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Identification and characterization of fungi associated with leaf spot/blight and melting-out of turfgrass in Algeria

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Summary. Symptoms of foliar blight were observed on turfgrass in Oran (Algeria), including yellow chlorotic patches on leaves during the 2020 summer (temperatures between 35 and 40°C). Symptoms extended downward from leaf tips and entire leaves became blighted, leading to irregular discoloured areas that later turned brown. Isolations from infected plants included 214 isolates identified as *Curvularia* or *Bipolaris*, based on morphological traits. Other isolates included *Fusarium, Myrothecium* and *Acremonium* spp. Three molecular loci, *ITS rDNA*, *gpd* and *tef1*, were amplified and sequenced. Morphological and multi-locus phylogenetic analyses revealed four fungal species viz. *B. sorokiniana*, *C. spicifera*, *C. verruculosa*, *C. geniculata*, and two additional *Curvularia* lineages, some of these fungi are reported are first records for Algeria. Koch's postulates were confirmed by inoculating potted turfgrass with spore suspensions of 16 isolates and re-isolating of the inoculated pathogens from symptomatic tissues. *Bipolaris sorokiniana* was the most virulent pathogen causing numerous foliar necrotic lesions similar to those observed in the field. Other isolates infected basal leaves only, and caused less severe symptoms. The results show that *Curvularia* species may be secondary pathogens infecting stressed plants, and that simultaneous occurrence of high temperatures and poor water quality have influenced disease progression. Correct identification of these pathogens is important for applying appropriate and timely disease management.

Keywords. *Bipolaris* spp., *Curvularia* spp., morphological characterization, multi-gene phylogeny, Koch's postulates.

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INTRODUCTION

Turfgrass production and management are multibillion-dollar industries that provide safe playing surfaces for sports fields and outdoor recreation areas, as well as economic opportunities for seed and sod producers, lawn care operators and landscapers (Stackhouse *et al*., 2020; Sithin, 2021).

Field turf is usually composed of two or more different grass species that may complement each other to provide functional and aesthetic improvements in turf quality (Zanelli *et al*., 2021). Seed mixtures of perennial ryegrass (*Lolium perenne* L.), smooth-stalked meadow grass/Kentucky bluegrass (*Poa pratensis* L.), and red fescue (*Festuca rubra* L.) are widely used to establish football pitches in temperate climate zones (Sherratt *et al*., 2017). These *Poaceae* are native to northern Africa, Europe and Asia, but are widely cultivated and naturalized around the world (Abdelguerfi and Abdelguerfi-Laouar, 2004; USDA-ARS, 2013). Some cultivars of these species have been shown to have good wintering, disease resistance and sodding characteristics (Wolski *et al*., 2021; Zanelli *et al*., 2021). In Algeria, these commercially available grass seed mixtures are imported from Europe, especially those used for the Oran Olympic Stadium playing field which was completed in 2019.

However, turfgrass production and use in sports fields are limited by several biotic stresses, with diseases being major limiting factors (Hatfield, 2017; Landschoot, 2021; Liu *et al*., 2023). Several leaf spot- or blight-causing fungi have been reported from turf under wet and warm weather conditions, including *Bipolaris sorokiniana* (Sacc.) Shoemaker and *Curvularia* spp. (Nelson, 1992; Martinez *et al*., 2020; Karunaratha, *et al*., 2021). Increased incidence and severity of these diseases on lawn grasses have been associated with high temperatures, large amounts of nitrogen, low mowing height (Falloon, 1976; Martinez *et al*., 2020; Landschoot, 2021), and other biotic stresses (Smiley *et al*., 2005). Helminthosporioid fungi such as *Bipolaris* and *Drechslera* have been associated with *Curvularia* disease symptoms when climatic conditions are favourable (Nelson, 1992; Martinez *et al*., 2020). These fungi cause root and crown rots which lead to "melting-out" symptoms in turf that typically follow the appearance of leaf spots (Martinez *et al*., 2020). The fungi survive on plant debris and diseased tissues at the soil surface (Smiley *et al*., 2005; Tan *et al*., 2018; Chamekh *et al*., 2019; Iturrieta-González *et al*., 2020; Al-Sadi, 2021), and their spores can be air- or seed-borne (Nelson, 1992; Almaguer *et al*., 2012; Santos *et al*., 2018; Al-Sadi, 2021). The dark pigmentation of conidia makes the pathogens resistant to damage by ultraviolet radiation (Corwin *et al*., 2007).

Bipolaris and *Curvularia* (*Pleosporaceae*, *Pleosporales*) include pathogens of many plants, particularly cereals and grasses with wide distribution, including bluegrass, maize, and oats (Sivanesan, 1987; Manamgoda *et al*., 2014; Marin Felix *et al*., 2017; Farr and Rossman, 2022). Many species of these genera are emerging opportunistic pathogens of animals and humans (Khan *et al*., 2000; Madrid *et al*., 2014; Manamgoda *et al*., 2015; Iturrieta-González *et al*., 2020; Pham *et al*., 2022; Thekkedath *et al*., 2022). Some species of *Bipolaris* and *Curvularia* are anamorphs of *Cochliobolus* (Sivanesan, 1987; Marin-Felix *et al*., 2020). They are characterized by septate and erect conidiophores, with sympodially proliferating conidiogenous cells, and pigmented phragmospores (Zhang *et al*., 2012). These fungi can be distinguished by conidial morphology (hila, septa, septum ontogeny, and wall structure) (Sivanesan, 1987).

Several methods have been used for diagnosing fungal pathogens of turfgrasses. Traditional diagnostic methods included symptomology, morphology, and microscopical identifications (Sivanesan, 1987). These have been augmented by nucleic acid detection such as PCR-based technologies (Stackhouse *et al*., 2020), since this group of fungi is not reliably identifiable using traditional techniques (Tan *et al*., 2018; Bhunjun *et al*., 2020). Variation in cultural and morphological characteristics, such as size and shape of conidia, and colony growth rate of isolates due to culture conditions, have been reported (Sun *et al*., 2003; Santos *et al*., 2018). These variations may lead to inaccurate pathogen identification (Manamgoda *et al*., 2015). In addition, phylogenetics studies based on the *ITS rDN*A region have limited utility for species identifications, especially among members of the *Pleosporales*. This marker provided little resolution for closely related *Curvularia* and *Bipolaris* species (Madrid *et al*., 2014; Bhunjun *et al*., 2020), indicating that additional markers are required for accurate identification within this group of fungi.

Berbee *et al*. (1999) conducted phylogenetic analyses of the *ITS rDNA* region and glyceraldehyde 3-phosphate dehydrogenase (*gpd*) gene sequences to assess the evolutionary relationships of *Cochliobolus*, *Pseudocochliobolus*, *Curvularia*, and *Bipolaris*. These phylogenies were later confirmed using additional loci such as the large subunit (LSU) and translation elongation factor (*tef1*) (Manamgoda *et al*., 2012). Two main monophyletic groups were then established. *Bipolaris* and *Cochliobolus* spp. were clustered in group 1. In group 2, species previously considered to be *Bipolaris, Cochliobolus*, or *Pseudocochliobolus* were reclassified as *Curvularia* (Manamgoda *et al*., 2012), with the most common species causing diseases on grasses. Some *Bipolaris* species were later reclassified as *Curvularia* (Jeon *et al*., 2015; Manamgoda *et al*., 2015; Marin-Félix *et al*., 2020). The *Curvularia-Bipolaris* complex currently comprises *Curvularia*, *Bipolaris, Cochliobolus*, and *Pseudocochliobolus* (Manamgoda *et al*., 2012; 2015; Deng *et al*., 2015; Marin-Félix *et al*., 2017), and the recently confirmed two species of *Exserohilum* in *Curvularia* (Hernández-Restrepo

et al., 2018; Marin-Félix *et al*., 2020). Currently, there are 45 accepted species of *Bipolaris* and more than 105 of *Curvularia* (Marin Felix *et al*., 2017; 2020; Bhunjun *et al*., 2020).

These fungi cause different symptoms depending on the host grass species (Landschoot, 2021), and some signs and symptoms of different pathogens may be similar, making diagnoses difficult. There are also several pathogens causing a disease with one name, allowing confusion when referring to fungicide resistances (Stackhouse *et al*., 2020). For example, turfgrass diseases related to leaf spots and leaf blights were formerly described as "Helminthosporium leaf spots and blights" or "Cochliobolus Diseases" (Nelson, 1992).

In Algeria, there is little information on occurrence of pathogens on cultivated grasses, and none on their presence in sports playing fields. For this reason, isolation and identification studies were carried out on the fungi found on symptomatic turfgrass at the Oran Olympic stadium. This research included pathogenicity tests to obtain basic data for development of effective disease management.

MATERIALS AND METHODS

Fungal isolates

Severe leaf blight and melting-out symptoms of turfgrass were observed in the Olympic stadium located in the Bir El Djir suburb of Oran, Algeria, during the summer of 2020. The turfgrass was composed by *Lolium perenne*, *Festuca rubra* and *Poa pratensis*. Suspected fungal causal agents were isolated from leaves, roots, stems, or seeds. Plant tissues were first immersed in water and then added to 2% sodium hypochlorite solutions for 2 min, followed by sterile distilled water for 5 min, and dried on sterilized paper towels. The tissue pieces were then placed onto potato carrot agar (PCA) or Czapek's agar and incubated at room temperature (24–28°C). Isolations from rhizosphere soil were carried out by the dilution plate method. Rhizosphere soil was separated from roots using a brush and was collected in a Petri dish. Dried soil (1 g) was then added to 9 mL of distilled water and vortex-homogenized for 5 min, and 1 mL of the mixture was then transferred into test tubes each containing 9 mL of sterilized distilled water. This operation was repeated twice. A 100 µL volume of a 1:1000 soil dilution suspension was then spread on Czapek's agar. Recovered fungi were examined using a stereo microscope, and fungal spores from mature colonies were transferred to PCA. Pure cultures of the fungi were prepared from hyphal tips. Fungus isolates were maintained on potato dextrose agar (PDA) slants at 4°C, and were preserved in 30% glycerol stock cultures in a deep freeze (-80°C) in the COMIC culture collection, Université d'Angers, France.

Morphology of isolated fungi

Microscopic features of 25 isolates (20 *Curvularia*, five *Bipolaris*), representing different morphological types, were examined by making direct mounts in 85% lactic acid from 5 to 12 d-old PCA cultures. Using a light microscope (Optika 190-B) and a calibrated micrometer, the lengths and width of approx. 50 conidia and 30 conidiophores of each isolate were assessed, together with other relevant morphological features. Preliminary identifications were made using the available literature (Sivanesan, 1987; Manamgoda *et al*., 2012; Madrid *et al*., 2014; Jeon *et al*., 2015). Colony features were studied on PDA, PCA, Czapek's agar and Malt extract agar (MEA), after growth at 25°C. Mycelial agar discs (5 mm diam.) were removed from edges of 6-d-old, actively growing cultures, and were inoculated centrally into 90 mm diam. agar plates each containing 15 mL of the respective media. The inoculated plates were kept in the dark at 4, 20, 25, 30, 35 or 40°C (± 0.1°C) for 7 days. The resulting colony colours were determined as described in *Methuen Handbook of Colour* (Kornerup and Wanscher, 1978). Radial colony growth rates were determined for each representative isolate. Two measurements of each colony were made at right angles to each other, and treatments were replicated three times for each of the four culture media.

