Pea Fusarium Wilt Races in Western Algeria

Aoumria MERZOUG[1], Lakhdar BELABID[1], Mokhtar YOUCEF-BENKADA[2], Fouzia BENFREHA[1] and Bassam BAYAA[3]

[1]Laboratory of Research on Biological Systems and Geomatics (LRSBG), Department of Agronomy, University of Mascara, Mascara, Algeria; [2]Plant Protection Laboratory, University of Mostaganem, Mostaganem, Algeria; [3]Faculty of Agriculture, University of Aleppo, Aleppo, Syria

Abstract

Merzoug A., Belabid L., Youcef-Benkada M., Benfreha F., Bayaa B. (2014): **Pea Fusarium wilt races in western Algeria**. Plant Protect Sci., **50**: 70–77.

The fungus *Fusarium oxysporum* f.sp. *pisi* (FOP), the pea wilt pathogen, causes appreciable yield losses under favourable environmental conditions in Algeria. Studies on the pathogen variability and distribution of races are essential to identify effective sources of resistance to this disease. In this study, a survey was conducted during the period 2007–2011 in four different agro-climatic zones. Pathogenic variability in 52 isolates of FOP, collected from different pea-growing areas of western Algeria, were evaluated using 7 genotypes as differential hosts. Results indicated that the disease was prevalent in all fields prospected and isolates obtained were assigned to Races 1, 2, 5 or 6 by their pathogenicity. It was found out that Races 1 and 2 were more common in all areas with 61.5 and 19.2%, respectively. This study is the first report of pea Fusarium wilt races distribution in Algeria.

Keywords: *Fusarium oxysporum*; physiological races; *Pisum sativum*; resistance

Pea (*Pisum sativum* L.) wilt, caused by *Fusarium oxysporum* f.sp. *pisi* Schlecht (Van Hall) Snyd. and Hans., has been reported wherever peas are grown commercially (Kraft & Pfleger 2001). The disease is often severe where short rotations with other crops are practiced. Under these conditions, when the pathogen has built up sufficient inoculum, and a susceptible cultivar is planted, severe crop losses occur (Kraft 1995). *Fusarium oxysporum* f.sp. *pisi* (FOP) is a significant and destructive pathogen of field pea worldwide (Haglund & Kraft 2001). The pathogen is soil-borne and survives as thick-walled chlamydospores, which remain viable in the soil for more than 10 years (Kraft 1995). FOP penetrates pea roots and infects the vascular system at any growth stage (Infantino *et al.* 2006). Infected plants often show an orange or dark red discolouration in the vascular tissue of the root and lower parts of the stem. Above ground symptoms consist of leaves yellowing that wilt and curl downward during the flowering to pod-fill stages. Early infection often results in seedling death, which may be obscured by the growth of adjacent plants. However, wilted mature plants are easily seen scattered throughout the field. The disease incidence may be reduced by using extended crop rotations and early planting, but the most practical and cost-effective management strategy is the use of resistant cultivars (Haglund & Kraft 2001).

FOP is known for its pathogenic variability (Kraft 1995). In addition, FOP is continually evolving, with new variants of the pathogen emerging (Kraft & Pfleger 2001). Eleven races of the pathogen have been described; however, considerable disagreement exists on races classification (Neumann & Xue 2002). The pathogen was named Race 1 of FOP Schl. (van Hall.) Snyd. and Hans. in 1935, Snyder described a new race of *F. oxysporum* that was capable of causing wilting to plants resistant to Race 1, and they labelled the pathogen Race 2. The disease was called "near-wilt" because it appeared later in the growing season, often only at full pod development. Plants infected with Race 2 are most often scattered throughout the field rather than being concentrated in specific areas as with Race 1. In addition, Race 2 is most prevalent in coarser-textured soils when soil tem-

peratures are near 25°C. Because symptoms caused by the Race 2 pathogen do not usually occur until plant maturity, the likelihood of seed transmission is greatly increased. In 1970; Race 5 was described in north-western Washington (Haglund & Kraft 1970), where all commercial cultivars resistant to Races 1 and 2 were susceptible. Because of the short crop rotations and favourable climate in that area for wilt evolution, a new race of wilt was again described from western Washington, which was pathogenic on cultivars and breeding lines resistant to Races 1, 2, and 5, and was named Race 6 (Haglund & Kraft 1979). Races 1 and 2 occur worldwide, while Races 5 and 6 have so far been important only in western Washington State, USA (Infantino *et al*. 2006; Bani *et al*. 2011).

