BREEDING RESISTANCE IN *APIUM GRAVEOLENS* TO *LIRIOMYZA TRIFOLII*: ANTIBIOSIS AND LINEAR FURANOCOUMARIN CONTENT

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Abstract

Apium graveolens L. (celery, var 'Conquistador'), *A. chilense*, 23 backcross accessions of *A. graveolens* x *A. chilense*, and two relatives were ewamined for resistance to a major pest of vegetable crops, *Liriomyza trifolii* (Burgess). All accessions were tested using a no-choice design in order to maximize feeding and oviposition. Resistance was evaluated by recording numbers of feeding/oviposition punctures, mines, pupae, and adults. Of these accessions, several appeared to have good-excellent leafminer resistance. Compared to all plants except the wild relatives, these plants had fewer feeding punctures and allowed significantly less survival during development. In order to document if the observed resistance was caused by the carcinogenic and mutagenic linear furanocoumarins, the amounts of psoralen, bergapten and xanthotoxin present in both leaves and petioles of each accession were quantified using high performance liquid chromatography. Results indicated that *L. trifolii* resistance was not due to these undesirable compounds, but some of the accessions were eliminated from further immediate consideration by unacceptably high concentrations. The implications for use in a breeding program for *A. graveolens* are discussed.

1. Introduction

Liriomyza species, including *L. trifolii*, are significant pests of celery and other vegetables throughout the world (Minkenberg and van Lenteren 1986; Trumble and Hare 1997). This species causes damage in several ways. First, the feeding/oviposition punctures and the mining reduce photosynthate production, resulting in a stunting of the plants (Trumble *et al.*, 1985). In celery this increases time to harvest, causing additional costs for pest and disease control, irrigation, land leases, etc. Second, at high population levels, larvae mine the petioles, which then are unmarketable (Trumble and Hare 1997). Finally, the insects can become contaminants (Trumble *et al.*, 1997). The larvae exit the mines prior to pupating, and physically attach themselves within spaces between petioles: many of these are not dislodged even if the plants are cooled with a cold water treatment following field packing.

When pesticides loose effectiveness, which happens rapidly with *L. trifolii* (Sanderson *et al.*, 1989), economic losses can be substantial. California's celery industry reportedly lost U.S. $20 million in a single year when no effective chemicals were commercially available (Anon. 1985). Parasites can provide an economically viable level of suppression for *L. trifolii*, but parasite populations are often disrupted by pesticide treatments for other celery pests (Trumble *et al.*, 1994). Thus, an alternative means of control such as plant-based resistance is desirable.

Breeding resistance to insects in *A. graveolens* is complicated by the tendency to select for plants with elevated concentrations of the phototoxic linear furanocoumarins (Diawara *et al.*, Diawara *et al.*, 1996; Trumble *et al.*, 1990). These compounds probably

evolved as protection against a several physical and biological stresses, including UV light, insects, and pathogens (Berenbaum 1981; Trumble and Millar 1997). In *A. graveolens*, these chemicals vary with location within the plant as well as by season (Diawara *et al.*, 1994). Unfortunately, the linear furanocoumarins are known to cross link DNA strands, leading to skin cancer, developmental mutations in insects (presumably humans as well), and a variety of other oncogenic, teratogenic and carcinogenic responses (Diawara and Trumble 1997). Responses are exacerbated by the presence of UV light (hence the term 'phototoxic'). In humans, a common response to either physical contact or ingestion is known as contact dermatitis, characterized by blistering skin lesions (Berkley *et al.*, 1986). The critical concentrations for an acute exposure dermatitis are approximately 18 µg/g fresh weight (Austad & Kavli 1983). For the chronic exposure dermatitis typically observed for celery harvesters or grocery store employees, as little as 9 µg/g fresh weight are required (Seligman *et al.*, 1987).

The research reported here had three primary objectives. The first objective was to determine if any of our available accessions of *Apium* species or their crosses exhibited resistance to *L. trifolii.* A second objective was to determine if any putative resistance observed was based on the mutagenic and carcinogenic linear furanocoumarins. Finally, we wanted document potential relationships between the various forms of linear furanocumarins. Specifically, did concentrations of one compound influence concentrations of another? In addition, were concentrations in the unmarketed leaves correlated with levels in the marketed petioles?