DNA extraction and PCR amplification from representative isolates

DNA was isolated from pure fungal cultures grown on PDA for 7 to 14 d, using the microwave method of Goodwin and Lee (1993). The *ITS rDNA* region, partial fragments of the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) and the translation elongation factor 1-α (*tef1*) genes were amplified, respectively, using fungal primers ITS1 and ITS4 (White *et al*., 1990), gpd1-gpd2 (Berbee *et al*., 1999), and EF1-983F-EF1-2218R (Rehner and Buckley, 2005). PCR amplifications of the extracted DNA (2 µL) was performed in a 50 µL reaction mixture containing: 75 mM Tris-HCl pH 9.0, 20 mM $(NH_4)2SO_4$, 0.01% (w/v) Tween 20, 1.5 mM $MgCl_2$, 200 µM of each deoxyribonucleotide triphosphate, 1 unit of thermostable DNA polymerase (GoTaq, Promega), and 400 nM of each relevant oligonucleotide primer. The reactions

were conducted in a T100™ thermal cycler (Bio-Rad), and thermal cycling parameters were as described in the above references. After complete amplification, the PCR products were analyzed with gel electrophoresis, using 1.2% agarose gel in 0.5× TBE buffer and ethidium bromide (0.5 µg mL^{-1}) as the staining agent. Successful products were sequenced by GATC Company (Germany). The obtained sequences were deposited in GenBank and accession numbers assigned.

Phylogenetic analyses

DNA sequences of eleven isolates and of related species retrieved from GenBank were concatenated and aligned using the MUSCLE algorithm in MEGA 7 (Kumar *et al*., 2016). Two datasets (sequences from *Curvularia* spp. or *Bipolaris* spp.) were made to perform separate phylogenetic analyses. The first set included five isolates pre-identified in *Bipolaris*, and in 34 *Bipolaris* species for which sequences at the three loci were available in GenBank. The second dataset included six isolates pre-identified in *Curvularia*, and in 98 *Curvularia* species for which sequences at the three loci were available in GenBank. Phylogenetic analyses were carried out using the maximum likelihood (ML) approach and IQTree v.1.6 software (Nguyen *et al*., 2015). The best evolutionary history of the fungi for each dataset was calculated using ModelFinder (Kalyaanamoorthy *et al*., 2017) and the Bayesian Information Criterion (BIC) selection procedure. The most suitable nucleotide substitution model for the "*Bipolaris*" dataset was TIM3 + F + I + G4, and for "*Curvularia*" was TIM2e + R4. The ML analyses were carried out with 1000 ultrafast bootstrap replicates, and only values above 70% were considered significant.

Pathogenicity tests

Pathogenicity of eleven morphologically distinct isolates was tested on potted turfgrass plants. Other isolates, including three of *Myrothecium*, one of *Fusarium* and one of *Acremonium*, were included to the tests for comparisons. A seed mixture (Rocalba®), containing 50% *Lolium perenne* (var. Jubilee), 35% *Festuca rubra rubra* (var. Relevant), and 15% *Poa pratensis* (var. Sunbeam) was sown into 10 cm diam. pots containing a 3:1 mixture of sterilized universal potting soil (Fertiplus®) and 25% sterilized sand. The pots with turfgrasses were maintained for 8 weeks in a greenhouse (nighttime 23–28°C, daytime 27–38°C). Each fungal isolate was grown on PCA for 14 d at room temperature. Conidia were removed from the surface of fungal colonies with 10 mL of sterile distilled water using a sterile glass rod. A 15 mL conidium suspension adjusted to 10^4 conidia mL^{-1} was then inoculated onto each pot of turfgrass, using a laboratory atomizer. Three replicates were used for each isolate treatment. Control pots of turfgrass were sprayed with sterile distilled water. The inoculated plants were covered with polyethylene plastic bags for 48 h to maintain high relative humidity (80–92%), and the bags were opened every second day for a few hours. The proportions (%) of necrotic leaf area (n. l. a.) were calculated after visual rating at 7 and 14 d, using the 0 to 9 disease severity scale of Falloon (1976). The experiment was repeated once, and the results presented are the averages of the two repeats, each with three replicates. Re-isolations from inoculated plants were carried out on PCA medium as described above, and isolates were compared to original cultures, providing evidence for fulfilment of Koch's postulates.

RESULTS

Foliar disease symptoms on the playing field in the Oran Olympic stadium were first observed at the beginning of July in the summer of 2020. Initially, symptomatic leaf blades appeared as off-colour and yellow, with dark brown to black spots. Symptoms extended downward from the tips of leaf blades and eventually blighted entire leaves, leading to irregular discoloured areas that later turned brown. By August, when temperatures ranged between 35 and 40°C, disease thinned the turf in large sections. Almost all the sampled plants showed two types of lesions characteristic of leaf blight and spot symptoms (Figure 1a). Leaf blight included elongated, large, brown/tan necroses generally on lower leaves of the infected plants (Figure 1 b and d), and leaf spots were small on leaf blades (Figure 1 e).

A total of 230 symptomatic samples were collected from damaged and healthy-appearing areas on borders and centre of the field. Numerous conidiophores and conidia were produced on lesions after 48 h of incubation on Czapek's agar at room temperature (24–26°C) (Figure 1c). Through isolation and microscope observations, 214 of 320 fungal colonies recovered from infected plants were identified as *Curvularia* or *Bipolaris*. *Curvularia* was isolated at high incidence (combined mean data for leaves, stems and roots, 54%), an *Bipolaris* was recovered from crowns and leaves at lower frequency (3%). *Fusarium* spp. were co-isolated with *Curvularia* and *Bipolaris* spp., representing 31% of isolates. Several other fungi were also isolated from symptomatic turfgrass tissues, and were considered as either saprophytes or as minor pathogens. These included *Myrothecium*

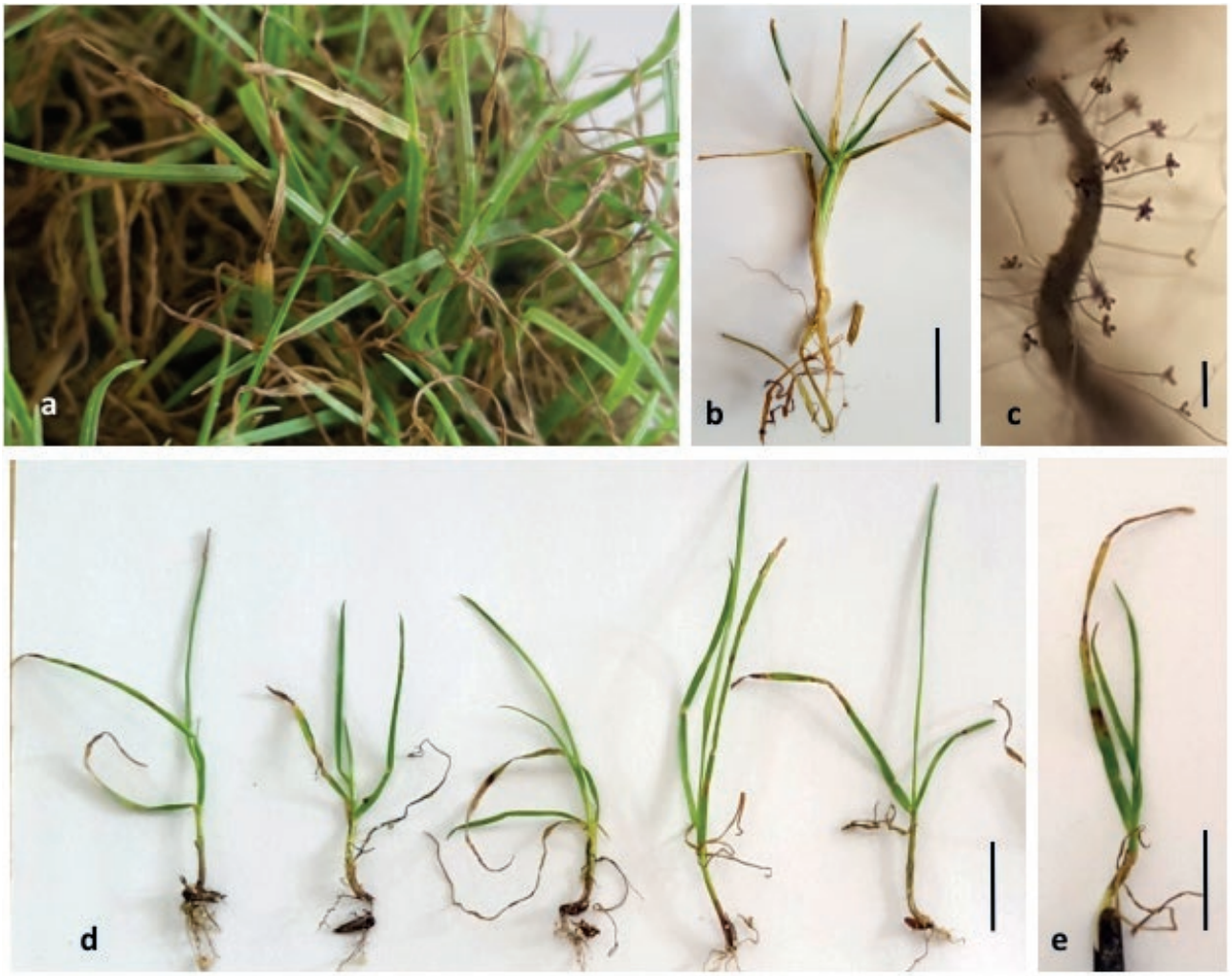

Figure 1. a, Leaf spot and melting out damage on field turfgrasses. **b**, tan to yellow crown and cut leaves. **c**, *Curvularia* conidia and conidiophores on dead leaves and stalks. **d** and **e,** black leaf spot and brown tip symptoms on naturally infected turfgrass. Scale bars: 50 µm (**c**); 10 mm (**b, d** and **e**).

spp., *Chaetomium* spp., *Penicillium* spp., *Trichoderma* spp. (10%), and *Acremonium* sp. (3%). All the fungi isolated from turfgrass rhizospheres were also isolated from aerial parts of turfgrass plants. Fewer *Bipolaris* spp. isolates were obtained from leaves, suggesting that the incubation temperature may have influenced their development and may have favoured proliferation of *Curvularia* spp. For this reason, growth assessments of different isolates were carried out on culture media under several different controlled temperatures.

Species identification

Twenty-five of 214 isolates with *Curvularia* and *Bipolaris* characteristics obtained from turfgrass tissues (leaves, roots, crowns, or seeds) and rhizospheres were purified, and these pure culture isolates were further characterized (Tables 1 and 2). Micromorphological data showed a distinct variation among the isolates based on conidium length and width (23–86 µm × 10–22 µm; n = 1250). The number of septa ranged between three and eight. The isolates had straight to curved conidia with variable hila. The presence or absence of protuberant hila was dependent on the conidium age; the hila were conspicuous on old conidia but not on young conidia. Macro-morphological characterization (colony texture), showed little variation between cottony and velvety, and colony pattern was either glabrous or raised.

