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REGULAR ARTICLE

PLANT SPECIFICITY OF NECROTROPHIC FUNGI ISOLATED ON WILD RADISH (*RAPHANUS RAPHANISTRUM* L.) IN TUNISIA

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SUMMARY

The specificity of several fungal species isolated from the wild radish (*Raphanus raphanistrum* L.) was evaluated on two Brassicaceae species, garden radish (*Raphanus sativus* L.) and rapeseed (*Brassica napus* L.) under controlled conditions. Most fungal species have attacked the three Brassicaceae plants, suggesting that wild radish, is a relay host for several diseases in garden radish and rapeseed field crops. However, two isolates belonging to, *Cercospora armoraciae* and *Colletotrichum higginsianum*, were pathogenic on wild radish plants but not on the other Brassicaceae species. These preliminary results indicate that these two fungi species may be considered as potential biocontrol agents against this weed in Tunisia. Further studies must be conducted on other plant species in order to determine the spectrum of infection of these two fungal species.

Keywords: Biological control, Brassicaceae, *Cercospora armoraciae*, *Colletotrichum higginsianum*, weed plants.

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1. Introduction

Wild radish (*Raphanus raphanistrum* L.) is a widespread weed in North Africa [11] which colonizes several crops including cereals and legumes [3-6], resulting in significant yield reductions. Indeed, a density of 80 plants m^{-2} causes a 50% decrease in wheat yield [10]. In a field of rapeseed (*Brassica napus* L.), a density of 64 plants per m^2 of wild radish reduced the crop yield between 77 and 91% [2]. Densities from 3 to 24 wild radish plants per m^2 in a lupine (*Lupinus angustifolius* L.) crop can cause a yield reduction varying from 27 to 66% [14]. The control of wild radish in crops is mainly achieved by cultural and chemical practices. However, the longevity of its seeds in the soil and the timing of germination [6] make it difficult to control by cultural methods. In addition, chemical control against wild radish is limited by the emergence of weed resistance to multiple herbicides [9-13]. Biological control using plant pathogens may be an alternative to control weeds for which conventional control methods are of limited effectiveness. Among plant pathogens, fungi are the most used microorganisms as biocontrol agents against weeds [5]. Indeed, most marketed bioherbicides have fungi-based formulation against specific host plants. Several studies have reported the potential of many fungi isolated from different weeds to be used as biocontrol agents against their hosts [21]. Currently, there are six mycoherbicides commercialized in various countries around the world, including DeVine®, Collego® and

Casst® against *Morrenia odorata*, *Aeschynomene virginica* and *Cassia obtusifolia*, respectively [4]. An earlier study made by Djébali and collaborators [6] has shown that wild radish in Tunisia is subject to the attack by several pathogenic fungi. The objective of this paper is to evaluate the host specificity of selected isolates of necrotrofic fungal species in order to identify those with potential as biological control agents against the wild radish.

2. Materials and methods

Plant material and growth conditions

Seeds of the Tunisian wild radish ecotype were collected in Hammam Saïala in north of the country. Seeds of the French wild radish ecotype, rapeseed (*Brassica napus* L.) (cv. INRA and cv. Australia) and garden radish (*Raphanus sativus* L.) (cv. Apolo) were provided by Dr. John K. Scott from ***Commonwealth Scientific and Industrial Research Organization*** (CSIRO, Montpellier). Garden radish and rapeseed, genetically closely related to wild radish [7-16], were used in this study to evaluate plant specificity of the selected fungal species isolated from the weed. Seeds were germinated in the dark at 20°C on wet filter paper with 600 ppm of gibberelic acid (Sigma). Germinated seeds were planted in an autoclaved (30 min at 121°C) clay soil compost mixture (25:75 %) contained in 770 cm^3 pots. Plants were grown in a growth chamber at 20°C with a 12 h photophase (100 µE) and were irrigated twice weekly with 0.25 g L^{-1} nutrient solution (AGLO SPEED: 18 units (U) of nitrogen, 18U P_2O_5, 18 U K_2O).

Preparation of inoculums and inoculation

To prepare fungal inocula, fungi were grown on PDA at 20°C with a 12 h photophase (100 µE). A conidial suspension was prepared by flooding the plates with sterile distilled water containing 0.05% Tween80 (Sigma) and scrapping the cultures. The resulting suspension was adjusted to 10^6 conidia ml^{-1}. Plants were inoculated by depositing the conidial suspension on the leaf surfaces using a sterile brush. Wild radish plants were inoculated at 5 to 11 leaf stage, the rapeseed plants at 4 to 9 leaf stage and the garden radish plants at 3 to 5 leaf stage. Each fungal isolate was inoculated on three plants. After inoculation, plants were kept for 2 days in a saturated atmosphere to stimulate infection, and then transferred to a growth chamber at 20°C, 80% humidity and 12 h photophase (100 µE).

