

A leaf spot disease of *Nymphaea alba* in the Netherlands

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Abstract

A destructive leaf spot disease was found on *Nymphaea alba* in the lakes near Kortenhoef, Netherlands. After comparison of fresh material and herbarium specimens the causal agent is named *Colletotrichum nymphaeae* (Pass.) van der Aa, comb. nov. Synonymy, morphology and cultural characters of the fungus are described and discussed. Some remarks on the relationship with several host plants are given and the provisional conclusion is that the species is a widespread parasite, but that the destructive attack in the Kortenhoef region is probably caused by a combination of various factors.

Introduction

In the beginning of July 1977 characteristic spotting was apparent on *Nymphaea alba* leaves from several parts of the lakes near Kortenhoef, in the centre of the Netherlands. On many of the affected leaves the spots were arranged in two lines, almost parallel to the midrib of the leaf (Fig. 1A). The spots at first were only a few millimeters in diameter and pale brown, but soon enlarged, fused and became dark brown to almost black (Fig. 1B). Leaves at this stage of attack easily broke off along the

Fig. 1. *Colletotrichum nymphaeae* on leaves of *Nymphaea alba*: A) Separate leaf spots. B) Further stage with coalescent leaf spots.

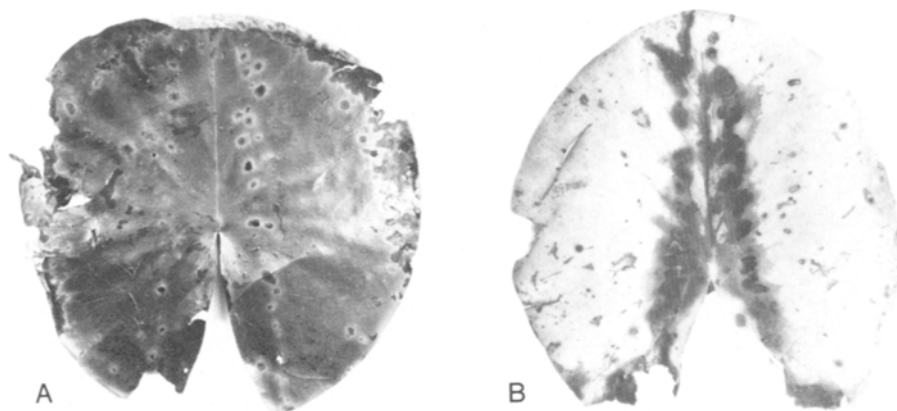


Fig. 1. *Colletotrichum nymphaeae* op bladeren van *Nymphaea alba*: A) Bladvlekken gescheiden. B) Later stadium waarbij de bladvlekken met elkaar versmelten.

affected 'lines' and only the midribs with small parts of the lamina remained. In some parts of the lakes numerous leaves attained this stage of damage in the middle of August.

In addition to the above pattern of arrangement, some leaves had scattered spots, which usually did not enlarge or fuse. These spots resembled the first type at an early stage of development.

From the very beginning of the attack a *Colletotrichum* species could be isolated from the inner parts of the leaves, after superficial disinfection. At a further stage numerous small, epiphyllous fruiting structures of this fungus could be observed on the spots. The species was tentatively identified as *Gloeosporium nymphaeorum* Allescher. The genus *Gloeosporium* however has not been accepted and Von Arx (1957, 1970) transferred most of the species into other genera. He had no material of this species available but mentioned its transfer to a separate genus, *Ovulariella*, by Bubák & Kabát (1912). The present study was based on a number of fresh specimens, isolates and herbarium collections of several species described in the last century, of which *Ascochyta nymphaeae* Pass. is the oldest, and this species is consequently transferred to the genus *Colletotrichum*.

***Colletotrichum nymphaeae* (Pass.) van der Aa, comb. nov.**

Ascochyta nymphaeae Pass. – Hedwigia 16: 120. 1877 (basionym).

