The Effects of Umbel Order, Soaking, and Scarification on Germination Inhibiting Substances in *Petroselinum crispum* L. and Other *Apiaceae* Seeds

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Abstract. Seed from six species of the *Apiaceae* and six parsley (*Petroselinum crispum* L.) cultivars with three seed lots of each parsley cultivar were tested for the presence of germination inhibiting substances. Aqueous leachate from seed of all six species inhibited germination of lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.). Leachate from root parsley seeds (*P. crispum tuberosum*) were least inhibitory, while leachate from celery and celeriac (both *Apium graveolens* L.) seeds were most inhibitory. Inhibitory concentrations in leachate varied by seed lot within a cultivar. Aqueous leachate of seeds from the primary umbels caused less inhibition of germination than did leachate from tertiary umbels. Washing parsley seeds in aerated water for 3 hours or more removed some of the germination inhibitory substance as indicated by the germination bioassay. An aqueous extract prepared from seedcoat tissue, removed during mechanical scarification, inhibited radish seed germination; inhibition was proportional to the duration of scarification and the amount of seedcoat tissue extracted. Parsley seeds scarified ≤ 60 minutes germinated at rates comparable to washed seeds, but longer scarification time reduced germination. Washing seeds of *Apiaceae* prior to commercial drying and cleaning may be a practical solution for removal of inhibitors.

Parsley seeding rates for commercial production have increased several-fold in recent years due to unreliable seed germination and poor field stand establishment (Dhillon and Latimer, 1986). Viable seeds that fail to germinate even under favorable environmental conditions may be considered dormant (Copeland and McDonald, 1995). Primary dormancy has been divided into two types, depending on the location of the controlling influence. Exogenous dormancy results from the absence of one of several favorable environmental germination factors such as light, water, or temperature. Endogenous dormancy results from inherent properties of the seed, such as inhibitory substances or immature seed embryos (Copeland and McDonald, 1995). Celery seed exhibit exogenous dormancy, whereas parsley has endogenous dormancy (Biddington and Thomas, 1978).

Chaturvedi and Muralia (1975) reported that seeds of several umbelliferous species germinated poorly (<10%) when soaked in aqueous extracts prepared from similar seeds, suggesting the presence of inhibitory sub-

stances. Kato et al. (1978) found that parsley seed contained coumarin (heraclenol), which inhibited seed germination. They postulated that this inhibitory substance occurred in the seedcoat. A method for removal of the outer parts of the seed was needed. Seed scarification initially was employed to determine the location of the inhibitory substance, as it breaks the impermeable layer of surface cells that form a moisture barrier (Hicks et al., 1989; Singer and Pitman, 1988). Seed scarification can be used with large round seeds having reasonably thin coats without causing damage to the embryo (Amalraj, 1985). However, due to the small size and contour of parsley seed, scarification has not been used commercially to remove seed coverings.

Seed position on the developing parent plant can affect seed germination. Thomas et al. (1979) found reduced germination of celery seed produced on secondary, tertiary, and quaternary umbels compared with those on primary umbels. Gray and Steckel (1982, 1983) reported that germination of carrot (*Daucus carota* L.) seed varied with umbel order; however, the effect of umbel order on parsley seed germination has not been studied.

The objectives of this study were to 1) evaluate the seeds of a number of species of *Apiaceae* for germination inhibitory substances; 2) determine the relationship of cultivar, seed lot, and umbel order to seed germination of parsley; and 3) evaluate the effects of seed leaching and scarification on parsley seed germination.

Evaluation of Apiaceae species for inhibitory substances. Seed samples were acquired from six members of the *Apiaceae*: 'Moss Curled' parsley, 'Scarlet Nantes' carrot, 'Florida 683' celery, 'Gians Prague' celery, 'Hamburg Rooted' root parsley, and 'Harris Model' parsnip. Seed samples were donated by Martin Rispen and Sons, Lansing, Ill., from 1991 stock that had received no chemical seed treatment. All seed were stored at 15 °C and 35% relative humidity (RH) until needed. A 200-g seed sample of each species was placed in a 2-L graduated cylinder containing 1 L of distilled water and vigorously aerated for 24 h at 27 °C using a brass, pyramid-shaped aerator placed at the bottom of the cylinder. This solution was then filtered using Agrel P-17 (spunbound polypropylene). Seeds from each sample were discarded and the leachate diluted to 1 L with distilled water. Thirty-three seeds of each type—six *Apiaceae* species, leaf lettuce (*Lactuca sativa* 'Slobolt'), and radish (*Raphanus sativus* 'Red King')—were equally spaced in individual polystyrene petri dishes (10 cm in diameter) containing a blue germination blotter (Hoffman Manufacturing Co., Albany, Ore.) moistened with 8 mL of leachate or 8 mL of distilled water. Seeds of the six *Apiaceae* species were prewashed (24 h) and air-dried to prevent self-inhibitory activity. Petri dishes were incubated at 27 °C in the dark. Duration of the test depended on time required for maximum germination of each species. Germination was defined as emergence of the radical through the seedcoat (Copeland and McDonald, 1995). After each test, nongerminated seeds were counted and recorded. Treatments were arranged as a randomized complete block with six replications.

