Evidence for an Expanded Host Range of *Fusarium oxysporum* f.sp. *raphani*

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The pathogenicity of four isolates of *Fusarium oxysporum* obtained from infected cultivated rocket (*Eruca vesicaria*) and wild (sand) rocket (*Diplotaxis tenuifolia*) was tested on the following cruciferous hosts: stock, radish, wild and cultivated rockets, and various species in the cabbage tribe: cabbage (*Brassica oleracea* var. *sabauda*), cauliflower (*Brassica oleracea* var. *botrytis*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), broccoli (*Brassica oleracea* var. *italica*), turnip (*Brassica rapa* var. *rapa*). The results indicated that isolates of *F. oxysporum* from cultivated and wild rocket belong to the *forma specialis raphani*. The isolates from rocket were pathogenic on cabbage, Brussels sprouts, broccoli, turnip, radish and stock; isolates of *F. oxysporum conglutinans* from cabbage and radish, and the isolate of *F. oxysporum* f.sp. *raphani* from rape obtained from the ATCC collection, were pathogenic on both cultivated and wild rocket.
KEY WORDS: Wild (sand) rocket; cultivated garden rocket; cruciferous crops; host range; Fusarium wilt.

INTRODUCTION

In spring of 2002, cultivated rocket (*Eruca vesicaria*) and wild rocket (also called sand rocket) (*Diplotaxis tenuifolia*) showing symptoms of a wilt disease were observed in several commercial greenhouses near Bergamo in northern Italy (6). Diseased plants were stunted and chlorotic, with red to brown or black streaks in the vascular system which extended from the chlorotic leaves into the crown and upper taproot. *Fusarium oxysporum* was isolated consistently from discolored vascular tissues of the symptomatic plants plated on a *Fusarium*-selective medium (7). The sudden appearance of this disease on 40 farms in the Lombardy region and later on other farms located in northern Italy demonstrated that it was due to the transmission of the pathogen from seeds (8,9).

Fusarium oxysporum f.sp. *conglutinans* (Wollenw.) Snyder & Hansen was first described as the causal agent of yellows and wilts of nearly all cultivated members of the cabbage tribe (cabbage, cauliflower, broccoli, Brussels sprouts, kohlrabi, kale, turnip) (1,10,11). On radish, Fusarium wilt was attributed to *Fusarium oxysporum* f.sp. *raphani* Baker, whereas on garden stock (*Matthiola incana*) a Fusarium wilt was considered to be caused by *F. oxysporum* f.sp. *matthioli* (1). Later, *F. matthioli* and *F. raphani* were renamed as different races of *F. oxysporum* f.sp. *conglutinans* (2).

Blank (3) compared 19 isolates of *F. oxysporum* f.sp. *conglutinans* from 11 states in the USA and demonstrated that this organism is uniform in pathogenicity on cabbage and six subspecies of *Brassica oleracea*. Bosland and Williams (4) examined an extensive collection of isolates of *F. oxysporum* from crucifer hosts in order to characterize

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populations of this pathogen, because of conflicting results from previous studies on the host range of crucifer isolates of *F. oxysporum*. They proposed the presence of four *formae speciales* from the previously described five races of *F. oxysporum* f.sp. *conglutinans*. Soil temperature has a strong influence on disease expression and ecotype variation has been shown between races 1 and 2 of *F. oxysporum* f.sp. *conglutinans* (5).

There were no reports of *F. oxysporum* on cultivated rocket in Europe before 2002 (7). A disease of *E. sativa* attributed to *F. oxysporum* f.sp. *erucae* was reported in India on wild rocket in 1973 (6). To the best of our knowledge, the disease has not yet been reported on wild rocket.

The objective of this study was to determine the *forma specialis* of isolates of *F. oxysporum* obtained from cultivated and wild rocket in Italy.

