Diallel Analysis of Resistance in Carrot to *Alternaria* Leaf Blight

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ADDITIONAL INDEX WORDS. *Daucus carota*, *Alternaria dauci*

ABSTRACT. Diverse carrot (*Daucus carota* L.) inbreds were evaluated as young plants in the greenhouse and mature plants in the field for resistance to the pathogen *Alternaria dauci* (Kühn) Groves and Skolko. Persistence of leaves after infection was the criterion used to estimate disease damage by the pathogen. Partial resistance was identified. A five-parent diallel evaluated for resistance indicated a preponderance of additive variation with some dominant gene action for resistance. Reciprocal cross differences were significant for certain crosses. Responses of most inbreds were useful in predicting resistance in their hybrids, but exceptions to this trend did occur. Field resistance ratings generally correlated well with resistance ratings obtained in greenhouses, even in grower fields where fungicides were applied.

Alternaria dauci (Kühn) Groves and Skolko is an important pathogen of carrot (*Daucus carota* L.) in most production areas of the world (Hooker, 1944; Netzer and Kenneth, 1969; Scott and Wenham, 1973; Strandberg, 1992a; Tahvonen, 1978). It incites alternaria leaf blight (ALB), a foliage blight that reduces photosynthetic area and, when severe, can reduce root yield and complicate harvesting. Reduced fungicide availability and higher costs have greatly affected leaf blight control. Thus, host resistance is desirable. Genetic variation for resistance and tolerance has been observed (Strandberg et al., 1972) and several sources have been identified (Strandberg, 1992b). Evaluation of half-sib families of carrot has indicated a broad-sense heritability of 40% to 82% within five carrot populations in two studies (Boiteux et al., 1993; Vieira et al., 1991). For breeders to effectively incorporate ALB resistance during the development of hybrid carrot cultivars, knowledge of combining ability for sources of resistance is needed. The objectives of this study were to evaluate a range of available carrot inbreds and their hybrids to determine combining ability of selected carrot inbred lines for response to *Alternaria dauci* using diallel analysis. For convenience, we will use the term resistance to refer to levels of response to the pathogen, but we are aware of the limitations associated with this term.

Materials and Methods

Twelve carrot inbred lines were evaluated for their response to *Alternaria dauci* following inoculation with spores of the pathogen in a greenhouse test over several months in Sanford, Fla., using the methods described by Strandberg (1988). These inbreds represent a broad range of carrot diversity used by the vegetable seed industry. Inoculum was produced as previously described and young plants were individually inoculated at the five- to six-leaf stage by briefly immersing the foliage in a spore suspension (1100 to 1400 spores/mL). Inoculated plants were maintained in nine small greenhouses with intermittent misting between 1800 and 0600 hr each day to promote disease development and spread. No

significant differences in disease response between greenhouses have been found (Strandberg, 1988). Response to the pathogen was measured by recording the interval between inoculation and death of leaves three, four, five, and six of each plant. These data were obtained by examining each plant daily beginning at 7 d after inoculation (DAI) and recording the date of death of the leaves of interest until 31 DAI; plants were removed from the experiment when these leaves were dead. Response ratings were obtained by multiplying the leaf number (three to six) by the number of days after inoculation that the leaf died and summing these values for each plant within a test population (leaves remaining alive at 31 DAI received the value of 31 in calculating this rating). This sum was divided by the number of plants in the population to obtain a mean rating: the disease damage rating (DDR). We assumed that the death of younger leaves was a more severe reaction to the pathogen, which merits a higher multiplier, but this notion has not been extensively investigated. In each test, ≈ 20 entries were evaluated. From 20 to 24 plants per entry were inoculated and placed on benches at random, but at 7 DAI, plants from each entry were grouped together to facilitate data collection. Uninoculated checks were included.

Five selected inbreds, B3475, B5280, B5344, B6253, and B9695, representing a broad range of resistance were crossed, including reciprocals, in a diallel mating design. Seed of three F_1 hybrids was not available. The resulting F_1 hybrids, their reciprocals, and the inbred parents were evaluated for ALB resistance as described above using a completely random design with three replicates. Diallel analysis was carried out using Griffing's Method 1, Model 1 (Burow and Coors, 1994; Griffing, 1956). Crosses were considered as fixed effects.

In addition to quantitative ALB ratings made in the greenhouse, subjective ratings were made in the field at Zellwood, Fla. (muck soil), in March 1993; Sanford, Fla. (Myakka fine sand soil), in April 1996; and Palmyra, Wis. (muck soil), in August 1993. Carrot seed was planted in October 1992, November 1995, and May 1993, respectively, in 5-m rows on 1-m centers with 60 to 95 plants/m. The 1993 Florida and 1993 Wisconsin trials were on growers' fields with standard fertilizer, herbicide and fungicide applications (including three to five applications of an iprodione fungicide). The 1996 Florida trials were planted with alternating and adjacent susceptible ('Danvers') infection rows inoculated with *A. dauci* at