Parsley cultivars, seed lot, and umbel order effects on inhibition of seed germination. Three lots of each of six different parsley cultivars were obtained from five seed companies (Alf Christianson, Mt. Vernon, Wash.; Asgrow Seed Co., Kalamazoo, Mich.; Stokes Seed Co., Buffalo, N.Y.; Martin Rispen and Sons; Siegers Seed Co., Grand Rapids, Mich.) Within cultivars, each lot represented a different production year or location. Seed samples had not been chemically treated. To determine the germination of each lot, seeds were tested for germination using the standards described in the rules for testing seed (Association of Official Seed Analysts, 1987). All seeds were then stored at 18 °C and 35% RH until needed.

The standard aerated water leachate was prepared from each of these lots in the same manner as in the first study. This extract was then diluted with distilled water to 25%, 50%, and 75% (by volume) and used for subsequent germination tests. The inhibitory effects of each seed leachate concentration were tested using the radish germination bioassay and procedures previously described.

Seed from selected umbels were obtained from Alf Christianson Seed Co. Prior to commercial harvesting, three field samples of the cultivar Garland were hand-harvested from the following umbel positions: primary, sec-

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ondary, and tertiary. In addition, a sample of combined umbel positions was taken to provide a replica of a commercial harvest. Samples from each field are referred to as field 1, field 2, and field 3. These samples were cleaned and then shipped. Upon receipt, the same standard germination test was conducted to determine the germination of each sample, and remaining seeds were placed in the same storage facility (15 °C, 35% RH).

The standard aerated water leachate was prepared in the same manner as in the first study and then diluted with distilled water to 25% and 50% (by volume). The inhibitory effects of each seed leachate concentration were tested using the radish germination bioassay with conditions as previously described. Germination was recorded after 48 h. The treatments were arranged as a randomized complete block with six replications.

Effects of duration of scarification. Thirty-gram samples ($\approx 18,000$ seeds) of parsley ('Moss Curled') were mechanically scarified using a Hoffman (Hoffman Manufacturing Co.) air-driven model SC-50 seed scarifier (15.9-cm-diameter cylinder). Air pressure for operation was 0.24 MPa. A medium-100 grade sandpaper (Norton Corp., Alberta, Canada) was used for the surface grid. Scarification time was 0, 30, 60, 90, and 120 min. At 30-min intervals, the sandpaper was removed and replaced with new sandpaper and mechanical scarification continued, depending on the treatment. Seedcoat residues from the scarification treatment were removed from the sandpaper using a 2.5-cm-wide paintbrush. Seedcoat material, which remained mixed with seed, was removed by using a no. 0 (0.30 mm) round dockage sieve, which retained the seed but allowed foreign material to pass through.

Seeds remaining after scarification were first weighed, counted, and a sample taken from each scarification time. This sample was tested for germination as previously described. Both scarified seeds and the seed covering residues from each scarification duration were placed separately in 100 mL of distilled water for 24 h and vigorously aerated. Seeds and seedcoat residues were then removed by filtration with Agryl P-17. The aqueous leachate was tested for inhibitory activity using the radish seed bioassay as previously described. Treatments were arranged as a randomized complete block with six replications. Analysis of variance was used in all experiments using the SAS system (SAS Institute, 1987).

Seed samples of both unscarified and scarified seed were photographed at 30 $\times$ magnification using an ISI 40 scanning electron microscope (Fig. 1).

Results and Discussion

Evaluation of Apiaceae species for inhibitory substances. Leachate from seed of each crop inhibited germination of seeds of the same crop as well as lettuce and radish seeds (Tables 1 and 2). Leachate from root parsley seeds appeared to be the least inhibitory and leachate from celery and celeriac seeds, most inhibitory. Radish was as effective as lettuce

Fig. 1. (top) Parsley seed before and (bottom) after 90 min of scarification. Photographs prepared using a scanning electron microscope. Horizontal bar = 1 mm.

