Phylogenetic Prediction of Alternaria Leaf Blight Resistance in Wild and Cultivated Species of Carrots

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ABSTRACT

Plant scientists make inferences and predictions from phylogenetic trees to solve scientific problems. Crop losses due to disease damage is an important problem that many plant breeders would like to solve, so the ability to predict traits like disease resistance from phylogenetic trees derived from diverse germplasm would be a significant approach to facilitate cultivar improvement. Alternaria leaf blight (ALB) is among the most devastating diseases of carrots (*Daucus* spp., Apiaceae) worldwide. Thus, new approaches to identify resistant germplasm to this disease are needed. In a study of 106 accessions of wild and cultivated *Daucus* and related genera, we determined plant height is the best explanatory variable to predict ALB resistance using a phylogenetic linear regression model. Using the estimated area under the disease progress curve, the most resistant species to ALB were the non-carrot relative *Ammi visnaga* (L.) Lam. and the wild carrot relative *D. crinitus* Desf. A permutation tail probability test was conducted considering phylogenetic signal to evaluate the strength of association between the *Daucus* phylogeny and ALB resistance. We found that species belonging to clade A, which includes carrots and other *Daucus* possessing $2n$ = 18, 20, or 22 chromosomes, are slightly more resistant to ALB than members of other clades of the *Daucus* phylogeny.

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Abbreviations: ALB, Alternaria leaf blight; AIC, Akaike information criterion; AUDPC, area under the disease progress curve; EBN, endosperm balance number; HSD, honestly significant difference; ML, maximum likelihood; PTP test, permutation tail probability test; PVY, *Potato virus Y*.

F EEDING a constantly increasing world population of 9.1 billion in year 2050, which is ~30% above today's population, will require increasing food production by 70% (FAO, 2009). This challenge can be addressed by employing plant breeding, with disease resistance breeding as a crucial component (Miedaner and Korzun, 2012). Alternative approaches for resistance breeding are needed, since there are increasing numbers of infectious crop diseases caused by fungi and oomycetes (Gawehns et al., 2013). Plant breeders have used germplasm resources for breeding based on assumptions and observations that they possess resistances to a variety of diseases. Therefore, germplasm characterization is of great importance to identify accessions with sufficient levels of disease resistance to improve yields, because resistance genes can be introgressed from wild species into elite varieties (Jansky, 2000; Gawehns et al., 2013; Piquerez et al., 2014).

A common method to identify disease-resistant accessions in germplasm collections is to screen genebank accessions exposed to the pathogen of interest in a greenhouse or field trial. However,

limitations exist, such as limited labor or experimental plots (Endresen, 2010). Other approaches, such as association studies between the trait of interest and ecogeographic factors, have been used to select germplasm of interest with varying levels of success. For example, Peeters et al. (1990) explored the association between salt tolerance and ecogeography to predict performance, obtaining only a weak association between these factors. Similarly, Hijmans et al. (2003) used a general linear model regression analysis to determine the association between frost tolerance of wild potatoes (*Solanum tuberosum* L.) with taxonomic, geographic, and ecogeographic factors, finding a strong association between frost tolerance and species, but that temperature at the accession collection site was a weak predictor of frost tolerance. Inconsistent and weak associations were also obtained between resistance to white mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] and ecogeography of collecting sites from 34 species of wild potato (Jansky et al., 2006). On the other hand, Endresen et al. (2011) investigated predictive association between biotic stresses and ecogeographic data for wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) landraces, concluding that ecogeographic distribution of resistance stem rust (caused by *Puccinia graminis* subsp. graminis Pers.:Pers.) in wheat and net blotch [caused by *Drechslera teres* (Sacc.) Shoemaker] in barley are associated with climatic factors.

Another plausible strategy to facilitate the accurate identification of germplasm with a trait of interest is to use taxonomic information of the species to focus on germplasm more likely to bear that trait, making the process of germplasm characterization more efficient by saving time and resources. Historically, it has been assumed that taxonomy has the ability to predict the presence of traits in a group for which the trait has been previously characterized in a representative subset of the group (Jansky et al., 2006; Spooner et al., 2009; Cai et al., 2011). The validation of this assumption was investigated by Jansky et al. (2008) inferring predictability of early blight [caused by *Alternaria solani* (E&M) Jones & Grout] in potato from the associations of taxonomic information and environmental variables, showing that monthly average precipitation in July was the most discriminating factor to predict resistance to early blight. In addition, Cai et al. (2011) conducted a test of taxonomic and biogeographic predictivity to resistance of *Potato virus Y* (PVY) in wild potato germplasm, finding that wild potato species with an endosperm balance number (EBN) of one shared stronger resistances to PVY than species with different EBN values. In addition, they showed that populations of wild potatoes from low elevations were more resistant than populations from high elevations, even though the mean of the predictors had a widespread and a low predictive value. Similarly, Spooner et al. (2009) studied the resistance to disease and insect pests in wild relatives of cultivated potato and concluded that a more effective approach

than taxonomic and biogeographic prediction is required for screening of germplasm collections.

Cultivated carrot (*Daucus carota* L. subsp. *sativus* Hoffm.) is one of the most popular and commonly consumed vegetables worldwide (Rubatsky et al., 1999). Modern carrot production uses mechanical harvesters, and strong healthy foliage is an important feature for an effective mechanical harvest. However, carrots are frequently affected by foliar diseases, including leaf blights caused by fungi *Alternaria dauci* (Kühn) Groves & Skolko and *Cercospora carotae* (Pass.) Solheim, and bacterial blight caused by *Xathomonas hortorum* (Pammel 1895) Dowson 1939 pv. *carotae* (Kendrick) Dye (Gugino et al., 2004; du Toit et al., 2005). Alternaria leaf blight (ALB), caused by *A. dauci*, has spread to all carrot production areas in the world and is considered the most destructive disease of carrots (Rubatsky et al., 1999; Vintal et al., 1999; Farrar et al., 2004), reducing yields by 40 to 60% (Ben-Noon et al., 2001). The most common methods of control include use of clean seed, crop rotation, cultivar selection, and fungicide applications. Partial resistance in some carrot cultivars offers limited protection but still requires frequent fungicide applications (Boedo et al., 2010). There has long been interest in genetic resistance and other nonfungicidal approaches to control of ALB (Farrar et al., 2004). Therefore, breeding for cultivars with higher levels of resistance to ALB, and durable resistance to *A. dauci*, is of major interest for carrot breeders (Boiteux et al., 1993; Boedo et al., 2008; Simon et al., 2008).

