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.

### 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.
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 (Kendrik and Middleton, 1959). The temperatures reached in this season in the Padrón greenhouses are around 24ºC, which is optimum for *Verticillium* growth and development of the disease according to Kendrik 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 (m²) | Irrigation | Disinfection in 1997 | No. of isolates of *V. dahliae*** |
|-------|------|-----------|------------|-----------------------|-----------------------------------|
|       |      |           |            |                       | 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  |
| 11    | OA   | 500       | furrow     | yes                   | 3  | 7  |
| 12    | GH   | 550       | drip       | yes                   | 6  | 8  |

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 (25ºC) 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.

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 bcd | 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 bcd | 45.9 bc |
| 8-5     | 65.0 bcde | 48.4 bcd  | 30.5 cdef | 42.6 bcd | 43.4 bcd |
| 12-10   | 52.6 bcdef | 55.7 abc  | 34.4 bdef | 42.2 cdef | 34.3 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 cdef | 38.4 cdef |
| 11-5    | 57.4 bcde | 41.1 cd   | 29.6 cdef | 34.1 cdef | 36.5 cdef |
| 12-6    | 50.2 cdef | 51.6 abcd | 33.5 bcde | 33.3 cdef | 36.5 cdef |
| 2-5     | 47.9 cdef | 53.3 abc  | 26.4 def  | 32.8 cdef | 35.2 cdef |
| 7-6     | 62.1 bcde | 47.3 abcd | 27.7 cdef | 31.3 cefg | 34.6 cdef |
| 1-1     | 46.1 ef   | 52.3 abcd | 29.4 cdef | 31.7 cdefg | 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 cefg | 34.1 cdef |
| 11-8    | 52.3 bcdef | 44.5 abcd | 29.9 bcdef | 31.2 cdefg | 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 bcde | 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 ≤ 0.05).
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 (Douira 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 pepper. 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.

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