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Cercospora beticola causes leaf and stem spots of New Zealand spinach (*Tetragonia tetragonoides*) in Brazil

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Summary. New Zealand (NZ) spinach (*Tetragonia tetragonoides*) is an important leafy vegetable crop in Brazil and other countries. This plant is used as a substitute for common spinach because it is rustic and tolerant to tropical and subtropical environmental conditions. It is often affected by a leaf and stem spot disease, which increases in severity during the warm climatic periods. *Cercospora tetragoniae* has been reported as the cause of this disease, but this is based on an early description of a *Cercospora*-like species on this host in Argentina, first named *Cercosporina tetragoniae* but later recombined into *Cercospora*. In the present study, isolates of *Cercospora*-like fungi were obtained from NZ spinach and beetroot plants in Brazil, and a multigene molecular study including the *act, cal, gapdh, his3,* ITS, and *tef1-α* regions was carried out to identify the causative pathogen. Additionally, morphological and cross inoculation studies were conducted with isolates obtained from diseased plants. The pathogen was confirmed as *Cercospora beticola*, a common and harmful pathogen of beetroot (*Beta vulgaris*). Cross-inoculations of isolates obtained from NZ spinach and beetroot showed that the isolates are infective to both hosts. This increases knowledge of epidemiology and management of this important disease. Several attempts to re-collect samples from the type locality in Argentina failed. NZ spinach is no longer grown at La Plata (Argentina), the type locality of *C. tetragoniae*. Therefore, the task of re-collecting the pathogen is still pending, for epitype designation and for a full clarification of the taxonomic status of *C. tetragoniae*. The possibility of the pathogen being seed-transmitted has been assessed, and evidence obtained justifies further assessment of this aspect.

Keywords. *Aizoaceae*, *Amaranthaceae*, Cercosporoid fungi, *Beta vulgaris*.

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INTRODUCTION

New Zealand (NZ) spinach (*Tetragonia tetragonoides, Aizoaceae*) is widely cultivated as a leafy vegetable. It is a semi-herbaceous, branched, succulent plant with a creeping growth habit and fleshy, triangular-shaped dark green leaves (Filgueira, 2000). It has a broad natural distribution ranging from sandy shorelines of eastern Asia to Australia and New Zealand, despite its

common name suggesting it to have a New Zealand origin (CABI, 2018). NZ spinach has been introduced and has become invasive in coastal habitats of Chile, Hawaii, Florida and California (CABI, 2018). The predominant "spinach" cultivated in Brazil is NZ spinach, and the area under cultivation was estimated to be approx. 1700 hectares, making it the fifth most important leafy vegetable in Brazil (Vilela and Luengo, 2017).

Little has been published on plant pathogens attacking NZ spinach. Only the leaf-spot fungus *Cercospora tetragoniae* (*Mycosphaerellaceae*) has been reported in association with this plant in Brazil (Viégas, 1945; Hino and Tokeshi, 1978; Mendes and Urben, 2019). In the first Brazilian report, Viégas (1945) provided a detailed description and illustration of the fungus on *T. tetragonoides* (as *T. exapansa*), based on samples collected in Campinas and São Paulo (state of São Paulo, Brazil). However, no pure cultures from Viégas's specimens or based on Hino and Tokeshi's records have been deposited in public culture collections (Viégas, 1945; Hino and Tokeshi, 1978).

In August 2017, NZ spinach plants growing in the Infectarium, a disease-demonstration garden on the Universidade Federal de Viçosa campus (Viçosa, Minas Gerais, Brazil) were observed with leaf and stem spot symptoms. These symptoms increased in incidence and severity as the host plants aged and as temperatures and humidity increased (Figure 1, A and B). The diseases started as few spots on few isolated leaves, and these became progressively more abundant on leaves and also occurred on tender stems. At final stages, most stems were girdled by coalescing necroses, and stem dieback led to death of aerials part of most affected plants. Slow recovery, from remaining root systems and seeds from the previous season, was observed in cool months in the same plots. This disease progression was repeated in following years, both in the Infectarium and in commercial vegetable gardens of Viçosa and in Rio de Janeiro (municipalities of Petrópolis, Nova Friburgo, and elsewhere).

This disease commonly occurs in these areas and wherever NZ spinach is cultivated in Brazil, and is the most damaging disease attacking these vegetable crops in several Brazilian states (R. W. Barreto, personal observations). Specimens of diseased NZ spinach were collected for preliminary examination, and a dematiaceous hyphomycete was regularly found associated with the necrotic host tissues. The present paper outlines results of an investigation that aimed to provide clarification of the aetiology of this disease observed in Brazil.