2. Materials and methods

2.1. Insects

Adult *L. trifolii* used in these trials were obtained from a celery field in Orange Co., Cal., and maintained in a laboratory colony reared *Phaseolus lunatus* (lima beans, *var* 'Henderson bush'). All *L. trifolii* adults used in this study were 3 d old, and had been fed only a 20% honey solution before exposure to the test plants. A plant species other *than A. graveolens* was chosen because Tavormina (1982) and Via (1984 a, b) demonstrated that a closely related species of *Liriomyza* developed a preference for the host species from which they had been reared.

2.2. Bioassays

All plants were standardized for bioassays by age and leaf area (after and Quiros 1988). *A. graveolens* L. (var. 'Conquistador'*)*, the related species *A. chilense* (A073), *A. nodiflorum* (A289) and *A. prostratum* (A230), as well as an F1 hybrid between celery 'T.U. 52-75' and *A. prostratum* and 23 backcross lines obtained by crossing the F1 hybrid back to the celery parent were examined for putative resistance to *L. trifolii. Apium prostratum* crosses were chosen as an earlier study suggested that this species contained leafminer resistance that was not always based in linear furanocoumarins (Trumble and Quiros 1988). The relatives *A. chilense* and *A. nodiflorum* were included as addition negative control plants because of strong resistance and high linear furanocoumarin contents observed previously (Trumble *et al.*, 1994). Within each accession and line, all plants were cloned to reduce variability. Plants were placed individually in 11.4 liter cylindrical containers and exposed to two pairs of adult *L. trifolii* for 2 h. Three replicates were evaluated, with each consisting of one plant from each of 27 accessions.

Because of 1) the large number of plants, 2) the labor involved in the assays, and 3) a desire to minimize type 1 errors on post hoc tests (Sokal and Rohlf, 1969), plants were split into four trials of 7 or 8 each. For comparison purposes, each trial included a control accession (var 'Conquistador'). Plants were held in an environmental chamber set for a photoperiod of 16:8 (L:D) and 26.7 ± 1 °C. After 3 d, feeding/oviposition punctures per plant were counted (as a measure of general attractiveness). The numbers of mines per plant were counted after 4-5 d (as a measure of attractiveness for oviposition). Plants then

were tilted on their sides so that larvae emerging from the leaves would drop into a tray filled with sand. Numbers of pupae were recorded daily (as a measure of suitability for larval development) until no live larvae remained in the foliage. Pupae from each plant were held for at least 2 weeks, at which time the numbers of adults per plant were recorded (as a measure of suitability for complete development). In addition, the ratio of pupae/mines (as a measure of mortality in the larval stage) and the ratio of adults/pupae (as a measure of mortality in the pupal stage) were calculated.

2.3. Linear furanocoumarin analyses

Two clones of each accession were split into leaf and petiole samples and prepared for analysis. The linear furanocoumarin contents were analyzed using an HPLC with a reverse-phase column as described in Diawara *et al.*, (1994) and Reitz *et al.*, (1997). Briefly, sample tubes were spiked with 5 µg of a synthetic internal standard, 7-benzyloxycoumarin (synthesized from commercially available 7-hydroxycoumarin (Aldrich Chemical, Milwaukee, Wi.). Plant samples were homogenized in d H_2O, extracted with toluene and the crude extract was partially purified by passage through a Extract Clean^R solid phase extraction cartridge tube (Alltech Assoc., Inc., Deerfield Ill., USA) and eluted with acetone in chloroform (95:5). The purified extracts were concentrated to dryness, then reconstitued in 250 µl of hexane. HPLC analyses were carried out with a Hewlett-Packard 1040 HPLC pump and an H.-P. 1050A diode array detector with a Chemstation data system (Hewlett-Packard, Avondale, Penn., USA). Peaks were monitored and quantified at 280 nm. An Alltech Econosil silica column (25 cm x 4.6 mm, 5µ particle size) with a 10 mm x 4.6 mm guard column filled with the same packing material were used, eluted isocratically with hexane:tetrahydrofuran (81:19).Specifically, we measured the µg/g fresh weight of psoralen, bergapten and xanthotoxin, the three most common furanocoumarins in celery (Diawara *et al.*, 1993; 1995). Specifically, we measured the µg/g fresh weight of psoralen, bergapten and xanthotoxin, the three most common furanocoumarins in celery (Diawara *et al.*, 1993; Diawara *et al.*, 1995).

