PROJECT NO.: ARS Final report

TITLE: Identification of Plant and Insect-Derived Odors Perceived by Pear Psylla Adults

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ACCOMPLISHMENTS:
The research conducted in this project fell primarily into one of two categories. First, we screened a number of synthetic and organic compounds for antifeedant or oviposition-deterrent properties against pear psylla. We tested compounds for effects on winterform feeding, post-diapause development, and oviposition (both morphs). A number of compounds caused large reductions in feeding and oviposition.

Second, we developed two bioassays (electroantennogram; pitfall trap) to test whether pear psylla adults perceive volatile compounds. The results are highly preliminary, but suggest that olfaction potentially mediates some interactions, either among insects (e.g., during mate-finding) or between host and insect (i.e., host-finding). The electroantennograph, in particular, looks to be a promising tool.

RESULTS:
Feeding assays. Several experiments were done to investigate the effect of various compounds on psylla feeding and ovarian development. In the first experiment, three treatments were applied to pear shoots: water, superior oil (1.5 %), or safer soap. Female psylla were collected from the field in January, 1995, and placed in arenas (3/shoot), with each arena having a single treated shoot. Psylla were allowed to feed for 4 days; after 4 days, honeydew pellets were counted. Psylla were then dissected to determine ovarian development. Significantly fewer pellets were found in arenas containing oil- and safer-treated shoots than in control arenas (Table 1). Ovarian development was significantly reduced on oil-treated shoots, although magnitude of the difference was fairly small (Table 1).

A second experiment tested the following compounds for effects on summerform feeding: "Garlic Barrier" (10% solution), Safer soap (2% solution), fenoxycarb (0.01 % solution), Neem extract (14 % solution), superior oil (0.25 % solution), sugar ester of Nicotiana gossei (0.15% solution), three Orchex oils (1.5% solution), and water extracts of several botanicals. Compounds that significantly reduced psylla feeding included the three orchex oils (Table 2) and water extracts of bitterbrush, Russian thistle, asparagus, pigweed, carrot tops, mint, and thinning apple (Table 4).

Oviposition assays. Summerform females were put into arenas (3/arena) that contained a pear seedling trimmed so that only two leaves remained. Two types of tests were conducted, choice and no-choice. In the choice tests, one of the two leaves was sprayed with water and the other was sprayed with one of the following: Orchex 796E (1.5% solution), "garlic barrier" (10% solution), safer soap (2% solution), fenoxycarb (0.01 % solution), Neem extract (14 % solution), superior oil (0.25 % solution), and water extract of several botanicals. Psylla were left in arenas for 48 hours. After 48 hours eggs on each leaf were counted. Oviposition was significantly reduced on leaves treated with several of the extracts (Tables 3 and 5).

No-choice tests were done to determine oviposition rates of summerform and winterform females on extract-treated tissue. For tests with summerforms, pear seedlings were trimmed as described above and the entire plant was treated with the extract. Three psylla were placed on each seedling, and eggs were counted after 48 hours. Winterforms were placed on pear shoots (3 psylla per shoot) that had been treated with safer soap, superior oil (1.5 %), or water. Eggs were counted after 4 days. Egglaying by summerforms on "garlic barrier"-treated seedlings (Table 2), Orchex oil-treated seedlings (Table 2), and thinning apple-treated seedlings (Table 4) was significantly less than egglaying on control seedlings. Although only a few eggs were deposited (due to psylla age), oviposition by winterform females was also significantly reduced on oil-treated pear shoots in no-choice tests (Table 1).

Field tests. Summerform females were confined in the field on foliage that had been sprayed either with water or with extract from thinning apple. After 5 days, branches were removed and eggs were counted. On the control foliage, 25.4 eggs were laid per female while 17.3 eggs per female were deposited on extract-treated foliage. In addition, no psylla mortality was observed in the control, whereas almost 11% of psylla died in the thinning apple treatment.

Summary: feeding and oviposition assays
We demonstrated that extracts from nonhost species applied to the surface of pear foliage caused reduced feeding and egglaying by both morphotypes of pear psylla. Results also indicate that a reduction in feeding (as estimated by honeydew production) is accompanied by a delay in ovarian development by winterform psylla. This result suggests that antifeedant compounds, if they are effective in the field, may also delay the onset of egg-laying by overwintered psylla. Results indicate that we may also be able to reduce egg-laying rates in the field through the application of selected compounds (e.g., thinning apple extracts).

Electroantennogram assay. We successfully attached pear psylla antennae to an electroantennograph, and challenged the antennae with odors from finely chopped pear leaves, solvent-extracted

males and females, and with several other readily availible chemical compounds. Although results were variable and sample sizes were small, a significant response of antennae to some odors (e.g., male antennal response to female extract) suggests that antennae detect volatiles (Table 6).