Ramularia nymphaeae Bres. – Hedwigia 33: (97). 1894; Sydow, Mycotheca Marchica 4077. 1894 = *Ovularia nymphaeae* (Bres.) Bres. in Krieger, Fungi Saxonici 1286. 1896.

Gloeosporium nymphaeorum Allesch. – Hedwigia 34: 276. 1895 = *Ovularia nymphaeorum* (Allesch.) Bres. & Allesch. in Allescher, Rabenh. Kryptog. Fl. 7: 511. 1903 = *Ovularia nymphaeorum* (Allesch.) in Lindau, Rabenh. Kryptog. Fl. 8: 241. 1905 = *Ovulariella nymphaeorum* (Allesch.) Bubák & Kabát – Hedwigia 52: 362. 1912.

Description on the host plant

Leaf spots roundish, initially 0.5–5 mm in diameter, later enlarging and coalescing to occupy larger areas of the leaf, initially pale brownish or greyish brown with a dark reddish brown to purplish brown margin, later dark brown to black, irregularly distributed over the upper leaf surface or arranged in rows, more or less parallel to the midrib.

Conidiogenous structures epiphyllous, whitish, arranged in narrow concentric rings, initially intraepidermal, 15–35 μm in diameter, composed of a cluster of thin-walled, hyaline to pale brownish, 4–10 μm wide hyphae, soon erumpent with a tuft of divergent conidiogenous cells (phialides) (Fig. 2a). In further stages of development these hyphal clusters can penetrate to the subepidermal tissues of the leaf and form 85–160 μm wide acervuli with a 10–25 μm thick basal tissue, composed of rather thin-walled, isodiametric or irregular, hyaline cells, 4–20 μm in diameter (Fig. 2b). More rarely the fruiting structures initiate as subepidermal, subglobose or pyriform, 30–60 μm wide, rather compact clusters, composed of a basal tissue with isodiametric, subhyaline or olivaceous-brown, 4–8 μm wide cells and an apical tissue of hyaline, elongated, parallel rows of cells from which the conidiogenous cells emerge. These initials resemble pycnidia but soon they open widely.

The conidiogenous cells are short conical or elongated, usually tapering towards

Fig. 2. *Colletotrichum nymphaeae*: a) Cross section through a leaf of *Nymphaea alba* with hyphomycetous type of sporulation. b) Cross section through an acervulus. c) Conidia.

