Resistance of several strawberry cultivars against three different pathogens

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Abstract

In addition to the agronomical characteristics of strawberries (Fragaria × ananassa Duch.), strawberry breeding programs should take into account its resistance to pathogens. The main goal of this study was to evaluate the resistance of strawberry cultivars against crown and root rot caused by Phytophthora cactorum (Lebert & Cohn) J. Schröt., Verticillium wilt caused by Verticillium dahliae Kleb. and V. albo-atrum Reinke et Berth., and angular leaf spot caused by Xanthomonas fragariae Kennedy and King, all of which are important diseases in strawberry plant nurseries in Spain. Ten strawberry cultivars were used for resistance testing against P. cactorum; nine cultivars were used for resistance testing against V. dahliae and V. albo-atrum; and eight strawberry cultivars were used for resistance testing against two strains of X. fragariae, IVIA349.9a and NCPPB 1469. These assays were conducted under greenhouse conditions for P. cactorum, and under growth chamber conditions for V. dahliae and V. albo-atrum and X. fragariae. ‘Sabrosa’, was classified as being resistant to P. cactorum and V. dahliae and V. albo-atrum and only ‘Sieger’ was classified as being resistant to both strains of X. fragariae. These results emphasize the importance of screening for disease resistance before using strawberry cultivars in commercial strawberry production in Spain.

Additional key words: angular leaf spot; crown rot; disease resistance; soil-borne pathogens; Verticillium wilt.

Resistencia de varios cultivares de fresa frente a tres patógenos diferentes

Los programas de mejora de la fresa (Fragaria × ananassa Duch.) deberían tener en cuenta, además de las características agronómicas de la fresa, su resistencia a patógenos. El objetivo principal de este estudio fue evaluar la resistencia de varios cultivares de fresa contra la podredumbre de corona y radicular causada por Phytophthora cactorum (Lebert and Cohn) J. Schröt., la Verticilosis causada por Verticillium dahliae Kleb. y V. albo-atrum Reinke et Berth., y la mancha angular causada por Xanthomonas fragariae Kennedy and King, todas las cuales son importantes enfermedades en los viveros de fresa en España. Diez cultivares de fresa, se utilizaron para evaluar la resistencia frente a P. cactorum; nueve cultivares se utilizaron para evaluar la resistencia frente a V. dahliae y V. albo-atrum y ocho cultivares fueron utilizados para probar la resistencia frente a dos cepas de X. fragariae, IVIA349.9a y NCPPB 1469. Estos ensayos se realizaron en condiciones de invernadero en el caso de P. cactorum, y en condiciones de cámara de crecimiento en los casos de V. dahliae y V. albo-atrum y X. fragariae. ‘Sabrosa’ fue clasificada como resistente a P. cactorum y V. dahliae y V. albo-atrum, mientras que sólo ‘Sieger’ se clasificó como resistente a ambas cepas de X. fragariae. Estos resultados señalan la importancia de evaluar la resistencia de los cultivares de fresa a las diferentes enfermedades presentes en España antes de utilizarlos en la producción comercial de fresas en España.

Palabras clave adicionales: mancha angular; patógenos de suelo; podredumbre de corona; resistencia a enfermedades; Verticilosis.

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Introduction

Strawberry is an important crop in Spain. In fact, Spain is the main European producer of fresh strawberries, whose production was 274,600 tonnes in 2010 (FAO, 2011; MARM, 2011). Californian and Floridian commercial nurseries in the USA supply more than 50% of the mother plants that are used in Spanish nurseries (Medina-Minguez, 2011). Each year, strawberry runners for transplanting are produced in nurseries in Castilla-León (north-central Spain), from where they are transported to the fruit production fields in other regions of Spain and to those in other European countries. The cultivation of strawberry plants (runners) is annual, with planting between April and May and the digging of fresh commercial strawberry runners throughout October (De Cal et al., 2004). In Spanish strawberry nurseries, pre-plant soil fumigation with mixtures of methyl bromide and chloropicrin was commonly used to the control of soil-borne diseases (López-Aranda et al., 2009). Since use of methyl bromide in Europe is phase-out (MBTOC, 2007), Spanish strawberry growers are under considerable pressure to seek an alternative method of efficient disease control of their strawberry crops. One potential solution to this phase-out is to develop disease-resistant strawberry cultivars.