MATERIALS AND METHODS

Isolation and morphological characterization of the pathogen

A sample of NZ spinach with leaf and stem spot symptoms at various stages of development was collected for laboratory examination, and selected parts with disease symptoms were dried in a plant press. Later, samples of NZ spinach bearing identical symptoms were obtained from a vegetable growing area in the separate geographic region of Petrópolis (state of Rio de Janeiro). A representative herbarium specimen from each source was deposited in the local herbarium of the Universidade Federal de Viçosa (Acc. Nos. VIC 44406, VIC 44456, VIC 44457, VIC 44458). A cercosporoid fungus was directly isolated from sporulating areas of lesions, by transfer of individual conidia onto potato dextrose agar (PDA; Kasvi) in Petri plates, using a sterile fine pointed needle, and pure cultures were obtained. Representative isolates of the fungus collected from NZ spinach at Viçosa and Petrópolis were deposited in the local culture collection (Acc. Nos. COAD 2380, COAD 2477), as well as pure cultures of *Cercospora beticola* obtained from diseased beetroot plants (*Beta vulgaris*) from the same localities (Acc. Nos. COAD2476, COAD2478).

In addition, fungal structures were scraped from the surfaces of the diseased NZ spinach tissues with a scalpel, and were mounted in lactoglycerol for microscope observations. Biometric data was compiled from at least 30 measurements of conidiophores and conidia. The samples were examined and images were captured using a light microscope (Olympus model BX 51) equipped with a digital image capture system (Olympus Q-Color 3™ camera). Morphology of colonies and colony pigmentation were observed after 7 d growth on PDA at 25°C under two fluorescent white and one NUV black light lamps (for 12 h each day), located 35 cm above the culture plates. Colony colour (Rayner, 1970) was assessed.

Detection of pathogen in seeds

A "blotter test" was carried out to preliminarily verify the speculation in Japan, in Table 1 of Hino and Tokeshi (1978), and in the present study in Brazil, that *Cercospora* spp. (including *C. tetragoniae*) occurring on plants in both countries may have been introduced at the same time through the transfer of "plant tissues or seeds". Packets of NZ spinach 'seed' (NZ spinach fruits which are used and treated as seeds for this crop), sold under three Brazilian brand names (Feltrin, Isla, Topseed) were acquired. Additionally, seeds obtained from plots at the Infectarium where NZ spinach showed disease symptoms

Table 1. Isolates included in the phylogenetic study reported in this paper. The newly generated sequences are underlined, and ex-type strains included in this study are indicated in bold font.