Isolates were classified into groups of close resemblance (Table 2), and 11 morphologically distinct isolates

Table 1. Details of the isolates used in this study, including GenBank accession numbers of the generated sequences of the ribosomal DNA region ITS, and the protein-coding genes *gpd* and *tef1*.

Isolate	Organ/ substrate	Species	GenBank accession numbers		
			ITS	gpd	tef1
NB838	Crown	*Bipolaris sorokiniana*	OP703618	OP709928	OP709939
NB839	Crown	*B. sorokiniana*	OP703619	OP709929	OP709940
NB840	Crown	*B. sorokiniana*	OP703620	OP709930	OP709941
NB841	Crown	*B. sorokiniana*	OP703621	OP709931	OP709942
NB842	Crown	*B. sorokiniana*	OP703622	OP709932	OP709943
NB843	Crown	*Curvularia spicifera*	OP703625	OP709933	OP709944
NB844	Crown	*C. spicifera*	OP703626	OP709934	OP709945
NB851	Leaf	*Curvularia* sp.	NA	NA	NA
NB853	Root	*Curvularia* sp.	NA	NA	NA
NB855	Rhizosphere	*Curvularia* sp.	OP703627	OP709935	OP709946
NB860	Leaf	*Myrothecium* sp.	NA	NA	NA
NB861	Leaf	*Myrothecium* sp.	NA	NA	NA
NB862	Leaf	*Myrothecium* sp.	NA	NA	NA
NB864	Leaf	*C. verruculosa*	OP703628	OP709936	OP709947
NB865	Leaf	*Curvularia* sp.	NA	NA	NA
NB866	Leaf	*Curvularia* sp.	NA	NA	NA
NB867	Leaf	*Curvularia* sp.	NA	NA	NA
NB870	Leaf	*Acremonium* sp.	NA	NA	NA
NB871	Root	*C. geniculata*	OP703629	OP709937	OP709948
NB872	Root	*Curvularia* sp.	NA	NA	NA
NB873	Root	*Curvularia* sp.	NA	NA	NA
NB874	Root	*Curvularia* sp.	OP703630	OP709938	OP709949
NB875	Leaf	*Fusarium* sp.	NA	NA	NA
NB876	Leaf	*Curvularia* sp.	NA	NA	NA
NB877	Leaf	*Curvularia* sp.	NA	NA	NA
NB878	Leaf	*Curvularia* sp.	NA	NA	NA
NB879	Leaf	*Curvularia* sp.	NA	NA	NA
NB880	Leaf	*Curvularia* sp.	NA	NA	NA
NB883	Seed	*Curvularia* sp.	NA	NA	NA
NB884	Seed	*Curvularia* sp.	NA	NA	NA

were selected among the groups for further molecular characterization. A phylogenetic tree from the combined dataset of ITS rDNA, *tef*1 and *gpd* sequences indicated more precise relationships and similarities with related sequences from recognized species within *Bipolaris* (Figure 2) and *Curvularia* (Figure 3).

Based on the combination of morphological characteristics and sequence data, six different lineages were distinguished, including *Bipolaris sorokiniana, Curvularia spicifera, C. verruculosa, C. geniculata* and two *Curvularia* spp., one related to *C. verruculosa* and the other to *C. mossaddeghii*. From available literature, *C. verruculosa* and *C. geniculata* are new records for fungi of Algeria. These species are described below, in alphabetic order.

Bipolaris sorokiniana (Sorokin) Shoemaker 1959

Five isolates (NB838, NB839, NB840, NB841, and NB842) exhibited similar colony morphology and colour. Colonies on PDA were velvety and radially creased, reaching 46.9 ± 7.5 mm diam. after 7 d, olive brown (4D4/4E7), with fluffy white mycelium and irregular white margins (Figure 4 a); reverse yellowish brown (5F4). Sporulation sparse until hyphae disturbed. Abundant, elevated aerial hyphae around colony margins and sparse aerial hyphae at colony centres were observed upon aging. On PCA, cottony, 68.5 ± 9.3 mm diam., olive (3D3) to olive brown (4D3), with abundant aerial mycelium with grayish surfaces and sometimes white patches (Figure 4 b); reverse olive brown (4D4/4F7). Colonies on Czapek's agar were velvety, 76.9 ± 3.4 mm diam., grayish yellow (4C7) to olive brown (4E6/

Table 2. Morphological characteristics of *Bipolaris* and *Curvularia* isolates obtained from turfgrass.

Isolate	Colony colour [a]	Conidium [b]				Conidiophore [b]			Molecular identification
		Mean length (µm) x ± SD	Mean width (µm) x ± SD	Septation	Shape	Mean length (µm) x ± SD	Mean width (µm) x ± SD	Septation	
NB838	Grayish yellow (4C7) to olive brown (4E6/ 4D7)	86.3 ± 4.9	21.9 ± 1.4	4–8	Straight. ellipsoidal to cylindrical	81.5 ± 15.4	7.1 ± 0.6	2.0–6	*B. sorokiniana*
NB842		67.9 ± 5.9	21.4 ± 1.4	3–8		75.4 ± 16.3	6.8 ± 0.5	2.0–6	*B. sorokiniana*
NB841		64.3 ± 5.4	20.4 ± 1.5	5–8		87.1 ± 25.8	7.1 ± 0.5	2.0–8	*B. sorokiniana*
NB840		66.7 ± 4.4	20.9 ± 1.1	4–7		113.5 ± 35.8	7.9 ± 0.6	2.0–9	*B. sorokiniana*
NB839		81.0 ± 7.0	22.9 ± 1.5	6–8		58.0 ± 12.3	6.9 ± 0.7	1.0–4	*B. sorokiniana*
NB844	Brownish orange (5C4/6B3)	29.9 ± 3.8	10.0 ± 0.8	3–4.0	Straight. obovoïd to ellipsoidal	67.6 ± 15.0	5.6 ± 0.4	4.0–9	*C. spicifera*
NB843		31.9 ± 0.8	10.3 ± 3.0	3–4.0		75.6 ± 17.5	5.4 ± 0.6	5.0–10	*C. spicifera*
NB866	Olive (3E7/3F6) or Light brown (6D4)	30.5 ± 2.4	12.1 ± 1.2	3	Ellipsoidal. unequal sided. straight to slightly curved	89.2 ± 20.2	5.8 ± 1.0	3.0–8	ND
NB864		25.6 ± 2.0	10.3 ± 0.9	3		122.8 ± 22.2	5.9 ± 0.6	5.0–12	*C. verruculosa*
NB873		30.4 ± 2.0	12.0 ± 1.0	3		107.5 ± 25.1	5.5 ± 0.9	4.0–12	ND
NB878		30.9 ± 3.0	12.0 ± 1.0	3		165.8 ± 84.9	4.9 ± 0.7	6.0–28	ND
NB874	Olive (2E2/ 2F3) or olive yellow (3C7/ 3 8D)	23.0 ± 1.2	12.2 ± 0.8	3	Ovoid. distinctly curved and ellipsoidal	130.7 ± 25.6	5.0 ± 0.2	6.0–12	*Curvularia* sp.
NB853		23.7 ± 1.9	11.5 ± 0.7	3		120.7 ± 29.2	4.2 ± 0.5	5.0–11	ND
NB884		28.0 ± 3.0	12.3 ± 0.8	3		96.7 ± 17.2	4.6 ± 0.6	4.0–10	ND
NB867	Grayish orange (5B3) to Light brown (6D6)	34.0 ± 6.8	10.4 ± 1.2	(3) 4 (5) (6)	Ellipsoidal. slightly curved	61.6 ± 29.0	4.2 ± 0.6	3.0–10	ND
NB876		32.9 ± 3.4	10.3 ± 0.8	4 (5)		76.7 ± 14.7	4.5 ± 0.6	3.0–8	ND
NB871		35.4 ± 5.0	11.2 ± 0.8	4 (5–6)		79.4 ± 21.1	4.2 ± 0.5	4.0–10	*C. geniculata*
NB872		30.0 ± 2.3	10.7 ± 0.9	(3) 4 (5)		98.5 ± 25.5	4.8 ± 0.4	4.0–12	ND
NB877	Light brown (6D5) to olive brown (4D5 / 4D6)	26.8 ± 2.1	12.0 ± 1.3	3	Distinctly curved	78.5 ± 12.2	5.0 ± 0.0	3.0–6	ND
NB880		27.9 ± 1.7	13.3 ± 1.1	3		128.1 ± 39.6	4.8 ± 0.4	4.0–11	ND
NB879		30.1 ± 2.7	11.3 ± 1.0	3	Ellipsoidal to ovoid. straight to slightly curved	61.5 ± 18.8	4.8 ± 0.5	3.0–7	ND
NB883		26.6 ± 1.3	12.1 ± 1.0	3		156.7 ± 46.5	5.1 ± 0.4	5.0–17	ND
NB855		26.0 ± 2.3	12.0 ± 1.0	3 (4)		134.9 ± 38.1	5.2 ± 0.4	4.0–13	*Curvularia* sp.
NB851		24.9 ± 1.9	11.1 ± 1.0	3 (5)		105.6 ± 30.2	4.9 ± 0.6	5.0–14	ND
NB865		27.0 ± 2.0	12.3 ± 0.9	3		126.6 ± 39.4	5.7 ± 0.7	4.0–21	ND

[a]: Characteristics on Czapek's agar. [b]: Microscopic features on PCA; ND, not determined.

4D7) (Figure 4 c), reverse same colour. On MEA, colony diam. 32.7 ± 6.3 mm, with cottony dense white margins; other characteristics same as PDA (Figure 4d). Conidiophores were simple, rarely branched, septate, (40) 83–201 µm long and 6–8 µm thick, pale to medium brown, occasionally with series of 2–4 geniculate, sympodial proliferations, bearing 1–3 conidia. Conidia formed singly at the tips of conidiogenous cells, and were pale to dark brown, mostly straight, ellipsoidal to cylindrical, rounded at the bases and apices, mostly with 5–6 (8) transverse septa, smooth walled, (47) 64–86 (98) µm long and (15) 21–27 µm wide (Figure 4, e to h). Germination was observed from only one distal cell on each conidium (Figure 3 g). Microconidiation was rarely observed, and these were hyaline, spherical cells at the extremities of conidia. Sexual morph not observed. Conidiophores emerging from the surfaces of dead infected plants, were rigid, brown, clustered or scattered, each with three conidiogenous loci, 77–143 µm long and 7–10 µm wide (Figure 4, h and i). Most conidia had 6–8 transverse septa, 65–75 µm. Other morphologies were as for growth on PCA.