The pathogenicity of Races 1, 2, 5, and 6 of FOP can be detected by their reaction on the differential varieties. The disease reaction of these differentials is based on a resistant response (no observable disease) and a susceptible reaction (dead or severely stunted chlorotic plants). Different screening methods for FOP resistance have been described although most of them only consider the disease incidence or the proportion of symptomless plants to classify accessions as resistant or susceptible (Sharma *et al*. 2010). Classification of isolates of FOP based on host-pathogen interactions is governed by the genetic makeup of both the host and pathogen (Kraft 1995). Four races of FOP are recognised based on differential pathogenicity on pea cultivars (McClendon *et al*. 2002).

As for many soil-borne pathogenic fungi, the use of fungicides is not necessarily effective in controlling Fusarium wilt (Sharma *et al.* 2010). As a consequence, control of this disease is achieved mainly by integration of different disease management procedures including agronomic and farming practices, soil disinfestation (Momma *et al.* 2010), biocontrol (Alabouvette *et al.* 2009), and breeding for resistance (Sharma *et al.* 2010).

The aim of the study was to identify and evaluate the importance and frequency of pea wilt disease, characterise physiological races of FOP and their geographical distribution in western Algeria.

MATERIAL AND METHODS

Survey and collection of isolates. The surveys were conducted from January to June in four different agro-climatic zones in western Algeria (coastal plains, interior plains, the High Plateaus, and the Sahara) (Table 1). Several pea fields were surveyed during the campaigns 2007–2011 at different stages of plant growth (seedling stage, flowering, and pod-formation). At each site (area), the percentage of diseased plants was calculated; the various symptoms were described as well as the stage of the plant was recorded (Rana *et al.* 2009). From each field, 10 plants showing wilting and yellowing symptoms were taken to be fully analysed in the laboratory. Wilt-affected plants were collected from all regions. They were dried in paper towels and transported to laboratory for pathogen isolation. Isolations were made from the rhizosphere, root, and stem pieces (five per plant). The pathogens associated with pea wilt samples were isolated, cultured on PDA, and incubated at 25°C for 7 days (Belabid & Fortaz 2002). The isolation method from the rhizosphere soil was the plate dilution method for the soils that easily detach from the roots. Fungi were purified by single sporing and identified (Watanabe 2002; Leslie & Summerell 2006).

To confirm the identity of FOP, the pathogenicity test of each isolate was performed by inoculating a susceptible variety (Little Marvel) as described by Haglund (1989).

Differential pea genotypes. The pathogenicity of Races 1, 2, 5, and 6 of FOP can be distinguished by their reaction on a set of differential lines. The differential lines of pea were originally obtained from the Plant Germplasm Introduction and Testing Research Station in Pullman, Washington, USA and the Morden Research Station, Agriculture and Agri-Food Canada. Seven differential lines of pea were used to determine the races of 52 FOP isolates. Race 1 causes wilting to the cultivar Little Marvel; Race 2 causes wilting to Little Marvel, Darkskin Perfection, and WSU 28, but not to New Era, New Season, WSU 23, and WSU 31; Race 5 causes wilting to all the above except WSU 23, WSU 28, and WSU 31; Race 6 causes wilting to all the above except New Season, WSU28, and WSU 31 (Haglund 1984).

Inoculum production. FOP isolates used for the determination of races are most aggressive on the susceptible variety. All cultures were single spored on 2% water agar, then increased on fresh PDA. To produce the inocula, the selected isolates were grown for 4 days on water agar at 12 h light (25°C) and 12 h dark (20°C) cycle. The cultures were then transferred to liquid sucrose medium (PS: potato extract from 200 g potato and 15 g sucrose) and incubated for 10 days on an orbital shaker at 200 rpm at room temperature (22°C). Conidia were recovered

Table 1. Frequencies (%) of pathogenic fungi species isolated from diseased pea over the 2007–2011 period by agro climatic zone