MATERIALS AND METHODS

Isolates of *F. oxysporum* Eight isolates of *F. oxysporum* (Table 1) obtained from cruciferous hosts were tested for pathogenicity on cultivars of several differential hosts (Table 2). Host plants tested included wild rocket (*Diplotaxis tenuifolia*), cultivated rocket (*Eruca vesicaria*), stock (*Matthiola incana*), radish (*Raphanus sativus*) and the following members of the cabbage tribe: cabbage (*Brassica oleracea* var. *sabauda*), cauliflower (*Brassica oleracea* var. *botrytis*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), broccoli (*Brassica oleracea* var. *italica*) and turnip (*Brassica rapa* var. *rapa*). Four isolates of *F. oxysporum* were obtained from the stem of infected wild rocket and cultivated rocket plants from four farms (7). One isolate of *F. oxysporum* f.sp. *raphani* (ATCC 16601), two isolates of *F. oxysporum* f.sp. *conglutinans* (ATCC 16600 and ATCC 52557) and one isolate of *F. oxysporum* f.sp. *matthioli* (ATCC 16602) were used as reference strains. The strains were maintained on potato dextrose agar at 8°C.

TABLE 1. Isolates of *Fusarium oxysporum* tested for their cruciferous host range

Isolate code[z]	Strains	Original host	Farm location in Italy
FusRuc 2	*F. oxysporum*	Wild rocket (*Diplotaxis tenuifolia*)	Consoli, Bergamo
FusRuc 9A	*F. oxysporum*	Wild rocket (*D. tenuifolia*)	Mangili, Bergamo
FusRuc 13/03	*F. oxysporum*	Cultivated rocket (*Eruca sativa*)	Berruto, Torino
FusRuc 6	*F. oxysporum*	Cultivated rocket (*E. sativa*)	Cunj, Bergamo
ATTC 16600[y]	*F. oxysporum* f.sp. *conglutinans*	Cabbage[x] (*Brassica oleracea*)	—
ATTC 16601	*F. oxysporum* f.sp. *raphani*	Radish[x] (*Raphanus sativus*) Rape[x] (*Brassica rapa*)	—
ATTC 52557	*F. oxysporum* f.sp. *conglutinans*	Cabbage[x] *B. oleracea*	—
ATTC 16602	*F. oxysporum* f.sp. *matthioli*	Stock[x] (*Matthiola incana*)	—

[z] FusRuc 2 and FusRuc 9A were obtained from wild rocket; FusRuc13/03 and FusRuc 6 from cultivated rocket.
[y] Source of ATCC (American Type Culture Collection) strains is from the website http://www.attc.org.
[x] Hosts from which the isolates deposited at ATTC were obtained.

Production of inoculum and pathogenicity test Each isolate of *F. oxysporum* was grown in shake culture (90 rpm) for 10 days on casein hydrolysate at 25°C with 12 h of fluorescent light per day. The culture suspension was then filtered through a single layer of cheese cloth. The concentration of spores and mycelium fragments was determined by hemacytometer and adjusted with deionized water to 1×10^6 CFU ml^{-1}.

TABLE 2. Differential cruciferous hosts used to test the range of isolates of *Fusarium oxysporum* obtained from wild rocket and cultivated rocket in Italy

Host code	Host	Cultivar/hybrid[z]	Seed company and location
1B	Cabbage (*Brassica oleracea* var. *sabauda*)	Vertus 2	Hortus (Forlì)
2A	Brussels sprout (*B. oleracea* var. *gemmifera*)	Perfection	Ortovivo (Forlì)
3C	Broccoli (*B. oleracea* var. *italica*)	D'Albenga Precoce	3G (Forlì)
3D	Broccoli (*B. oleracea* var. *italica*)	Romanesco Natalino	Hortus (Forlì)
4A	Cauliflower (*B. oleracea* var. *botrytis*)	Palla di Neve	Ortovivo (Forlì)
4B	Cauliflower (*B. oleracea* var. *botrytis*)	Precoce di Toscana	Blumen (Piacenza)
4C	Cauliflower (*B. oleracea* var. *botrytis*)	di Sicilia Violetto	3G (Forlì)
5A	Turnip (*Brassica rapa* var. *rapa*)	Piatta Collo Viola	Hortus (Forlì)
6C	Radish (*Raphanus sativus*)	Flamboyant 3	Ortovivo (Forlì)
7B	Stock (*Mathiola incana*)	Violaciocca gigante di Nizza	Blumen (Piacenza)
8A[z]	Wild rocket (*Diplotaxis tenuifolia*)	Hybrid 21/04	Orosem (Bergamo)
8B[z]	Cultivated eruca (*Eruca vesicaria*)	Hybrid 10/04	Galassi (Forlì)

[z] Only hosts 8A and 8B were hybrids.

