Eur J Plant Pathol (2008) 122:369–383
DOI 10.1007/s10658-008-9301-6

Vegetative compatibility grouping in *Botrytis cinerea* using sulphate non-utilizing mutants

N. Korolev · Y. Elad · T. Katan

Received: 25 June 2007 / Accepted: 3 March 2008 / Published online: 28 March 2008

Abstract Twenty-one strains of *Botrytis cinerea* isolated from six plant species on ten sites throughout Israel, as well as a strain from France, were tested for vegetative and mycelial incompatibility, pathogenicity, resistance to the fungicides carbendazim and iprodione, and colony morphology. Selenate-resistant mutants were isolated from the strains as spontaneous, fast-growing sectors arising from restricted colonies on medium amended with sodium selenate with a mean frequency of 0.04 sectors/colony; 81% of the sectors were sulphate non-utilizing (*sul*) mutants. One hundred and four *sul* mutants were divided into two complementary groups: resistant (66 mutants) and sensitive to chromate. Based on compatibility reactions between chromate-resistant and chromate-sensitive *sul* mutants, 12 strains were compatible only with themselves and were each classified as belonging to different vegetative compatibility groups (VCGs). Nine strains were each compatible with one to three other strains, and were assembled into three multi-member VCGs. Mycelial incompatibility between wild-type strains (barrage), in the form of a zone of dark pigmentation or sparse mycelium with or without dark pigmentation of the agar along the line of confrontation, was observed for 70% of the inter-strain pairings. There was no correspondence in compatibility between strains revealed by two approaches: strains in different VCGs did not necessarily produce a barrage. However, self-compatibility was observed both as heterokaryon formation between complementary *sul* mutants and as an absence of barrages between mycelia of wild-type strains; wild-type strains belonging to the same VCG did not exhibit strong barrages, although weak antagonistic reactions were observed. Strains in two multi-member VCGs showed the same patterns of resistance to carbendazim and iprodione; the third multi-member VCG contained isolates with different patterns of resistance. Four morphological types were revealed among wild-type strains: conidial (five strains), sclerotial (six strains), intermediate (ten strains), and mycelial (one strain). On bean leaves, conidial strains were more aggressive than sclerotial strains.

Keywords Barrage · *Botrytis cinerea* · Carbendazim · Iprodione · Morphological type · *Phaseolus vulgaris* · Pathogenicity · Sulphate non-utilizing mutants · VCG

N. Korolev (✉) · Y. Elad · T. Katan
Department of Plant Pathology and Weed Research,
Volcani Center, ARO,
Bet Dagan 50250, Israel
e-mail: vpptlg@volcani.agri.gov.il

Introduction

Botrytis cinerea, the anamorph of *Botryotinia fuckeliana* is an important pathogen that affects a large

number of economically significant crops (Elad et al. 2004). Genetic variability in *B. cinerea* populations is very high, and the populations often resemble a mix of different strains without any correlation between the origins and properties of the strains (Alfonso et al. 2000; Kerssies et al. 1997; Vialta et al. 1999). Vegetative compatibility provides an opportunity for population sub-structuring. Strains that are capable of forming viable heterokaryons with each other are referred to as vegetatively compatible and are members of the same vegetative compatibility group (VCG; Leslie 1993). Members of the same VCG can undergo hyphal fusion, with the potential for transferring nuclear and cytoplasmic elements. VCGs have been very useful for identifying clones of fungi that are largely asexual, and VCGs often correlate with pathogenicity and other traits (Korolev and Katan 1999; Korolev et al. 2000, 2001). Direct tests of heterokaryon formation involve establishment of a stable prototrophic heterokaryon under conditions in which neither of the two complementary auxotrophic mutants could survive or form wild-type growth. Nitrate non-utilizing (Nit) mutants have been widely used for such direct tests in many fungi (Leslie 1993) since the first such attempt by Puhalla (1985). Nit mutants of *B. cinerea* have been described (Delcan and Melgarejo 2002; Levis et al. 1997; Weeds et al. 1998; White et al. 1998). Delcan and Melgarejo (2002) were the first to describe both *nit1* and nitM mutants, and to show complementation between *nit1* and nitM mutants that originated from the same isolates. However, their mutants were unstable. Beever and Parkes (2003) devised a method facilitating recovery of both *nit1* and nitM pairs of *B. cinerea*. They showed the existence of multiple VCGs in *B. cinerea*, with all six field strains examined being in different groups. Subsequent studies have shown that a large number of VCGs exist within *B. cinerea* populations. To date, 59 VCGs have been recognized among 82 field strains of the fungus in New Zealand (Beever and Weeds 2004; Weeds and Beever 2004).

Nitrate non-utilizing mutants are resistant to chlorate, and were obtained on media amended with chlorate, the toxic analogue of nitrate. Selenate is the toxic analogue of another common nutrient, sulphate, and can be used to obtain selenate-resistant (*sel*) mutants (Arst 1968). Weeds et al. (1998) described *sel* mutants in *B. cinerea*, some of which were putative sulphate non-utilizing. Information about the use of sulphate non-utilizing (*sul*) mutants (Harp and Correl 1998) in VCG studies is limited: two *sul* mutants recovered from a strain of *Fusarium oxysporum* were able to complement one another by forming a heterokaryon when co-cultured on minimal medium (MM) amended with sulphate (Correll and Leslie 1987), whereas multiple *sul* mutants from a strain of *Magnaporthe grisea* were unable to complement each other, and the mutants were classified as carrying the mutations at a single locus (Harp and Correll 1998). Weeds et al. (1998) demonstrated the ability of a *sul* mutant to complement a *nit1* mutant generated from the same strain. Complementation between *sul* and Nit mutants was shown also in *F. oxysporum* and *Fusarium solani* (Jacobson and Gordon 1988).

Heterokaryon formation between auxotrophic mutants (both Nit and *sul* mutants are auxotrophs) is the direct method for assessment of vegetative compatibility between different strains. The barrage phenomenon is regarded as the opposite of prototrophic vegetatively compatible heterokaryon formation (Leslie 1993). Barrages occur between vegetatively incompatible strains of many fungi when hyphae of incompatible strains grow into each other and interact in an antagonistic manner, with numerous lethal hyphal fusions and the formation of a layer of dark pigment in the barrage area. Vegetatively compatible strains do not interact in this manner, but grow into each other without altering their morphology (Leslie 1993). Such mycelial incompatibility has been found to be widespread in *B. cinerea* (Beever and Parkes 1993, 2003; Delcan and Melgarejo 2002).

We hypothesize that *sul* mutants of *B. cinerea* might be divided into complementary groups, and that the complementary mutants might be useful for classifying strains into VCGs, and that the correspondence between VCGs and strain properties might be established. Therefore, the objectives of the present study were (1) to recover *sul* mutants of *B. cinerea* and characterize mutants according to their ability to complement each other; (2) to determine the VCG diversity in a sample of an Israeli population of *B. cinerea*, by using complementation between different *sul* mutants or incompatibility reactions among wild-type strains; (3) to estimate diversity among a collection of *B. cinerea* strains with regard to pathogenicity, colony morphology, and resistance to

fungicides; and to search for possible correlations between the latter properties and VCGs.

