

Seed Transmission of *Fusarium oxysporum* f.sp. *lactucae*

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Twenty-seven seed samples belonging to the lettuce cultivars most frequently grown in Lombardy (northwestern Italy), in an area severely affected by Fusarium wilt of lettuce, were assayed for the presence of *Fusarium oxysporum* on a Fusarium-selective medium. Isolations were carried out on subsamples of seeds (500 to 1500) belonging to the same seed lots used for sowing, and either unwashed or disinfected in 1% sodium hypochloride. The pathogenicity of the isolates of *F. oxysporum* obtained was tested in four trials carried out on lettuce cultivars of the butterhead type, very susceptible to Fusarium wilt. Nine of the 27 samples of seeds obtained from commercial seed lots used for sowing in fields affected by Fusarium wilt were contaminated by *F. oxysporum*. Among the 16 isolates of *F. oxysporum* obtained, only one was isolated from disinfected seeds. Three of the isolates were pathogenic on the tested cultivars of lettuce, exhibiting a level of pathogenicity similar to that of the isolates of *F. oxysporum* f.sp. *lactucae* obtained from infected wilted plants in Italy, USA and Taiwan, used as comparison. The results obtained indicate that lettuce seeds are a potential source of inoculum for Fusarium wilt of lettuce. The possibility of isolating *F. oxysporum* f.sp. *lactucae*, although from a low percent of seeds, supports the hypothesis that the rapid spread of Fusarium wilt of lettuce observed recently in Italy is due to the use of infected propagation material. Measures for prevention and control of the disease are discussed.

KEY WORDS: Lettuce; Fusarium wilt; disease management.

INTRODUCTION

Fusarium wilt of lettuce, recently observed in Italy (4), has emerged as a major production problem in Lombardy (northwestern Italy), where every year cropping of lettuce is carried out repeatedly in the same soil. Symptoms were first observed on cv. 'Salad Bowl' at thinning, when seedlings (30 days old) appeared wilted. Affected plants were stunted, and developed yellowed leaves and brown or black streaks in the vascular system. A similar disease was described in Japan in 1967 (10), in the United States in 1993 (8) and in Taiwan in 1998 (7). In Japan and Taiwan the causal agent was identified as *F. oxysporum* f.sp. *lactucae*, whereas in the USA the pathogen was described as *F. oxysporum* f.sp. *lactucum*. Most recently, Fusarium wilt was described in the California coastal district (6) and in Italy (4). Californian and Italian isolates of *F. oxysporum* f.sp. *lactucae* showed pathogenicity similar to Japanese race 1 and it has been proposed that *F. oxysporum* f.sp. *lactucum* is identical to *F. oxysporum* f.sp. *lactucae* (3,6). Such isolates of *F. oxysporum* behaved similarly on all the tested cultivars (5).

The recent outbreaks of Fusarium wilt in northern Italy occurred on spring and summer leaf lettuce, particularly on cultivars belonging to the Batavia type and grown for processed lettuce (11). Circumstantial evidence from lettuce wilt surveys in the area suggested that

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the sudden appearance of this disease was due to the transmission of the pathogen by seeds. The present study was undertaken to ascertain the extent of and the variation in occurrence of *F. oxysporum* f.sp. *lactucae* in lettuce seed.

MATERIALS AND METHODS

Seed infection evaluation Lettuce seed samples belonging to the cultivars most frequently grown in the area (Table 1), were obtained from commercial seed lots used for sowing in farms affected by the disease during autumn of 2002. The proportion of infected lettuce plants in the different fields varied between 20% and 60% after 2 years of continuous, intensive cropping. Twenty-seven seed samples were collected and assayed for the presence of *F. oxysporum* on a *Fusarium*-selective medium (9).

Subsamples consisting of 500 to 1500 seeds were tested in petri dishes (25 seeds per dish) containing Komada's selective medium. Isolations were carried out on seeds unwashed or disinfected by soaking for 1 min in 1% sodium hypochloride. Seeds infected by *F. oxysporum* were surrounded by characteristic fast-growing, fluffy white fungal colonies that produced a red pigment. Identifications were confirmed by conidial morphology.

