Eur J Plant Pathol (2011) 129:591–608
DOI 10.1007/s10658-010-9723-9

Screening of *Botrytis cinerea* isolates from vineyards in Israel for resistance to fungicides

Nadia Korolev · Mukhiddin Mamiev · Tirza Zahavi · Yigal Elad

Accepted: 15 November 2010 / Published online: 28 December 2010

Abstract Plots in two vineyards in the Golan Heights, Israel were treated with six botryticides during three growing seasons with 3 applications per season. Applications of fenhexamid, pyrimethanil and cyprodinil + fludioxonil were effective, resulting in 52–65% and 53–63% mean reduction in grey mould incidence and severity, respectively. Carbendazim, fluazinam and iprodione were ineffective or slightly effective. Five hundred and sixteen *B. cinerea* isolates were collected from infected berries or trapped from the air in the vineyards, and profiles of sensitivity to benomyl, fenhexamid, fluazinam, fludioxonil, iprodione and pyrimethanil were established for each of the isolates based on a mycelial growth test. Seventy-four percent of the isolates were sensitive to the six tested fungicides, and the other 26% of the isolates were classified into 10 phenotypes characterized by resistance to one or more fungicides. Resistant isolates showed fitness parameters similar or reduced in comparison to sensitive isolates. Resistance to benzimidazoles and to dicarboximides was the most frequent (up to 25%) and apparently pre-existed in the populations tested. Increased frequency of benzimidazole resistance, but not dicarboximide resistance, was observed following the 3 years of applications of the fungicides. High level resistance to pyrimethanil was present at a frequency of about 2% in both vineyards in the first 2 years of the sampling survey and reached 10% in the third year at Site 2. A few isolates were resistant to fenhexamid or fludioxonil (0.8 or 0.2%, respectively). No strong resistance to fluazinam was detected, although numerous, less sensitive isolates, presumably possessing multi-drug resistance traits, were recovered at higher frequency from the plots treated with fluazinam than from the untreated plots.

Keywords Benomyl · Fenhexamid · Fluazinam · Fludioxonil · Iprodione · Pyrimethanil

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

T. Zahavi
Extension Service, Ministry of Agriculture,
Kiryat-Shemone, Israel

Introduction

Grey mould, caused by *Botrytis cinerea* Pers.:Fr., causes damage to grapes (*Vitis vinifera*) in vineyards throughout the world. The first signs of disease on grapes often appear during the bloom period, when the fungus attacks the flower parts. These early infection sites are a source of inoculum that can cause severe bunch rot near harvest, although not all fruit infections at harvest are the result of floral and latent infections during the bloom period. The infected fruit may become covered with greyish-tan conidia of the

fungus. Berry stalks and cluster stems may be invaded, causing them to shrivel, and berries that have split or have been punctured are often attacked by other organisms (Elmer and Michailides 2004).

Chemical control remains an important method for managing grey mould, although its efficacy may be limited by the development of pathogen resistance. The fungicides used in this study belong to different families of synthetic botryticides and can be classified into five categories according to their biochemical modes of action (Leroux 2004): (i) the anti-microtubule toxicants, specifically the benzimidazole fungicides carbendazim and benomyl; (ii) fungicides whose toxicity can be reversed by applications of amino acids, specifically the anilinopyrimidine fungicides cyprodinil and pyrimethanil; (iii) a toxicant affecting fungal respiration, specifically the phenylpyridinamine fungicide fluazinam; (iv) compounds affecting osmoregulation, specifically the phenylpyrrole fungicide fludioxonil and the dicarboximide fungicide iprodione; and (v) a sterol biosynthesis inhibitor, specifically the hydroxyanilide fungicide fenhexamid.

Benzimidazole and dicarboximide fungicides have been used since the 1970s, and the intensive use of these site-specific fungicides has led to the rapid selection of resistant strains in various countries including Israel (Elad et al. 1992; Leroux 2004). Modern botryticides with different modes of action (anilinopyrimidines, hydroxyanilides, phenylpyridinamines and phenylpyrroles) were introduced in the 1990s (Leroux 2004). Resistance to these fungicides in *Botrytis* has been documented in California, Chile, France, Japan, Switzerland and other regions (Baroffio et al. 2003; Esterio et al. 2007; Förster et al. 2007; Ma and Michailides 2005; Leroux 2004; Myresiotis et al. 2007), but has never been characterized in Israel. The current research was initiated, in part, to address growers' concerns regarding control failures, and the presence and prevalence of fungicide-resistant populations of *B. cinerea* in Israeli vineyards. The objectives of the research were: (i) to determine if resistance to the modern fungicides fenhexamid, fluazinam, fludioxonil and pyrimethanil as well as resistance to older fungicides benomyl and iprodione exists in vineyards in the Golan Heights; (ii) to characterize the *B. cinerea* isolates from two vineyards for the level of sensitivity to the above-mentioned fungicides and for fitness parameters; (iii) to determine the efficacy of the fungicides in controlling bunch rot in the two vineyards, and (iv) to monitor the influence of the fungicide applications over three growing seasons on the sensitivity of *B. cinerea* populations.