Disease assessment

Disease severity of the tested fungal species was estimated 15 days post inoculation for the three Brassicaceae plant species and was calculated as the ratio of (infected leaves / total number of inoculated leaves) x 100 [6].

Statistical analysis

Data were subjected to an analysis of variance (ANOVA) using the Statistica software version 5.1 (www.statsoft.com) and the means were compared using the LSD test at $P \leq 0.05$.

3. Results and discussion

Results of the analysis of variance showed that the fungal species, the plant cultivar and their interaction have a very highly significant effect on the total variability of disease severity, with the fungal species having the highest effect (81.07%) (Table 1). The plant cultivar contributed only by 14.01% to the total variability of the disease severity (Table 1), which may suggest that the response to the pathogen attacks was similar among the three used Bassicacea species, which can be explained by their common genetic back-grounds [7-16].

Table 1. Analysis of variance of the disease severity of several fungal pathogens on three Brassicaceae species 15 days post inoculation.

Effect	df Effects	MS Effects	df Error	MS Error	F	CTV (%)
Fungus specie (Fu)	8	13070.09	90	5.3	2465.93	81.07***
Plant cultivar (Pl)	4	2259.52	90	5.3	426.30	14.01***
Interaction (Fu x Pl)	32	792.55	90	5.3	149.53	4.92***

df: degree of freedom for different effects. MS: mean square. F: index of Fisher-Snedecor. CTV: contribution to the total variability in %. ***: P <0.001 (LSD test).

The two ecotypes of wild radish were susceptible to the attack of most tested fungal species, except for the French one which was resistant to the attack of *Alternaria* sp. (Table 2). The reaction of the wild radish to fungal attacks was dependent on the used ecotype. Indeed, the Tunisian wild radish ecotype was more susceptible to the attack by the three species of *Alternaria* and *Colletotrichum higginsianum*, while the French ecotype was more susceptible to the attack by *Stemphylium herbarum*, *Phoma lingam*, *Cercospora armoraciae* and *Cladosporium cladosporioides*. The two wild radish ecotypes showed the same level of sensitivity to *Ascochyta* sp. The INRA rapeseed cultivar was resistant to the attack by most of tested fungi species except for *Alternaria raphani*, *Alternaria* sp. and *Phoma lingam* isolates. The Australian rapeseed cultivar showed resistance only to *Alternaria* sp. attack.

Table 2. Plant specificity of the tested fungal species on wild radish (*Raphanus raphanistrum*), rapeseed (*Brassica napus*) and garden radish (*Raphanus sativus*).

Fungus specie	Plant (cultivar, origin)	DS (%) ♣
Alternaria brassicicola	Wild radish (Hammam-saïala, Tunisia)	39.9 a
	Wild radish (Vendres, France)	10 d
	Rapeseed (Australia)	22.2 b
	Rapeseed (INRA, France)	0 e
	Garden radish (Apolo, France)	20 b
Alternaria raphani	Wild radish (Hammam-saïala, Tunisia)	85.7 a
	Wild radish (Vendres, France)	62.5 c
	Rapeseed (Australia)	85.7 a
	Rapeseed (INRA, France)	75 b
	Garden radish (Apolo, France)	80 ab
Alternaria sp.	Wild radish (Hammam-saïala, Tunisia)	37.5 b
	Wild radish (Vendres, France)	0 d
	Rapeseed (Australia)	0 d
	Rapeseed (INRA, France)	20 c
	Garden radish (Apolo, France)	50 a
Stemphylium herbarum	Wild radish (Hammam-saïala, Tunisia)	10.3 b
	Wild radish (Vendres, France)	20 a
	Rapeseed (Australia)	20 a
	Rapeseed (INRA, France)	0 c
	Garden radish (Apolo, France)	20 a
Phoma lingam	Wild radish (Hammam-saïala, Tunisia)	87.5 b
	Wild radish (Vendres, France)	100 a
	Rapeseed (Australia)	66.6 c
	Rapeseed (INRA, France)	100 a
	Garden radish (Apolo, France)	100 a