The latest comprehensive taxonomic monograph of *Daucus* by Sáenz Laín (1981) recognized 21 species divided into five sections: *Anisactis* DC., *Chrysodaucus* Thell., *Daucus* L., *Meoides* Lange, and *Platyspermum* DC. *Daucus* species maintained in germplasm banks could provide additional sources of genetic resistance for ALB that would benefit cultivated carrot. With this in mind, it will be useful for carrot breeders to identify resistant carrot genotypes by using predictivity approaches. The purpose of the present study is (i) to evaluate resistance to ALB among *Daucus* species, and (ii) to investigate the association between the *Daucus* clades and ALB scores. To this end, we employed the phylogenetic tree generated by Arbizu et al. (2014b), using 94 nuclear orthologs, and screened 106 accessions of wild and cultivated carrots. We conducted an evaluation of carrot vigor and ALB disease resistance in the field, then developed a trait evolution model using the *Daucus* maximum likelihood (ML) tree (Arbizu et al., 2014b) and a phylogenetic linear regression model. The most significant explanatory variable was predicted by using the ALB scores. Finally, to determine the strength of association between ALB resistance and the classification of carrots, a permutation tail probability (PTP) test was conducted, employing the phylogenetic signal as the criterion.

MATERIALS AND METHODS

Plant Material

We evaluated 91 accessions of *Daucus* and 15 accessions of the related genera *Ammi*, *Astrodaucus*, *Caucalis*, *Oenanthe*, *Orlaya*, *Pseudorlaya*, *Rouya*, *Torilis*, and *Turgenia* for a total of 106 accessions collected from 21 countries (Supplemental Table S1). All accessions were obtained from the United States National Plant Germplasm System, maintained at the North Central Regional Plant Introduction Station in Ames, IA. Further details of the accessions examined in this study are available at the Germplasm Resources Information Network (GRIN, https://npgsweb.ars-grin.gov/gringlobal/search.aspx).

Field Experimental Design and Disease Phenotyping

All 106 accessions were direct seeded by hand in 1-m × 3-m observation plots at the University of Wisconsin Hancock Agricultural Research Station in Hancock, WI, with two replications per accession. Natural ALB infestation occurs on carrots in this research station; therefore, plots were not artificially inoculated. Nitrogen fertilizer was applied at the beginning and middle of the growing season. Plots were weeded by hand and hoe, and plants were hand thinned to 5 to 6 cm in the row, leaving approximately 50 to 60 carrots in each plot. An ALB susceptible cultivar, 'Heritage', was used as infection plots that are reliably attacked by naturally occurring populations of *A. dauci*. In addition, 'Bolero', a less susceptible cultivar, was also included as a control. Presence of *A. dauci* conidia was confirmed by Tas (2016) following the protocol described by Strandberg (1983). Subjective ALB ratings were scored for each accession plot by examining leaves in 10 random sites within each plot six times over the entire growing season using the following scale: 0 = no visible disease damage, 1 = up to 25% disease damage, 2 = 26 to 50%, 3 = 51 to 75%, 4 = >75%. Plant height and plant width characters were scored in the field 50 d after sowing by measuring 10 plants per plot, capturing the normal range of variation. Briefly, plant height was scored using a ruler from the base of the plant to the highest point. Leaves that snapped at their base or were near the ground were not considered to measure plant width; measurements were scored at the widest part. All evaluations were recorded by the same individual.

Statistical Analysis

We analyzed our disease scores with R version 3.3.1 (R Core Team, 2016). Means were calculated using the *ddply* function in the *plyr* package (Wickman, 2011). A descriptive statistical analysis was conducted to verify the mean, median, standard deviation, and range of values. Box plots were used to visualize comparisons across accessions and to check for outliers that may represent erroneous entries. Accessions were classified into their corresponding species names according to the most recent molecular and morphological studies (Arbizu et al., 2014a, 2014b, 2016a, 2016b; Spooner et al., 2014). A quantitative summary of ALB intensity over the growing season was determined with the area under the disease progress curve (AUDPC). Values of AUDPC were calculated using the *audpc* function in the *Agricolae* package version 1.2-4 (de Mendiburu, 2016) in R. We also conducted an ANOVA of the AUDPC values to evaluate differences between species. The AUDPC mean comparisons were conducted by using Tukey's honestly significant difference (HSD) test with the function *HSD.test* in the *Agricolae* package.

A phylogenetic linear regression was employed to predict ALB scores in wild and cultivated species of carrots considering a ML tree obtained by Arbizu et al. (2014b), plant height, and plant width. First, we fitted six phylogenetic linear regression models (lambda, Brownian Motion, Kappa, Ornstein-Uhlenbeck model with the ancestral state at the root having the stationary distribution, Ornstein-Uhlenbeck model with an ancestral state to be estimated at the root, and Early Burst) using the *phylolm* function in the *phylolm* package (Ho and Ané, 2014) in R, with only ALB scores recorded 93 d after sowing (harvest time) as the dependent variable, and plant height and plant width as predictors (i.e., explanatory variables). We then performed model selection based on the Akaike information criterion (AIC). That is, the model with the lowest AIC value was chosen. A backward stepwise model selection for phylogenetic linear model was conducted using the function *phylostep* in the *phylolm* package. Finally, since we were interested in determining whether certain clades of our ML tree contain entries that possess higher levels of ALB resistance, a comparison among *Daucus* clades (Fig. 1) was performed as follows: (i) clade A vs. clade B + outgroup, (ii) clade B vs. clade A + outgroup, (iii) clade A vs. clade B, and (iv) subclade A′ vs. the remaining species in clade A + clade B + outgroup. Comparisons were performed using a PTP test to determine whether our data contain phylogenetic structure (Baum and Smith, 2013). The ALB scores were permuted 1000 times using R, randomly assigning states to taxa. Permuted datasets were subjected to phylogenetic signal estimation using the *phylolm* function. Phylogenetic signal was then visualized using the function *hist* in R. If phylogenetic signal for the original dataset (not permuted), which is Pagel's $\Lambda = 0.98$, is higher than all of the permuted datasets, the original dataset can be said to have significant phylogenetic signal, determining differences among the clades that were compared. Then, for those comparisons that were significant, an ANOVA of the ALB scores recorded 93 d after sowing (harvest time) was performed, grouping the accessions according to the clade to which they belonged (Fig. 1). Briefly, *Daucus* is contained within two main clades, A and B, and within clade A, there is a subclade named A′ comprising the subspecies of *D. carota*, *D. syrticus* Murb., and *D. sahariensis* Murb., all with $2n = 18$ chromosomes (Fig. 1). Finally, ALB mean comparisons among clades were conducted by using Tukey's HSD test with the function *HSD.test* in the *Agricolae* package.

