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Short communication

An oospore-forming strain of *Peronospora statices* on cultivated *Limonium* in the UK, the Netherlands and Italy

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Abstract

Peronospora statices is reported on cultivated *Limonium sinuatum* plants for the first time in the UK, the Netherlands and Italy. This is the first record of a downy mildew on a host in the *Plumbaginaceae* in these three countries. A description of this rare fungus and the disease it causes, including the first details of the unusual morphology of its oogonial walls, is provided. In the UK outbreak, the disease was controlled by the fungicide furalaxyl. The implications of the occurrence of an oospore-forming strain of this potentially highly destructive fungus are discussed.

Background

In mid-February 1995, a consignment from the Netherlands of 405 plants of statice, *Limonium sinuatum,* consisting of cvs Misty Pink, Misty White and Misty Blue, was received at a nursery in Cornwall (SW England). The plants were grown in two polytunnels, 6 m wide and 30 m long with good ventilation, being open at each end and on a marked slope. The plants were irrigated by low level spray. Symptoms of a mildew disease were first seen on one-year old plants and spread to two-year old plants, with the result that 100% showed similar symptoms on 7 July 1995 when the nursery was inspected. The nursery had grown statice plants since 1992; these had always been obtained from the Netherlands, although mildew symptoms had never been seen before. Initially, the mildew caused localised leaf necrosis which eventually spread to the entire leaf. The old growth remained attached to plants, leaving a collar of dead leaves around the stem. The first year plants were most severely affected with some small patches of plants severely affected.

At first, the infection was treated with Fungaflor (Hortichem Ltd.; active ingredient imazalil) with little or no effect. At week 23, Fongarid 25 WP (Ciba Agriculture; active ingredient furalaxyl) was applied to plants, followed by two further sprays at weekly intervals and a final spray two weeks later. These treatments appeared to control the disease and new growth was free from infection. In early November 1995, the plants were cut down and all the material was carefully removed and burnt.

Plant material from this UK outbreak was sent to the International Mycological Institute for identification of the fungus during June and July, 1995 (IMI 367976 and 368485). Material was also obtained in 1995 from an outbreak of the disease in the Netherlands on *L. sinuatum* cv. Misty Blue (IMI 368514). At the same time, a specimen of downy mildew on *Limonium* sp. was received for identification by the IMI from Pescia, Italy (IMI 368673) following disease outbreaks which had occurred in Pescia (PT) and Montecarlo (LU) in 1995. These outbreaks were subsequently reported by Vettori and Carrai (1995) as occurring on *Limonium* × *hybridum* cvs Beltlaard and Ocean

Blue and on *Limonium altaica* cv Emile. They noted that

> Symptoms appeared both on the edge, or in the centre of the leaf, and enlarged to the whole leaf quickly. Necrotic lesions were often surrounded by [a] chlorotic halo; in the former phase, oil-like spots appeared on leaves, looking like those caused by downy mildew on grapevine.
>
> (translation by Vettori and Carrai).

Preparation of material for microscopic observation

Material from the UK collection was mounted in lactic acid, examined under the light microscope at $\times 1000$ magnification and the dimensions of forty conidiophores and conidia were measured and averaged. Material was also examined using Nomarski differential interference contrast (DIC) optics to observe the surface of the conidium and conidiophores (Figure 1A, B). Small pieces of infected tissue were prepared for examination with a low temperature scanning electron microscope (Hitachi S570) according to the method of Hall and Humphreys-Jones (1989) (Figure 1C). Leaf sections were also cleared to reveal oospores by soaking in 10% (w/v) NaOH at 25 °C for 48 h, followed by neutralisation with 1M HCl and three washes in distilled water. Cleared leaves were then mounted in lactic acid, observed with the light microscope and the dimensions of 20 oogonia, oospores and oospore walls were measured and averaged (Figure 1D–F).

Description of the fungus

Leaf lesions brown, bounded by veins; felt of conidiophores borne on the undersurface of the leaf. *Conidiophores* arborescent, branching 5–6 times dichotomously in the upper half to one third, (112-) 210 ± 64.0 (-332) μm long from base to first branch, trunk 7–10 μm wide, smooth, bases straight or with a slight swelling, tips 3–8 μm long, usually unequal in length, reflexed at 90° or more with blunt ends. *Conidia* ovoid to globose, often conical towards the point of attachment to the conidiophore, wall very pale brown, translucent, pedicel very short, (16.0-) 19.5 ± 0.97 (-22.4) × (12.8-) 14.5 ± 1.80 (-15.2) μm; under the light microscope, the surface of conidia appeared lightly roughened (microtuberculate), which was more easily demonstrated using the S.E.M. (Figure 1C); a smooth annulus was visible around the pedicel at its break point from the conidiophore. *Oogonia* were abundant in leaf tissue, (sub)globose, infrequently ovoid, pale yellow, 41.8 ± 2.37 μm diameter (average of 20 crossed diameters), thin-walled, densely pitted. *Antheridia* translucent, usually seen attached to the oogonia, ovoid, to irregularly elongated, usually lobed, large, 5–6 × 15–20 μm. *Oospores* (sub)globose, translucent when young, becoming yellow-brown or brown on ageing, 32.0 ± 1.54 μm diameter with a thin, deep brown, irregularly wrinkled, ridged or tuberculate, outer spore wall (exosporium) and a thick (4–5 μm), more lightly pigmented inner spore wall (endosporium), the innermost surface of which was irregularly sinuous. The ooplast was not readily visible.