Received for publication 17 July 1997. Accepted for publication 7 Jan. 1998. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Mean alternaria leaf blight damage ratings in a five-parent carrot diallel and representative ratings for seven other inbreds evaluated with the same procedures.^z

	Males				
	B5280	B5344	B6253	B3475	B9695
Females	Rating				
Diallel cross					
B5280	308 def ^y	278 ghi	368 a	---	297 efgh
B5344	313 cde	224 k	337 bc	---	---
B6253	332 bcd	279 ghi	285 fgh	315 cde	306 defg
B3475	306 defg	269 hij	349 ab	268 hij	231 k
B9695	309 def	280 fgh	314 cde	231 k	251 ijk
Other inbreds tested					
F524	281 fgh				
B2566	245 jk				
B3080	276 hi				
B3180	298 efgh				
B3640	270 hij				
B4367	267 hij				
B6274	230 k				

^zDisease damage rating which reflects the average number of days leaves three, four, five, and six survived after inoculation with the pathogen.

^yValues followed by the same letter are not significantly different at $P = 0.05$ by Duncan's multiple range test.

Table 2. Diallel analysis for general combining ability (GCA), specific combining ability (SCA), and reciprocal effects in five carrot inbreds, their hybrids and reciprocals for alternaria leaf blight resistance.

Source of variation	df	Mean squares
Treatments	24	4,003 ^z
GCA	4	92,397 ^z
SCA	10	17,112 ^z
Reciprocals	10	4,124 ^z
Error	48	286

^z $P < 1\%$

about the four-leaf stage. Inoculum (10^4 to 10^5 spores/mL) was produced in the laboratory (Strandberg, 1988) and applied only to infection rows with a compressed air sprayer. For all three field trials, subjective ALB ratings were made at harvest time using the following scale: 0 = no visible disease damage; 1 = few lesions mostly on older leaves; 2 = numerous lesions on older leaves, scattered on younger leaves; 3 = abundant lesions, some defoliation and leaf damage; 4 = abundant lesions, moderate leaf damage and defoliation; 5 = severe leaf damage and defoliation.

Results and Discussion

The response of carrot inbreds to *Alternaria dauci* was highly variable (Table 1). Leaves of susceptible inbreds died within 20 d of inoculation, whereas more resistant inbreds retained more leaves for longer periods. B5280 was more resistant to *Alternaria dauci* than other carrot inbreds tested over a wide range of trials. Uninoculated plants displayed no disease symptoms. Although young plants from greenhouse evaluation were not grown to maturity, ALB attack early in plant growth retards future plant growth significantly.

Highly significant genotypic differences were observed for ALB resistance in carrot. General combining ability (GCA) and specific combining ability (SCA) contributed significantly to this variation (Table 2), although the GCA sum of squares was 2.2 times as large as SCA sum of squares. Thus, additive genetic variation was very important but nonadditive variation should also be considered. In an evaluation of three half-sib families, Vieira et al. (1991) found broad-sense heritabilities ranging from 45% to 82% whereas additive genetic variance ranged from 7% to 18%. Boiteux et al. (1993) found a narrow-sense heritability of 40% for 'Brasilia' half-sib families.

Table 3. Mean Alternaria leaf blight disease damage ratings and general combining ability (GCA) effects of five carrot inbreds and their reciprocal diallel crosses.

Parent	Inbred score	Midparent score ^z	GCA effect	Hybrid score using parent as	Observed hybrid score ^y
B5280	308	283	+55	Female	315
				Male	314
B6253	288	274	+42	Female	342
				Male	308
B5344	224	252	+21	Female	277
				Male	302
B3475	268	268	-59	Female	282
				Male	289
B9695	251	261	-60	Female	284
				Male	278
LSD _{0.05}	31		44		27

^zMean rating of inbred indicated with every other inbred.

^yMean score of crosses using the inbred indicated.

Differences in mean *Alternaria* disease damage ratings (DDR) were significant among reciprocal hybrids (Tables 1 and 2). Specific crosses demonstrated significant reciprocal differences but a discernible trend (e.g., general effects in one direction of cross or for certain inbreds) could not be established (Table 3). For example, hybrids using B6253 as a female parent demonstrated significantly less disease damage than those using B6253 as a male, whereas B5344 transmitted more resistance (lower DDR) as a male parent than as a female parent. The fact that seed was not available for reciprocal cross evaluation in 3 of the 10 hybrid combinations limits general observations that can be made about reciprocal effects.

Observed hybrid DDR scores were always greater than midparent DDR scores by 14 to 68 units (Table 3). Observed DDR scores for a given hybrid were also comparable to or greater than those of the more resistant inbred parent especially in hybrids of B6253 and B5344 (Table 3). The only exceptions to this generalization were

Table 4. Mean *alternaria* leaf blight estimates of specific combining ability obtained in a five-parent carrot diallel.