Fungal species	Strain number	Host name	GenBank Acc. No.					
			ACT	CAL	GAPDH	HIS3	ITS	tef1
Cercospora apii	**CBS 116455**	*Beta vulgaris*	AY840450	AY840417	MH496173	AY840384	AY840519	AY840486
	CCTU 1086	*Cynanchum acutum*	KJ885928	KJ885767	MH496176	KJ886089	KJ886411	KJ886250
	CCTU 1215	*Cynanchum acutum*	KJ885929	KJ885768	MH496177	KJ886090	KJ886412	KJ886251
Cercospora apiicola	**CBS 116457**	*Apium* sp.	AY840467	AY840434	-	AY840401	NR119526	AY840503
	CPC 11642	*Apium* sp.	DQ233393	DQ233419	-	DQ233441	DQ233341	DQ233367
Cercospora armoraceae	**CBS 250.67**	*Armoracia rusticana*	JX143053	JX142807	MH496181	JX142561	JX143545	JX143299
	CBS 555.71	*Coronilla varia*	JX143058	JX142812	MK531772	JX142566	JX143550	JX143304
Cercospora asparagi	AS16-01	*Asparagus officinalis*	KY549091	KY549093	-	KY549095	KY549097	KY549101
	AS16-02	*Asparagus officinalis*	KY549092	KY549094	-	KY549096	KY549098	KY549102
Cercospora beticola	**CBS 116456**	*Beta vulgaris*	AY840458	AY840425	MH496185	AY840392	NR_121315	AY840494
	CCTU 1088	*Sonchus asper*	KJ885945	KJ885784	MH496191	KJ886106	KJ886428	KJ886267
	CCTU 1089	*Plantago lanceolata*	KJ885946	KJ885785	MH496189	KJ886107	KJ886429	KJ886268
	COAD 2380	*Tetragonia tetragonoides*	OQ944120	MH469231	OQ944127	OQ944129	MG780415	MN517124
	COAD 2476	*Beta vulgaris*	OQ944121	MT561868	OQ944124	OQ944130	MT555312	MN517125
	COAD 2477	*Tetragonia tetragonoides*	OQ944122	MT561866	OQ944125	OQ944131	MT555313	-
	COAD 2478	*Beta vulgaris*	OQ944123	MT561867	OQ944126	OQ944128	MT555314	MT561869
Cercospora celosiae	CBS 132600	*Celosia argentea* var. *Cristata*	JX143080	JX142834	-	JX142588	JX143570	JX143326
Cercospora coniogrammes	**CBS 132634**	*Coniogramme japonica* var. *Gracilis*	JX143095	JX142849	-	JX142603	NR_147260	JX143341
	CPC 25070	*Hypolepis mitis*	KT037599	KT037466	-	-	KT037517	KT037477
Cercospora cf. *citrulina*	CBS 119395	*Musa* sp.	JX143089	JX142843	-	JX142597	EU514222	JX143335
	CBS 132669	*Musa* sp.	JX143090	JX142844	-	JX142598	-	JX143336
Cercospora gamsiana	CCTU 1074	*Malva neglecta*	KJ885943	KJ885782	MH496276	KJ886104	KJ886426	KJ886265
	CCTU 1035	*Malva sylvestris*	KJ885940	KJ885779	MH496277	KJ886101	KJ886423	KJ886262
	CCTU 1109	*Malva sylvestris*	KJ885948	KJ885787	MH496278	KJ886109	KJ886431	KJ886270
Cercospora cf. *malloti*	MUCC 575	*Cucumis melo*	JX143138	JX142892	-	JX142646	JX143625	JX143384
	MUCC 787	*Mallotus japonicus*	JX143139	JX142893	-	JX142647	JX143626	JX143385
Cercospora mercurialis	**CBS 550.71**	*Mercurialis perennis*	JX143141	JX142895	-	JX142649	JX143628	JX143387
	CBS 551.71	*Mercurialis ovata*	JX143142	JX142896	-	JX142650	JX143629	JX143388
	IRAN 3949C	*Mercurialis annua*	MT843620	MT843648	MT843715	MT843673	MT804381	MT843593
Cercospora cf. *richardiicola*	CCTU 1004	*Bidens tripartita*	KJ886036	KJ885875	MH496295	KJ886197	KJ886519	KJ886358
	CBS 132627	*Ajuga multiflora*	JX143153	JX142907	-	JX142661	JX143640	JX143399
Cercospora samambaiae	**CPC 24673**	*Thelypteris dentata*	KT037596	KT037463	-	KT037555	KT037514	KT037474
	COAD 1427	*Pteris deflexa*	KT037590	KT037457	-	-	KT037508	KT037468
Cercospora cf. *sigesbeckiae*	CBS 132641	*Persicaria orientalis*	JX143166	JX142920	-	JX142674	JX143653	JX143412
	IRAN 3832C	*Glycine max*	MT186115	MT186086	MT186131	MT186076	MT338034	MT186099
	IRAN 3837C	*Sesamum indicum*	MT186120	MT186088	MT186136	MT186080	MT338039	MT186104
	VIC 39069	*Commelina benghalensis*	-	KY287250	-	-	KY351634	KY287251

(Continued)

Table 1. (Continued).

Fungal species	Strain number	Host name	GenBank Acc. No.					
			ACT	CAL	GAPDH	HIS3	ITS	tef1
Cercospora tetragoniae	HL T-1	*Tetragonia tetragonoides*	LC579811	LC579812	-	-	-	LC579813
	HL Tt-1	*Tetragonia expansa*	LC589278	LC589277	-	-	MT095118	LC589279
Cercospora violae	**CBS 251.67**	*Viola tricolor*	JX143250	JX143004	MH496322	JX142758	JX143737	JX143496
	CPC 5368	*Viola odorata*	JX143251	JX143005	-	JX142759	JX143738	JX143497
Cercospora zeae-maydis	CBS 117756	*Zea mays*	DQ185097	DQ185109	-	DQ185121	DQ185073	DQ185085
	CBS 117757	*Zea mays*	DQ185098	DQ185110	-	DQ185122	DQ185074	DQ185086
Cercospora zeina	**CPC 11995**	*Zea mays*	DQ185105	DQ185117	-	DQ185129	DQ185081	DQ185093
	CPC 11998	*Zea mays*	DQ185106	DQ185118	-	DQ185130	DQ185082	DQ185094
Cercospora cf. *zinniae*	CBS 132624	*Zinnia elegans*	JX143272	JX143026	-	JX142780	JX143756	JX143518
	CBS 132676	*Zinnia elegans*	JX143273	JX143027	-	JX142781	JX143757	JX143519
Cercospora zebrina	CCTU 1039	*Alhagi camelorum*	KJ886062	KJ885901	MH496323	KJ886223	KJ886545	KJ886384
	CCTU 1185	*Vicia* sp.	KJ886066	KJ885905	MH496333	KJ886227	KJ886549	KJ886388
	CCTU 1012	*Medicago* sp.	KJ886061	KJ885900	MH496328	KJ886222	KJ886544	KJ886383
Septoria provencialis	CBS 118910	*Eucalyptus* sp.	JX143276	JX143030	JX142538	JX142784	DQ303096	JX143522