Regions	Departments	Wilt	*F. oxysporum*	FOP	*F. solani*	*Rhizoctonia* sp.	*Fusarium* sp.	*Ascochyta sp.*	*Cladosporium* sp.	*Alternaria* sp.	*Stemphylium* sp.	*Sclerotinia* sp.	*Pythium* sp.
Coastal plains	Ain Temouchent	27.93	48.50	38.50	19.00	9.25	10.16	18.20	30.05	8.70	14.60	0.77	10.25
	Mostaganem	7.30	27.37	17.37	2.42	5.35	8.60	16.46	25.42	3.86	18.35	0.50	5.25
	mean	17.62	37.94	27.94	10.71	7.30	9.38	17.33	27.74	6.28	16.48	0.64	7.75
Interior plains	Tlemcen	11.02	44.50	31.45	21.00	10.20	17.50	15.35	6.20	11.50	5.66	3.75	4.02
	Cheliff	16.58	5.20	7.20	12.75	34.58	7.20	13.70	0.00	5.50	3.40	3.00	0.25
	Relizane	33.98	22.35	18.35	19.60	16.20	12.25	2.75	2.34	9.37	5.58	2.50	0.66
	Mascara	14.93	15.38	12.38	20.61	1.53	20.35	11.53	12.30	7.69	4.16	4.08	3.07
	Sidi Belabes	16.58	19.25	15.25	12.80	15.30	14.00	14.56	5.20	10.20	4.12	1.30	0.77
	mean	18.62	21.34	16.93	17.35	15.56	14.26	11.58	5.21	8.85	4.58	2.93	1.75
High Plateaus	Tiaret	14.70	14.00	13.30	25.00	9.00	9.60	2.90	0.00	2.70	0.00	3.20	0.00
	Saida	9.66	12.78	6.20	22.60	9.75	12.06	5.85	2.15	6.05	2.00	3.60	0.82
	mean	12.18	13.39	9.75	23.80	9.38	10.83	4.38	1.08	4.38	1.00	3.40	0.41
Sahara	Adrar	13.75	32.50	10.50	20.00	2.05	12.75	1.25	0.00	2.75	0.00	0.00	0.00
	mean	13.75	32.50	10.50	20.00	2.05	12.75	1.25	0.00	2.75	0.00	0.00	0.00
Total mean		15.54	26.29	16.28	17.97	8.57	11.81	8.63	8.50	5.56	5.51	1.74	2.48

Wilt – frequencies (%) of yellowing-wilting symptom in all fields prospected; FOP – frequencies (%) of forma specialis *pisi* among *Fusarium oxysporum* isolated

and adjusted to a concentration of approximately 5×10^6 conidium/ml by the aid of a haemocytometer (Haglund & Kraft 2001). The spores were tested for germination and viability on 2% water agar.

Inoculation and disease scoring. The pathogenicity of 52 isolates of FOP and one non-pathogenic isolate was determined by inoculating each of the differential lines. Seeds of each test line were surface-disinfested with a 2% sodium hypochlorite solution for 5 min before planting them in autoclaved vermiculite, and inoculated using the root prune and dip technique (Newman & Xue 2002). When seedlings grown in vermiculite were 10-days old and had produced 4–5 nodes, they were inoculated as follows: plants were pulled out from vermiculite, and the roots were cut at approximately 4 cm below the cotyledon attachment, dipped into the inoculum to cover the cotyledons for 5 min and then transplanted into pots containing soil-sand-peat (1:1:1, v/v/v); plants were watered as needed. Control plants were treated in the same way and were immersed in sterile water. Greenhouse temperatures were maintained at 20–24°C, and all plants were grown under available light. Susceptible lines died 21–28 days after inoculation (Newmann & Xue 2002; Bani *et al.* 2011). Plants were visually assessed at the 10–12 nodes stage, 28 days after inoculation, using the following 0–5 rating scale: 0 = no symptoms; 1 = chlorosis or wilting of one basal leaf, pale yellow green, downward curling of leaf margins and stipules; 2 = chlorosis or wilting of some basal leaves, no stunting; 3 = chlorosis or wilting of several basal leaves, slight stunting and yellowing of most leaves; 4 = chlorosis or wilting of most leaves, heavy stunting and drying of lower leaves, and 5 = death of the seedling. The scale was modified (Newmann & Xue 2002). Values were averaged for the four plants per pot and three pots were used for each isolate/line combination. Disease scores of 0, 1, and 2 were considered resistant (R) responses, while scores of 3, 4, and 5 were considered susceptible (S). After the evaluation of the reaction of the differential lines, the isolation of pathogen was performed in order to confirm the nature of the symptoms.

