

Preliminary evaluation of *Septoria urticae* Rob et Desm as a biological control agent of *Urtica urens* L

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Summary — Plants of *Urtica urens* L (annual nettle) showing symptoms of severe foliar necrosis were collected in the horticultural belt of the city of La Plata (Province of Buenos Aires, Argentina). The agent causing the disease was identified as *Septoria urticae* Rob et Desm. Due to the severe lesions caused by the pathogen, its potential as a biocontrol agent of *U urens* was studied. Routine phytopathological techniques were used in order to evaluate the bioherbicide potential of the pathogen in the greenhouse on plants of *U urens* and other species of the Urticaceae family, such as *Boehmeria nivea* (L) Gaud, *Pilea cadierei* Gagn et Guil and *Parietaria officinalis* L. Using variance analysis it was shown that the pathogen caused a high percentage of defoliation and a reduction in dry weight (DW) of nettle plants, apart from significantly diminishing the seed production (number and DW) in the greenhouse. *B nivea* and *P cadierei* did not show any symptoms of the disease, whereas *P officinalis* was slightly affected by the fungus. This study suggests that the pathogen could play an important role as a biocontrol agent since it reduces *U urens* production.

***Septoria urticae* / *Urtica urens* / biological control / bioherbicide**

Résumé — Évaluation préliminaire de *Septoria urticae* Rob et Desm comme agent de contrôle biologique de *Urtica urens* L. Des plantes de *Urtica urens* montrant des symptômes de nécrose foliaire sévère ont été ramassés dans la ceinture horticole de la ville de La Plata (Province de Buenos Aires, Argentina). L'agent qui cause la maladie a été identifié comme *Septoria urticae* Rob et Desm. À cause des lésions sévères provoquées par le pathogène, on a étudié son efficacité comme agent de lutte biologique contre *U urens*. Des techniques phytopathologiques de routine ont été employées pour évaluer le potentiel bioherbicide du pathogène en serre sur des plantes de *U urens* et d'autres espèces de la famille Urticaceae, telles que *Boehmeria nivea* (L) Gaud, *Pilea cadierei* Gagn et Guill et *Parietaria officinalis* L. L'analyse de variance a montré que le pathogène a causé un haut pourcentage de défoliation et une réduction du poids sec (DW) de plantes d'ortie, en plus d'une diminution significative de la production de semences en serre. *B nivea* et *P cadierei* n'ont pas montré de symptômes de la maladie, tandis que *P officinalis* a été légèrement affecté par le champignon. Cette étude suggère que le pathogène pourrait jouer un rôle important comme agent de biocontrôle, parce qu'il réduit la production de *U urens*.

***Septoria urticae* / *Urtica urens* / contrôle biologique / bioherbicide**

INTRODUCTION

Urtica urens, an annual plant of European origin, has become naturalized in different parts of the world. It grows in gardens, on road verges, railway tracks and pastures and is an important weed in potatoes, maize, alfalfa, vegetables and grass crops (Marzocca, 1976). In the horticultural belt of the city of La Plata, *U urens* plants

showing symptoms of a severe foliar necrosis were collected. The agent causing the disease was identified as *Septoria urticae*, which has been reported to attack *U dioica* L and *U urens* in France, Belgium, Italy, Great Britain and Australia (Saccardo, 1884), *U gracilis* in the USA (USDA, 1960) and *U urens* in South America (Viégas, 1961). In Argentina, Spegazzini (1898) described for the first time *S urticae* as a parasite on *U urens* leaves.

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This paper records the efficacy of *S urticae* as a potential agent for biocontrol of *U urens*.

MATERIALS AND METHODS

The pathogen was isolated from *U urens* leaves which were collected in the field and were naturally infected. These leaves were kept for 48 h in an incubation chamber in order to allow cirrhi emergence from the pycnidia which were present in the necrosed tissue. The spore masses were seeded by means of a sterile needle on Martin's medium (Martin, 1950). After 4–5 d the colonies which developed were transferred to slant tubes with PDA medium for sporulation. The cultures were incubated in a growth chamber at 18 ± 2 °C for 25 d with alternating light (3 500 lux–dark cycles of 12 h plus the addition of near UV light (365 nm) to induce sporulation.

Fungus determination was performed by comparing the symptoms on *U urens* besides the morphology and biometric characteristics of *S urticae* to those described by Saccardo (1884) and Spegazzini (1913). The etiology was confirmed by examining the following specimens: *Septoria urticae*, Italy, Conegliano Jun 1876, leg C Spegazzini, LPS 10.728, ex Saccardo, Myc Ven 1.002 on *Urtica urens*; Argentina, La Plata; Sep 1906, leg C Spegazzini, LPS 10.729, on the same host.

For pathogenicity tests, *U urens* plants transplanted from the field and with an average height of 5.5 cm were used. These plants were grown in 14-cm high and 12-cm diameter pots in a greenhouse under controlled conditions (temperature: minimum mean, 10 °C; maximum mean, 20 °C; relative humidity: 60–80). Twenty d after transplantation the inoculation was carried out by applying a spore suspension on leaves with the use of a soft bristle brush (Fall, 1951). In order to determine the possible susceptibility to *S urticae* of other species of Urticaceae, plants of *Boehmeria nivea* (L) Gaud, *Pilea cadierei* Gagn et Guill and *Parietaria officinalis* L, also obtained through transplantation, were inoculated. The inoculum was obtained by adding sterile distilled water in the tubes with sporulating colonies of *S urticae* and by rubbing their surface with a sterile bent needle. After filtering through a nylon sieve the suspension was adjusted to 1.2×10^6 spores/ml by adding sterile water and using the Neubauer hemacytometer (Jairo Castaño, 1986).

