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PATHOLOGIE VÉGÉTALE

Colletotrichum destructivum O'Gara, causal agent of a new disease on *Lotus tenuis* Waldst. et Kit.

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SUMMARY

A new disease caused by *Colletotrichum destructivum* O'Gara on *Lotus tenuis* Waldst. et Kit. has been studied for the first time. The anthracnose was found in field plants in the province of Buenos Aires, Argentina. The pathogen was isolated, cultured on PDA and identified by its morphobiometrical and cultural characters. The symptoms of the disease and causal agent characteristics are described. The fungus was inoculated on some economically important legumes to test its pathogenicity. The presence of *C. destructivum* was studied on 25 *L. tenuis* seed samples from different areas. The results showed that one isolate caused wilt and death of *L. tenuis* plants, whereas the other two isolates caused only light symptoms.

Additional key words : *Anthracnose, legumes.*

RÉSUMÉ

Colletotrichum destructivum *O'Gara, agent d'une nouvelle maladie de* Lotus tenuis *Waldst. et Kit.*

Cette étude présente pour la première fois l'anthracnose de *Lotus tenuis* Waldst. et Kit., causée par *Colletotrichum destructivum* O'Gara. Cette maladie a été découverte sur des plantes cultivées en provenance de la Province de Buenos Aires, République Argentine. L'agent pathogène a été isolé, cultivé sur PDA et identifié sur la base de sa spécificité pathogénique et de ses caractères morphologiques, biométriques et culturaux. Les symptômes de la maladie ainsi que les caractéristiques de l'agent causal sont décrits.

Afin de vérifier son degré de virulence, le champignon a été inoculé à d'autres légumineuses d'importance économique. La présence de *C. destructivum* a été analysée sur 25 échantillons de semences de *L. tenuis* de différentes provenances. Les essais ont permis de montrer que l'un des isolats a provoqué le flétrissement et la mort des plantes de *L. tenuis*, tandis que deux autres ont causé seulement des symptômes légers.

Mots clés additionnels : *Anthracnose, légumineuses.*

I. INTRODUCTION

The perennial legume *Lotus tenuis* Waldst. et Kit., native to Europe, easily adaptable to flood-plains and to alkaline-saline soils, has become naturalized in the « Pampa Deprimida » of the province of Buenos Aires (MONTES, 1980). Its adaptability plus its high nutritive value (VIDAL & PIERGENTILI, 1963 ; VONESCH, 1968) has made it available as a forage plant.

Several wilted plants of *L. tenuis* presenting necrotic symptoms on aerial organs were found in the Experimental Field of the Faculty of Agronomy of the Universidad Nacional of La Plata. A fungus identified as *Colletotrichum destructivum* O'Gara was isolated from the infected material.

This fungus has been cited on several species of *Leguminosae* (LOPEZ-MATOS, 1962 ; MASSENOT & RAYNAL, 1973 ; TIFFANY & GILMAN, 1954) ; however, there is no mention of its parasitism on *L. tenuis*.

The aims of this work were to describe for the first time this anthracnose on the above-mentioned forage plant, to isolate and identify the etiological agent, to verify its effect upon other economically important legumes and to detect the presence of the fungus in *L. tenuis* seeds from different areas.

II. MATERIALS AND METHODS

The infected plants were found in the province of Buenos Aires. The pathogen was isolated from leaf and stems cuts presenting necrotic symptoms.

The material was disinfected with 70° alcohol and mercuric chloride 0.1 %, washed with sterile distilled water and afterwards cultured on 2 % potato dextrose agar (PDA).

To identify the fungus, morphobiometrical and cultural studies were conducted on single-spore colonies grown on the same media, in a climatized chamber at 20° ± 2 °C, under a 12 h light + 12 h darkness photoperiod. Mycelium color was described according to RAYNER's (1970) color chart.