RESULTS

Surveys that were carried out in the four different agro-climatic zones in western Algeria have identified the presence of wilting symptoms; plant symptoms consisted in chlorotic leaflets, which curl downward and become flaccid. The plant eventually wilts and turns a yellowish-brown colour. Often, the above- and below-ground vascular system turns a light yellow to brick-red colour and the lower subterranean portion of the stem becomes larger than normal. The disease can attack pea plants at any growth stage, we have observed it attacking plants at a very young stage. In growing regions, it appears to be worse during hotter periods of the growing season, early infections may cause plant death; late infections reduce the number of harvests.

Significant differences in the frequencies of the disease are observed between vegetative stages of the plant and the regions, there is an increase in the percentages of diseases in all areas with the age of the plant (Merzoug *et al.* 2009, 2011). Yellowing-wilting symptom is observed during all stages of the plant growth, in the majority of fields and in all the years of prospecting with frequencies ranging 7.30–33.98% for the entire western region of Algeria. Pea culture at the internal plains is the most vulnerable with 18.61% (33.98% being the maximum in Relizane) (Table 1).

The study of the microflora showed significant variation, quantitatively and qualitatively, in the fungal population in pea rhizosphere. Some number of pathogens was associated with wilting and yellowing symptoms. The most dominant species for all regions surveyed was *F. oxysporum* with an average of 26.29%, followed by *F. solani* (17.97%). The coastal plains have the highest percentage of *F. oxysporum* (37.94%), in the High Plateaus *F. solani* predominated (23.80%). In addition to the species of *Fusarium*, the analysis allowed us to detect a significant number of fungal species that are located at the rhizosphere and stem of diseased plants with variable frequencies from one region to another. The Sahara is the most species-poor area quantitatively as well as qualitatively (Table 1).

The first symptoms of artificial inoculation on the susceptible line with isolates of *F. oxysporum* collected from different fields surveyed appeared after 7 days, the plants were completely destroyed between the 10th and 26th day, the symptoms were similar to those described in the field. Similarly, cultures derived from re-isolations made from inoculated seedlings were morphologically identical to their respective parent cultures. We confirmed the presence of f.sp. *pisi*. Among isolates of FO, 16.28% proved to be forma specialis *pisi* (Table 1). From more than one hundred isolates characterised, 52 isolates were selected for the study of races characterisation based on regions and cultural variability.

Characterisation of races of FOP. Variability in cultural characteristics which was observed among isolates varied from sparse to abundant, whereas the mycelium in culture remained cottony-floccose, cottony-dense, aerial, felted or mucous (ropy). The majority of the isolates had abundant and floccose cultural growth. Colour of the mycelium also varied from white, whitish pink, whitish yellow, dark purple, light purple to purplish red. The results obtained show the predominance of aerial type followed by a cottony type with light pigmentation (white or purple), there was also the presence of mucous (ropy) types with mauve pigmentation which becomes increasingly dark (dark purple or violet to black) in the medium (Table 2).

Occurrence and distribution of races of FOP. Current classification of pathogen races is based on host response using differential lines and defined reproducible inoculation procedures. Wilt ratings were taken 5 weeks following planting of pea seeds and 3–4 weeks following inoculation with cultures of FOP, ratings were based on the number of plants killed or expressing typical symptoms. In our study the majority of susceptible plants were killed by the isolates of FOP. Results obtained showed the presence of four races (1, 2, 5 and 6) of FOP, which have been identified in western regions of Algeria. The levels of the races found in western Algeria from isolates collected from infected peas plants are shown in Table 2. Races 1 and 2 were more common in all areas with 61.47 and 19.2%, respectively. Race 6 is represented by 11.52%, it is absent at the Sahara. For Race 5, only two isolates (A21 and M42) were identified in coastal plains where the pea crop is early sown compared to other regions.