RESULTS

Alternaria Leaf Blight Screening

Severity of ALB damage was visually confirmed, as well as the presence of *A. dauci* fungi in the research plot by microscopic evaluation by Tas (2016). Supplemental Table S1 lists the disease scores recorded for each accession. At harvest time, complete resistance (disease score = 0) was observed in all four accessions of species *D. crinitus*. In addition, 11 accessions of *Daucus*, and two related species [*Ammi visnaga* (L.) Lam. and *Torilis arvensis* (Huds.) Link]
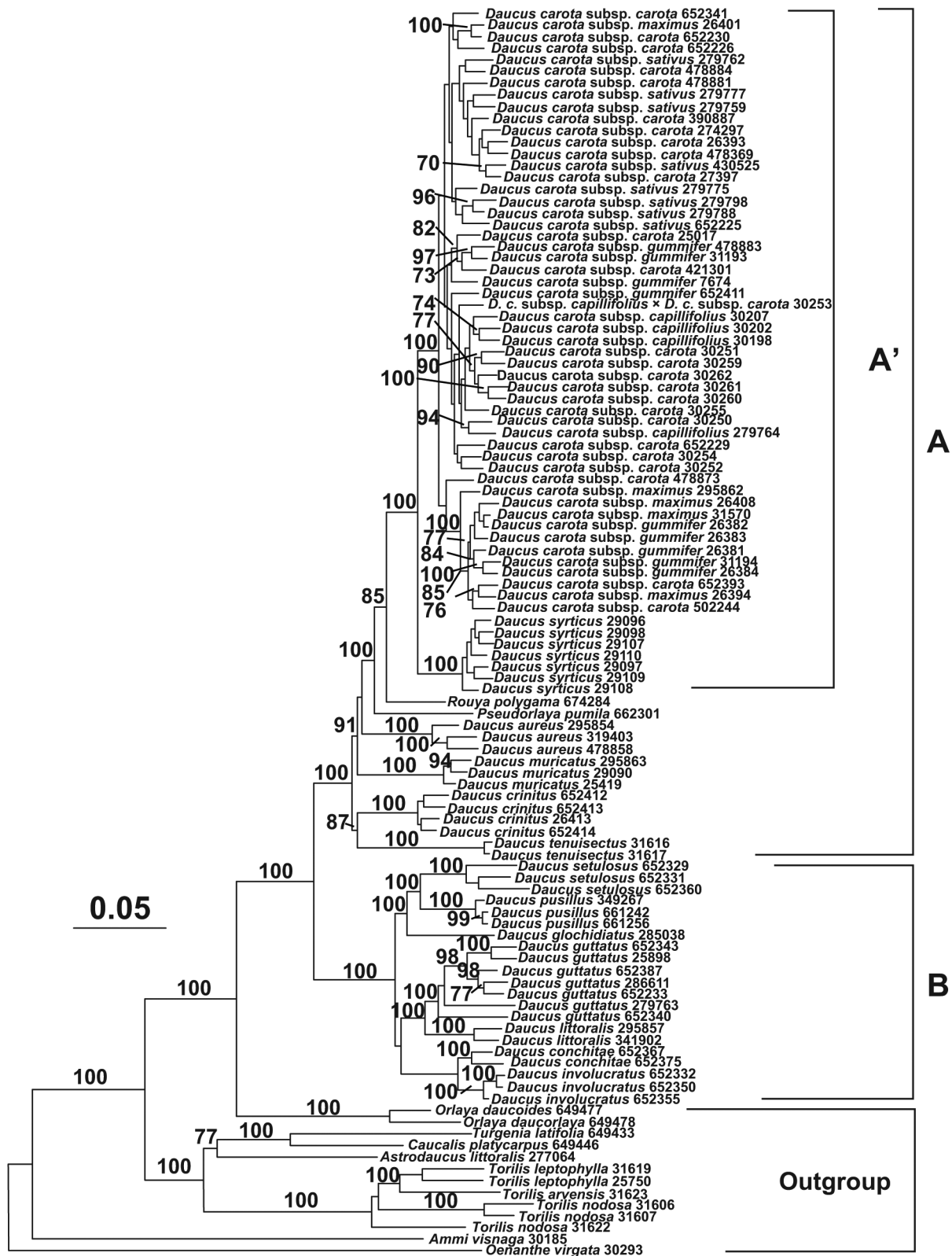
Fig. 1. Phylogeny of *Daucus* obtained from Arbizu et al. (2014b) using a maximum likelihood analysis based on 94 nuclear orthologs and 107 accessions. Species identities have been corrected in the *Daucus carota* complex and *D. syrticus* in subclade A′, *Rouya polygama* in clade A, and members of the *D. guttatus* complex in clade B, according to Arbizu et al. (2014a, 2016a, 2016b).

showed partial resistance (disease score = 2). Thirty-four accessions had ALB symptoms on >75% of the foliar area (disease score = 4), demonstrating very low levels of resistance against *A. dauci*. Further, 77 d after sowing (i.e.,

~10 wk), 75 accessions had ALB symptoms on <50% of the foliar area, indicating partial resistance. However, at harvest time (93 d after sowing), they were predominantly scored with a value of 3 or 4, showing a progress on the

spread of ALB. Variation among accessions for ALB score for two subspecies of *D. carota* was visualized using box plots (Fig. 2). Two members of the *D. carota* complex, subsp. *capillifolius* and subsp. *sativus*, illustrate the interaccession variation (Fig. 2) that exists in our dataset. Species were ranked according to AUDPC value (Table 1) to determine the resistance to ALB. Two species, *Amni visnaga* and *D. crinitus*, showed very high levels of resistance; on the other hand, *D. littoralis* Sibthorp & Smith and *Pseudorlaya pumila* (L.) Grande possess the lowest significant levels of resistance to ALB. Among the *D. carota* complex (Fig. 3), subsp. *capillifolius* and subsp. *maximus* exhibited the highest and subsp. *sativus* the lowest significant levels of susceptibility (Fig. 3). *Daucus syrticus*, which is the closest species to the subspecies of the *D. carota* complex (Arbizu et al., 2016a), had a higher AUDPC value compared with those subspecies (Fig. 3).

