

Short Communication: Virulence of different isolates causing verticillium wilt of pepper in the Padrón region

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Abstract

A survey of verticillium wilt of pepper was carried out in farms of the Padrón region, including both greenhouses and fields. Pepper plants cv. Padrón showing wilt symptoms were collected in three successive sampling sessions performed from May to October 1997. A total number of 79 isolates of *Verticillium dahliae* Kleb were obtained. The high frequency of farms with plants infected with *V. dahliae*, especially at the end of the crop season, was related to a high persistence of the fungus during the whole of the 1990s. Twenty-two isolates were tested for pathogenicity, and all of them were able to induce wilting in cv. Padrón pepper plants. Dry weight and stem length data of inoculated plants showed that there was a high variability in the virulence of the isolates tested, and significant differences were observed among them.

Key words: *Verticillium dahliae*, *Capsicum annuum*, pathogenicity, Galicia.

Resumen

Virulencia de diferentes aislados causantes de la verticilosis del pimiento en la comarca de Padrón

Se realizó una prospección en explotaciones de pimiento con síntomas de verticilosis de la comarca de Padrón (provincia de A Coruña), tanto al aire libre como en invernadero, consistente en tres muestreos sucesivos entre los meses de mayo y octubre de 1997. Se obtuvieron 79 aislados de *Verticillium dahliae* Kleb. El alto número de explotaciones en las que se detectó *V. dahliae*, sobre todo al final de la temporada de producción, fue unida a una elevada persistencia durante la década de 1990. Al evaluar el comportamiento patogénico de 22 aislados de *V. dahliae* se vio que todos fueron capaces de reproducir la enfermedad en las plantas de pimiento de Padrón. El análisis del peso seco y la longitud del tallo de las plantas inoculadas puso de manifiesto una gran variabilidad en cuanto a la virulencia de los aislados ensayados, encontrándose diferencias significativas entre ellos.

Palabras clave: *Verticillium dahliae*, *Capsicum annuum*, patogenicidad, Galicia.

The Padrón pepper, like most cultivated peppers, belongs to the *Capsicum annuum* L. species. The special characteristics of this cultivar seem to be related to the geographical, edaphic and climatological conditions of the Padrón region (Coruña province) and to some degree of genetic isolation as a consequence of self-fertilisation and farmers' conservation of seeds (Estrada *et al.*, 2000).

One of the most serious pathological problems in pepper cultivation is verticillium wilt, caused by the deuteromycete fungus *Verticillium dahliae* Kleb. (Nuez *et al.*, 1996; Tsror *et al.*, 2000). It has been reported to be the main pathological problem of peppers grown in open fields in the Ebro valley (Palazón and Palazón,

1989), and has also been reported on a number of Galician farms (Saavedra, 1993).

Infection of the plant starts at the root, either directly through the epidermis or through lesions. The main symptoms observed in affected plants are partial or total wilting due to a loss of turgidity in the tissues, curling of the adult leaves, defoliation, discoloration of aerial organs, reduced overall plant growth and darkening of the vascular bundles (Palazón and Palazón, 1989; Nuez *et al.*, 1996).

In order to compare the epidemiological situation of verticillium wilt of Padrón pepper in the localities of Herbón and Padrón, we sampled during the different stages of the productive cycle, to isolate and identify the fungus causing the verticillium wilt and to classify the isolates in relation to their pathogenicity and virulence against Padrón pepper.

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Received: 12-04-02; Accepted: 04-10-02.

Samples were taken from a total of 12 farms (9 greenhouses and 3 open-air farms) in three successive sampling periods, from the beginning of May to the beginning of October 1997 (Table 1). In each farm, 10 Padrón pepper plants with symptoms of the disease were collected.

For isolation of the fungi, samples were taken from different parts of the plants corresponding to the base of the stem, the neck and base of the root. The surface of the fragments was disinfected with sodium hypochlorite at 0.5% for 5 minutes and inoculated onto plates with PDA medium (Potato Dextrose Agar). *Verticillium* determination was done according to Smith's (1965) and Isaac's (1967) criteria and the following aspects were studied: presence of microsclerotia, presence and characteristics of microconidia and conidiophores, and cardinal temperatures. Two numbers were assigned to the different isolates: the first referred to the farm and the second to the sampled plant number.

The presence of *Verticillium dahliae* was detected in all the sampling sessions (Table 1) and a total of 79 isolates were obtained in the three sampling sessions performed. No other species of *Verticillium* was detected.

Symptoms corresponding to *V. dahliae* attack were scarce in the first sampling session and the main ones were partial wilting and dwarfism. These became more severe in the second and third sampling session and were accompanied later by other symptoms such as defoliation and vascular necrosis.

In the third sampling session (September and October), coinciding with the end of production, there

was an increase in the number of farms in which *V. dahliae* was detected. This increase could be related with temperature since this factor is closely related to the incidence of verticillium wilt (Kendrick and Middleton, 1959). The temperatures reached in this season in the Padrón greenhouses are around 24°C, which is optimum for *Verticillium* growth and development of the disease according to Kendrick and Middleton (1959) and Barriuso *et al.* (1992).