were harvested and were included in this study. Polystyrene germination boxes were cleaned internally with 70% ethanol and then each lined with two layers of sterile blotter paper and were moistened with sterile water. Seeds were surface disinfected by immersion in 70% alcohol for 1 min, followed by immersion in 1% sodium hypochlorite for 1 min, and then rinsing in sterile tap water. The seeds were then placed within the boxes 1 2 cm spacings, using sterile forceps. An aliquot of a 5 ppm dichlorophenoxyacetate (2,4-D) solution was then added to each box to stop seed germination. The boxes were then maintained for 7 d at 25°C under a 12 h photoperiod. The seeds were then examined under a stereoscopic microscope to assess for presence of fungal conidiophore fascicles and conidia. Confirmation of the identity of the fungi was through observation of morphology, as described above. Fifty seeds from each source were evaluated in this preliminary assessment.

DNA extraction, PCR amplification and sequencing

Representative single conidium isolates of the fungus obtained from necrotic NZ spinach tissues and from beetroot (see Table 1) were grown on PDA (Kasvil) at 25°C under a 12 h photoperiod for 1 week, and genomic DNA was extracted, as described by Duarte *et al.* (2016). The primers ITS4 and ITS5 (White *et al.*, 1990) were used to amplify the ITS region and the 5.8S rRNA gene. Additionally, five informative gene fragments were amplified, including actin (*act*), calmodulin (*cal*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), histone3 (*his3*), and translation elongation factor 1-alpha (*tef1-α*), with the respective primer pairs ACT-512F/ACT-783R (Carbone and Kohn, 1999), CAL-228F/CAL2Rd (Carbone and Kohn, 1999), GDF1/GDR1 (Guerber *et al.*, 2003), CYLH3F/CYLH3R (Crous *et al.*, 2004), and EF1-728F/EF1-986R (Carbone and Kohn, 1999).

PCR products were analyzed on 2% agarose electrophoresis gels stained with GelRed™ (InstantAgarose™) in a 1× TAE buffer, and were visualized under UV light to check for amplification extent and purity. PCR products were purified and sequenced by Macrogen Inc. (http://www.macrogen.com).

Phylogenetic analyses

The resulting nucleotide sequences were edited with the DNA Dragon software (https://www.dnadragon.com/index.php). All sequences were checked

manually, and nucleotides with ambiguous positions were clarified using primer sequences in both directions. Resulting sequences were deposited in GenBank (www.ncbi.nlm.nih.gov), and are described in Table 1. Sequences obtained from GenBank datasets and the novel sequences generated during this study were aligned using MEGA X (Kumar *et al*., 2018). Appropriate models were selected for each gene partition using MrModeltest ver. 2.3 (Nylander 2004). Based on the results of MrModeltest, the evolutionary model K80+G was applied to *act*; K80 was used with the ITS partitions; HKY+G was applied to *cal* and *his3* regions; and GTR+G was applied to *gapdh* partition.

Phylogenetic analyses were based on a concatenated dataset of *act*, *cal*, *gapdh*, *his3*, and ITS regions, which were combined using SequenceMatrix (Vaidya *et al*., 2011). To assess relationships between isolates, two independent algorithms were used: Maximum-Likelihood (ML) and Bayesian inference (BI), both present in the CIPRES web portal (Miller *et al*., 2010). ML analyses used RAxML v. 8.2.12 (Stamatakis, 2014), and bootstrap values (BS) were determined after 1000 bootstrap samples. BI analyses were performed using MrBayes ver. 3.2.1 (Ronquist *et al*., 2012) and applying the substitution models listed above. The Markov chain Monte Carlo (MCMC) method was used to search for the best tree topology. Two simultaneous and independent analyses were performed, each with four chains. MCMCs were run for 5,000,000 generations, and trees were sampled every 500th generation, until convergence was reached. The first 25% of trees were discarded as the burn-in phase. The remaining 7,500 trees from each run generated the consensus tree, from which posterior probabilities values (PPs) were obtained.