Materials and methods

Fungicides

Technical grade benomyl, fenhexamid, fluazinam, fludioxonil, iprodione and pyrimethanil were used in the laboratory tests. The fungicides benomyl (Benlate 50 WP; DuPont, Wilmington, DE), fenhexamid (Teldor SC 500; Bayer Crop Science, Monheim, Germany), fluazinam (Ohayo SC 500; Ishihara Sangyo Kaisha, Japan), fludioxonil (Celest SC 230; Syngenta Crop Protection, Basel, Switzerland), iprodione (Rovral 50 WP; Bayer Crop Science) and pyrimethanil (Mythos SL 300; Bayer Crop Science) were evaluated in the greenhouse studies. The fungicides carbendazim (Delsene 50 WP, DuPont, France), fenhexamid, fluazinam, iprodione and pyrimethanil, as well as a combination treatment of cyprodinil and fludioxonil (Switch WG; Syngenta Crop Protection, 37.5% cyprodinil a.i. and 25% fludioxonil a.i.) were used in our field study.

Growth media

Potato dextrose agar (PDA; Difco Laboratory, Detroit, MI) was used for the routine culturing of fungal isolates. PDA amended with 0.15 $g l^{-1}$ chloramphenicol was used for isolating *B. cinerea* from diseased grape berries. The minimal media (CDA) derived from the Difco Czapek-Dox recipe (Yourman and Jeffers 1999) and CDA amended with the different fungicides were used to test *B. cinerea* strains for sensitivity to fungicides. This media contained: 3 $g l^{-1}$ $NaNO_3$, 1 g l^{-1} KH_2PO_4, 1 $g l^{-1}$ K_2HPO_4, 0.5 $g l^{-1}$ $MgSO_4 \times 7H_2O$, 0.5 $g l^{-1}$ KCl, 0.01 $g l^{-1}$ $FeSO_4 \times 7H_2O$, 15 $g l^{-1}$ agar and 10 $g l^{-1}$ glucose. Appropriate volumes of stock fungicide suspensions were added to molten CDA for final active ingredient concentrations of 0.001–10.0 μg ml^{-1} (six concentrations as described in "Sensitivity tests"). Stock fungicide suspensions were stored at 4°C and were refreshed each month. Botrytis spore trap media (BSTM) (Edwards and Seddon 2001) and BSTM amended with the different fungicides were

used for trapping *B. cinerea* conidia from the air. The BSTM contained: 0.1 gl^{-1} $NaNO_3$, 0.1 gl^{-1} K_2HPO_4, 0.2 gl^{-1} $MgSO_4 \times 7H_2O$, 0.1 gl^{-1} KCl, 0.2 gl^{-1} chloramphenicol, 0.02 gl^{-1} pentachloronitrobenzene (PCNB, Quintozene, Uniroyal Chemicals, Kharagpur, India), 0.02 gl^{-1} maneb (Maneb 80 WP; Cerexagri Inc., King of Prussia, PA), fenarimol (Rubigan 120 EC; Dow Agrosciences, Indianapolis, IN) at 0.1 ml l^{-1}; 5 gl^{-1} tannic acid; 2 gl^{-1} glucose and 20 gl^{-1} agar. The pH was adjusted to 4.5 with 1 M NaOH before the agar was poured.