In Spanish strawberry breeding programs, agronomical characteristics are usually considered but resistance to main pathogens is only considered when strawberry cultivars have been previously selected by its agronomical characteristics. Furthermore the selection of new cultivars has been made in the last decades in soils where methyl bromide was largely used as soil disinfectant method, and then soils are free of the main soilborne pathogens (Duhart et al., 2000). Therefore nowadays the Spanish strawberry breeding industry should consider the development of resistant cultivars to main pathogens while considering the agronomical characteristics of cultivars.

The oomycete P. cactorum is the causative microorganism for crown and root rot in cultivated strawberries, and the causative agent of leather rot of fruit in wild (Fragaria vesca L.) and commercial (F. × ananassa) strawberries (Mass, 1998). During the last few years, this soil-borne pathogen has become widespread, especially in Europe (Eikemo et al., 2000; Duncan, 2002), and has become a limiting factor for successful strawberry production. Three reasons have been submitted to explain the increased incidence of this disease: (i) the extensive use of a few highly susceptible strawberry genotypes in most strawberry-producing areas (Harris et al., 1997; Stensvand et al., 1999), (ii) the introduction of the pathogen into pathogen-free strawberry nurseries by asymptomatic infected plants, and (iii) the limited effect of new soil fumigants, other than methyl bromide, against this pathogen (De Cal et al., 2004; López-Aranda et al., 2009). Crown rot resistance in strawberries is now well-documented, and several screening methods to identify such cultivars have been developed (van Rijbroek et al., 1997; Redondo et al., 2009).

The two fungi V. dahliae and V. albo-atrum are also important strawberry pathogens in Spain. Verticillium wilt is difficult to completely control even with soil fumigation (Martin & Bull, 2002), and repeated soil fumigations may be necessary to control the pathogen. Since their microsclerotia can survive in the soil for many years, crop rotation is less effective for controlling Verticillium wilt than other control methods, such as soil fumigation. Cross-infection does occur and the existence of pathotypes of V. dahliae and V. albo-atrum with different levels of pathogenicity has been described (Horiuchi et al., 1990). It is also known that quantitative resistance to Verticillium spp. is present in some strawberry cultivars, and that this resistance originated from wild, non-cultivated strawberry species, such as F. chiloensis L., F. virginiana Duch., and F. ovalis (Lehm.) Rydb. (Ebihara et al., 2010).

Angular leaf spot incited by X. fragariae is another important disease in strawberry nursery production. This disease affects only Fragaria spp., and is most commonly transmitted by planting infected stock (Maas et al., 1995). No effective chemical control is available, and the disease can only be managed by avoiding the introduction of contaminated planting material into nurseries (Maas et al., 1995). The European and Mediterranean Plant Protection Organization (EPPO) have listed X. fragariae as an A2 quarantine pathogen (EPPO, 2010). Therefore, nurseries that wish to export strawberry plants to European countries must maintain high phytosanitary standards in order to exclude X. fragariae. Resistance to X. fragariae has been reported in some Fragariae spp. (Lewers et al., 2003), and strawberry cultivars reportedly vary in their susceptibility to angular leaf spot (Maas et al., 2000).

The main goal of this study was to evaluate the resistance of strawberry cultivars against crown and root rot caused by P. cactorum, Verticillium wilt caused by V. dahliae and V. albo-atrum and angular leaf spot.
caused by *X. fragariae*, all of which are important diseases in strawberry plant nurseries in Spain.

**Material and methods**

**Plant material and plant micropropagation**

The strawberry plants used in this study were obtained from the Fragaria germplasm collection of the Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA)-Centro de Churriana, Málaga, Spain. Resistance testing was done using different strawberry cultivars (Table 1). The cultivars ‘Frau Mieze Schindler’, ‘Sieger’, ‘Deutsch Evern’, and ‘Africa’ are historical or pioneer cultivars that are not in current use, but whose genetic base is more expanded than the cultivars currently grown, namely ‘Camarosa’, ‘Ventana’, ‘Aguedilla’, and ‘Sabrosa’ (Candonga®) (Gil-Ariza et al., 2009). Two cultivars were used as controls for each pathogen. These control cultivars were different for each pathogen according to their susceptibility or resistance to each pathogen. For *P. cactorum*, ‘Senga Sengana’ was used as the crown rot-resistant cultivar (Stensvad et al., 1999; Eikemo et al., 2000) and ‘Surprise Halles’ was used as the crown rot-susceptible cultivar (Pitrat & Risser, 1977). ‘Pandora’ was used as the Verticillium wilt-resistant cultivar and ‘Carisma’ was used as the Verticillium wilt-susceptible cultivar (Redondo et al., 2009). For *X. fragariae* only, ‘Camarosa’ was used as the control because of its high susceptibility to this pathogen (Xue et al., 2005).