Materials and methods

Media

The MM used in this study was a modified Vogel's medium N (Korolev et al. 2006; Vogel 1964). MM or MM amended with sodium selenate at 1 g l^{-1} and taurine at 100 mg l^{-1} were used to recover and subculture selenate-resistant mutants. MM amended with *L*-methionine at 0.5 g l^{-1} was used for partial phenotyping of *sel* mutants (Harp and Correll 1998). MM amended with potassium chromate at 1 g l^{-1} and *L*-methionine at 0.04 g l^{-1} was used for partial phenotyping of *sul* mutants (Weeds et al. 1998). MM supplemented with Triton 100 at 0.5 ml l^{-1} was used to generate slow-growing, compact colonies of *B. cinerea* (Grindle 1979). Potato dextrose agar (PDA; Difco Laboratory, Detroit) was used for routine culturing, for comparing growth among the various strains, for preparing inoculum for pathogenicity tests and for testing mycelial incompatibility (barrage). Malt extract agar (MEA; Difco Laboratory, Detroit) amended with NaCl at 40 g l^{-1} (Beever and Parkes 1993, 2003) and an additional 15 g l^{-1} of agar (Difco) was used for testing mycelial incompatibility (barrage). A defined MM modified from the Difco Czapek-Dox agar (CDA) recipe according to Yourman and Jeffers (1999), and CDA amended with carbendazim and iprodione at 0.5, 1.0, 5.0, 50.0 and 500.0 μg of active ingredient ml^{-1} were used to test parental strains for resistance to fungicides (Korolev et al. 2006).

Strains of *B. cinerea*

Twenty-one *B. cinerea* strains from six plant species were collected from open fields or greenhouses in ten sites throughout Israel in 1997 and 1998. Strain B4 originated from France (Table 1). Each strain was stored on PDA slants under 15% glycerol at −20°C. One monoconidial culture was prepared from each strain and stored on PDA at 4°C during this work. The representative strains have been lodged in the Centraalbureau voor Schimmelcultures (CBS) collection (the Netherlands) as follows: B1=CBS 120090, B6=CBS 120091, B11=CBS 120092, and B33=CBS 120093.

Colony morphology and growth rate

Colony morphology and growth rate of *B. cinerea* strains on PDA were compared. An agar plug colonized by mycelium of the appropriate strain was placed in the centre of a 9 cm diam plate and incubated in the dark at 20°C to 22°C. The rates of growth, densities of conidia, and numbers of sclerotia per plate were determined. The colony diameters were measured after 4 days, and ratings for conidial and sclerotial production on the plate were determined after 5, 12, 20 and 28 days, according to a scale of 0 to 5, where 0 = no conidia or sclerotia produced, and 1 to 4 correspond to increasing area of the colony covered with conidiophores and conidia, ranging from sparse to completely covered, or to numbers of sclerotia per colony ranging from 1 to >100. For conidial production, 1=<25% of the colony area; 2=25 to 50%; 3=51 to 75%; and 4>75%. For sclerotial production, 1=1 to 9 sclerotia per colony; 2=10 to 50; 3=51 to 100; and 4>100. The area-under-growth-curve (AUGC) was calculated according to Campbell and Madden (1990) and expressed as a percentage of the maximum possible AUGC for the 28-day growth period. The experiment was arranged as a completely randomized block design (CRD) with three replications (plates) per treatment (strain), and was repeated once. *B. cinerea* colonies on PDA showed some degree of heterogeneity, and the mycelial blocks showing the prevalent colony morphology were used to derive the new colonies for the second repetition of the experiment. The two experiments did not differ significantly according to contrast *t*-test and were combined. Data on colony morphology were subjected to the hierarchical clustering algorithm using Ward's minimum variance method (Romesburg and Clarles 1990) with colony diameter, density of conidia and number of sclerotia specified as *Y* variables, and strain as a label variable, in order to create clusters of similar mean values. Each of the clusters was then used to represent a morphological type, and the morphological types were subjected to one-way analysis of variance (ANOVA) and, when a significant *F* value was observed, pairs of means were subjected to the contrast *t*-test. All tests were performed at $P \leq 0.05$ with JMP 5.0 software (Statistical Analysis Systems

Table 1 *B. cinerea* strains listed by VCG and origin

VCG	Strain[a]	Host plant	Region	Location	Cropping system
1	B1	Pepper	Central Israel	Volcani Center	Greenhouse
2	B4	Grape	France	Bordeaux	Vineyard
3	B6	Strawberry	Central Israel	Volcani Center	Greenhouse
4	B13	Cucumber	Southern Coastal Plain	Netiv Haasara	Greenhouse
5	B14	Strawberry	Central Coastal Plain	Kalansua	Low tunnels
6	B15	Cucumber	Central Coastal Plain	Baka el Garbia	Greenhouse
7	B30	Grape	Judean Hills	Ofra	Vineyard
8	B31	Grape	Judean Hills	Ofra	Vineyard
9	B34	Pepper	Central Israel	Volcani Center	Greenhouse
10	B35	Pepper	Southern Coastal Plain	Besor	Greenhouse
11	BP1	Tomato	Judean Hills	El Arub, Hebron	Greenhouse
12	B18	Tomato	Southern Coastal Plain	Hazav	Greenhouse
13	B16	Cucumber	Central Coastal Plain	Ahituv	Greenhouse
13	B2	Tomato	Central Israel	Volcani Center	Greenhouse
13	B33	Eggplant	North West Negev	Besor	Greenhouse
14	B7	Strawberry	Central Israel	Kfar Menahem	Greenhouse
14	B21	Grape	Judean Hills	Ofra	Vineyard
14	B11	Cucumber	Southern Coastal Plain	Netiv Haasara	Greenhouse
14	B12	Cucumber	Southern Coastal Plain	Netiv Haasara	Greenhouse
15	B3	Eggplant	Central Israel	Volcani Center	Greenhouse
15	B20	Grape	Judean Hills	Ofra	Vineyard
NT	B19	Grape	Judean Hills	Ofra	Vineyard

NT = not tested

[a] All strains except B4 were isolated from plants in Israel.

Institute, Inc., Cary, NC, USA). Mycelial blocks showing unusual (not prevalent) colony morphology were also used to derive the new colonies. Data on the derived colonies' morphology were recorded and compared with the corresponding data for colonies derived from mycelial blocks with prevalent morphology.

Pathogenicity tests

Twenty-two wild-type *B. cinerea* strains were compared for pathogenicity on bean (*Phaseolus vulgaris* cv. Hilda) using two methods: inoculation of detached leaves with conidial suspensions, and inoculation of intact plants with mycelial discs (whole plant assay). For the detached leaf assay, leaves were cut from 2 to 3 week-old plants, and placed on 0.8% agar amended with chloramphenicol at 30 mg l^{-1} in 30 cm diam Petri dishes. To promote infection, the suspension was supplemented with half-strength potato dextrose broth (PDB; Difco Laboratory, Detroit, MI, USA). Non-inoculated control leaves were treated with half-strength PDB. The dishes were covered to maintain high humidity, and kept in a growth chamber under 12 h/day light at 22°C to 24°C. The lesion diameter was measured 72 h after inoculation. Each leaf was inoculated with three to six (depending on the leaf size) 10-µl drops of suspension, containing 10^6 conidia $ml^{-1}$of the appropriate *B. cinerea* strain, and three leaves were used for each strain. The same plants were utilized for the intact plant assay, following leaf removal for the detached leaf assay. Discs of 5 mm diam PDA colonized by mycelium, were cut from 3 day-old cultures, and put (one disc per leaf) on the upper surface of five intact leaves of each of three plants per strain. The inoculated plants were placed under high humidity at 22°C to 24°C, under two transparent polypropylene covers. The diameter of each lesion was measured 4 days after inoculation. Strain B11 formed uniformly extensive lesions and was thus chosen as a reference strain. Mean lesion diameter caused by this strain was taken as 100%, and lesion diameter caused by other strains was determined relative to this strain. Each experiment was arranged as a CRD with 15 replications (lesions) per

treatment (strain). The experiment with detached leaves was repeated three times, and that with whole plants was repeated once. To compare experiments or strains (within each set of experiments), data were analyzed by ANOVA and when a significant F value was observed, pairs of means were subjected to contrast t-tests. All tests were performed at $P \leq 0.05$ with JMP 5.0 software.