Field trials, spray programs and monitoring procedures

The experiment was conducted in the 2006 to 2008 growing seasons at two commercial vineyards: At Site 1 (Ortal), the experiment was conducted on 22-year-old vines of cv. Sauvignon Blanc. At Site 2 (Sha'al), the experiment was conducted on 11-year-old plants of cv. Pinot Noir. These two sites are ≈ 5 km apart, in the Golan Heights ≈ 20 km east of the city of Kiryat-Shemone (33°12′27″N and 35°34′11″E) about 800 m above sea level, on dark volcanic soils. There was no rainfall in the area from June through September in 2006, 2007 and 2008, and the weather was hot and dry. The main fungicides used commercially in the vineyards before this study commenced were benzimidazole and dicarboximide fungicides, which had been used for a long period, and anilinopyrimidine fungicides which were used during the last year before the trial. At each site, treatment plots were arranged in a randomized complete block design with four replicates; each plot included six to nine vines; the same plants were treated for 3 years in a row. Fungicides for the control of *B. cinerea* were applied with a hand-held gun sprayer at 100 psi in a volume of 1000 lha^{-1}. These fungicide treatments were applied three times each season with a 2-week interval beginning in late July at veraison (onset of ripening). The plots were treated at doses recommended for commercial control of vines grey mould as follows: Delsene 50 WP 1.0 kg ha^{-1} (50% a.i.; 0.1%); Mythos SL 300 2.5 kg ha^{-1} (30% a.i.; 0.25%); Ohayo SC 500 (50% a.i.), Rovral 50 WP (50% a.i.) and Teldor SC 500 (50% a.i.) 1.5 kg ha^{-1} (0.15%) and Switch WG (37.5% a.i. cyprodinil and 25% a.i. fludioxonil) 1.0 kg ha^{-1} (0.1%). Disease incidence was evaluated three times each season, beginning 10–12 days after the first spray; for a second time 7–10 days after the first evaluation, and for a third time 7–10 days later by examining 30 clusters from each plot each time. Disease incidence was calculated as the percentage of clusters showing any disease symptoms. Disease severity was assessed as the percentage of symptomatic berries per cluster (starting with 1% grade and then at an incremental step of 10%: 1, 10, 20 % ...) (Savary et al. 2009), and mean disease severity was calculated for each plot by averaging severity estimates for each rated cluster.

Strains of *B. cinerea* from diseased grape berries and from the air in vineyards

Diseased berries were collected randomly from each treatment, shortly before harvest. A small piece (approximately 2×2 mm) of PDA amended with chloramphenicol was placed in contact with a conidiating fruit lesion. The agar piece with the adhering conidia was then placed in the center of a Petri dish containing PDA with chloramphenicol and incubated at 20°C in the dark. After 3–4 days, an agar plug from the leading edge of the formed colony was transferred to the center of a new Petri dish containing PDA. The dishes were placed on a bench in the laboratory for 10–14 days. Conidia were harvested from the dishes by pipetting 3 ml of a sterile aqueous solution of 0.01% Tween 80 into each dish, rubbing the colonies gently with sterile disposable spreader, and then using a pipette to transfer the conidial suspensions into sterile glass tubes through a layer of Miracloth (Calbiochem; Inc. La Jollia, CA). An equal volume of sterile 30% glycerol in water was added to each tube, resulting in a final concentration of 15% glycerol. Conidial suspensions were transferred to 2-ml cryogenic vials and stored in an ultra-low-temperature freezer at −80°C.

B. cinerea was also trapped from the air in the two vineyards during August and September of 2007 and 2008, in 9-cm-diameter plates containing plain BSTM (BSTM as described in "Growth media") or fungicide-amended BSTM. Fungicides were added to BSTM at the concentrations preventing the formation of colonies of sensitive, but not of resistant isolates on this media. To define these concentrations, circa 100 conidia were spread on 9-cm-diam. plates filled with BSTM and BSTM amended with the fungicides at four concentrations ranging in a tenfold

dilution series (μg a.i. ml^{-1}) starting from EC_{50} value for sensitive isolate: benomyl 0.025, 0.25, 2.5 and 25.0; fenhexamid and fludioxonil 0.005, 0.05, 0.5 and 5.0; iprodione 0.2, 2.0, 20.0 and 200.0; and pyrimethanil 0.05, 0.5, 5.0 and 50.0. These cultures were incubated for 7–10 days at 20°C, and the colonies were then counted. Three resistant isolates (where available) and three isolates sensitive to each fungicide obtained from grapes in this work or from other hosts in Israel (Table 1), were used. Three plates were used for each fungicide concentration, and the experiment was repeated twice. The concentrations preventing the formation of sensitive but not resistant to fungicides colonies were 100 times the EC_{50} values of the isolates sensitive to benomyl, iprodione, fenhexamid and pyrimethanil; and 1000 times the EC_{50} values of the isolates that were sensitive to fludioxonil. No fluazinam-resistant isolates were available in our collection of isolates from plants, and we did not try to recover them from the air. The media were found to be effective in preliminary experiments in which *B. cinerea* conidia were collected from artificially infested air (data not shown). Plates with plain BSTM and BSTM amended with the fungicides at concentrations preventing growth of sensitive isolates were exposed in the vineyards near midday, when the concentration of conidia is expected to reach a peak (Fitt et al. 1985). Three plates of each medium were placed on the ground in each vineyard plot and were left open for 1 h, after which the plates were closed and incubated for 10 d at 20°C in the dark. The colonies on each plate were then counted, the representative colonies transferred to CDA, grown, saved as described, and further tested to confirm any apparent fungicide resistance as described in "Sensitivity tests".