Parent	B5344	B6253	B3475	B9695
B5280	-39	-6	+56*	+49*
B5344		-13	+50*	+73*
B6253			+48*	+31
B3475				---

*Significant at the 0.05 level of probability

Table 5. Mean *alternaria* leaf blight damage ratings for 12 carrot inbreds grown in the field with adjacent inoculated infection rows or natural infection.^z

Inbred	Wisconsin ^y 1993	Florida ^x 1993	Florida ^w 1996
B5280	0.5	0.5	1.9
B5344	1.5	2.0	4.0
B6253	2.0	1.5	3.1
B3475	4.0	2.5	4.8
B9695	3.0	2.5	4.5
F524	---	1.0	---
B2566	3.0	2.0	3.6
B3080	---	1.5	---
B3180	1.0	1.0	---
B3640	2.5	1.5	---
B4367	1.5	1.5	3.9
B6274	2.5	2.5	4.3

^zSubjective disease damage ratings where 0 = no visible disease damage; 1 = few lesions mostly on older leaves; 2 = numerous lesions on older leaves, scattered on younger leaves; 3 = abundant lesions, some defoliation and leaf damage; 4 = abundant lesions, moderate leaf damage and defoliation; 5 = severe leaf damage and defoliation.

^yNatural infection, one judge.

^wNatural infection, one judge.

^xInfection rows, three judges.

Table 6. Phenotypic correlations for *alternaria* leaf blight damage ratings from quantitative greenhouse evaluation and subjective field evaluation in Wisconsin and Florida.

	Wisconsin 1993	Florida 1993	Florida 1996
Quantitative rating	-0.46 ^{NS}	-0.81	-0.69 ^{**}
Wisconsin 1993		0.84 ^{**}	0.78*
Florida 1993			0.94 ^{**}

^{NS},*,** Nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

in B5280 x B5344, B3475 x B9695, and B9695 x B3475. These observations suggest that dominant gene action can confer resistance. Alternatively, this could also infer heterosis per se may be an important component of resistance. The low disease ratings for reciprocal hybrids B9695 x B3475 and B3475 x B9695 (scores of 231) compared to 268 for B3475 and 251 for B9695 (Table 1) may support the suggestion of dominance for resistance, presuming that B3475 and B9695 lack dominant resistant alleles.

The relative contribution of individual parents to ALB resistance was estimated by comparison of GCA effects (Table 3). Inbred B5280 had a significant positive GCA effect and high hybrid DDR whereas inbreds B3475 and B9695 had significant negative GCA effects and low mean hybrid DDRs. DDR values of inbred B5344 and the midparent DDR were less than that of either inbred B3475 or B9695, but B5344 hybrids (averaged over reciprocals) performed similarly to B3475 and B9695 hybrids.

Estimates of SCA indicated several crosses performed better in regard to ALB than would be expected from the GCA of the parents involved (Table 4). Crosses between B5280, the most resistant parent, and B3475 and B9695, had significant SCA effects (+56 and +49, respectively). Interestingly, crosses between the most susceptible parent, B5344, and B3475 and B9695 also had significant SCA effects (+50 and +73, respectively). Such results suggest that both dominance and epistasis are important in ALB resistance, at least in some carrot hybrid combinations. No crosses had SCA effects significantly <0.

The results of this study indicate that ALB resistance in carrot hybrids can be partially predicted from inbred performance. However, certain hybrid combinations differed significantly from expectations based on strictly additive gene action. In general, hybrid DDRs observed were greater than those expected from midparent predictions, which is useful in hybrid carrot cultivar development. This suggests that dominant gene action may contribute to resistance. To test this idea the transmission of ALB resistance in a recurrent selection program can better elucidate the nature of gene action suggested by this study since evidence for dominant gene action could then be evaluated over several generations of selection. Such a program could also yield additional ALB resistant carrot inbreds.

Resistance to ALB in young carrot plants evaluated in greenhouses is convenient, particularly where conditions do not favor disease development in the field, but may not fully reflect plant performance over an entire season of field exposure to the pathogen. To make this comparison ALB ratings were made in the field (Table 5). Field ratings generally agreed with the resistance ratings obtained in the greenhouse. The two inbreds with the best greenhouse ratings, B5280 and B3180, also had the best field ratings. Furthermore, those inbreds with greenhouse ratings under 270 (the more susceptible 6 of the 12 tested) always had field ratings in the more susceptible half of inbreds evaluated. Yet several susceptible inbreds from the greenhouse evaluation demonstrated relatively mild ALB symptoms in some field evaluations (e.g., B4367-WI 1993, FL 1993, B5344-WI 1993). Even in grower fields where fungicides were applied, relative resistance and susceptibility to ALB was evident.

Correlations were generally high among ALB evaluations (Table 6) although less consistently for those with the Wisconsin field test. Whether this represents uneven field disease attack, strain differences in the pathogen, or other factors cannot be determined.

More extensive field evaluations throughout plant establishment and growth would be desirable to follow disease development and more definitively identify resistant and susceptible

genetic stocks. Strain differences in this pathogen are not documented but may warrant further evaluation. Other aspects of resistance such as leaf persistence after infection, rate of lesion development, sporulation levels, and leaf infection rate could also be evaluated in greenhouses to provide a more complete picture of the nature of field resistance to *Alternaria dauci*.

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