The resulting trees were visualized in FigTree (Rambaut, 2012). ML and BI topologies were compared, and the BI topology was adopted. The BI tree was exported to graphic software, and BS values greater than 70%, or PP values greater than 0.95, were maintained. *Septoria provencialis* (isolate CBS 118910) served as the outgroup for the phylogenetic analyses.

Pathogenicity tests

Inocula of isolates COAD 2380 (obtained from NZ spinach) and COAD 2476 (from beetroot) were cultivated using the "biphasic method" (Jackson *et al*., 1996) with modifications. Aliquots (100 mL each) of potato dextrose (PD) were placed in separate 250 mL capacity flasks, and were then autoclaved for 20 min at 121°C. After cooling, each flask was seeded with five 1 cm diam. disks obtained from the margin of an actively growing PDA colony of one of the isolates. The flasks were then placed on an orbital shaker (Marconi®-MA420) set at 130 rpm and 25 +/- 2°C, and then incubated for 30 d. The flasks were drained and the mycelium in each was separated. The mycelium masses were suspended in sterile water, triturated with a mortar, and then transferred onto potato carrot agar (PCA; Johnston and Booth, 1983) in Petri plates. The plates were then incubated under the conditions described above. After 14 d, the surface of each plate was flooded with 10 mL of sterile water, scraped with a rubber spatula, and the resulting material was filtered through cheesecloth. The resulting conidium suspensions were adjusted to 1.4×10^6 conidia mL^{-1}.

Four one-month-old healthy NZ spinach plants, grown from seeds (Isla Sementes) in separate 2 L capacity pots containing a mixture of pasteurized soil and manure, were used in pathogenicity tests. Two plants were sprayed until runoff with the conidium suspension and two plants were sprayed with sterile tap water as inoculation controls.

Additionally, two healthy 2-month-old beetroot plants were also inoculated with isolate COAD 2380 conidium suspension, and two NZ spinach plants were inoculated with isolate COAD 2476. All the plants were then left in a dew chamber for 48 h, then transferred to a greenhouse bench, where they were observed each day for disease symptoms.

RESULTS AND DISCUSSION

Molecular identification of isolates

Phylogenetic studies combining *act*, *cal*, *gapdh*, *his3*, and ITS regions were based on 50 *Cercospora* taxa and the outgroup *Septoria provencialis*. The combined alignment comprised 1846 characters with gaps (187 for *act*, 237 for *cal*, 686 for *gapdh*, 306 for *his3*, and 430 for ITS). Previous studies have shown that it is important to include the *cal* and *gapdh* regions in analyses of such *Cercospora* taxa (Groenewald *et al*., 2013; Bakhshi and Zare, 2020). The combined data obtained in the present study confirmed this.

Phylogenetic analyses indicated that the *Cercospora* isolates obtained from *T. tetragonoides* (isolates COAD 2380 and COAD 2477) and *B. vulgaris* (isolates COAD 2476 and COAD 2478) formed a monophyletic and well supported clade with *C. beticola* (BS/PP = 96/1) (Figure 1). The clade containing the isolates under study, as well as the *C. beticola* isolates, was separated from *C. apii*, *C. apiicola*, *C. asparagi*, and *C. gamsiana* (Figure 1). These results demonstrate that the fungus from NZ spinach examined in this study was *C. beticola*.