The strawberry plants were propagated *in vitro* using a previously described protocol (López-Aranda et al., 1994). The use of micropropagated material can be allowed to initiate *in vitro* cultures at any time of year because it does not depend on the production of runners from the mother plant. Furthermore *in vitro* propagation for continuous clonal plant production was initiated from meristematic tissues, which were free from viruses and other potential pests or pathogens. For each cultivar, strawberry shoots were subcultivated every six weeks. At each subculture, the new plants were first separated, and then individually cultured. This process was repeated three times until the required number of plants for each assay was reached. The individual *in vitro* micropropagated plants were carefully washed in running water, and then transplanted into 4 cm × 4 cm polystyrene trays containing a 1:1 (by volume) mix of sterile peat (Gebr. BRILL substrate, GmbH & Co. KG, Germany) and perlite (Perlita expandida, Europerlita Española S.A., Barcelona). The plants were acclimatized to *ex vitro* conditions in a greenhouse for resistance testing against *P. cactorum* and in a growth chamber for resistance testing against Verticillium wilt and *X. fragariae*. For this purpose, the trays were first placed inside a polyethylene tunnel, whose relative humidity (RH) was 100% for two weeks. At the end of the two weeks, the tunnel was slowly demolished over two weeks by progressive removal of the polyethylene tunnel so that the RH was same as that of the room.

| Table 1. Strawberry cultivars used in this study and their origins |
|---------------------------------------------------------------|
| **Name**        | **Type**            | **Pedigree** | **Developer**                  | **Country** |
| Africa           | Historical cultivar | Unknown      | 1870                           | France      |
| Deutsch Evern   | Historical cultivar | Unknown      | Johannes Böttner 1902          | Germany     |
| Frau Mieze Schindler | Historical cultivar | Lucida Perfekta × Johannes Müller | 1919 Germany |
| Sieger           | Historical cultivar | Kaiser SamLing × Laxton’s Noble | Johannes Böttner 1987 Germany |
| Aguedilla       | Commercial cultivar | Camarosa × Sel. 67-35-167 | IFAPA-INIA-IPI FNM-AEVPF 1998 | Spain       |
| Camarosa        | Commercial cultivar | Douglas × Cal 85.218-605 | UCA 1992                       | USA         |
| Sabrosa (Candonga®) | Commercial cultivar | Sel. 92.28 × Sel. 86.032 | PLANASA                        | Spain       |
| Ventana         | Commercial cultivar | Cal 93.170-606 × Cal 92.35-601 | UCA 1997                      | USA         |
| Surprise Halles | Susceptible to *P. cactorum* | Unknown | Guyot of Dijon 1925 France | Spain       |
| Senga Sengana   | Tolerant to *P. cactorum* | Sieger × Makee (or reverse) | Sengbush 1954 Germany | Germany     |
| Carisma         | Susceptible to *Verticillium* spp. | Oso grande × Vilanova | IFAPA-INIA-IPI 1998 Spain | Spain       |
| Pandora         | Tolerant to *Verticillium* spp. | (Von Humboldt × Redstar) × Morton Down | 1988 | United Kingdom |

1 Information from Darrow (1966). 2 UCA, University of California; IFAPA, Instituto Andaluz de Investigación y Formación Agraria y Pesquera, Málaga; INIA, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid; IVIA, Instituto Valenciano de Investigaciones Agrarias, Valencia; FNM, Fresas Nuevos Materiales, Huelva; AEVPF, Asociación Española de Viveristas de Plantas de Fresa, Madrid; PLANASA, Plantas de Navarra SA, Valtierra.
The plants were then transplanted into plastic pots that contained the identical substrate, and grown in the greenhouse or in growth chambers until required for resistance testing.

**Isolates**

Four *P. cactorum* isolates, two isolates of *V. dahliae* and *V. albo-atrum*, and two *X. fragariae* isolates were used as inoculum in this study. The *P. cactorum* isolates, CH971 and CH972, were obtained from strawberry plants (*F. vesca*) with crown rot from Málaga, Andalusia, Spain. The *P. cactorum* isolates, CH1020 and CH1021, were obtained from strawberry plants (*F. × ananassa*) with crown rot from Huelva, Andalusia, Spain. All isolates were conserved in IFAPA’s plant pathogen collection, and were stored on Oatmeal agar (OMA, Difco, Detroit, MI, USA) at 15°C for short-term storage, and underwater at 4°C for long-term storage.

