

Mycelial compatibility groups and pathogenic diversity in *Sclerotium rolfii* populations from sugar beet crops in Mediterranean-type climate regions

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The population structure of *Sclerotium rolfii* from autumn-sown sugar beet crops in Mediterranean-type climate regions of Chile, Italy, Portugal and Spain was determined by analyses of mycelial compatibility groups (MCGs) and pathogenicity to 11 economically important plant species. Twelve MCGs (i–xii) were identified among 459 *S. rolfii* isolates. MCG iii was the most prevalent group in all countries except Italy. MCG i, the most abundant group (64.7% of isolates) was identified in Portugal and Spain. The remaining MCGs were restricted to various regions within one country (ii, vi, ix) or different countries (v), or to specific localities (iv, vii, viii, x, xi, xii). MCGs iv, vii and x each comprised one isolate. Fields extensively sampled in southern Spain were infected with one to three MCGs. Plant species differed in susceptibility to MCG tester isolates with a MCG by species interaction. Cluster analyses allowed selection into five MCG groupings and grouped plant species into species-groups 1 (broccoli, chickpea, sunflower, tomato) and 2 (cotton, pepper, sugar beet, watermelon). MCG groupings 1 (i, ix), 2 (ii, iii, vi, viii) and 5 (x, xii) were moderately virulent to species-group 1 and mildly virulent to species-group 2. MCG groupings 3 (iv, v, xi) and 4 (vii) were mildly virulent to both species-groups. Across MCG groups, species were rated highly susceptible (chickpea, sunflower), susceptible (cotton, pepper, tomato, watermelon), moderately resistant (broccoli, melon, sugar beet) and resistant (corn, wheat). Establishing the MCG population structure and virulence variability among *S. rolfii* isolates should help in the management of sclerotium root rot diseases.

Keywords: *Beta vulgaris*, cluster analysis, host range, MCG

Introduction

Sclerotium rolfii is the mycelial stage of the basidiomycete *Athelia rolfii*, a worldwide soilborne plant pathogenic fungus that lacks a conidial stage. The host range of *S. rolfii* comprises over 500 plant species, mostly dicotyledons, but also some monocotyledons (Harlton *et al.*, 1995; Okabe *et al.*, 1998; Cilliers *et al.*, 2000). The main disease symptoms caused by this pathogen include crown and root rot, stem canker or damping-off. The resulting disease is referred to as southern blight, southern stem rot, or sclerotium root rot (Aycock, 1966; Punja, 1985). *S. rolfii* produces abundant coarse, white mycelia on infected host tissues and forms sclerotia (Punja, 1985). The fungus overwinters in soil by means of sclerotia, as well as by mycelia in infected plants or infested plant debris (Punja, 1985). It can form a sexual stage, but this

rarely occurs in nature and its role in the life cycle of the fungus is unknown (Nalim *et al.*, 1995).

Sclerotium rolfii has been reported from nearly every country between northern and southern latitudes of 38°. However, disease epidemics are most severe in warm temperate and subtropical regions that favour sclerotial germination, mycelial growth, infection and subsequent disease development (Aycock, 1966; Punja, 1985). These climatic conditions occur in the Mediterranean basin, southern California and Chile, where outbreaks of *S. rolfii* often occur on autumn-sown sugar beet (*Beta vulgaris*) crops.

Disease surveys conducted in Andalusia, southern Spain, in 2004 showed that sclerotium root rot of sugar beet is widespread in autumn-sown crops in the southernmost part of the region, causing 5–80% yield loss (AIM-CRA, 2005). Similarly, the disease severely affects sugar beet crops in central and southern Portugal (M. Paim, Associação para Desenvolvimento da Beterraba, Portugal, personal communication) and the central area of Chile (regions VI to VII) (R. Paillalef-Monnrad, IANSAGRO, Chile, personal communication).

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As a cosmopolitan soilborne plant pathogen, knowledge of its genetic diversity and distribution in infested areas would be useful for the management of sclerotium root rot diseases. Characterization of fungal populations into mycelial compatibility groups (MCGs) has been widely used for indirectly assessing the genetic variability among isolates of fungal plant pathogens (Leslie, 1993). MCGs play an important role both in defining field populations of fungi and facilitating genetic exchange in fungal species. This is particularly relevant for fungi for which sexual reproduction has a minimal impact on the disease cycle (Kohn *et al.*, 1991; Leslie, 1993) because MCGs help to preserve the identity of genetically dissimilar mycelia and restrict the exchange of cytoplasm, genetic material and extra-chromosomal elements (Caten, 1972). Mycelial incompatibility results in separate and distinct gene pools, which may differ in ecological, physiological and pathological traits. Mycelial compatibility studies have been used to estimate genetic variability or relatedness within many fungal species of the Basidiomycota such as *Sclerotium cepivorum* (Earnshaw *et al.*, 2000) and *Rhizoctonia solani* (Carling *et al.*, 2002; Ciampi *et al.*, 2008), as well as ascomycetes such as *Fusarium* spp. (Puhalla, 1985; Elias *et al.*, 1993; Chulze *et al.*, 2000; Bayraktar *et al.*, 2010), *Cryphonectria* spp. (van Heerden & Wingfield, 2001), *Verticillium dahliae* (Korolev *et al.*, 2000; Collado-Romero *et al.*, 2006) and *Sclerotinia sclerotiorum* (Kohn *et al.*, 1991).

Mycelial compatibility was demonstrated to occur between field isolates of *S. rolfsii*, and has been used to designate MCGs (Punja & Grogan, 1983) as well as to assess the genetic variability among *S. rolfsii* populations (Punja & Grogan, 1983; Harlton *et al.*, 1995; Nalim *et al.*, 1995; Cilliers *et al.*, 2000, 2002; Okabe & Matsumoto, 2000; Almeida *et al.*, 2001; Punja & Sun, 2001; Sarma *et al.*, 2002; Adandonon *et al.*, 2005). These studies suggested that MCGs in *S. rolfsii* can be associated with either the host source or geographical area of isolates, but also a single MCG can comprise isolates from diverse geographical origins and host sources. The variability of mycelial compatibility among *S. rolfsii* populations occurring in particular geographic regions or specific crops has also been demonstrated. Studies in Brazil (Almeida *et al.*, 2001), India (Sarma *et al.*, 2002) and South Africa (Cilliers *et al.*, 2002) indicated that a considerable genetic variability existed among *S. rolfsii* isolates from various hosts and localities without clear correlation between the MCG to which they belonged and their host source or geographic origin (Almeida *et al.*, 2001; Sarma *et al.*, 2002), although a certain relationship between MCGs and host source of isolates was suggested for *S. rolfsii* populations in South Africa (Cilliers *et al.*, 2002). On the other hand, in peanut, wide variation was reported among isolates within a field in Texas, USA (Nalim *et al.*, 1995) and Japan (Okabe & Matsumoto, 2000), with a single peanut field harbouring up to three (Okabe & Matsumoto, 2000) or five (Nalim *et al.*, 1995) MCGs.

Control of sclerotium root rot diseases is difficult because of the extensive mycelial growth, persistence in soil, genetic variability of populations and wide host range of *S. rolfsii*. In sugar beet, no resistant cultivars are available and attempts to control the disease with fungicides or biological control agents have been unsuccessful (Lal *et al.*, 1997). However, a degree of resistance to *S. rolfsii* was found in some cultivars of cowpea (Fery & Dukes, 2002), alfalfa (Pratt & Rowe, 2002), peanut (Branch & Breneman, 1999), pepper, sweet potato (Dukes *et al.*, 1983) and chickpea (Akram *et al.*, 2008). Reports from California and India indicate that the pathogen inoculum density can be effectively reduced by rotations of sugar beet with crops that are slightly susceptible to *S. rolfsii*, such as alfalfa, asparagus, barley, corn and wheat (Schneider & Whitney, 1996). This same strategy was also effective with rotations of susceptible carrot with sweet potato or buckwheat (Jenkins & Averre, 1986) and peanut with bahiagrass, corn, cotton (Johnson *et al.*, 1999) or wheat (Minton *et al.*, 1991). Knowledge about the genetic and virulence diversity in local populations of *S. rolfsii* associated with different crops is a key component for the management of sclerotium root rot diseases, particularly through the use of host resistance and crop rotation in a given region. In addition, knowledge of the host range of *S. rolfsii* populations present in a given area is essential for recommending suitable crops as an alternative to sugar beet production. Unfortunately, information of that nature is lacking in areas of Mediterranean-type climate. Furthermore, the sugar beet production area in the Mediterranean Basin has steadily decreased in Italy, Portugal and southern Spain during the last few years, being replaced by vegetable crops that can be potentially affected by sclerotium root rot.

In the present study, a large collection of *S. rolfsii* isolates from sclerotium root rot-affected sugar beet crops in four countries with a Mediterranean-type climate and eight intensively sampled fields in southern Spain were used to: (i) assess *S. rolfsii* MCG diversity and geographical distribution; (ii) determine within-field diversity and prevalence of *S. rolfsii* MCGs in sugar beet fields of southern Spain; (iii) determine any correlation that might exist between MCGs of *S. rolfsii* isolates from sugar beet and their pathogenicity and virulence to 11 agricultural crops; and (iv) determine the pathogenic variability within MCGs on three selected susceptible hosts.

Materials and methods

Sampling and isolation of the pathogen

Two sampling strategies were used to account for variability of *S. rolfsii* on different spatial scales. Sugar beet field plots located in southern Spain were intensively sampled to determine variability at field level. For that purpose, an area of c. 0.06 ha in each field plot was divided into a regular grid of 80 (4 × 2-m) quadrants, and one affected sugar beet root was sampled from each quad-

rant. For the second spatial scale, whole fields in Chile, Italy and Portugal were arbitrarily selected to determine pathogen variability among fields with at least four arbitrarily chosen affected roots sampled from each field. In all cases, the sampled area was representative of the sugar beet fields affected by sclerotium root rot in each country.

