Aggressiveness and Fungicide Sensitivity of *Alternaria dauci* from Cultivated Carrot

P. M. Rogers, Graduate Research Assistant, and W. R. Stevenson, Professor and Extension Plant Pathologist, Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706

ABSTRACT

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Isolates of *Alternaria dauci* causing Alternaria leaf blight (ALB) were collected from commercial carrot (*Daucus carota* var. *sativus*) fields in northeastern North America during 2004. Twenty-two isolates representing a range of genetic diversity were analyzed for their aggressiveness on three commercial carrot varieties (Bolero, Enterprise, and Heritage) varying in disease susceptibility as well as their in vitro response to three fungicides (azoxystrobin, chlorothalonil, and boscalid) commonly used for ALB control. Severity of leaf and petiole blight and leaf chlorosis varied among isolates and carrot varieties in each of three experiments. Visible differences in disease severity, which ranged from 10.9 to 45.1% of the leaf area affected, were apparent 16 days after inoculation. Intensity of chlorosis correlated strongly with blight severity among all isolates. Significant differences were noted among carrot varieties in response to ALB. These varieties may prove useful as differentials capable of distinguishing isolates because variety by isolate interactions were detected. Inhibition of conidial germination ranged from 0.01 to 0.37 µg/ml for azoxystrobin, 0.009 to 0.08 µg/ml for chlorothalonil, and 0.09 to 0.59 µg/ml for boscalid. On average, isolates were more sensitive to chlorothalonil than to azoxystrobin and boscalid. No significant correlation was noted between fungicide sensitivity and aggressiveness. These data provide evidence for phenotypic diversity among *A. dauci* isolates collected from areas of commercial carrot production.

Alternaria leaf blight (ALB), caused by *Alternaria dauci* (Kühn) Groves & Skolko, is an omnipresent and destructive foliar disease of carrots (*Daucus carota* var. *sativus* Hoff.) (8,9,23,30,38). Disease development is favored by warm temperatures and prolonged leaf wetness; in areas where conducive environments persist, foliage may rapidly succumb to blight (14,23,36). Premature loss or collapse of foliage may result in significant yield reductions in carrot fields due to the impairment of photosynthesis. Additionally, blighted or weakened foliage can limit harvestability, as leaves or petioles may break when mechanically lifted, causing roots to remain in the field (27). For these reasons, commercial carrot growers employ multiple management strategies for ALB control, including the use of pathogen free seed, crop rotation, planting of disease resistant varieties, disease forecasting models, and most notably, the application of protective fungicides (4,8,14,24,27).

Research on carrot breeding and the evaluation of variety performance have led

to improvements in our understanding of disease susceptibility among carrot breeding lines (6,21,27,35). Efforts to develop varieties with resistance to ALB have met with limited success due in part to low heritability and the apparent complexity of resistance traits. Inoculation experiments in controlled environments have been developed to screen carrot germplasm for resistance to ALB (7,21,37). Moderate levels of resistance to *A. dauci* have been observed, and a small number of resistant varieties are available. In addition, resistant varieties require less fungicide input to achieve similar levels of disease control when compared to more susceptible material (2,3,28). Collectively, this suggests that the host may play a significant role in determining the variation in disease response to ALB observed in the field. However, the influence of pathogen diversity on disease development has not been elucidated.

Accompanying these developments have been concerted efforts to evaluate fungicide efficacy for ALB control (2,3,16,26,39). The identification of effective compounds such as azoxystrobin, boscalid, and pyraclostrobin and the development of weather-based forecasting techniques have led to the deployment of Integrated Pest Management (IPM) programs for ALB (2,4,26). Concerns have been expressed regarding the long-term efficacy of azoxystrobin, pyraclostrobin, and boscalid and

the selection for resistant individuals within a fungal population (12,13,19,20,32). Monitoring *A. dauci* isolates is necessary to better understand population responses to these compounds and the potential for continued control of ALB (5).

Within populations of *Alternaria* spp., variation in virulence as well as genetic, morphological, and biochemical traits has been identified (1,18,25,29,30,42,43). One study assessed genetic variability among isolates of *A. dauci* in carrot fields in California in order to identify potential sources of inoculum (17). Sequence analysis of the intergenic spacer region (IGS) revealed conserved and variable domains of the IGS, which allowed isolates to be grouped into 10 genotypes. To date, no research has assessed large-scale diversity of virulence in *A. dauci* populations or determined the interaction of a collection of isolates with different host genotypes. Additionally, research aimed at characterizing the sensitivity of *A. dauci* isolates to commonly used fungicides has not been reported.