Fungicide resistance

Carbendazim (Delsene 50DF, E.I. DuPont de Nemours and Co., Wilmington, DE, USA) and iprodione (Rovral 50 WP, Rhone-Poulenc, Lyon, France) were used to assess resistance of the *B. cinerea* strains to the benzimidazole and dicarboximide classes of fungicides. Appropriate volumes of stock fungicide suspensions were added to molten CDA to give final concentrations of 0.5, 1.0, 5.0, 50.0, and 500.0 μg of active ingredient ml^{-1}. Mycelial plugs of 5 mm diam (one plug per plate), taken from the edge of a 5 day-old colony of each strain on PDA, were placed in the centre of 9 cm diam plates containing fungicide-amended media or non-amended medium as a control. Cultures were incubated at 20°C to 22°C in the dark, and the diameter of each colony was measured after 4 days. The experiment was arranged as a CRD with three replications (plates) per strain per fungicide per concentration, and was repeated once. The experiments did not differ significantly according to ANOVA ($P \leq 0.05$) and were combined. The effective fungicide concentration that inhibited mycelial growth by 50% (EC_{50}) was calculated by fitting a dose–response function to a logistic model using an inverse prediction option with the JMP 5.0 software.

Selenate-resistant *sul* mutants

The *sul* mutants were generated as described previously (Weeds et al. 1998; Korolev et al. 2006). Plugs were taken from the growing edge of 3 to 5 day-old colonies on PDA and placed on MM amended with sodium selenate (1 g l^{-1}) and taurine (100 mg l^{-1}) at six to ten points in 9 cm diam plates, and incubated at 20°C to 22°C in the dark. Selenate-resistant sectors that emerged from the restricted colonies were evident after 2 to 4 weeks. The sectors were transferred to fresh selenate medium and then to MM. The ability of selenate-resistant mutants to utilize sulphur was determined on MM and MM amended with L-methionine (0.5 g l^{-1}). Sectors that grew as thin expansive colonies with little or no aerial mycelium on MM, but similarly to the wild-type on L-methionine-amended MM were considered to be *sul* mutants. Two *B. cinerea* strains were subjected to UV irradiation (UV-C 254 nm, Vilber Lourmat, France; Korolev et al. 2006). Slow compact colonies of *Botrytis* that developed on MM supplemented with Triton 100 were counted and the UV dosage was adjusted to kill 95% of the conidia. The irradiated conidia were then spread on MM amended with sodium selenate at 1 g l^{-1}. After 10 to 14 days, growing colonies were transferred to fresh selenate medium to confirm their resistance, and then to MM.

Phenotyping of *sul* mutants and VCGs

MM amended with potassium chromate at 1 g l^{-1} and methionine at 0.04 g l^{-1} was used for partial phenotyping of the *sul* mutants. Mutants that grew on this medium were regarded as chromate-resistant, and other mutants as chromate-sensitive. To determine if *sul* mutants from a given strain were able to complement each other, the *sul* mutants, including chromate-sensitive and chromate-resistant mutants whenever both phenotypes were recovered, were paired in all possible combinations. Pairs of mycelial plugs were placed approximately 3 cm apart (one pair per plate) on MM in 5 cm diam plates, and incubated at 20°C to 22°C in the dark. Each pairing was repeated at least three times, with two plates in each replication. The plates were monitored for up to 28 days. Complementation of paired *sul* mutants was indicated by the formation of dense aerial mycelium at the contact zone between the two mutants. When mutants of two different strains formed a heterokaryon, the parent strains were assigned to the same VCG.

Mycelial incompatibility (barrage)

Mycelial incompatibility was tested by observing the interaction zone between paired colonies of wild-type *B. cinerea* strains on PDA and on MEA amended with NaCl. Pairs of actively growing mycelial plugs taken from 4 day-old colonies on PDA were placed 3 cm apart in 5 cm diam Petri plates (one pair per plate) and incubated at 20°C to 22°C in the dark. Each pairing was performed at least three times, with

two plates in each replication, and each isolate was paired with itself as a control. The pairings were examined 2 weeks after inoculation. Strains that formed dark pigmentation or exhibited sparse mycelium, with or without dark pigmentation, along the line of confrontation were considered incompatible. Incompatibility between two strains was registered as strong if it was evident and stable in replications; it was registered as weak if the line of confrontation was vague and/or it was inconsistent in the replications.

Results

Morphological and cultural variations among *B. cinerea* strains

Variation among strains in colony morphology were evident (Fig. 1). Cluster analysis of the morphological traits assessed for the strains (colony diameter, density of conidia and number of sclerotia) revealed four distinct clusters representing four morphological types of *B. cinerea*: conidial (five strains), sclerotial (six strains), intermediate (ten strains), and mycelial (one strain). The five most abundantly conidiating isolates (mean AUGC for the 28-day period=73% of the maximal possible AUGC) formed relatively small numbers of sclerotia (mean AUGC=31%) and, conversely, six weakly conidiating isolates (mean AUGC=28%) formed numerous sclerotia (mean AUGC=71%). Isolates forming abundant conidia and isolates forming numerous sclerotia grew faster than isolates with intermediate morphology (Table 2). Phenotypic differences between strains were reproducible as long as typical mycelial blocks (blocks showing the prevalent colony morphology) were used to derive colonies. However, the morphological feature of a strain could be changed by selecting a different phenotype in a series of transfers. For example, a mycelial block with rather abundant conidia taken from the periphery of a weakly conidiating colony and transferred to PDA, produced a colony with more conidia than its parent.

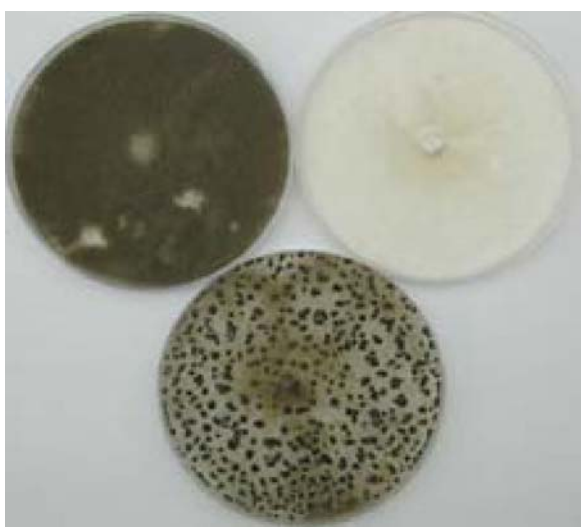

Fig. 1 Morphological differences among *B. cinerea* wild-type isolates. *Top left*: strain B2 with abundant conidiophores and conidia. *Top right*: strain BP1, which is mycelial. *Bottom*: strain B13 with abundant sclerotia. Strains were grown on PDA in 9 cm diam plates at 20°C to 22°C in the dark; photograph was taken 2 weeks after inoculation

Pathogenicity of wild-type *B. cinerea* strains

All strains tested produced lesions on bean leaves, although the size of the lesions varied. The strains differed in aggressiveness; the relative infection rate (compared with B11) ranged from 15% to 126% in the detached leaf assay, and from 40% to 127% in the whole plant assay. The aggressiveness of 15 of the 22 isolates differed significantly among experiments, although strain aggressiveness remained relatively low (B13, B15) or relatively high (B14, B18, B30, and other) across the experiments (Table 3). Strains that produced abundant conidia (conidial morphological type) were on average more aggressive than strains that formed abundant sclerotia (sclerotial morphological type). Strains with intermediate colony morphology showed intermediate aggressiveness in two experiments, whereas these strains did not differ significantly from the conidial or sclerotial morphological types in the other four experiments (Table 4).