Curvularia geniculata (Tracy & Earle) Boedijn 1933
Isolate NB871 formed velvety colonies on PDA, reach-

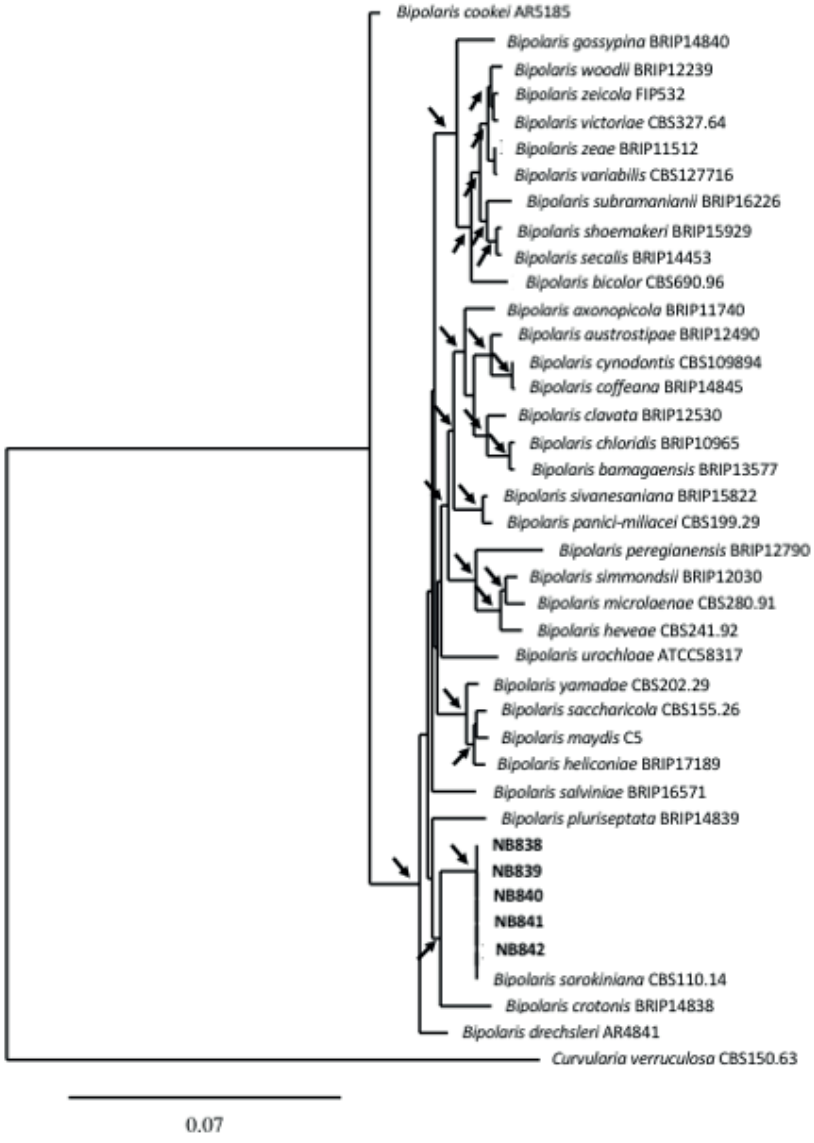

Figure 2. Phylogenetic tree reconstructed using the maximum likelihood method, from the alignment of *ITS rDNA*, *gpd* and *tef1* sequences of *Bipolaris* isolates. The tree was rooted with *Curvularia verruculosa* CBS 150.63. Bootstrap support values greater than 0.7 are indicated by arrows near nodes. The scale bar indicates the expected number of substitutions per position.

ing 56.9 ± 2.8 mm diam., after 7 d at 25°C. The upper colony surfaces were olive brown (4F7) (Figure 5 a), with white irregular borders. On PCA, colonies were velvety, 73.4 ± 1.7 mm diam., olive brown (4E5/4E6) (Figure 5 b), with olive brown (4E3) reverse sides. On Czapek's agar, velvety, 78.0 ± 4.1 mm diam., and grayish orange (5B3) (Figure 5 c). On MEA, velvety to floccose, 68.9 ± 3.0 mm diam., yellowish-gray (4B2) margins with olive brown (4F6) centres, other colony characteristics the same as on PDA (Figure 5 d). Conidiophores on PCA were geniculate at the apices, unbranched, septate, (42) 79–143 µm long and 3–5 µm wide. Conidia were ellipsoidal, slightly curved, light brown, (28) 35–50 µm long, 10–12 µm wide, 4 to 6 septate, and each with an enlarged central cell and a punctate wall (Figure 5, e to h). Microconidia and sexual stages were not observed. Conidium germination was from one or two distal cells (Figure 5 i). Conidiophores emerging from the surface of dead infected leaves were

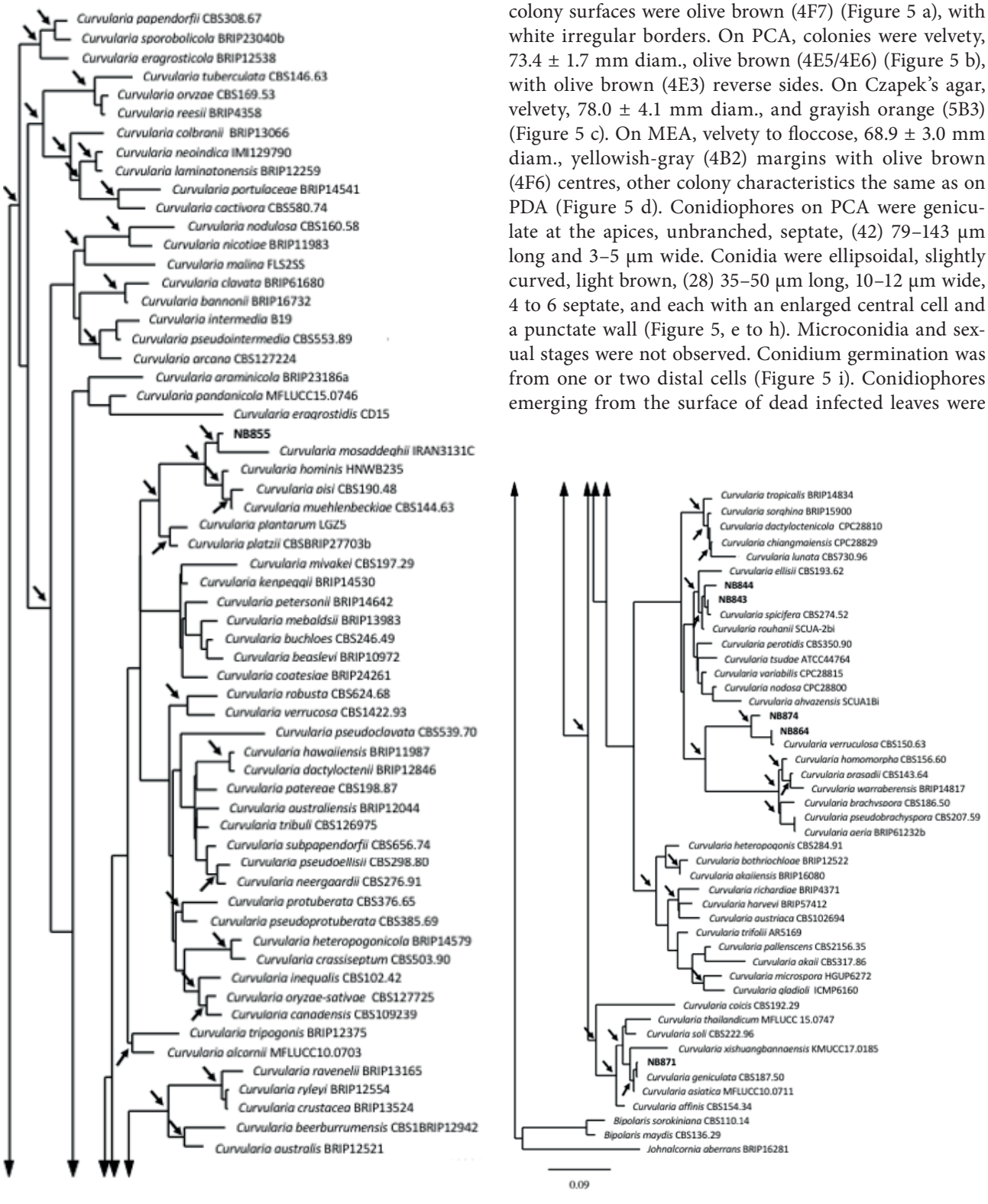

Figure 3. Phylogenetic tree reconstructed using the maximum likelihood method, from the alignment of *ITS rDNA*, *gpd* and *tef1* sequences of *Curvularia* isolates. The tree was rooted with *Bipolaris* and *Johnalcornia* spp. Bootstrap support values greater than 0.7 are indicated by arrows near nodes. The scale bar indicates the expected number of substitutions per position.

