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Ascochyta hyalospora (Cooke & Ell.) comb. nov. in seeds of Chenopodium quinoa

G. H. BOEREMA[1], S. B. MATHUR[2] and PAUL NEERGAARD[2]

[1]Plant Protection Service (PD), Wageningen
[2]Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen

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Abstract

Ascochyta hyalospora (Cooke & Ell.) comb. nov. was encountered in seeds of *Chenopodium quinoa*; infection ranged from 7.8 to 26.3% in six samples from Bolivia. The fungus produced abundant pycnidia on ungerminated seeds and abnormal seedlings. Description of morphology and a list of synonyms are given. In pathogenicity tests the fungus caused leaf spots and stem necroses in *C. quinoa* and *C. album*.

Introduction

Chenopodium quinoa Willd. is an important plant species in western South America, the seeds being used as food. The plant is also commonly used as a test plant for plant viruses. During seed health testing of *C. quinoa* obtained from Bolivia, *Ascochyta* pycnidia were observed on ungerminated seeds and seedlings showing infection. In this paper we have described the fungus which was found to be identical with species previously described in North America. Its nomenclature is discussed and its pathogenicity established. Appropriate seed health testing procedures are defined.

Materials and methods

The six seed samples used in this investigation (Table 1) came from La Paz and Oruro, the two important *Chenopodium* growing areas of Bolivia. Sample No. 3 was collected from a farm, the others from experimental stations. The samples were harvested in August 1974 and tested in November 1974 by the methods given below.

1. Blotter method. Of each sample 200 seeds were tested. Twenty-five untreated seeds were equally spaced on three layers of water-soaked blotters in plastic petri dishes of 9 cm diameter and incubated at 20 °C under 12 hour alternating cycles near-ultraviolet light and darkness for seven days.

After incubation the seeds were examined under a stereoscopic microscope and the seedlings and ungerminated seeds were observed for pycnidial production.

2. Agar plate method. Of each sample 200 seeds were tested. Seeds were first pre-

treated with 1 % chlorine for 5 minutes before being plated on potato-dextrose agar (Difco) in plastic petri dishes, 10 seeds per dish. Incubation conditions were the same as in the blotter method.

3. Germination test. Germination of the seeds was tested by the Between Paper method 'BP' (I.S.T.A., 1976). Seeds were rolled in water-soaked filter paper and kept at 20 °C. Normal seedlings, abnormal seedlings and non-germinated (dead) seeds were counted after seven days. All abnormal seedlings and ungerminated dead seeds were examined microscopically for the presence of pycnidia of *Ascochyta* and infection of other fungi.

4. Pathogenicity test. Heavily sporulating *Ascochyta* cultures on oatmeal agar (C.M.I., 1968) were used for testing pathogenicity on fullgrown plants of *Chenopodium quinoa* and *Chenopodium album* L. A conidium suspension for inoculation was prepared by shaking dislodged ripe pycnidia in tap water and was applied with an atomizer. Controls were treated with tap water.

Results and discussion

1. Identity of the fungus. On oatmeal agar the fungus produces a dark mycelial mat with scattered globose to subglobose pycnidia, usually 175–250 μm in diameter (Fig. 1a). The opening in a pycnidium develops towards the end of the growing process and therefore may be interpreted as a porus instead of an ostiole. Sometimes short dark hyphae occur around the porus. The ellipsoidal or cylindrical conidia are relatively large, usually 20–30 $\times$ 8–12 μm. Initially they are hyaline but in mature condition yellowish-pale brown and then usually provided with one, or sometimes two septa (Fig. 1b, c). The size of the conidia is influenced by the growth conditions.