Table 2. Continued

Fungus specie	Plant (cultivar, origin)	DS (%) ♣
Ascochyta sp.	Wild radish (Hammam-saïala, Tunisia)	28.6 a
	Wild radish (Vendres, France)	28.6 a
	Rapeseed (Australia)	12.5 c
	Rapeseed (INRA, France)	0 d
	Garden radish (Apolo, France)	25 b
Cercospora armoraciae	Wild radish (Hammam-saïala, Tunisia)	25.4 b
	Wild radish (Vendres, France)	71.4 a
	Rapeseed (Australia)	0 c
	Rapeseed (INRA, France)	0 c
	Garden radish (Apolo, France)	0 c
Colletotrichum higginsianum	Wild radish (Hammam-saïala, Tunisia)	31.3 a
	Wild radish (Vendres, France)	25 b
	Rapeseed (Australia)	0 c
	Rapeseed (INRA, France)	0 c
	Garden radish (Apolo, France)	0 c
Cladosporium cladosporioides	Wild radish (Hammam-saïala, Tunisia)	25 b
	Wild radish (Vendres, France)	55.5 a
	Rapeseed (Australia)	20 c
	Rapeseed (INRA, France)	0 d
	Garden radish (Apolo, France)	25 b

♣ DS: Disease severity (%) = (number of infected leaves / number of inoculated leaves) x 100. Means followed by the same letter are not significantly different at $P \leq 0.05$ (LSD test).

Damages caused by most fungal species on wild radish, rapeseed and garden radish were low to moderate, except for *A. raphani* and *P. lingam* which showed severe symptoms on leaves of the three Brassicaceae species. The three *Alternaria* species (*A. brassicicola*, *A. raphani* and *Alternaria* sp.) were able to infect plants of rapeseed and garden radish with varying levels. This result suggests that wild radish is a relay host for these fungal species in field crops of these two Brassicaceae. In addition to, given the early emergence of this weed in the fields [6], it is a host for the multiplication of *Alternaria* primary inoculums, which increases the damage caused by this fungus on rapeseed and garden radish crops. Indeed, Winter and Gindrat [23] reported that wild radish is a relay host for *Alternaria* disease in rapeseed field crops in Switzerland. Similarly, the fungal species *Phoma lingam*, *Stemphylium herbarum*, *Ascochyta* sp. and *Cladosporium cladosporoides* showed no specific attack against wild radish. These species are known to infected several crops. In fact, *P. lingam* infects several crop species of the genus Brassica [18]. *Stemphylium herbarum* attack alfalfa (*Medicago sativa* L.) leaves [12] causing brown spots surrounded by pale yellow halo. *Cladosporium cladosporioides* infects cropped and spontaneous plants such as *Phaseolus vulgaris* [1] and *Mitracarpus hirtus* [15], respectively.

Inoculation test of *Cercospora armoraciae* on the three Brasscicacea species showed that the fungus infects only wild radish plants (Table 2) causing macerated spots on leaves. The attacked leaves turn yellow and eventually die. In the literature, several *Cercospora* species are described to attack specifically their hosts, such

as *Cercospora rodmanii* against *Eichhornia crassipes* (water hyacinth) [5]. *Colletotrichum higginsianum* also showed specific attack against the two wild radish ecotypes (Table 2). This fungus causes necrotic brown to dark lesions surrounded by a yellow margin. Several *Colletotrichum* species were formulated as mycoherbicides including *Colletotrichum gloesporioides* f.sp. *Cuscutae*, *C. gloesporioides* f.sp. *Aeschynomene* and *C. gloesporioides* f.sp. *malvae* [8-17-22]. Despite their specificity, the two fungi *C. armoraciae* and *C. higginsianum* showed a low level of attack on the two wild radish ecotypes (Table 2). Accordingly, this low level of infection was reported for several fungal biological control agents, particularly for *Colletotrichum* species [17]. Several methods have been adopted to enhance the pathogenicity of the biocontrol agents. Vurro [19] showed that the combined use of non-specific toxins with specific biological agents can enhance the virulence of *Colletotrichum* species. Watson and Ahn [20] studied the possibility of enhancing the virulence of *Colletotrichum coccodes* against *Abutilon theophrasti*. Co-inoculation of *Colletotrichum coccodes* with three strains of *Pseudomonas* spp. has significantly increased the severity of the attack and reduced the time of the onset of symptoms [20]. The same authors [20] reported that spraying weeds with *Colletotrichum coccodes* combined to calcium chelators increase the virulence of this fungus.

This study showed that both *Cercospora armoraciae* and *Colletotrichum higginsianum* are two promising biocontrol agents against the wild radish in Tunisia. Further studies must be conducted to evaluate the specificity of these fungi on a broad range of plant species and to improve their virulence on the weed through the use of different formulations under natural conditions.

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