For isolation of *S. rolfsii*, root tissues with symptoms were washed under running tap water, surface-sterilized in 0.5% NaOCl for 1 min and blotted dry between sterile filter papers. Pieces of surface-sterilized tissues (2–4 mm²) were plated onto potato dextrose agar (PDA, Difco Laboratories) amended with 0.25 mL 85% lactic acid and incubated at 25 ± 1°C in the dark for 2–5 days. Pure cultures forming sclerotia were obtained from each root sample. Single-sclerotia cultures obtained from pure cultures were incubated to form abundant sclerotia and were allowed to dry at room temperature. Sclerotia from dry cultures were collected, dried and stored in paper bags at room temperature until use.

Isolate collection

A total of 459 *S. rolfsii* isolates were obtained from sclerotium-root-rot-affected sugar beet crops sampled at a total of 18 localities from Chile (four localities, 22 isolates), Italy (one locality, one isolate), Portugal (five localities, 63 isolates) and Spain (eight localities, 373 isolates) from 2004 to 2007 (Table 1).

Determination of mycelial compatibility groups

A single sclerotium from each of the *S. rolfsii* isolates was placed on a PDA plate and incubated in the dark at

25 ± 1°C for 5 days. Then, small plugs from the inner colony were transferred to modified Patterson's medium (MPM) in new plates (90-mm diameter) and incubated for 5 days in the same conditions. This MPM allows improved assessment of mycelial interactions among *S. rolfsii* isolates (E. Remesal & J. A. Navas-Cortés, unpublished results). Mycelial discs from the edges of actively growing MPM colonies of isolates were paired on MPM plates. Three isolates were paired on a 90-mm-diameter plate by placing discs 50 mm apart and incubating at 25 ± 1°C in the dark. There were four replicated plates per pairing. All isolates were paired with themselves to ensure self-compatibility. All pairings were repeated twice. Pairings were examined macroscopically after 5 and 10 days of incubation for the presence of an antagonism zone in the region of mycelial contact (Punja & Grogan, 1983) and the presence of a coloured red line in the reaction zone between the colonies (Fig. 1a,b). Mycelial compatibility reactions showed no antagonism and were distinguished by merging colonies with no detectable coloured red line between the interacting colonies (Fig. 1c). MCGs were identified based on data from compatible reactions among tested isolates. Because of the high number of *S. rolfsii* isolates and difficulty of pairing them in all possible combinations, MCGs were defined first at field plot level, pairing isolates from a plot in all possible combinations. Then, one tester isolate was arbitrarily selected from each of the identified MCGs for further analyses. Representative tester isolates from all the identified MCGs in each field plot were paired in all possible combinations (Nalim *et al.*, 1995).

The diversity of MCGs in each country and/or field plot in the study was estimated by the Shannon–Weaver

Table 1 Geographic origin and mycelial compatibility group (MCG) of 459 *Sclerotium rolfsii* isolates infecting sugar beet used in this study

Country	Geographic origin		No. of isolates	MCG (no. of isolates) ^a	Isolate code
	Province	Locality			
Chile	VI	Colchagua	1	vii (1)	Sr1
	VII	Curicó	2	iii (2)	Sr2, Sr3
		Linares	8	viii (3), ix (1), xii (4)	viii: Sr4–Sr6; ix: Sr7; xii: Sr8–Sr11
	VIII	Chillán	11	iii (1), ix (9), x (1)	iii: Sr12; ix: Sr13–21; x: Sr22
Spain	Cádiz	Arcos Ftra.	48	i (48)	Sr23–Sr70
		Jerez Ftra.	50	i (43), iii (7)	i: Sr71–Sr113; iii: Sr114–Sr120
		Vejer Ftra.	35	i (19), v (16)	i: Sr121–Sr139; v: Sr140–Sr155
	Córdoba	Fuente Palmera	37	i (37)	Sr156–Sr192
		Posadas	31	i (4), ii (17), iii (10)	i: Sr193–Sr196; ii: Sr197–Sr213; iii: Sr214–Sr223
	Huelva	Paterna Campo	64	i (62), xi (2)	i: Sr224–Sr285; xi: Sr286, Sr287
	Seville	Lebrija	28	i (3), iii (25)	i: Sr288–Sr290; iii: Sr291–Sr315
		Los Palacios	80	i (79), ii (1)	i: Sr316–Sr394; ii: Sr395
Italy	Bari	Bari	1	iv (1)	iv: Sr396
Portugal	Lisbon	Vila Franca de Xira	26	i (2), iii (5), v (10), vi (8)	i: Sr406, Sr407; iii: Sr408–Sr413; v: Sr414–Sr423; vi: Sr424–Sr431
		Portalegre	Avis	12	iii (12)
		Benavila	8	iii (8)	Sr444–Sr451
		Elvas	10	iii (10)	Sr452–Sr461
	Santarém	Coruche	7	iii (6), vi (1)	iii: Sr462–Sr467; vi: Sr468

^aMCG typing was done in this study.

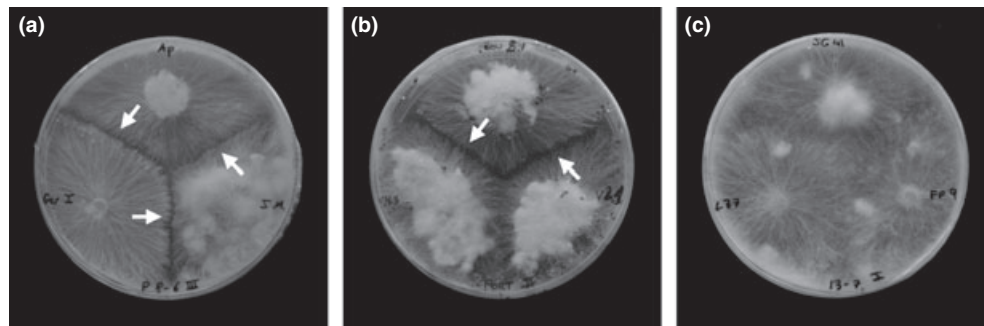


Figure 1 Mycelial interactions between isolates of *Sclerotium rolfsii* tested on modified Patterson's medium: (a) and (b) incompatible reactions [different mycelial compatibility groups (MCGs)]; (c) compatible reactions (same MCG). Arrow indicates incompatible reaction zone.

diversity index: $H' = -\sum p_i \times \ln p_i$, where p_i is the frequency of the i th MCG (Shannon & Weaver, 1963).

Pathogenic diversity

Plant species

Pathogenicity and virulence of *S. rolfsii* isolates were determined on 11 cultivated plant species of eight botanical families, including Amaranthaceae: sugar beet (*Beta vulgaris* cv. Markus); Asteraceae: sunflower (*Helianthus annuus* cv. SH-25); Brassicaceae: broccoli (*Brassica oleracea* var. *italica* cv. Romanesco); Cucurbitaceae: melon (*Cucumis melo* L. var. *cantalupensis* cv. Piel de Sapo) and watermelon (*Citrullus lanatus* cv. Sugar Baby); Fabaceae: chickpea (*Cicer arietinum* cv. P-2245); Malvaceae: cotton (*Gossypium hirsutum* cv. Theka); Poaceae: corn (*Zea mays* cv. Girona) and bread wheat (*Triticum aestivum* cv. Pinzón); and Solanaceae: pepper (*Capsicum annuum* cv. Cristal) and tomato (*Solanum lycopersicum* cv. Early-Pack). Seeds were obtained from commercial seed companies. These plant species were selected as representative of the host range of the pathogen as well as of mono- and dicotyledonous species that could be an alternative to sugar beet crops in Mediterranean-type climate areas.

Experimental design

Three experiments (I–III) were carried out in a growth chamber to determine the reaction of plant species to *S. rolfsii* isolates. Experiments I and II were first performed to determine reaction of the 11 plant species to inoculation with 12 tester isolates representative of each of the 12 identified MCGs. Because of space limitations in the growth chamber, experiments I and II consisted of six and five plant species, respectively. To test for reproducibility of reactions to the 12 MCG tester isolates, sunflower and tomato were common plant species in the two experiments.

In experiment III, a total of 23 *S. rolfsii* isolates representative of five of the more abundant MCGs identified, which comprised 5% of the total number of isolates in the study, were used to assess within-MCG variation in pathogenicity and virulence. Isolates tested included 12 isolates for MCG i, four for MCG iii, three for MCG v and two for each of MCG vi and MCG xii. For this experi-

ment, sunflower, tomato and watermelon were selected as susceptible hosts based on the consistency of their reaction to tester MCG isolates in experiments I and II.

In all three experiments, there were eight replications (eight pots, one plant per pot) of each treatment combination of plant species and *S. rolfsii* isolate. Each experiment was repeated partially and preliminary analyses of common treatments indicated no significant differences ($P > 0.05$) among experiments (see below); consequently, data were pooled for further analyses.

For each plant species, seeds were surface-sterilized in 2% (v/v) NaOCl for 30 s, and germinated on autoclaved layers of filter paper in moist chambers at $25 \pm 1^\circ\text{C}$ in the dark for 2 days. Germinated seeds, selected for uniformity (length of radicle = 0.5–1 cm), were sown in sterile plastic pots filled with an autoclaved (121°C , 1 h, twice, on two consecutive days) soil mixture (silt:peat, 2:1).

Sclerotium rolfsii inoculum, plant inoculation and growth conditions

Inoculum for experiments consisted of infested oat (*Avena sativa*) seeds. One hundred grams of seeds were moistened in 50 mL water and autoclaved in 1-L flasks for 60 min at 121°C . The sterilized seeds were then infested using 10 mycelial discs, 5 mm in diameter, from the growing edge of a *S. rolfsii* colony on PDA and incubated at $25 \pm 1^\circ\text{C}$ in the dark for 2 weeks.

