Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

OPEN ACCESS

Citation: Moumni M., Mancini V., Allagui M.B., Murolo S., Romanazzi G. (2019) Black rot of squash (*Cucurbita moschata*) caused by *Stagonosporopsis cucurbitacearum* reported in Italy. *Phytopathologia Mediterranea* 58(2): 379-383. doi: 10.14601/Phytopathol_Mediter-10624

Accepted: April 3, 2019

Published: September 14, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Josep Armengol Forti, Polytechnical University of Valencia, Spain.

New or Unusual Disease Reports

Black rot of squash (*Cucurbita moschata*) caused by *Stagonosporopsis cucurbitacearum* reported in Italy

Marwa MOUMNI[1,2,3], Valeria MANCINI[1], Mohamed Bechir ALLAGUI[3], Sergio MUROLO[1], Gianfranco ROMANAZZI[1,*]

[1] Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy
[2] National Agricultural Institute of Tunisia, 43 Avenue Charles Nicolle, 1082 Tunis, Tunisia
[3] Laboratory of Plant Protection, National Institute for Agronomic Research of Tunisia, University of Carthage, Rue Hédi Karray, 2080 Ariana, Tunisia
*Corresponding author: g.romanazzi@univpm.it

Summary. *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*) can affect cucurbits through induction of black rot. This pathogen produces irregular white spots covered with pycnidia on infected cucurbit fruit. Twenty squash fruit (cv. Butternut) with black rot symptoms were collected in Italy from two locations: Osimo (AN) and Montacuto (AN), in the Marche region. Several fungal colonies were isolated from these fruit, the morphological features of which corresponded to *S. cucurbitacearum*. This identification was confirmed using multiplexing of three microsatellite markers and by sequence analysis using internal transcribed spacers. The sequence identity for the internal transcribed spacer regions was greater than 98% compared with sequences of *S. cucurbitacearum* in the NCBI database. This is the first report of *S. cucurbitacearum* on *Cucurbita moschata* fruit with black rot symptoms in Italy.

Keywords. Butternut squash, black rot, ITS sequencing, microsatellite markers.

INTRODUCTION

Black rot (BR) of cucurbits is caused by the fungal pathogen *Stagonosporopsis cucurbitacearum* (Fr.) Aveskamp, Gruyter & Verkley (Aveskamp *et al.*, 2010) (anamorph *Phoma cucurbitacearum* (Fr.) Sacc.), synonym *Didymella bryoniae* (Fuckel) Rehm, which is one of the most important pathogens on cucurbits worldwide (Li *et al.*, 2015; Yao *et al.*, 2016). *Stagonosporopsis cucurbitacearum* and *S. citrulli* can infect several species of Cucurbitaceae, including watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) (Rennberger and Keinath, 2018; Babu *et al.*, 2015; Huang and Lai, 2019), muskmelon (*Cucumis melo* L.) (Nuangmek *et al.*, 2018), squash (*Cucurbita maxima* Duch, *Cucurbita moschata* Duch) (Keinath, 2014), and pumpkin (*Cucurbita pepo* L.) (Grube *et al.*, 2011). These fungi can cause infection of the stems, leaves, roots, seeds, and fruit of these host plants (Keinath, 2011).

Infected fruit manifest large irregular-shaped spots and black rot (Choi *et al.*, 2010). Fruiting bodies are found in the oldest parts of lesions, because *S. cucurbitacearum* is a necrotrophic fungus (Keinath, 2014). Keinath (2011) reported that *S. cucurbitacearum* produces black mycelia inside melon and giant pumpkin (*C. maxima*) fruit. The ideal conditions for disease development include humidity greater than 90%, leaf wetness and temperatures from 16 to 24°C (Park *et al.*, 2006; Seebold, 2011). BR can reduce preharvest and postharvest yields (de Neergaard, 1989), and cause up to 15% fruit loss (Keinath, 2000). *Stagonosporopsis cucurbitacearum* has only been reported once in Italy, in 1885 on *C. melo* L., and it was described as *Didymella melonis* Pass. by Giovanni Passerini (Corlett, 1981). Our investigations aimed to identify the causal agent of black rot symptoms on squash fruit.