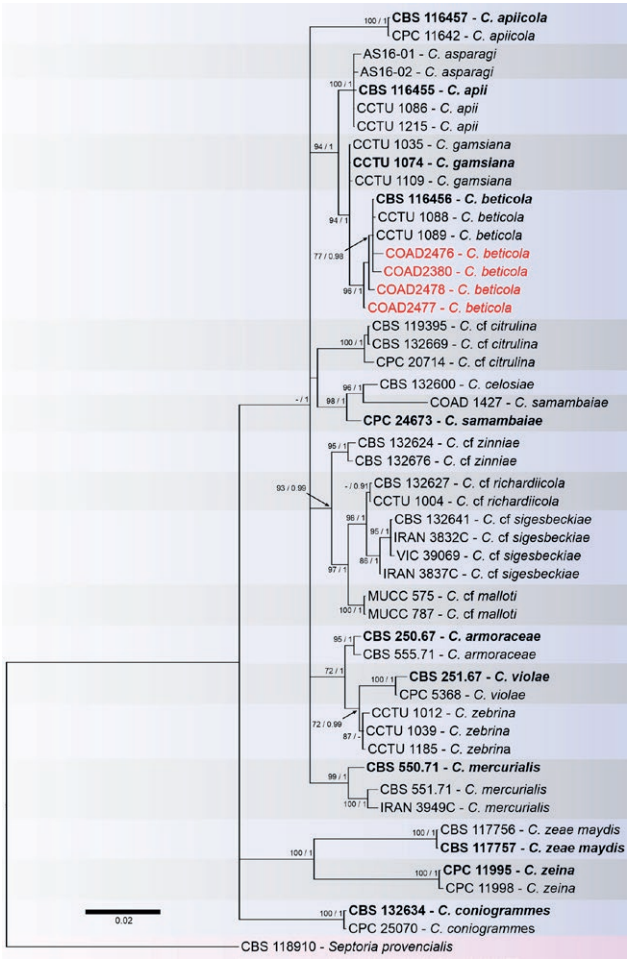

Figure 1. Consensus tree of selected *Cercospora* species, with topology from Bayesian analysis of the combined *act*, *cal*, *gapdh*, *his3*, and ITS regions. Numbers before and after slashes, respectively, represent likelihood bootstrap and posterior probabilities values. The tree is rooted with *Septoria provencialis* (isolate CBS118910). Isolates collected and included in this study are in red font, and ex-type isolates are in bold font. Scale bar indicates 0.02 expected changes per site.

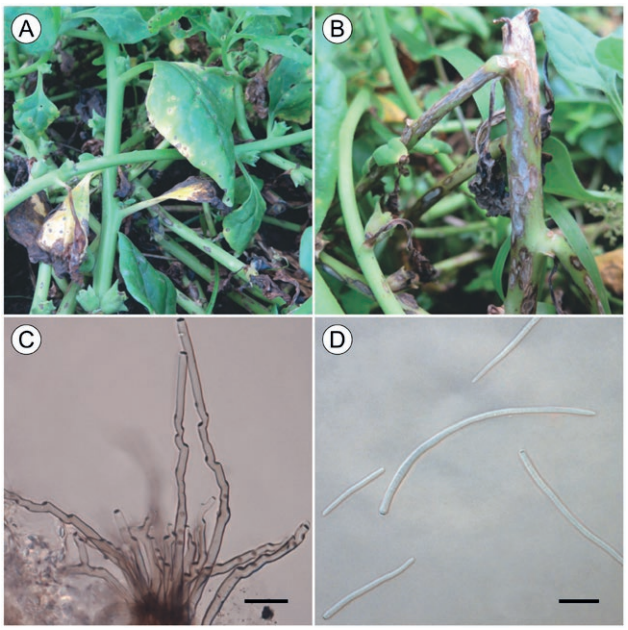

Figure 2. *Cercospora beticola* on leaves and stems of *Tetragonia tetragonoides*. A, leaf spots. B, stem spots. C, geniculate conidiophores with conspicuous conidiogenous loci. D, subcylindrical hyaline pluriseptate conidia with thickened and darkened scars. Scale bars = 30 µm.

Taxonomy

Morphology of the fungus on *T. tetragonoides* was recognized at early stages of this study as typical of the broad assemblage of fungi placed by Crous and Braun (2003) in *Cercospora apii sensu lato*, a group including *C. beticola*.

Cercospora beticola Sacc., *Nuovo Giornale Botanico Italiano* 8 (2): 189 (1876), Fig. 2 A-D.

Symptoms. Leaf lesions starting as small dark brown dots, circular becoming irregular to sub-circular on leaves and elongated on stems, white to grey, each centrally, surrounded by a dark brown rim, 1–3 mm diam., later coalescing and leading to yellowing of leaves, causing premature defoliation; on stems necrotic lesions similar to those on leaves (but somewhat elongated), progressively girdling the stems and causing branch dieback (Figure 2, A and B).

Morphology. Mycelium intra- and intercellular, hyphae branched, septate, pale brown, 2–5 µm wide. Stromata sub-epidermal, irregular, 13–40 × 13–38 µm, and dark brown. Conidiophores cylindrical, fasciculate, 65–162 × 2–5 µm, 2–6 septate, grey-brown at the bases, becoming paler towards the apices, smooth. Conidiogenous cells terminal, integrated, cylindrical, 7–20 × 2–5 µm, pale brown, smooth. Conidiogenous loci conspicuous, 1 to 2 per cell, 2–3 µm diam., thickened and darkened. Conidia solitary, acicular to sub-cylindrical, straight to slightly curved, or sinuous, hyaline, smooth, 55–252 × 2.5 µm, 6–32 septate, each attenuating from base towards the subacute tip, sub-truncate at the base, with a thickened and darkened hilum.

In culture. PDA colonies slow-growing (2 cm diam. after 14 d), flat, cottony, dense and smoke grey centrally, sparser and grey olivaceous towards the periphery, with irregular borders, and olivaceous black reverse sides; not sporulating.