2.4. Statistical analyses

Fisher's Protected Least Significant Difference Test was used to compare the various measures of potential resistance (Super ANOVA, Berkeley, CA). If necessary, data were normalized with an arcsine (square root) transformation prior to analysis. Data were then transformed back for presentation. To determine if linear furanocoumarin concentrations in the leaves were predictive of concentrations in the petioles, regressions (with furanocoumarin levels in leaves as the independent variable) were conducted using StatView 4.01 (Abacus Concepts, Berkeley, CA).

3. Results and discussion

3.1. Bioassays

Trials numbered 1, 2, and 4 had control plants (*A. graveolens* var 'Conquistador') with similar numbers of punctures, but control plants in test number 3 had nearly twice as many punctures (Table 1). This can occur if the environmental conditions, particularly temperature, change slightly during plant exposure (Trumble and Quiros 1988). Because the exposures were conducted on laboratory benches, some fluctuation was expected. Nonetheless, the relative number of punctures between the control plants and *A. chilense* or *A. prostratum* was consistent, allowing valid comparisons within and between trials.

In trial 1, no lines showed substantial promise for *L. trifolii* antixenosis and/or antibiosis (Table 1). As compared to the controls, none of the lines had significantly fewer numbers of mines, pupae, or adults. One line, 91A498-11, supported more mines per plant than the controls. In addition, no significant differences in larval mortality (measured as ratio of pupae per mine) or pupal mortality (measured as adults per pupae)

were observed.

In trial 2, only line 91A498-25 appeared promising. Although this line did not have significantly fewer punctures or mines per plant compared to the commercial control plants, all larvae feeding on 91A498-25 died prior to pupation (Table 1). This would obviously limit the pest population expansion if the variety were planted over large areas.

In trial 3, the F1 hybrid 89A775-8, and lines 91A498-3, 10, and 13 had *L. trifolii* antixenosis and/or antibiosis responses (Table 1). While only 91A498-3 had significantly fewer punctures, all of these accessions had significantly reduced numbers of mines, pupae, and adults as compared to the commercial control. In particular, 89A775-8 and 91A498-10 did not allow any insects to reach the adult stage. In both of these latter accessions, most of the leafminers died in the larval stage, thus reducing overall damage and preventing larvae from creating exit holes that can allow pathogen entry.

Trial 4 contained only a single line which could have resistance potential against *L. trifolii*. The ratios of 'pupae per mine' and 'adults per pupa' were significantly lower for line 91A498-5 than for the controls (Table 1). Over a season, this could lower the population growth of *L. trifolii*, perhaps resulting in fewer pesticide applications.

3.2. Furanocoumarin analyses

The concentrations of linear furanocoumarins in the lines was quite variable (Table 2). Concentrations of all furanocoumarins combined varied from lows of less that 4 μg/g of leaf to over 445 μg/g. With the exception of *A. prostratum*, all accessions and breeding lines had higher total linear furanocoumarins in the leaves than in the petioles. This is important, as celery is marketed as just petioles without leaves in the U.S.A. and many other countries. Regressions examining possible relationships between concentrations in the leaves and concentrations in the petioles found a weakly predictive relationship (Table 3). Thus, while high levels in the leaves could be a potential problem for farmers and harvesters, the petioles for some accessions did not have equivalently high concentrations.

In general, psoralen was the least common of the three furanocoumarins, but accessions 89A775-8 and 91A498-25 had exceptionally high concentrations. The commercial control had more bergapten than xanthotoxin, which was consistent with previous reports (Diawara *et al.*, 1994; Diawara *et al.*, 1995). However, no pattern was evident for relative concentrations of bergapten and xanthotoxin in the backcrosses derived from crosses between *A. prostratum* and *A. graveolens*. In addition, no relationship was observed for bergapten levels in leaves versus petioles (Table 3). There was a weak relationship ($r^2 = 0.44$) between xanthotoxin concentrations in leaves versus petioles. No significant relationship was found between levels of bergapten and xanthotoxin in leaves (Table 3).

