Pestycydy/Pesticides, 2009, (1-4), 51-56.
ISSN 0208-8703

Preventing fruit infestation by codling moth neonates with *Artemisia* extracts

Kevin DURDEN, Samantha SELLARS and Maciej A. PSZCZOLKOWSKI*

Department of Agriculture, Missouri State University,
9740 Red Spring Road, Mountain Grove, MO 65711, USA
**e-mail: MPszczolkowski@missouristate.edu*

Abstract: In preliminary experiments, we show that extracts from *Artemisia absinthium*, and *Artemisia arborescens x absinthium* "Powis Castle" have deterrent properties against neonate larvae of codling moth, *Cydia pomonella*. By partitioning crude extracts and comparative High Performance Thin Layer Chromatography we suggest candidate feeding deterrents for further analysis.

Keywords: *Cydia pomonella*, apple, common wormwood

INTRODUCTION

The codling moth *Cydia pomonella* (CM) is a major pest of apple, and if not controlled may eliminate up to 80% of the crop, which translates to $ 40 billion, worldwide, annually. Current control practices include sprays with azinphos-methyl or other insecticides, "attract and kill", and mating disruption. Azinphos-methyl has already been banned in the European Union and will be banned in the USA by 2012. Other insecticides (spinetoram, neonicotinoids) are at initial stages of implementation, mating disruption is expensive and its efficacy is limited by immigration of gravid females from adjacent areas [1], "attract and kill" is ineffective in insecticide resistant populations [2]. The grower has limited options of this insect control.

Neonatal behavior of codling moth suggests alternative control strategies. Because female codling moths oviposit on foliage of apple trees [3], neonates must search for fruit to burrow into [4]. Manipulation with neonate searching behavior may provide an alternative approach to current strategies for codling moth management [5]. Repelling or deterring the neonates from fruit may be one of such strategies.

Plant-derived chemicals that have insect-deterrent properties have received attention from scientists [6]. In particular, Hwang et al. [7] found that wormwood, *Artemisia vulgaris* contains chemicals that repel mosquitoes. Suomi et al. [8] found that *Artemisia absynthium* extracts, at 10 mg/ml, prevent fruit infestation by CM neonates. However, the active ingredient was not suggested nor identified.

Here, we confirm that extracts from *A. absinthium* have deterrent properties against CM neonates, and in a dose-dependent manner. We also found one more a member of the genus *Artemisia* that has insect deterrent properties and three have no deterrent or repellent properties against CM neonates. By partitioning crude extracts and comparative High Performance Thin Layer Chromatography (HPTLC) we suggest candidate CM feeding deterrents for further analysis.

MATERIALS AND METHODS

Plants and Crude Plant Extracts: *Artemisia absinthium, Artemisia vulgaris*, *Artemisia californica, Artemisia arborescens x absinthium* "Powis Castle", and *Artemisia ludoviciana* "Silver King" were tested. *A. absinthium* was purchased from London Apothecary, Mansfield MO, USA, as a dried powder. Remaining *Artemisia* plants were harvested from the rain garden at Missouri State University Fruit Experiment Station, Mountain Grove, MO, in August, 2008. Foliage was dried, powdered and extracted with denaturated alcohol. Extracts were transferred into pre-weighed tubes, dried in a SpeedVac rotary evaporator and re-suspended to the desired concentration (ranging from 0.3 to 10 mg/ml).

Partitioning: *A. absinthium* extract was separated into hexane and alcohol soluble fractions. In a centrifuge tube, 400 µl of crude alcoholic extract was mixed with 100 µl of water, and vortexed briefly. Next, 500µl of hexane was added, and the tube vortexed for 30 seconds. The mixture was allowed to rest 10 minutes at room temperature, and then centrifuged for 10 minutes at 2000 G. The hexane fraction (upper portion) was removed and placed into a pre-weighed centrifuge tube. The alcohol fraction (lower) was placed into another pre-weighed centrifuge tube. The samples were dried and reconstituted to the concentration of 10 mg/ml in dehydration alcohol.

Insects and Bioassay: Codling moth pupae were obtained from YARL Wapato, WA and kept in 16:8 L:D photoperiodic regime. The extracts were tested as described elsewhere [9]. Deterrence index was calculated according to Jones [10] by dividing the number of CM neonates feeding in apple plugs treated with *Artemisia* extracts by the number of CM neonates feeding in control plugs, treated with dehydration alcohol only, subtracting this figure from 1, and multiplying the result by 100.

HPTLC: Extracts were applied onto Merck 10 x 10 cm silica gel 60 F_{254} glass plates with Camag's Nanomat 4 HPTLC plate spotter. Plates were developed for 6 minutes in Camag's Horizontal Development Chamber using a mixture of toluene, ethyl acetate and acetic acid (8:2:0.2, *V/V*) a protocol supplied by Camag. Plates were viewed in Camag's UV viewing chamber at UV_{366} and digitally photographed using a Nikon D70 camera, with a Micro Nikkor 60 mm lens.

Image capturing and Processing: Images were captured with Nikon Capture Control Software, converted to JPEG, cropped to size, reduced to 100 dpi using Adobe Photoshop and converted to TIFF for measuring retardation factor (R_f) with NIH Scion Image.

Statistics: Exact Fisher's test ($\alpha = 0.05$) was used in all assays to test the null hypothesis that neonates do not discriminate between plugs or apples treated with plant extract and those treated with alcohol (i.e., 50% of the neonates choose treated plugs or apples and 50% of the neonates choose control plugs or apples).