Phylogenetic Linear Regression Analyses

The six models of trait evolution tested in the present study had AIC values ranging from 221.5 to 227.5. The Ornstein-Uhlenbeck model with an ancestral state to be estimated at the root (OUfixed) had the lowest AIC value. Therefore, we continued our stepwise model selection for phylogenetic linear model with OUfixed, obtaining plant height as the only significant explanatory variable (AIC = 220.4); plant width was not considered as significant to

be included in the model. Supplemental Table S2 lists the predicted ALB scores, using plant height as the predictor (i.e., explanatory variable). Predicted ALB scores ranged from 3.1 to 4.0, indicating that no partial resistance was observed or >50% of disease damage was present in accessions of *Daucus* and related genera.

Comparison among *Daucus* clades (Materials and Methods) to determine the presence of ALB resistance revealed that only two comparisons were significantly different, with the phylogenetic signal of the permuted dataset consistently lower than the phylogenetic signal with the original dataset: (i) clade A vs. clade B + outgroup, and (ii) clade A vs. clade B. Similarly, a multiple comparison procedure (Tukey's HSD, significance level = 0.1) showed that entities of clade A are slightly more resistant to ALB when it is compared with members that belong to (i) clade B + outgroup ($p = 0.08$), and (ii) clade B ($p = 0.03$). As mentioned above, *D. crinitus*, which belongs to clade A, has the highest resistance to ALB. To determine if the significant difference found within clade A vs. clade B + outgroup and vs. clade B is not influenced only by the very high resistance of *D. crinitus*, we excluded

Table 1. Area under the disease progress curve (AUDPC) in ascending order for 25 species and five subspecies.

Species	AUDPC of Alternaria leaf blight†
Ammi visnaga	4f
Daucus crinitus	6.69f
Astrodaucus littoralis	37.75ef
Daucus glochidiatus	49ef
Daucus carota subsp. *capillifolius*	55.75ef
Orlaya daucorlaya	57ef
Daucus carota subsp. *maximus*	58.25ef
Torilis arvensis	59ef
Oenanthe virgata	60.5ef
Daucus carota subsp. *carota*	62.88ef
Caucalis platycarpos	67def
Daucus guttatus	70.46de
Daucus carota subsp. *gummifer*	70.66de
Torilis nodosa	72.42cde
Daucus carota hybrid	72.5cde
Daucus carota subsp. *sativus*	78.97cde
Daucus pusillus	82.42bcde
Rouya polygama	88.25bcde
Torilis leptophylla	88.75bcde
Daucus syrticus	88.79bcde
Daucus involucratus	92.83bcde
Daucus aureus	94.25bcde
Daucus setulosus	97.33abcde
Daucus tenuisectus	101.88abcde
Daucus conchitae	108abcd
Daucus muricatus	120.33abcd
Orlaya daucoides	121abcd
Turgenia latifolia	129.5abc
Daucus littoralis	129.88ab
Pseudorlaya pumila	157.75a

† Values followed by the same letter were not significantly different at $p = 0.05$.
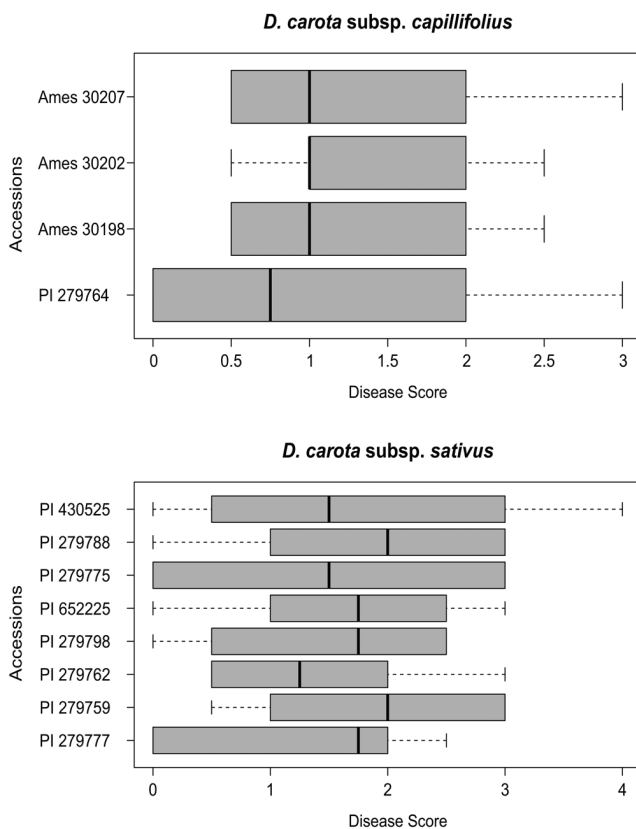