Material examined. Brazil: Minas Gerais, Viçosa, on *Tetragonia tetragonoides*, 10 November 2017, G. Kolesza (VIC 44406, culture COAD 2380).

Additional material. Brazil: Minas Gerais, Viçosa, on leaves of *Beta vulgaris*, 26 April 2018, G. Kolesza (VIC 44456, culture COAD 2476); Rio de Janeiro, Petrópolis, Bonfim, on *Tetragonia tetragonoides*, 16 April 2018, R. W. Barreto (VIC 44457, culture COAD 2477); Rio de Janeiro, Petrópolis, Bonfim, on leaves of *Beta vulgaris*, 16 April 2018, R. W. Barreto (VIC 44458, culture COAD 2478).

At 13 d after inoculation, typical symptoms equivalent to those observed in the field appeared on the two inoculated plants of NZ spinach, and on the beetroot plants, but not on the inoculation control plants. Conidiophores, fascicles and conidia of *Cercospora beticola* were present on the necrotic tissues. A fungus was reisolated from diseased tissues, and colonies obtained were identical to those of the inoculated fungus originally obtained from NZ spinach. The cross-inoculations of COAD 2380 and COAD 2476 resulted in typical *Cercospora* leaf spot symptoms, both on NZ spinach (Figure 3, A and B) and beetroot (Figure 3, C and D).

Some conidiophore fascicles of *Cercospora beticola*, each bearing abundant acicular conidia, were present in all of the examined NZ spinach seed lots, including on freshly collected seeds from the plots where the disease was first observed. Incidence of the fungus on seeds was small, ranging from two to four seeds per batch of 50 seeds. This was confirmation of the earlier assessments of Hino and Tokeshi (1978), and justifies further studies on the potential for dissemination of this important disease through infected and marketed seeds (fruits).

Since Spegazzini's first description of the fungus on NZ spinach as *Cercosporina tetragoniae*. based on a specimen collected in La Plata (Argentina), and Siemaszko's recombination into *Cercospora,* this fungus has been examined by experts on the taxonomy of *Cercospora* and allied fungi. As Siemaszko's recombination appeared in an obscure publication, it escaped Chupp's (1954) monograph. Chupp went on to propose the superfluous recombination *C. tetragoniae*. The holotype material, deposited at LPS (Fungarium Instituto Spegazzini, La Plata), was re-examined by Chupp (1954), Sutton and Pons (1980), and Braun (2000). Although these authors recognized the type material as being scarce, they found some conidiophores and conidia on it and confirmed the identity of *C. tetragoniae* as a member of *Cercospora*. Braun (2000) emphasized that this species is indistinguishable from *C. apii sensu lato*, which is a broad morphological concept proposed by Crous and Braun (2003) which included *C. beticola*. Crous and Braun (2003) introduced the concept of "compound species" which each consisted of morphologically indistinguishable species with different races (host ranges), that were genetically uniform or heterogeneous, with different degrees of biological specialization. They also proposed that genetically and morphologically clearly distinguishable taxa should be treated as separate species. Crous and Braun (2003) proposed that *C. tetragoniae* should be regarded as a synonym of *C. apii*. *Cercospora* species on NZ spinach were not included in the later, critical publication by Groenewald et al. (2005), which led to re-establishment of *C. beticola*. In 2015, the name *C. tetragoniae* reappeared in the literature, along with description and illustration of the fungus based on holotype, but with no mention of the earlier proposal of this to be regarded as a synonym of *C. apii sensu lato* (Braun *et al.,* 2015). The present authors agree with Braun (2000), and also consider *C. tetragoniae* indistinguishable from *C. apii sensu lato* (the assemblage containing *C. beticola*). Fungus morphology and host symptoms both indicate that *C. tetragoniae* is a synonym of *C. beticola*. Nevertheless, for final clarification of this nomenclatural issue, the fungus should be recollected from the type locality of *C. tetragoniae* in La Plata for definitive molecular studies.

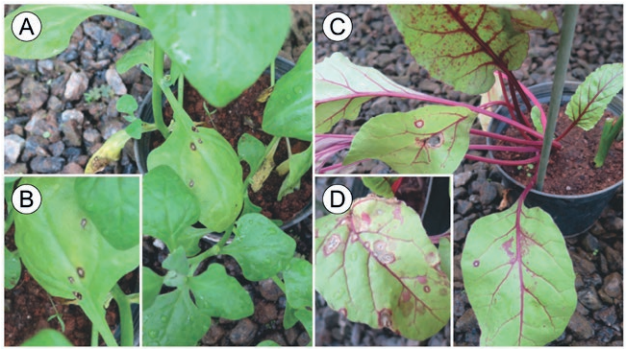

Figure 3. Results from cross-inoculation tests between *Cercospora beticola* isolates obtained from *Tetragonia tetragonoides* (COAD 2380) and beetroot (COAD 2476). A and B, *T. tetragonoides* plants inoculated with *C. beticola* isolate COAD 2476, and C and D, beetroot plants inoculated with *C. beticola* COAD 2380, after 2–3 weeks from inoculations.