Inoculations were performed with 3 isolates of the pathogen isolated from 3 naturally infested *L. tenuis* plants. A second culture obtained by re-isolating from isolate 2 was also used. *Lotus tenuis, L. corniculatus* L., *L. pedunculatus* Cav. from Argentina and England, *Glycine max* (L.) Merril cv. Hood, cv. Asgrow 4268 and cv. Calland, *Medicago sativa* L., *Trifolium repens* L. and *T. pratense* L., were inoculated with isolate 1. Isolate 2 was inoculated only on *L. tenuis*. Isolate 2 re-isolate and isolate 3 were inoculated on *T. repens, T. resupinatum* L., *T. alexandrinum* L., *M. sativa* L., *M. polymorpha* L., *Melilotus albus* Desr., *M. albus* var. *officinalis* (L.) Desr., *Phaseolus lathyroides* L., *P. erythroloma* Mart. ex Benth., *Leucaena leucocephala* (Lam.) De Wit., *L. esculenta* Benth., *Lotus tenuis* and *L. corniculatus*.

In every case, 15 plants of each legume grown in 3 pots (12 cm diameter) were infected ; 5 plants were the non-infected controls. The plants grew outdoors ; when they were one month-old they were sprayed with conidial suspension in sterile distilled water with a 1.5×10^6 spore/ml concentration. Inoculated plants and controls were kept in a moist chamber for 72 h and after in a growth chamber at 19°-28 °C and 85 % relative humidity.

Twenty-five samples of *L. tenuis* seeds from different areas of Buenos Aires were analyzed by the blotter test (I.S.T.A.), (NEERGAARD, 1974) to verify the presence of *C. destructivum*.

III. RESULTS

A. Symptomatology and etiology

The first symptom revealing the anthracnose in *L. tenuis* plants was total wilting of most of the canopy in contrast with green areas not yet infected. The leaves, yellowed or dried up, remained attached on the dead stems until they fell down. A great number of black spots with acervuli were easily observed on the greyish-brown internodal surface.

On PDA, the fungus developed round colonies, initially hyaline, slimy, with filamentous edges. On the 3rd day mycelium in the center of colonies darkened, turning from buff into olivaceous, and initiated several black acervuli in concentric rings. Big masses of salmon-colored conidia appeared progressively upon the acervuli, from the center towards the edges of the colony. Old cultures turned to olivaceous black or to black, and were covered with greyish exudates.

Conidia hyaline, unicellular, elliptical, obovoid, cylindrical or clavate, with both apices rounded or with acute basal one. Dimensions 7.5-16.87 (12.57) × 2.82 − 5.62 (4.11) µm.

Acervuli, rounded and black, with standard diameter varying from 130 to 280 µm, presented high number of setae (fig. 1). Setae were elongated and conical, 1-3-septate, colored isabelline or sepia, with a length of 75-150 (104.37) × 2.82 − 3.75 (3.33) µm width.

Figure 1

Acervuli of C. destructivum, *with setae and spores, isolated from* L. tenuis *(× 360).*

Acervules, avec soies et spores, de C. destructivum *isolé de* L. tenuis *(× 360).*

All these characteristics refered the isolated fungus to *Colletotrichum destructivum* O'Gara.

Cultural characteristics of the different isolates of *C. destructivum* were similar. In successive subcultures, isolate 1 was observed to stop sporulation, forming only sterile mycelium, whereas the other isolates presented no variation in sporulation.

B. Inoculations

L. tenuis plants infected with *C. destructivum* isolate 1 had lesions on leaves, stems and petioles after 10 days. Elongated necrotic reddish-brown spots could be observed girdling the stem in different zones extending from the apex to the crown. Green areas above each necrosis

finally dried, causing curling and wilting of terminal shoots (fig. 2). Dead stems were covered with abundant black spots which were identified under stereobinocular microscope as groups of setae belonging to the subepidermical acervuli (fig. 3). Simultaneously, leaves suffered from serious chlorosis followed by amphigenous, isolated or confluent rounded spots, light- to dark-brown. The development and spread of these necrotic spots over the leaf surface led to foliole death and fall. The defoliation became more severe as the anthracnose symptoms increased.

