Incidence and Pathogenicity of *Didymella americana* on Table Beet in New York

Niloofer Vaghefi, School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology Section, Cornell University, Geneva, NY 14456; **Julie R. Kikkert**, Cornell Cooperative Extension, Canandaigua, NY 14424; and **Sarah J. Pethybridge**, School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology Section, Cornell University, Geneva, NY 14456

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New York is the second largest producer of table beet (*Beta vulgaris* ssp. *vulgaris*) in the United States. In 2015, a *Didymella* sp. was isolated from table beet fields across New York. Foliar symptoms included dark sunken spots, large necrotic patches with indefinite margins, and necrotic, round-to-oval lesions (Fig. 1). The objective was to identify the *Didymella* sp. associated with table beet, and assess pathogenicity.

Diseased leaves ($n = 10$ to 20) were collected from each of four fields in Batavia, Kendall, Elba, and Keeseville, NY, in 2015. If pycnidia were observed, a single pycnidium was transferred to a tube containing sterile distilled water (SDW), and vortexed. The spore suspension was spread over 2% (w/v) water agar (WA), and incubated at room temperature ($23 \pm 2^\circ\text{C}$) under a 12-h photoperiod. A single spore was transferred to clarified V8 medium (CV8) the following day. If no pycnidia were observed, leaves were surface-sterilized in 0.08% (v/v) sodium hypochlorite for 30 s, and rinsed in SDW three times. Symptomatic tissue sections (3×3 mm) from lesion margins were transferred to WA, and incubated as above. After two days, resultant mycelia were transferred to CV8, and single-conidium cultures were established. Isolation frequencies of *Didymella* sp. were 27% (Batavia), 56% (Kendall), 66% (Elba), and 9% (Keeseville).

Six isolates were selected for species identification (Table 1). Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The 28S subunit (LSU) and internal transcribed spacer (ITS) of the nrDNA, partial β -tubulin (*tub2*), and RNA polymerase II second largest subunit (*rpb2*) were amplified (Gorny et al. 2016), and sequenced (GenBank Accession Nos. KY070278 to KY070301; Table 1). LSU and ITS sequences of all isolates were identical to *D. americana* reference strains: CBS 185.85 (Chen et al. 2015) and NRRL 66312 (Gorny et al. 2016). The *rpb2* sequences of all isolates, except for P-020, were identical to those of the reference strains. The *tub2* sequences showed the highest variability (up to 5 bp) compared to CBS 185.85 and NRRL 66312. Bayesian analysis (Ronquist and Huelssenbeck 2003) conducted on the concatenated alignment (LSU, 878; ITS, 460; *tub2*, 270; and *rpb2*, 596) supported identification as *D. americana* (Fig. 2).

The pathogenicity of four isolates (K-001, K-004, R63-012, and P-020) was assessed on table beet variety ‘Ruby Queen’. Prior to inoculation, 6-week-old plants were incubated in a mist chamber, at 18°C with a 12-h photoperiod for 2 weeks. Conidial suspensions (10^5 spores/ml + 0.2% (v/v) Tween-20) were ob-

tained from 15-day-old CV8 cultures. Approximately 10 ml of inoculum was sprayed onto each of 15 replicate plants per isolate.

FIGURE 1

Foliar disease symptoms associated with *Didymella americana* on table beet in New York: (A) dark sunken lesions; (B) necrotic lesions with dark concentric rings, which are also typical of *Pleospora betae*; (C, D) Pycnidia of *Didymella americana* on lesions.

FIGURE 2

Reconstructed phylogeny of *Didymella americana* isolates from table beet in New York, 2015, based on a 50% majority rule consensus tree using Bayesian Inference (BI) analysis of the combined LSU, ITS, *tub2*, and *rpb2* sequences. The BI posterior probabilities are presented above the branch. The bar indicates the number of substitutions per site. The tree is rooted with *Phoma herbarum* (CBS 615.75 = PD 73/665 = IMI 199779).

Corresponding author: S. J. Pethybridge. Email: sjp277@cornell.edu.

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TABLE 1
Didymella strains used in phylogenetic analysis. GenBank accessions produced in this study are indicated in bold.