Pitfall assays. Extracts of pear tissue (seedlings: SF, shoots: WF) were applied to red rubber septa and placed in arenas containing psylla and an identical untreated septa. After several minutes, the number of psylla on each septa was observed. Septa treated with tissue extract always had more psylla on them than untreated septa. It was not clear, however, if the response was due to volatile materials (i.e., an olfactory response), or was due to chemicals present on the surface of septa (i.e., a taste response). A pit-fall olfactometer was designed to discriminate between these two explanations. The olfactometer consisted of a petri dish with two holes drilled through the bottom. A vial was attached at each hole. An odor source was then placed in the bottom of one vial; psylla responding to the source fell into the vial. We tested several combinations of pear leaves and psylla as odor sources, and also tested solvent extracts of these sources. Although more psylla responded to test odors than the control in some of the tests (Table 7), data were too variable and sample sizes too small to make any definite conclusions. However, we believe that the assay techniques could prove to be useful, should this type of research continue.

Summary: electroantennograph and pitfall assays. The data gathered so far are hardly conclusive, but do suggest that pear psylla perceives volatile compounds. Additionally, many of the initial difficulties in developing a useful bioassay have now been overcome, and the electroantennogram bioassay (in particular) looks very promising for pursuing questions about psylla olfactory capabilities. We suggest that this research area merits additional attention.

Table 1. 24-hr no-choice tests of egglaying and production of honeydew pellets by winterform pear psylla females in cages containing pear shoots treated with various compounds.

Treatment	n	Mean[a] (per female)		Ovarian Score	
		Eggs	Pellets	Mean[a]	Range
Control	30	0.8a	2.0a	6.5a	4.8 - 7.0
M-Pede	30	0.4a	1.5 b	6.3 b	5.3 - 7.0
Supreme Oil	30	0.1 b	1.1 c	6.2 c	4.8 - 7.0

[a] Means followed by the same letter are statistically similar ($P < 0.05$; Least Significant Difference Test).

Table 2. 24-hr no-choice tests of oviposition and production of honeydew pellets by summerform pear psylla females in cages containing pear seedlings treated with water or test compounds.

Treatment	n	Mean[a]	
		Eggs/Female	Pellets/Female
Experiment 1			
Control (water)	15	12.9a	1.7ab
Fenoxycarb	15	12.8a	0.9 bc
Supreme Oil	15	12.8a	2.4a
Acyl Ester	15	10.5a	1.5ab
M-Pede	15	9.9a	1.5ab
Neem	15	9.7a	0.1 bc
Garlic Barrier	15	3.8 b	1.2 bc
Orchex 796E	15	1.8 b	0.6 c
Experiment 2			
Control (water)	10	7.6a	5.4a
Orchex 796E	10	0.2 b	1.0 b
Orchex 692	10	0.6 b	0.5 b
Orchex WS2928	10	0.4 b	0.9 b

[a] Means followed by the same letter are statistically similar ($P < 0.05$; Least Significant Difference Test).

Table 3. 24 hr egglaying by summer- and winterform pear psylla females allowed a choice between water-treated leaves (column A) and leaves treated with various test compounds (column B).

Comparison			Eggs/Female		
A	B	n	A	B	P[a]
SUMMERFORM					
Water	Garlic Barrier	10	4.8	2.1	0.0008
Water	Neem	10	1.6	0.9	0.21
Water	Supreme Oil	10	1.6	0.2	0.02
Water	M-Pede	10	5.3	0.1	0.002
Water	Acyl Ester	10	1.6	2.4	0.33
Water	Fenoxycarb	10	2.8	1.8	0.32
Water	Orchex 796E	10	2.1	0.0	0.046
WINTERFORM					
Water	Supreme Oil	12	2.9	0.3	0.02
Water	M-Pede	12	4.6	0.9	0.001

[a] P-statistic from paired sample t-test.

Table 4. 24-hr no-choice tests of oviposition and production of honeydew pellets by summerform pear psylla females in cages containing pear foliage treated with extracts of various local plant species.

			Mean[a]	
Assay	Treatment	n	Eggs/Female	Pellets/Female
1	Water	10	4.4 bc	4.2ab
	Bitterbrush	10	10.1a	1.6 c
	Cedar	10	5.4abc	5.0a
	Kochia	10	2.3 c	2.4 bc
	Mallow	10	8.3ab	3.5abc
2	Water	10	2.5 bc	6.3a
	Russian Thistle	10	1.8 bcd	2.6 bc
	Bindweed	10	6.0a	3.8ab
	Asparagus	10	3.1ab	2.8 bc
	Pigweed	10	0.7 cd	2.4 c
	Thinning Apple	10	0.2 d	2.0 c

Table 4. Continued.

