RESISTANCE OF DIFFERENT ROCKET CULTIVARS TO WILT CAUSED BY STRAINS OF *FUSARIUM OXYSPORUM* UNDER ARTIFICIAL INOCULATION CONDITIONS

G. Gilardi[1], G. Chen[2], A. Garibaldi[1], C. Zhiping[2] and M.L. Gullino[1]

[1]*Centre for Innovation in the Agro-Environmental Sector AGROINNOVA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy*
[2]*Department of Ecology and Ecological Engineering, College of Resources and Environmental Sciences, China Agricultural University, Beijing, China*

SUMMARY

Serious losses occur in cultivated (*Eruca vesicaria*) and wild (*Diplotaxis tenuifolia*) rocket varieties infected by *Fusarium oxysporum* f.sp. *raphani* in north-western Italy. Forty-four varieties of cultivated and wild rocket were chosen for resistance tests carried out under glass from June 2004 to June 2005, for the evaluation of resistance to Fusarium wilt. Roots of 30-day-old plants were inoculated by dipping in a conidial suspension (10^6 CFU/ml) of the pathogen. Three trials were conducted using as inoculum strain Fus Ruc 9A isolated from a wild rocket variety and strain Fus Ruc 13/03, isolated from cultivated rocket; both were strains of *Fusarium oxysporum* f.sp. *raphani*. Fus Ruc 9A was found to be the more virulent, and the majority of rocket varieties were susceptible or highly susceptible to it. Some rocket varieties showed resistance to Fus Ruc 13/03. In an overall disease index evaluation, varieties 7/02, 2/03, 5/03, 6/03, 7/03, 11/03, 12/03, 20/03, 24/03, 6/04, and 13/04 were at least partially resistant. The data obtained indicate that only a few of the rocket varieties available in Italy are resistant to Fusarium wilt. Moreover, differences in the host range of the two strains of *F. oxysporum* f.sp. *raphani* used suggest the presence of different races of the pathogen.

Key words: Fusarium oxysporum f.sp. *raphani*; disease resistance; *Eruca vesicaria*; *Diplotaxis tenuifolia.*

INTRODUCTION

Rocket is a vegetable increasingly grown in Italy and widely used in the Mediterranean cuisine as salad and as dish decoration. Two types of rocket are available on the Italian market: *Eruca vesicaria* (synonym *E. sativa*) known as "ruchetta" or cultivated garden rocket, and *Diplotaxis tenuifolia*, wild rocket (Santamaria *et al.*, 2002). Wilt caused by *Fusarium oxysporum* was reported on cultivated rocket (*Eruca sativa*) in India (Chatterjee and Rai 1974) and later in Italy (Garibaldi *et al.*, 2003). Fusarium wilt was observed in Italy in several commercial plastic houses near Bergamo on both cultivated and wild rocket (Garibaldi *et al.*, 2003). Diseased plants are stunted and chlorotic, with brown or black streaks in the vascular system. *Fusarium oxysporum* f. sp. *raphani* is the causal agent on both cultivated and wild rocket (Garibaldi *et al.*, 2006). The pathogen is transmitted through infected seed (Garibaldi *et al.*, 2004b).

The existence of significant differences in varietal response of cultivated and wild rocket to Fusarium wilt was not known hitherto. Three trials were conducted under glass in order to evaluate the susceptibility of the most common varieties of cultivated and wild rocket to Fusarium wilt.

MATERIALS AND METHODS

Isolates and inoculum production. Two strains of *F. oxysporum* f.sp. *raphani* (Catti *et al.*, 2007; Garibaldi *et al.*, 2006), coded Fus Ruc 9A and Fus Ruc 13/03, isolated from infected plants of *D. tenuifolia* and *E. vesicaria* obtained in two different infested areas in northern Italy (Bergamo and Turin provinces respectively) were chosen from a collection of forty six isolates obtained during 2002-2004. The two isolates, previously tested for pathogenicity showed medium-high virulence on both hosts (Catti *et al.*, 2007; Garibaldi *et al.*, 2006).

The isolates were maintained on PDA at 8°C in a cold chamber. For inoculum production, they were grown as previously reported (Garibaldi *et al.*, 2004a). The concentration of conidia and mycelium fragments was determined by hemacytometer and adjusted with deionized water to a final concentration of 10^6 CFU (colony forming units) /ml, which was adopted in all trials.