The morphological and cultural characters of the fungus point to the genus *Ascochyta* Lib., which has recently been redefined by Boerema & Bollen (1975). According to the Saccardoan system (Saccardo, 1884), *Ascochyta* comprised fungi which occur on leaves with pycnidia having 1-septate conidia. The present concept of the genus, however, includes similar fungi which also occur on stems (in the Saccardoan system erroneously arranged under *Diplodina* = *Discula*) and pycnidial fungi with occasionally 2- or 3-septate conidia (by Saccardo usually arranged under *Stagonospora* and sometimes under *Phleospora* = *Septoria*).

The pycnidial and conidial characteristics of the fungus on inoculated stems of *Chenopodium quinoa* and *Chenopodium album* agree with those in vitro, but the conidia are usually somewhat slender (Fig. 1d). The conidia found in the pycnidia on leaf spots obtained by inoculation in the two *Chenopodium* species were significantly longer than those formed in vitro (up to 35 μm) and moreover often had two or three septa (Fig. 1e).

In Europe many pycnidial fungi with 1-3-septate hyaline conidia have been described from Chenopodiaceae, but their conidia are significantly smaller than those of the present fungus. However, two species described in the United States of America, completely agree with our fungus:

The first species was described by Cooke and Ellis (1878) from stems of an unidentified *Chenopodium* species collected in Newfield, New Jersey, and named *Diplodia hyalospora* (conidia: 1-, sometimes 2-septate; 20–26 $\times$ 9 μm). This binomial

Fig. 1. *Ascochyta hyalospora:* a) Pycnidium in vitro on oatmeel agar (× 200); b) Conidiogenous cells of pycnidium on oatmeal agar (× 1000); c) Conidia from pycnidium on oatmeal agar (room temperature, ca 15 h daylight and 9 h dark regime) (× 400); d) Conidia from pycnidium on inoculated stem of *Chenopodium quinoa* (× 400); e) Conidia from pycnidium on leafspot of inoculated leaf of *Chenopodium quinoa* (× 400).