Variability in spatial and temporal distribution of ALB symptoms within a field or growing region has been observed (26). Such patterns are not easily explained by environment or host genotype alone and present additional difficulty for disease management. Pockets of disease with much higher severity may be a result of individual pathogen genotypes with increased aggressiveness. Specific interactions of isolates with host genotypes may also explain, in part, the variation in susceptibility of carrot varieties across fields, farms, or growing regions. The objectives of this study were to (i) examine the aggressiveness of *A. dauci* isolates on carrot varieties differing in disease susceptibility, (ii) identify and characterize isolate by carrot variety interactions, and (ii) examine the in vitro sensitivity of *A. dauci* to three fungicides used to control ALB.

MATERIALS AND METHODS

Inoculations were carried out on 60-day-old carrot plants (five- to six-leaf stage) under greenhouse conditions as described by Strandberg (38). Briefly, conidia of each isolate were sprayed with an atomizer onto eight carrot plants per pot for each of four carrot varieties. Inoculated plants were exposed to continuous leaf wetness and high relative humidity (90 to 100% RH) in a mist chamber for 24 h. Colonization by *A. dauci* was allowed to develop

Corresponding author: P. M. Rogers
E-mail: peter.rogers@unhems.com

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until macroscopic symptoms were visible (~7 days). Reisolation of the pathogen followed visual assessments of disease symptoms.

Leaf sampling and fungal isolation.

Sixteen hundred isolates of *A. dauci* were obtained during August and September 2004 from 50 ALB symptomatic leaves gathered arbitrarily from commercial carrot fields in Michigan, New Jersey, and Wisconsin. These locations corresponded to six carrot-growing regions (three from Wisconsin, two from Michigan, and one from New Jersey) and represented a range of geographic, field, cultivar, and fungicide histories. From each leaf, three lesions removed with a cork borer were surface disinfested and placed on 1.5% water agar (WA) amended with streptomycin at 50 µg/ml and tetracycline at 15 µg/ml to allow sporulation and positive identification (30,31,34,36). Monoconidial isolates from individual lesions were transferred to clarified V8 (CV-8) media in 9-cm-diameter polystyrene petri plates according to Strandberg (36) and placed under continuous light exposure to four 20-watt fluorescent Hi-vision cool-white tubes (General Electric, Nela Park, OH) at 20°C for 7 days. Sterilized filter paper (1-cm squares) was then placed on the medium surface surrounding growing single-spore colonies. The squares were collected 5 to 6 days later when conidia had formed and the fungus had grown through the paper. Isolates were then stored on the paper at -20°C.

Isolates included in this study were chosen from a larger set of *A. dauci* isolates

previously examined and analyzed for intraspecific genetic diversity and variation in morphological characters (P. M. Rogers Z. K. Atallah, and W. Stensson, in press). Twenty-two isolates of *A. dauci* were selected in an effort to represent a range in genotypes and geographic distribution across six carrot growing regions (Table 1). Two isolates of *A. solani*, a close relative to *A. dauci*, were isolated from potato and included to provide a control outgroup and allow comparison in fungicide response as reported previously (19,20). All 24 isolates were included in aggressiveness and fungicide response studies and subjected to the same analysis.

Variety selection and plant growth.

Carrot varieties representing a known range of susceptibility to ALB were selected for this study based on the disease response and performance observed in field trials and the frequency of use by the Wisconsin carrot industry (26,28). Varieties Heritage (Seminis Vegetable Seeds, Oxnard, CA), Enterprise (Seminis), and Bolero (Vilmorin Seed Co., LaMenitre, France) were selected to represent susceptible, intermediate, and resistant categories, respectively. A wild *D. carota* (Queen Anne's lace) accession collected from the central Wisconsin carrot production region (Hancock, WI) was also included to evaluate the role wild carrot may play in *A. dauci* diversity and carrot production.

Carrot seeds (17) were sown in autoclaved 15-cm-diameter plastic pots in a glasshouse, with each pot containing a sterilized metro mix/sand mixture (1:1

ratio; Scotts metro mix/sand). Seeds were allowed to germinate and grow for 30 days after planting (DAP), at which point carrot seedlings were thinned to eight plants per pot. Plants were then grown for 30 additional days until they reached the five- to six-leaf stage (60 DAP). Glasshouse conditions were maintained at 20 to 23°C under a photoperiod of 12 h for the entire growth period. Plants were watered on alternating days until 20 DAP, and then daily through the remainder of the growing process. Beginning at 20 DAP, 100 ml of a modified Hoagland's solution was added to each pot once each week to maintain adequate nutrient levels.