Assay	Treatment	n	Mean[a] Eggs/Female	Mean[a] Pellets/Female
3	Water	10	10.6a	2.8a
	Alfalfa	10	10.1a	3.2a
	Nightshade	10	14.0a	3.1a
	Potato	10	6.4a	2.7a
	Sagebrush	10	19.6a	1.2a
4	Water	10	13.9a	12.6a
	Carrot top	10	13.7a	6.6 b
	Mint	10	12.3a	3.1 b
5	Water	15	52.2a	12.4a
	Thinning Apple	15	11.0 b	2.8 b

[a] Means followed by the same letter are statistically similar ($P < 0.05$; Least Significant Difference Test).

Table 5. Oviposition by summerform pear psylla females allowed a choice between water-treated leaves (column B) and leaves treated with test compounds (column A).

Comparison A	Comparison B	n	Eggs/Female A	Eggs/Female B	p[a]
Bitterbrush	Water	10	7.1	5.4	0.48
Cedar	Water	10	4.4	9.6	0.10
Buffalo Gourd	Water	10	3.9	8.3	0.02
Hop Cone	Water	10	1.9	8.2	0.03
Hop Leaves	Water	10	5.4	6.6	0.55
Kochia	Water	10	2.1	9.5	0.007
Mallow	Water	10	2.3	6.2	0.04
Asparagus	Water	10	2.9	7.6	0.098
Bindweed	Water	10	8.3	7.3	0.77
Pigweed	Water	10	4.9	5.6	0.76
Russian Thistle	Water	10	3.1	8.7	0.01
Thinning Apple	Water	25	2.0	12.0	0.0001
Alfalfa	Water	10	1.0	1.2	0.70

Table 5. Continued.

Comparison			Eggs/Female		
A	B	n	A	B	p[a]
Carrot Top	Water	10	1.9	7.2	0.06
Mint	Water	10	2.0	1.1	0.46
Nightshade	Water	10	1.1	1.2	0.66
Mustard	Water	10	1.0	1.3	0.97
Potato	Water	10	1.6	2.4	0.57
Sagebrush	Water	10	0.8	2.1	0.24

[a] P-statistic from paired sample t-test.

Table 6. Electroantennogram response of pear psylla to various odors.

Morph/Sex	Treatment	Antennal Response (mV)	n	p[a]
SF Males	Live Females	0.015	8	*
SF Males	SF Female Ex. (0.1 FE)	0.033	13	**
SF Males	SF Male Ex. (0.1 ME)	0.006	2	NS
SF Males	12:OH	0.022	8	*
SF Males	14:OH	0.018	13	**
SF Males	15:OH	0.032	17	**
SF Males	16:OH	0.024	13	**
SF Males	alpha farnesene	0.032	10	*
SF Males	Chopped Leaves	0.000	2	NS
SF Males	Seedling Extract	0.042	2	NS
SF Females	SF Female Ex. (0.1 FE)	0.024	4	NS
SF Females	SF Male Ex. (0.1 ME)	0.009	6	NS
SF Females	12:OH	0.011	6	NS
SF Females	14:OH	0.007	6	NS
SF Females	15:OH	0.006	6	NS
SF Females	16:OH	0.007	6	NS
SF Females	alpha farnesene	0.009	9	NS
SF Females	Chopped Leaves	0.006	2	NS
SF Females	Seedling Extract	0.007	4	NS
WF Males	WF Female Ex. (0.1 FE)	0.013	7	*
WF Males	alpha farnesene	0.006	2	NS

[a] Significant response (mV > 0) indicated by * ($P < 0.05$) and ** ($P < 0.01$).

Table 7. Response of pear psylla adults to various odors sources in a pitfall bioassay.

Comparison		Test Insect			% Response	
A	B	Morph	Sex	N	A	B
Seedling Extract	Control	SF	Mixed	1	67	33
Seedling + SF females	Control	SF	Mixed	1	86	14
Seedling + WF females	Control	WF	Male	1	71	29
10 WF females	Control	WF	Male	8	54	46
Pear Shoot	Apple	SF	Male	4	75	25
Pear Shoot	Apple	SF	Female	6	57	43
Pear Shoot	Control	WF	Male	8	56	44
Shoot + 10 WF females	Control	WF	Male	8	38	62
Bud Extract	Control	WF	Female	19	42	58
10 WF Males	Control	WF	Female	5	60	40
10 WF Females	Control	WF	Male	3	100	0
10 WF Females + Shoot	Control	WF	Male	5	20	80
10 WF Males + Shoot	Control	WF	Female	5	20	80
5 WF Males + 5 WF Fem.	Control	WF	Male	5	40	60
Alpha-farnesene	Control	WF	Mixed	19	42	58

PUBLICATIONS

Horton, D.R., T.M. Lewis & T.J. Weissling. 1995. Reduction in feeding by diapausing and postdiapause pear psylla (Homoptera: Psyllidae) caused by extract from buffalo gourd. J. Entomol. Soc. Brit. Columbia (in press).

Weissling, T.J., T.M. Lewis, L.M. McDonough & D.R. Horton. Reduction in pear psylla (Homoptera: Psyllidae) oviposition and feeding by application of selected compounds. Can. Entomol. (at the journal).