Plants were inoculated at the two-leaf stage. For inoculation, two selected oat seeds heavily and homogeneously colonized with mycelia of an isolate were buried at a depth of 0.5 cm and a distance of 0.5–1 cm from the plant stem. Plants in pots with non-infested seeds served as controls. Plants were incubated in a walk-in growth chamber adjusted to $28 \pm 1^\circ\text{C}$ with a 14-h photoperiod of fluorescent light of $360 \mu\text{E m}^{-2} \text{s}^{-1}$ and 60–90% relative humidity for 18 days. Plants were watered as needed to maintain field capacity and fertilized weekly with 100 mL 0.1% hydro-sol fertilizer solution (Haifa Chemicals, Ltd, 20-5-32 N-P-K + micronutrients).

Disease assessment and data analyses

Disease reaction was characterized by the incidence of dead plants, established as the number of dead plants at the end of the experiment, 18 days after inocula-

tion. *S. rolfsii* isolates were classified in terms of virulence based on the disease incidence (DI) that they induced: highly virulent ($DI \geq 66\%$), moderately virulent ($66 < DI \geq 33\%$), mildly virulent ($33 < DI > 0\%$) and non-pathogenic ($DI = 0\%$). Similarly, plant species were classified according to DI values as: highly susceptible ($DI \geq 66\%$), susceptible ($66 < DI \geq 33\%$), moderately resistant ($33 < DI > 0\%$) or resistant ($DI = 0\%$).

The number of dead plants per treatment was used for statistical analyses. Data were analysed with the GENMOD procedure using the binomial distribution and the logit as link function in SAS (version 9.2, SAS Institute Inc.) (Agresti, 2002). Least squares means were computed for all response variables to allow multiple comparisons between/among treatments by using the SAS macro MULT (<https://www.uni-hohenheim.de/bioinformatik/beratung/toolsmacros/sasmacros/mult.sas>) (Piepho, 2004). Linear single-degree-of-freedom contrasts were computed to test the effect of selected experimental treatment combinations. The stability of disease reactions in the plant-species by MCG combination was explored by cluster analyses of the number of dead plants to identify associated groupings of MCGs and plant species in two separate analyses. To find functional groupings of correlated MCGs or plant species, respectively, an agglomerative clustering based on the Spearman correlation matrix was performed among MCGs and among plant species using the Ward clustering method. For MCGs, the optimum number of clusters was estimated on the basis of the average silhouette width according to the Mantel statistic. Thus, the number of clusters in which the within-group mean intensity of the link of the objects (MCG) to their groups was highest (i.e. with the largest average silhouette width) indicated the optimum cluster number. A new dendrogram was then produced representing the identified groupings of MCGs (Borcard *et al.*, 2011). All calculations for cluster analyses were made using R version 2.13.0 (R Foundation for Statistical Computing, <http://www.R-project.org/>) with the CLUSTER (Maechler *et al.*, 2005) and VEGAN (Oksanen *et al.*, 2011) packages.

The degree of virulence variation within each of five MCGs tested in experiment III was estimated by a separate likelihood analysis using the GENMOD procedure of SAS, as described above, followed by multiple comparison of mean number of dead plants induced by the *S. rolfsii* isolates tested. In addition, the disease reaction induced by the tester isolate of each of the MCGs was compared with that of each of the rest of isolates within the same MCG using linear single-degree-of-freedom contrast at $P < 0.05$.

Homogeneity of disease reactions across experiments

The consistency of results between experiments I and II was tested by a preliminary analysis in which the number of dead plants for treatments common to both experiments (i.e. the plant species sunflower and tomato inoculated with each of 12 MCG-representative isolates) were analysed with the GENMOD procedure of SAS as described

above. The likelihood ratio test indicated no significant effects of the two experimental runs ($\chi^2 = 0.35$, $P = 0.5536$), or their interaction with MCG isolate ($\chi^2 = 9.22$, $P = 0.6017$), plant species ($\chi^2 = 2.21$, $P = 0.1367$), or both factors ($\chi^2 = 13.14$, $P = 0.2844$).

Similarly, homogeneity of disease reactions in experiments I, II and III was tested with the same methodology as indicated above using the number of dead plants of common treatments in all three experiments (i.e. the plant species sunflower and tomato inoculated with isolates representative of each of MCGs i, iii, v, vi and xii included in experiment III). The likelihood ratio test indicated no significant effects of the three experimental runs ($\chi^2 = 0.27$, $P = 0.8740$), or their interactions with MCG isolate ($\chi^2 = 4.25$, $P = 0.8336$), plant species ($\chi^2 = 2.05$, $P = 0.3595$), or both factors ($\chi^2 = 11.53$, $P = 0.1732$).

Results

Mycelial compatibility groups

Twelve MCGs were identified among the 459 isolates of *S. rolfsii* tested in the study. MCGs were designated in roman numerals from i to xii. The MCGs of isolates and their geographical locations are shown in Table 1. Mycelial compatibility among isolates of a MCG was characterized by their mycelia intermingling at the zone of interaction without aversion (Fig. 1c). Conversely, isolates assigned to different MCGs showed aversion at the interaction zone, together with thinned mycelium and formation of a red reaction line (Fig. 1a,b). All 459 isolates were self-compatible, showing a reaction similar to that described for compatible isolates. Replicated experiments and replicated pairings within each experiment produced identical results.

Distribution and frequency of the 12 identified MCGs differed among sampling regions and countries. MCGs i and iii were the most prevalent among sampled isolates, representing 64.71 and 18.95% of the isolates, respectively. MCG i was identified only in the Iberian Peninsula, being present in all eight locations sampled in southern Spain and one location sampled in central Portugal (Table 1; Fig. 2). MCG iii was less abundant than MCG i but was the most widely distributed MCG, being identified in 10 of the 18 sampled locations from all sampled countries [Chile (two), Portugal (five) and southern Spain (three)] except Italy (Table 1; Fig. 2). MCG v comprised 5.66% of the isolates and was restricted to two distant field plots located in Vejer de la Frontera (Cádiz province) and Vila Franca de Xira (Lisbon province) in southern Spain and central Portugal, respectively. MCG ii comprised 3.92% of the isolates from two locations in Córdoba and Seville provinces in southern Spain. The remaining eight MCGs accounted for 6.75% of the isolates and were locally distributed. MCGs ix, vi, xii, viii and xi comprised 10, nine, four, three and two isolates, respectively; among these five multimember MCGs, MCGs viii, ix and xii were identified only in three loca-

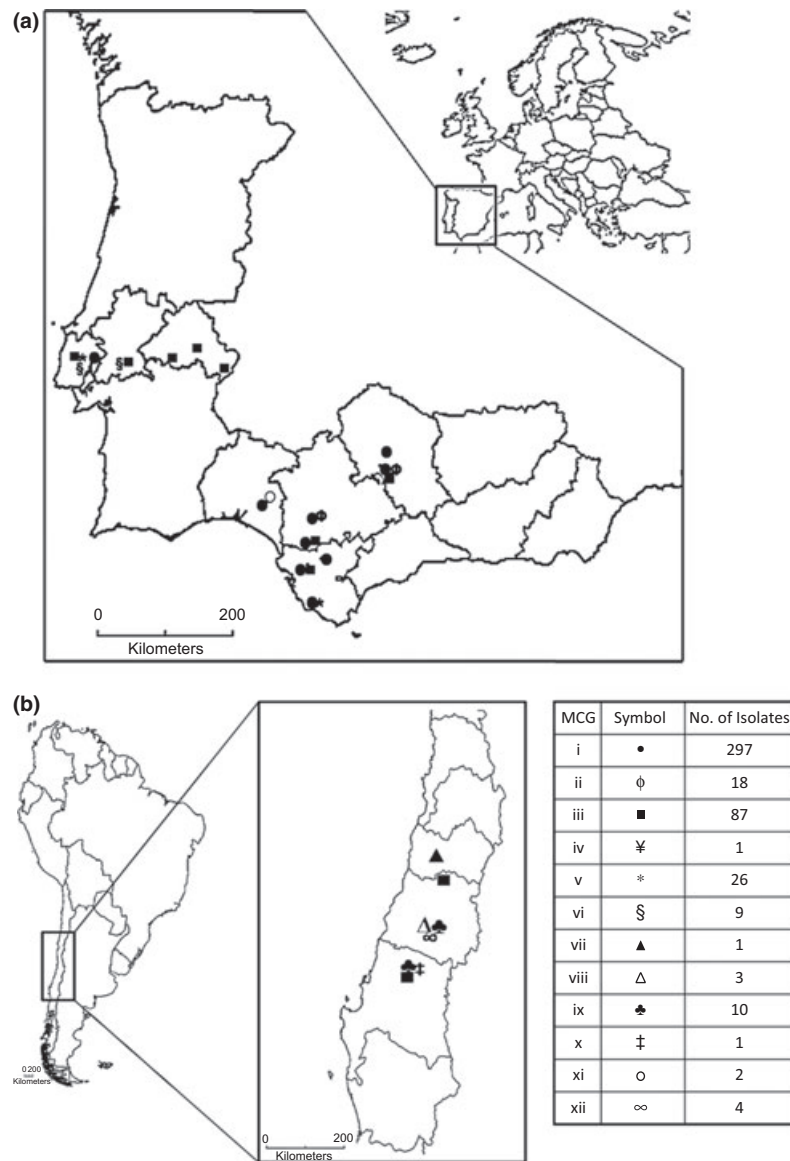


Figure 2 Geographic distribution of mycelial compatibility groups (MCGs) identified among isolates of *Sclerotium rolfsii* infecting sugar beet in (a) Iberian Peninsula (Spain and Portugal); and (b) Chile.

tions in Chile, MCG vi in two locations in Portugal, and MCG xi in one location in Spain. MCGs iv, vii and x each consisted of a single isolate, identified in Bari (southern Italy), Colchagua (Chile) and Chillán (Chile), respectively.