In a previous survey carried out in 1991 and 1992 (Saavedra, 1993), the presence of *V. dahliae* was detected in farms 1, 2, 3, 4 and 7, permitting us to deduce that these farms had verticillium wilt almost constantly over this decade. This persistence of *V. dahliae* in the Padrón farms is because the farmers did not solarise the soil to destroy the microsclerotia and also due to the repetition of pepper crops during several years in the same plot, increasing the infectious potential. In general, all reported cases refer to microsclerotia as the main form in which the fungus is conserved in the soil. Isaac (1967) indicated a survival of 6 to 14 years of these organs in normal crop rotations and up to 4 years in the total absence of hosts in the rotation, with the potential to germinate when the environmental conditions are more favourable (Pegg, 1974).

A total of 22 of the isolates obtained (30%) were studied to determine their pathogenicity and degree of virulence. This percentage was chosen taking into account the number of isolates per farm. Hence, in farms with the greatest number of isolates (9 or more), 3 were studied and in the rest of the farms 1 or 2.

Table 1. Characteristics of the surveyed farms and the number of isolates of *Verticillium dahliae* collected in 3 sampling sessions

Farms	Type	Area (m ²)	Irrigation	Disinfection in 1997	No. of isolates of <i>V. dahliae</i> **		
					1*	2*	3*
1	GH	700	drip	no		9	6
2	GH	400	furrow	no		1	2
3	GH	1000	drip	no		1	2
4	GH	550	drip	yes			1
5	GH	1700	furrow	yes			
6	GH	1200	drip	yes		1	1
7	OA	4500	furrow	no			2
8	OA	2100	furrow	no		7	2
9	GH	1600	drip	yes	1		
10	GH	360	furrow	yes	2	9	3
11	OA	500	furrow	yes		3	7
12	GH	550	drip	yes	6	8	9

GH: greenhouse. OA: open air. * Sampling session. ** Each isolate was obtained from a different plant.

Pathogenicity experiments were carried out on 16 day-old plants of the Padrón pepper variety in the phenological stage of 4 true leaves. The plants, in groups of 10, were inoculated with the different isolates of *V. dahliae*, according to the method of Tello *et al.* (1991), that consists in dipping the roots in an «inoculum unit» for 20 min. This «inoculum unit» was obtained by milling a PDA petri dish, totally covered with the mycelia from the isolate to be inoculated, in 400 ml of distilled water. Another group of 10 plants was treated with sterile water instead of the inoculum and corresponded to the control group. After carrying out the inoculation, the plants were transplanted to 10 well-trays with a sterilised substrate composed of potting soil and vermiculite in a ratio of 2:1 (v/v). The experiments were conducted in a chamber with controlled temperature (25°C) and photoperiod (16 hours light and 8 hours darkness).

Plants were checked regularly for symptoms produced by each of the isolates of *V. dahliae*, and the stem length of inoculated plants was recorded over 30 days. After this period, different organs were separated from the plants (roots, hypocotyl, epicotyl and leaves) and the dry weight was determined.

The data were studied by one way analysis of variance (ANOVA) and Duncan's test with a significance of 5%. The data were transformed by the logarithmic function in base 10 prior to their analysis. The statistical analysis was done with the help of the Statgraphics Plus 4.0 software for Windows.

In all cases, *V. dahliae* was reisolated from experimentally inoculated plants that had developed symptoms of the disease.

Table 2 shows that the epicotyl and the leaves were the organs most affected by the disease followed by the hypocotyl and the root. On the other hand, there was considerable variability in the virulence of the isolates, with significant differences between some of these. The least and most virulent isolates were clearly 2-9 and 1-3, respectively. Isolates with an intermediate virulence could be found between these two extremes. Another important point is that, in some cases, there was a great variability between the isolates from the same farm. For example, from farms 2 and 10, extremely high virulent isolates were obtained (2-5 and 10-8) and also ones with an especial low virulence (2-9 and 10-10).

The measurements of stem growth of the plants inoculated with isolates 1-3 and 2-9 confirm the dry

Table 2. Relative dry weight* of the organs and the whole plant of Padrón pepper plants inoculated with different isolates of *Verticillium dahliae*