In addition to the four previously described races, two new or unknown types of FOP were recovered, they were identified in the interior plains only. Although these two unknowns (UR1 – isolate C24 and UR2 – isolate SB5) were similar in cultural morphology to Race 1, they were quite different in their reactions with the differential lines and may, therefore, be new races. Their differential reactions require further study before any definitive descriptions can be made. The isolate URl caused the death of all differential lines. For isolate UR2, only two

Table 2. Geographical origin and characteristics of races of *Fusarium oxysporum* f.sp *pisi* in western Algeria

No. of isolate	Regions	Departments	Location of sampling areas	Dates of collection	Isolated portion	Cultural variability		Races
						morphotype	pigment	
A1	coastal plains	Ain Temouchent	Ain Tolba-1	2010	stem	cottony-floccose	whitish	R1
A2			Ain Tolba-2	2011	stem	cottony-floccose	whitish yellow	R1
A16			Benisaf-1	2010	root	aerial	whitish	R6
A18			Benisaf-2	2011	stem	cottony-dense	dark purple	R2
A19			Benisaf-3	2010	stem	cottony-floccose	whitish	R1
A21			Benisaf-4	2010	stem	felted	whitish	R5
A22			Benisaf-5	2010	stem	aerial	dark purple	R6
M42		Mostaganem	Farnaka	2009	stem	aerial	light purple	R5
M43			Bouguirat	2009	stem	felted	dark purple	R2
M44			Anonyme-1	2007	root	cottony-dense	whitish pink	R1
M45			Anonyme-2	2007	stem	aerial	whitish	R1
TL24		Tlemcen	Sebdou1	2010	stem	aerial	light purple	R6
TL27			Sebdou2	2010	rhizosphere	cottony-floccose	whitish yellow	R1
TL28			Ouled Mimoune1	2010	rhizosphere	cottony-floccose	light purple	R6
TL29			Ouled Mimoune2	2010	stem	felted	light purple	R1
TL31			OuledMimoune3	2011	rhizosphere	cottony-floccose	whitish pink	R1
TL32			Ouled Mimoune4	2011	stem	felted	whitish yellow	R1
C20	interior plains	Chlef	Aine Mrane-1	2009	root	mucous	light purple	R1
C21			Aine Mrane-2	2009	stem	aerial	light purple	R1
C24			Aine Mrane-3	2010	rhizosphere	cottony-floccose	whitish	UR1
R17			SidiMhammed Ben Ali	2009	root	felted	whitish pink	R1
R26		Relizane	Mazouna-1	2009	root	mucous	dark purple	R2
R27			Elgattar	2009	stem	aerial	light purple	R1
R28			Mazouna-2	2010	stem	cottony- dense	whitish	R1
R29			Yallel	2009	stem	aerial	whitish	R1
R31		Mascara	Ain Errahma-1	2009	stem	cottony-floccose	whitish pink	R1
R32			Ain Errahma-2	2010	root	aerial	purplish red	R6
Ma6			Tighennif	2007	stem	aerial	dark purple	R2
Ma9			Messaâdia	2007	rhizosphere	mucous	dark purple	R2
Ma13			Elbordj	2008	stem	aerial	whitish	R1
Ma15			Mamounia-1	2009	rhizosphere	mucous	violet to black	R2
Ma19			Mamounia-2	2009	root	felted	whitish	R1
Sb1		Sidi Belabes	Sfisef-1	2009	stem	aerial	light purple	R1
Sb3			Sfisef-2	2009	stem	aerial	dark purple	R2
Sb4			ITGC Sbelabes	2010	stem	felted	whitish pink	R1
Sb5			ITGC Sbelabes	2010	rhizosphere	felted	whitish	UR2
Sb11			Lamtar-1	2010	rhizosphere	cottony-dense	whitish	R1
Sb14			Lamtar-2	2011	stem	felted	light purple	R1
T46	High Plateaus	Tiaret	Rahouia-1	2009	stem	felted	light purple	R1
T47			Rahouia-2	2009	root	aerial	dark purple	R2
T48			Rahouia-3	2009	root	felted	purple	R1
T49			ITGCSebaîne	2009	stem	cottony-floccose	whitish pink	R6
T50			ITGCSebaîne	2009	stem	felted	light purple	R1
T51			Sebaîne-1	2009	stem	cottony-floccose	whitish pink	R1
T52			Sebaîne-2	2009	stem	aerial	light purple	R1
S56		Saida	ITGCSaida	2008	stem	aerial	whitish	R1
S58			ITGCSaida	2008	stem	felted	whitish yellow	R1
Ad60	Sahara	Adrar	Aougroute-1	2009	root	cottony-floccose	whitish	NPR
Ad62			Aougroute-2	2009	stem	aerial	purplish red	R2
Ad63			Aougroute-3	2009	root	cottony-dense	whitish	R1
Ad66			Zaouiet Kounta-1	2009	root	felted	whitish pink	R1
Ad67			Zaouiet Kounta-2	2009	root	aerial	dark purpule	R2

