI-16-82 |
| | Chicorium intybus (L.)<br>Chicory | VII-26-82 |
| | Chrysothamnus nauseosus (Pallas)<br>Rabbitbrush | IX-23-82 |
| | Erigeron canadensis (L.)<br>Horseweed | IX-15-82 |
| | Madia glomerata (Hooker)<br>Tarweed | VIII-10-82 |

| | |
|---|---|
| Matricaria matricarioides (Lessing) Pineapple Weed | VII-7-82 |
| Tanacetum vulgare (L.) Tansy | IX-6-82 |
| Taraxacum officinale (Weber) Dandelion | VIII-16-82 |
| Tragopogon porrifolius (L.) Salsify | VI-15-82 |

[a]The Pinaceae, Liliaceae, and Aristolochiaceae were collected in Latah County, Idaho; all others were collected in Whitman County, Washington.

[b]Names from Hitchcock and Cronquist (1973).

## RESULTS AND DISCUSSION

Because the female codling moth does not always oviposit directly on the young fruit, neonatal larvae are exposed to many environmental hazards. Thus, these newly emerged larvae must search for food, resulting in a depletion of energy resources, an increase in exposure to predation, parasitism, and pathogens and desiccation due to high temperatures and low relative humidity. These latter climatic conditions are especially prevalent in the central basin of Washington. Exposure to these hazardous situations makes the first larval instar the "weak link" in the life cycle of the codling moth.

Among the 25 plant species tested, a number of extracts showed considerable promise as feeding deterrents for neonatal larvae of the codling moth. There were significantly ($P<0.001$) fewer larvae found on the apple plugs treated with the chloroform extracts of *Artemisia absinthium*, *Chrysothamnus nauseosus*, and *Tanacetum vulgare* than were found on the control plugs treated only with chloroform. *Tanacetum vulgare*, for example, is closely related to the chrysanthemums which are known for their insecticidal properties (Wodehouse 1971). The main components of the volatile oil of *T. vulgare* were identified as bicylic monoterpenoids, borneol (Brewer and Ball 1981), β-thujone and ℓ-camphor (Gibbs 1974). The latter repels moths (Windholz *et al.* 1976), and tansy extracts have proven to be particularly obnoxious to insects (Lewis and Elvin-Lewis 1977). Tansy oil diluted in alcohol has been used as a mosquito repellent (Crockett 1977).

In addition, a polyacetylene (trans-dehydromatricaria ester) has been isolated from *T. vulgare* leaves (Bohlman *et al.* 1973). Polyacetylenes are often associated with composites such as *Chrysothamnus nauseosus* where they have been credited with antifeeding activity against *Leptinotarsa decemlineata* (Rose *et al.* 1980). For example, dihydromatricaric acid is a polyacetylene that is a known defense secretion used by a cantharid beetle, *Chauliognathus lecontei* (Meinwald *et al.* 1968). Seven of the 25 plants tested are known to contain polyacetylenes, including *Matricaria matricarioides* (Lessing) whose generic name implies "a place where something rotten is generated" (Borrer 1960). Extracts from only 2 of the 7 polyacetylene-containing plants tested, *T. vulgare* and *C. nauseosus*, exhibited antifeeding activity against neonatal codling moth larvae. This is not surprising since so many insects feed upon plants of the Compositae. Absinthins, dimeric sesquiterpenoids isolated from *Artemisia absinthium*, inhibited feeding by larvae of *Spodoptera littoralis* (Boisduval) (Wada and Munakata 1971), and chloroform extracts from this plant deterred 90% of the codling moth larvae from feeding on treated apple plugs. Sesquiterpenoids from *Parabenzoin trilobum* (L.) (Wada *et al.* 1968) and *Aneura pinguis* (L.) (Goodwin 1971) have provided antifeedant activity against several insects, but not all sesquiterpene-containing plants deter feeding. *Achillea millefolium*, for instance, is known to contain at least 4 sesquiterpenoid structures (Yoshiaka *et al.* 1973), and yet chloroform extracts of yarrow had no effect on *C. pomonella* larvae.

Two plants which contained chloroform extracted materials that were effective feeding deterrents were *Veratrum californicum* (Durand) and *Allium sativum* (L.). *V. californicum* contains teratogenic steroid alkaloids which cause cyclopian and related cephalic malformations in lambs born to ewes that ate the plants (Binns *et al.* 1963). These defects were also found to occur in other animals eating the plant (Keller 1975). For this reason, *V. californicum* was viewed as containing potentially hazardous materials, and was not investigated further. However, mixed alkaloidal preparations of *Veratrum* and *Schoenocaulon* have been used as insecticides (Kingsbury 1964). *A. sativum*, garlic, although known to be an effective insect repellent (Nasseh 1982) was considered too odoriferous for pre-harvest application to an apple crop.

**TABLE 2.** Percentage of larvae actively feeding on apple cores treated with chloroform extracts of various plants or found dead within the arena after 24 h.

| Plant species | % Feeding on treated apple cores | % Mortality |
|---|---|---|
| *A. sativum* | 2 | 8 |
| *A. absinthium* | 9 | 10 |
| *C. nauseosus* | 12 | 10 |
| *G. viscossissimum* | 20 | 8 |
| *M. glomerata* | 12 | 8 |
| *P. monticola* | 14 | 12 |
| *S. dulcamara* | 12 | 18 |
| *T. vulgare* | 3 | 16 |
| *T. majus* | 12 | 10 |
| *V. californicum* | 2 | 4 |

[a]When only solvent-treated apple cores were used, 90% of the larvae penetrated the epidermis and mortality averaged 4% for 15 arenas of 10 larvae each.

**TABLE 3.** Location of 10 larvae placed in each arena after 24 h. Data represent 15 arenas for each plant species.

| Location in Arena | Plant Species | | | | | |
|---|---|---|---|---|---|---|
| | *A. absinthium* | | *C. nauseosus* | | *T. vulgare* | |
| | $\bar{x}$ | S.D. | $\bar{x}$ | S.D. | $\bar{x}$ | S.D. |
| Treated Core | 2.07a | 1.03 | 2.33a | 1.05 | 1.93a | 1.10 |
| Control Core | 4.67b | 0.90 | 5.07b | 0.96 | 5.40b | 1.12 |
| Wandering | 3.27a,b | 1.28 | 2.60a | 1.24 | 2.67a | 1.05 |

Means in the same column followed by the same letter are not significantly different ($P<0.001$).

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**KEY WORDS**

Codling moth, neonate, plant extracts, feeding deterrents, *Artemisia*, *Chrysothamnus*, *Tanacetum*

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