Inoculum preparation and inoculation procedure.

One filter paper disk containing conidia of each *A. dauci* isolate was placed on 25 ml of clarified V8 medium in 9-cm-diameter polystyrene petri plates and held for 10 days at 20°C with continuous exposure to four 20-watt fluorescent Hi-vision cool-white tubes. Conidia were gently dislodged from the agar surface with 10 ml of sterile distilled H₂O and agitation with a glass rod. Conidial suspensions were adjusted to 1.0 × 10⁴ spores/ml. The surfactant Tween 20 (Acros Organics, Morris Plains, NJ) adjusted to a concentration of 0.5% was added to each suspension.

Each pot containing eight plants was sprayed until runoff (~30 ml per pot) using an atomizer pressurized with compressed air at 137.9 kPa. Following inoculation, plants were placed in a mist chamber for 24 h without light at 22°C to maintain leaf wetness and 95 to 100% RH to promote spore germination and infection. Carrot plants were then returned to the same environment used for initial plant growth for the remainder of the experiment.

Treatments and experimental design.

Treatments consisted of 22 *A. dauci* isolates, two *A. solani* isolates, and distilled water + 0.5% Tween 20 only. Each of three carrot varieties and wild carrot were inoculated with each treatment, and all treatments were replicated three times for a total of 300 pots per experiment. The experiment was designed as a randomized complete block, with glasshouse benches blocked by carrot variety and each treatment randomized within the block. The experiment was conducted three times during 2006–07.

Disease assessment and statistical analysis. Foliar disease severity (%) was scored according to the disease damage index published by Strandberg, and the percent leaf area affected for eight plants per pot was recorded (38). In addition, severity of petiole symptoms (symptom severity for each of eight plants) was based on a 0 to 5 scale where 0 = 0%, 1 = 0 to 9%, 2 = 10 to 19%, 3 = 20 to 39%, 4 = 40 to 59%, and 5 = >60% of the petiole affected, and an estimate of the extent of chlorosis surrounding the lesions (0 = no

Table 1. Source of isolates of *Alternaria dauci* and *A. solani* evaluated in this study for aggressiveness on carrot and sensitivity to three fungicides

Isolate	State and region	Carrot variety	Culture collection code	Fungicide exposure ^a
<i>Alternaria dauci</i>				
04-68	WI-South	Gold King	...	CH
BST493	WI-Central	Bolero	...	CH
BST503	WI-Central	Bolero	...	CH
CDST611	WI-North	Canada	...	CH
CST536	WI-North	Carson	...	CH
CST542	WI-North	Carson	CBS122043	CH
E15D260	WI-Central	Enterprise	...	CH, AZ
E15D481	WI-Central	Enterprise	...	CH, AZ
EST433	WI-Central	Enterprise	...	CH
HRS437	WI-North	Fontana	...	CH, AZ
HST401	WI-Central	Heritage	...	CH
HST403	WI-Central	Heritage	CBS122046	CH
HST420	WI-Central	Heritage	...	CH
IND902	WI-North	Indiana	CBS122044	CH
IND904	WI-North	Indiana	...	CH
MF1002	MI-Central	Fontana	...	CH, AZ
MF1003	MI-Central	Fontana	CBS122048	CH, AZ
MNB014	MI-West	Nandrin	...	CH
MNB024	MI-West	Nandrin	...	CH
NJ170	NJ-Bridge	Fontana	CBS122047	CH, AZ
NJ182	NJ-Bridge	Fontana	CBS122045	CH, AZ
NJ201	NJ-Bridge	Fontana	...	CH, AZ
<i>A. solani</i> 21	WI	Norkotah potato	...	CH, AZ, BC
<i>A. solani</i> 98	WI	Norkotah potato	...	CH

^a Notation signifies field exposure of each isolate to at least one application of the fungicides chlorothalonil (CH), azoxystrobin (AZ), or boscalid (BC) and is suggestive of perhaps more than one or both.

chlorosis; 1 = visible, 1 to 5% of leaf area; 2 = moderate, 5 to 25% of leaf area; 3 = severe, >25% of leaf area) was recorded. Observations were recorded 8 and 16 days postinoculation to observe any differences in symptom development over time. Following data collection, leaves from one plant for each isolate were collected, surface disinfested, and placed on water agar for 7 days to allow sporulation and species identification according to Simmons (33,34).