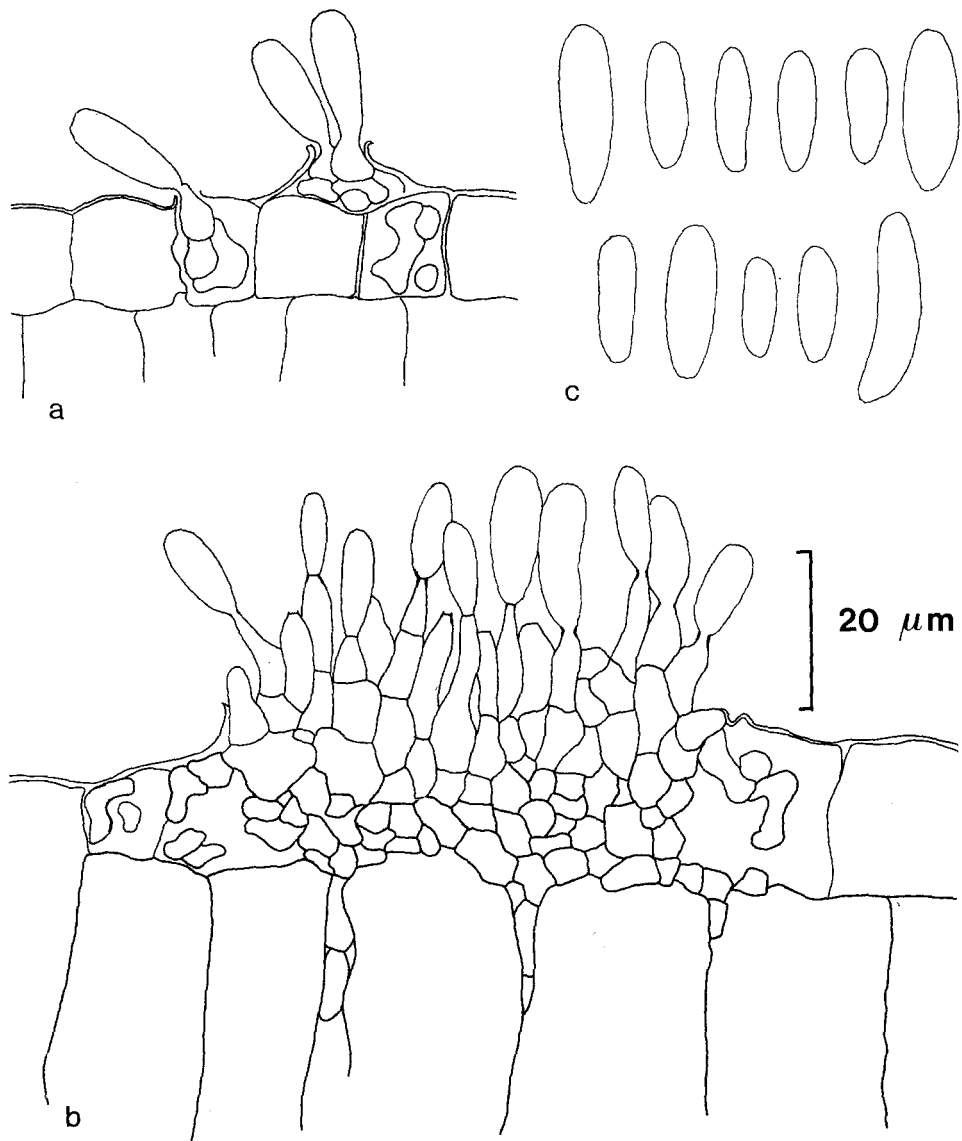


Fig. 2. *Colletotrichum nymphaeae*: a) Doorsnede door een blad van *Nymphaea alba* met hyphomycet-achtige groeiwijze. b) Doorsnede door een acervulus. c) Conidiën.

the tip, $12-30(-38) \times 2-7 \mu\text{m}$, forming conidia in basipetal succession. The conidia are one-celled, hyaline, ellipsoidal or cylindrical, straight or slightly curved, rounded at the apex, somewhat attenuated, occasionally truncate at the base, $11.8-23.0 \times 4.2-5.6 \mu\text{m}$. Setae, which are characteristic of many species of *Colletotrichum*, are not formed.

Description in pure culture

Colonies growing rather slowly, attaining a diameter of 4–5 cm on CMA, MEA and OA and 5–7 cm on PDA in 14 days at 20 °C in daylight. On MEA the colonies have a lobate, on the other media a finely fimbriate margin. In fresh isolates no aerial mycelium is developed; the submerged mycelium is composed of hyaline, septate, repeatedly branched hyphae, locally turning greyish to olivaceous-brown, especially on OA, where the colony attains a dark spotted appearance. In older cultures the colony turns dark olivaceous to olivaceous-black with a shade of dark slate blue on MEA.

Conidiogenous structures are formed from the very beginning and are macroscopically visible from the 5th day onwards. They are at first completely hyphomycetous, with conidiogenous cells borne singly or in small groups directly on the germinating conidia or on the germ tubes (Fig. 3b); in older cultures melanconiacious structures are formed, with the conidiogenous cells more densely aggregated on a basal tissue, which is composed of isodiametric, hyaline to subhyaline, occasionally pale olivaceous, thin-walled cells, 4–15 μm in diameter. Conidiogenous cells and conidia as on the host plant. The conidiogenous structures are usually arranged in a concentric pattern and produce peach to flesh coloured slimy conidial droplets.