Regarding sampled countries, six, five and four MCGs were identified in Chile (MCGs iii, vii, viii, ix, x and xii), Spain (MCGs i, ii, iii, v and xi) and Portugal (MCGs i, iii, v and vi), respectively. The isolate sampled in southern Italy was assigned to MCG iv and occurred in this location only. Accordingly, the highest Shannon–Weaver H' index of diversity, 1.49, was estimated in Chile, decreasing to 0.95 and 0.74 in Portugal and Spain, respectively. In southern Spain, MCG i was present in all locations

sampled, whereas MCGs iii and v were identified in Cádiz, in the southwest of the region, MCG xi was found only at Huelva in the northwest of the region, and MCGs ii and iii occurred at Córdoba and Seville, in the centre of the region in the Guadalquivir valley (Fig. 2).

At the field level, the eight intensively sampled fields in four provinces in southern Spain showed a high degree of MCG homogeneity (Table 1; Fig. 3). The number of MCGs per field ranged from one to three and MCG i was present in all fields. A single field located at Posadas (Córdoba province) consisted of MCGs i, ii and iii, 12.9, 54.8 and 32.3% of a total of 31 isolates, respectively, and yielding a Shannon–Weaver index of $H' = 0.96$. Two MCGs were identified in five fields; they

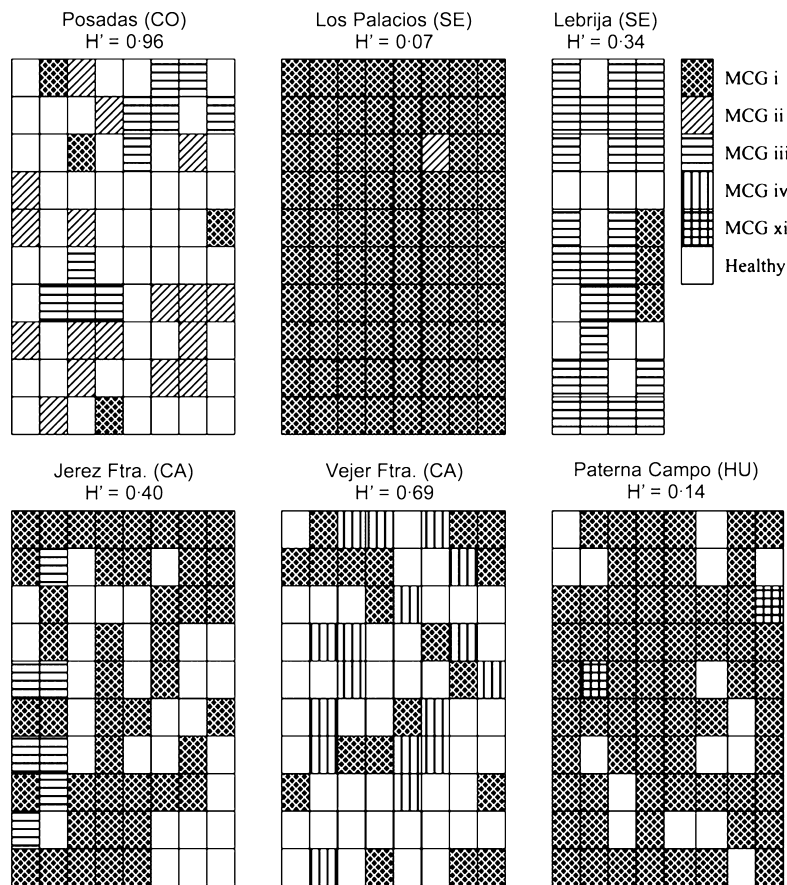


Figure 3 Schematic distribution of mycelial compatibility groups (MCGs) of *Sclerotium rolfsii* within six intensively sampled sugar beet fields in southern Spain, and Shannon–Weaver diversity index: H' . The sampled area (640 m²) was divided into 80 subplots, except for the field at Lebrija, in which the sampled area was 320 m² divided into 40 subplots. Provinces sampled in southern Spain: CA, Cádiz; CO, Córdoba; SE, Seville; and HU, Huelva.

included MCG i together with either MCGs ii (1.3% of isolates, $H' = 0.07$), iii (two fields, 14.0 and 89.3% of isolates, $H' = 0.40$ and $H' = 0.34$, respectively), v (45.7% of isolates, $H' = 0.69$) or xi (3.1% of isolates, $H' = 0.14$) (Table 1; Fig. 3). In those fields, *S. rolfsii* isolates of a MCG tended to form discrete clusters of different sizes distributed along the plot, but there was no discernible spatial pattern for individual MCGs (Fig. 3).

Pathogenic diversity

Susceptible reactions consisted of initial pale green and wilted leaves, followed by complete collapse and death of plants. Most plant mortality occurred within the first 8 days after inoculation. Symptoms developed neither in non-inoculated controls nor in resistant plants. Overall, MCGs iii and MCGs vi had the widest host range, with their isolates being pathogenic to all plant species tested, except for wheat and corn, which were resistant to all 12 MCGs.

Isolates of MCGs i, ii, vii, ix and x were pathogenic to eight plant species, whereas isolates of MCGs viii, xi and

xii were pathogenic to seven plant species, and those of MCGs iv and v were pathogenic to six (Fig. 4a). Three of the 12 tested plant species, chickpea, pepper and watermelon, were infected by all 12 MCGs, whereas cotton and sunflower were infected by 11 MCGs, tomato by 10, sugar beet by nine, broccoli by eight and melon by only six MCGs (Fig. 4b). Melon plants were not infected by six of the 12 MCGs tested. DI values were low for the remaining MCGs, reaching 12.5% in inoculations with the representative isolate of each of MCGs iii to vi, and 25 and 37.5% with those of MCG ii and MCG i, respectively, and were not included in the statistical analyses (data not shown).

Global likelihood ratio analysis of the number of dead plants for the combinations of isolates representative of each of MCGs i to xii and the remaining eight plant species indicated a significant effect of MCG ($\chi^2 = 33.37$, $P = 0.0005$), plant species ($\chi^2 = 68.04$, $P < 0.0001$) and the MCG by plant-species interaction ($\chi^2 = 104.17$, $P < 0.0213$) (data not shown). To further analyse the MCG by plant-species interaction, functional groupings of MCGs were first identified using clustering silhouettes.

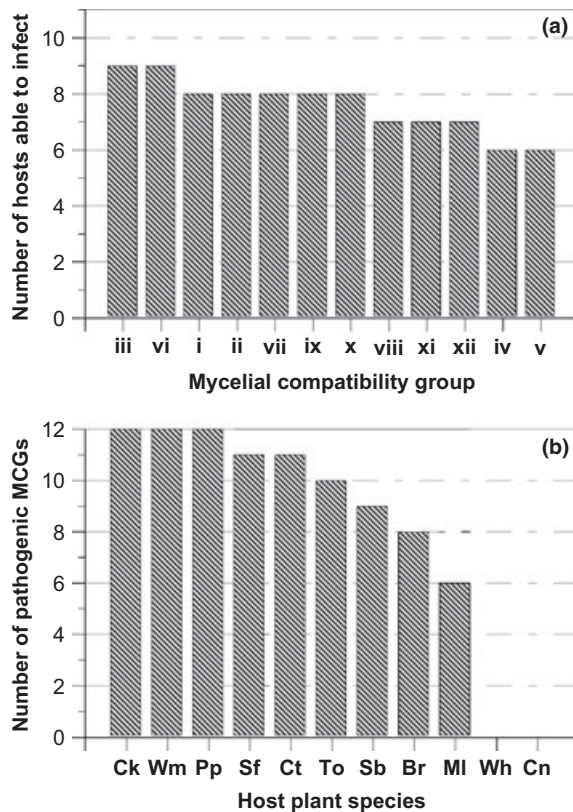


Figure 4 Disease reactions of 11 plant species to infection by 12 *Sclerotium rolfsii* isolates representative of mycelial compatibility groups (MCGs). (a) Number of plant species to which each MCG were pathogenic. (b) Number of MCGs pathogenic to each plant species. Plant species: Br, broccoli; Ck, chickpea; Cn, corn; Ct, cotton; MI, melon; Pp, pepper; Sb, sugar beet; Sf, sunflower; To, tomato; Wh, wheat; and Wm, watermelon.