Variation in resistance to fungicides among *B. cinerea* strains

Of the 22 *B. cinerea* strains, ten were sensitive to the two fungicides evaluated and 12 showed resistance to one or both of the fungicides (Table 2). The mean EC_{50} for strains resistant and sensitive to carbendazim were >100 and <0.5 μg ml^{-1}, respectively, and for

Table 2 Colony morphology and resistance to fungicides of *B. cinerea* strains

Morphological type[a]	Strain[b]	Density of conidia[c]	Number of sclerotia[c]	Colony diam (mm)[c]	Sensitivity to fungicides	
					Carbendazim	Iprodione
Conidial	B2 (VCG13)	91 (0.9)	22 (5.0)	50 (0.9)	R	S
	B4	71 (4.9)	43 (5.9)	53 (1.5)	R	S
	B1	64 (5.1)	28 (5.3)	55 (1.1)	S	S
	B16 (VCG13)	63 (5.8)	11 (4.2)	61 (1.9)	R	S
	B30	63 (0.9)	24 (5.1)	57 (2.2)	S	S
	Mean	73 a	28 c	55 a		
Intermediate	B14	56 (5.5)	32 (5.1)	44 (1.8)	S	S
	B35	53 (3.1)	71 (4.3)	47 (0.5)	S	S
	B15	49 (2.3)	36 (3.6)	55 (2.3)	R	R
	B18	46 (5.7)	42 (4.5)	52 (0.4)	R	R
	B12 (VCG14)	45 (4.1)	48 (5.4)	49 (1.9)	R	S
	B11 (VCG14)	41 (5.6)	38 (4.9)	52 (2.1)	S	S
	B3 (VCG15)	40 (3.3)	46 (5.0)	53 (2.2)	R	R
	B7 (VCG14)	39 (4.1)	22 (1.7)	49 (1.5)	S	S
	B33 (VCG13)	23 (0.9)	75 (1.0)	51 (2.3)	R	S
	B31	22 (3.3)	60 (4.3)	52 (1.2)	S	S
	Mean	41b	52b	50b		
Sclerotial	B34	62 (2.8)	49 (5.6)	42 (1.6)	R	R
	B20 (VCG15)	32 (5.4)	59 (2.9)	57 (1.7)	R	R
	B21 (VCG14)	30 (4.7)	64 (3.8)	66 (1.9)	S	R
	B19	22 (0.9)	74 (3.6)	57 (0.9)	S	S
	B6	22 (0.9)	65 (5.7)	56 (1.6)	R	R
	B13	18 (3.5)	72 (5.2)	71 (1.1)	S	S
	Mean	31 c	71 a	58 a		
Mycelial	BP1	5 (2.0)	9 (0.8)	59 (1.3)	S	S

R = resistant, *S* = sensitive.

[a] Strains in each morphological type belong to the same cluster of mean values using the hierarchical clustering algorithm by Ward's minimum variance method with colony diameter, density of conidia and number of sclerotia specified as Y variables, and strain number as a label variable.

[b] Strains are listed in descending order of density of conidia produced. Strains belonging to multi-member VCGs are indicated.

[c] Each strain was incubated on PDA in 9 cm diam Petri plates at 20 to 22°C. Diameter of each colony (mm) was measured 4 days after placing a 5 mm diam agar plug colonized by mycelium of the appropriate strain in the centre of the plate. Conidial and sclerotial numbers were measured after 5, 12, 20 and 28 days of incubation, on a scale of 0 to 5, where 0 = no conidia or sclerotia produced, and 1 to 4 correspond to increasing area of the colony covered with conidiophores and conidia ranging from sparse to completely covered; or to numbers of sclerotia per colony ranging from 1 to >100. The AUGC was expressed as a percentage of the maximum possible AUGC. Values for each isolate are means of six replicates with the standard error enclosed in parentheses. In each column, mean values with different letters are significantly different according to contrast *t*-tests ($P \leq 0.05$).

strains resistant and sensitive to iprodione were >20 and <2 μg ml^{-1}, respectively. Six strains were resistant to both carbendazim and iprodione, five were resistant to carbendazim only, and one was sensitive to carbendazim and resistant to iprodione. Five strains assigned to the conidial morphological type were sensitive to both fungicides or resistant to carbendazim only. Strains sensitive to both fungicides and strains resistant to carbendazim only were equally pathogenic (86.4% vs 85.7% respectively, $P=0.508$; ten isolates sensitive to both fungicides vs five isolates resistant to carbendazim only). Strains resistant to two fungicides and a strain resistant to iprodione only belonged to the sclerotial or intermediate morphological types (Table 2). As a group, these strains were less aggressive to beans than strains sensitive to both fungicides (mean lesion size 70.1% vs 86.4%, respectively, $P \leq 0.001$; seven isolates

Table 3 Mean lesion size on bean leaves inoculated with strains of *B. cinerea* compared with that of reference isolate B11

VCG	Strain	Lesion size on bean leaves (% of lesion size caused by strain B11)					
		Detached leaves				Attached leaves	
		Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
1	B1	76.1 bc A	72.3 bc A	68.7 c A	62.4 cd A	87.8 b A	88.8 bc A
2	B4	93.1 ab A	79.4 bc A	NT	91.7 ab A	108.3 a A	77.5 c B
3	B6	68.0 c B	98.9 b A	65.7 c B	80.2 bc AB	60.5 c A	64.7 cd A
4	B13	14.6 e C	25.4 e B	28.9 e B	39.6 e A	40.5 d B	79.9 c A
5	B14	81.9 bc AB	94.8 bc A	69.2 c B	82.7 b AB	94.7 ab B	124.8 a A
6	B15	31.1 d B	51.4 d A	39.4 e B	57.5 d A	55.9 c B	71.3 c A
7	B30	83.7 b B	126.0 a A	74.2 bc B	90.6 ab B	80.1 b	NT
8	B31	NT	NT	82.3 b A	90.4 ab A	NT	126.9 a
9	B34	80.5 bc A	73.4 bc AB	62.0 cd AB	58.3 d B	NT	62.7 d
10	B35	78.2 bc A	NT	74.6 bc A	81.7 b A	NT	109.1 b
11	BP1	86.2 b A	69.7 cd A	78.4 bc A	81.3 b A	74.7 bc	NT
12	B18	NT	NT	88.8 b B	96.6 ab A	102.7 ab A	80.0 c B
13	B16	NT	NT	69.2 c B	96.1 ab A	92.5 b A	98.8 b A
13	B2	87.1 b A	96.2 bc AB	80.7 bc AB	71.7 c B	105.3 ab A	95.1 b A
13	B33	80.6 bc A	84.8 bc A	51.9 d B	64.9 cd B	NT	54.3 d
14	B11	100.0 a A	100.0 b A	100.0 a A	100.0 a A	100.0 ab A	100.0 b A
14	B12	80.6 bc A	75.7 bc A	55.6 d B	60.1 cd B	106.0 ab A	80.2 c B
14	B21	74.9 bc A	64.4 bc A	70.1 c A	70.2 c A	91.0 b A	96.4 b A
14	B7	NT	NT	76.5 bc A	81.9 b A	110.0 a A	88.8 bc B
15	B3	90.1 b A	NT	82.3 b A	81.0 b A	75.6 bc A	79.3 c A
15	B20	87.1 b A	50.3 cd C	70.9 c B	88.5 b A	100.0 ab A	99.7 b A
NT	B19	NT	NT	NT	NT	102.7 ab A	80.0 c B

In each column, values with different lower-case letters are significantly different according to contrast *t*-tests ($P<0.05$). For each strain, means followed by the same upper-case letter refer to mean comparisons among experiments (in a given set of experiments) and are significantly different according to contrast *t*-tests ($P\leq0.05$).
NT = not tested.

Table 4 Mean diameters of lesions on bean leaves caused by strains of *B. cinerea* from different morphological groups

Experiment[a]	Number of lesions	Mean lesion diameter for the morphological group (cluster)[b] (% of lesion diameter caused by reference strain B11)		
		Conidial	Intermediate	Sclerotial
1	263	85.1 A[c]	77.8 B	64.9 C
2	281	93.3 A	68.5 B	63.9 B
3	230	72.9 A	70.7A	63.9 B
4	317	80.8 A	80.3 A	68.9 B
5	309	89.6 A	93.8 A	77.7 B
6	238	98.5 A	91.1 B	80.3 C
Total	1,638	87.4	80.8	69.9

[a] Experiments 1 to 4: inoculation of detached leaves with a conidial suspension of each strain. Experiments 5 and 6: inoculation of attached leaves with mycelial agar block of each strain (see details for each strain in Table 3).
[b] See Table 2 for definition of morphological cluster.
[c] For each experiment (horizontal row), letters refer to differences between morphological clusters. Values with different upper-case letters are significantly different according to contrast *t*-tests ($P\leq0.05$).

resistant to iprodione vs ten isolates sensitive to both fungicides).