Fig. 2. Box plots showing interaccession variation for Alternaria leaf blight scores for *Daucus carota* subsp. *capillifolius* and subsp. *carota*.
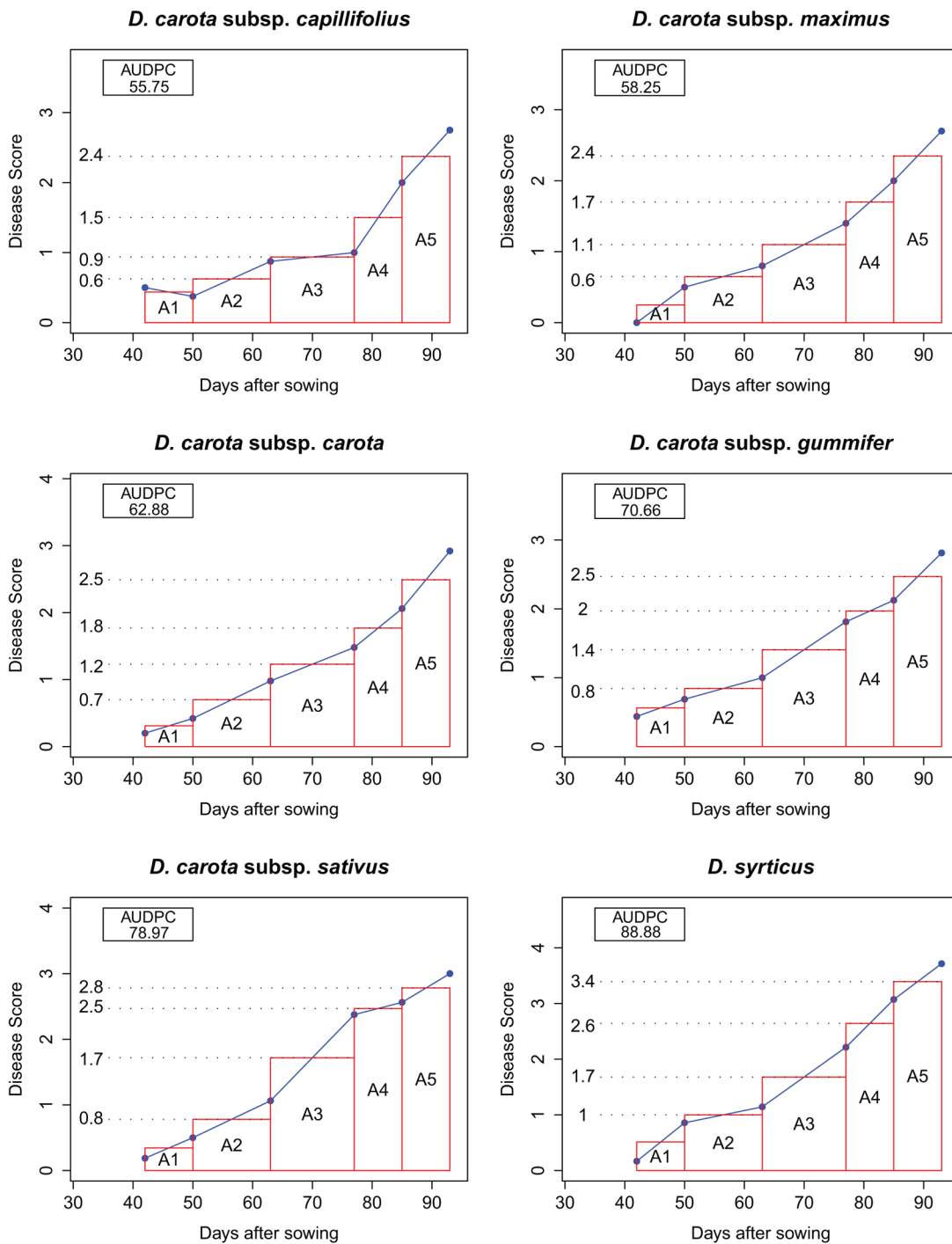
Fig. 3. Measurement of Alternaria leaf blight throughout a growing period (i.e., area under the disease progress curve [AUDPC]) of 93 d for subspecies of *D. carota* and *D. syrticus*.

it from a new round of analyses using the PTP test. When *D. crinitus* is taken out of the analysis, the phylogenetic signal of the permuted dataset is still lower than the phylogenetic signal with the original dataset. However, this value without *D. crinitus* tends to be closer to the phylogenetic signal of the original dataset when comparing clade A vs. clade B + outgroup. In addition, the ANOVA test indicated that resistance to ALB of clade A is not significantly different from other clades ($p = 0.27$). On the contrary, the ANOVA test showed differences for ALB resistance among members of clade A vs. clade B considering a significance level of 0.1 ($p = 0.06$).

DISCUSSION

A phylogenetic tree is a hypothesis of evolutionary histories based on one or more criteria (here, nuclear orthologs). Hypotheses of evolutionary histories have been used by the scientific community for various purposes (Rønsted et al., 2012; Baum and Smith, 2013) and have been assumed to be useful to predict the presence of traits of interest

in a group for which the trait has been characterized in only a subset of the group (Jansky et al., 2006; Spooner et al., 2009; Cai et al., 2011), such as disease resistance (Khiutti et al., 2015). To date, predictivity studies have been conducted in potato (Jansky et al., 2006, 2008, 2009; Spooner et al., 2009; Cai et al., 2011; Chung et al., 2011; Limantseva et al., 2014; Khiutti et al., 2015), the family Amaryllidaceae (Rønsted et al., 2012), the genus *Euphorbia* (Ernst et al., 2016), and barley (Endresen, 2010; Endresen et al., 2011). However, this is the first phylogenetic predictivity investigation on *Daucus* or any other member of the Apiaceae.

Calculation of the AUDPC has been widely used by epidemiologists to assess quantitative resistance in many crop cultivars (Jeger and Viljanen-Rollinson, 2001). Since ALB is a polycyclic disease (many infection cycles in a season), it is recommended to calculate the AUDPC to summarize the disease scores (Fry, 1978). We here identified *A. visnaga* ($2n = 20, 22$) and *D. crinitus* ($2n = 22$) as the most resistant species to ALB. These two species do not belong to the primary genepool for carrot breeding, which possess $2n = 18$. However, it is worth exploring the high antimicrobial capacity of *D. crinitus* against *Candida albicans* (C.P. Robin) Berkhout and *Staphylococcus aureus* Rosenbach (Bendiabdellah et al., 2013), supporting the traditional medicinal application of this plant. As a result, we consider that, similar to the study of Camadro et al. (2008), *D. crinitus* should be tested to establish the feasibility of hybridization with the cultivated carrot. On the other hand, we report that *D. littoralis* and *Pseudorlaya pumila* are the two least resistant species. Interestingly, we found that, despite the higher resistance of *Ammi visnaga*, the ALB resistance level as a whole in the outgroup clade of *Daucus* (Fig. 1) was not high. This may be explained by the virulence of the pathogen *A. dauci*, which was reported to be capable of infecting not only wild carrots, but also other wild Apiaceae (Neergaard, 1977; Soteros, 1979; Boedo et al., 2012). Among the subspecies of *D. carota* in clade A′ (Fig. 1), subsp. *capillifolius* and subsp. *maximus* present significantly higher levels of resistance to ALB than the other subspecies, demonstrating new sources of resistance to ALB for carrot breeding programs. Previous studies showed that interspecific crosses between subsp. *capillifolius* and other subspecies have been successful (McCollum, 1975, 1977). A recent study (Arbizu et al., 2016a) also proposed the use of subsp. *maximus* as a new source of genes for the development of new carrot cultivars. Further, subsp. *carota* possess the second highest level of resistance. This result is congruent with the study conducted by Tas (2016), where 812 accessions of carrots (mainly subsp. *carota*) were evaluated for ALB disease resistance, concluding that complete resistance was not found, with <40 accessions exhibiting partial resistance to ALB (disease scores ≤2).