The *V. dahliae* isolate, VER13, was obtained from strawberry plants (*F. × ananassa*) in Segovia, Spain, and the *V. albo-atrum* isolate 20103, was obtained from alfalfa (*Medicago sativa* L.) in Aragón, Spain and was kindly provided by Dr. Rafael González Torres, Servicio de Investigación Agraria (SIA), Aragón, Spain. These two isolates were maintained in 20% glycerol at –80°C for long-term storage, and were grown on potato dextrose agar (PDA) (Difco, Detroit, MI, USA) at 20 ± 2°C for seven days for inoculum production.

The *X. fragariae* strain NCPPB 1469 was isolated from *F. × ananassa* in the USA. The *X. fragariae* IV1A 349.9a strain was isolated from *F. × ananassa* in Benifayó, Valencia, Spain (López et al., 1985) and kindly provided by Dr. María Milagros López (IVIA, Valencia). Both strains were maintained in 30% glycerol at –80°C for long-term storage and grown on Wilbrink-N medium whose composition (per litre distilled water) was 10 g sucrose, 5 g protease peptone type 3, 0.5 g K₂HPO₄, 0.25 g MgSO₄, 0.25 g NaNO₃, and 15 g agar (Koike, 1965), and incubated at 24°C for three to five days for inoculum production.

**Testing the resistance of strawberry cultivars against *P. cactorum***

Prior to testing for resistance, the strawberry plants were inoculated with the *P. cactorum* isolates, and the isolates were then re-isolated, as recommended by Eikemo et al. (2000). Each *P. cactorum* inoculum was prepared by growing each re-isolated *P. cactorum* isolate on OMA at 24°C for ten days in the dark. The methods that were used for inducing sporangial and zoospore production were identical to those described by Redondo et al. (2009). Briefly, 5-mm mycelial plugs of each 10-day-old isolate were transferred to Petri dishes that contained 20 mL of sterile Petri’s mineral solution, and then maintained at 22°C for seven days under fluorescent lighting. Sterile Petri’s mineral solution was added during the production of the zoospores if necessary (Redondo et al., 2009). At the end of the incubations, the zoospore-containing solutions from the different isolates were each passed through a sterile glass cloth, and the filtrates from the different isolates were then pooled. The number of zoospores in the pooled filtrate was then counted using a cell counting chamber and a light microscope. The concentration of the zoospore-containing filtrate was then adjusted with Petri’s mineral solution so that its final concentration was 10⁴ zoospores mL⁻¹. The strawberry plants were inoculated with the isolates of *P. cactorum* by immersing their roots in the zoospore-containing filtrate for 30 min. Non-inoculated or control plants had their roots immersed in zoospore-free Petri’s mineral solution for 30 min. Twenty inoculated and non-inoculated plants of each strawberry cultivar, ‘Frau Mieze Schindler’, ‘Sieger’, ‘Deutsch Evern’, ‘Africa’, ‘Camarosa’, ‘Ventana’, ‘Aguedilla’, ‘Sabrosa’, ‘Senga Sengana’, and ‘Surprise Halles’, were used for resistance testing. After inoculation, the plants were transplanted to plastic pots containing a 1:1 (by volume) mix of sterile peat and perlite, and then grown in a greenhouse at 18 to 24°C and 60 to 80% RH for ten days under natural lighting.

Disease severity in each plant was then scored for presence of disease every 3 days for ten days on a scale from 0 to 4, where a score of (a) 0 was given to healthy plants (0 to 24%); (b) 1 was given to plants with apparent wilting of their leaves (25 to 49%); (c) 2 was given to plants with severe wilting symptoms (50 to 74%); (d) 3 was given to plants with general wilting and several dry leaves (75 to 90%); and (e) 4 was given to dead plants (100%). In addition, the pathogen was re-isolated from the crown and root tissues using a selective medium, P₅ARP (Jeffers & Martin, 1986). Two independent resistance assays using the zoospore-containing filtrate were performed.
Testing the resistance of strawberry cultivars against Verticillium wilt