MATERIALS AND METHODS

Fungal isolation

Twenty fruit of squash (*C. moschata*; cv. Butternut) with symptoms of black rot were collected from Osimo (AN) and Montacuto (AN), in the Marche region of Italy in September and October 2018. Small infected pieces of squash peel (≈2 mm) were cut from the fruit, sterilized with 1% sodium hypochlorite solution for 2 min, washed three times with sterilized distilled water, placed into 90 mm diam. Petri dishes containing water agar (Bacteriological agar; Liofilchem), and incubated at 24°C. After 7 d, pycnidia were excised from developing fungus colonies, placed into Petri dishes containing potato dextrose agar (Liofilchem), and incubated at 24°C. Identification of the fungus was carried out according to the colour and shape of the colonies, and to the size of the conidia produced from pycnidia (50 conidia measured).

DNA amplification and phylogenetic studies

The fungal genomic DNA was extracted from 100 mg of mycelia of isolates grown on potato dextrose agar as pure cultures, following the protocol proposed by Varanda *et al.* (2016). The DNA was amplified in a rapid PCR based assay for distinguishing the three morphologically similar species (*S. cucurbitacearum*, *S. citrulli*, and *S. caricae*) by multiplexing of the three microsatellite markers *Db01*, *Db05* and *Db06* (Brewer *et al.*, 2015). The primer pairs ITS_1 and ITS_4 (White *et al.*, 1990) were then used to amplify the internal transcribed spacers (ITS). The PCR products were separated on 1.5% agarose electrophoresis gels, stained with Red Gel (Biotium), and visualized, with images captured using an imaging system (Gel Doc XR; BioRad). Selected PCR amplicons were purified and sequenced by Genewiz, and the sequences have been deposited with Genbank (accession numbers: isolates ID1, MK330934; ID3, MK330935; ID9, MK330936; for ITS regions). The nucleotide sequences were subjected to Blast analysis to determine the relative similarities with other sequences available in Genbank.

RESULTS AND DISCUSSION

In the two surveyed locations in Italy, black rot symptoms occurred on butternut squash fruit. The initial symptoms were brown circular spots with exudates on the fruit surfaces (Figure 1A). Over time, the spots became white and were covered with black fruiting bodies (Figure 1B, C). After 8 d incubation on water agar, some pycnidia were seen (using a stereomicroscope) to be developing in rows on the fruit peel (Figure 2A). On crushing the pycnidia, the conidia inside were cylindrical, mostly non-septate and a few one-septate, and measuring 4 to 11 μm × 2 to 5 μm (Figure 2B, C). The isolates on potato dextrose agar showed white mycelia on the colony upper surfaces top and black mycelia on the undersides. These morphological characteristics coincided with those known for *S. cucurbitacearum* (Keinath *et al.*, 1995; Koike, 1997; Choi *et al.*, 2010; Keinath, 2013).

Morphological identification was supported by the multiplex amplification using the primers *Db01*f/r, *Db05* f/r and *Db06* f/r, which yielded two amplicons (220 bp and 280 bp), characteristic for *S. cucurbitacearum*. The presence of an amplicon of 280 bp and the lack of an amplicon of about 360 bp indicated that three isolates (ID1, ID3, and ID9) were *S. caricae* or *S. citrulli*, as reported by Brewer *et al.* (2015) (Figure 3). Blast analysis showed 98% to 99% similarity for the ITS regions compared to other sequences of *S. cucurbitacearum* already in the NCBI database, as shown in Table 1. Therefore, the isolates ID1, ID3, and ID9 from butternut squash are confirmed as *Stagonosporopsis cucurbitacearum*.

Stagonosporopsis spp. is a major pathogen of cucurbits worldwide, and it occurs everywhere these crops are grown (Stewart *et al.*, 2015; Mancini *et al.* 2016; Nuangmek *et al.*, 2018). Gummy stem blight and BR can affect all part of cucurbit plants, including stems, leaves, roots, seeds, and fruit. This pathogen is seed- and soilborne, and it can remain for long periods in the seeds and in the soil. Infected seed has continued to spread