In a hanging drop of water the conidia usually germinate immediately after first becoming one septate. The germ tubes usually arise from the apex of the conidium or near it. They are hyaline, septate, rarely branched, 2–6(10) μm thick and form

Fig. 3. *Colletotrichum nymphaeae*, germinating conidia: a) In a hanging drop, showing the formation of appressoria. b) On malt agar after 3 days.

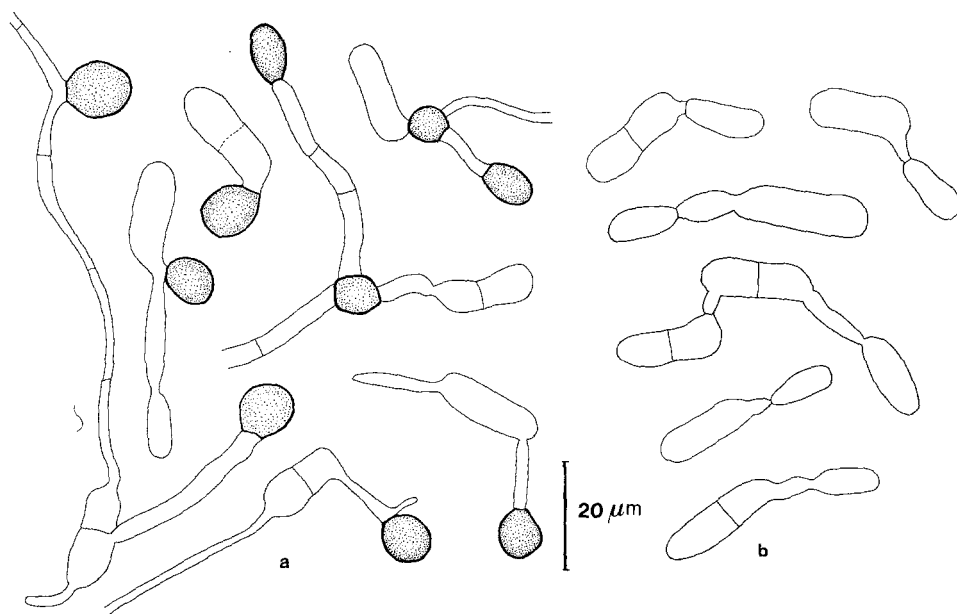


Fig. 3. *Colletotrichum nymphaeae*, ontkieming der conidiën: a) In hangende druppel, met appressoriën-vorming. b) Op mout-agar na 3 dagen.

olivaceous-brown to dark brown, subglobose to pyriform, rather thick-walled appressoria, which are 8–12, seldom up to 18 μ m in diameter (Fig. 3a).

Material examined

Herbarium specimens:

Ascochyta nymphaeae Pass., Rabenhorst, Fungi Europaei exsiccati, Klotzschii herbarii vivi mycologici continuatio, Edit. III: 2251, ad folia Nymphaeae albae, Parma, leg. G. Passerini, 1877; paratypus (CBS 00769).

Gloeosporium nymphaearum Allesch., Pilzherbar A. Allescher, auf *Nymphaea alba*, München, Bot. Garten, leg. A. Allescher, Sept. 1894; with handwritten descriptions by Allescher; holotypus (M).

Ovularia nymphaeae Bres., Krieger, Fungi Saxonici 1286, auf *Nymphaea alba*, bei der Schweizmühle, leg. W. Krieger, 25 Aug. 1895 (U).

Colletotrichum nymphaeae (Pass.) van der Aa, Herb. mycol. H. A. van der Aa 6040, on leaves of *Nymphaea alba*, Kortenhoef, near Wijde Blik, leg. J. Daams & H. A. van der Aa, 12 Aug. 1977 (CBS); Herb. mycol. H. A. van der Aa 6045, on leaves of *Nymphaea* cultivar, Wageningen, pond in garden, leg. D. Mulder, August 1977 (CBS); Herb. mycol. H. A. van der Aa 6046, on leaves of *Nymphaea alba*, collected in the Kortenhoef area and sent to the Phytopathological Laboratorium 'Willie Commelin Scholten', Baarn, 12 Aug. 1977 (CBS).