Strain number ^x	Species	Host	Location	LSU	GenBank Accessions		
					ITS	<i>rpb2</i>	<i>tub2</i>
CBS 128.93 = PD 79/140 (R)	<i>Didymella</i> <i>chenopodii</i>	<i>Chenopodium quinoa</i>	Peru	GU238055	GU237775	KT389602	GU237591
CBS 185.85 = PD 80/1191 (R)	<i>D. americana</i>	<i>Zea mays</i>	USA	GU237990	FJ426972	KT389594	FJ427088
CBS 391.93 = PD 80/87	<i>D. protuberans</i>	<i>Spinacia oleracea</i>	The Netherlands	GU238015	GU237858	KT389621	GU237566
F-003	<i>D. americana</i>	<i>Beta vulgaris</i> ssp. <i>vulgaris</i>	Keeseville, NY	KY070284	KY070278	KY070296	KY070290
K-001	<i>D. americana</i>	<i>B. vulgaris</i> ssp. <i>vulgaris</i>	Kendall, NY	KY070286	KY070280	KY070298	KY070292
K-004	<i>D. americana</i>	<i>B. vulgaris</i> ssp. <i>vulgaris</i>	Kendall, NY	KY070285	KY070279	KY070297	KY070291
NRRL 66312	<i>D. americana</i>	<i>Phaseolus lunatus</i>	NY	KU948539	KT264181	KU948540	KT264183
R63-012	<i>D. americana</i>	<i>B. vulgaris</i> ssp. <i>vulgaris</i>	Batavia, NY	KY070288	KY070282	KY070300	KY070294
R63-023	<i>D. americana</i>	<i>B. vulgaris</i> ssp. <i>vulgaris</i>	Batavia, NY	KY070289	KY070283	KY070301	KY070295
P-020	<i>D. americana</i>	<i>B. vulgaris</i> ssp. <i>vulgaris</i>	Elba, NY	KY070287	KY070281	KY070299	KY070293

^xCBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; NRRL = United States Department of Agriculture – Agricultural Research Service, Culture Collection; PD = Plant Protection Service, Wageningen, the Netherlands. R indicates reference isolates according to Chen et al. 2015.

FIGURE 2

Didymella americana symptoms on table beet (A) 20 days, (B) 25 days, and (C) 30 days after inoculation; (D) pycnidia in concentric circles; (E) chlamydospores, and (F) conidiospores formed on V8; and a 10-day-old colony on (G) potato dextrose agar, (H) V8 agar, and (I) oatmeal agar (scale bars = 20 µm).

Fifteen control plants were sprayed with the same volume of SDW + 0.2% Tween-20. After inoculation, plants were covered with clear plastic bags for 3 days. Disease incidence (DI) was defined as the number of diseased plants / total number of plants $\times$ 100. Four weeks after inoculation, in order to complete Koch's postulates, isolations were conducted from lesions as previously described. The trial was conducted in a completely randomized design and repeated once.

In trial 1, necrotic lesions analogous to field samples were observed on two of the plants inoculated with K-001 (DI = 13%) and one inoculated with R63-012 (DI = 6%; Fig. 3). Isolations gave rise to *D. americana*. In trial 2, two plants inoculated with K-001 and four with P-020 and K-004 showed symptoms. Isolations from plants inoculated with P-020 and K-004 gave rise to *Pleospora betae*, which is known to be seedborne (Harveson et al. 2009). Isolations from the two plants inoculated with K-001 resulted in *D. americana* (DI = 13%).

This information augments knowledge of the fungi that contribute to foliar disease of table beet in New York. Low disease incidence following inoculations suggests *D. americana* may be a weak and/or opportunistic pathogen of table beet. In the United States, *D. americana* has been reported on corn, soybean (Aveskamp et al. 2009), wheat (Boerema et al. 2004), and lima bean

(Gorny et al. 2016), all found in typical rotations with table beet in western New York. Although the incidence and severity of disease caused by *D. americana* on table beet is not sufficient to warrant implementation of control strategies specific to *D. americana*, this provides useful information for assessing inoculum risk for susceptible field crops cultivated in rotation with table beet.

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