Selection of selenate-resistant mutants

The 22 wild-type strains of *B. cinerea* did not grow on MM amended with sodium selenate at 1 g l^{-1}. Therefore, fast-growing sectors arising from the inoculum plugs were considered to be *sel* mutants. The 128 *sel* mutants were recovered from 2,881 colonies (mean frequency of 0.04 sectors per colony). On MM with sulphate as the sole sulphur source, 104 of the *sel* mutants (81%) grew as thin, expansive colonies with little or no aerial mycelium, and were considered to be *sul* mutants (Fig. 2). All *sul* mutants grew normally on MM with L-methionine or PDA. The mean frequency of *sul* recovery ranged from 0.01 to 0.14 sectors/colony depending on strain. Strain B19 produced numerous *sel* sectors, but these never formed thin expansive colonies on MM, thus being *sel* but not *sul*. Strains B15 and B33 were exposed to UV irradiation, which resulted in a high yield of *sel* mutants from B15 and fewer from B33, as >500 *sel* colonies were recovered from strain B15 (i.e. a mutation rate of 1 in 4.7×10^4 conidia), whereas only 11 *sel* colonies were recovered from strain B33 (i.e. a mutation rate of 1 in 3.7×10^6 conidia). No resistant mutants were determined in 1.0×10^7 viable conidia of each strain plated on MM amended with sodium selenate (i.e., background mutation rate <1 in 10^7 viable conidia). Seventy-six percent of the resistant mutants recovered from both isolates did not form thin expansive colonies on MM, thus being *sel* but not *sul*.

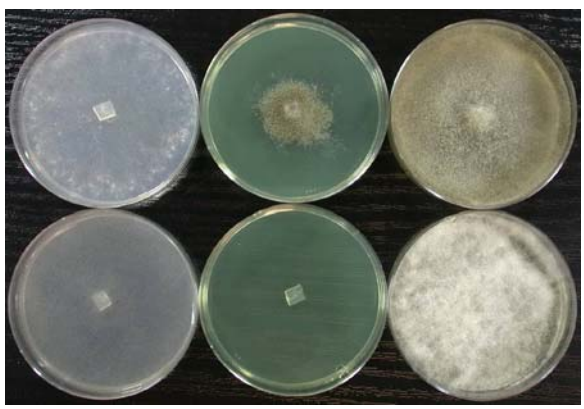

Fig. 2 Growth of sulphate non-utilizing (*sul*) mutants of *B. cinerea* on three media. From *left* to *right*: MM, MM amended with potassium chromate (1 g l^{-1}) and L-methionine (0.04 g l^{-1}), and PDA. *Top*: chromate-resistant mutant B3Sul3; *bottom*: chromate-sensitive mutant B3Sul8. Cultures were grown in 5 cm diam plates at 20°C to 22°C in the dark for 10 days when the photograph was taken

Phenotyping of *sul* mutants and intra-strain pairings

Out of 104 spontaneous *sul* mutants recovered from the 21 *B. cinerea* strains, 38 were chromate-sensitive, and the remainder grew on media amended with chromate (Fig. 2). To determine if *sul* mutants from a given isolate were able to complement each other physiologically, mutants originating from the same strain (including chromate-resistant and chromate-sensitive mutants, if both phenotypes were available) were paired in all possible combinations. Wild-type growth, indicating heterokaryon formation, was always observed between chromate-sensitive and chromate-resistant mutants derived from the same parent strain (Fig. 3). The character of heterokaryotic growth depended on the isolate and the mutant (Fig. 4). Complementary pairs of *sul* mutants from each strain (where available) that exhibited the ability to form strong heterokaryons with each other were chosen as tester strains for VCG determination in inter-strain pairings.

Vegetative compatibility grouping with *sul* mutants

Heterokaryosis was always observed between different *sul* mutants derived from the same parent strain, whereas heterokaryon formation between complementary *sul* mutants derived from different strains was uncommon. A strong incompatibility reaction was observed for 93% of the inter-strain pairings, whereas either strong or weak compatible reactions were observed for only 7% (Table 5). Based on strong compatibility reactions, 12 strains were compatible with themselves only, and were classified as single-member VCGs (VCGs 1 to 12). Other strains were assembled into three multi-member VCGs (VCGs 13 to 15; Table 1). Not all strains in VCG14 were compatible with each other, but they were included in the same group because mutants of B7, B12, and B21 produced heterokaryons with compatible mutants of strain B11. No correlations were evident between VCGs and strain host or geographic origin, i.e., with

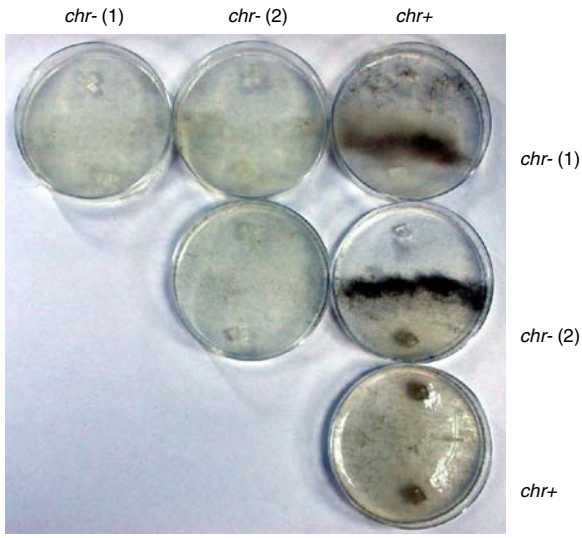

Fig. 3 Heterokaryon formation between chromate-sensitive (chr-) and chromate-resistant (chr+) sulphate non-utilizing (*sul*) mutants of *B. cinerea*, and absence of heterokaryon formation when mutants were paired with themselves or with those carrying the same type of mutation. The photograph shows results for two chromate-sensitive and one chromate-resistant mutant of strain B4, paired on MM in all possible combinations in 5 cm diam plates at 20°C to 22°C in the dark. Photograph was taken 12 days after inoculation

the exception of two strains in VCG14, strains in three multi-member VCGs originated from different hosts and places. Three strains in VCG13 exhibited resistance to carbendazim and susceptibility to iprodione, the two strains in VCG15 were resistant to both fungicides, and only VCG14 contained different phenotypes, i.e., susceptibility to either one or both of the fungicides. Each multi-member VCG contained isolates belonging to different morphotypes (Table 2). Strains in multi-member VCGs showed high aggressiveness to bean; differences among strains were insignificant or inconsistent among replications (Table 3).

Mycelial incompatibility among *B. cinerea* strains (barrage)

No antagonistic interactions were observed when strains were paired with themselves, whereas a barrage, in the form of a zone of dark pigmentation or sparse mycelia, with or without dark pigmentation along the line of confrontation, was observed in 70% of the pairings between strains (Fig. 5; Table 5). Reactions of incompatibility were observed on both PDA and MEA amended with NaCl, although the black zone on MEA was narrower and more evident than that on PDA. Strains differed in the extent of compatibility, from being incompatible with 90% of isolates (B19, BP1) to being compatible with 80% of isolates (B14); 34% of the combinations of strains produced a weak or inconsistent antagonistic interaction. There was no strong incompatibility reaction (barrage) between mycelia of wild-type strains in the three multi-member VCGs. However, strains from different VCGs did not necessarily produce barrages (Table 5).