Examination of leaves of infected plants from the Netherlands and Italy with the light microscope showed that the pathogen on these plants was morphologically identical to the one on the UK material and that oospores were also present in leaf tissues.

Discussion

This disease has also been reported on plants of *Limonium tataricum*, a dried flower crop, in Bács-Kiskun County, Hungary by Virányi and Szántó (1990) who attributed it to *Peronospora statices*. They observed symptoms of the disease on flowering and on transplanted (but not flowering) plants. The leaves of diseased plants became yellow, shrunken and developed a bluish-grey layer on their lower surfaces and had distorted flower stalks, but it is not clear whether they were killed entirely, as were the plants in this study. Considerable loss of plants from this disease was observed by Virányi and Szántó (1990) who recommended early control.

Three names are available for *Peronospora* species on the *Plumbaginaceae*; *Peronospora constantineanui* Săvul. & Rayss, *P. limonii* Simonyan and *P. statices* Lobik, which is the earliest of the three names. Săvulescu (1948) retained *P. constantineanui* on the basis of its general aspect, longer and thinner conidiophores, larger conidia and a different felt colour. However, Constantinescu (1991) compared the type specimens of these three species, but found no significant difference between them and suggested that the names were synonymous. Ul'yanishchev (1967) also considered that *P. constantineanui* was synonymous