These assays were done using previously described protocols in Redondo et al. (2009). In this investigation, one isolate of V. dahliae VER13, and one isolate of V. albo-atrum, 20103, were used as the inoculum, which was prepared by incubating the two isolates on PDA in Petri dishes at 20 ± 2°C for seven days in the dark. A conidial suspension of the two isolates was prepared by first scratching the plates to detach the conidia from the PDA, and then flooding the plates with 10 mL of sterile distilled water. The strawberry plants were inoculated with the conidia by immersing their roots in the conidial suspensions (10^7-10^8 conidia mL⁻¹) for 30 min. Non-inoculated or control plants had their roots immersed in sterile distilled water for 30 min. Twenty inoculated and non-inoculated plants of each strawberry cultivar, ‘Sieger’, ‘Deutsch Evern’, ‘Africa’, ‘Camarosa’, ‘Ventana’, ‘Aguedilla’, ‘Sabrosa’, ‘Carisma’, and ‘Pandora’, were used for resistance testing.

After inoculation, each strawberry plant was first placed into pots that contained one litre of sterile peat, and then incubated in growth chambers at 18 to 24°C and 60 to 80% RH under fluorescent lighting (100 µE m⁻² s⁻¹, 16-hour photoperiod) for 35 days. The strawberry plants were watered with tap water for the duration of the assay. Therefore, disease severity in each plant was scored for presence of disease weekly on a scale of 0 to 5, where a score of (a) 0 was given to healthy plants (0%); (b) 1 was given to plants whose lower leaves were yellowing (1 to 24%); (c) 2 was given to plants whose lower leaves were dead (25 to 49%); (d) 3 was given to plants whose upper leaves showed signs of wilting (50 to 74%); (e) 4 was given to plants with necrotic leaves and were sometimes stunted in stature (75 to 99%); and (f) 5 was given to dead plants (100%). At the end of 35-day observation period, the crowns of all plants that showed signs of disease were cut into two pieces, and then cultured in an incubator for 7 to 15 days at 20 ± 2°C in the dark in order to recover the pathogen. The pathogens were identified by observing fungal structures under a light microscope. Two independent resistance assays were performed.

Testing the resistance of strawberry cultivars against X. fragariae

Resistance to X. fragariae of the different cultivars was evaluated on micropropagated strawberry plants that were generated, as described in subsection “Testing the resistance of strawberry cultivars against P. cactorum”. Each bacterial strain was grown in Wilbrink-N medium (Koike, 1965), as described in subsection “Isolates”. The final optical density of the bacterial suspension was adjusted spectrophotometrically (SmartSpec™ Plus spectrophotometer, BioRad Laboratories Ltd., Ontario, Canada) to 0.1 at an optical density of 600 nm, which corresponded to approximately 10^9 colony-forming units mL⁻¹. The plants were inoculated by pressure infiltration of the abaxial surface of the leaflets using a previously described protocol (Maas et al., 2000). Three plants each of the cultivars ‘Sieger’, ‘Frau Mieze Schindler’, ‘Deutsch Evern’, ‘Africa’, ‘Camarosa’, ‘Ventana’, ‘Aguedilla’, and ‘Sabrosa’, were inoculated with each bacterial strain on three different leaves. Each leaf was inoculated at four different points, for a total of 12 inoculation points per plant. The control for this assay was three plants of each cultivar that were inoculated with sterile water at four different inoculation points on three different leaves. After inoculation, plants were grown in growth chambers at 18 to 24°C and 60 to 80% RH under fluorescent lighting (100 µE m⁻² s⁻¹, 16-h photoperiod) for three weeks. The inoculation sites were graded every week using a previously described scoring system (Maas et al., 2000): 0 - no reaction; 1 - water-soaked lesions at the inoculation site; 2 - central necrosis of the inoculation site; 3 - water-soaked lesions that extended beyond the inoculation site; 4 - necrosis that had spread beyond the inoculation site; 5 - extensive necrosis beyond the inoculation site. Two independent resistance assays to the two strains of X. fragariae were performed.

Data analysis

The disease severity of each plant caused by each pathogen in each experiment was plotted as a disease progress curve. The area under this disease progress curve (AUDPC) was calculated as described Campbell & Madden (1990) for each experiment and each pathogen using an Excel spreadsheet. Data were statistically analysed by analysis of variance. When the F-test was significant at p = 0.05, the means were compared by Student-Newman-Keul’s multiple range test (Snedecor & Cochram, 1980). All experiments were repeated twice. When there were not significant differences between the results of the repeated experiments,
the data from each experiment were pooled. When there was quantitative significant difference between the results of the repeated experiments, the data from each experiment were not pooled and only one of them was shown.