Susceptibility test. Forty-four cultivated and wild rocket varieties obtained from different seed companies in Italy were tested (Table 1). Seeds were sown in a soil mixture (peat: compost broad bark: sand, 60: 20: 20 vol/vol steamed for 30 min at 80°C) in plug trays main-

Corresponding author: M.L. Gullino
Fax: +39.011.6709307
E-mail: marialodovica.gullino@unito.it

Table 1. List of rocket varieties tested.

Variety code	Species	Varieties	Type	Seed company and location
2/04	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Agrisem (Torino)
3/04	*Eruca vesicaria*	Rucola coltivata	Cultivated	Agrisem (Torino)
2/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Franchi (Bergamo)
5/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Franchi (Bergamo)
15/03	*Diplotaxis tenuifolia*	Selvatica extra	Wild	Franchi (Bergamo)
11/04	*Eruca vesicaria*	Foglia frastagliata	Cultivated	Galassi (Cesena)
12/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Isi Sementi (Forlì)
4/04	*Diplotaxis tenuifolia*	My way	Wild	Isi Sementi (Forlì)
5/04	*Diplotaxis tenuifolia*	Runway	Wild	Isi Sementi (Forlì)
6/04	*Diplotaxis tenuifolia*	Foglia frastagliata	Wild	Isi Sementi (Forlì)
7/04	*Diplotaxis tenuifolia*	Foglia intera	Wild	Isi Sementi (Forlì)
8/04	*Eruca vesicaria*	Rucola coltivata	Cultivated	Isi Sementi (Forlì)
10/04	*Eruca vesicaria*	Rucola coltivata	Cultivated	La Semiorto Sementi (Salerno)
9/04	*Diplotaxis tenuifolia*	Foglia frastagliata	Wild	La Semiorto Sementi (Salerno)
10/02	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Mazzocchi (Milano)
4/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Mazzocchi (Milano)
7/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Mazzocchi (Lodi)
13/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Mazzocchi (Milano)
14/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Mazzocchi (Milano)
1/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Mazzocchi (Milano)
8/02	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Mazzocchi (Milano)
7/02	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Orosem (Bergamo)
9/02	*Eruca vesicaria*	Rucola coltivata	Cultivated	Orosem (Bergamo)
3/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Orosem (Bergamo)
6/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Orosem (Bergamo)
9/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Orosem (Bergamo)
11/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Orosem (Bergamo)
16/03	*Diplotaxis tenuifolia*	Rucola selvatica	Wild	Orosem (Bergamo)
17/03	*Diplotaxis tenuifolia*	Foglia d'ulivoSAHL14	Wild	Orosem (Bergamo)
18/03	*Diplotaxis tenuifolia*	Foglia frastagliataSAAH279	Wild	Sais (Cesena)
19/03	*Eruca vesicaria*	Foglia largaSAAL205	Cultivated	Sais (Cesena)
20/03	*Diplotaxis tenuifolia*	Foglia frastagliata SAZH789	Wild	Sais (Cesena)
21/03	*Eruca vesicaria*	Foglia lobata SAAL165	Cultivated	Sais (Cesena)
22/03	*Eruca vesicaria*	Foglia lobata SAAL110	Cultivated	Sais (Cesena)
23/03	*Diplotaxis tenuifolia*	Foglia d'ulivo n.3242	Wild	Sais (Cesena)
24/03	*Diplotaxis tenuifolia*	Foglia frastagliata n.3241	Wild	Sais (Cesena)
25/03	*Eruca vesicaria*	Foglia frastagliata n.3237	Cultivated	Sais (Cesena)
26/03	*Eruca vesicaria*	Foglia larga n.3238	Cultivated	Sais (Cesena)
1/04	*Eruca vesicaria*	Rucola coltivata	Cultivated	Agrisem (Torino)
12/04	*Eruca vesicaria*	Foglia d'ulivo	Cultivated	Sais (Cesena)
13/04	*Eruca vesicaria*	Foglia frastagliata	Cultivated	Sais (Cesena)
14/04	*Eruca vesicaria*	Foglia lobata	Cultivated	Sais (Cesena)
15/04	*Eruca vesicaria*	Foglia larga	Cultivated	Sais (Cesena)

Table 2. Differences among trials as expressed by disease index.

