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Wilt and vascular root rot (*Pythium tracheiphilum*) of lettuce in Bahía Blanca, Argentina

Marchitamiento y podredumbre vascular (*Pythium tracheiphilum*) de lechuga en Bahía Blanca, Argentina

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Abstract. A new disease of lettuce has been observed in the surroundings of Bahía Blanca, Argentina. The symptoms include dwarfing, general chlorosis, wilting, root rot, and leaf blight, sometimes followed by plant death. *Pythium tracheiphilum* Matta was found to be consistently associated with those symptoms. The morphological and morphometric characteristics of this oomycete are described. Isolates obtained from field-infected lettuce plants were inoculated to lettuce and caused similar symptoms as those found in natural infections. Seedling emergence was also severely affected following experimental inoculation. Koch's Postulates were fulfilled by recovering the fungus from inoculated plants. The new disease shows a high destructive potential but currently has a limited prevalence and incidence.

Keywords: *Pythium tracheiphilum*; Lettuce; *Lactuca sativa*; Argentina.

Resumen. En los cultivos de lechuga del cinturón hortícola de Bahía Blanca se detectó una nueva enfermedad caracterizada por detención del crecimiento, clorosis, marchitez, podredumbre foliar y podredumbre vascular de la raíz, a veces seguido de muerte. El oomicete *Pythium tracheiphilum* Matta estuvo constantemente asociado con esta sintomatología. Se describe la morfología de este hongo que fue aislado de plantas enfermas de lechuga y estudiado in vitro. En ensayos de inoculación artificial en lechuga, se produjeron síntomas similares a los observados en infecciones naturales y se logró reaislar el hongo dando cumplimiento a los Postulados de Koch. *Pythium tracheiphilum* produjo además, en condiciones experimentales, la muerte de plántulas de lechuga en preemergencia. Hasta la fecha, la prevalencia e incidencia de esta nueva enfermedad es limitada pero se advierte de su potencial destructivo.

Palabras clave: *Pythium tracheiphilum*; Lechuga; *Lactuca sativa*; Argentina.

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most grown horticultural crops worldwide. Diseases caused by fungi, bacteria, viruses and other agents are an important adverse factor to lettuce production and marketing (Davis et al., 1997; Blancard et al., 2005). A compilation of the lettuce diseases recorded in Argentina is given by Wolcan (2014).

In autumn 1999, disease symptoms unknown to date were observed in lettuce crops of the green belt of the city of Bahía Blanca in the southern Pampas region, Province of Buenos Aires. Affected plants showed poor growth, wilting and root rot. The type of symptoms suggested that we were dealing with the vascular wilt caused by the oomycete *Pythium tracheiphilum* Matta. This fungus has been reported on lettuce and other plants in Europe, North and South America, and Australia (Tortorolo, 1985; Hall, 1991; Kumar et al., 2007). In Argentina, *P. tracheiphilum* has been indicated to occur on lettuce in Buenos Aires Province (Resnik, *s.a.*), without further information. We initiated studies with the objective of identifying the causal agent affecting lettuce in Bahía Blanca. A preliminary report has been presented (Kiehr et al., 2000).

MATERIALS AND METHODS

Roots of naturally infected lettuce plants of the Crespa and Mantecosa types were rinsed with tap water during 12 to 15 h and superficially disinfested with NaOCl (0.8% active chlor) during 1 min, dried with sterile paper and cut into small pieces which were transferred to Petri dishes with potato dextrose agar (PDA).

Inoculation trials were carried out on plants of lettuce cv. Grand Rapids. In the first trial, plants in the rosette stage were inoculated, placing small agar portions colonized with the fungus on: (a) non-wounded basal leaves, (b) the crown at soil level, wounded with a sterile needle, and (c) the non-wounded crown; (d) non-wounded non-inoculated, control plants were also included. The inoculation sites were covered with moistened cotton and the plants, five per treatment, were placed in a humid chamber. After two days they were transferred to the greenhouse.

The second trial, with plants in the two-leaf stage, was similar to the first one. The inoculum was placed on (a) non-wounded leaves and (b) the non-wounded crown tissue at the soil-line; (c) control plants remained non-inoculated. There were four plants per treatment.

In the third inoculation trial, 20-day-old fungal cultures in PDA were mixed with twice autoclaved soil, at the rate of one Petri dish (9 cm) per 500 cm^3 of soil. Eighteen seeds of lettuce cv. Grand Rapids were sown per pot. As a non-inoculated check, pots with uninfested autoclaved soil were sown in the same way. The pots were placed in the greenhouse and plant emergence and survival were recorded every two days. There were two treatments (Inoculated, Non-inoculated) with four replicates each, distributed at random. We used the Median test to compare the data of surviving plants.