Table 1. The influence of aqueous leachate from seeds of six species of *Apiaceae* on inhibition of germination of seeds of the same species.*

Source of leachate and seeds	% Germination		% Inhibition	Germination time (days)
	Distilled H ₂ O	Leachate		
<i>Petroselinum crispum</i>	60	28*	53	10
<i>Daucus carota sativus</i>	76	22*	71	6
<i>Apium graveolens dulce</i>	62	18*	71	11
<i>A. graveolens rapaceum</i>	72	30*	58	10
<i>P. crispum tuberosum</i>	79	47*	41	12
<i>P. sativa</i>	68	20*	71	12

*Data are means for six replications.

*Significantly different from distilled H₂O control by LSD at $P \leq 0.05$, after arcsin transformation of percentage data.

Table 2. The influence of aqueous leachate from seeds of six species of *Apiaceae* on inhibition of germination of 'Red King' radish and 'Slobolt' lettuce.^a

Source of leachate	Percent germination ^b	
	Radish	Lettuce
Control (distilled H ₂ O)	81	100
<i>Petroselinum crispum</i>	44*	38*
<i>Daucus carota sativus</i>	31*	31*
<i>Apium graveolens dulce</i>	26*	28*
<i>A. graveolens rapaceum</i>	22*	27*
<i>P. crispus tuberosum</i>	43*	48*
<i>P. sativa</i>	20*	42*

^aData are means for six replications.

^bGermination time: radish 48 h, lettuce 72 h

*Significantly different from distilled H₂O control by LSD at $P \leq 0.05$, after arcsin transformation of percentage data.

in detecting inhibitory activity. The duration of each bioassay was determined by the length of time that seeds normally required for radicle emergence. When germination was observed for an additional period of time, no increase in germination was recorded.

Parsley cultivar, seed lot, and umbel order effects on seed germination. Seed lots within a cultivar differed significantly in the amount of inhibitory activity. For example, standard aerated water leachate from 'Bravour' showed little inhibition at 25% concentration, regardless of the seed lot (Table 3). However, at higher concentrations all seed lots of 'Bravour' differed significantly from each other. Seed age appeared to have little consistent effect; for example, with 'Bravour' lots 7547 and 7987 both harvested in 1991, lot 7987 had significantly greater inhibitory activity. However, as the seeds aged inhibitory activity increased.

Seed from primary umbels contained the least amount of inhibitory activity, while seeds produced later contained increasing levels of activity (Table 4).

Effects of duration of scarification. The irregular shape of unscarified seed (Fig. 1, top) made the removal of the seedcoat difficult. The initial oval shape of scarified seed (Fig. 1, bottom) indicates that most tissue was removed from the ends of the seed, although material was also abraded from the sides.

The aqueous leachate from seedcoat material from scarified parsley seeds inhibited germination of radish seeds, regardless of scarification time (Table 5). Aqueous leachate from material collected after 90 or 120 min completely inhibited germination. The activity of the aqueous leachate collected from seeds declined as scarification was prolonged (Table 5). This indicates that inhibition was proportional to the amount of seedcoat remaining. To insure that scarification did not damage the embryo, scarified parsley seeds were germinated. Parsley seed germination declined after >60 min of scarification, indicating damage to the embryo (Table 5).

'Moss Curled' was the primary cultivar investigated due to its extensive commercial usage. The large variation between lots within cultivar suggested that differences in inhibitory levels were the result of differences in

seed development, environment, or harvest maturity that influences umbel order (Table 3). These findings are not consistent with those of Gray and Steckel (1992), which showed cultivar differences were significant in carrots.

Duration of seed storage appeared to have little effect on the level of inhibitory activity. However, the range of seed ages was not great, and most were harvested from 1989 to 1991. The oldest lot, 'Bravour', was produced in 1981 and resulted in 81% to 90% inhibition of radish seed germination at the 50% and 100% concentrations from the leachate extracts.

Seeds from primary umbels contained less inhibitor than did seeds from secondary or tertiary umbels (Table 4). Thus, conventional seed harvesting procedures that mix seeds from all umbel positions may have a considerable influence on the amount of inhibitor present. Gray and Steckel (1982, 1983) found that umbel order affected both the vigor and germination of carrot seed. (However, our study indicated no loss in vigor or germination.) In addition, they found that the numbers

of umbels per plant and seeds per umbel could be affected by soil N level, irrigation, and plant density. Management practices favoring a higher percentage of primary umbels should lower inhibitory activity.

An established bioassay for detecting inhibitory activity in seed of many crops uses lettuce seed (Berrie et al., 1968). However, different cultivars require exact conditions of temperature and light for optimal germination. Our studies indicate that radish seeds are as effective as lettuce seeds for a bioassay. Radish seeds germinate rapidly and results can be obtained in as little as 48 h. However, freshly harvested radish seeds exhibit some dormancy. The most desirable assay would be to use the seed of the respective crop; however, the time required for germination of most umbelliferous crops is considerably longer than that of either radish or lettuce. In addition, germination percentages for these crops are generally low when compared to radish or lettuce; thus, inhibitory effects are more difficult to detect at low concentrations.