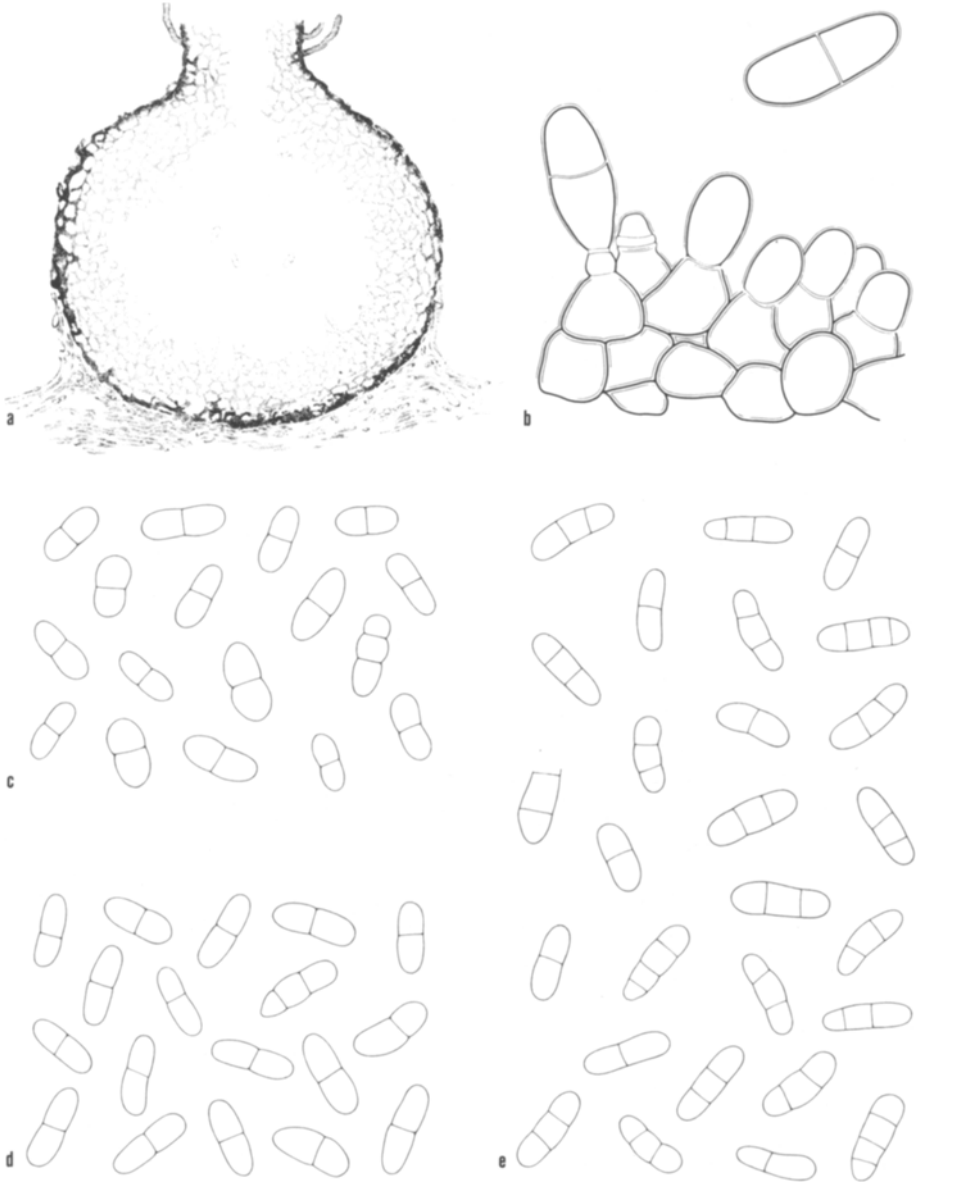

Fig. 1. Ascochyta hyalospora: a) Pycnide in vitro op havermout-agar (× 200); b) Conidiogene cellen van pycnide op havermout-agar (× 1000); c) Conidia van pycnide op havermout-agar (kamertemperatuur, per etmaal ca. 15 uur daglicht); d) Conidia van pycnide op geïnoculeerde stengel van Chenopodium quinoa (× 400); e) Conidia van pycnide op bladvlek van geïnoculeerd blad van Chenopodium quinoa (× 400).

seems contradictory, because the genus *Diplodia* belongs to the 'Phaeosporae' (with dark conidia). In the Latin diagnosis of the species the conidia are called 'hyalinis', but on a hand-written label attached to the holotype specimen (NY) is noted 'spores nearly hyaline, pale yellowish'.

The second species, *Phleospora chenopodii* Ellis & Kellerman (1888; holotype in NY), is described from leaf spots on *Chenopodium album*, Manhattan, Kansas (conidia: 3-septate; 20–35 (mostly 20–25) × 8–11 μm).

The first-mentioned species was transferred to the genus *Diplodina* by Saccardo (1884), who renamed it *Diplodina ellisii* Sacc. Without doubt Saccardo changed the epithet to avoid, in his opinion, the inappropriate combination '*Diplodina hyalospora*' (according to Saccardo's definition *Diplodina* belongs to the 'Hyalosporae' in contrast to the phaeosporous *Diplodia*). On transference of this species to the genus *Ascochyta*, as proposed below, we should have also preferred a more suitable epithet, because the conidia are explicitly non-hyaline being yellowish of pale brown. However, according to Art. 62 of the Seattle Code (Stafleu et al., 1972) a legitimate epithet must not be rejected merely because it is inappropriate or disagreeable.

ASCOCHYTA HYALOSPORA (Cooke & Ell.) Boerema, Mathur & Neergaard comb. nov.

≡ *Diplodia hyalospora* Cooke & Ell. *in* Grevillea 7: 5. (Sept.) 1878.

≡ *Diplodina ellisii* Sacc. *in* Sylloge Fung. 3: 417. 1884.

≡ *Phleospora chenopodii* Ell. & Kell. *in* J. Mycol. 4: 26. 1888.