Living cultures:

CBS 526.77, isolated from leaf of *Nymphaea alba*, Kortenhoef, Netherlands, August 1977 by H. A. van der Aa (from Herb. mycol. H. A. van der Aa 6040). Several further isolates from leaves in the same area.

Taxonomic position of the fungus

The fungus is classified in *Colletotrichum* because of the structure of the conidiomata, the phialidic conidiogenous cells and the presence of appressoria on the germ tubes of the conidia. It is close to the plurivorous *Colletotrichum gloeosporioides* Penz.; the size and shape of the conidia fall within the wide range of this species (Von Arx, 1970). On the other hand, *Colletotrichum nymphaeae* differs in that it often grows in a hyphomycetous manner, both on the host plant and in pure culture, and in its apparent restriction to Nymphaeaceae.

The hyphomycetous appearance of the fungus is the reason that it has repeatedly been described in the hyphomycete genera *Ramularia* and *Ovularia*. Sometimes the young fruiting structures are subglobose and pycnidium-like and therefore the fungus was described by Passerini (1977) in the genus *Ascochyta*. The melanconiacous nature of the mature fruitbodies of *Ascochyta nymphaeae* Pass. was already recognized by Diedicke (1915) and later confirmed by several other authors (Grove, 1937; Mel'nik, 1977).

Hemmi and Kawase (1954) described a fungus pathogenic on *Nymphaea* spec. in Japan under the name *Gloeosporium* (*Colletotrichum*) *nymphaeae* Hemmi & Kawase. The generic name *Colletotrichum* was placed in brackets as the authors were of the opinion that *Colletotrichum* would differ from *Gloeosporium* only by the presence of setae in the former. They considered this as an unstable character and preferred the generic name *Gloeosporium*. From their description the fungus appears to be a typical species of *Colletotrichum*, which differs from *C. nymphaeae* by the constant melanconiacous nature of the fruitbodies and presence of dark brown, thick-walled,

up to 76 μ m long setae. From their description it may be conspecific with *Colletotrichum gloeosporioides* Penz.

Host plants and pathogenicity

Colletotrichum nymphaeae has been reported under its synonyms on several species of *Nymphaea* and on *Nuphar luteum*. Allescher (1899) observed it on *Nymphaea alba* (the type host) and on *N. capensis*, near Coimbra, Portugal. Lindau (1905) recorded the species on *Nymphaea alba*, *N. flava* and *N. odorata*. The present author has only seen the fungus on *Nymphaea alba* and a *Nymphaea* cultivar.

In the Kortenhoef area, where *Nuphar luteum* grows close to the affected *Nymphaea* vegetation, many leaf spots of *Nuphar luteum* were investigated but *Colletotrichum nymphaeae* was not found. In older, zonated spots on leaves of *Nuphar luteum*, *Phoma macrostoma* Mont., a plurivorous species, was occasionally found. Grove (1935) mentioned *Ascochyta nymphaeae* Pass. on *Nuphar luteum*, but from the description this also may have been a *Phoma* species, close to *P. macrostoma*. In 1937 Grove classified *Ascochyta nymphaeae* Pass. as *Gloeosporium nymphaearum* Allesch., which was found on both *Nymphaea* and *Nuphar* in England and was considered to be the cause of a severe disease.