The cultivars were classified into four resistance categories for each pathogen using values of AUDPC in the following way: (a) resistant (R) was given to cultivar whose AUDPC was not significantly different to that observed in disease-resistant control cultivar; (b) tolerant (T) was given to cultivars whose AUDPC was significantly greater than that observed in disease-resistant control cultivar and significantly lesser than that observed in disease-susceptible control cultivar; (c) susceptible (S) was given to cultivar whose AUDPC was not significantly different to that observed in disease-susceptible control cultivar; (d) highly susceptible (HS) was given to cultivars whose AUDPC was significantly greater than that observed in disease-susceptible control cultivar.

Results

Resistance of strawberry cultivars against *P. cactorum*

Significant differences in resistance to *P. cactorum* among the ten strawberry cultivars were observed. *P. cactorum* was isolated and identified as the cause of crown rot in the diseased plants at the end of the assay.

The susceptible (‘Surprise Halles’) and the resistant (‘Senga Sengana’) control cultivars reacted as expected. Specifically, the disease severity in the crown rot-susceptible cultivar, ‘Surprise Halles’ displayed greater than 80% at the end of the assay (Fig. 1) and the AUDPC was large (803.8) (Table 2). In contrast, the crown rot-resistant cultivar, ‘Senga Sengana’ displayed a percentage of disease severity lesser than 50% at the end of the assay (Fig. 1) and the AUDPC was small (321.9) (Table 2).

When the differential resistance of the strawberry cultivars to *P. cactorum* was determined based on AUDPC, ‘Deutsch Evern’ and ‘Ventana’ were categorized as being highly susceptible (HS) to *P. cactorum* (Table 2). The remaining of the tested cultivars were categorized as being tolerant (T) to *P. cactorum* (Table 2).

Resistance of strawberry cultivars against *Verticillium wilt*

The disease severity of *Verticillium wilt* in each of the nine strawberry cultivars is displayed in Fig. 2. Significant differences in resistance to *Verticillium wilt* among the nine strawberry cultivars were observed. The susceptible (‘Carisma’) and the resistant (‘Pandora’) controls reacted as expected. The first symptoms of *Verticillium wilt* were observed in ‘Camarosa’, seven days after inoculation. Disease severity in ‘Camarosa’ was always the highest during the 35-day observation period (Fig. 2). The disease severity for ‘Africa’, ‘Aguedilla’, and ‘Carisma’ was between 15 and 25% and for ‘Deutsch Evern’, ‘Pandora’, ‘Sieger’, and ‘Ventana’ was the lowest (< 10%) the duration of the assay (Fig. 2). *V. dahliae* and *V. albo-atrum* were isolated and identified as the cause of *Verticillium wilt* from the diseased plants that were grown for 35 days under controlled conditions.

![Figure 1. Disease severity (%) incited by a mixture of four isolates of *Phytophthora cactorum* (CH971, CH972, CH1020 and CH1021) in ten different strawberry cultivars, Africa (○), Aguadilla (◼), Camarosa (▲), Sabrosa (Candonga®) (◊), Deutsch Evern (+), Frau Mieze Schindler (●), Senga Sengana (♦), Sieger (+), Surprise Halles (□), and Ventana (◊). Plants were grown in pots containing a 1:1 (v:v) mix of sterile peat and perlite in a greenhouse at 18 to 24°C and 60 to 80% relative humidity under natural light for ten days. Evaluations of disease severity were done at 4, 7 and 10 days. Disease severity was scored as 100% in the dead plants. Values are the mean of 20 replicates.](https://example.com/figure1.png)
When the differential resistance of the strawberry cultivars against Verticillium wilt was determined based on AUDPC, the most susceptible (S) cultivars were ‘Africa’, ‘Aguedilla’ and ‘Camarosa’ (Table 2). Of the nine tested cultivars, only ‘Sabrosa’ was categorized as being resistant (R) (Table 2). The remaining of the tested cultivars was categorized as being tolerant (T) cultivars to Verticillium wilt (Table 2).