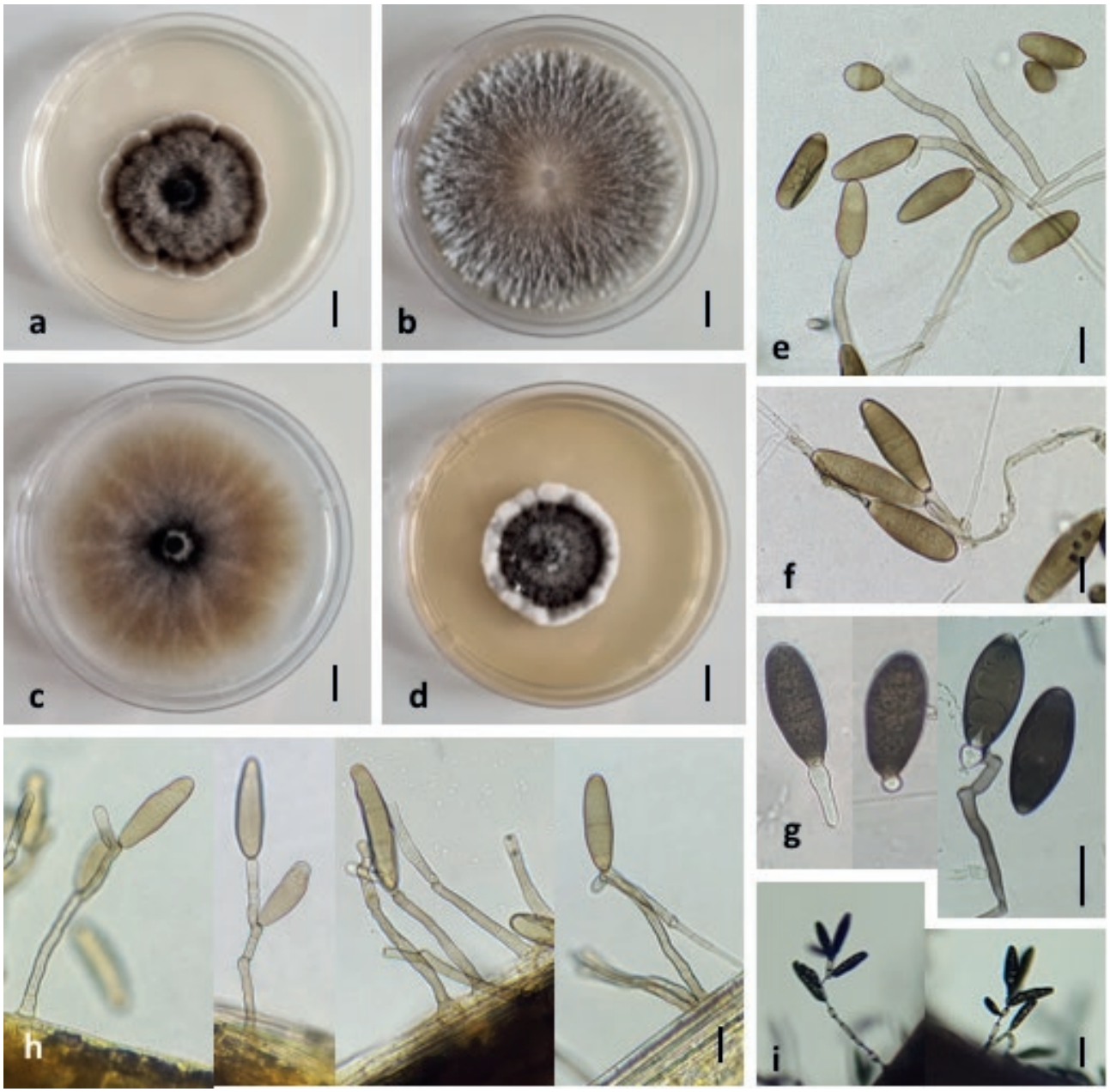

Figure 4. *Bipolaris sorokiniana*. Colony on: **a**, PDA, **b**, PCA, **c**, Czapek's agar, **d**, MEA. **e** and **f**, conidiophores and conidia on PCA. **g**, germinating conidia. **h**, conidiophores and conidia produced on leaf host. **i**, conidiophore proliferation on host tissue 21 days after inoculation. Scale bars: 10mm (**a** to **d**); 25µm (**e** to **h**); 50 µm (**i**).

rigid, brown, scattered, each with 3–7 (15) conidiogenous loci, 67–99 (133) µm long and 3.8–5.5 µm wide (Figure 5 j). Most conidia had 4–5 transverse septa, were 33–44 µm long and 9–13 µm wide. Hila were not protuberant.

Curvularia spicifera (Bainier) Boedijn 1933

Colonies of two isolates (NB843, NB844), were velvety with cottony centres, 56.6 ± 1.1 mm diam. after 7 d at 25°C, irregular, olive brown (4F7), and slightly white at the margins (Figure 6 a), sporulating abundantly when grown on PDA at 25°C; reverse sides yellowish brown (5F4). Colonies on PCA were velvety to floccose and olive (3F3/ 3F7), 65.4 ± 2.2 mm diam. (Figure 6b). On Czapek's agar, velvety with glabrous irregular margins, 65.4 ± 4.8 mm diam., brownish to grayish orange (5C4/6B3) (Figure 6 c); reverse sides same color. On

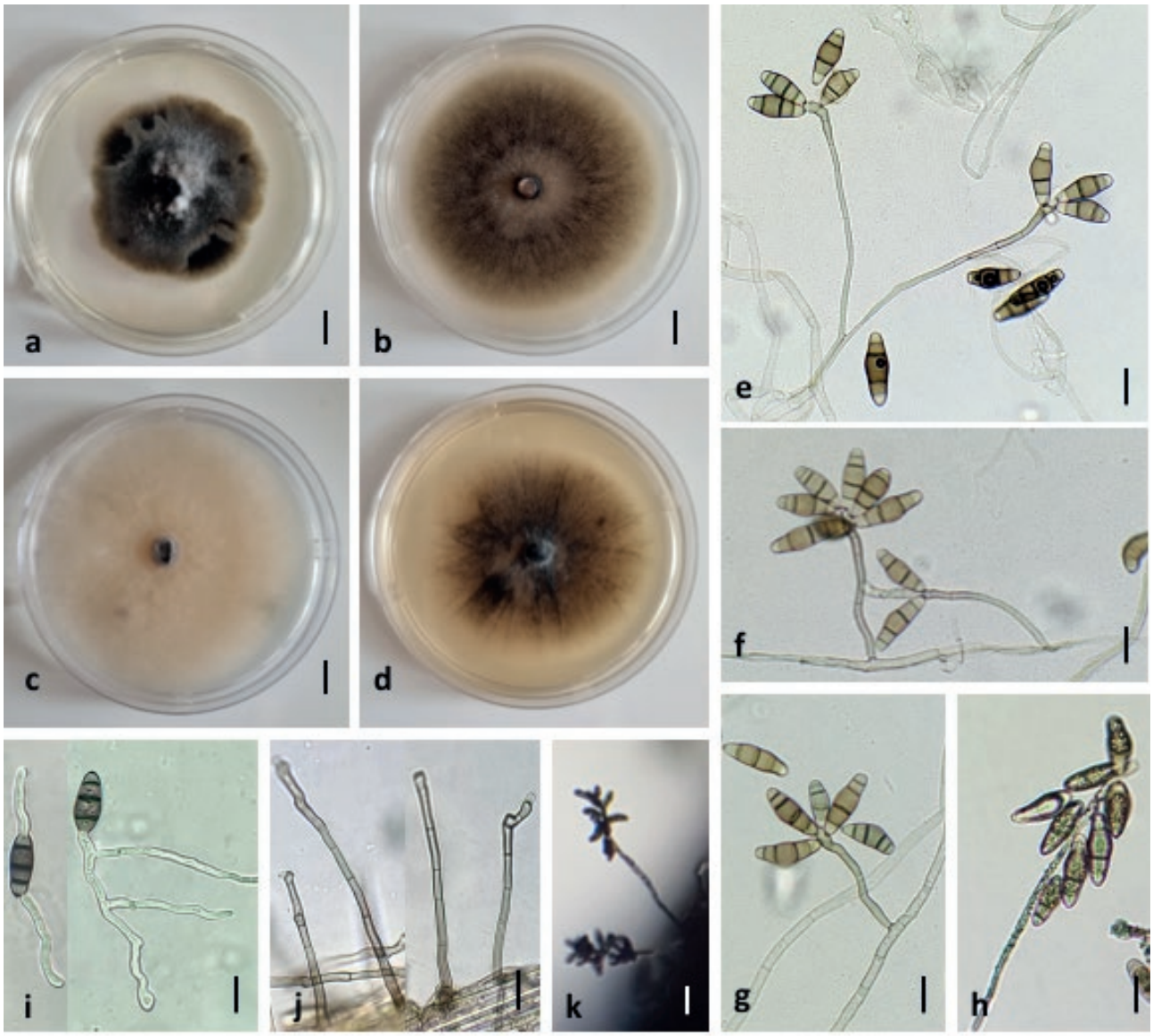

Figure 5. *Curvularia geniculata* (NB871). Colony on: **a**, PDA, **b**, PCA, **c**, Czapek's agar, **d,** MEA. **e–g**, conidiophores and conidia on PCA. **h**, conidia and conidiophore ornamentation. **i**, conidia germination. **j** and **k**, conidiophores and sporulation pattern on leaf host 21 days after inoculation. Scale bars 10 mm (**a** to **d**); 25 µm (**e–j**); 50 µm (**k**).

MEA, cottony compact, 58.4 ± 1.3 mm diam., grayish green (27F5) with dull green (27D3) margins (Figure 6 d), reverse sides yellowish brown (5E5/ 5F4). Conidiophores on PCA were erect, mostly straight, rarely geniculate in the upper parts, (42) 71–120 long × 4–6 µm wide, brown at the base, paler towards the apices, septate with cell walls thicker than those of vegetative hyphae. Conidiogenous cells were integrated, terminal or intercalary, with sympodial proliferations (15–25), pale brown to brown, with darkened scars (Figure 6 e and k). Conidia obovoid to ellipsoid, straight, (23) 30–40 µm long and 8–12 µm wide, terminal cells usually light brown at the apices, third cells often more swollen, 3 distoseptate with punctate cell walls (Figure 6 g); hila not protuberant. Sexual morph not observed. Microconidiation was observed as forming hyaline, spherical cells at the ends of phragmoconidia (Figure 6, f to j). Conidiophores emerging from the surfaces of dead infected leaves, rigid, brown, scattered, with 3–7 (15) conidiogenous loci, 67–99 (133) µm long and 3.8–5.5 µm wide. Most conidia with 4–5 transverse septa were 33–44 µm long and 9–13 µm wide (Figure 6, h and i).