RESULTS

Symptoms. Lettuce plants infected in the field appear isolated or in small patches. They are smaller than neighboring healthy plants, slightly chlorotic, presenting wilting (Fig. 1 A) and necrosis of leaves; eventually they die. In the beginning, the symptoms may appear on only one side of the plant.

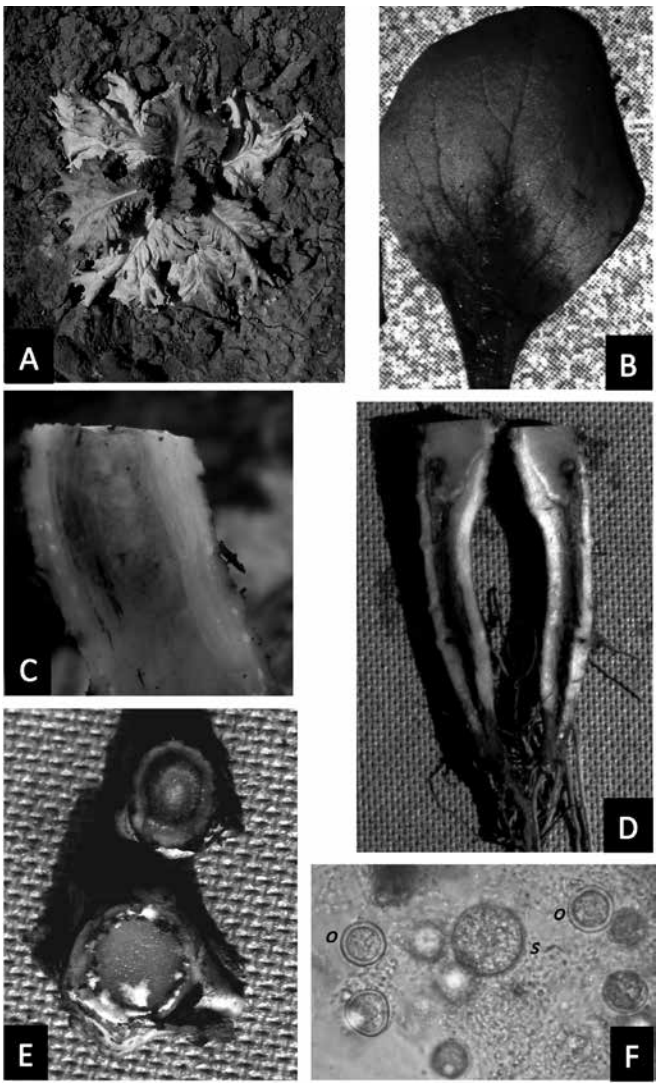

Fig. 1. (A-E): Symptoms on lettuce. (A) Wilting; (B) leaf rot (experimental infection); (C-E): vascular rot of the tap root; (D) *Pythium tracheiphilum*: sporangia (s) and oospores (o).
Fig. 1. (A-E): Síntomas en lechuga. (A) Marchitez; (B) podredumbre foliar (infección experimental); (C-E): podredumbre vascular de la raíz principal; (D) *Pythium tracheiphilum*: esporangios (s) y oosporas (o).

Lateral roots of diseased plants are poorly developed. The tap roots externally show a reddish-brown discoloration ascending from the basal part upwards. Internally, the distal part of the root may be affected totally by the rot, while in the proximal part, in the beginning only the vascular tissue shows a dark necrotic coloration, with small black striations. Later, the necrosis may extend to the central cylinder, and finally to the cortical tissue (Fig. 1 C-E). In some plants, the lower leaves may develop a wet rot accompanied by a faint whitish down, consisting of the mycelium and sporangia of the fungus.

Description of the fungus. *Pythium tracheiphilum* Matta: Mycelium hyaline, nonseptate. Sporangia are formed on infected plant tissue and, in large numbers, on PDA; terminal, sometimes intercalary, spherical to subspherical, 17.5 - 32 µm, sometimes elongated or irregular (Fig. 1 F); germinating directly via a germinating tube. Zoospores were not observed.

Oogonia formed in two to three weeks old cultures on PDA, smooth, intercalary or terminal, with one, rarely two monoclinous antheridia.

Oospores plerotic, spherical, smooth, 10.5 - 19 µm (Fig. 1 F).

Pathogenicity tests

First trial: Plants in the rosette stage, inoculated on their non-wounded basal leaves (treatment a), developed rot of petioles and lamina, as early as two days after inoculation. This rot rapidly progressed to the other aerial plant parts developing a generalized dark-brown necrosis (Fig. 1 B). Plants inoculated on the wounded crown tissue (treatment b) developed a rot which advanced towards the roots. On the infected tissue many sporangia of *P. tracheiphilum* were observed. Plants inoculated on the non-wounded crown (treatment c) and non-inoculated control plants (treatment d) did not become infected.