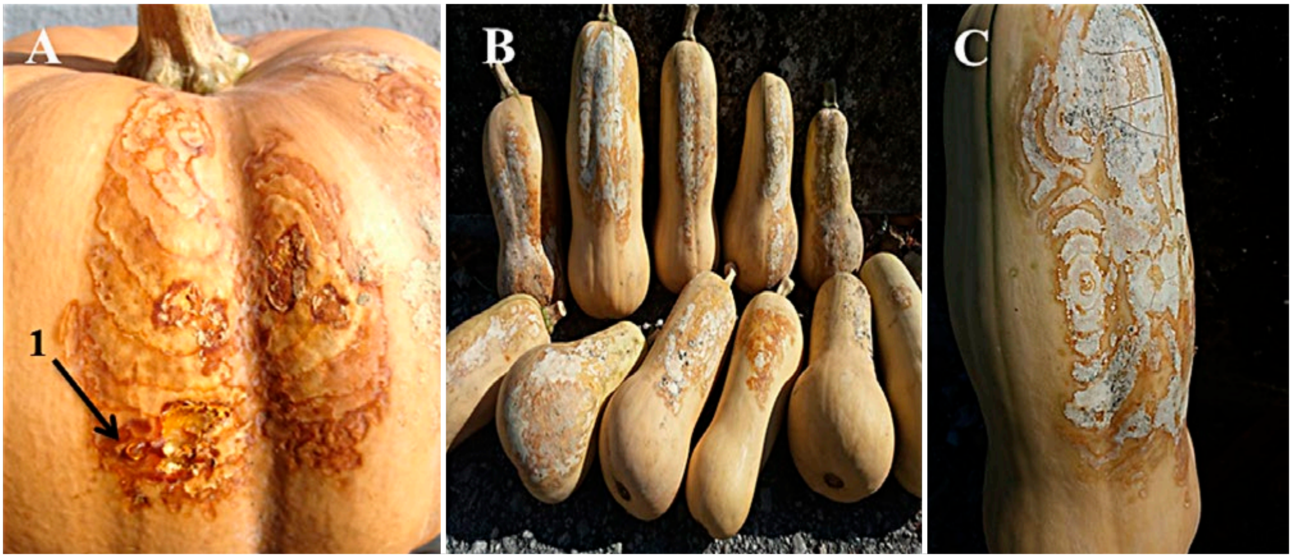

Figure 1. Typical symptoms of black rot caused by *Stagonosporopsis cucurbitacearum* on butternut squash fruit. **A,** Infected fruit showing exudate (arrow 1). B and C, Irregularly circular and white spots covered by pycnidia.

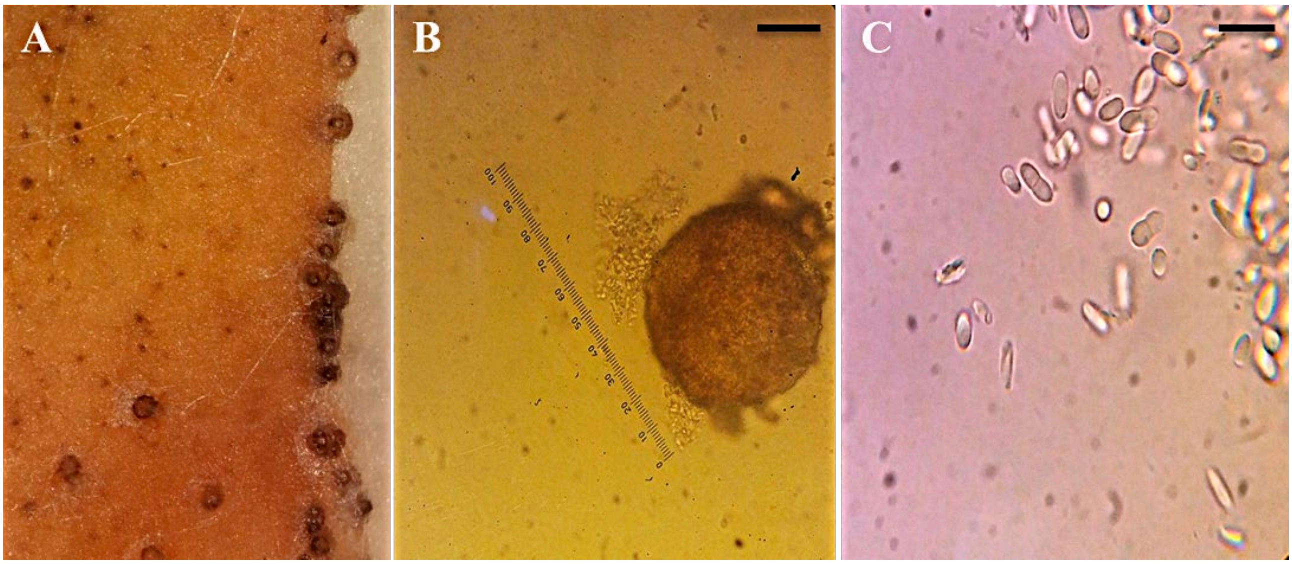

Figure 2. Morphological characteristics of *Stagonosporopsis cucurbitacearum*. A, Row of pycnidia on peel from a squash fruit under the stereomicroscope. B, Pycnidia under the microscope. Scale bar = 50 μm. C, Aseptate and one-septate pycnidiospores. Scale bar = 10 μm.