In this analysis, four to seven cluster groupings could be appropriate according to the silhouettes' mean width. This allowed selection of five functional MCG groupings among the 12 MCGs of the study which included: MCG grouping 1 (MCGs i and ix), MCG grouping 2 (MCGs ii, iii, vi and viii), MCG grouping 3 (MCGs iv, v and xi), MCG grouping 4 (MCG vii) and MCG grouping 5 (MCGs x and xii) (Fig. 5a). Similarly, plant species could be grouped into two plant species (SP) groups, namely SP group 1 (chickpea, broccoli, sunflower and tomato) and SP group 2 (cotton, pepper, sugar beet and watermelon) according to the two-first-order cluster groups identified in the cluster analysis (Fig. 5b). Thereafter, a new analysis was performed using MCG groupings and SP groups as independent variables, which indicated significant effects of both factors ($P \leq 0.0495$), as well as of their interaction ($P = 0.0012$) (Table 2). This interaction was the result of disease incidence induced by isolates of MCG groupings 1, 2 and 5 being higher ($P \leq 0.0319$) on SP group 1 than on SP group 2, whereas isolates of MCG groupings 3 and 4 induced similar disease incidence

($P \geq 0.0902$) on SP groups 1 and 2 (Table 2). Additionally, a separate analysis was performed for each SP group to further assess the effects of MCG groupings on disease incidence induced by *S. rolfsii* isolates on the plant species included in each SP group. Results indicated no significant ($P \geq 0.05$) MCG-grouping by plant-species interaction for the two SP groups (Table 3). Overall, for SP group 1, disease reaction to inoculation was significantly influenced both by the MCG grouping and plant species ($P < 0.0001$) but not by their interaction ($P = 0.2026$) (Table 3). DI was highest ($P < 0.05$) for isolates of MCG groupings 1, 2 and 5, which showed moderate virulence, and decreased significantly ($P < 0.05$) for MCG groupings 3 and 4, which were mildly virulent (Fig. 6a). Among plant species, DI was highest ($P < 0.05$) on chickpea, which was categorized as highly susceptible, decreased significantly ($P < 0.05$) on sunflower, which was categorized as susceptible, and was least ($P < 0.05$) on tomato and broccoli, which were considered moderately resistant with no significant differences ($P \geq 0.05$) between these two species (Fig. 6a). No significant differences ($P = 0.0681$) occurred among MCG groupings on SP group 2 (Table 3; Fig. 6b). Across plant species in SP group 2, disease incidence was highest ($P < 0.05$) on cotton and pepper, which showed susceptible reactions, and decreased significantly ($P < 0.05$) to a moderately susceptible reaction on sugar beet. DI on watermelon was intermediate between those occurring on the three species of SP group 2 and did not differ significantly ($P \geq 0.05$) from any of them (Fig. 6b).

Finally, the disease reaction induced by isolates of MCGs included in each of the four multimember MCG groupings (i.e. MCG groupings 1, 2, 3 and 5) on SP groups 1 and 2 was fairly homogeneous ($P \geq 0.0566$). This supports the consistency of the five MCG groupings identified in the cluster analysis.

Within-MCG pathogenic variability

There was little variation in the disease reaction induced on sunflower, tomato and watermelon by *S. rolfsii* isolates belonging to the same MCG (Fig. 7). Thus, likelihood analyses showed no significant differences ($P \geq 0.5350$) among *S. rolfsii* isolates belonging to either MCG iii, v, vi or xii (Table 4). Moreover, disease reactions induced by isolates of each of these four MCGs were not significantly different ($P \geq 0.05$) from those in experiments I and II. An exception to this occurred for isolates of MCG i, which showed significant differences between each other ($P < 0.0001$) (Table 4, Fig. 7). In any case, of the 12 MCG i isolates tested, only the disease reaction induced by isolate Sr033 differed significantly ($P = 0.0068$) from that induced by the MCG i type isolate Sr078 (Fig. 7). Comparison of the disease reaction induced by this isolate with those induced by the rest of the MCG i isolates indicated that Sr033 was more virulent to all three plant species (Fig. 7). For all five MCGs tested, there were no significant ($P \geq 0.4638$) isolate by plant-species interactions (Table 4).

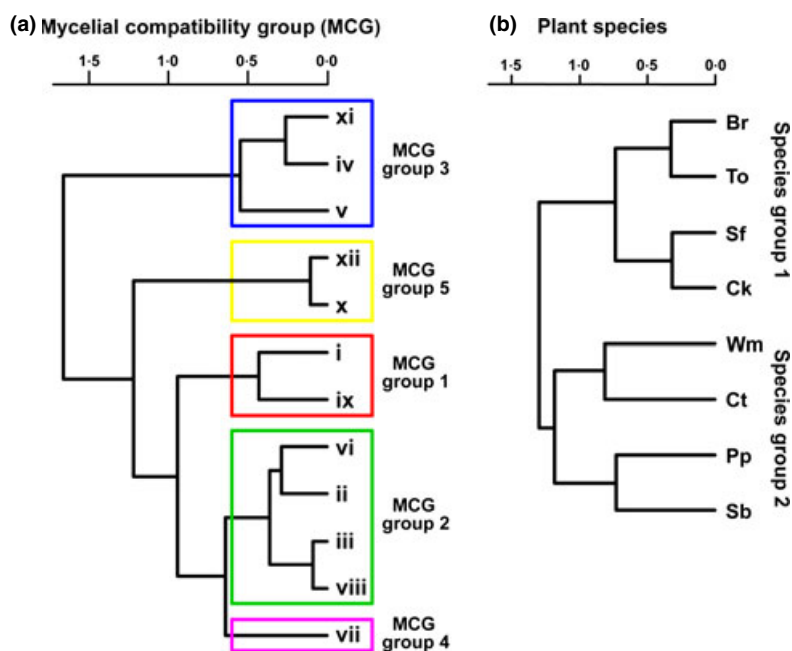


Figure 5 Dendrogram showing results of cluster analyses of disease reaction to infection by 12 *Sclerotium rolfii* isolates representative of (a) mycelial compatibility groups (MCGs) and (b) eight plant species. Agglomerative cluster analyses were performed based on the Spearman correlation matrix calculated using number of dead plants among MCGs or among plant species using the Ward method. MCG groupings were estimated on the basis of the average silhouette width according to the Mantel statistic. Plant species: Br, broccoli; Ck, chickpea; Ct, cotton; Pp, pepper; Sb, sugar beet; Sf, sunflower; To, tomato; and Wm, watermelon.

Table 2 Global likelihood ratio statistics to test the effects of groupings of *Sclerotium rolfii* mycelial compatibility groups (MCG groupings) on sclerotium root rot incidence in groups of plant species (SP groups)

Source of variation ^a	d.f.	χ^2	$P > \chi^2$
MCG grouping	4	22.36	0.0002
SP group	1	3.86	0.0495
MCG grouping*SP group	4	18.07	0.0012
Contrasts			
MCG grouping 1: SP group 1 vs. 2	1	13.53	0.0002
MCG grouping 2: SP group 1 vs. 2	1	4.60	0.0319
MCG grouping 3: SP group 1 vs. 2	1	2.87	0.0902
MCG grouping 4: SP group 1 vs. 2	1	0.24	0.6249
MCG grouping 5: SP group 1 vs. 2	1	4.70	0.0301

^aMCG groups included: MCG grouping 1: MCGs i and ix; MCG grouping 2: MCGs ii, iii, vi and viii; MCG grouping 3: MCGs iv, v and xi; MCG grouping 4: MCG vii; and MCG grouping 5: MCGs x and xii. SP group 1: chickpea, broccoli, sunflower and tomato; SP group 2: cotton, pepper, sugar beet and watermelon.

Discussion

Genetic and pathogenic characterization of *S. rolfii* isolates from different agricultural regions can provide important insights into the epidemiology and management of the diseases they cause. In this study, the structure of *S. rolfii* populations obtained from a single host crop (autumn-sown sugar beet) and regions of the Iberian Peninsula (Portugal and Spain), Italy and Chile sharing Medi-

Table 3 Likelihood ratio statistics to test the effects of groupings of *Sclerotium rolfii* mycelial compatibility groups (MCG groupings) on sclerotium root rot incidence in plant species of two groups of species (SP groups)

MCG grouping	Source of variation ^a	d.f.	Species group 1		Species group 2	
			χ^2	$P > \chi^2$	χ^2	$P > \chi^2$
Global	MCG-group (A)	4	33.30	<0.0001	8.73	0.0681
	Species (B)	3	27.03	<0.0001	8.41	0.0382
1	A*B	12	15.76	0.2026	17.91	0.1184
	MCG (A)	1	0.05	0.8280	0.49	0.4818
2	Species (B)	3	22.28	<0.0001	0.93	0.8186
	A*B	3	1.77	0.6207	5.48	0.1398
3	MCG (A)	3	6.56	0.0874	6.88	0.0759
	Species (B)	3	46.34	<0.0001	12.05	0.0072
4	A*B	9	9.41	0.3999	9.93	0.3565
	MCG (A)	2	0.29	0.8650	0.09	0.9571
5	Species (B)	3	21.41	<0.0001	2.69	0.4412
	A*B	6	2.36	0.8840	11.38	0.0773
4	Species	3	0.66	0.8837	5.41	0.1439
	MCG (A)	1	3.63	0.0566	0.15	0.7025
5	Species (B)	3	12.43	0.0060	11.56	0.0090
	A*B	3	0.54	0.9107	0.83	0.8417

^aMCG groupings included: MCG grouping 1: MCGs i and ix; MCG grouping 2: MCGs ii, iii, vi and viii; MCG grouping 3: MCGs iv, v and xi; MCG grouping 4: MCG vii; and MCG grouping 5: MCGs x and xii. SP group 1: chickpea, broccoli, sunflower and tomato; SP group 2: cotton, pepper, sugar beet and watermelon.