Cercospora beticola is a broad-spectrum pathogen attacking 42 host species in 20 genera of several plant families (Crous and Braun, 2003), including *Acanthaceae*, *Apiaceae*, *Amaranthaceae*, *Asteraceae*, *Plumbaginaceae*, *Rosaceae*, *Malvaceae*, *Plantaginaceae*, *Polygonaceae*, *Martyniaceae*, *Pedaliaceae* and *Solanaceae* (Farr and Rossman, 2021). *Cercospora beticola* is known as the etiological agent of the most important foliar disease of beetroot (Tedford *et al*., 2018), and regarded as the most important disease of beetroot in Brazil (Carmelo-Gacia *et al*., 2016). This pathogen also causes severe leaf spot of Swiss chard (Soylu *et al*., 2003), a form of *Beta vulgaris*, and of spinach (*Spinacia oleracea*) (Mukhtar *et*

al., 2019). NZ spinach is likely to be an additional host of the broad host ranged *C. beticola*.

Despite *C. tetragoniae* being mentioned in previous reports, no molecular information linked to publications on this species is available. The lack of molecular data from *C. tetragoniae* led us to attempt to obtain the topotypic material of this fungus, but without success despite several attempts. Since the early 1900s, interest by vegetable growers of La Plata and cool areas of Argentina in production of NZ spinach has vanished.

Searches for *C. tetragoniae* in the NCBI nucleotides database identified sequences associated with two isolates listed as *C. tetragoniae*. These isolates were listed as obtained from *T. tetragonoides* and *T. expansa* (a synonym of *T. tetragonoides*), and were referred to as part of a study to be published in the future, which would report the occurrence of leaf spots caused by *C. tetragoniae* on *T. tetragonoides* in Taiwan. When incorporated into the present study phylogenetic analysis, these *C. tetragoniae* isolates formed a well-supported and distinct clade from the present study isolates (Figure 1). It is not clear whether the Taiwanese isolates represent "true *C. tetragoniae*" until further information on these isolates becomes available.

There is no previous record of *C. beticola* affecting NZ spinach, other *Tetragonia* spp., or any other member of the *Aizoaceae*. Records of *Cercospora* (either as *Cercospora* sp. or *Cercospora tetragoniae*) on *T. tetragonoides* (or its synonym *Tetragonia expansa*) in Farr and Rossman (2021), and the New Zealand list of fungi (Landcare, 2020), among other databases, are based on herbarium records or names appearing in pathogen lists, which are not accompanied by taxonomic or phytopathological information. There are numerous published records of *C. tetragoniae* on *T. tetragonoides* [= *T. expansa*] from Africa, Asia, South, Central and North America, listed in Braun *et al.* (2015). Strangely the authors of these records have ignored the previous view of Braun (2000) that *C. tetragoniae* was a late synonym of *C. apii*. In New Zealand, *C. tetragoniae* was collected for the first time in 2008 on NZ spinach (Landcare, 2018). *Cercospora beticola* had been recorded much earlier from New Zealand, but on Swiss chard (Dingley, 1969) and beetroot (Pennycook, 1989).

The results of the cross-inoculation study performed here, involving one NZ spinach isolate (COAD 2380) and one beetroot isolate (COAD 2476), confirmed that NZ spinach can be a host for *C. beticola*, and that one crop host may serve as the inoculum source for disease outbreaks on the other. This could be of relevance for crop management, since both crops are often cultivated in the same vegetable gardens or in neighboring areas.

Although the present study is preliminary and prospective, demonstration of occurrence of the leaf spot pathogen of NZ spinach in 'seeds' deserves further investigation in Brazil and elsewhere. Pittner *et al.* (2016), showed that *C. beticola* impaired beetroot seed quality, leading to loss of viability of 'seeds', and poor germination and emergence after sowing, and contributed to disseminating the pathogen over long distances. It is likely that the same applies to this fungus on NZ spinach.

Considering the relevance of NZ spinach as an internationally important vegetable crop, broader surveys should be carried out, including isolation and characterization of *Cercospora*-like fungi associated with leaf spots on NZ spinach in other countries. These would further clarify the relevance of *C. beticola* as a pathogen for this crop, and clarify the diversity of cercosporoid species associated with NZ spinach.

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