The ability to predict disease resistance to ALB in carrots would benefit farmers through reduced use of agrochemicals. In addition, plant scientists may find useful our results showing the predictor of carrot height, as stimulated here by the studies of Turner et al. (2016, 2017). Additionally, our analysis predicted that no strong resistance (disease score = 0 or 1, Supplemental Table S2) to ALB existed on carrot germplasm examined here. A plausible explanation is the presence of a high disease pressure in the research field where this study was conducted. Plant height has been commonly used on several statistical models as a key variable to evaluate yield in corn (*Zea mays* L.; Mourtzinis et al., 2013). Other studies indicate that the severity of a foliar disease caused by *Septoria tritici* Desm. shows a relationship with date of heading and plant height in winter wheat (Tavella, 1978; Lovell et al., 1997). Relative to this study, further research is needed to determine if other top size traits in carrots can help to predict resistance to ALB.

A detailed screening of traits from germplasm, like the data generated in the present study, is frequently used in association mapping studies (Clotault et al., 2010; Jourdan et al., 2015). We here employed phylogenetic signal as a criterion to test the strength of association because it is a statistical dependence among the traits of species due to their phylogenetic relationships (Revell et al., 2008). We only found significant association between clade A vs. clade B + outgroup and clade A vs. clade B regarding resistance to ALB. Similarly, Tas (2016) studied possible associations between ALB severity and domestication status, flowering habit, leaf glossiness, storage root color, and geographic origin and found only a slight correlation between increased ALB severity in purple-, red-, or white-colored root relative to orange and yellow roots. Studies in potato showed weak or inconsistent association of disease scores and taxonomy or geography (Jansky et al., 2006, 2008, 2009; Spooner et al., 2009; Cai et al., 2011; Chung et al., 2011; Limantseva et al., 2014; Khiutti et al., 2015). Perhaps resistance to ALB evolved rapidly so that no phylogenetic signal could be detected. Tas (2016) reported little correlation between ALB disease phenotypes of carrots and their geographic origins. However, using the phylogenetic signal proposed here would be useful, and stronger levels of association may be obtained.

Carrot is the second most popular vegetable in the world after potato (Heywood, 2014), and it is the economically most valuable member of the Apiaceae. Extensive research is being conducted to understand the genetic control of ALB resistance in carrots (Boiteux et al., 1993; Simon and Strandberg, 1998; Le Clerc and Pawelec, 2009; Le Clerc et al., 2015). Here we provide evidence that taxa belonging to clade A (Fig. 2, *D. carota* subsp. *capillifolius* and subsp. *maximus*) may provide new sources of resistance to ALB.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

References

Arbizu, C., K.R. Reitsma, P.W. Simon, and D.M. Spooner. 2014a. Morphometrics of *Daucus* (Apiaceae): A counterpart to a phylogenomic study. Am. J. Bot. 101:2005–2016. doi:10.3732/ajb.1400252

Arbizu, C., H. Ruess, D. Senalik, P.W. Simon, and D.M. Spooner. 2014b. Phylogenomics of the carrot genus (*Daucus*, Apiaceae). Am. J. Bot. 101:1666–1685. doi:10.3732/ajb.1400106

Arbizu, C.I., S.L. Ellison, D. Senalik, P.W. Simon, and D.M. Spooner. 2016a. Genotyping-by-sequencing provides the discriminating power to investigate the subspecies of *Daucus carota* (Apiaceae). BMC Evol. Biol. 16:234. doi:10.1186/s12862-016-0806-x

Arbizu, C.I., P.W. Simon, F. Martínez-Flores, H. Ruess, M.B. Crespo, and D.M. Spooner. 2016b. Integrated molecular and morphological studies of the *Daucus guttatus* complex (Apiaceae). Syst. Bot. 41:479–492. doi:10.1600/036364416X691948

Baum, D.A., and S.D. Smith. 2013. Tree thinking: An introduction to phylogenetic biology. Roberts and Co., Greenwood Village, CO.

Bendiabdellah, A., M. El Amine Dib, N. Meliani, A. Muselli, D. Nassim, B. Tabti, and J. Costa. 2013. Antibacterial activity of *Daucus crinitus* essential oils along vegetative life of the plant. J. Chem. 2013:1–7. doi:10.1155/2013/149502

Ben-Noon, E., D. Shtienberg, E. Shlevin, H. Vintal, and A. Dinoor. 2001. Optimization of chemical suppression of *Alternaria dauci*, the causal agent of Alternaria leaf blight in carrots. Plant Dis. 85:1149–1156. doi:10.1094/PDIS.2001.85.11.1149

Boedo, C., S. Benichoua, R. Berruyerc, S. Bersihanda, A. Dongoa, P. Simoneaua et al. 2012. Evaluating aggressiveness and host range of *Alternaria dauci* in a controlled environment. Plant Pathol. 61:63–75. doi:10.1111/j.1365-3059.2011.02494.x

Boedo, C., R. Berruyer, M. Lecomte, S. Bersihand, M. Briard, V. Le Clerc et al. 2010. Evaluation of different methods for the characterization of carrot resistance to the alternaria leaf blight pathogen (*Alternaria dauci*) revealed two qualitatively different resistances. Plant Pathol. 59:368–375. doi:10.1111/j.1365-3059.2009.02218.x

Boedo, C., V. Le Clerc, M. Briard, P. Simoneau, M. Chevalier, S. Georgeault, and P. Poupard. 2008. Impact of carrot resistance on development of the *Alternaria* leaf blight pathogen (*Alternaria dauci*). Eur. J. Plant Pathol. 121:55–66. doi:10.1007/s10658-007-9241-6