Analysis of variance (ANOVA) was conducted for foliar blight severity, petiole severity, and extent of chlorosis using PROC GLM in PC SAS version 8.1 (SAS Institute Inc., Cary, NC). Mean separation for each treatment variable was compared using Fisher's least significant difference (LSD). In addition, a multifactorial ANOVA was conducted, including carrot variety and isolate as variables, to examine the possibility of variety by isolate interactions. To examine the relationships among symptom variables, correlations were determined between all disease measurements. Means for each isolate and cultivar were tested for homogeneity of variance across all three experiments, and based on those tests, some data were combined.

Fungicide sensitivity. Fungicide sensitivity of isolates was investigated in the laboratory to determine the effective concentration to inhibit spore germination by 50% (EC₅₀) for each isolate and to compare three fungicides commonly used for ALB control. Response to fungicide active ingredients served as a marker to assess diversity among *A. dauci* isolates and test the efficacy of compounds to control ALB. The fungicide exposure history of isolates included in this experiment ranged from those exposed to multiple applications of chlorothalonil and azoxystrobin to those that may not have been exposed to either of these fungicides (Table 1). To our knowledge, none of the isolates included in this assay had been exposed to boscalid, and they likely represent a baseline response to this compound.

The same 22 *A. dauci* isolates assayed for aggressiveness (Table 1) were used for fungicide evaluations. Conidia were harvested as described previously. In all germination tests, a conidial suspension of 1×10^4 (50 μ l) was spread on the surface of water agar amended with fungicide. Germination was assessed for 50 conidia at each fungicide concentration after incubation for 4 h at 20°C.

Tests at the discriminatory dose of 0.01 μ g/ml were performed twice for a subset of isolates in order to determine the precision of the relative germination test. The fungicide concentrations of each active ingredient used for this assay included 0, 0.001, 0.01, 0.1, 1, and 10 μ g/ml. A conidium was rated as germinated if a germ tube was equal to or greater than the length of the conidium or if an appressorium formed at

the tip of the germ tube. Isolate sensitivities are expressed as relative germination, defined as the ratio of conidia germinating in the presence of fungicide to those germinating in the absence of the fungicide $\times$ 100.

Technical grade active ingredients of the fungicides were dissolved and stored in acetone. Treatments for sensitivity assays included water agar containing acetone only, acetone plus each fungicide, and in the case of azoxystrobin, methanol plus salicylic hydroxamic acid (SHAM) or methanol plus SHAM with azoxystrobin in acetone, at each aforementioned concentration. SHAM was included to block the alternative oxidase pathway, as described earlier (12,13). For all isolates, EC₅₀ values were determined and comparisons were made among isolates. EC₅₀ values were calculated by regressing percent relative germination against the log concentration of the fungicide (μ g/ml) for each isolate. Data were graphed and a best fit line using the linear model (R^2 between 80.5 and 88.3%) was used to estimate EC₅₀ values. The polynomial equation provided a slightly tighter best fit line (R^2 between 89 and 99.1%) for population response to each active ingredient, and EC₅₀ values for each isolate were calculated using this procedure. Each isolate–fungicide combination was replicated four times in each experiment. Mean EC₅₀ values for all isolates were compared using ANOVA, and Fisher's LSD was generated for mean

separation. The experiment was conducted a total of three times. The homogeneity of variance among EC₅₀ values allowed for the combination of data from all experiments.

In addition, EC₅₀ values provided a tool to compare effective doses to labeled fungicide rates used for control of *A. dauci* under field conditions. Field rates commonly applied for ALB control were converted to μ g/ml in order to compare EC₅₀ values for each isolate to concentrations of each fungicide used in commercial production. Correlation coefficients were sought between EC₅₀ values for each fungicide active ingredient to examine the likelihood for cross resistance with these fungicides and between EC₅₀ values and aggressiveness parameters.

RESULTS

Aggressiveness among isolates. Aggressiveness varied significantly ($P < 0.001$) among *A. dauci* isolates 8 and 16 days postinoculation; data are only shown for the latter in the interest of space (Table 2). Significant differences were detected among strains in all measured categories (leaf blight severity, petiole symptom severity, and chlorosis intensity), and significant positive correlations (leaf blight $R^2 > 0.885$; petiole $R^2 > 0.825$; chlorosis $R^2 > 0.872$) were noted for data from repeated experiments. Data for each aggressiveness category were tested for homogeneity of variance as well as rank order of isolates.