the pathogen around the world (Keinath, 2011). Seedborne pathogens can reduce of the quantity and quality harvested fruits and/or seeds, and their management is crucial for profitable production (Mancini *et al*., 2014). On cantaloupe, field losses due to *S. cucurbitacearum* can reach 100% under conditions conducive to infection (Nuangmek *et al*., 2018), and on watermelon, Gummy stem blight and BR can cause significant production losses, both in the field and postharvest (Maynard and Hopkins, 1999). No commercial cultivar of any cucurbit species has resistance to Gummy stem blight (Keinath, 2017).

Somai *et al.* (2002) have already reported *S. cucurbitacearum* for butternut squash in the United States of America. In Italy, this pathogen has been reported on *C. melo* (Corlett, 1981). To our knowledge, this is the first report of *Stagonosporopsis cucurbitacearum* on squash in Italy.

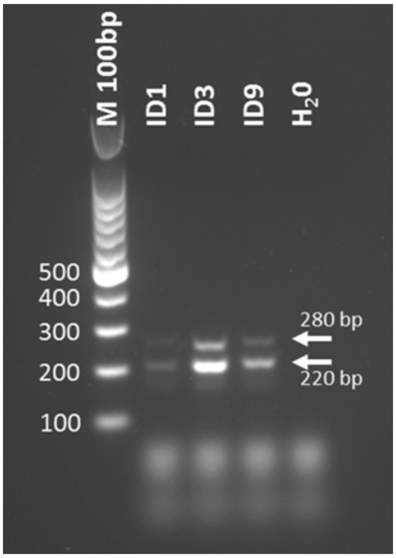

Figure 3. Polymerase chain reaction (PCR)-based marker for distinguishing *Stagonosporopsis cucurbitacearum*. Lane M is a 100-bp ladder with sizes of visible fragments indicated. Three fungal isolates (ID1, ID3 and ID9) from butternut squash fruit were analyzed with PCR-based markers using three sets of primers (Db01, Db06 and Db06) in a single reaction. Two amplicons of 220 and 280 bp were produced and no fragment of 360 bp was visible, despite the presence of the microsatellite locus Db01.

Table 1. Comparison of sequence similarities of *Stagonosporopsis cucurbitacearum* isolates with sequences already in the NCBI database.

Fungal species	Isolate number	NCBI accession no.	Nucleotide similarity (%)	Query cover
S. cucurbitacearum	ID1	EU167573	99%	100%
S. cucurbitacearum	ID3	MG009202	98%	100%
S. cucurbitacearum	ID9	KF990402	99%	99%

LITERATURE CITED

Aveskamp M.M., de Gruyter J., Woudenberg J.H.C., Verkley G.J.M., Crous P.W., 2010. Highlights of the *Didymellaceae*: a polyphasic approach to characterize *Phoma* and related pleosporalean genera. *Studies in Mycology* 65: 1–60.

Babu B., Kefialew Y.W., Li P.F., Yang X.P., George S., … Paret M.L., 2015. Genetic characterization of *Didymella bryoniae* isolates infecting watermelon and other cucurbits in Florida and Georgia. *Plant Disease* 99: 1488–1499.

Brewer M.T., Rath M., Li H.X., 2015. Genetic diversity and population structure of cucurbit gummy stem blight fungi based on microsatellite markers. *Phytopathology* 105: 815–824.

Choi I.Y., Choi J.N., Choi D.C., Sharma P.K., Lee W.H., 2010. Identification and characterization of the causal organism of gummy stem blight in the muskmelon (*Cucumis melo* L.). *Mycobiology* 38: 166–170.

Corlett M., 1981. A taxonomic survey of some species of *Didymella* and *Didymella*-like species. *Canadian Journal of Botany* 59: 2016–2042.

de Neergaard E., 1989. Studies of *Didymella bryoniae* (Auersw.) Rehm: development in the host. *Journal of Phytopathology* 127: 107–115.

Grube M., Fürnkranz M., Zitzenbacher S., Huss H., Berg G., 2011. Emerging multi-pathogen disease caused by *Didymella bryoniae* and pathogenic bacteria on Styrian oil pumpkin. *European Journal of Plant Pathology* 131: 539–548.