Seeds of each of the host plants (Table 2) were obtained from different seed companies, sown in a steamed soil mixture (peat, compost broadleaf bark and clay, at a ratio of 60:20:20 vol/vol, respectively) in plug trays (Oktpac 160, Arca, Bergamo, Italy) and maintained at 25°C, with 12 h of fluorescent light per day. Roots of 15-day-old plants were washed, trimmed to a length of 5 cm, and dipped in 200 ml of spore suspensions of the appropriate isolate for 10 min. Inoculated plants were then transplanted into soil heated for 30 min at 70°C placed in 1500 ml pots. The control plants of each species and cultivar were prepared similarly but soaked in deionized water. Four replicates of 15 plants were used for each host, arranged in a randomized complete block design. Six trials were carried out in a glasshouse, with the minimum temperatures ranging from 19 to 22°C, and the maximum temperatures from 30 to 34°C. Each trial lasted from 32 to 42 days.

Plants were checked for disease development, starting 10 days after inoculation, at 7–10-day intervals and wilted plants were counted. At the end of the experiments, re-isolation was carried out from inoculated and control plants. Data are expressed as percent of dead plants and as disease index (0–100). A disease index of 0 corresponded to healthy plant; 25, vascular discoloration, slight leaf chlorosis and growth reduced by 25% in comparison with healthy control; 50, vascular discoloration, chlorosis and strong growth reduction; 100, dead plant.

Data were processed statistically by means of variance analysis (univariate Anova) and with Tukey's test on data from each trial.

RESULTS AND DISCUSSION

Typical symptoms of Fusarium wilt were first observed 10 to 20 days after inoculation. All six trials provided statistically similar results, as shown by univariate Anova from each trial by considering separately the two variables, trial and isolate (Tables 3 and 4). Results of inoculations of cabbage, cauliflower, Brussels sprouts, broccoli, turnip, stock, radish, wild rocket and cultivated rocket with isolates of *F. oxysporum* from wild and cultivated rocket, are reported in Table 5. The isolates of *F. oxysporum* from rocket were

pathogenic on cabbage, Brussels sprouts, broccoli, cauliflower, turnip and stock. The cabbage *Fusarium* strains (ATCC 16600 and 52557) caused wilting not only of cabbage and others members of the cabbage tribe, but also of wild and cultivated rocket (Table 5). The radish *Fusarium* isolate (ATCC 16601) caused wilting of cabbage, Brussels sprouts, broccoli, cauliflower, turnip, radish, wild rocket, cultivated rocket and stock. The stock *Fusarium* isolate (ATCC 16602) was most aggressive on stock but caused wilting also on cabbage, Brussels sprouts, broccoli, turnip, stock and cultivated rocket, but was not pathogenic on wild rocket (Tables 5 and 6).

TABLE 3. Homogeneous groups among trials, expressed as a function of percent of dead plants and disease index of isolates tested on different hosts

No.	Trial		Average temp.	% Dead plants	Disease index
	Start	End	(°C)		
1	1/04/04	7/05/04	25	b	b[z]
2	17/05/04	16/06/04	28	a	a
3	23/07/04	31/08/04	28	b	b
4	23/09/04	29/10/04	27	b	b
5	1/04/05	6/05/05	24	c	c
6	18/04/05	13/05/05	25	c	c

[z]Within a column, the same letters do not differ significantly based on Tukey's test ($P<0.05$). Data were analyzed using arcsine square root values.

TABLE 4. Homogeneous groups among tested isolates of *Fusarium oxysporum*, expressed as function of percent of dead plants and of disease index on different hosts.

F. oxysporum isolate code[z]	% Dead plants	Disease index
FusRuc 6	b[y]	b
FusRuc 13/03	b	b
FusRuc 9A	b	b
FusRuc 2	b	b
F. oxysporum f.sp. *conglutinans* ATTC 16600	d	d
F. oxysporum f.sp. *raphani* ATTC 16601	b	b
F. oxysporum f.sp. *conglutinans* ATTC 52557	c	c
F. oxysporum f.sp. *matthioli* ATTC 16602	a	a

[z]FusRuc 2 and FusRuc 9A were obtained from wild rocket**,** and FusRuc 13/03 and FusRuc 6 from cultivated rocket.