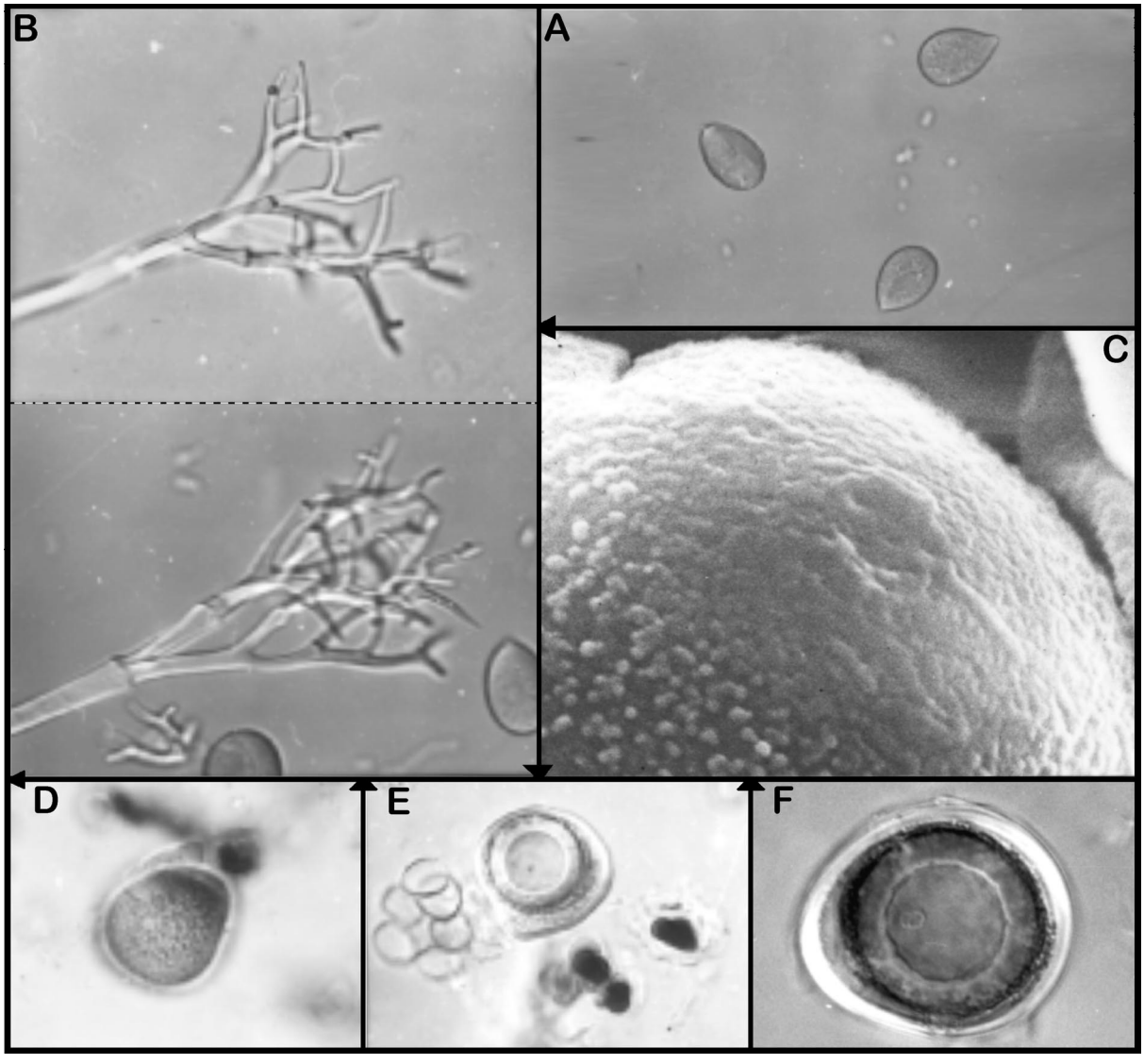

Figure 1. *Peronospora statices* from cultivated *Limonium sinuatum*. (A) Conidia. (B) Smooth conidiophores. (C) Conidium showing microtuberculate surface, annulus and detachment scar. (D) Oogonium with pitted wall and attached antheridum. (E) Oogonium showing thin (detached) exosporium and thick endosporium and attached antheridium (arrow). (F) Oospore showing irregularly sinuous inner wall. (C) was taken using low temperature S.E.M.; the rest with Nomarski DIC optics. —— Bar = 15 μm for A; 10 μm for B, E–H; 0.95 μm for C.

with *P. statices* (as *P. staticis*). Therefore, the name *Peronospora statices* is used to describe the species on *Limonium sinuatum* described here.

Peronospora statices was first described by Lobik (1928, p. 18 & Table1, Figure 1a–c) on *Statice gmelinii* (=*Limonium gmelinii*) growing on the island of Malyi Gokhai ('Little' Gokhai) during an investigation of the mycoflora of flooded areas of the river Kuma. This river flows from the Caucasus to the Caspian Sea, 200 km south of Astrakhan and the site may now be in Checheniya. Lobik (1928) gave the dimensions of conidia as 16.5–23 $\times$ 13.2–18 μm which are very similar to those reported here. The slight roughening of the conidial surface was not recorded by Lobik (1928), but has also been recorded in *Peronospora sordida* Berk. & Broome (Hall and Humphreys-Jones, 1989) and *Peronospora sparsa* Berk. (Constantinescu, 1996). The smooth annulus around the pedicel at its break point was also not recorded by Lobik (1928),

but was observed in *Peronospora sordida* (Hall and Humphreys-Jones, 1989).

There are records of *P. statices* in France on *Limonium vulgare* Miller subsp. *serotinum* (=*Statice limonium*) (Viennot-Bourgin, 1956, as *Peronospora constantineanui*); in Hungary on *Limonium tataricum* (Virányi and Szántó, 1990); in Azerbaidjan, in the Caucasus (Stavropol) on *Limonium meyeri* (Ul'yanishchev, 1967; with a description in Russian); in Romania on *L. vulgare* subsp. *serotinum* (Săvulescu and Rayss, 1932; as *P. constantineanui*) and on *Limonium gmelinii* (Constantinescu and Negrean, 1983, p. 549); and in Armenia on *Limonium meyeri* (Simonyan, 1976; as *Peronospora limonii*). *Peronospora statices* was recorded in Russia by Jaczewski and Jaczewski (1931) on *Statice gmelinii* (=*Limonium gmelinii*), but, as no location was given, it is most likely a repetition of the original host record by Lobik (1928).

There are no reports of oogonia or oospores in the original description by Lobik (1928), in any of the records of its occurrence listed here and they were not found in five collections from Romania (from the Danube delta and eastern Romania) examined by Constantinescu (pers. comm.). The tuberculate nature of the oospore wall places it in the section *Calothecae* of de Bary and Fischer's scheme (see Gustavsson, 1959), but the irregularly pitted nature of the oogonium is a feature which has not been recorded in oospores of the genus *Peronospora* before. Oogonia are produced in downy mildews by the mechanisms of heterothallism, primary or secondary homothallism, heterokaryosis or by host, chemical or environmental induction (Hall, 1996). However, the reason for the occurrence of sexual reproduction in this strain is unknown and the prevalence of sexually-reproducing strains in this species is unclear, since material in previous records may not have been examined for the presence of oospores.

P. statices appears to be mainly confined to western Asiatic Russia and Romania, where it is rare, and there are no previous records of its occurrence (or of the occurrence of synonymous species) in the UK (Francis and Waterhouse, 1988), in the Netherlands (Oudemans, 1904; Anon., 1961) or in Italy (Ciferri, 1961). The first occurrence of an oospore-forming strain on statice in three separate European countries suggests that the downy mildew may have been distributed in infected stock.

A sexually-reproducing strain of this fungus with such destructive potential must have serious implications for the cut flower industry and requires advice about control measures to be more widely known. The initial application of an unsuitable fungicide by the grower demonstrates the importance of the correct identification of a pathogen and an understanding of its biology for effective control. Experience in the UK clearly demonstrates the effectiveness of furalaxyl against the disease. In the Hungarian outbreak, Ridomil Plus 50WP (copper oxychloride and metalaxyl; Ciba Agriculture), Mikal C 64 WP and Sandofan C were moderately effective in controlling the disease in a pot experiment with plants of *Limonium tataricum*, but Cursate Super CZ and copper oxychloride 50WP were ineffective (Virányi and Szántó, 1990).

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