UR – unknown race; NPR – non-pathogenic race

cultivars, Little Marvel and Dark skin Perfection, were susceptible.

DISCUSSION

For the pathogens associated with wilt, *F. oxysporum* was the major cause of wilt disease in all agroclimatic zones followed by *F. solani*. The association of *F. oxysporum*, *F. solani, Rhizoctonia* sp. with wilt in legumes in Algeria and other countries has already been reported (Belabid *et al*. 2000; Neumann & Xue 2002; Rana *et al*. 2009). The widespread occurrence of the disease and association of soil-borne pathogens indicated the possibility of increased disease incidence in the future, if suitable management practices are not adopted. This study has demonstrated that the population of FOP in western Algeria was highly variable.

When isolates grown on acidified PDA (pH 5), Race 2 secreted a purplish black pigment into the medium whereas Races 1, 5, and 6 produced little or no pigment, similar results were observed (Sharma *et al*. 2005). Lin *et al*. (1984) considered the mucous (ropy) types of isolates with a purplish red pigmentation might be one variant of Race 2, since it could be obtained from old cultures of the same group. In our study, we have noted that isolates A22 and R32 identified as Race 6 presented an aerial mycelium with whitish to light purple pigment during the first two weeks after which they began to secrete a dark purple pigment resembling that of Race 2. Similarly, this result was recorded for the Danish Race 6 isolates, which had floccose white to light peach aerial mycelium. The reverse of the colony was similar in colour for about 12 days subsequently; a dark purple pigment was excreted into the medium. In pigmentation the same result was observed for the English and Danish isolates, suggesting that they belong to the same phylogenetic group and that this group may be widespread in Europe (Bodker *et al*. 1993; Kraft & Pfleger 2001). In the literature, Race 1 has aerial mycelium with little or no pigmentation when grown on PDA medium adjusted to pH 4.5. The aerial mycelium may be in strands, and sporulation is sparse. Few to no macroconidia and only limited numbers of microconidia are produced on PDA. Race 2 is also aerial, and mycelium may form strands. Pigmentation, light purple to black in most cultures, occurs on acid PDA. Sporulation is profuse, with production of abundant macro- and microconidia. Linear growth rates of Races 1, 5, and 6 were approximately equal to each other and were slower than those of Race 2.

The differentials lines or cultivars used in this study have been used quite frequently, suggesting that they are particularly useful for evaluating pathogenic variability in various parts of the world. Races 1 and 2 of the wilt pathogen occur in most of the pea growing areas worldwide; they are present and significant in the European and Mediterranean region (OEPO/EPPO 1994). Race 6 has been reported in Europe, while all four races are found in Australia and the four specific races of pea *Fusarium* wilt have been identified and studied in the USA (Haglund & Kraft 2001) and Canada (Neumann & Xue 2002). Races 1 and 2 were the only economically important ones in the United States until Race 5 appeared in north-western Washington in 1963, followed by Race 6 (Haglund & Kraft 2001). Races 1 and 2 occur worldwide, while Races 5 and 6 are only important in western Washington State and their impact has lessened as fresh pea production has moved out of western Washington.

The standard control differentials lines have been used successfully for many years; their reactions to Race 2 have recently been questioned. Intermediate reactions of the standard differential lines confound results from individual experiments.

In the majority of wilt diseases in the regions surveyed, peas are probably binds to the existence of a permanent inocula in the soil maybe due to the fact that crop rotation is very short or absent in most departments, poor sowing conditions, agriculture practices, the use of susceptible cultivars (Merveille de Kelvedon, Latcha local variety, Grand vert, Onward) to different races of FOP in most areas, infected seed is suspected of carrying the pathogen, it is not considered likely.