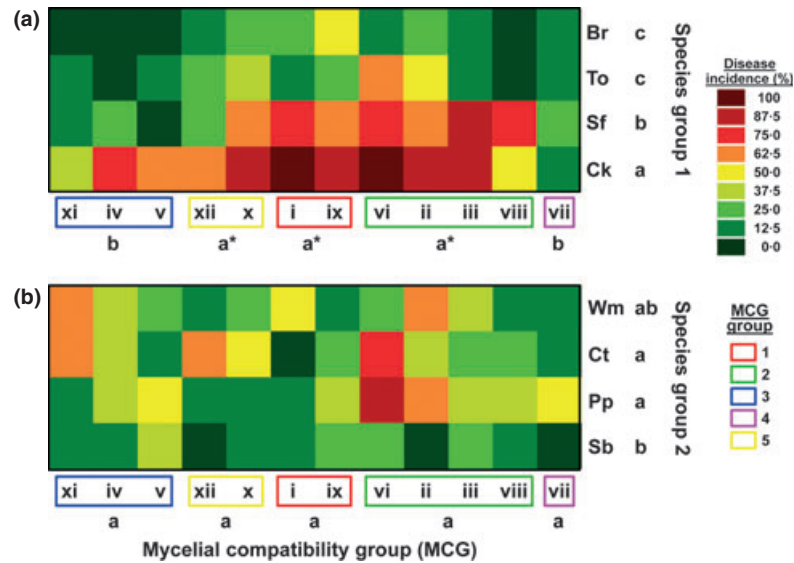


Figure 6 Two-way plots showing results of likelihood analyses of disease reaction estimated by the number of dead plants to infection by 12 *Sclerotium rolfisii* isolates representative of mycelial compatibility groups (MCGs) grouped into five MCG groupings (x-axis) and eight plant species (y-axis) grouped into species-group (SP) 1 (a) and SP group 2 (b). Plant species: Br, broccoli; Ck, chickpea; Ct, cotton; Pp, pepper; Sb, sugar beet; Sf, sunflower; To, tomato; and Wm, watermelon. For each SP group, MCG groupings or plant species followed by a different letter showed significant differences on the least squares means at $P < 0.05$. MCG groupings with an asterisk indicate a significantly higher least squares mean for disease incidence induced on SP group 1, compared to that on SP group 2 at $P < 0.05$.

terrestrial-type climate were characterized by means of MCG analysis and pathogenicity to 11 economically important plant species cultivated in those regions. It was demonstrated that differences in virulence occur among isolates from certain locations and that these differences can be associated with genetic diversity based on MCGs of isolates. In addition, variation at field level was assessed by intensive sampling of plots at several locations in southern Spain.

A system of mycelial incompatibility was previously demonstrated to occur among field isolates of *S. rolfisii* and was used to designate MCGs (Punja & Grogan, 1983). This study was able to identify up to 12 MCGs among 459 *S. rolfisii* isolates originating from a wide geographic range but a single host crop. This is apparently novel work in *S. rolfisii* because previously published work has involved *S. rolfisii* isolates from: (i) widely distant geographic areas and diverse host sources (Punja & Grogan, 1983; Harlton *et al.*, 1995; Punja & Sun, 2001); (ii) a restricted region and diverse hosts (Cilliers *et al.*, 2000, 2002; Almeida *et al.*, 2001; Sarma *et al.*, 2002); and (iii) a single region and host plant (Nalim *et al.*, 1995; Okabe & Matsumoto, 2000; Adandonon *et al.*, 2005). Two abundant and prevalent MCGs (MCGs i and iii) were found. Some other MCGs were: (a) less prevalent but present in various regions of different countries (MCG v) or regions within a country (MCGs ii, vi and ix); (b) restricted to specific localities (MCGs viii, xi and xii); or (c) single-isolate member groups (MCGs iv, vii and x).

The results agree with most previous reports of a single *S. rolfisii* MCG comprising isolates from widely distant geographic areas of Brazil (Almeida *et al.*, 2001), India

(Sarma *et al.*, 2002), South Africa (Cilliers *et al.*, 2000) and the USA (Harlton *et al.*, 1995; Punja & Sun, 2001). In this same context, the existence of MCGs at high frequencies with wide geographic distribution was also reported for *S. sclerotiorum* infecting canola (*Brassica napus*) through Canada, where a single MCG representing 18% of the isolates was found in all three distant provinces sampled (Kohli *et al.*, 1992).

In southern Spain, *S. rolfisii* was reported infecting sugar beet crops in some localities in Córdoba and Seville provinces in the centre of Andalusia in the early 1940s (Benlloch, 1943). By 1944, the pathogen had also been reported in Cáceres province in the Extremadura region of southwest Spain, close to the Portuguese border (Domínguez García-Tejero, 1951). Interestingly, this coincided with when the crop sowing date was brought forward from spring to early winter (Morillo-Velarde *et al.*, 2003), which appears more favourable for disease development (Aycock, 1966; Punja, 1985). In autumn sowings, several important changes were implemented in the husbandry of sugar beet, which included complete mechanization of harvesting using heavy equipment. Thus, movement of equipment across fields, together with transportation of harvested roots from fields to distant processing factories, may have facilitated dispersal of the pathogen in soil particles and could be responsible for the rapid spread of MCGs i and iii through Andalusia (Fig. 2). On the other hand, sugar beet has been extensively grown in Portugal since the late 1990s (FAOSTAT, 2010), and sclerotium root rot was of great concern in the major growing areas of central and southern Portugal in 2003, at which time a disease prevalence of 21% of fields

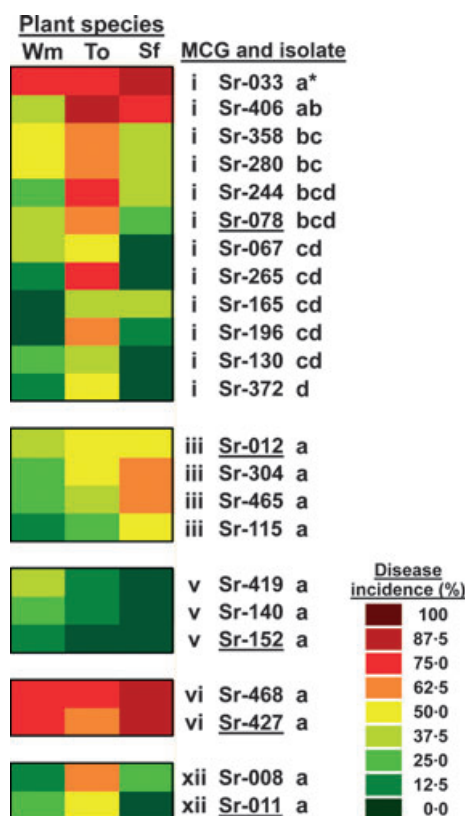


Figure 7 Two-way plot showing results of likelihood analyses of disease reaction estimated by the number of dead plants of sunflower (Sf), tomato (To) and watermelon (Wm) (x-axis) to infection by 23 *Sclerotium rolfsii* isolates representative of five of the more prevalent mycelial compatibility groups (MCG) (y-axis) identified in this study. Isolates tested included 12 isolates for MCG i, four isolates for MCG iii, three for MCG v, and two for each of MCG vi and MCG xii. The type isolate for each MCG is underlined. For each MCG, isolates followed by a different letter showed significant differences in the least squares means at $P < 0.05$. Isolates with an asterisk indicate significantly different least squares means from that of the type isolate at $P < 0.05$.

accounted for 16.6% of the cultivated area being affected (M. Paim, Associação para Desenvolvimento da Beterraba, Portugal, personal communication). In Chile, sugar beet has been grown since the early 1950s and *S. rolfsii* was first reported in the central regions affecting diverse crops in 1981 (Esterio & Auger, 1982) and on sugar beet in the Metropolitan region and V region in 1983 (Acuña, 1985). Thereafter, the pathogen spread to the southern regions reaching the VIII region in 1999 (R. Paillalef-Monnrad, IANSAGRO, Chile, personal communication). MCG iii was the most prevalent group in the current study, being present in two of four, three of eight and all five locations sampled in Chile, Spain and Portugal, respectively. This suggests that MCG iii may have been spread extensively within and among cultivated areas by cultural practices, vehicle movements, planting material, containers, etc. The existence of *S. rolfsii* isolates of the

Table 4 Likelihood ratio statistics to test the pathogenic variability among *Sclerotium rolfsii* isolates belonging to different mycelial compatibility groups (MCG)

MCG	Source of variation ^a	d.f.	χ^2	$P > \chi^2$
MCG i	Isolate (A)	11	41.83	<0.0001
	Species (B)	2	28.42	<0.0001
	A*B	22	20.64	0.5431
MCG iii	Isolate (A)	3	2.18	0.5350
	Species (B)	2	6.93	0.0312
	A*B	6	1.32	0.9708
MCG v	Isolate (A)	2	0.57	0.7522
	Species (B)	2	2.99	0.2246
	A*B	4	0.41	0.9819
MCG vi	Isolate (A)	1	0.07	0.7889
	Species (B)	2	1.68	0.4307
	A*B	2	0.17	0.9185
MCG xii	Isolate (A)	1	0.30	0.5864
	Species (B)	2	8.10	0.0174
	A*B	2	1.54	0.4638

^aIsolates tested included 12 isolates for MCG i, four isolates for MCG iii, three for MCG v, and two for each of MCG vi and MCG xii. Plant species tested included sunflower, tomato and watermelon.

same MCG in different distant countries was also reported by Harlton *et al.* (1995), who identified MCG 8 in the USA (Alabama, California and Maryland) and Pakistan; and MCG 1 in the USA (California, Georgia and North Carolina) and Mexico. Similarly, Kull *et al.* (2004) reported MCG 8 of *S. sclerotiorum* in soyabean fields in several states in the USA, on soyabean in Switzerland and on canola in Canada.

The present results are also consistent with those of other authors, suggesting a correlation between MCG and geographical source of isolates, as *S. rolfsii* isolates originating from the same locality tended to form a distinct MCG (Punja & Grogan, 1983; Harlton *et al.*, 1995; Cilliers *et al.*, 2000, 2002; Punja & Sun, 2001; Sarma *et al.*, 2002). In fact, three of the 12 MCGs identified (MCGs ii, vi and ix) were found only at two close localities and six were present at a single locality. This was the case of MCG iv, which was identified exclusively in Bari (Italy), MCGs vii, viii, x and xii, which were present in each of four different localities in Chile, and MCG xi, which was identified only in Huelva (Spain) (Fig. 2). In a comparable study, Nalim *et al.* (1995) found that a rather small number of MCGs existed within single peanut fields in Texas. Localization of unique *S. sclerotiorum* MCGs was also observed in vegetable-growing regions in New Zealand (Carpenter *et al.*, 1999) and on winter canola in Ontario, Canada (Kohn *et al.*, 1991). Explanations for unique low-frequency MCGs in a sampling area may include recent MCG introductions or random mutation events. The emergence of new genotypes localized in single fields may be an indication of MCGs or clones becoming adapted to specific field microclimates or hosts, and are less likely to be the result of genetic exchange and recombination (Ben-Yephet & Bitton, 1985; Hambleton

et al., 2002). The existence of single-member MCGs was also reported with variable frequency in different geographic areas and/or host sources (Punja & Grogan, 1983; Harlton *et al.*, 1995; Nalim *et al.*, 1995; Cilliers *et al.*, 2000; Okabe & Matsumoto, 2000; Almeida *et al.*, 2001; Punja & Sun, 2001; Sarma *et al.*, 2002).