Discussion

Selenate, a toxic analogue of inorganic sulphate, is inhibitory in many biological systems (Arst 1968). Selenate-resistant mutants with altered sulphate transport and assimilation have been characterized in several fungal genera, including *Acremonium* (Vialta et al. 1999), *Aspergillus* (Arst 1968), *Botrytis* (Weeds et al. 1998), *Fusarium* (Correll and Leslie 1987),

Fig. 4 Variation in intra-strain complementary heterokaryotic growth of *B. cinerea*. From *left* to *right*: Heterokaryons formed between *chr*+ and *chr*− mutants of strains B3 and B31 (*top*); and B3 and BP1 (*bottom*). Different mutants of B3 were used. The pairings were performed on MM in 5 cm diam plates at 20°C to 22°C in the dark. Photograph was taken 12 days after inoculation

Table 5 Complementary heterokaryon formation among *sul* phenotypes (above the diagonal) and barrage formation among wild-type strains (under the diagonal) of *B. cinerea*

	B1	B2	B3	B4	B6	B7	B11	B12	B13	B14	B15	B16	B18	B19	B20	B21	B30	B31	B33	B34	B35	BP1
B1		−	−	−	−	−	−	−	−	−	−	+/−	−	...	−	−	−	−	−	−	−	−
B2	+		−	−	−	−	−	−	−	−	−	+	−	...	−	−	−	−	−	−	−	−
B3	+	+/−		−	−	−	−	+/−	−	−	−	−	−	...	+	−	−	−	−	−	−	−
B4	+	+	+		−	−	−	+/−	−	−	−	−	−	...	−	−	−	−	−	−	−	−
B6	−	−	−	+/−		−	+/−	−	−	−	−	−	−	...	−	−	−	−	−	−	−	−
B7	+	−	+/−	+	+/−		+	+	−	−	−	−	−	...	−	−	−	+/−	−	−	−	−
B11	−	−	+	−	+/−	−		+	−	−	−	−	−	...	−	+	−	−	−	−	−	−
B12	+	+	+	+/−	+/−	−	+/−		−	−	−	−	−	...	−	−	−	−	−	−	−	−
B13	−	+	+	−	+/−	−	−	+/−		−	−	−	−	...	−	−	−	+/−	−	−	−	−
B14	−	−	−	−	−	−	−	+/−	−		−	−	−	...	−	−	−	−	−	−	−	−
B15	−	−	−	+/−	−	+	+/−	+/−	+/−	−		−	−	...	−	−	−	−	−	−	−	−
B16	−	+/−	+	+	+/−	+	+/−	+/−	+/−	+/−	+		+/−	...	−	−	−	−	+	−	−	−
B18	−	+	−	+	−	+/−	+	+/−	+	+/−	−	+		...	−	−	−	−	−	...	−	−
B19	+	+	+	+	+	+/−	+/−	+	−	+	+	+	+		...	...	...	...	...	...	...	...
B20	−	+	+/−	+	−	+/−	+/−	−	+/−	−	+/−	−	−	+		−	−	−	−	−	−	−
B21	−	+/−	+	+	+/−	−	+/−	+/−	+/−	−	+/−	−	+	+/−	+/−		−	−	−	...	−	−
B30	+/−	+	+/−	+	+/−	+	−	+	+/−	−	+	+	+	+	+	+		−	−	−	−	−
B31	+/−	+	+	−	+/−	+/−	+/−	+/−	+/−	−	+	+/−	+/−	+/−	+/−	+	+/−		−	−	...	−
B33	−	+/−	+/−	+/−	−	−	−	−	−	−	−	+/−	−	−	+/−	−	+/−	+/−		−	−	−
B34	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...		−	−
B35	−	+/−	+/−	+/−	+/−	+	−	+/−	−	−	+/−	−	+	+	+/−	+	−	+	−	...		...
BP1	+	+	+	+	+	+	+	+/−	+/−	−	+	+	+	+	+	+	+	+/−	−	...	+	

Above the diagonal: + = Dense prototrophic growth; +/− = weak prototrophic growth; − = prototrophic growth absent; ... = not tested. Dense prototrophic growth was observed in all intra-strain pairings tested. Under the diagonal: + = Strong incompatible reaction; +/− = weak or inconsistent reaction; − = absence of incompatible reaction; ... = not tested. Absence of incompatible reaction was observed in all intra-strain pairings tested.

Magnaporthe (Harp and Correll 1998), and *Neurospora* (Marzluf 1970). Chromate, another toxic analogue of inorganic sulphate, is also transported into the fungal cell by sulphate permease, and is hypothesized to be toxic because intracellular accumulation of the compound causes death, possibly through strong oxidizing effects (Roberts and Marzluf 1971). Five classes of *sul* mutants have been described in *Aspergillus nidulans*: one comprising chromate-resistant mutants, and four different chromate-sensitive genotypes (Arst 1968). Similarly, chromate-resistant, sulphate transport-affected mutations in *Neurospora* were mapped to a single genetic locus, whereas chromate-sensitive mutations were encoded by several loci (Marzluf 1970). Selenate-resistant mutants, both resistant and sensitive to chromate, were identified in *B. cinerea* (Weeds et al. 1998). In the present study, *sel* mutants were recovered spontaneously in the sector method from the 21 *B. cinerea* strains grown in the presence of sodium selenate. Eighty-one percent of the *sel* mutants were also *sul*. UV mutagenesis increased the yield of selenate-resistant mutants, but most of them were normal in sulphate assimilation (*sel* but not *sul*). Similarly, 4-nitroquinoline 1-oxide mutagenesis yielded exclusively chlorate-resistant mutants normal in nitrate assimilation (Crn but not Nit) in *B. cinerea* (Weeds et al. 1998). Based on growth of the mutants on chromate-amended media, *sul* mutants were divided into two phenotypes: chromate-resistant and chromate-sensitive. Vigorous heterokaryotic growth was obtained with all intra-strain pairings of different *sul* phenotypes, demonstrating that: (1) chromate-sensitive and chromate-resistant *sul* mutants complemented one another and could be used for vegetative compatibility studies in *B. cinerea*, and (2) heterokaryon self-compatibility was common in this collection of strains from Israel. Self-compatibility in *B. cinerea* strains has been demonstrated by heterokaryon formation between Nit mutants (Beever and Parkes 2003; Delcan and Melgarejo 2002), and by complementation between *sul*

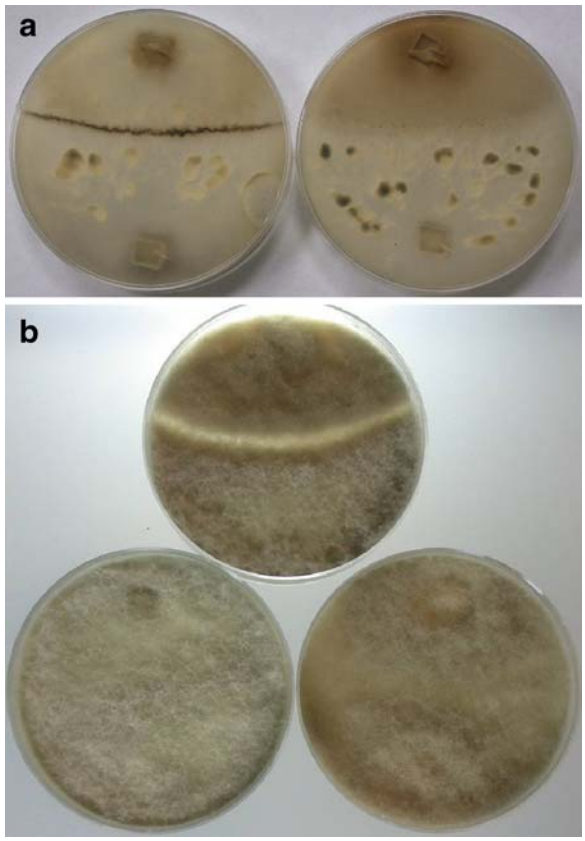

Fig. 5 Barrage formation between incompatible strains of *B. cinerea*. **a** Pigmented interaction zone between incompatible strains B4 and B21 (*left*), and absence of a barrage between compatible strains B7 and B21 (*right*). **b** Interaction zone in the form of sparse mycelium between incompatible strains B4 and B7 (*top*), and absence of barrage when the strains were paired with themselves (*bottom*) on PDA in 5 cm diam plates maintained at 20°C to 22°C in the dark. Photograph was taken 10 days after inoculation

and Nit mutants derived from the same strain (Weeds et al. 1998). Self-compatibility is usual but not universal among fungi, e.g., self-incompatibility is common in the entomopathogen *Verticillium lecanii* (Korolev and Gindin 1999).