Considerable variation in the pathogenicity of different isolates of FOP has been reported. Most studies have not used the same, or the same number of differential genotypes, or have been conducted under different environmental conditions, so comparison of results between studies is difficult. The environmental factors, temperature, light, and humidity were stable in all trials and their effects were not examined in the present study. However, it has been shown that even slight changes in environmental conditions can significantly affect the expression of plant infection.

In this study, some races were specific to a single region while others were found in various regions. The most common race was recorded throughout all the regions. This might be due to seed transmission of FOP, since infected pea is important for natural spread of the pathogen. In Algeria, the transfer of pea seed from one region to another is common and uncontrolled. Seed transmission will only occur if

growers keep seeds from known infected plants; Race 1 can be transmitted occasionally by seeds when harvested from wilt-infested fields. The probability of seed transmission of Race 2, when the pathogen attacks a pea plant at flowering to pod development stages, is much higher than for Races 1, 5, or 6 which usually kill a susceptible plant before blooming. Masheshwari *et al.* (1981) isolated FOP from surface disinfested seeds of six varieties grown in the Hoshiarpur district of Punjab (India), where pea root rot and wilt are a problem and climatic characteristics of the regions are in most cases favourable to the disease development. Infected plants may look normal at low temperatures, but at soil temperatures of 20°C and above, wilt develops rapidly, resulting in the collapse of the entire aerial part. The disease could cause appreciable yield losses under favourable environmental conditions; it is a major yield-limiting factor in the dry-temperate zone.

In conclusion, this study allowed us to put in evidence the presence of pea wilt in all regions surveyed, a geographic distribution map of four races of the FOP at the western Algerian regions has been compiled. Races of the fungus and the population types present in the area need to be evaluated for disease control management. Specifically this information assists when examining newly bred cultivars and for the development of new resistant cultivars. The first stage of any disease control program involves the examination of the disease and its distribution. Growers may choose cultivars according to the race predominating in the soil in a particular region. Resistance to Fusarium wilt is not a substitute for good cultural practices but must be used in combination with them, when possible and early to minimize future losses. Incorporation of useful resistance sources effective against diverse races into adapted genotypes will be necessary to minimise future losses. Effective seed hygiene is also very important in order to prevent spread and introduction of highly virulent strains of the pathogen into new areas.

Acknowledgement. We thank Dave Stout, Biological Science Technician (Plants) USDA, ARS, WRPIS, Plant Germplasm Introduction and Testing Research Station in Pullman, Washington University, USA, and Dr Robert Conner, Modern Research Station Agriculture and Agri-Food, Canada for providing seeds of pea differential genotypes.

References

Alabouvette C., Olivain C., Migheli Q., Steinberg C. (2009): Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. New Phytologist, **184**: 529–544.

Bani M., Rubiales D., Rispail N. (2011): A detailed evaluation method to identify sources of quantitative resistance to *Fusarium oxysporum* f.sp. *pisi* race 2 within a *Pisum* spp. germplasm collection. Plant Pathology, **61**: 532–542.

Belabid L., Fortas Z. , Dalli D., Khiare M., Amdjad D. (2000): Importance du flétrissement et la pourriture racinaire de la lentille dans le nord-ouest Algérien. Cahiers Agricultures, **9**: 515–518.

Belabid L., Fortas Z. (2002): Virulence and vegetative compatibility of Algerian isolates of *Fusarium oxysporum* f.sp. *lentis*. Phytopathologia Mediterranea, **41**: 179–187.

Bodker L., Lewis B.G., Coddington A. (1993): The occurrence of a new genetic variant of *Fusarium oxysporum* f.sp. *pisi*. Plant Pathology, **42**: 833–838.

Haglund W.A. (1984): Fusarium wilts. In: Hagedorn D.J. (ed.): Compendium of Pea Diseases. APS, St. Paul: 22–24.

Haglund W.A. (1989): A rapid method for inoculating pea seedlings with *Fusarium oxysporum* f.sp. *pisi*. Plant Disease, **73:** 457–458.

Haglund W.A. , Kraft J.M. (1970): *Fusarium oxysporum* f.sp. *pisi* race 5. Phytopathology, **60**: 1861–1862.

Haglund W.A., Kraft J.M. (1979): *Fusarium oxysporum* f.sp. *pisi* race 6: Occurrence and distribution. Phytopathology, **69**: 818–820.