Table 2. Effect of *Alternaria dauci* and *A. solani* on the development of blight symptoms on carrot^a 16 days after inoculation

Isolate	Leaf blight severity (%)	Petiole blight severity (%)	Chlorosis intensity (%)
<i>Alternaria dauci</i>			
04-68	20.6	6.2	9.3
BST493	36.1	12.8	30
BST503	22.1	7.5	14.9
CDST611	29.5	8.9	20.7
CST536	17.8	5.6	11.9
CST542	15.7	4.3	4.8
E15D260	37.4	11.8	20.2
E15D481	33.1	8.4	16.2
EST433	25.9	7.9	20.1
HRS437	27.5	9.3	15.1
HST401	11	3.2	4.3
HST403	45.1	17.8	36.2
HST420	20.9	6.4	12.5
IND902	22.1	7.1	8.4
IND904	24.1	7.4	11.2
MF1002	27.9	6.3	9.5
MF1003	13.6	3.2	4.9
MNB014	36.2	9.9	19.2
MNB024	40.5	13.2	29.4
NJ170	11.5	4.4	7.6
NJ182	29.1	6.5	23
NJ201	23.7	5.9	9.2
<i>A. solani</i> 21	0	0	0
<i>A. solani</i> 98	0	0	0
Control	0	0	0
P value	<0.001	<0.001	<0.001
LSD	3.8	2.1	4.9

^a Data are means of three reps across three repeated trials using carrot varieties Heritage, Enterprise, and Bolero, and wild *Daucus carota* (Queen Anne's lace).

Because statistical differences between experiments were not observed, data from the three experiments were combined. Leaf blight severity, petiole severity, and chlorosis intensity data collected on day 16 were combined within each category for all experiments (Table 2). No symptoms were noted in any of the experiments for the control treatment of distilled H₂O + Tween 20 or on carrots inoculated with the two *A. solani* isolates.

Aggressiveness among isolates displayed a continuous range from 11 to 45%, 3.2 to 14.8%, and 4.3 to 36.2% for leaf blight severity, petiole symptom severity, and chlorosis intensity, respectively (Table 2). Leaf blight severity and chlorosis inten-

sity were greatest for isolate HST403 and least for isolate HST401. There were significant positive correlations between leaf blight severity and petiole severity ($R^2 = 0.948$), as well as leaf blight severity and chlorosis intensity ($R^2 = 0.903$), and petiole severity and chlorosis intensity ($R^2 = 0.933$). In general, isolates that caused high levels of leaf blight severity also displayed high levels of petiole blight severity and chlorosis (Table 3).

Varietal differences and isolate by variety interactions. Significant differences were recorded in leaf blight severity and petiole severity response to *A. dauci* isolates among carrot varieties (Fig. 1). Collectively across all isolates, Heritage and

Enterprise were the most susceptible varieties, wild carrot was relatively less susceptible, and Bolero was the most resistant variety as measured by leaf blight severity ($P < 0.001$). Varieties demonstrated this general rank across all three experiments. However, wild carrot demonstrated the greatest variability in response (14.5 to 22.9%) to all isolates among experiments. Mean leaf chlorosis was greater in Heritage (18.4%), Enterprise (18.2%), and wild carrot (11.1%) than in Bolero (4.1%).

Statistical differences in leaf blight severity, petiole symptom severity, and chlorosis intensity were noted among isolates and varieties (Fig. 2). Significant variety by isolate interactions ($P \leq 0.001$) were detected among isolates and varieties for leaf blight severity (Fig. 2), petiole symptom severity, and chlorosis intensity (data not presented). Leaf blight severity ranged from 64.2% for isolate HST403 to 18.8% for isolate HST401 on Heritage. Conversely, on Bolero, the most aggressive isolate was E15D481, while the least aggressive was HST401.

In most cases, the susceptibility ranking of varieties did not change by isolate. However, in a few cases, the rank order of susceptibility among varieties varied for different *A. dauci* genotypes. This resulted in particular isolates displaying an affinity for cultivated varieties from which they were originally isolated prior to this study (Table 1 and Fig. 2). Isolates BST503 and BST493, obtained from variety Bolero, were not significantly more aggressive on the susceptible variety Heritage when compared to the resistant variety Bolero. However, isolate E15D481, isolated from Enterprise, was more aggressive on Bolero and Enterprise when compared to Heritage or the wild carrot. Isolates EST433, HST403, HRS437, and E15D481 demonstrated a significantly increased aggressiveness on the carrot variety from which they were collected when compared with inoculation on other varieties.