The other hosts inoculated artificially with the same isolate of the fungus presented only light necrotic spots on leaves, stems and petioles, except in the cases of *Phaseolus vulgaris* and the 3 *Glycine max* cvs., which were not affected.

Isolate 2 caused symptoms similar to those above mentioned, though of less importance. Some folioles presented necrotic spots ; the folioles from the lower part of the plant presented a chlorotic aspect. Re-isolated of isolate 2 was not effective when infected *L. tenuis* and the other hosts.

On *L. tenuis, L. corniculatus, Medicago polymorpha, Leucaena esculenta, L. leucocephala, Phaseolus lathyroides* and *P. erythroloma*, isolate 3 gave slight symptoms, scattered lesions on some folioles and stems.

C. Seed analysis

Only one sample, from the area of Chascomús, revealed 1 % fungus contamination. The other samples presented no contamination with *C. destructivum*.

IV. DISCUSSION AND CONCLUSION

Differences in pathogenicity between different isolates of *C. destructivum* have already been demonstrated by RAYNAL (1977) and DEBBAGH (1957). Accordingly, infections with different isolates of the microorganism on the same host resulted here both in different kinds of symptoms and infection intensity.

CHILTON (1943) studied exhaustively the variability of *C. destructivum* in culture. MASSENOT & RAYNAL (1973) also referred to the frequent loss of sporulation capacity on artificial media. The present work confirmed alterations in the aspect of the old colonies compared to the younger ones. Sporulating capacity in the successive subcultures was not constant either.

The mentioned alterations could be related to genetically different entities, such as mutations of the original culture (CHILTON, 1943). These genetical alterations could affect the fungus infectivity, as observed in the infection with re-isolate of isolate 2.

Curling and death of stems were the most severe injuries caused by *C. destructivum*. This corresponds with TIFFANY & GILMAN's (1954) observations on lucerne and *Melilotus* sp. They suggested a systemic infection of wilted stems ; this could likewise explain the phytopathological process observed on *L. tenuis*.

Morphobiometric, cultural and pathogenic characteristics of the fungus led to its identification as *C. destructivum*, anamorph of *Glomerella glycines* Lehman & Wolf (MANANDHAR *et al.*, 1986), cited as etiological agent of the anthracnose upon *L. tenuis*. It causes death of *L. tenuis* young plants with damping-off

Figure 2

A : Lotus tenuis *terminal shoot artificially inoculated. Curling and wilting caused by the anthracnose can be observed (× 1).*
B : Control.

A : Extrémité d'une tige de Lotus tenuis *inoculée artificiellement, montrant l'enroulement et le flétrissement provoqués par l'anthracnose (× 1).*
B : Témoin.

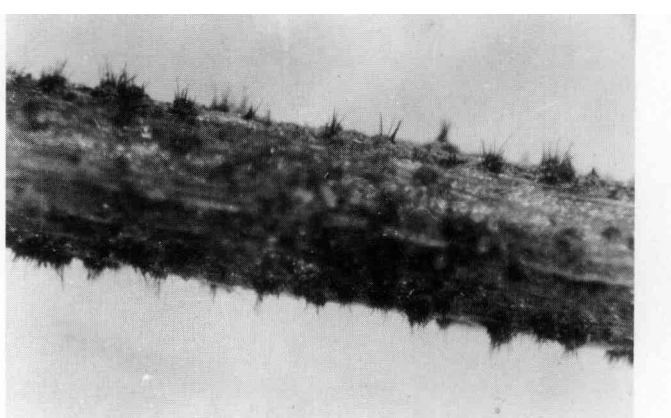

Figure 3

Stem portion of Lotus tenuis *naturally infected, with several acervuli (× 22).*

Portion de tige de L. tenuis *infectée naturellement montrant plusieurs acervules (× 22).*

and top-killing. In severe infections of adult plants, foliage wilt can be partial or total ; if total, it causes host death.

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