Haglund W.A., Kraft J.M. (2001): Fusarium wilt. In: Kraft J.M., Pfleger F.L. (eds): Compendium of Pea Diseases and Pests. APS Press, St. Paul: 13–14.

Infantino A., Kharrat M., Riccioni L., Coyne C.J., Kevin E., Phee M.C., Grunwald N.J. (2006): Screening techniques and sources of resistance to root diseases in cool season food legumes. Euphytica, **147**: 201–221.

Kraft J.M. (1995): Fusarium wilt of peas (a review). Agronomie, **14**: 561–567.

Kraft J.M., Pfleger F.L. (2001): Compendium of Pea Diseases and Pests. 2nd Ed. The American Phytopathological Society, St. Paul.

Leslie J.F., Summerell B.A. (2006): The Fusarium Laboratory Manual. Vol. 2, Issue 10. Blackwell Publishing Ltd., Oxford: 101–117.

Lin Y.S., Sun W., Pie-Han Wong (1984): Fusarium root rot and wilt of garden pea in Taiwan. Journal of Agricultural Research of China, **33**: 395–405.

Masheshwari S.K., Gupta J.S., Jhooty J.S. (1981): Effect of various cultural practices on the incidence of the wilt and root rot of pea. Indian Journal of Agricultural Sciences, **15**: 149–151.

McClendon T., Inglis A., McPhee E., Coyne J. (2002): DNA markers linked to Fusarium wilt race 1 resistance in pea. Journal of American Society and Sciences, **127**: 602–607.

Merzoug A., Benfreha F., Taleb M. (2009)**:** Les principales maladies fongiques du Petit Pois (*Pisum sativum*) et Pois chiche (*Cicer arietinum*) dans le nord ouest Algérien. In: Colloque international sur la gestion des risques phytosanitaires, 9–11 Novembre 2009, Marrakech, Maroc. Proceedings V1: 115–123.

Merzoug A., Belabid L., Benfeha F. (2011): Recensement des principales espèces fongiques pathogènes sur la culture du petit Pois (*Pisum sativum*) dans le nord ouest Algérien. In: Colloque International «Nouvelles Espèces Végétales et Microbiennes décrites en Algérie de 1962–2010», 18–20 Octobre 2011, Oran, Algérie.

Momma N., Momma M., Kobara Y. (2010): Biological soil disinfestation using ethanol: effect on *Fusarium oxysporum* f.sp. *lycopersici* and soil microorganisms. Journal of General Plant Pathology **76**: 336–344.

Neumann S., Xue A.G. (2002): Reactions of field pea cultivars to four races of *Fusarium oxysporum* f.sp. *pisi*. Canadian Journal of Plant Sciences, **83:**377–379.

OEPP/EPPO (1994): Organisation Européenne et Méditerranéenne pour la Protection des Plantes: Principe de bonne pratique phytosanitaire, pois. OEPP/EPPO Bulletin, **24**: 233–240. Available at http://onlinelibrary.wiley.com/doi/10.1111/epp.1994.24.issue-2/issuetoc

Rana U., Sharma A., Paul Y., Dev Sharma K. (2009): Survey for pea diseases and identification of fungi associated with wilt and root rot complex in Himachal Pradesh. Journal of Mycology and Plant Pathology, **39**: 416–421.

Sharma P., Sharma K.D., Sharma R., Plaha P. (2005): Genetic variability in pea wilt pathogen *Fusarium oxysporum* f.sp. *pisi* in north western Himalayas. Indian Journal of Biotechnology, **5:** 298–302.

Sharma A., Rathour R., Plaha P. (2010): Induction of Fusarium wilt (*Fusarium oxysporum* f.sp. *pisi*) resistance in garden pea using induced mutagenesis and *in vitro* selection techniques. Euphytica, **173**: 345–356.

Watanabe T. (2002): Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. 2nd Ed. Lewis Publishers, CRC Press, Florida: 270– 276.

Received for publication March 18, 2013
Accepted after corrections October 17, 2013

Corresponding author:

Dr Aoumria Merzoug, University of Mascara, Department of Agronomy, Laboratory of Research on Biological Systems and Geomatics (LRSBG), P.O. Box 305, Mascara 29000, Algeria; E-mail: merzougaoumria@hotmail.com