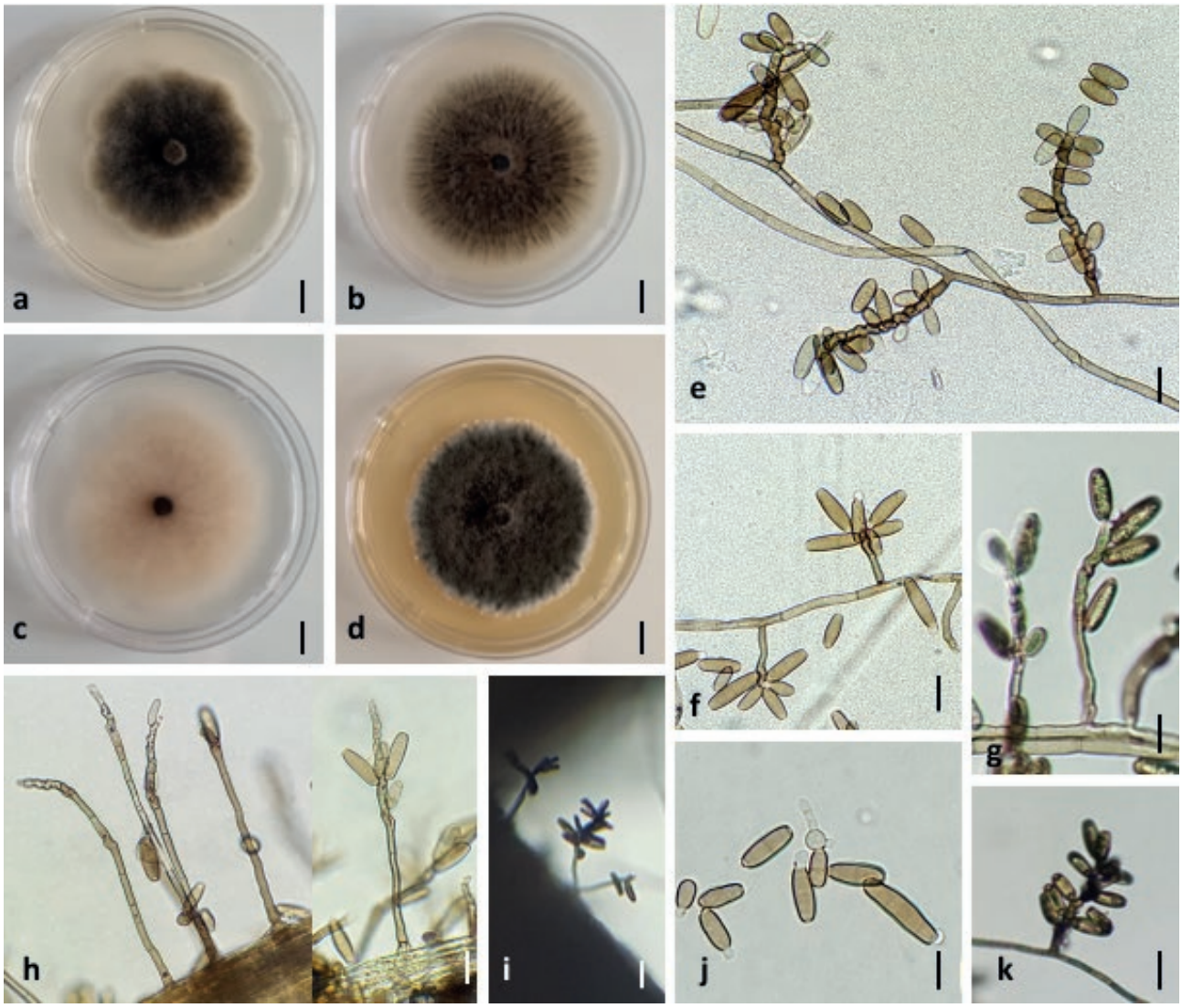

Figure 6. *Curvularia spicifera*. Colony on: **a**, PDA, **b**, PCA, **c**, Czapek's agar, **d**, MEA. **e,** conidiophores, conidiogenous cells and conidia on PCA. **f** and **j**, microconidiation and conidia germination. **g**, conidia ornamentation. **h** and **i**, conidiophores and conidia on leaf host. **k**, sporulation pattern on center of 7 days PCA colony. Scale bars: 10 mm (**a** to **d**); 25 µm (**e**, **f**, **g**, **h** and **j**); 50 µm (**i** and **k**).

Curvularia verruculosa Tandon & Bilgrami ex M.B. Ellis 1962

Isolate NB864 produced powdery colonies that developed gentle radial folds as they aged, reaching 34.8 ± 2.2 mm diam. after 7 d at 25°C on PDA (Figure 7 a); reverse sides olive brown (4F8). On PCA, glabrous to velvety, flattened olive (3F6), 71.5 ± 1.3 mm diam. (Figure 7 b), and reverse sides olive (3F3). On Czapek's agar, glabrous with irregular margins, 59.8 ± 1.3 mm diam., olive (3E7/3F6) (Figure 7 c); reverse sides same colour. On MEA, 35 ± 0.7 mm diam., grayish green (28E4/ 28F6), reverse sides brownish gray (6F2), other characteristics as on PDA (Figure 7 d). Conidiophores on PCA were septate, simple but rarely branched, erect or curved, pale, geniculate close to the apices, thick-walled, (85) 122 to 155 µm long and 5 to 6 µm wide. Conidia were each ellipsoidal, unequal sided, straight to slightly curved, three-septate, rounded at both ends, with a rough verrucose wall, (21) 25–30 µm long and (8) 10–12 µm wide, light to dark brown, the subterminal third cell larger and darker than other cells (Figure 7, e and f). Microconidial and sexual stages were not observed. Germination was from two distal cells (Figure 7 g). Conidiophores emerged from the surfaces of dead infected leaves, and were rigid, brown, clustered, with 4–15 conidiogenous loci, 134–268 µm long and 6–10 µm wide (Figure 7 i). Most conidia with 2–3 transverse septa,

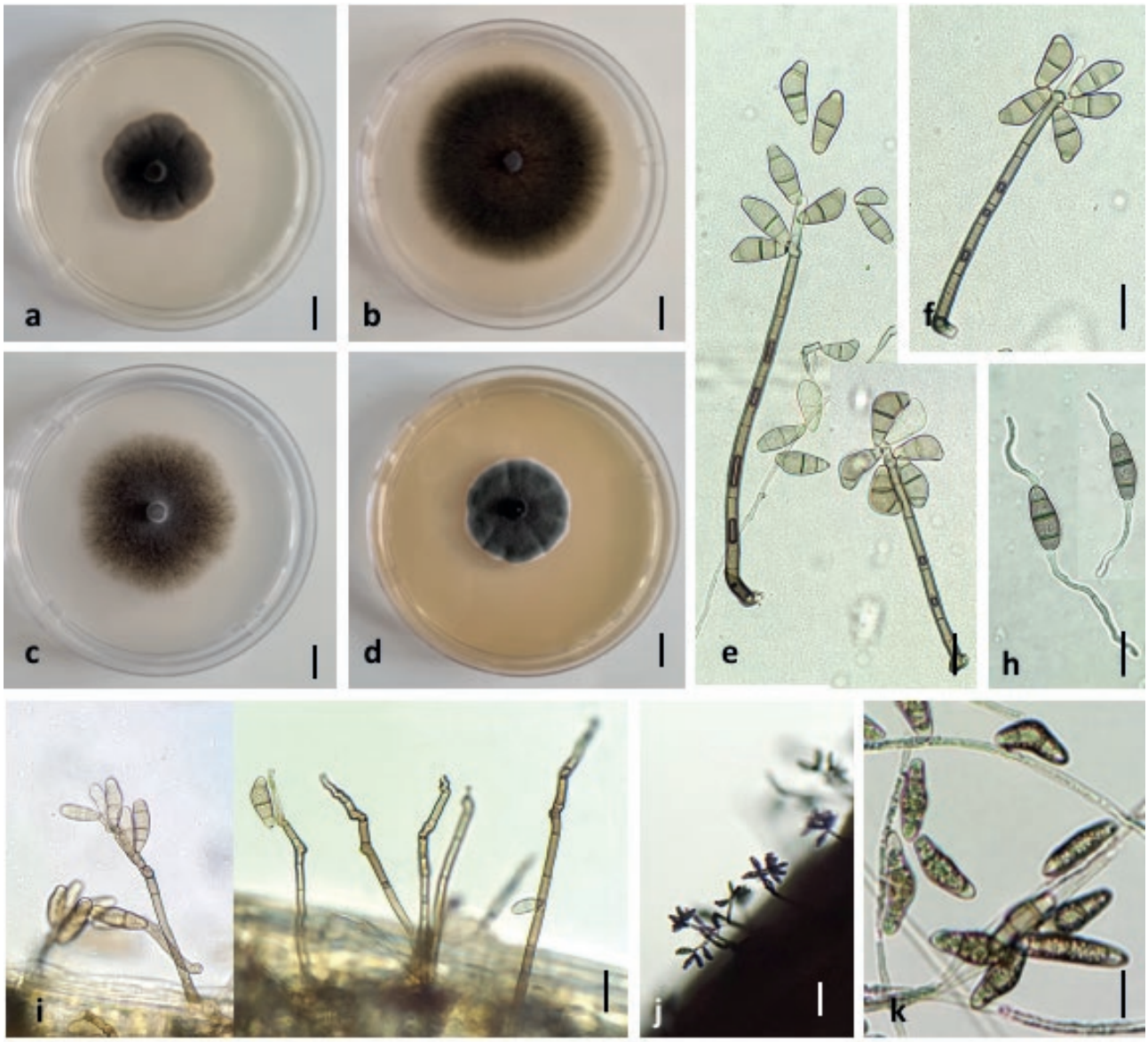

Figure 7. *Curvularia verruculosa* (NB864). Colony on: **a**, PDA, **b**, PCA, **c**, Czapek's agar, **d**, MEA. **e** and **f**, conidiophores, conidia and sporulation pattern on PCA. **g**, conidia germination, **h**, conidiophores and conidia formed on leaf host. **i** and **j**, sporulation on leaf host. **k**, conidia and hypha ornamentation. Scale bars 10 mm (**a** to **d**); 25 µm (**e**, **f**, **h**, **i**, **j** and **k**); 50 µm (**g**).

27–38 µm long and 11–15 µm wide. Hila not protuberant. The conidium terminal cells were paler and less verrucose than the central cells (Figure 7 k).

***Curvularia* spp. morphological group IV**

One isolate (NB874) formed olive brown (4E5), velvety to powdery and folded colonies on PDA. reaching 66.5 ± 1.7 mm diam. after 7 d at 25°C. The colonies were circular, gray at the centres (3B2), effuse and white (3B1) towards the periphery, with fimbriate margins (Figure 8 a); reverse sides olive brown (4F8/ 4E3). On PCA, 71.5 ± 1.3 mm diam., olive (3F4), velvety (Figure 8 b); reverse sides olive (3F4) to olive gray (3E2). On Czapek's agar, glabrous with velvety centres, 44.8 ± 1.7 mm diam., olive (2E2/2F3) (Figure 8 c); reverse sides the same colour. On MEA, 73.8 ± 0.5 mm diam., and colony characteristics as on PDA (Figure 8 d). Conidiophores on PCA usually simple, straight or slightly geniculate, brown, with cell walls often thicker than those of the vegetative hyphae, (85) 130–193 µm long and 4–5 µm wide. Conidiogenous cells intercalary, polytretic, proliferating sympodially (Figure 8, e and f), slightly swollen. Conidia ovoid, ellip-