In this study, inter-strain incompatibility was widespread among *B. cinerea* strains. Strong heterokaryon formation was observed in only 3.4% of pairings, and an additional 3.9% produced weak complementation. The 22 strains were assigned to 12 single-member VCGs and three multi-member VCGs. Sexually reproducing populations of ascomycetes are expected to have high VCG diversity (Glass et al. 2000). *B. cinerea* is the anamorph of the ascomycete *B. fuckeliana*, and the species showed a high level of vegetative incompatibility in previous studies. Using Nit mutants, 59 VCGs were recognized amongst 82 strains of *B. cinerea* from New Zealand, with few strains in the same group. Along with an additional seven VCGs found among progenies of sexual crosses between two strains, these results suggested that more than six *vic* genes controlled vegetative compatibility in *B. cinerea*, since six *vic* genes would generate 2^6=64 VCGs (Beever and Weeds 2004; Weeds and Beever 2004). Our data on VCG diversity in Israel (tested by *sul* mutants) agree with those reported for New Zealand (tested by Nit mutants), i.e., about 80% of the VCGs are composed of a single strain, and the remainder contained two to four strains per VCG. The large number of VCGs identified, and the small proportion that contained more than one strain suggest that sexual recombination occurs in populations of *B. fuckeliana* in Israel, although apothecia are seldom found in nature and have never been found in areas with a warm dry climate, such as Israel or southern Spain (Beever and Weeds 2004; Raposo et al. 2001; Yunis and Elad 1989).

The four strains in the multi-member VCG14 were not mutually compatible, but were grouped together because of their compatibility reactions with mutants of the bridging strain that was compatible with three other isolates. The occurrence of bridging strains and cross-VCG compatibility is well known in *F. oxysporum* (Katan and Katan 1999; Katan et al. 1991; Vakalounakis and Fragkiadakis 1999). In ascomycetous fungi, vegetative incompatibility has been determined by a number of *vic* genes that exist in two or more allelic states, and generally, one allelic difference between strains can cause incompatibility (Glass et al. 2000). The existence of cross-VCG compatibility indicates that not all alleles have to be similar to allow a compatible reaction between strains. Cross-VCG compatibility may contribute to difficulties in VCG classification of strains of *B. cinerea*. It should be noted that VCG grouping based on both strong and weak complementation could lead to a different VCG classification in this work: only six isolates would be regarded as single-member VCGs, whereas others could be combined into two groups comprising five and ten isolates, related directly or via bridging strains.

Mycelial incompatibility (barrage) is widespread in populations of *B. cinerea* (Beever and Parkes 2003; Delcan and Melgarejo 2002). In the present study, a barrage between wild-type *B. cinerea* strains, in the

form of a zone of dark pigmentation or of sparse mycelia with or without dark pigmentation along the line of confrontation, was observed in 36% of the combinations of strains, and a weak antagonistic reaction was observed for an additional 34% of the pairings. No visible adverse reaction was noticed in 30% of the pairings. There was no correspondence in compatibility between strains revealed by the two different experimental approaches, since strains from different VCGs did not necessarily produce barrages. However, self-compatibility was observed both as heterokaryon formation between complementary *sul* mutants and as an absence of barrages between mycelia of wild-type strains; wild-type strains belonging to the same VCG did not exhibit strong antagonistic reactions, although weak antagonistic interactions could occur. In another study, single-ascospore strains of *B. cinerea*, after a series of backcrosses, were complemented by Nit mutants but still produced a dark interaction line, and the authors concluded that it was premature to equate mycelial and vegetative incompatibility in *B. cinerea* (Beever and Weeds 2004). The lack of a direct correlation between mycelial and vegetative compatibility was reported in *Sclerotinia sclerotiorum* and *Neurospora crassa* (Ford et al. 1995), and genetic control of barrage formation in *N. crassa* was found to operate independently from that of heterokaryon incompatibility and mating type (Micali and Smith 2003).

Diversity in colony morphology among *B. cinerea* strains is widely known. Typically, the strains are classed as mycelial, sporulating (conidial) or sclerotial, with sub-types, and may differ in saprophytic or parasitic fitness (Beever and Weeds 2004). We described the growth characteristics of 22 strains (21 from Israel) on PDA, and identified four morphological types: conidial, which produce abundant conidia and rather few sclerotia; sclerotial, which produce abundant sclerotia and fewer conidia than the conidial type; and intermediate, which produce both conidia and sclerotia, but generally fewer of either of the former; one strain developed aerial mycelia with a negligible amount of conidia, and was classified as mycelial. The morphological types differed in pathogenicity on bean leaves: as groups, strains forming abundant conidia were more aggressive than those with abundant sclerotia; strains with intermediate colony morphology had intermediate aggressiveness. Previous studies also have shown that different morphological types of *B. cinerea* differed in virulence, with the sclerotial type more virulent on strawberry, and the mycelial type more virulent on flax (Lorbeer 1980). Resistance to dicarboximides also may affect pathogenicity: strains resistant to iprodione were less aggressive in our study than sensitive strains. Fitness of dicarboximide-resistant *B. cinerea* strains assessed by survival of sclerotial was lower than fitness of sensitive isolates (Raposo et al. 2000).

The diversity and instability in colony morphology among *B. cinerea* strains is attributed to the heterokaryotic nature of the fungus: monoconidial isolates derived from a single parent often differ morphologically from the parent and from each other. The large number of VCGs observed in the field suggests that in nature fusion of genetically different strains in the same VCG is uncommon, which is inconsistent with the wide occurrence of heterokaryons (Beever and Weeds 2004). The authors suggest that the main mechanism for heterokaryon generation is mutation within long-lived somatic lineages. In the imperfect fungus *Verticillium dahliae*, known for morphological instability, the presence or absence of microsclerotia in progenies derived from single conidia (resulting in mycelial or microsclerotial morphological types) is at least partially controlled by mitochondria which are self-replicating and transmitted via the cytoplasm of the conidia (Heale 1988). A similar mechanism may play a role in the control of sclerotia production and morphological diversity in *B. cinerea*. The possibility to change the *B. cinerea* colony morphology by transferring mycelial blocks in several passages supports this idea.

The main findings of this research are the demonstration that *sul* mutants are useful in defining VCGs in *B. cinerea*, and that there are numerous VCGs in a sample of the population (14 amongst 21 strains). Mycelial incompatibility (barrage) was common between strains but the groups defined using barrage did not closely match those defined by complementation. No correlations were detected between VCGs and strain host or geographic origin or colony morphology or pathogenicity. Pathogenicity depended on the morphological characteristics of strains.

Acknowledgements This research was supported, in part, by a fellowship from the Israeli Ministry of Immigration and by the research fund of the Chief Scientist of the Israeli Ministry of

Agriculture and Rural Development, project no. 132-1106-03. Contribution No 502/06 from the ARO, Volcani Center, Institute of Plant Protection.

References

Alfonso, C., Raposo, R., & Melgarejo, P. (2000). Genetic diversity in *Botrytis cinerea* populations on vegetable crops in greenhouses in south-eastern Spain. *Plant Pathology*, *49*, 243–251.

Arst, H. N. (1968). Genetic analysis of the first steps of sulphate metabolism in *Aspergillus nidulans*. *Nature*, *219*, 268–270.