[y]Within a column, the same letters do not differ significantly based on Tukey's test ($P<0.05$). Data were analyzed using arcsine square root values.

On the basis of results obtained in this study, it is concluded that Fusarium wilt of both cultivated and wild rocket is incited by *F. oxysporum* f.sp. *raphani.* Although it was not possible to evaluate the original rocket strain of *F. oxysporum* obtained in India (6), it is probable that also such strain does not belong to the *forma specialis erucae,* but to the *forma specialis raphani.*

Bosland and Williams (4), on the basis of their study carried out on an extensive collection of isolates of *F. oxysporum* from crucifers, by examining pathogenicity, isozyme polymorphism, vegetative compatibility and geographic origin, recommended renaming races 1, 2, 3, 4 and 5 of *F. oxysporum* f.sp. *conglutinans* as *F. oxysporum* f.sp. *conglutinans* race 1, *F. oxysporum* f.sp. *raphani, F. oxysporum* f.sp. *matthioli* race 1, *F. oxysporum* f.sp. *matthioli* race 2, and *F. oxysporum* f.sp. *conglutinans* race 2, respectively. The authors observed a strong influence of environmental conditions on the results of pathogenicity

TABLE 5. Pathogenicity, on different hosts, of isolates of *Fusarium oxysporum* obtained from wild and cultivated rocket, of *F. oxysporum* f.sp. *conglutinans* from radish and cabbage, of *F. oxysporum* f.sp. *raphani* from radish and rape, and of *F. oxysporum* f.sp. *matthioli* from stock, expressed as percent of dead plants (average of six trials)

Host code	Inoculated plant	Cultivar of inoculated plant	% Dead plants							
			Isolate of *F. oxysporum*[z]							
			FusRuc 2	FusRuc 9A	FusRuc 13/03	FusRuc 6	f.sp. *raphani* ATTC 16600	f.sp. *conglutinans* ATTC 16601	f.sp. *conglutinans* ATTC 52557	f.sp. *matthioli* ATTC 16602
1B	Cabbage	Vertus 2	36.5 bc[y]	31.9 a	48.9 b	30.0 ab	98.5 c	23.3 ab	91.3 ef	11.1 a
2A	Brussels sprouts	Perfection	24.8 abc	44.4 a	28.7 ab	46.3 b	88.9 bc	27.8 ab	91.3 f	7.8 a
3C	Broccoli	D'Albenga Precoce	4.4 ab	7.8 a	12.8 ab	1.1 a	53.1 b	3.7 a	37.0 b	1.1 a
3D	Broccoli	Romanesco Natalino	0 a	10.0 a	2.8 a	3.9 a	59.8 b	7.2 a	48.3 bc	4.4 a
4A	Cauliflower	Palla di Neve	5.4 ab	10.0 a	3.3 a	6.7 a	88.3 bc	8.3 a	74.3 def	0 a
4B	Cauliflower	Precoce di Toscana	7.2 ab	8.1 a	5.9 a	8.1 a	14.8 a	0 a	28.0 ab	1.1 a
4C	Cauliflower	di Sicilia Violetto	7.0 ab	12.8 a	14.8 ab	6.3 a	88.5 bc	3.3 a	60.9 cd	1.1 a
5A	Turnip	Piatta Collo Viola	47.8 c	33.9 a	32.6 ab	26.7 ab	86.7 bc	21.7 ab	60.7 def	7.8 a
6C	Radish	Flamboyant 3	9.8 ab	26.7 a	17.6 ab	9.4 a	53.7 b	58.1 b	12.6 a	1.1 a
7B	Stock	Violaciocca gigante di Nizza	28.0 abc	15.6 a	21.3 ab	29.1 ab	56.1 b	27.2 ab	41.5 bc	57.8 b
8A	Wild rocket	hybrid 21/04	34.3 abc	33.3 a	37.8 ab	44.6 b	55.2 b	42.8 ab	16.9 ab	0 a
8B	Cultivated rocket	hybrid 10/04	85.2 d	84.1 b	89.3 c	80.7 c	70.4 bc	45.7 ab	54.6 cde	5.6 a

[z] FusRuc 2 and FusRuc 9A were obtained from wild rocket; FusRuc 13/03 and FusRuc 6 from cultivated rocket.