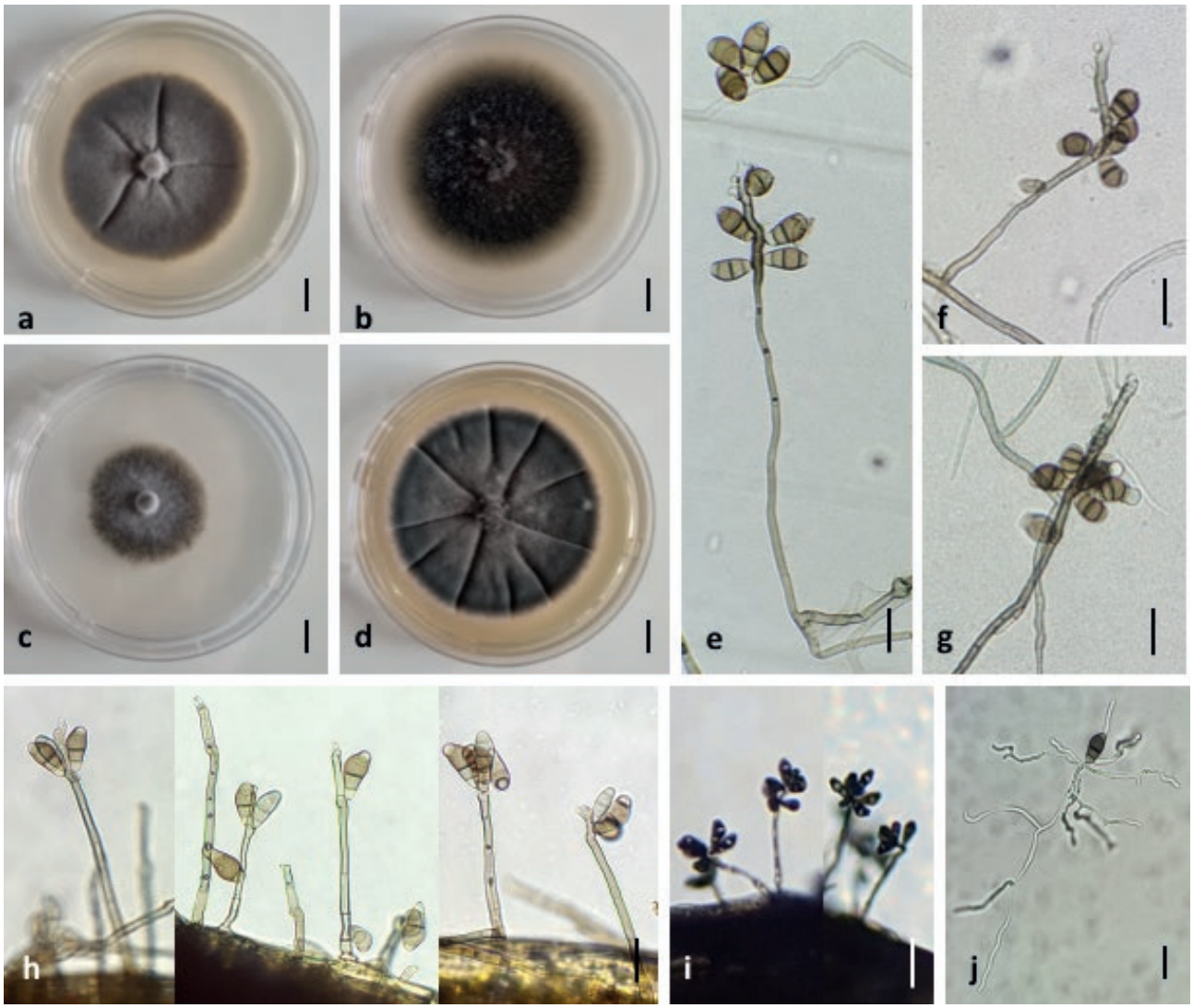

Figure 8. *Curvularia* sp. morphotype group IV (NB874). Colony on: **a**, PDA, **b**, PCA, **c**, Czapek's agar, **d**, MEA. **e** and **f**, conidiophores, conidiogenous cells and conidia on PCA. **g**, microconidiation. **h**, conidia and conidiophores on leaf host. **i**, sporulation pattern on leaf host 21 days after inoculation. **j**, conidium germination. Scale bars 10 mm (**a** to **d**); 25 µm (**e**, **f**, **g**, **h** and **j**); 50 µm (**i**).

tical, smooth-walled and curved at the subterminal cells, three septate, four celled, 20–25 µm long and 10–13 µm wide, brown, with each third cell unequally sided and larger than the others, apical and basal cells subhyaline. Hila non-protruding. Microconidiation observed, forming pale brown, globose cells at the upper parts of conidia (Figure 8 g). Sexual morph not observed. Conidiophores emerging from the surfaces of dead infected leaves, rigid, brown, scattered, with 2–5 conidiogenous loci, 105–288 µm long and 5–7 µm wide. Most of conidia with 3 transverse septa were 27–33 µm long and 10–15 µm wide (Figure 8, h and i). Germination was observed from one or two distal cells of conidia (Figure 8 j). Hila were protuberant on mature conidia. Based on the multilocus phylogeny, this isolate was strongly related to *C. verruculosa* but was significantly separated from this species. *gpd* and *ITS rDNA* sequences were similar to that of *C. americana*, but no *tef1* sequence is available for this species in GenBank.

Curvularia spp. morphological group VI

Isolate NB855 had velvety, olive brown (4E5) with grayish beige (4C3) colony surfaces, which were 77.3 ± 1.7 mm diam. on PDA after 7 d at 25°C (Figure 9 a). The periphery of each colony was floccose and olive with a fimbriate margin; reverse sides olive brown (4F5). Colonies on PCA were funiculose, olive (3F5), and were 76 ± 0.8 mm diam. (Figure 9 b), with reverse sides olive (3F4).

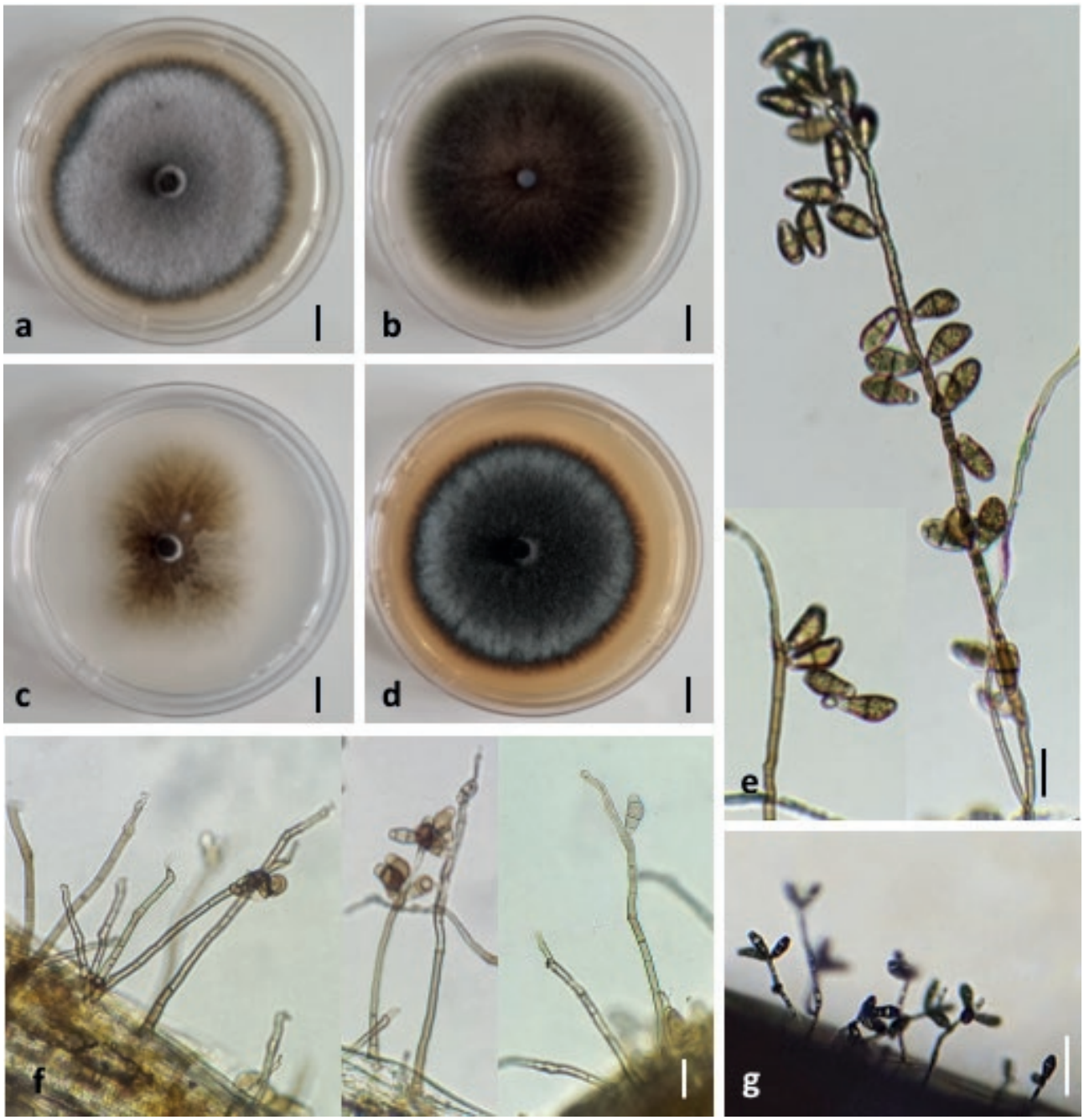

Figure 9. *Curvularia* sp. morphotype group VI (NB855). Colony on: **a**, PDA, **b**, PCA, **c**, Czapek's agar, **d**, MEA. **e**, Conidiophores and conidia ornamentation on PCA. **f**, conidia and conidiophores on leaf host. **g**, sporulation pattern on leaf host 21 days after inoculation. Scale bars 10mm (a to d); 25µm (e–f); 50µm (g).

On Czapek's agar, glabrous, and olive brown (4D5/4D6) with irregular transparent margins, and 67.8 ± 3.8 mm diam. (Figure 9 c); reverse sides same colour. On MEA, 72.8 ± 0.5 mm diam., brownish gray (5D2) centres and yellowish brown (5F5) margins (Figure 9 d), and other characteristics same as on PDA. Conidiophores on PCA were simple or branched, geniculate towards the apices, brown, septate, with cell walls thicker than those of the vegetative hyphae, (78) 135–263 µm long and 5–6 µm wide. Conidiogenous cells intercalary, polytretic, proliferating sympodially, subcylindrical to irregular with dark conidiogenous loci. Conidia ellipsoid to ovoid,