Beever, R. E., & Parkes, S. L. (1993). Mating behavior and genetics of fungicide resistance of *Botrytis cinerea* in New Zealand. *New Zealand Journal of Crop and Horticultural Science*, *21*, 303–310.

Beever, R. E., & Parkes, S. L. (2003). Use of nitrate non-utilizing (Nit) mutants to determine vegetative compatibility in *Botryotinia fuckeliana* (*Botrytis cinerea*). *European Journal of Plant Pathology*, *109*, 607–613.

Beever, R. E., & Weeds, P. L. (2004). Taxonomy and genetic variation of *Botrytis* and *Botryotinia*. In Y. Elad, P. Williamson, P. Tudzinski, & N. Delen (Eds.), *Botrytis*: *Biology*, *pathology and control* (pp. 29–52). Dordrecht: Kluwer Academic.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. New York: Wiley.

Correll, J. C., & Leslie, J. H. (1987). Recovery of spontaneous selenate resistant mutants from *Fusarium oxysporum* and *Fusarium moniliforme*. *Phytopathology*, *77*, 1710.

Delcan, J., & Melgarejo, P. (2002). Mating behaviour and vegetative compatibility in Spanish populations of *Botryotinia fuckeliana*. *European Journal of Plant Pathology*, *108*, 391–400.

Elad, Y., Williamson, B., Tudzynski, P., & Delen, N. (2004). *Botrytis* spp. and diseases they cause in agricultural systems—an introduction. In Y. Elad, P. Williamson, P. Tudzinski, & N. Delen (Eds.), *Botrytis*: *Biology*, *pathology and control* (pp. 1–8). Dordrecht: Kluwer Academic.

Ford, E. J., Miller, R. V., Gray, H., & Sherwood, J. E. (1995). Heterokaryon formation and vegetative compatibility in *Sclerotinia sclerotiorum*. *Mycological Research*, *99*, 241–247.

Glass, N. L., Jacobson, D. J., & Shiu, P. T. K. (2000). The genetics of hyphal fusion and vegetative incompatibility in filamentous fungi. *Annual Review of General*, *34*, 165–168.

Grindle, M. (1979). Phenotypic differences between natural and induced variants of *Botrytis cinerea*. *Journal of General Microbiology*, *111*, 109–120.

Harp, T. L., & Correll, J. C. (1998). Recovery and characterization of spontaneous selenate-resistant mutants of *Magnaporthe grisea*, the rice blast pathogen. *Mycologia*, *90*, 954–963.

Heale, J. B. (1988). *Verticillium* spp., the cause of vascular wilts in many species. *Advances in Plant Pathology*, *6*, 291–312.

Jacobson, D. J., & Gordon, T. R. (1988). Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. *Phytopathology*, *78*, 668–672.

Katan, T., & Katan, J. (1999). Vegetative compatibility grouping in *Fusarium oxysporum* f. sp. *radicis-lycopersici* from the UK, the Netherlands, Belgium and France. *Plant Pathology*, *48*, 541–549.

Katan, T., Zamir, D., Sarfatti, M., & Katan, J. (1991). Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology*, *81*, 255–262.

Kerssies, A., Bosker-van-Zessen, A. I., Wagemakers, C. A. M., & van Kan, J. A. L. (1997). Variation in pathogenicity and DNA polymorphism among *Botrytis cinerea* isolates sampled inside and outside a glasshouse. *Plant Disease*, *81*, 781–786.

Korolev, N., & Gindin, G. (1999). Vegetative compatibility in the entomopathogen *Verticillium lecanii*. *Mycological Research*, *103*, 833–840.

Korolev, N., & Katan, T. (1999). Vegetative compatibility grouping in *Verticillium nigrescens* and *V. tricorpus*. *Mycological Research*, *103*, 65–76.

Korolev, N., Katan, T., & Elad, Y. (2006). Use of selenate-resistant strains as markers for the spread and survival of *Botrytis cinerea* under greenhouse conditions. *Phytopathology*, *96*, 1195–1203.

Korolev, N., Katan, J., & Katan, T. (2000). Vegetative compatibility groups of *Verticillium dahliae* in Israel: Their distribution and association with pathogenicity. *Phytopathology*, *90*, 529–536.

Korolev, N., Perez-Artes, E., Bejarano-Alcazar, J., Rodrigues-Jurado, D., Katan, J., Katan, T., et al. (2001). Comparative study of genetic diversity of *Verticillium dahliae* from cotton in Spain and Israel. *European Journal of Plant Pathology*, *107*, 443–456.

Leslie, J. F. (1993). Fungal vegetative compatibility. *Annual Review of Phytopathology*, *31*, 127–151.

Levis, C., Fortini, D., & Brygoo, Y. (1997). Transformation of *Botrytis cinerea* with nitrate reductase gene (niaD) shows a high frequency of homologous recombination. *Current General*, *32*, 157–162.

Lorbeer, J. W. (1980). Variation in *Botrytis* and *Botryotinia*. In J. R. Coley-Smith, K. Verhoeff, & W. R. Jarvis (Eds.), *The biology of Botrytis* (pp. 19–39). London: Academic.

Marzluf, G. A. (1970). Genetic and metabolic controls for sulfate metabolism in *Neurospora crassa*: Isolation and study of chromate-resistant and sulfate transport-negative mutants. *Journal of Bacteriology*, *102*, 716–720.

Micali, O. C., & Smith, M. L. (2003). On the independence of barrage formation and heterokaryon incompatibility in *Neurospora crassa*. *Fungal General Biology*, *38*, 209–219.

Puhalla, J. E. (1985). Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany*, *63*, 179–183.

Raposo, R., Gomez, V., Urrutia, T., & Melgarejo, P. (2000). Fitness of *Botrytis cinerea* associated with dicarboximide resistance. *Phytopathology*, *90*, 1246–1249.

Raposo, R., Gomez, V., Urrutia, T., & Melgarejo, P. (2001). Survival of *Botrytis cinerea* in southern Spanish greenhouses. *European Journal of Plant Pathology*, *107*, 229–236.

Roberts, K. R., & Marzluf, G. A. (1971). The specific interaction of chromate with the dual sulfate permease systems of *Neurospora crassa*. *Archives of Biochemical Biophysics*, *142*, 641–659.

Romesburg, H., & Clarles, A. (1990). *Cluster analysis for researchers* (p. 340). Malabar: Krieger.

Vakalounakis, D. J., & Fragkiadakis, G. A. (1999). Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. *Phytopathology*, *89*, 161–168.

Vialta, A., Catani, C. F., Junior, R. B., & Azevedo, J. L. (1999). Isolation and characterization of selenate resistant mutants of *Acremonium chrysogenum*. *Brazil Archives of Biological Technology*, *42*, 369–374.

Vogel, H. J. (1964). Distribution of lysine pathways among fungi: evolutionary implications. *American Naturalist*, *98*, 435–446.

Weeds, P. L., & Beever, R. E. (2004). Vegetative compatibility groups in Botryotinia fuckeliana. Abstract. In: XIII International *Botrytis* Symposium, Antalya, Turkey, p. 78. Izmir, Turkey: Syngenta Crop Protection.

Weeds, P. L., Beever, R. E., & Long, P. L. (1998). New genetic markers for *Botrytis cinerea* (*Botryotinia fuckeliana*). *Mycological Research*, *102*, 791–800.

White, G. J., Dobinson, K., & Traquair, J. A. (1998). Selection of nitrate-nonutilizing mutants in *Verticillium*, *Alternaria*, and *Botrytis*. *Canadian Journal of Plant Pathology*, *20*, 340.

Yourman, L. F., & Jeffers, S. N. (1999). Resistance to benzimidazole and dicarboximide fungicides in greenhouse isolates of *Botrytis cinerea*. *Plant Disease*, *83*, 569–575.

Yunis, H., & Elad, Y. (1989). Survival of dicarboximide-resistant strains of *Botrytis cinerea* in plant debris during summer in Israel. *Phytoparasitica*, *17*, 13–21.