Boiteux, L.S., P.T. Della Vecchia, and F.J.B. Reifschneider. 1993. Heritability estimate for resistance to *Alternaria dauci* in carrot. Plant Breed. 110:165–167. doi:10.1111/j.1439-0523.1993.tb01230.x

Cai, X.K., D.M. Spooner, and S.H. Jansky. 2011. A test of taxonomic predictivity: Resistance to *Potato virus Y* in wild relatives of the cultivated potato. Phytopathology 101:1074–1080. doi:10.1094/PHYTO-02-11-0060

Camadro, E.L., M.A. Cauhépé, and P.W. Simon. 2008. Compatibility relations between the edible carrot *Daucus carota* and *D. pusillus*, a related wild species from the Argentinian Pampas. Euphytica 159:103–109. doi:10.1007/s10681-007-9462-y

Chung, Y.S., K. Holmquist, D.M. Spooner, and S.H. Jansky. 2011. A test of taxonomic and biogeographic predictivity: Resistance to soft rot in wild relatives of cultivated potato. Phytopathology 101:205–212. doi:10.1094/PHYTO-05-10-0139

Clotault, J.E., E. Geoffriau, E. Lionneton, M. Briard, and D. Peltier. 2010. Carotenoid biosynthesis genes provide evidence of geographical subdivision and extensive linkage disequilibrium in the carrot. Theor. Appl. Genet. 121:659–672. doi:10.1007/s00122-010-1338-1

de Mendiburu, F. 2016. agricolae: Statistical procedures for agricultural research. R package version 1.2-4. R Foundation for Statistical Computing. http://CRAN.R-project.org/package=agricolae (accessed 22 Oct. 2016).

du Toit, L.J., F.J. Crowe, M.L. Derie, R.B. Simmons, and G.Q. Pelter. 2005. Bacterial blight in carrot seed crops in the Pacific Northwest. Plant Dis. 89:896–907. doi:10.1094/PD-89-0896

Endresen, D.T.F. 2010. Predictive association between trait data and ecogeographic data for Nordic barley landrace. Crop Sci. 50:2418–2430. doi:10.2135/cropsci2010.03.0174

Endresen, D.T.F., K. Street, M. Mackay, A. Bari, and E. De Pauw. 2011. Predictive association between biotic stress traits and eco-geographic data for wheat and barley landraces. Crop Sci. 51:2036–2055. doi:10.2135/cropsci2010.12.0717

Ernst, M., C.H. Saslis-Lagoudakis, O.M. Grace, N. Nilsson, H.T. Simonsen, J.W. Horn, and N. Rønsted. 2016. Evolutionary prediction of medicinal properties in the genus *Euphorbia* L. Sci. Rep. 6:30531. doi:10.1038/srep30531

FAO. 2009. How to feed the world in 2050. FAO. http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf (accessed 15 Nov. 2016).

Farrar, J.J., B.M. Pryor, and R.M. Davis. 2004. Alternaria diseases of carrot. Plant Dis. 88:776–784. doi:10.1094/PDIS.2004.88.8.776

Fry, W.E. 1978. Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato late blight. Phytopathology 68:1650–1655. doi:10.1094/Phyto-68-1650

Gawehns, F., B.J.C. Cornelissen, and F.L.W. Takken. 2013. The potential of effector-target genes in breeding for plant innate immunity. Microb. Biotechnol. 6:223–229. doi:10.1111/1751-7915.12023

Gugino, B.K., J. Carroll, J. Chen, J. Ludwig, and G. Abawi. 2004. Carrot leaf blight diseases and their management in New York. Cornell Univ. Coop. Ext. http://vegetablemdonline.ppath.cornell.edu/factsheets/Carrot_Leaf_Blight.pdf (accessed 25 Nov 2016).

Heywood, V.H. 2014. The socio-economic importance of the Apiales. J. Fac. Pharm. Istanbul Univ. 44:113–130.

Hijmans, R.J., M. Jacobs, J.B. Bamberg, and D.M. Spooner. 2003. Frost tolerance in wild potato species: Unraveling the predictivity of taxonomic, geographic, and ecological factors. Euphytica 130:47–59. doi:10.1023/A:1022344327669

Ho, L.T., and C. Ané. 2014. A linear-time algorithm for Gaussian and non-Gaussian trait evolution models. Syst. Biol. 63:397–408. doi:10.1093/sysbio/syu005

Jansky, S., R. Simon, and D.M. Spooner. 2009. A test of taxonomic predictivity: Resistance to the Colorado potato beetle in wild relatives of cultivated potato. J. Econ. Entomol. 102:422–431. doi:10.1603/029.102.0155

Jansky, S.H. 2000. Breeding for disease resistance in potato. Plant Breed. Rev. 19:69–155.

Jansky, S.H., R. Simon, and D.M. Spooner. 2006. A test of taxonomic predictivity: Resistance to white mold in wild relatives of cultivated potato. Crop Sci. 46:2561–2570. doi:10.2135/cropsci2005.12.0461

Jansky, S.H., R. Simon, and D.M. Spooner. 2008. A test of taxonomic predictivity: Resistance to early blight in wild relatives of cultivated potato. Phytopathology 98:680–687. doi:10.1094/PHYTO-98-6-0680

Jeger, M.J., and S.L.H. Viljanen-Rollinson. 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theor. Appl. Genet. 102:32–40. doi:10.1007/s001220051615

Jourdan, M., S. Gagné, C. Dubois-Laurent, M. Maghraoui, S. Huet, A. Suel et al. 2015. Carotenoid content and root color of cultivated carrot: A candidate-gene association study using an original broad unstructured population. PLoS One 10:e0116674. doi:10.1371/journal.pone.0116674

Khiutti, A., D.M. Spooner, S.H. Jansky, and D.A. Halterman. 2015. Testing taxonomic predictivity of foliar and tuber resistance to *Phytophthora infestans* in wild relatives of potato. Phytopathology 105:1198–1205. doi:10.1094/PHYTO-02-15-0046-R

Le Clerc, V., S. Marques, A. Suel, S. Huet, L. Hamama, L. Voisine et al. 2015. QTL mapping of carrot resistance to leaf blight with connected populations: Stability across years and consequences for breeding. Theor. Appl. Genet. 128:2177–2187. doi:10.1007/s00122-015-2576-z