2. Recording of the natural infection of A. hyalospora in seeds. The fungus attacked both seeds and seedlings, the effect being more pronounced on blotter, where it freely produced pycnidia on the seed coat of ungerminated seeds and seed coats adhering to seedlings, than on agar. The pycnidia were mostly produced superficially but in some cases even in the inner tissues of the seed. The fungus caused mild to severe browning in the root and hypocotyl. Some of the severely infected seedlings died.

Infection counts made on blotter and agar are presented in Table 1; they are generally much higher on blotter than on agar. Two of the samples showing 7.8 and 13.0% infection on blotter, respectively, gave no infection on agar. It is possible that the chlorine pretreatment before plating seeds on agar has eliminated most of the superficial infection. In order to check this, chlorine-pretreated seeds were plated on blotters in a subsequent experiment and the infection percentages were compared with those of untreated seeds. Chlorine treatment reduced the infection of the fungus significantly also on blotters (Table 2).

3. Germination. All six samples showed reduced germination; the percentage of normal seedlings ranged between 64 and 85 (Tab. 3). Abnormal seedlings were categorised according to the Handbook for Seedling Evaluation (Wellington, 1970). Five kinds of abnormalities were observed. The most common abnormalities encountered were:

a) seedlings with small cotyledons and short primary roots, and

b) seedlings with small primary roots and short hypocotyls.

The abnormal seedlings and dead seeds were examined microscopically and in almost all cases they were found to be infected by *Ascochyta hyalospora*.

Table 1. Percentage infection of *Ascochyta hyalospora* in seed samples of *Chenopodium quinoa* from Bolivia.

Sample No.	Blotter method	Agar plate method
1	24.0	4.5
2	26.3	9.0
3	7.8	0
4	9.0	4.0
5	21.0	6.0
6	13.0	0

Tabel 1. Percentage Ascochyta hyalospora in zes zaadmonsters Chenopodium quinoa uit Bolivia bij de filtreerpapier-methode (2de kolom) en de agar-methode (3de kolom).

Table 2. Effect of chlorine pretreatment on the infection counts of *Ascochyta hyalospora* incubated on blotter.

Sample No.	Untreated seeds	Chlorine pretreated seeds
1	31.0	7.0
2	27.5	6.0
3	6.5	1.0
4	9.5	4.5
5	22.5	8.5
6	14.0	6.0

Tabel 2. Effect van voorbehandeling met chloor op het infectiepercentage Ascochyta hyalospora in zes monsters geïncubeerd op filtreerpapier. Kolom 2: niet-voorbehandelde zaden. Kolom 3: voorbehandelde zaden.

Table 3. Percentage germination of infected *C. quinoa* seed using the Between Paper ('BP') method.

Sample No.	Normal seedlings	Abnormal seedlings	Dead seeds
1	71	24	5
2	64	14	22
3	81	16	3
4	85	10	5
5	75	15	10
6	78	18	4

Tabel 3. Kiemkrachtpercentage van zes monsters C. quinoa-zaad bij de zgn. BP (tussen papier)-methode. Kolom 2: normale kiemplanten. Kolom 3: abnormale kiemplanten. Kolom 4: niet gekiemde (dode) zaden.

4. Pathogenicity. The inoculation of full-grown plants of *Chenopodium quinoa* and *C. album* resulted in typical leaf spots and stem necroses, with abundant development of pycnidia (Fig. 2). The control plants remained healthy.

Fig. 2. *Chenopodium quinoa* inoculated with a conidium suspension of *Ascochyta hyalospora:* a) Whitish leaf spots, 5 days after inoculation (ca × 3/4); b) Necrotic leaf spots with pycnidia, 10 days after inoculation (× 1); c) Dead stem part with pycnidia (× 1).