Fungicide sensitivity. Based on the homogeneity of variance of relative conidial germination, data were combined across all experiments for each fungicide. Statistically significant differences ($P = 0.002$) in EC₅₀ values were detected for *A. dauci* isolates for each of the three fungicides (Table 4). When tested against azoxystrobin, EC₅₀ values ranged from 0.03 to 0.30 µg/ml, and the mean value across the population was 0.08 µg/ml. Nineteen of the 22 isolates were highly sensitive (EC₅₀ ≤ 0.10 µg/ml). Conidial germination of one isolate, CST542, occurred at a greater concentration of azoxystrobin than the germination of conidia of all other isolates (Table 4). Of the eight isolates previously exposed to azoxystrobin, the conidia germination of only one, NJ182 with an EC₅₀ = 0.11 µg/ml, fell outside the highly sensitive range (≤0.10 µg/ml), but did not differ significantly from 0.10 µg/ml. Frequency

Table 3. Correlation coefficients^a for comparison of aggressiveness and fungicide sensitivity measurements for 22 isolates of *Alternaria dauci*

	Blight severity	Petiole severity	Chlorosis intensity	EC ₅₀ -AZ ^b	EC ₅₀ -CH
Blight severity	...				
Petiole severity	0.95	...			
Chlorosis intensity	0.90	0.93	...		
EC ₅₀ -AZ	-0.22	-0.20	-0.20	...	
EC ₅₀ -CH	-0.04	0.01	-0.10	0.14	...
EC ₅₀ -BC	-0.15	-0.17	0.06	0.19	-0.13

^a Data were combined data sets across repeated replicated experiments.

^b Mean estimated concentration of 50% inhibition of conidial germination for 22 isolates of *A. dauci*. AZ = azoxystrobin, CH = chlorothalonil, BC = boscalid.

Fig. 1. Foliar blight severity, petiole severity, and chlorosis intensity for each carrot variety used as differentials to examine variation in aggressiveness among *Alternaria dauci* isolates following inoculation. Data are presented for all *A. dauci* isolates tested combined for each variety. Bars represent the mean value for three replicates for each of 22 *A. dauci* isolates combined. Error bars represent Fisher's least significant difference at $P < 0.001$.

distribution of isolates showed a normal distribution of *A. dauci* isolates centered around a log concentration of -1.50 ($EC_{50} = 0.06$) with a single outlier CST542 (Fig. 3).

Conidial germination of *A. dauci* was generally more sensitive to chlorothalonil than to azoxystrobin (Table 4). Differences in mean EC_{50} values were noted among isolates ($P = 0.028$) with a range defined by isolates HST401 ($0.03 \mu\text{g/ml}$) and 04-68 ($0.23 \mu\text{g/ml}$). Mean value for the response to chlorothalonil across the population was $0.06 \mu\text{g/ml}$, and only isolates CST542, IND904, and 04-68 had EC_{50} values significantly higher than $0.06 \mu\text{g/ml}$. Of these isolates, 04-68 had an EC_{50} value 3.8 times greater than the popu-

lation average and 2.3 times greater than the next closest isolate (Table 4). Responses of isolates of *A. solani* did not differ significantly from the *A. dauci* population mean sensitivity to chlorothalonil. The frequency distribution graph of chlorothalonil data shows a population skewed to the left and centered on -1.80 log concentration units ($EC_{50} = 0.04$) (Fig. 3).

Population responses to boscalid were very different for *A. dauci* isolates as the mean EC_{50} values on boscalid-amended media were substantially higher than azoxystrobin and double that of chlorothalonil (Table 4). Mean EC_{50} values ranged from 0.03 to $0.65 \mu\text{g/ml}$ with a population mean of $0.13 \mu\text{g/ml}$. Any isolate with an

$EC_{50} \leq 0.20 \mu\text{g/ml}$ (the mean EC_{50} of $0.13 \mu\text{g/ml}$ plus the LSD of 0.07) was considered sensitive, and four *A. dauci* isolates displayed values significantly higher than $0.20 \mu\text{g/ml}$. Of these isolates, NJ182 had an $EC_{50} = 0.65 \mu\text{g/ml}$, more than three-fold higher than the $0.20 \mu\text{g/ml}$. Four isolates required fungicide concentrations significantly greater than $0.20 \mu\text{g/ml}$ to inhibit germination of half of the spores (Fig. 3).