Isolates and their preservation The isolates obtained from seeds were coded as listed in Table 1. The Italian isolate FOL 4 of *F. oxysporum* f.sp. *lactucae* was obtained in 2002 from infected lettuce plants collected at Bergamo (northwestern Italy). The isolate ATCC 76616 of *F. oxysporum* f.sp. *lactucum* was obtained from the American Type Culture Collection (ATCC) and was isolated from infected plants in California (8). The isolates FOL 40 and FOL 18 (supplied by Dr. J.H. Huang) belonged respectively to Taiwan type 1 and 2 of *F. oxysporum* f.sp. *lactucae*. The various strains were maintained on PDA at 8°C.

Inoculum production and pathogenicity test The strains were grown in shake culture for 10 days on casein hydrolysate at 25°C under 12 h of fluorescent light per day. The culture suspension was then filtered through a single layer of cheesecloth. The concentration of spores and mycelium fragments was determined with a hemacytometer and adjusted with deionized water to 1×10^6 CFU (colony-forming units) ml⁻¹.

Seeds of cvs. 'Cappuccio Lido' (Royal Sluis Italia Spa), 'Cappuccina Ballerina' (ISEA Spa), 'Cappuccina Macre' (Bra 1421; Vilmorin Italia Srl) and 'Regina di Maggio' (Consorzio di Parma), all belonging to the butterhead type and very susceptible to *Fusarium* wilt (5), were sown in a steamed soil mixture (peat, compost broadleaved bark and clay, 60:20:20 v/v) in plug trays 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 for 10 min in the pathogen spore suspension prepared as described above. Inoculated plants were then transplanted into steamed soil (30 min at 70°C) in pots (1.5 l volume). Control plants were prepared similarly but soaked in plain deionized water. Three to five replicates were used, with each replicate consisting of three to six plants. Four trials were carried out: two under growth chamber conditions and two in a glasshouse. In the growth chamber, plants were maintained at 28°C and 12L:12D, fluorescent light. In the glasshouse, the minimum temperature ranged between 19° and 22°, and the maximum between 30° and 34°C.

Typical symptoms of *Fusarium* wilt started to be visible 8 days after artificial inoculation. Plants were checked for disease development and wilted plants were counted. The data are expressed as percent dead plants 20 days after the artificial inoculation.

RESULTS AND DISCUSSION

Nine of 27 samples of lettuce seeds, obtained from seed companies, were contaminated by *F. oxysporum* (Table 1). Sixteen isolates of *F. oxysporum*, isolated from the different seed lots, were coded (Table 1), maintained in culture, and tested for their pathogenicity on lettuce. Only one isolate, code no. 4-11, was obtained from disinfected seeds. Three of the isolates obtained were pathogenic on the tested cultivars of lettuce (Table 2), with inoculated lettuce plants showing typical wilt symptoms. The level of pathogenicity evinced by the three isolates was similar to that of the isolates of *Fusarium* spp. obtained in Italy, USA and Taiwan (Table 2).

TABLE 1. Evaluation of the presence of *Fusarium oxysporum* in lettuce seeds (in **bold**, the single case of isolation of *F. oxysporum* from disinfected seeds)

Lettuce cultivar (sample no.)	Number of <i>F. oxysporum</i> colonies detected/number of seeds tested	Isolate code
Salad bowl (4)	2/1500	4 b, 4-9
Salad bowl (4)	1/500	4-11
Salad bowl (1)	1/500	1a; 1b
Salad bowl Red (23)	0/1500	–
Salad bowl (25)	0/1500	–
Batavia (6)	1/1500	6 b
Salad bowl (7)	2/1500	7a; 7 e
Salad bowl (24)	0/1500	–
Batavia (5)	0/1500	–
Salad bowl (26)	1/1500	26 c
Lattughina Bionda Ricciolina (1/03)	1/500	1/03 a
Lattughina Bionda Ricciolina (2/03)	0/500	–
Batavia-B (3/03)	0/500	–
Batavia-B (4/03)	1/500	4/03 c
Salad Bowl (5/03)	0/500	–
White Salad Bowl (6/03)	0/500	–
White Salad Bowl (7/03)	3/500	7/03 a; 7/03 b; 7/03 c
White Salad Bowl (8/03)	2/500	8/03 a; 8/03 d
White Salad Bowl (9/03)	0/500	–
White Salad Bowl (10/03)	0/500	–
Salad Bowl (11/03)	0/500	–
Lattughina Bionda Ricciolina (12/03)	0/500	–
White Salad Bowl (13/03)	0/500	–
White Salad Bowl (14/03)	0/500	–
White Salad Bowl (15/03)	0/500	–
Green Salad Bowl (16/03)	0/500	–
White Salad Bowl (17/03)	0/500	–
Salad Bowl (18/03)	0/500	–