Similar regressions were analyzed to determine if bergapten, xanthotoxin or the combination of furanocoumarins affected leafminer feeding, mining, number of pupae per plant, or number of adults per plant. For this portion of the analysis we used only lines in the 91A series, which had similar plant architecture and a known genetic background. We found no significant relationships ($P>0.27$, for all possible combinations). Thus, at the concentrations found in these plants, linear furanocoumarins had no discernable impact on *L. trifolii* survival or host acceptance. Thus, the lines demonstrating resistance did not have that resistance based on linear furanocoumarins, and could be candidates for resistance breeding. However, those accessions with excessively high furanocoumarin content (essentially above concentrations in current commercial celery) would require additional backcrossing to celery and selection for furanocoumarin content reduction and improvement of horticultural traits.

3.3. Plant selection

Commercial celery often contains approximately 30 μg/g of furanocoumarins in the leaves (Trumble and Quiros 1988; Diawara *et al.*, 1995). Given this approximate value for leaves, and a concern that any accessions selected do not exceed the chronic

dermatitis concentration of 9 μg/g in petioles (Seiligman *et al.*, 1987), the primary candidates for immediate consideration were accessions 91A498-3 and 91A498-13. The accession 91A498-10 was somewhat less desirable, having low levels in the petioles but unacceptably higher levels in the leaves. However, any of these the lines must undergo at least three more backcrosses to develop horiculturally acceptable lines, which likely will result in some furanocoumarin reduction.

Two additional caveats apply. First, the plants should be field tested to see if environmental conditions outside the greenhouse induce furanocoumarin production (see Diawara *et al.*, 1994). Second, the mechanism of resistance should be identified. Releasing a variety without knowing the mechanism for resistance assumes that there will be no consequences to the consumer. The consequences of an erroneous assumption could be substantial.

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Tables

1. Plant acceptance and suitability parameters for *Liriomyza trifolii* on selected *Apium* accessions and lines[a].

Trial No.	Accession or line	No. of Punctures	No. of Mines	No. of Pupae	No. of Adults	Pupae/ Mine	Adults/ pupa
1	Control	120.00 bc	19.50 ab	16.00	14.25	0.781	0.917
	91A498-8	50.50 ab	21.00 ab	15.50	12.50	0.752	0.801
	91A498-9	40.50 a	15.20 a	14.50	13.00	0.963	0.865
	91A498-11	146.00 c	41.20 b	25.75	25.75	0.733	0.893
	91A498-12	38.70 a	21.00 ab	19.33	15.00	0.848	0.749
	91A498-15	63.50 ab	27.00 ab	21.00	17.00	0.801	0.824
	91A498-16	53.20 ab	14.00 a	12.50	11.25	0.877	0.878
2	Control	87.00 bcd	33.25 b	25.75 bc	19.25 bc	0.774	0.750
	A. nodiflorum	0.75 a	0.00 a	0.00 a	0.00 a	-	-
	A. prostratum	3.00 a	0.00 a	0.00 a	0.00 a	-	-
	A. chilense	73.00 bc	18.75 ab	15.75 abc	8.50 ab	1.250	0.797
	91A498-18	139.00 de	47.00 b	44.00 c	25.50 c	1.160	0.907
	91A498-27	94.50 cd	23.75 ab	10.50 ab	7.75 bc	0.787	0.754
3	Control	308.50 b	73.50 d	59.50 d	44.00 c	0.814 c	0.737
	A. chilense	85.75 a	36.00 abc	30.50 bc	26.75 bc	0.651 bc	0.659
	89A775-8	173.5 ab	25.5 ab	0.50 a	0.00 c	0.01 a	-
	91A498-3	104.50 a	30.00 ab	17.75 ab	15.50 ab	0.423 bc	0.679
	91A498-7	212.00 ab	59.50 cd	36.25 bcd	29.00 bc	0.606 bc	0.826
	91A498-10	302.50 b	17.25 a	0.00 a	0.00 a	-	-
	91A498-13	253.75 b	48.75 bcd	16.50 ab	13.25 ab	0.327 ab	0.696
	91A498-21	311.25 b	68.28 d	47.75 cd	38.00 c	0.686 c	0.829
4	Control	116.50 bc	26.00 bcd	26.00 bc	21.50 bc	1.000 b	0.829 bc
	A. prostratum	11.75 a	0.00 a	0.00 a	0.00 a	-	-
	91A498-1	139.00 bc	41.67 d	36.33 c	25.67 c	0.865 b	0.651 bc
	91A498-2	137.25 bc	16.75 abc	14.00 abc	11.75 abc	0.981 b	0.888 c
	91A498-5	30.33 ab	5.33 ab	5.00 ab	4.33 ab	0.312 a	0.289 a
	91A498-20	35.50 ab	15.75 abc	15.25 abc	11.50 abc	0.702 b	0.543 ab
	91A498-23	35.67 ab	5.67 ab	4.33 ab	4.00 ab	0.852 b	0.933 c
	91A498-28	211.25 c	35.75 cd	32.00 c	25.00 c	0.916 b	0.791 bc

[a] Plants within columns and within tests followed by different letters are signficantly different at the $P<0.05$ level (Fisher's Protected LSD Test). Control plants were *commercial A. graveolens* var 'Conquistador'.