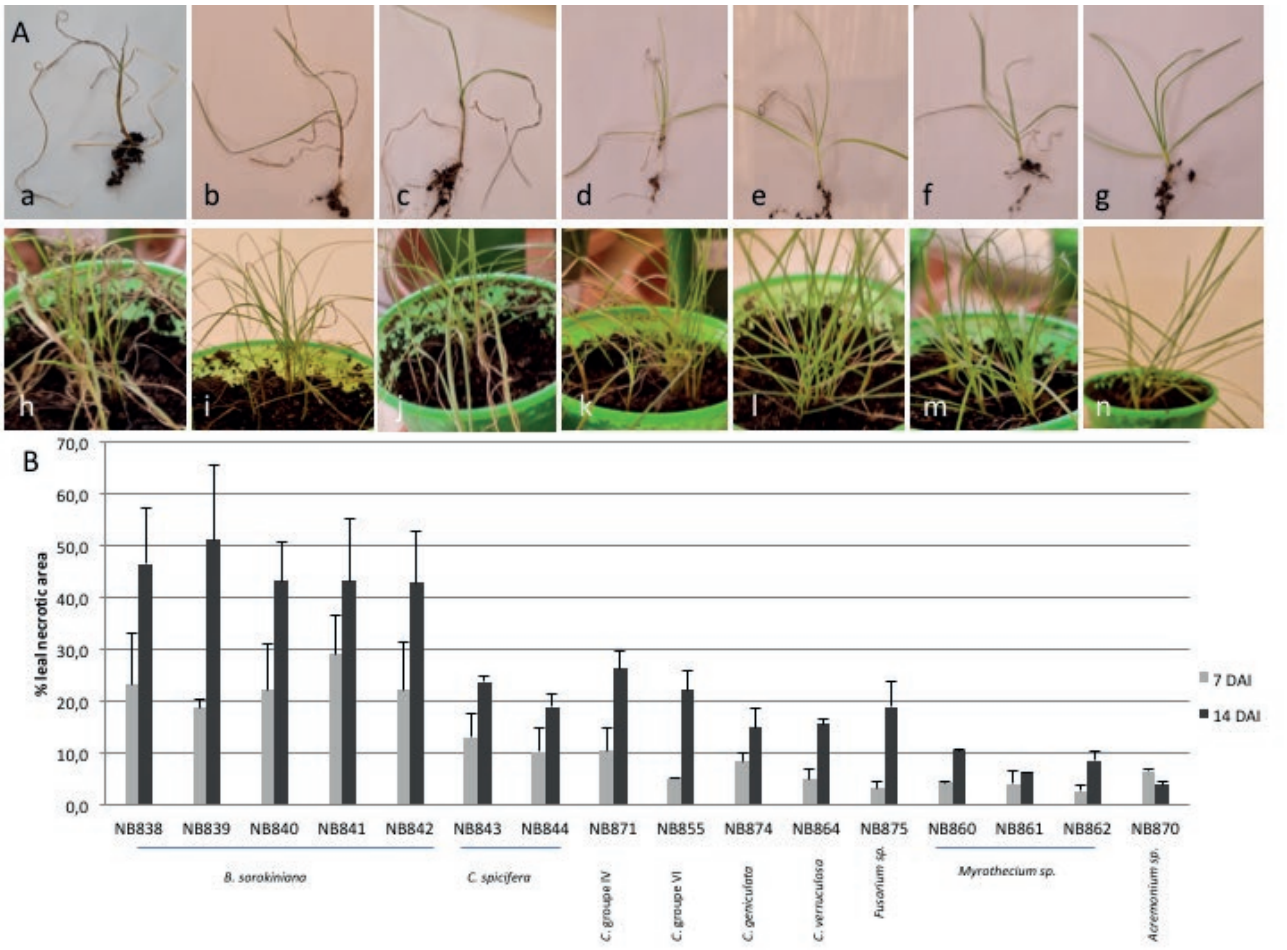

Figure 10. A, leaf spot and blight symptoms on six-week-old turfgrass leaves under greenhouse conditions 14 days after inoculation with: **a** and **h**, *Bipolaris sorokiniana* (NB840), **b** and **I**, *C. spicifera* (NB843), **c** and **j**, *C. geniculata* (NB871), **d** and **k**, *Curvularia* group IV (NB874), **e** and **i**, *Curvularia* group VI (NB855), **f** and **m**, *C. verruculosa* (NB864). **g** and **n**, negative control. **B**, percentage of leaf necrotic area recorded at 7 and 14 DAI on potted turfgrass.

straight to slightly curved, (21) 26–30 µm long and (10) 12–14 µm wide, 3–4 celled, each with the third intermediate cells usually verruculose and darker than the others, brown, and apical and basal cells pale brown (Figure 9 e). Microconidial and sexual stages were not observed. Conidiophores emerging from the surfaces of dead infected leaves, rigid, brown, scattered or clustered, with 3–11 conidiogenous loci, 87–150 (188) µm long and 5–8 µm wide (Figure 9, f and g). Most conidia with 3 transverse septa, 22–30 µm long and 8–14 µm wide. Hila sometimes slightly protuberant. The multilocus phylogeny showed that this strain grouped closely to *C. mosaddeghii,* but formed a separate branch.

Three *Curvularia* isolates (NB869, NB873, and NB877) produced sexual morphs on PDA after 4 weeks at 20°C. Sterile ascomata were solitary or arising in groups, immersed or erumpent, dark brown or black, and were 600–950 (1050) µm long and 200–400 (550) µm wide, thick-walled, surrounded by hyphae and conidiophores arising in groups from the bodies and necks. The internal cells were angular to globose, and hyaline.

Colony radial growth assessments at different temperatures for representative isolates from each of the six morphological groups showed that the optimum temperature ranges of *B. sorokiniana* were 25–30°C, but *C. spicifera* and *Curvularia* group IV grew most rapidly between 25 and 35°C. The other morphological groups had maximum growth at 30°C. *Curvularia* morphotype group VI strain NB855 displayed more rapid growth than other species groups. None of the isolates exhibited radial growth at 4°C after 7 d incubation. At 40°C, mycelial growth was less rapid than at the other temperatures for all the assessed isolates.

Pathogenicity assessments

Under greenhouse conditions, turfgrass plants inoculated with *Curvularia* spp. and *Bipolaris* conidium suspensions developed small brown spots on leaves 7 d after inoculation (DAI). These spots enlarged with time, and closely resembled disease lesions observed in the field. At 14 DAI, *Curvularia* isolates induced leaf tip dieback and chlorosis, mostly on basal leaves (Figure 10 A). *Bipolaris sorokiniana* isolates produced dark, irregular brown spots that increased in size and coalesced, leading to an extensive blighting of entire leaf blades and tillers that turned tan and brown (Figure 10 A, a to h). At 21 DAI, leaves inoculated with *B. sorokiniana* isolates began disintegrating ("melting"), and thereafter infections spread to the crown and root areas, which induced the "melting out" phase of the plants.

Results presented in Figure 10 B showed that *B. sorokiniana* isolates were more aggressive than the other inoculated isolates. Mean proportions of leaf necrotic area ranged from 43.3% to 51.3% (overall mean = 45.5 ± 3.5%) for plants inoculated with *B. sorokiniana*, and from 14.9% to 26.3% (overall mean = 20.3 ± 4.6%) for *Curvularia* spp. inoculations. Minor symptoms ranging from 3.9% to 19% (overall mean = 7.3 ± 5.8%) were induced by isolates of *Myrothecium*, *Fusarium*, or *Acremonium*. Little difference was detected in aggressiveness among the *B. sorokiniana* isolates. Sporulation was observed in the host lesions after 7 to 14 d, and the respective pathogens were reisolated from 90 to 100% of diseased leaves sampled from each trial. Plants sprayed with sterile distilled water remained symptomless (Figure 10 A, g and n).

DISCUSSION

Accurate identification of *Bipolaris* and *Curvularia* species causing leaf spots and blight of turfgrass is important, due to potential species level variations in pathogenicity characteristics. Overall, using characteristics of conidia, *Curvularia* sp. isolates were easily distinguished from *B. sorokiniana* isolates, and from *C. spicifera* that had atypical, straight, short conidia that were similar to those of *Bipolaris*. *Curvularia* is characterized by conidia with enlarged intermediate cells that contribute to the characteristic curvatures, while in *Bipolaris* conidia curvature is continuous throughout conidium lengths. Conidia of *Bipolaris* are also usually longer than those of *Curvularia* (Marin-Felix *et al*., 2017). Krizsan *et al*. (2015) showed that the most important discriminative features between species were the shapes and septation of the conidia. However, some *Curvularia* species have short and straight conidia with intermediate conidial characteristics, making species identification difficult. These species may seem different from the type genus *Curvularia* that has euseptate and curving conidia.

Morphological variation was demonstrated when conidia are produced on different substrates (Sivanesan, 1987; Sun *et al*., 2003). Presence or absence of protuberant hila within the one species has been observed in relation to conidium age (Santos *et al*., 2018). In addition, Differences in mycelium colour can also occur when comparing subcultures from one colony. This shows that colony colour alone should not be a characteristic for species identification (Santos *et al*., 2018). Due to ambiguities in morphological characteristics, DNA sequences of multiple loci are widely used for accurate species identification, and determining new species (Jeon *et al*., 2015; Tan *et al*., 2018; Marin-Félix *et al*., 2017, 2020; Iturrieta-González *et al*., 2020; Zhang *et al*., 2020; Connally *et al*., 2021). These studies have shown that morphological criteria of several species do not correlate with molecular identification, highlighting the usefulness of combining sequence data from *ITS rDNA*, *gpd* and *tef1* to correctly delineate species within *Curvularia* and *Bipolaris* genera (Marin-Félix *et al*., 2017; 2020). Using this set of sequences, the present study confirmed that some of the isolates from the Oran Olympic stadium turfgrass corresponded to *B. sorokiniana* and *C. spicifera*, and other isolates were of four other *Curvularia* species, i.e., *C. geniculata*, *C. verruculosa* and two undetermined *Curvularia* species.

The presence of *Curvularia* spp. associated with grass diseases has been reported world-wide. These fungi have been found in Argentina and the United States of America (Roane and Roane, 1997; Goldring *et al*., 2007; Roberts and Tredway, 2008; Martinez and Pearce 2020); in Europe, in Portugal (Sivanesan, 1987; Coelho *et al*., 2020); in Asia, including Iran and China (Ahmadpour *et al*., 2013; Xin-hua *et al*., 2019); in Oceania, including New Zealand and Australia (Falloon 1976; McKenzie 1978; Pennycook, 1989; Tan *et al*., 2018); and in Southern Africa, in: Zimbabwe (Sivanesan, 1987). *Bipolaris sorokiniana* is a plurivorous species, and has been reported as a phytopathogen of almost a hundred *Poaceae*, including cultivated and wild species (Farr and Rossman, 2022), and from different countries (Karunarathna *et al*., 2021). In Algeria, *B. sorokiniana* has been isolated from leaves of lentil (El Amine *et al*., 2021) and maize (Zibani *et al*., 2022).

Bipolaris sorokiniana was found in small proportions of turfgrass crowns samples in the present study. The low frequency of isolation of this pathogen may be

related to the incubation conditions that were applied, and to strong competition with the fast growing *Curvularia* species. Temperature is an important environmental factor that affects fungal activity and pathogenicity. There is an optimal level at which the growth and activity of fungi are greatest; above and below that level, growth and activity are reduced. In the present study experiments, optimum growth of *Curvularia* was at 30 – 35°C, whereas *B. sorokiniana* isolates grew most rapidly at 25 – 30°C. Rapid coloization of substrates *Curvularia* probably hampered development of *B. sorokiniana*. This indicates that alternative methods (e.g. nucleic acid-based detection from symptomatic plants) should be used for precise and rapid disease diagnoses.

Although six species of fungi were identified on turfgrass, the presence of a pathogen alone does not denote disease. Differences in pathogenicity among these species was detected in the present study, Koch's postulates were fulfilled. Plant trials indicated that *Curvularia* species are likely to be minor turfgrass pathogens, in contrast to *B. sorokiniana*. *Curvularia* spp. are typically considered to be weak pathogens of several plant hosts, or as saprobes (Smiley *et al*., 2005; Manamgoda *et al*., 2012; Ayoubi *et al*., 2017).

Simultaneous occurrence of high temperatures and poor water quality may influence disease progression. Incidence of lawn blight diseases can be ascribed to environmental factors (climate, atmospheric pollution, soil texture), mineral supply, water quality, and plant factors (growth stage sensitivity, varietal response to the stress). The Mediterranean climate is characterized by long drought periods that occur in the North (Margat and Vallé, 2000). The results of the present study are consistent with those of Dicklow and Madeiras (2021). They reported *Curvularia* as a stress pathogen of turf resulting from high temperatures or summer stress, which may be secondary to other pathogens or caused by poor cultural practices. A possible explanation is that lesions caused by *B. sorokiniana* are invaded by *Curvularia* which is aggressive at high temperatures. The occurrence of these fungi together causes greater disease than with one species alone.

The present study has confirmed that *B. sorokiniana* is a potential threat for production of animal fodder and in rangeland grasses, where pathogen damage can severely affect pasture and lawn quality. In sports fields specifically, assessments for this pathogen may allow for disease prevention. This will aid appropriately swift disease management to be applied, as different pathogens and their strains can respond differently to chemical treatments. This will prevent further spread of disease, and reduce losses due to diseases.

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