Isolate	Roots	Hypocotyl	Epicotyl	Leaves	Plant
2-9	95.9 a	58.3 ab	71.7 a	68.7 a	70.2 a
4-3	73.6 abcd	60.9 ab	49.3 abc	55.6 ab	56.6 ab
7-7	88.6 ab	62.8 ab	59.5 ab	49.4 bc	54.1 ab
10-10	79.8 abc	64.7 ab	42.8 bcd	47.1 bcde	50.6 bc
3-3	65.9 bcde	49.2 abcd	39.5 bcde	49.6 bcd	49.6 bc
6-10	55.5 bcdef	67.2 a	43.0 abcd	42.5 bcde	45.9 bc
8-5	65.0 bcde	48.4 bcd	30.5 cdef	42.6 bcde	43.4 bcd
12-10	52.6 def	55.7 abc	34.4 bcdef	42.2 cdef	43.4 cde
8-6	74.3 abcd	52.5 abcd	37.0 bcdef	39.0 cdef	42.5 bcd
10-1	66.6 bcde	52.8 abcd	29.8 ef	35.2 defg	38.4 cdef
11-5	57.4 bcde	41.1 cd	29.6 cdef	34.1 defg	36.5 cdef
12-6	50.2 cdef	51.6 abcd	33.5 bcde	33.3 defg	36.5 cdef
2-5	47.9 cdef	53.3 abc	26.4 def	32.8 defg	35.2 cdef
7-6	62.1 bcde	47.3 abcd	27.7 cdef	31.3 efg	34.6 cdef
1-1	46.1 ef	52.3 abcd	29.4 cdef	31.7 defg	34.6 cdef
8-2	76.4 bcdef	37.2 d	26.2 f	31.5 fg	34.4 def
1-8	57.7 bcdef	48.2 abcd	22.6 def	31.7 efg	34.1 cdef
11-8	52.3 bcdef	44.5 abcd	29.9 bcdef	31.2 defg	34.1 cdef
11-6	51.0 bcdef	47.8 abcd	23.2 def	27.5 fg	30.8 def
12-8	40.3 ef	45.0 abcd	19.6 def	28.8 fg	30.3 def
10-8	45.5 def	41.9 bcd	19.8 f	25.5 g	28.2 ef
1-3	35.5 f	47.3 abcd	20.6 def	25.2 g	27.5 f

* The data are expressed in % dry weight of the plants belonging to each group relative to the control group inoculated with sterile water. Different letters in the same column reflect significant differences between treatments ($p \leq 0.05$).

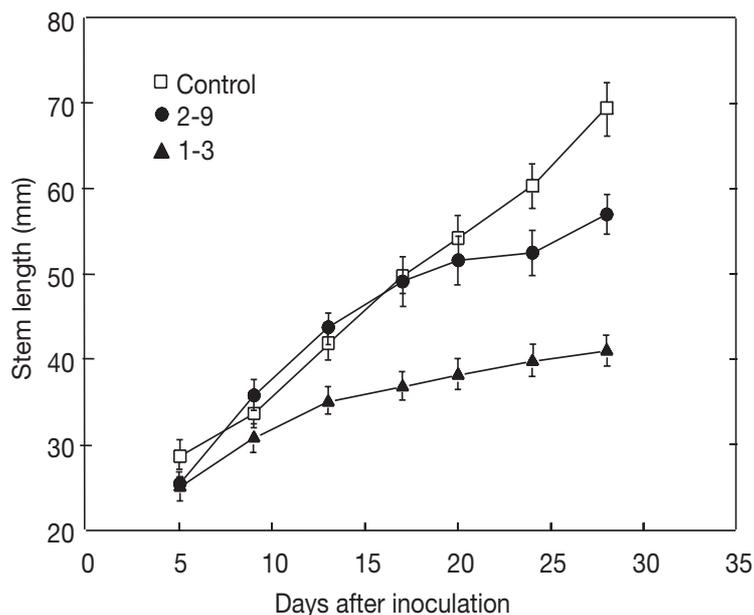


Figure 1. Growth of stem in pepper plants cv. Padron inoculated with two isolates of *Verticillium dahliae*. The error bars correspond to a standard error (n = 10).

weight results. Figure 1 shows clear differences in the growth of pepper plants inoculated with the most virulent isolate, 1-3, and the least virulent isolate, 2-9. The plants inoculated with isolate 1-3 hardly exceeded 40 mm in length at the end of the experiment while plants inoculated with 2-9 reached almost 60 mm, and the control plants reached a height of 70 mm. This slower growth of the plants inoculated with isolate 1-3 was the consequence of an early appearance of symptoms of the disease, finally reflected in a smaller dry weight than that of plants inoculated with isolate 2-9 (Table 2).

It is known that *Verticillium* can infect a wide range of organisms. More specifically, the hosts of *V. dahliae* include crops of cotton, tomato, pepper, aubergine, potato, mint and several cucurbitaceous species, ornamental woody shrubs and fruit trees (Heale, 1988; Bejarano-Alcázar *et al.*, 1996; Tsror *et al.*, 2000). Possibly, the isolates of *V. dahliae* with the least virulence are not specific to pepper. In fact, isolate 4-3 came from a farm in which pepper and tomato plants were grown together and this strain could be more specific to tomato than to pepper or could be a strain with little host specificity. However, several cases have been described of strains of *V. dahliae* specific to pepper (Doura *et al.*, 1995; Riley and Bosland, 1997). The most virulent group of isolates tested, headed by 1-3, could, therefore, correspond to isolates specific to pep-

per. In any case, in order to assign host specificity to an isolate or a group of isolates, or even the possible establishment of races of *V. dahliae* among these isolates would require further experiments to be conducted that include other host plants and different pepper cultivars.

Acknowledgments

This work has been financed by the DGSEIC (project AGF99-0301) and the XUGA (project PGDIT-99AGR-10301). The authors would like to thank Jesus Collar Urquijo, Delegate of the Consellería de Política Agroalimentaria e Desenvolvemento Rural for the province of A Coruña, for his help in identifying the isolates.

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