2. Concentration of linear furanocoumarins in *Apium* accessions and lines[a].

Accession or line	Plant Part	µg/g fresh weight			
		Psoralen	Bergapten	Xanthotoxin	Total
Control	leaf	0.00	2.20	1.10	3.30
	petiole	0.00	2.21	0.75	2.96
A. prostratum	leaf	0.00	37.38	29.42	66.80
	petiole	0.00	47.38	124.90	172.28
89A775-8	leaf	93.23	34.50	42.09	169.82
	petiole	49.40	30.10	42.00	121.50
91A498-1	leaf	0.00	18.08	5.94	24.02
	petiole	0.00	5.61	1.61	7.22
91A498-2	not available				
91A498-3	leaf	0.00	26.61	6.85	33.46
	petiole	0.00	4.64	1.61	6.25
91A498-4	leaf	0.00	39.86	67.90	53.88
	petiole	0.00	18.24	11.16	29.40
91A498-5	leaf	0.00	27.89	39.46	67.35
	petiole	0.00	2.45	4.43	6.88
91A498-7	leaf	0.00	7.89	21.59	29.48
	petiole	0.00	2.41	3.26	5.67
91A498-8	leaf	0.00	10.67	8.85	19.52
	petiole	0.00	1.46	3.15	4.61
91A498-9	leaf	0.00	32.46	2.30	34.76
	petiole	0.00	2.58	1.03	3.61
91A498-10	leaf	0.00	26.70	24.23	50.93
	petiole	0.00	8.66	16.40	25.06
91A498-11	leaf	0.00	62.79	6.96	69.75
	petiole	0.00	2.54	1.46	4.00
91A498-12	leaf	0.00	6.26	0.91	7.17
	petiole	0.00	3.22	0.48	3.70
91A498-13	leaf	0.00	21.67	3.34	25.01
	petiole	0.00	4.19	1.31	5.50
91A498-14	leaf	1.96	12.66	5.98	20.60
	petiole	0.00	0.59	1.10	1.69
91A498-15	leaf	2.61	19.94	56.59	76.53
	petiole	0.00	8.17	3.58	11.75
91A498-16	leaf	0.00	22.07	6.73	28.80
	petiole	0.00	3.90	0.91	4.81
91A498-18	leaf	0.00	27.38	3.48	30.86
	petiole	7.42	7.99	2.40	17.81
91A498-20	leaf	0.00	18.08	26.09	44.17
	petiole	0.00	6.83	8.68	15.51
91A498-21	leaf	31.13	38.89	76.10	146.12
	petiole	3.66	17.96	10.94	32.56
91A498-23	leaf	0.00	7.49	2.50	9.99
	petiole	0.00	3.49	3.24	6.73
91A498-25	leaf	221.40	29.99	195.95	447.34
	petiole	66.84	37.01	68.27	172.12
91A498-27	leaf	2.89	11.11	15.65	29.65
	petiole	1.68	1.82	0.87	4.37
91A498-28	leaf	0.00	12.48	4.75	17.23
	petiole	0.00	5.46	0.67	6.13

[a] Concentrations in µg/g fresh weight.

3. Regression analyses showing relationships between for linear furanocoumarins in various plant parts[a].

Independent Variable	Dependant Variable	Degrees freedom	F-Value	P-Value	Regression Coefficient
bergapten in leaf	bergapten in petiole	1,17		0.101	ns
xantotoxin in leaf	xantotoxin in petiole	1,18		0.001	0.44
bergapten in leaf	xantotoxin in leaf	1,17		0.179	ns
total furanocoumains in leaf	total furanocoumains in petiole	1,18		0.002	0.44

[a]only three accessions contained psoralen, so no analyses were conducted separately for psoralen. However, psoralen concentrations were included in the analysis of 'total furanocoumarins'