[y] Within a column, values followed by a common letter do not differ significantly based on Tukey's test ($P<0.05$). Data were analyzed using arcsine square root values.

TABLE 6. Summary of the reaction of different hosts to different isolates of *Fusarium oxysporum* f.sp. *conglutinans* from radish and cabbage, of *F. oxysporum* f.sp. *raphani* from radish and rape, and of *F. oxysporum* f.sp. *matthioli* from stock and from wild and cultivated rocket on different hosts (average of six trials)

Host code	Inoculated plant	Cultivar of inoculated plant	Host reaction[z]							
			Isolate code of *F. oxysporum*[y]							
							f.sp. *raphani*	f.sp. *conglutinans*	f.sp. *conglutinans*	f.sp. *matthioli*
			FusRuc 2	FusRuc 9A	FusRuc 13/03	FusRuc 6	ATTC 16601	ATTC 16600	ATTC 52557	ATTC 16602
1B	Cabbage	Vertus 2	S[z]	S	S	S	S	HS	HS	PR
2A	Brussels sprouts	Perfection	S	S	S	S	S	HS	HS	PR
3C	Broccoli	D'Albenga precoce	R	PR	PR	R	R	S	S	R
3D	Broccoli	Romanesco natalino	R	PR	R	PR	PR	HS	S	R
4A	Cauliflower	Palla di neve	R	PR	R	PR	PR	HS	HS	R
4B	Cauliflower	Precoce di Toscana	R	PR	PR	PR	R	PR	S	R
4C	Cauliflower	di Sicilia Violetto	R	PR	S	R	R	HS	HS	R
5A	Turnip	Piatta collo viola	S	S	S	S	PR	HS	HS	PR
6C	Radish	Flamboyant 3	PR	S	PR	PR	S	HS	PR	R
7B	Stock	Violaciocca gigante di Nizza	S	PR	S	S	S	HS	S	HS
8A	Wild rocket	Hybrid 21/04	S	S	S	S	S	S	S	R
8B	Cultivated rocket	Hybrid 10/04	HS	HS	HS	HS	S	HS	HS	PR

[z]R, resistant (disease index 0–10); PR, partially resistant (disease index 11–25); S, susceptible (disease index 26–60); HS, highly susceptible (disease index 61–100).
[y]FusRuc 2 and FusRuc 9A were obtained from wild rocket, and FusRuc 13/03 and FusRuc 6 from cultivated rocket.

tests, with an influence on the results obtained. In particular, soil temperature influenced disease severity and an ecotypic variation was observed between *F. oxysporum* f.sp. *conglutinans* race 1 and *F. oxysporum* f.sp. *conglutinans* race 2, with race 2 having the ability to cause disease below 14°C, whereas race 1 was avirulent at temperatures lower than 18°C.

In our study, air temperatures varied between 24 and 28°C in the different trials: statistical analysis carried out showed that, by separately analyzing trials and isolates, the interaction trial × isolate is not significant at $P>0.05$: thus, the differences observed in the various trials did not significantly affect the behavior of the tested isolates. Tukey's test enabled grouping of the trials into three homogeneous groups (Table 3). Similarly, when the variable isolates was considered, Tukey's test – carried out by elaborating the percent of dead plants and the Disease Index – did group the tested strains into four homogeneous groups (with strains FusRuc 6, FusRuc 9, FusRuc 2 and FusRuc 13/03 belonging to the same group of the isolate *F. oxysporum* f.sp. *raphani* ATCC 16601). The isolates *F. oxysporum* f.sp. *matthioli* ATCC 16602, *F. oxysporum* f.sp. *conglutinans* ATTC 16600 and ATTC 52557 were grouped together (Table 4).

Since both wild and cultivated rocket are being grown more and more, with an overall acreage of 750 ha in Italy, there is a strong risk of spread of *F. oxysporum* f.sp. *raphani* to different cruciferous hosts.

At present, the vegetative compatibility and molecular characteristics of strains of *F. oxysporum* used in this study as well as by other authors, are under investigation, in order to evaluate the possibility of physiological races within *F. oxysporum* f.sp. *raphani*.

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