Trial	Start of the trial	Average temperature (°C)	Disease index
1	August 2004	29	b*
2	October 2004	25	a
3	May 2005	26	a

*The same letters do not differ significantly based on the non-parametric Kruskal-Wallis's test (p=0.05)

tained at 25°C. Roots of 30 day old plants were trimmed to a length of 5 cm, and dipped for 10 min in conidial suspensions of the two strains, prepared as previously described. Inoculated seedlings were transplanted to containers (10 litres) filled with steamed soil. Control plants were prepared similarly but dipped in sterile deionised water. Ten seedlings per container were transplanted in each trial. Each container was considered as a replicate. Three replicates were used in each trial. Plants were maintained in a glasshouse at a temperature ranging from 22 to 34°C. Symptoms appeared 7-10 days after inoculation. The trial was repeated three times. Each trial lasted approximately 35 days.

Disease evaluation and statistical analysis. Plants were evaluated weekly for disease development and wilted plants were counted. The final disease rating took place from 4 to 6 weeks after inoculation. Two indicators were applied to evaluate resistance: the percentage of dead plants and a disease index (DI) from 0 to 100. An index of 0 corresponded to healthy plants; 12.5 to plants growing regularly with slight vascular discoloration; 25 to vascular discoloration, slight leaf chlorosis and reduced growth; 50 to vascular discoloration, chlorosis and growth reduction; 75 to extended vascular discoloration, strong leaf chlorosis and strong growth reduction; 100 to dead plants. Four levels of resistance rate (Garibaldi *et al.*, 2004a) were applied to classify resistance or susceptibility: resistant (R; disease index 0-10), partially resistant (PR; disease index 11-30), susceptible (S; disease index 31-60), highly susceptible (HS; disease index 61-100).

All data were arc-sin-transformed and analysed with the non-parametric Kruskal-Wallis test. In addition, data from the second and third trials were processed by analysis of variance (univariate Anova) and by Tukey's test (Table 5).

RESULTS AND DISCUSSION

The inoculation method adopted gave good disease incidence in all trials, and provided a useful screening system for resistance to Fusarium wilt. Symptoms started

Table 3. Reaction of rocket varieties after artificial inoculation with *Fusarium oxysporum* f.sp. *raphani* (strains Fus Ruc 9A and Fus Ruc 13/03) expressed as dead plants (%) and disease index (0-100) during the first trial.

Variety code	Strain Fus Ruc 9A			Strain Fus Ruc 13/03		
	% dead plants	Disease index (0-100)		% dead plants	Disease index (0-100)	
7/02	23.3	41.7	a-g*	20.0	30.0	a-c
8/02	50.0	62.5	a-n	23.3	35.0	a-e
9/02	36.7	53.8	a-m	26.7	41.7	a-e
10/02	26.7	43.3	a-g	13.3	23.3	a-c
1/03	13.3	29.2	a-c	13.3	22.9	a-c
2/03	13.3	27.9	ab	6.7	16.3	ab
3/03	26.7	42.9	a-g	6.7	17.9	ab
4/03	30.0	43.3	a-g	13.3	22.9	a-c
5/03	13.3	26.3	ab	10.0	19.2	ab
6/03	30.0	43.3	a-g	13.3	24.6	a-c
7/03	26.7	42.9	a-g	13.3	27.1	a-c
9/03	23.3	37.5	a-d	3.3	14.2	a
11/03	33.3	45.0	a-h	6.7	16.3	ab
12/03	16.7	32.5	a-c	6.7	15.8	ab
13/03	30.0	43.8	a-g	6.7	15.8	ab
14/03	70.0	83.3	f-n	30.0	42.9	a-e
15/03	80.0	88.3	h-n	40.0	50.8	a-e
16/03	63.3	71.7	c-n	26.7	42.9	a-e
17/03	70.0	79.6	d-n	66.7	75.0	de
18/03	40.0	49.2	a-l	13.3	24.6	a-c
19/03	90.0	91.7	n	43.3	49.2	a-e
20/03	33.3	50.4	a-l	10.0	21.7	ab
21/03	76.7	81.3	d-n	23.3	32.5	a-d
22/03	90.0	91.3	i-n	30.0	37.5	a-e
23/03	93.3	96.7	mn	36.7	49.2	a-e
24/03	23.3	47.9	a-i	10.0	24.2	a-c
25/03	90.0	92.5	l-n	43.3	51.3	a-e
26/03	66.7	80.8	d-n	36.7	48.3	a-e
1/04	73.3	76.7	d-n	20.0	30.0	a-c
2/04	26.7	38.3	a-e	26.7	37.9	a-e
3/04	76.7	80.4	d-n	10.0	22.5	a-c
4/04	100.0	100.0	n	90.0	65.0	c-e
5/04	76.7	81.3	d-n	33.3	46.3	a-e
6/04	26.7	40.8	a-f	3.3	13.3	a
7/04	3.3	22.1	a	10.0	18.8	ab
8/04	83.3	85.4	g-n	33.3	42.9	a-e
9/04	60.0	69.2	b-n	30.0	40.0	a-e
10/04	100.0	100.0	n	70.0	75.4	e
11/04	33.3	45.4	a-h	6.7	18.3	ab
12/04	70.0	82.1	e-n	33.3	41.7	a-e
13/04	43.3	59.6	a-n	20.0	34.2	a-e
14/04	73.3	82.1	e-n	33.3	41.7	a-e
15/04	93.3	96.3	mn	26.7	36.3	a-e