There are two types of attack: one rather destructive with enlarging spots arranged in lines, more or less parallel to the midrib of the leaf, and another less destructive with restricted spots scattered irregularly over the lamina. The latter pattern is found in most of the herbarium specimens, the fresh collections from Wageningen and some of the leaves from Kortenhoef. *Colletotrichum nymphaeae* has been found in many European countries since the late 19th century and the attack occasionally has caused some damage. However, an attack with the characteristic parallel arrangement of spots, as found in Kortenhoef, has never been mentioned and it is very likely that some other organism or circumstances are responsible for the early infection and destructive development of the fungus. An attack by animals could not be detected but this can probably be found at a very early stage of leaf development. When leaf spots appear in the Kortenhoef area small holes are visible in the centre of many spots, suggesting that an arthropod has been active. It can be provisionally concluded that the fungus is a wide-spread parasite but that the destructive attack in Kortenhoef is caused by a combination of factors of which the first is unknown.

Samenvatting

Een bladplekkenziekte van Nymphaea alba in Nederland

Gedurende de zomermaanden van 1977 werd in het Kortenhoefse plassen gebied een ziekte geconstateerd bij de Witte waterlelie. Vanaf het moment dat de symptomen zichtbaar waren kon uit de bladeren een schimmel worden geïsoleerd, die in een later stadium ook op de bladplekken fructificeerde. De betrokken schimmel bleek een reeds lang bekende parasiet te zijn die onder verschillende namen beschreven was. Uit dit onderzoek is gebleken dat de schimmel thuishoort in het geslacht *Colletotrichum*, hetgeen resulteerde in de nieuwe combinatie *Colletotrichum nymphaeae* (Pass.) van der Aa. Een beschrijving van de schimmel op de plant en in reïncultuur

wordt gegeven, waarbij tevens aandacht is besteed aan de conidiënkleming.

Hoewel deze studie op de eerste plaats een taxonomische en beschrijvende wil zijn, worden nog enkele opmerkingen gemaakt over de ziekte als zodanig. De voorlopige conclusie wordt getrokken dat de schimmel weliswaar een mogelijk tot deze waardplantfamilie beperkte parasiet is, maar dat het ernstige schadebeeld dat zich bij Kortenhoef voordeed waarschijnlijk het gevolg is van een combinatie van factoren, waarbij de eerste factor, mogelijk een insect, niet bekend is.

References

- Allescher, A., 1899. In: Rabenhorsts Kryptogamenflora: Die Pilze Deutschlands, Österreichs und der Schweiz, Abt. 6, Fungi imperfecti: hyalin-sporige Sphaerioideen. Liefg. 64-69: 321-704.
- Arx, J. A. von 1957. Revision der zu *Gloeosporium* gestellten Pilze. Verh. K. ned. Akad. Wet. Afd. Natuurk., Reeks 2, 61 (3): 1-153.
- Arx, J. A. von, 1970. A revision of the fungi classified as *Gloeosporium*. Bibliotheca Mycologica 24: 203 pp., Lehre.
- Bubák, F. & Kabát, J. E., 1912. Mykologische Beiträge IV. Hedwigia 52: 340-363.
- Diedicke, H., 1915. Pilze VII, Sphaeropsideae, Melanconieae. KryptFl. Mark Brandenburg 9: 962 pp.
- Grove, W. B., 1935. British stem- and leaf-fungi (Coelomycetes). 1: 488 pp. Cambridge University Press.
- Grove, W. B., 1937. British stem and leaf-fungi (Coelomycetes) 2: 406 pp. Cambridge University Press.
- Hemmi, T. & Kawase, Y., 1954. On a new anthracnose of Waterlily caused by *Gloeosporium nymphaeae* sp. n. Bull. Naniwa Univ., Sér. B, 4: 1-6.
- Lindau, G., 1905. In: Rabenhorsts Kryptogamenflora: Die Pilze Deutschlands, Oesterreichs und der Schweiz, Abt. 8, Fungi imperfecti: Hyphomycetes (erste Hälfte). Liefg. 95-98: 177-432.
- Mel'nik, V. A., 1977. Opredeitel' gribov roda *Ascochyta* Lib. Izd. Nauka, Leningrad: 245 pp.
- Passerini, G., 1877. *Ascochyta nymphaeae* Passer. Hedwigia 16: 120.

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