The population of the pathogen in Chile was particularly diverse. The area sampled for the study in that country was similar in size to that in southern Spain. However, MCGs identified in Chile accounted for half of the 12 MCGs identified in the study, of which five were found only in this country. Moreover, a single location in Chile could contain up to three different MCGs, as occurred in Linares (MCGs viii, ix and xii) and Chillán (MCGs iii, ix and x). The existence of considerable genetic variability among *S. rolf sii* isolates was also reported in Brazil, where 13 compatibility groups were identified using 30 isolates from 14 states and 11 hosts (Almeida *et al.*, 2001).

At field-plot level, a relatively low number of MCGs was found within each of the eight sugar beet fields sampled in the Guadalquivir Valley in southern Spain. In these fields, three, two and one MCGs were identified in one, five and two fields, respectively. MCG i was always present, being unique and predominant in two and four fields, respectively (Figs 2 and 3). Interestingly, the number of MCGs in a field was not related to the number of *S. rolf sii* isolates sampled in it. Thus, for a similar sampled area the number of isolates ranged from 28 to 80, the field harbouring three MCGs being represented by 31 isolates. These results do not fully agree with those reported by Nalim *et al.* (1995), who studied four peanut fields in Texas and found one, three and five MCGs in one, two and one field, respectively. However, none of the MCGs reported by Nalim *et al.* (1995) were prevalent across fields, as only one MCG was present in two fields, while the others were specific to each field. In contrast, the results of the present study are closer to those of Okabe & Matsumoto (2000) in peanut fields in Japan. These authors analysed seven peanut fields and identified four MCGs, with up to three MCGs occurring in a single field and one MCG being dominant in most of the fields sampled. In similar studies on *S. sclerotiorum*, Kohn *et al.* (1991) found much higher variability within two canola fields in Ontario, Canada, where they identified 26 and six MCGs from 30 and 33 isolates, respectively, with two MCGs being predominant in one field, and only one MCG being common to the two fields. Similar results were described for the MCG population structure of *S. sclerotiorum* on soyabean in Argentina and Illinois (USA) (Kull *et al.*, 2004). The present observations and those of Nalim *et al.* (1995) and Okabe & Matsumoto (2000) are coherent with a 'founder effect' and may reflect the mode of reproduction of *S. rolf sii*, which does not produce conidia and spreads by mycelial growth. Whilst *S. rolf sii* does have a perfect stage, its role in nature is not fully understood. In contrast, sexual reproduction in *S. sclerotiorum* and a greater selection for specific MCGs in populations of this fungus com-

pared with that in *S. rolf sii* may account for the smaller number of *S. rolf sii* MCGs found per field in this study. Thus, in the six sugar beet fields in southern Spain comprising more than one *S. rolf sii* MCG, the MCGs appeared scattered within distinct disease foci with no discernible spatial pattern. This agrees with reports on *S. rolf sii* in peanut fields in Texas (Nalim *et al.*, 1995) or *S. sclerotiorum* on soyabean in Illinois (Kull *et al.*, 2004).

The results demonstrate that high pathogenic variability occurs among isolates representative of *S. rolf sii* MCGs as well as in the degree of susceptibility among the tested plant species. To our knowledge this is the first genetic and virulence characterization of *S. rolf sii* populations. Overall, MCGs vi and ii were highly virulent, MCGs i, iii, ix and x moderately virulent, and MCGs iv, v, vii, viii, xi and xii were mildly virulent. However, that range in virulence was not directly related to the pathogenic spectrum of a given MCG.

The results also showed a low level of pathogenic variability among *S. rolf sii* isolates within a MCG. In fact, for five of the most abundant MCGs identified in this study, isolates within a MCG were similarly virulent to three plant species. Only one out of 12 MCG i isolates tested caused a disease reaction that differed significantly from that induced by the MCG i type isolate, Sr078, being more virulent to all three plant species, sunflower, tomato and watermelon (Fig. 7). These results suggest that a correlation may exist between MCG typing and virulence trait.

Among plant species, chickpea and sunflower were highly susceptible; cotton, pepper, tomato and watermelon moderately susceptible; broccoli and sugar beet mildly susceptible; and corn and wheat were resistant. A degree of variability in host plant reaction to a *S. rolf sii* isolate from sugar beet in Pakistan was reported by Yaqub & Shahzad (2005): mungbean, sugar beet and sunflower were highly susceptible; cabbage, lentil, tomato and sweet pumpkin were mildly susceptible; and cauliflower was completely resistant. To some extent, these results agree with those of Flores-Moctezuma *et al.* (2006) who evaluated the reactions of 12 plant species to a set of 20 non-MCG-typed *S. rolf sii* isolates collected from different agricultural and ecological areas in Mexico. These authors found that gooseberry, lentil, pumpkin, radish and tomato were susceptible to all tested isolates, whereas the degree of susceptibility of the remaining seven species varied widely with the isolate. In the present study, sugar beet cv. Markus was susceptible, moderately susceptible and resistant to isolates from one, eight and three MCGs, respectively. Similarly, Sharma *et al.* (1991) found that only one of five *S. rolf sii* isolates from different sugar beet-growing areas in northwest India was highly virulent to sugar beet in artificial inoculations.

The results of this study have important implications for the management of sclerotium root rot of sugar beet in Mediterranean-type climates, particularly in relation to crop rotation and choice of appropriate crops for use

in infested soils previously cropped to sugar beet. Crop rotations are essential to maintain crop productivity and reduce the build-up of soilborne plant pathogens and diseases, which can devastate crops grown in multiple consecutive years (Cook, 2000). Generally, the degree of control is based on the level of susceptibility or resistance of crops involved and the sequences of cropping. In the present study, monocotyledonous species (wheat and corn) were resistant to all MCGs tested and thus are suitable for rotation with susceptible crops. These results agree with those of Boyle (1967), who indicated a reduction in the incidence of sclerotium root rot in peanut fields following a monocotyledonous crop, as well as with those of Minton *et al.* (1991), who reported that disease incidence in a wheat-peanut rotation was lower than that in a fallow-peanut rotation. Similarly, disease incidence was the lowest in peanut following 2 years of bahiagrass, intermediate following 2 years of corn or cotton, and highest in continuous peanut (Johnson *et al.*, 1999). Nevertheless, results of this present study suggest that *S. rolfsii* isolates in any of the identified MCGs were able to cause different levels of plant mortality in at least six of the nine susceptible plant species tested. This, together with the occurrence of up to three different MCGs within a field plot or locality differing in pathogenic and virulence profiles, make it difficult to recommend crop rotation as a sole control measure. It would be of interest to evaluate the risk of occurrence of severe epidemics on a given crop based on the prevalence of a given MCG of the pathogen. This would be of importance for southern Spain and Portugal, where vegetable crops such as carrots, peppers, tomato, etc. are becoming alternative crops for sugar beet as a result of the sugar beet production area being drastically reduced or nearly eliminated, respectively. Consequently, sclerotium root rot diseases can become an important threat for vegetable crop production in these areas, as was recently reported for pepper fields in southern Spain (Remesal *et al.*, 2010).

Studies are in progress to assess the molecular diversity existing within *S. rolfsii* MCGs and to determine any genetic relationships that might exist among them.

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References

- Acuña R, 1985. Pudrición radicular causada por *Sclerotium rolfsii* Sacc., en cultivos de remolacha en la región central del país. *Simiente* 55, 34–5.
- Adandonon A, Aveling TAS, van der Merwe NA, Sanders G, 2005. Genetic variation among *Sclerotium* isolates from Benin and South Africa, determined using mycelial compatibility and ITS rDNA sequence data. *Australasian Plant Pathology* 34, 19–25.
- Agresti A, 2002. *Categorical Data Analysis*, 2nd edn. Hoboken, NJ, USA: John Wiley & Sons, Inc.
- AIMCRA, 2005. *Memoria de los Trabajos Efectuados en la Campaña 2003/04*. Siembra Otoño 2002 Zona Sur. Valladolid, Spain: Asociación para la Mejora del Cultivo de la Remolacha Azucarera (AIMCRA).
- Akram A, Iqbal SM, Rauf CA, Aleem R, 2008. Detection of resistant sources for collar rot disease in chickpea germplasm. *Pakistan Journal of Botany* 40, 2211–5.
- Almeida AMR, Abdelnoor RV, Calvo ES, Tessman D, Yorinori JT, 2001. Genotypic diversity among Brazilian isolates of *Sclerotium rolfsii*. *Journal of Phytopathology* 149, 493–502.
- Aycock R, 1966. Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. North Carolina Agricultural Experiment Station Technical Bulletin No. 174.
- Bayraktar H, Türkkan M, Dolar FS, 2010. Characterization of *Fusarium oxysporum* f. sp. *cepae* from onion in Turkey based on vegetative compatibility and rDNA RFLP analysis. *Journal of Phytopathology* 158, 691–7.
- Benlloch M, 1943. El "mal del esclerocio" en los remolachares de algunas vegas andaluzas. *Boletín de Patología Vegetal y Entomología Agrícola* 12, 221–8.
- Ben-Yephet Y, Bitton S, 1985. Use of a selective medium to study the dispersal of ascospores of *Sclerotinia sclerotiorum*. *Phytoparasitica* 13, 33–40.
- Borcard D, Gillet F, Legendre P, 2011. *Numerical Ecology with R*. New York, USA: Springer.
- Boyle LW, 1967. A factorial study of certain schemes of peanut culture. *University of Georgia Agriculture Experimental Station Research Bulletin* 18, 11–2.
- Branch WD, Brenneman TB, 1999. Stem rot disease evaluation of mass-selected peanut populations. *Crop Protection* 18, 127–30.
- Carling DE, Kuninaga S, Brainard KA, 2002. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. *Phytopathology* 92, 43–50.
- Carpenter MA, Frampton C, Stewart A, 1999. Genetic variation in New Zealand populations of the plant pathogen *Sclerotinia sclerotiorum*. *New Zealand Journal of Crop and Horticultural Science* 27, 13–21.
- Caten CE, 1972. Vegetative compatibility and cytoplasmic infection in fungi. *Journal of General Microbiology* 72, 221–9.
- Chulze SN, Ramirez ML, Torres A, Leslie JF, 2000. Genetic variation in *Fusarium* section *Liseola* from no-till maize in Argentina. *Applied and Environmental Microbiology* 66, 5312–5.