### Resistance of strawberry cultivars against X. fragariae

Fig. 3 describes the disease progression curves for angular leaf spot disease caused by the X. fragariae strains, IVIA 349.94a (Fig. 3A) and NCPPB 1469 (Fig. 3B), in the eight tested strawberry cultivars. Disease symptoms caused by X. fragariae strain IVIA 349.94a were first observed in ‘Deutsch Evern’, ‘Camarosa’, and ‘Sabrosa’, seven days after their inoculation (Fig. 3A). For X. fragariae NCPPB 1469, all cultivars, except ‘Sieger’, showed disease symptoms,
seven days after their inoculation (Fig. 3B). Significant differences in the AUDPCs of angular leaf spot disease caused by the two strains of *X. fragariae* were found (Table 2). ‘Sieger’, ‘Aguedilla’, and ‘Africa’ were categorized as being resistant (R) to *X. fragariae* strain IVIA 349.9a. One historical cultivar, ‘Sieger’, and one commercial cultivar, ‘Ventana’, were categorized as being resistant (R) to *X. fragariae* strain NCPPB 1469 (Table 2). Disease susceptibility to the two bacterial strains was the same for ‘Camarosa’, ‘Sabrosa’, ‘Frau Mieze Schindler’, and ‘Deutsch Evern’ when they were compared to susceptible control (‘Camarosa’) and were categorized as being susceptible (S) (Table 2).

**Discussion**

In this investigation, the differential resistance of various strawberry cultivars to three diseases that cause serious losses of strawberry crops in Spain was determined under greenhouse conditions for *P. cactorum* and under growth chamber conditions for Verticillium wilt and *X. fragariae*. None of the strawberry cultivars that underwent resistance testing against *P. cactorum*, *V. dahliae* and *V. albo-atrum*, and *X. fragariae* were resistant to each of the three tested pathogens. This failure to find a universal disease-resistant cultivar may be related to the limited size of the strawberry collection that we tested in this study. However, we found that (i) ‘Sabrosa’ (Candonga®) was resistant cultivar to both *P. cactorum* and Verticillium wilt, (ii) ‘Frau Mieze Schindler’, ‘Sieger’, ‘Africa’, ‘Camarosa’, and ‘Aguedilla’ were tolerant cultivars to *P. cactorum*, and (iii) ‘Sieger’ was resistant to *X. fragariae*.

Strawberry production in Spain involves a sequence of nursery propagation steps of which one step is pre-plant soil fumigation. In response to environmental and health concerns about the widespread use of pesticides, there is now considerable pressure to find alternative control strategies for crop diseases in integrated pest management (Reuveni, 1995). Plant resistance is the most powerful control measure against diseases and pests (Horiuchi et al., 1990; Eikemo et al., 2003). However, there are no strawberry cultivars with acceptable fruit quality and shelf life that are resistant to the diseases that were investigated in this study (Pertot et al., 2008). Hence, the development of *Verticillium*, *Phytophthora*, and *Xanthomonas* resistant strawberry cultivars has now become a major breeding goal in Spain because of the European ban on the use of methyl bromide as a soil fumigant. Furthermore, commercially available alternatives to methyl bromide, such as chloropicrin or 1,3-dichloropropene (1,3-D) (De Cal et al., 2004; López-Medina et al., 2007; López-Aranda et al., 2009), either used alone or combined, will also be banned in the near future as a result of these European Union regulations (Garcia-Mendez et al., 2008).

Another important task in disease control on strawberry is to identify sources of resistance with the purpose of using them in the creation of new commercial
cultivars. In this investigation, the four historical or pioneer cultivars, ‘Frau Mieze Schindler’, ‘Sieger’, ‘Deutsch Evern’, and ‘Africa’ were selected because they were commonly used in earlier European breeding programs. The four commercial cultivars that were used in the present study were selected because of their importance for Spanish strawberry industry: ‘Camarosa’ and ‘Ventana’ are the most produced cultivars, and ‘Aguedilla’ and ‘Sabrosa’ were developed for use in Spanish breeding programs by the Spanish Ministry of Agriculture and Planasa SA, respectively (López-Aranda et al., 2005; Maxwell, 2010). According to our results, ‘Sabrosa’, ‘Frau Mieze Schindler’, ‘Sieger’, ‘Africa’, ‘Camarosa’, and ‘Aguedilla’ could be used as parents to increase the average resistance in the resulting offspring for newly-developed commercial strawberry cultivars against P. cactorum. In addition, ‘Sabrosa’ could also be used as a parent to increase the average resistance in the resulting offspring for newly-developed commercial strawberry cultivars against Verticillium wilt, and ‘Sieger’ could also be used as a parent to increase the average resistance in the resulting offspring for newly-developed commercial strawberry cultivars against X. fragariae.