Le Clerc, V., and A. Pawelec. 2009. Genetic architecture of factors underlying partial resistance to alternaria leaf blight in carrot. Theor. Appl. Genet. 118:1251–1259. doi:10.1007/s00122-009-0978-5

Limantseva, L., N. Mironenko, O. Shuvalov, O. Antonova, A. Khiutti, L. Novikova et al. 2014. Characterization of resistance to *Globodera rostochiensis* pathotype Ro1 in cultivated and wild potato species accessions from the Vavilov Institute of Plant Industry. Plant Breed. 133:660–665. doi:10.1111/pbr.12195

Lovell, D.J., S.R. Parker, T. Hunter, D.J. Royle, and R.R. Coker. 1997. Influence of crop growth and structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat. Plant Pathol. 46:126–138. doi:10.1046/j.1365-3059.1997.d01-206.x

McCollum, G.D. 1975. Interspecific hybrid *Daucus carota* × *D. capillifolius*. Bot. Gaz. 136:201–206. doi:10.1086/336803

McCollum, G.D. 1977. Hybrids of *Daucus gingidium* with cultivated carrots (*D. carota* subsp. *sativus*) and *D. capillifolius*. Bot. Gaz. 138:56–63. doi:10.1086/336899

Miedaner, T., and V. Korzun. 2012. Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathology 102:560–566. doi:10.1094/PHYTO-05-11-0157

Mourtzinis, S., F.J. Arriaga, K.S. Balkcom, and B.V. Ortiz. 2013. Corn grain and stover yield prediction at R1 growth stage. Agron. J. 105:1045–1050. doi:10.2134/agronj2012.0393

Neergaard, P. 1977. Seed pathology. Halsted Press, New York. doi:10.1007/978-1-349-02842-9

Peeters, J.P., H.G. Wilkes, and N.W. Galwey. 1990. The use of ecogeographic data in the exploitation of variation from gene banks. Theor. Appl. Genet. 80:110–112. doi:10.1007/BF00224023

Piquerez, S.J.M., S.E. Harvey, J.L. Beynon, and V. Ntoukakis. 2014. Improving crop disease resistance: Lessons from research on *Arabidopsis* and tomato. Front. Plant Sci. 5:671. doi:10.3389/fpls.2014.00671

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Revell, L.J., L.J. Harmon, and D.C. Collar. 2008. Phylogenetic signal, evolutionary process, and rate. Syst. Biol. 57:591–601. doi:10.1080/10635150802302427

Rønsted, N., M.R.E. Symonds, T. Birkholm, S.B. Christensen, A.W. Meerow, M. Molander et al. 2012. Can phylogeny predict chemical diversity and potential medicinal activity of plants? A case study of amaryllidaceae. BMC Evol. Biol. 12:182. doi:10.1186/1471-2148-12-182

Rubatsky, V.E., C.F. Quiros, and P.W. Simon. 1999. Carrots and related vegetable Umbelliferae. CABI Publishing, New York.

Sáenz Laín, C. 1981. Research on *Daucus* L. (Umbelliferae). Anales del Jardín Botánico de Madrid 37:481–533.

Simon, P.W., R.E. Freeman, J.V. Vieira, L.S. Boiteux, M. Briard, T. Nothnagel et al. 2008. Carrot. In: J. Prohens, M.J. Carena and F. Nuez, editors, Handbook of crop breeding, Vol. 1: Vegetable breeding. Springer-Verlag, GmBH, Heidelberg, Germany p. 327–357.

Simon, P.W., and J.O. Strandberg. 1998. Diallel analysis of resistance in carrot to Alternaria leaf blight. J. Am. Soc. Hortic. Sci. 123:412–415.

Soteros, J.J. 1979. Pathogenicity and control of *Alternaria radicina* and *A. dauci* in carrots. N. Z. J. Agric. Res. 22:191–196. doi:10.1080/00288233.1979.10420860

Spooner, D.M., M.P. Widrlechner, K.R. Reitsma, D.E. Palmquist, S. Rouz, Z. Ghrabi-Gammar et al. 2014. Reassessment of practical subspecies identifications of the USDA *Daucus carota* L. germplasm collection: Morphological data. Crop Sci. 54:706–718. doi:10.2135/cropsci2013.04.0231

Spooner, D.M., S.H. Jansky, and R. Simon. 2009. Tests of taxonomic and biogeographic predictivity: Resistance to multiple disease and insect pests in wild relatives of cultivated potato. Crop Sci. 49:1367–1376. doi:10.2135/cropsci2008.04.0211

Strandberg, J.O. 1983. Infection and colonization of inflorescences and mericarps of carrot by *Alternaria dauci*. Plant Dis. 67:1351–1353. doi:10.1094/PD-67-1351

Tas, P.M. 2016. Evaluating resistance to *Alternaria dauci* and related traits among diverse germplasm of *Daucus carota*. Ph.D. diss., Univ. of Wisconsin-Madison, Madison, WI.

Tavella, C.M. 1978. Date of heading and plant height of wheat varieties, as related to septoria leaf blotch damage. Euphytica 27:577–580. doi:10.1007/BF00043184

Turner, S.D., P.L. Maurizio, W. Valdar, B.S. Yandell, and P.W. Simon. 2017. Dissecting the genetic architecture of shoot growth in carrot (*Daucus carota* L.) using a diallel mating design. bioRxiv 115519 doi:10.1101/115519

Turner, S.D., N.D. Miller, E.P. Spalding, and P.W. Simon. 2016. Image-based phenotyping and Bayesian analysis of a diallel mating design for top size in carrot (*Daucus carota* L.). Nat. Assoc. Plant Breeders. https://www.plantbreeding.org/files/napb/annual-meeting-2016-abstract-book.pdf (accessed 25 Nov 2016).

Vintal, H., E. Ben-Noon, E. Shlevin, U. Yermiyahu, D. Shtienberg, and A. Dinoor. 1999. Influence of rate of soil fertilization on alternaria leaf blight (*Alternaria dauci*) in carrots. Phytoparasitica 27:193–200. doi:10.1007/BF02981458

Wickman, H. 2011. The split-apply-combine strategy for data analysis. J. Stat. Softw. 40:1–29.