Field application rates for each fungicide were compared to EC_{50} values calculated for each isolate in vitro. Rates applied for these fungicides in the field were 10 to 100 times more than the highest in vitro EC_{50} values. The range of sensitivity observed for these isolates did not fall

Fig. 2. Severity of *Alternaria* leaf blight, petiole severity, and chlorosis intensity of four carrot varieties inoculated with 22 isolates of *Alternaria dauci* and two isolates of *A. solani* under controlled conditions. Bars represent the means of three replicates and three repeated experiments. Diagonal bars are variety Bolero (resistant), black solid bars are Enterprise (moderate), horizontal bars are Heritage (susceptible), and white solid bars are wild carrot.

outside the normal use rates of each product for ALB control.

Coefficients of correlations among aggressiveness parameters revealed strong positive relationships between blight severity, petiole symptom severity, and chlorosis (Table 3). No strong correlations existed between measures of aggressiveness and fungicide response for each fungicide. Cross resistance between the fungicides was not observed as measured by the negative correlations noted among chlorothalonil, azoxystrobin, and boscalid (Table 3).

DISCUSSION

Diversity within *A. dauci* was studied using two sets of biologically relevant phenotypic characters in an attempt to identify and characterize variability among strains isolated from different geographic regions of carrot production. Research described in this study provides evidence for intra-species variability, most notably in aggressiveness on carrot varieties varying in disease susceptibility. Isolate response measured among differential carrot varieties revealed multiple examples of isolate by variety interactions. In addition, response of *A. dauci* to labeled fungicide chemistries appears well within the range expected for control. These findings provide useful information applicable to ALB resistance breeding programs, cultivar deployment strategies, and

evaluation of ALB fungicide management approaches.

Under controlled conditions, it was possible to efficiently inoculate carrot plants and consistently produce typical ALB symptoms allowing separation of isolates based on aggressiveness. This method appears amenable to both screening carrot varieties for response to ALB as well as utilizing carrot lines as differentials to examine variation in isolate aggressiveness. These findings are substantiated by results recorded for *A. brassicicola*, where distinctions in isolate aggressiveness were apparent on *Cakile maritima* (41). Similarly, differences in aggressiveness of *A. solani* on potato have been observed (29,42). In addition, mustard host differentials were established for studying variability of aggressiveness of *A. brassicae* (10,18). Collectively, it appears that diversity in isolate aggressiveness and resulting disease symptoms are evident within *A. dauci* as well as other related members of the genus.

Analysis of variety effects on *A. dauci* aggressiveness revealed significant isolate by variety interactions. Not only could isolates be categorized as more or less aggressive across all four varieties, but particular isolates demonstrated increased aggressiveness on or preference for particular host genotypes. Some individual isolates were most aggressive on the host genotype from which they were originally

isolated. Two isolates, EST433 and E15D481, were collected from the variety Enterprise prior to this study and were more aggressive on Enterprise when compared to Heritage, normally considered to be more susceptible. More interestingly, two isolates collected from the resistant cultivar Bolero caused as much or more severe disease symptoms on Bolero than on Enterprise or Heritage. Although these isolates were not among the most aggressive of the entire population, they did demonstrate tendencies of increased aggressiveness on Bolero.

Isolates HST401 and HST403 were collected from the same region, field, and cultivar and represented the range in aggressiveness across all isolates in each category. In our companion study regarding genetic diversity of *A. dauci*, we found >73% of the genetic diversity distributed within a region. Similar observations have been made for other fungi (11,15). This implies that strains with different levels of aggressiveness can be found within a region or field and that diversity within *A. dauci* may be present inside a small geographic or spatial distribution. Such evidence may suggest biologically important variation at the field or farm level, and more detailed hierarchical sampling of isolates by such stratifications is justified.

Chlorosis intensity varied considerably among isolates and also among varieties, resulting in large visible differences in chlorotic areas and damage to foliage. Differences in chlorosis intensity among isolates suggest that isolates may vary in the production or secretion of fungal toxins. This finding is in agreement with studies examining toxin production and host selective toxins in *Alternaria* spp. (40). Three of the four most aggressive isolates identified in this study also produced significantly higher chlorosis levels, resulting in substantial tissue damage. Differences in chlorotic response of carrot varieties here and evidence of complex biochemical diversity of compounds produced by *Alternaria* spp. demonstrated by other authors are suggestive of the involvement of toxic fungal metabolites (25,40).