Fusarium wilt represents a potential threat to lettuce production in Italy. The disease has been detected on spring and summer leaf lettuce of the Batavia type and grown for processing. Many of the most widely grown lettuce varieties, of the Romaine and Batavia types, are susceptible to *Fusarium* wilt (5). Identifying the primary source of inoculum is of critical importance for effective disease management. With the recent increase in usage of commercial lettuce seed produced outside Italy, there is greater potential for introducing and disseminating the pathogen into areas where it has not been reported previously.

TABLE 2. Pathogenicity of isolates of *Fusarium oxysporum* in different trials on lettuce, butterhead type, evaluated by artificially inoculating 15-day-old plants (cv. Lido) by root dipping

Isolate code	Origin	Identified as	% Dead plants 20 days after inoculation in Trial no.				
			1	2	3		4
			Cappuccio Lido	Cappuccio Ballerina	Cultivar Cappuccina Macre Regina di Maggio		Cappuccina Macre
1 a	Lettuce seed	<i>F. oxysporum</i>	0	0	0	0	0
1 b	Lettuce seed	<i>F. oxysporum</i>	100	100	100	100	60
6 b	Lettuce seed	<i>F. oxysporum</i>	0	0	0	0	0
7 a	Lettuce seed	<i>F. oxysporum</i>	0	0	0	0	0
7e	Lettuce seed	<i>F. oxysporum</i>	100	100	100	100	53
4 b	Lettuce seed	<i>F. oxysporum</i>	100	100	100	100	40
26 c	Lettuce seed	<i>F. oxysporum</i>	0	0	n.t.	n.t.	n.t.
4-9	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	n.t.	n.t.	0
4-11	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	n.t.	n.t.	0
1/03	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	0	0	0
4/03	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	0	0	0
7/03 a	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	0	0	0
7/03 b	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	0	0	0
7/03 c	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	13	10	0
8/03 a	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	0	0	0
8/03 d	Lettuce seed	<i>F. oxysporum</i>	n.t.	n.t.	13	10	0
FOL 4	Lettuce plant, Bergamo	<i>F. lactucae</i>	100	100	100	100	67
FOL 40	Taiwan Type 1	<i>F. lactucae</i>	100	100	100	100	n.t.
FOL 18	Taiwan Type 2	<i>F. lactucae</i>	100	100	100	100	n.t.
ATCC 76616	ATCC	<i>F. lactucum</i>	100	100	100	100	67

n.t. = not tested

This paper provides evidence that the causal agent of Fusarium wilt, *F. oxysporum* f.sp. *lactucae*, is seed-transmitted, which suggests that seeds may be important in disseminating the pathogen. The results of this study do not provide information on the effects of *F. lactucae* on the quality and germination ability of lettuce seeds, but do indicate that the seeds are a potential source of inoculum for development of Fusarium wilt. The rapid

spread of the disease that occurred in northwestern Italy in 2002, leads us to hypothesize that the pathogen was introduced into Italy *via* infected seeds.

Further research should be carried out to determine the epidemiological significance of seedborne inoculum as well as efficient methods to eliminate this threat to lettuce production. The use of *F. oxysporum* f.sp. *lactucae*-free certified propagation material will become an essential qualification for worldwide distribution of this crop. Seed dressing with permitted and effective fungicides should also represent one more option for disease control within an integrated disease management approach. In addition, physical methods could be used for seed disinfection. At present, a method based on the use of aerated steam, effective for the control of seedborne diseases of cereals (2), is being tested. Only one isolate of *F. oxysporum* was obtained from disinfected seeds and it was not pathogenic. Thus we may speculate that the pathogen is an external contaminant of seeds, and if so, seed disinfection should help to reduce the dissemination of the pathogen.

Since the conventional pathogen detection techniques may lack the sensitivity required to detect seedborne pathogens, the detection threshold of *F. lactucae* in lettuce seeds could be enhanced by using molecular techniques, such as polymerase chain reaction, which has proven useful in the case of Fusarium wilt of basil (1).

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