The controls were brushed with distilled water. With the purpose of collecting the achenes that had fallen up to the time of evaluation, *U urens* plants were surrounded with a cellophane paper cone made with an 80-cm diameter circle and fastened with yarn to the stem base. Inoculated plants were checked for infection every 7 d for 4 consecutive wk and disease confirmed by microscopic examination of leaf spots for pycnidia. Twenty-eight d after inoculation all healthy and inoculated plants were harvested and the number and DW of collected achenes, number and DW of

adult leaves, number of leaves with lesions and DW of stems and roots were evaluated. For DW determination, samples were over dried at 70 °C for 48 h until constant weight was achieved. In order to determine differences between treatments and controls, an analysis of variance for a 1-way design was carried out. Taking into account the *F* value, the data were also tested according to Student's *t*-test.

RESULTS

Etiology

On isolation, the microorganisms that causes the disease was identified as *Septoria urticae*. This fungus forms *pycnidia* within the leaf tissue which are epiphyllous, globose, brown to black, and 75–90 µm in diameter. Pycnospores are filiform, straight or curved, 2–6 septate, hyaline, 0.9–1.9 (1 µm) x 22.5–63.8 (40.7 µm), slightly rounded at the apex and usually blunt at the base.

Two wk after inoculation, small dispersed chlorotic spots were observed on the leaves, coinciding with the infection points. These preliminary symptoms later altered to become light brown necrotic spots which began to extend and join up among themselves, mainly from the edge towards the centre of the lamina. On maturity the spots fused to cover the whole leaf. On these lesions several black points, corresponding to the pathogenic development were formed. Once the tissues became dry they turned dark brown and brittle, and became detached from the affected sections. However, on some leaves the symptoms were limited to isolated spots. The petioles also showed light brown spots, but these were elongated and encircling. As the disease progressed, in many cases complete wilting of the leaves followed by intense defoliation was noted. These symptoms were similar to those observed in the plants which were naturally infected in the field. The effect of the infection on the growth and production of nettle seeds is recorded in table I.

Statistically significant differences were found between the number and DW of leaves, stems and roots, and achenes of infected plants and the controls. Using Student's *t*-test the significances obtained by Fisher's test were confirmed. The overall DW, reduced by 40% in the inoculated plants, shows the severe damage caused to them by *Septoria urticae*, which is reflected by

Table I. Effect of *Septoria urticae* infection on growth and seed production of *Urtica urens*.

	No of leaves ^a per plant	No of leaves with lesions	No of achenes per plant	DW of achenes per plant (g)	DW of leaves per plant (g)	DW of stems and roots per plant (g)
Infected plants	16.6 ± 4.97 ^b	15.5 ± 5.61	62.1 ± 38.23	0.08 ± 0.04	0.27 ± 0.14	0.41 ± 0.22
Non-infected plants (control)	23 ± 5.16*	2 ± 0.82***	163 ± 9.02***	0.18 ± 0.03***	0.46 ± 0.05*	0.61 ± 0.12 NS

^a Each value represents the average of 20 plants harvested after 28 d of initiation treatment; ^b standard deviation; * significant at *P*: 0.05 (*F* and *t*); *** significant at *P*: 0.001 (*F* and *t*); NS: non significant (*F* and *t*).

the intensity of the lesions/leaf. However, the total number of leaves/plant was not greatly reduced by the infection.

Inoculation in other species

B nivea and *P cadierei* did not show any symptoms of the disease, whereas *P officinalis* was only slightly affected by the fungus. The leaves of the latter showed small chlorotic areas that later formed isolated necrotic spots without affecting plant growth.

CONCLUSIONS

In the present study the pathogen contributed towards 28% defoliation; 62% reduction in the number of achenes/plant and 40% reduction in DW of *U urens* plants. Although the reduction in the number of leaves per plant was significant (*P*: 0.05) it was small when compared to the reduction in the total DW/plant. The low DW content appeared to be closely related to the alteration in the vegetative metabolism due to the marked intensity of foliage necrosis caused by *S urticae*.

U urens is a plant that multiplies through seeds, the number of which was considerably reduced due to the infection. Thus, for the following year, a weed population decrease could be expected within the invaded crop. The antagonism toward the useful plants would become even lower by reduction of the volume of foliage and roots, which would also reduce the use of air space, water and nutrients. As regards inoculation in other plants, although the degree of susceptibility to the fungus was small in *P officinalis*

and null in the 2 remaining Urticaceae, further pathogenicity studies should be carried out.

Septoria is a highly specific genus (Punithalingam and Wheeler, 1965; Cunfer, 1984) but the formation of biotypes with a wide spectrum of virulence in different species, varieties and even botanical genera (Prestes and Hendrix, 1978; Rufty *et al*, 1981; Krupinsky, 1985), condition the use of *S urticae* as a biocontrol agent.

The results obtained are considered promising and the potential of *S urticae* as a biological control agent of *U urens* should be tested in the field.

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