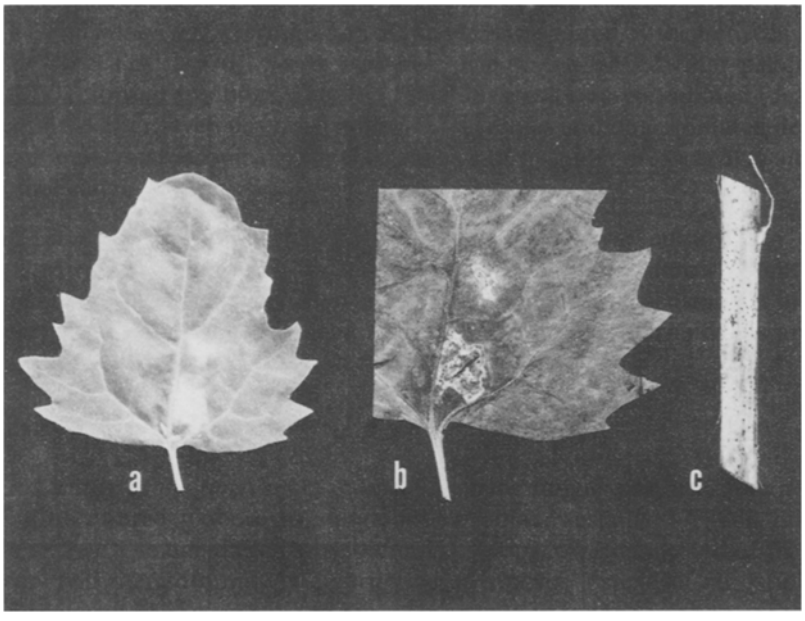

Fig. 2. Chenopodium quinoa geïnoculeerd met een sporensuspensie van Ascochyta hyalospora: a) Witte bladvlekken, 5 dagen na inoculatie (ca. × 3/4); b) Necrotische bladvlekken met pycniden, 10 dagen na inoculatie (× 1); c) Dood stengeldeel met pycniden (× 1).

Samenvatting

Ascochyta hyalospora (Cooke & Ell.) comb. nov. bij zaden van Chenopodium quinoa

In een zestal zaadmonsters van *Chenopodium quinoa* uit Bolivia bleek 7,8 tot 26,3% van de zaden geïnfecteerd te zijn met een pycniden-vormende schimmel die qua morfologie en kenmerken in vitro thuishoort in het geslacht *Ascochyta*. De genoemde infectiepercentages werden gevonden bij de filtreerpapier-methode; toepassing van de agar-methode gaf lagere percentages (Tabel 1), hetgeen bleek te berusten op de bij de agar-methode toegepaste voorbehandeling met chloor (Tabel 2).

De sporen van de schimmel, die aanvankelijk hyalien maar bij rijpheid geel–lichtbruin getint zijn, variëren in afmetingen en aantal septen afhankelijk van de groeiomstandigheden resp. het substraat (Fig. 1). De schimmel bleek identiek te zijn met de in de 19de eeuw door Noordamerikaanse onderzoekers beschreven *Diplodia hyalospora* Cooke & Ell. (later herdoopt tot *Diplodina ellisii* Sacc.) en *Phleospora chenopodii* Ell. & Kell. Op de eerstgenoemde naam is de voorgestelde nieuwe combinatie *Ascochyta hyalospora* (Cooke & Ell.) gebaseerd.

Een deel der geïnfecteerde zaden kiemde niet, andere brachten abnormale kiemplanten voort (Tabel 3). Een en ander ging samen met een overvloedige produktie van pycniden.

Inoculatie van volgroeide planten van *Chenopodium quinoa* en *C. album* met een sporensuspensie van de schimmel resulteerde in typische bladvlekken en stengelnecroses (Fig. 2).

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Addresses

G. H. Boerema: Plantenziektenkundige Dienst, Geertjesweg 15, Wageningen, the Netherlands.

S. B. Mathur and Paul Neergaard: Danish Government Institute of Seed Pathology for Developing Countries, 78, Ryvangs allé, 2900 Hellerup – Copenhagen, Denmark.