*Each value is a mean of 3 replicates. Means of the same column, followed by the same letter, do not significantly differ following Tukey's test (p=0.05).

Table 4. Reaction of rocket varieties after artificial inoculation of two strains of *Fusarium oxysporum* f. sp. *raphani* in trials 2 and 3.

Variety code	Disease Index (0-100)				Average	Tukey's Test	Reaction*
	Strain Fus Ruc 9A		Strain Fus Ruc 13/03				
	Trial 2	Trial 3	Trial 2	Trial 3			
7/02	37.1	20.8	20.0	19.2	24.3	a-d**	PR
8/02	39.2	42.5	36.7	53.3	42.9	a-e	S
9/02	33.3	18.3	33.8	47.1	33.1	a-e	S
10/02	37.9	34.2	31.3	28.8	33.0	a-e	S
1/03	34.6	19.2	38.3	28.3	30.1	a-d	S
2/03	40.6	22.5	21.1	32.5	29.2	a-d	PR
3/03	60.4	33.3	21.7	13.8	32.3	a-e	S
4/03	47.1	31.7	45.0	37.9	40.4	a-e	S
5/03	27.5	12.5	27.5	31.7	24.8	a-d	PR
6/03	22.1	19.2	9.6	30.0	20.2	ab	PR
7/03	24.2	12.5	15.0	41.7	23.3	ab	PR
9/03	37.5	15.0	14.2	56.7	30.8	a-d	S
11/03	35.0	14.2	26.3	32.1	26.9	a-d	PR
12/03	40.4	13.3	18.8	15.0	21.9	ab	PR
13/03	29.2	7.5	58.3	45.8	35.2	a-e	S
14/03	30.0	15.0	52.5	65.8	40.8	a-e	S
15/03	53.3	44.2	65.8	59.2	55.6	a-g	S
16/03	29.2	10.8	30.4	57.5	32.0	a-e	S
17/03	45.8	50.8	84.6	72.5	63.4	c-g	HS
18/03	45.8	30.8	52.1	50.8	44.9	a-f	S
19/03	68.3	44.2	61.2	81.7	63.8	d-g	HS
20/03	25.8	18.3	17.5	10.0	17.9	a	PR
21/03	67.9	52.5	19.6	26.2	41.5	a-e	S
22/03	60.8	50.0	24.6	28.3	40.9	a-e	S
23/03	80.8	76.3	50.8	76.7	71.2	e-g	HS
24/03	26.3	23.3	9.6	35.0	23.5	ab	PR
25/03	57.5	30.0	35.0	31.7	38.5	a-e	S
26/03	50.8	37.5	68.1	76.7	58.3	b-g	S
1/04	44.2	34.2	22.1	46.7	36.8	a-e	S
2/04	40.8	35.0	35.0	35.8	36.7	a-e	S
3/04	42.1	18.3	22.5	50.0	33.2	a-e	S
4/04	100.0	81.7	67.9	100.0	87.4	g	HS
5/04	92.5	80.0	86.3	80.0	84.7	fg	HS
6/04	42.9	15.0	10.4	26.7	23.8	a-c	PR
7/04	25.8	24.2	50.4	36.7	34.3	a-e	S
8/04	85.0	61.7	31.3	55.8	58.4	b-g	S
9/04	49.2	30.8	44.6	43.3	42.0	a-e	S
10/04	96.7	83.3	65.0	100.0	86.3	g	HS
11/04	54.2	46.7	17.1	21.7	34.9	a-e	S
12/04	45.4	30.8	73.8	73.8	55.9	a-g	S
13/04	26.3	21.7	24.2	33.8	26.5	a-d	PR
14/04	35.8	36.7	29.2	28.3	32.5	a-e	S
15/04	66.7	46.7	60.8	80.8	63.8	d-g	HS