- Ciampi MB, Meyer MC, Costa MJN, Zala M, McDonald BA, Ceresini PC, 2008. Genetic structure of populations of *Rhizoctonia solani* anastomosis group-1 IA from soybean in Brazil. *Phytopathology* 98, 932–41.
- Cilliers AJ, Herselman L, Pretorius ZA, 2000. Genetic variability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. *Phytopathology* 90, 1026–31.
- Cilliers AJ, Pretorius ZA, van Wyk PS, 2002. Mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. *South African Journal of Botany* 68, 389–92.
- Collado-Romero M, Mercado-Blanco J, Olivares-García C, Valverde-Corredor A, Jiménez-Díaz RM, 2006. Molecular variability within and among *Verticillium dahliae* vegetative compatibility groups determined by fluorescent amplified fragment length polymorphism and polymerase chain reaction markers. *Phytopathology* 96, 485–95.
- Cook RJ, 2000. Advances in plant health management in the twentieth century. *Annual Review of Phytopathology* 38, 95–116.
- Domínguez García-Tejero F, 1951. Distribución en España de las plagas y enfermedades de la remolacha. *Boletín de Patología Vegetal y Entomología Agrícola* 18, 181–204.
- Dukes PD, Ferry RL, Jones A, 1983. Evaluating peppers, cowpeas, sweet potato, and tomatoes for resistance to southern blight incited by *Sclerotium rolfsii* Sacc. *Phytopathology* 73, 785–6.
- Earnshaw DM, McDonald MR, Boland GJ, 2000. Interactions among isolates and mycelial compatibility groups of *Sclerotium cepivorum* and cultivars of onion (*Allium cepa*). *Canadian Journal of Plant Pathology* 22, 387–91.
- Elias KS, Zamir D, Lichtman-Pleban T, Katan T, 1993. Population structure of *Fusarium oxysporum* f. sp. *lycopersici*: restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility group is an indicator of evolutionary origin. *Molecular Plant-Microbe Interactions* 6, 565–72.
- Esterio M, Auger J, 1982. Presencia de *Pellicularia rolfsii* (Curzi) West (*Sclerotium rolfsii* Sacc.) en la zona central del país. *Simiente* 52, 32.
- FAOSTAT, 2010. FAOSTAT production statistics of crops. [Accessed September 01 2011: <http://faostat.fao.org/site/567/default.aspx#ancor>].
- Fery RL, Dukes PD Sr, 2002. Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance. *Crop Protection* 21, 403–8.
- Flores-Moctezuma HE, Montes-Belmont R, Jiménez-Pérez A, Nava-Juárez R, 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments. *Crop Protection* 25, 195–201.
- Hambleton S, Walker C, Kohn LM, 2002. Clonal lineages of *Sclerotinia sclerotiorum* previously known from other crops predominate in 1999–2000 samples from Ontario and Quebec soybean. *Canadian Journal of Plant Pathology* 24, 309–15.
- Harlton CE, Uvesque CA, Punja ZK, 1995. Genetic diversity in *Sclerotium (Athelia) rolfsii* and related species. *Phytopathology* 85, 1269–81.
- van Heerden SW, Wingfield MJ, 2001. Genetic diversity of *Cryphonectria cubensis* isolates in South Africa. *Mycological Research* 105, 94–9.
- Jenkins SF, Averre CW, 1986. Problems and progress in integrated control of southern blight of vegetables. *Plant Disease* 70, 614–9.
- Johnson AW, Minton NA, Breneman TB et al., 1999. Bahiagrass, corn, cotton rotations, and pesticides for managing nematodes, diseases, and insects on peanut. *Journal of Nematology* 31, 191–200.
- Kohli Y, Morrall RAA, Anderson JB, Kohn LM, 1992. Local and trans-Canadian clonal distribution of *Sclerotinia sclerotiorum* on canola. *Phytopathology* 82, 875–80.
- Kohn LM, Stasovski E, Carbone I, Royer J, Anderson JB, 1991. Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. *Phytopathology* 81, 480–5.
- Korolev N, Katan J, Katan T, 2000. Vegetative compatibility groups of *Verticillium dahliae* in Israel: their distribution and association with pathogenicity. *Phytopathology* 90, 529–36.
- Kull LS, Pedersen WL, Palmquist D, Hartman GL, 2004. Mycelial compatibility grouping and aggressiveness of *Sclerotinia sclerotiorum*. *Plant Disease* 88, 325–32.
- Lal RJ, Srivastava SN, Agnihotri VP, 1997. Epidemiology and management of *Sclerotium* root rot of sugarbeet: a threatening disease. In: Agnihotri VP, Sarbhoy AK, Singh DV, eds. *Management of Threatening Plant Diseases of National Importance*. New Delhi, India: Malhotra Publishing House, 161–78.
- Leslie JF, 1993. Fungal vegetative compatibility. *Annual Review of Phytopathology* 31, 127–50.
- Maechler M, Rousseeuw P, Struyf A, Hubert M, 2005. *Cluster Analysis: Basics and Extensions: An R Library*. [Accessed September 01 2011: <http://cran.r-project.org/web/packages/cluster/>].
- Minton NA, Csinos AS, Lynch RE, Breneman TB, 1991. Effects of two cropping and two tillage systems and pesticides on peanut pest management. *Peanut Science* 18, 41–6.
- Morillo-Velarde R, Bermejo JL, Ayala J, Moreno A, Gutiérrez M, Márquez L, 2003. *Remolacha Azucarera de Siembra Otoñal*. Normas Técnicas de Cultivo. Sevilla, Spain: Junta de Andalucía, Consejería de Agricultura y Pesca.
- Nalim FA, Starr JL, Woodard KE, Segner S, Keller NP, 1995. Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. *Phytopathology* 85, 1507–12.
- Okabe I, Matsumoto N, 2000. Population structure of *Sclerotium rolfsii* in peanut fields. *Mycoscience* 41, 145–8.
- Okabe I, Morikawa C, Matsumoto N, Yokoyama K, 1998. Variation in *Sclerotium rolfsii* isolates in Japan. *Mycoscience* 39, 399–407.
- Oksanen J, Guillaume Blanchet F, Kindt R et al., 2011. *Vegan: community ecology package*. R package version 1.17-11. [Accessed September 01 2011: <http://vegan.r-forge-project.org/>].
- Piepho HP, 2004. An algorithm for a letter-based representation of all-pairwise comparisons. *Journal of Computational and Graphical Statistics* 13, 456–66.
- Pratt RG, Rowe DE, 2002. Enhanced resistance to *Sclerotium rolfsii* in populations of alfalfa selected for quantitative resistance to *Sclerotinia trifoliorum*. *Phytopathology* 92, 204–9.
- Puhalla JE, 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* 63, 179–83.
- Punja ZK, 1985. The biology, ecology and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology* 23, 97–127.
- Punja ZK, Grogan RG, 1983. Hyphal interactions and antagonism among field isolates and single-basidiospore

- strains of *Athelia (Sclerotium) rolfsii*. *Phytopathology* **73**, 1279–84.
- Punja ZK, Sun LJ, 2001. Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* (teleomorph *Athelia rolfsii*) and *Sclerotium delphinii*. *Mycological Research* **105**, 537–46.
- Remesal E, Lucena C, Azpilicueta A, Landa BB, Navas-Cortes JA, 2010. First report of southern blight of pepper caused by *Sclerotium rolfsii* in Southern Spain. *Plant Disease* **94**, 280.
- Sarma BK, Singh UP, Singh KP, 2002. Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia* **94**, 1051–8.
- Schneider CL, Whitney ED, 1996. Sclerotium root rot. In: Whitney ED, Duffus JE, eds. *Compendium of Beet Diseases and Insects*. St Paul, MN, USA: APS Press, 22–3.
- Shannon CE, Weaver W, 1963. *The Mathematical Theory of Communication*. Urbana, IL, USA: University of Illinois Press.
- Sharma BS, Pathak VN, Bhatnagar K, 1991. Morphological, cultural and pathogenic variations in *Sclerotium rolfsii* Sacc. causing root rot of sugarbeet. *Cryptogamie Mycologie* **12**, 71–9.
- Yaqub F, Shahzad S, 2005. Pathogenicity of *Sclerotium rolfsii* on different crops and effect of inoculum density on colonization of mungbean and sunflower roots. *Pakistan Journal of Botany* **37**, 175–80.