Isolates showed a range of sensitivity responses within and among the fungicides azoxystrobin, chlorothalonil, and boscalid. EC₅₀ values revealed that chlorothalonil was more effective than azoxystrobin or boscalid at reducing conidial germination across the population as a whole. EC₅₀ values calculated for each fungicide and isolate combination were not outside the range of values according to other published studies (13,20,22). Results of *A. dauci* sensitivity to azoxystrobin and boscalid were very similar to results published for the baseline sensitivity of *A. solani*, and none of the *A. dauci* isolates here exhibited values comparable to *A. solani* isolates deemed less sensitive by Pasche et al. (19). To our knowledge, fun-

Table 4. Inhibition of germination of conidia of *Alternaria dauci* and *A. solani* isolates in response to exposure to three fungicides

Isolate	Azoxystrobin EC ₅₀ ^a (µg/ml)	Chlorothalonil EC ₅₀ (µg/ml)	Boscalid EC ₅₀ (µg/ml)
<i>Alternaria dauci</i>			
04-68	0.05	0.23	0.04
BST493	0.06	0.06	0.04
BST503	0.04	0.04	0.06
CDST611	0.04	0.03	0.08
CST536	0.10	0.04	0.28
CST542	0.30	0.10	0.04
E15D260	0.07	0.04	0.07
E15D481	0.05	0.03	0.08
EST433	0.03	0.04	0.09
HRS437	0.04	0.04	0.05
HST401	0.06	0.03	0.05
HST403	0.12	0.08	0.32
HST420	0.05	0.03	0.05
IND902	0.06	0.08	0.05
IND904	0.06	0.09	0.06
MF1002	0.07	0.04	0.03
MF1003	0.06	0.06	0.38
MNB014	0.09	0.05	0.15
MNB024	0.07	0.05	0.08
NJ170	0.10	0.04	0.10
NJ182	0.11	0.04	0.65
NJ201	0.06	0.05	0.04
<i>A. solani</i> 21	0.09	0.04	0.27
<i>A. solani</i> 98	0.13	0.06	0.33
Control	0	0	0
<i>P</i> value ^b	0.002	0.028	<0.001
LSD	0.04	0.03	0.07

^a The estimated concentration of each fungicide that inhibited the germination of 50% of the conidia (EC₅₀) was calculated for each isolate in vitro by regressing percent relative germination against the log concentration of the fungicide active ingredient (µg/ml). Each isolate-fungicide combination was replicated four times in each of three experiments.

^b Fisher's LSD. Data are the means from three replicated experiments.

gicide sensitivity values presented here represent baseline values useful for future comparisons if reduced performance should be observed with these fungicides in the field.

To date, we have neither observed nor know of any reports of reduced perform-

ance of these fungicide compounds in commercial carrot fields. It would appear that use of a high resistance risk compound such as the QoI fungicide azoxystrobin under labeled recommendations and in rotation with chlorothalonil has not resulted in significant reduced sensitivity

among isolates. We also did not see any positive cross resistance or cross reaction among fungicide compounds evaluated herein, as evidenced by very low or even negative correlation coefficients (Table 3). This is further confirmed by the absence of an obvious trend in fungicide response for those isolates previously exposed to chlorothalonil or azoxystrobin, as we failed to identify any reduced sensitivity in relation to previous fungicide exposure history. Identification of variation in response to these compounds does not seem to be of importance to current fungicide management programs, and it would be expected that the continued use of these compounds at prescribed rates under label recommendations, in rotation with other modes of action, will remain effective.

These results may have significant implications for management of ALB. Apparently, *A. dauci* is quite diverse in its aggressiveness and reaction to host genotype even at the field level, which may explain much of the variability in disease symptoms observed within a field, farm, or production region. Producers should be cautious about carrot variety selection and rotation of varieties across farms and regions, since host genotype may be an important selection factor for isolate aggressiveness. Future studies should establish universal host genotypes as differentials to distinguish among isolates and focus more closely on pathogen factors which contribute to differences in aggressiveness among isolates. Considering the number of known host-specific toxins in the genus *Alternaria*, it also seems appropriate to pursue the role of toxins produced by *A. dauci* in host-pathogen interactions.

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Fig. 3. Frequency distribution of in vitro response of 22 *Alternaria dauci* and two *A. solani* isolates to azoxystrobin, chlorothalonil, and boscalid labeled for *Alternaria* leaf blight control. The log concentration of 50% control of conidial germination for each fungicide was calculated for each isolate based on the mean of three replicates and two repeated experiments.

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