*R: resistant (Disease index 0-10); PR: partially resistant (Disease index: 11-30); S: susceptible (Disease index: 31-60); HS: highly susceptible (Disease index: 61-100).
** The Tukey's test was applied on mean value obtained in these 2 trials (trials 2 and 3) not different significantly on the base of the Kruskal-Wallis's results. Means of the same column, followed by the same letter, do not significantly differ following Tukey's test (p=0.05).

Table 5. Significant differences between two isolates of *Fusarium oxysporum* f.sp. *raphani* expressed as % of dead rocket plants and as disease index for trials 2 and 3.

F. oxysporum f.sp. *raphani* isolate	% Dead plants	Disease index
Fus Ruc 9A	a	a*
Fus Ruc 13/03	b	a

*Within a column, the same letters do not differ significantly based on the non-parametric Kruskal-Wallis's test (p=0.05).

to show 7-10 days after inoculation, and the percentage of dead plants reached a maximum between 4 and 6 weeks after inoculation. In the first trial, carried out in summer, when a maximum temperature of 34°C could easily be reached in the glasshouse, symptoms developed very quickly. Kruskal-Wallis's test showed that trials 2 and 3 were not significantly different from each other but that trial 1 was different from the others probably due to the high temperatures experienced (Table 2). All trials showed that strain Fus Ruc 9A was more virulent than Fus Ruc 13/03. In the first trial, some rocket varieties (7/02, 10/02, 3/03, 4/03, 6/03, 7/03, 9/03, 11/03, 12/03, 13/03, 18/03, 20/03, 24/03, 1/04, 3/04, 6/04 and 11/04) were partially resistant to Fus Ruc 13/03, but susceptible or highly susceptible to Fus Ruc 9A (Table 3). The varieties 1/03, 2/03, 5/03 and 7/04 were partially resistant and showed the same reaction to both strains (Table 3). Kruskal-Wallis's test showed the absence of differences among strains in trials two and three (Table 5). Evaluation of disease index data for all trials revealed partial resistance to *F. oxysporum* f.sp. *raphani* in varieties 7/02, 2/03, 5/03, 6/03, 7/03, 11/03, 12/03, 20/03, 24/03, 6/04 and 13/04 (Table 4). When temperatures ranged between 25-26°C, the most susceptible varieties of cultivated rocket were 15/04, 10/04 and 19/03, and of wild rocket, 17/03, 23/03, 4/04 and 5/04 (Table 4). Not even one of the tested rocket varieties was found totally resistant to Fusarium wilt: this fact could lead to rapid spread of the disease in many areas where this crop is grown. Categorising host reactions (resistant, partially resistant, susceptible, highly susceptible) can be useful for indicating how a cultivar will respond when grown in an area that is favourable for disease development.

The inconsistency, in terms of wilt-resistance, of some rocket varieties observed in some trials could be explained by the known intrinsic variability of rocket. It is a challenge for the breeder to select single plants which might be resistant. Moreover, there is also a certain variability in the pathogen. Kruskal-Wallis's test showed the absence of differences between Fus Ruc 9A and Fus Ruc 13/03 in trials 2 and 3 when the disease index indicator was used (Table 5). However, a pronounced difference in virulence was observed when the percentage of dead plants was evaluated (Table 5). The different behaviour of the two strains and the different host range shown might suggest the presence of different races of the pathogen. This should be further investigated.

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