In this investigation was found that ‘Sabrosa’ was resistant to P. cactorum. We also found that the level of resistance against P. cactorum of three historical cultivars, ‘Frau Mieze Schindler’, ‘Sieger’, and ‘Africa’, and two commercial cultivars, ‘Camarosa’ and ‘Aguedilla’, was also high, although lower than that of the control, ‘Senga Sengana’. Overall, the resistance responses to crown rot of the cultivars were comparable to those that have been previously reported (Pitrat & Risser, 1977; Bell et al., 1997). Although some European strawberry breeding programs select for crown rot resistance when developing new cultivars (Eikemo et al., 2003), information on the genetic control of strawberry resistance to the crown rot pathotype of P. cactorum is lacking. Finally, the degree of resistance against crown rot also seems to be dependent on the plant’s age, the season of the year, and the physiological state of the plant (Pitrat & Risser, 1977; Eikemo et al., 2000).

In this study, ‘Sabrosa’ was classified as Verticillium-resistant cultivar because it AUDPC was not significantly different to that of ‘Pandora’, the Verticillium wilt-resistant cultivar control. Redondo et al. (2009) previously reported ‘Aguedilla’ resulted more resistant to V. dahliae than ‘Camarosa’. Here, the results showed both of cultivars as being susceptible to a mixture of V. dahliae and V. albo-atrum. Other researchers have also reported varietal differences for resistance to Verticillium wilt (Ebihara et al., 2010). In previous studies, Shaw et al. (1997) suggested large differences among the plants treated with different conidial concentrations, so genotypes originally classified as intermediate in resistance performed more like susceptible types at higher conidial concentration (10^6 conidia mL⁻¹). In this work, the conidial concentration was 10⁷-10⁸ conidia mL⁻¹.

The results showed differences in the level of the resistance of cultivars against the two strains of X. fragariae. Specifically, ‘Sieger’ was classified as being resistant (R) against both strains of X. fragariae, whereas ‘Aguedilla’ and ‘Africa’ were classified as being resistant against X. fragariae strain IVIA 349.94a and ‘Ventana’ was classified as being resistant against X. fragariae strain NCPPB 1469. The strain NCPPB 1469 of X. fragariae was more aggressive than the strain IVIA 349.94a of X. fragariae. Resistance to X. fragariae has been reported in other Fragaria species. Kennedy & King (1962a, b) found that the disease severity of the diploid F. vesca cultivar ‘Alpine’ is low when compared to that of other Fragaria spp., when determined under greenhouse and field conditions. Hazel (1981) reported that of the several Fragaria spp. that were evaluated by inoculation, only F. moschata Duch. are disease-resistant and some F. virginiana clones displayed moderate degrees of tolerance. More recently, Maas et al. (2000, 2002) reported that two genotypes, a native F. virginiana and an F. virginiana × F. × ananassa hybrid, were highly resistant to the four pathogenic strains of X. fragariae that were identified by Pooler et al. (1996). These data suggest that resistance to angular leaf spot can be incorporated into cultivars by recurrent selection (Maas et al., 2000).

‘Sabrosa’ is the most important strawberry cultivar in Spain, and in this study this cultivar has been categorized as being susceptible to both strains of X. fragariae. ‘Camarosa’ is used in Spanish production areas because of its good agronomical characteristics, although this cultivar was highly susceptible to the strawberry pathogens that were assayed in this study. ‘Aguedilla’ was the first strawberry cultivar produced in the research project, INIA CC01-0008-C3 with increased adaptability to the environmental conditions of Huelva and other Spanish production areas. In the present work, this cultivar was categorized as being susceptible to Verticillium wilt, resistant to P. cactorum and X. fragariae IVIA349.94a, and tolerant to X. fragariae NCPPB 1469.
Therefore, we emphasize the importance of considering both the agronomical characteristics of strawberries and the resistance of strawberries to their main pathogens in commercial strawberry breeding programmes. These studies will require the use of naturally infected soils in order to determine whether the observed resistance of the strawberry cultivars that we found in this investigation is adequate for their commercial production.

In conclusion, the role of susceptible cultivars in the spread of diseases underlies the importance of screening for disease resistance before the introduction and use of strawberry cultivars in commercial strawberry production. In addition, the planting of disease-resistant strawberry cultivars will reduce the number of chemical treatments in the commercial production of strawberries.

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