Second trial: Plants in the two-leaf-stage, inoculated on the non-wounded leaves (treatment a), developed initial symptoms identical to those in the rosette stage; however, after a few days, the rot descended to the crown and roots and the plants died. Plants inoculated in the non-wounded crown (treatment c) developed crown rot followed by plant death. Non-inoculated control plants (treatment c) remained healthy. *Pythium tracheiphilum* was re-isolated from the inoculated plants thus fulfilling Koch's postulates.

Third trial: The first seedlings emerged four days after sowing, and the number of seedlings emerged was significantly higher in the non-inoculated than in the inoculated treatment (median test, p=0.028); in the following days, the differences were even greater (Fig. 2). In the non-inoculated control, the highest emergence rate (45.8%) was reached at day 12, while in the inoculated substrate the highest rate (8.3%) was reached at day 8 after sowing. There was some post-emergence death of seedlings in the inoculated treatment.

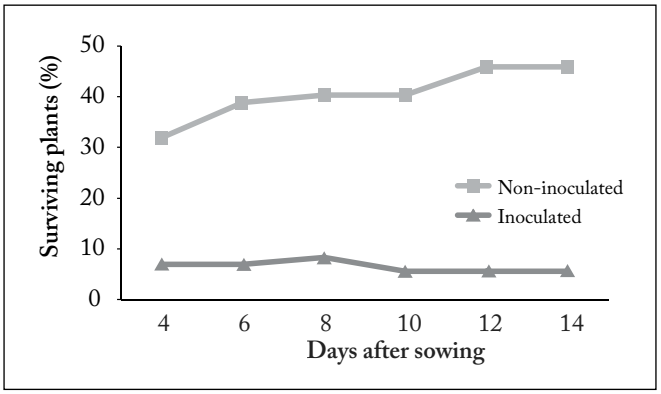

Fig. 2. Survival of lettuce seedlings (inoculated and non-inoculated with *Pythium tracheiphilum*) from day 4 to day 14 after sowing.
Fig. 2. Supervivencia de plántulas de lechuga (inoculadas y no-inoculadas con *Pythium tracheiphilum*), entre los 4 a 14 días después de la siembra.

Distribution and disease intensity. Wilting and root rot of lettuce due to *P. tracheiphilum* was found in five fields and one greenhouse of the green belt of Bahía Blanca. Lettuce types Mantecosa, Crespa, and Latina were found to be infected. In no case the incidence recorded was higher than 10%.

DISCUSSION

The causal agent of the wilt and vascular rot disease of lettuce was identified as *Pythium tracheiphilum* (Pythiacae) on the basis of its morphology, morphometry, symptoms produced and pathogenicity tests. Although this fungus has been included before in a list of disease agents in this country (Province of Buenos Aires), without further information (Resnik, *s.a.*), this is the first description of *P. tracheiphilum* in Argentina.

Pythium tracheiphilum was first described in Italy and later in other countries of Europe, Australia, and North America (Hall, 1991; Kumar et al., 2007). In South America it has been found on lettuce in Venezuela (Tortolero, 1985) and on oca (*Oxalis tuberosa*) in Peru (Icochea et al., 1994).

With the isolates of *P. tracheiphilum* of Bahía Blanca, studied by us, only direct germination of sporangia was observed; the formation of vesicula and zoospores has never been seen. In many European isolates, direct germination seems to predominate also (Kröber, 1985; Messiaen et al., 1995).

In Argentina, as well as in other countries, *P. tracheiphilum* causes dwarfing, wilting and sometimes death of lettuce plants once the vascular system of the tap root has been invaded; in addition, expanding infections may occur on the leaves. The fungus may penetrate through wounds but also into non-wounded tissue, especially on very young plants. As shown in experimental inoculations, the fungus is also able to cause damping-off, mainly in pre-emergence. However, we have not

seen these disease symptoms in the field, and we do not know how important the damping-off stage in lettuce may be.

So far, no systematic surveys have been carried out in the lettuce crops of the Bahía Blanca region. Nevertheless, the wilt and vascular root rot disease has been identified in five fields and one greenhouse of this region. In some of those fields, the disease has been observed repeatedly during several years. This indicates that *P. tracheiphilum* is endemic in the green belt of Bahía Blanca and, according to Resnik (s.a.), perhaps in other localities of the Province of Buenos Aires, and also in other regions of Argentina.

Lettuce is the most affected crop by *P. tracheiphilum* worldwide. However, other crops suffer damping-off and other diseases caused by this agent as this fungus has an extremely ample host range. Those crops include artichoke (Messiaen et al., 1995), spinach (Larsson, 1994), Chinese cabbage (Møller & Hockenhull, 1997), rice (Cother & Gilbert, 1993), and even *Pinus* spp. (Paul et al., 1992).

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