Huang C.J., Lai Y.R., 2019. First report of *Stagonosporopsis citrulli* causing gummy stem blight of watermelon in Taiwan. *Journal of Plant Pathology* 101: 417 (doi 10.1007/s42161-018-0192-x).

Keinath A.P., Farnham M.W., Zitter T.A., 1995. Morphological, pathological, and genetic differentiation of *Didymella bryoniae* and *Phoma* spp. isolated from cucurbits. *Phytopathology* 85: 364–369.

Keinath A.P., 2000. Effect of protectant fungicide application schedules on gummy stem blight epidemics and marketable yield of watermelon. *Plant Disease* 84: 254–260.

Keinath A.P., 2011. From native plants in central Europe to cultivated crops worldwide: the emergence of *Didymella bryoniae* as a cucurbit pathogen. *Hortscience* 46: 532–535.

Keinath A.P., 2013. Diagnostic guide for gummy stem blight and black rot on cucurbits. Online. *Plant Health Progress* doi: 10.1094/PHP-2013-1024-01-DG.

Keinath A.P., 2014. Differential susceptibility of nine cucurbit species to the foliar blight and crown canker phases of gummy stem blight. *Plant Disease* 98: 247–254.

Keinath A.P., 2017. Gummy stem blight. In: *Compendium of Cucurbit Diseases and Pests 2nd Ed* (A.P. Keinath, W.M. Wintermantel, T.A. Zitter, ed.), American Phytopathological Society, St, Paul, MN, 59.

Koike S.T., 1997. First report of gummy stem blight, caused by *Didymella bryoniae*, on watermelon transplants in California. *Plant Disease* 81: 1331.

Li P.F., Ren R.S., Yao X.F., Xu J.H., Babu B., … Yang X.P., 2015. Identification and characterization of the causal agent of gummy stem blight from muskmelon and watermelon in east China. *Journal of Phytopathology* 163: 314–319.

Mancini V., Romanazzi G., 2014. Seed treatments to control seedborne fungal pathogens of vegetable crops. *Pest Management Science* 70: 860–868.

Mancini V., Murolo S., Romanazzi G., 2016. Diagnostic methods for detecting fungal pathogens on vegetable seeds. *Plant Pathology* 65: 691–703.

Maynard D.N., Hopkins D.L., 1999. Watermelon fruit disorders. *HortTechnology* 9: 155–161.

Nuangmek W., Aiduang W., Suwannarach N., Kumla J., Lumyong S., 2018. First report of gummy stem blight caused by *Stagonosporopsis cucurbitacearum* on cantaloupe in Thailand. *Canadian Journal of Plant Pathology* 40: 306–311.

Park S.M., Jung H.J., Kim H.S., Yu T. S., 2006. Isolation and optimal culture conditions of *Brevibacillus* sp. KMU-391 against black root pathogens caused by *Didymella bryoniae*. *Korean Journal of Microbiology* 42: 135–141.

Rennberger G., Keinath A.P., 2018. Susceptibility of fourteen new cucurbit species to gummy stem blight caused by *Stagonosporopsis citrulli* under field conditions. *Plant Disease* 102: 1365–1375.

Seebold K.W., 2011. Gummy stem blight and black rot of cucurbits. Cooperative extension service, University of Kentucky, College of Agriculture, PPFS-VG-08.

Somai B.M., Dean R.A., Farnham M.W., Zitter T.A., Keinath A.P., 2002. Internal transcribed spacer regions 1 and 2 and random amplified polymorphic DNA analysis of *Didymella bryoniae* and related *Phoma* species isolated from cucurbits. *Phytopathology* 92: 997–1004.

Stewart J.E., Turner A.N., Brewer M.T., 2015. Evolutionary history and variation in host range of three *Stagonosporopsis* species causing gummy stem blight of cucurbits. *Fungal Biology* 119: 370–382.

Varanda C.M.R., Oliveira M., Materatski P., Landum M., Clara M.I.E., Félix M.d.R., 2016. Fungal endophytic communities associated to the phyllosphere of grapevine cultivars under different types of management. *Fungal Biology* 12: 1525–1536.

White T.J., Bruns T., Lee S., Taylor J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White ed.), Academic Press, San Diego, CA, USA, 315–322.

Yao X., Li P., Xu J., Zhang M., Ren R., … Yang X., 2016. Rapid and sensitive detection of *Didymella bryoniae* by visual loop-mediated isothermal amplification assay. *Frontiers in Microbiology* 7: 1372.