These studies indicate that germination

Table 3. Influence of aqueous leachates from seeds of three lots of each of six cultivars of parsley on inhibition of germination of radish seed.

Cultivar	Lot no.	Production (month/year)	Percent inhibition of radish seed			Significance of R^2	
			Concn of leachate (%) ^a				
			25	50	100	L	Q
Forest Green	G66A	10/89	48 b	77 d-f	99 a-c	**	**
	247	8/90	7 ef	55 gh	83 fg	**	**
	GM629	10/91	8 ef	17 e	80 fg	**	**
Dark Green Italian	VW011	6/89	63 b	92 ab	99 ab	**	**
	VGW308	5/90	80 a	95 a	100 a	**	NS
	238	11/91	20 cd	79 c-e	100 a	**	**
Evergreen	1239	12/89	50 b	89 bc	100 ab	**	**
	1241	6/90	19 cd	67 e-g	92 de	**	**
	1240	12/90	19 cd	79 c-e	89 ef	**	**
Moss Curled	VGP022	7/90	20 cd	56 gh	95 c-e	**	**
	VGN5026	7/91	21 c	66 e-g	98 a-d	**	**
	VGW054	1/92	19 cd	64 fg	100 a	**	**
Plain	VGW640	12/89	32 c	61 gh	100 a	**	NS
	VGW013	6/90	29 c	85 b-d	99 ab	**	**
	VGW316	7/91	10 de	22 i	100 a	**	**
Bravour	8113	7/81	8 ef	90 ab	95 b-d	**	**
	7547	10/91	3 f	15 i	30 h	**	**
	7987	10/91	9 ef	48 h	12 g	**	**
Mean			26	64	90	**	**

^aSee text for preparation.

^bMean separation within columns by LSD ($P \geq 0.05$), after arcsin transformation of percentage data. Data are means of four replications.

NS, **Nonsignificant or significant within rows at $P \leq 0.01$.

Table 4. Influence of aqueous extracts of parsley seed, cv. Evergreen, sampled from three umbel positions, on inhibition of germination of radish seed.

Field	Umbel position	Germination (%)	Percent inhibition		
			Concn of leachate (%) ^z		Significance of R ²
			25	50	
1	Combined	74	56 de	83 b	*
	Primary	73	29 f	47 c	*
	Secondary	74	92 b	97 a	*
	Tertiary	76	96 a	99 a	NS
	Secondary/Tertiary	73	73 c	99 a	*
2	Primary	72	65 cd	77 b	*
	Secondary	70	95 ab	99 a	NS
3	Primary	68	52 d	60 c	NS
Mean			70	83	*

^aSee text for preparation.

^bMean separation within columns by LSD ($P \geq 0.05$), after arcsin transformation of percentage data. Data are means of four replications.

NS, **Nonsignificant or significant at $P \leq 0.05$, respectively, within rows.

Table 5. Effects of scarification time on germination of parsley seed and of aqueous leachates from parsley seedcoat tissues and scarified seed on germination of radish seed.

Scarification time (min)	Seedcoat removed/ 30-g seed (g)	Germination of remaining seed (%)	Inhibition of radish seed germination	
			Seedcoat leachate (%)	Seed leachate (%)
Control	0.00	77 a ^z	0 d ^z	100 a ^z
30	2.83	73 ab	39 c	57 b
60	3.79	72 ab	81 b	42 c
90	5.37	68 b	100 a	23 d
120	5.84	47 c	100 a	28 c
Linear		**	**	**

^zMean separation within columns by LSD ($P \geq 0.05$), after arcsin transformation of percentage data. Data are means of six replications.

**Significant at $P \leq 0.01$.

inhibitors in parsley are located in the seed coverings (Table 5); however, some of the inhibitor may have been present in the endosperm or other seed tissue. No effort was made to separate the seedcoat residue from endosperm residue. Scarification not exceeding 60 min did not adversely affect seed germination. The inhibitory substance is water soluble and can be removed from the seed by washing for 3 h (Table 5). Heydecker and Gibbins (1978) demonstrated that biological activity begins in most seeds after stage 1 inhibition. Parsley seed imbibed water through 12 h (data not shown), yet most of the inhibitory activity was measured in the aqueous extract by 3 h. These results, combined with removal of inhibitors by dry scarification, indicate that the inhibitory substance is not a result of metabolic activity, but is present in dry seed.

In the past, scarification has been performed to overcome impermeability, allowing imbibition and germination to take place (Hicks et al., 1989; Singer and Pitman, 1988). Our results indicate that scarification also can be used to remove germination inhibitors.

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