RESULTS AND DISCUSSION

Our assay shows that *A. absynthium* has dose-dependent deterrent effects on CM neonate feeding (Table 1). This result corroborates earlier work by Suomi et al, [8]. At 10 mg/ml concentration, extracts from *A. arborescens x absinthium* deterred CM neonates as well (Table 2). *A. vulgaris*, *A. californica* and *A. ludoviciana* "Silver King" do not deter feeding (Table 2).

Table 1. Dose-dependent deterrent effects of crude *A. absinthium* extract

Concentration mg/ml	**Number of neonates tested**	**Number of neonates feeding on apple**	
		Treated with extract	*Treated with solvent*
0.3	20	10	10
1	28	14	14
3	29	9	20
10	36	5***	31

***P<0.001, Fisher exact test.

Table 2. Deterrent effects of crude extracts obtained from five members of *Artemisia* family. Each extract was tested at 10 mg/ml concentration

Species of *Artemisia*	**Number of neonates tested**	**Number of neonates feeding on apple**	
		Treated with extract	*Treated with solvent*
A. absinthium	36	5***	31
A. vulgaris	24	12	12
A. californica	18	7	11
A. arborescens x absinthium	16	1*	15
A. ludoviciana	15	7	8

***P < 0.001, *P < 0.05 in Fisher exact test

Chromatography showed four distinct bands in deterrent A. *absynthium* crude extract (Figure 1A). These bands were also present in another deterrent extract, one from *A. arborescens x absinthium* (Figure 1A). A distinct band placed at R_f 0.5 was absent in non-deterrent crude extracts from *A. vulgaris*, *A. californica* and *A. ludoviciana*, (Figure 1A), and initially was thought to contain a candidate for CM feeding deterrent.

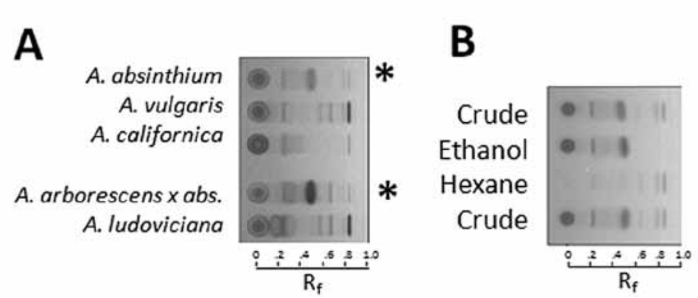

Figure 1. A. Comparative chromatogram of crude extracts (10 mg/ml) from five members of *Artemisia* family. Extracts that prevent fruit infestation by codling moth neonates are marked with asterisks. Remaining extracts had no deterrent properties. B. Comparative chromatogram of crude *A. absinthium* extracts, ethanol fraction, and hexane fraction after partitioning.

However, the results of our experiments where *A. absynthium* crude extract was partitioned to hexane and ethanol, and partitioned fractions were further bioassayed for deterrence and subjected to HPTLC, do not support this initial hypothesis. Hexane fraction contained the chemical corresponding to bands with $R_f > 0.6$ (Figure 1B) and deterred 5 out of 38 neonates from entering the fruit ($P < 0.001$, $N = 33$, Fisher exact test). Ethanol fraction contained chemicals represented by a wide band with R_f 0.5. Statistical analysis indicates that this fraction was not deterrent ($P = 0.082$, $N = 30$, Fisher exact test).

Based on our current report we hypothesize that insect deterrent compounds contained in *A. absinthium* crude alcoholic extract are non-polar, dissoluble in hexane, and locate in upper parts of our chromatograms. We plan to continue research on this putative insect deterrent by subjecting the hexane fraction to more sophisticated analytical procedures that would allow identifying *A. absinthium* insect deterrents. Such compounds, which probably are secondary metabolites of this plant, could be perhaps manufactured commercially and become an alternative to currently used sprays against CM neonates. Identification of such a polar substance could also open an avenue to genetically modifying the apple so this deterrent could be expressed in wax layer of the fruit, making it naturally repulsive to codling moth. (Wax layer, together with insecticide residues, is removed from commercial apples, and the fruit is re-waxed with paraffin and shellac prior to reaching the consumer, thus *Artemisia* deterrent could be removed too prior to fruit consumption).

Acknowledgements

We would like to thank Ms. Pauline Anderson and Mr. Jim Harris (YARL, Wapato, WA, USA) for their technical assistance. A part of this research was supported by MSU Graduate Assistantship.

REFERENCES

[1] Carter N., Mating Disruption for Management of Insect Pests, Ontario, Ministry of Agriculture Food & Rural Affairs, 2003; http://www.omafra.gov.on.ca/english/crops/facts/03-079.htm

[2] Poullot D., Beslay D., Bouvier J-C., Sauphanor B., Pest Manag. Sci., 2001, 57, 729-736.

[3] Jackson, M.D., Ann. Entomol. Soc. Am., 1979, 72, 361-368.

[4] Jackson, M.D., *ibid.*, 1982, 75, 284-289.

[5] Pszczolkowski M.A., Ann. Warsaw Univ. of Life Sc.-SGGW, Horicult. and Landsc. Architect., 2007, 28, 7-18.

[6] Jacobson M., Glossary of plant derived insect deterrents, CRC Press, Boca Raton, Florida 1990.

[7] Hwang Y-S., Wu K-H., Kumamoto J., Axelrod H., Mulla M.S., J. Chem. Ecol.,

1985, 11, 1297-1306.
[8] Suomi, D., Brown J.J., Akre R.D., J. Entomol. Soc. Brit. Columbia, 1986, 83, 12-18.
[9] Durden K., Brown J.J., Pszczolkowski, M.A., *ibid.*, 2008, 105, 83-88.
[10] Jones G., J. Exp. Biol., 1952, 29, 372-86.