A total of 516 isolates of *B. cinerea* from two Golan Heights vineyards (332 isolates from diseased berries and 184 isolates from plain BSTM exposed to the air) were recovered and saved. Additionally, sixty *B. cinerea* isolates from the laboratory collection, which were recovered from different hosts and locations in Israel in previous work (Korolev et al. 2009), were included in this study (Table 1).

Sensitivity tests

For 73 isolates (13 isolates isolated from grapes from Site 1 or Site 2 in June 2006 prior to fungicide treatments, and 60 isolates from other hosts and locations in Israel; Table 1), the concentration that causes 50% inhibition of mycelial growth (EC_{50} value) was determined by growing *B. cinerea* isolates on CDA amended with six concentrations of the fungicides. The concentrations of the conidial suspensions were standardized to 10^5 conidia/ml. Twenty μl of each suspension was pipetted onto PDA discs (10 mm diameter, 4 mm thick). The discs were incubated in a Petri dish for 17 h at 20°C in the dark. During this time, more than 90% of the conidia germinated and formed young, homogeneous mycelia with no conidia, which was then used as inoculum (Hilber and Hilber-Bodmer 1998). The agar discs containing the mycelia were then inverted onto plates (three discs per plate) containing CDA media without any fungicide, or amended with each of the fungicides at five concentrations in a tenfold dilution series and one additional concentration close to the estimated EC_{50} of sensitive isolates (μg a.i. ml^{-1}) based on data of preliminary experiments: benomyl at 0.001, 0.01, 0.025, 0.1, 1.0 and 10; fenhexamid at 0.001, 0.005, 0.01, 0.1, 1.0 and 10; fluazinam at 0.001, 0.01, 0.05, 0.1, 1.0 and 10.0; fludioxonil at 0.001, 0.005, 0.01, 0.1, 1.0 and 10.0; iprodione at 0.001, 0.01, 0.1, 0.5, 1.0 and 10.0; and pyrimethanil at 0.001, 0.01, 0.05, 0.1, 1.0 and 10.0. Each plate was inoculated with 3 replicate discs and then incubated for 3 days at 20°C in the dark. Each experiment was repeated once. Mean colony diameter was measured and expressed as percentage of mean diameter of the untreated control, and EC_{50} values, discriminating doses (DD), and relative mycelial growth (RG) were defined. The DD was defined as the concentration at which *B. cinerea* isolates could be separated into two groups: those inhibited in the presence of the fungicide and those not inhibited, and considering the baseline sensitivity and DD values described earlier: benomyl, fenhexamid, fluazinam, fludioxonil and pyrimethanil were used at DD of 0.1 μg ml^{-1}, and iprodione at 1.0 μg ml^{-1} (Baroffio et al. 2003; Beever et al. 1989; Hilber and Hilber-Bodmer 1998; Leroux et al. 1999). Fungicide sensitivity categories (sensitive and resistant) were defined as follows: benomyl, fenhexamid, fluazinam, fludioxonil and pyrimethanil resistant if $EC_{50} \geq 0.1$ μg ml^{-1}; and iprodione resistant if $EC_{50} \geq$ 0.8 μg/ml. The RG was defined as the difference between the mean colony diameter on the media amended with fungicide and the diameter of the agar

Table 1 Isolates of *Botrytis cinerea* used in this study

Host common name (Scientific name)	Origin	Year of isolation	No. of isolates
Isolates from the Golan Heights experimental sites [a]			
Grape (*Vitis vinifera*)	Ortal (Site 1)	2006–2007	133
	Sha'al (Site 2)	2006–2008	383
Additional isolates of different origins [b]			
Cucumber (*Cucumis sativus*)	Ahituv, Baka el Garbia, Nativ Haasara	1997–2006	13
Eggplant (*Solanum melongena*)	Bet Dagan	1997	1
Grape (*Vitis vinifera*)	Kefar Nahush, Lakhish, Ofera	1997–2006	9
Lisianthus (*Eustoma grandiflorium*)	Besor	2005–2006	25
Bell pepper (*Capsicum annuum*)	Bet Dagan	1997	1
Ruscus (*Ruscus hypophyllum*)	Bet Halevy, Benei Zion, Ein Vered, Herut	2006	9
Strawberry (*Fragaria*×*Ananassa*)	Bet Dagan	1997	1
Tomato (*Solanum lycopersicum*)	Besor	2005	1
Total			576

[a] At Site 1, 93 isolates originate from diseased berries and 40 isolates were trapped from the air in plates containing selective media (Edwards and Seddon 2001). At Site 2, 239 isolates originate from diseased berries and 144 isolates were trapped from the air on selective media. Thirteen isolates from Site 1 or Site 2 (recovered from diseased plants in 2006 before starting spray program) and 60 isolates of different origin (below) were tested for sensitivity to fungicides on a range of concentrations, whereas the rest of isolates from Site 1 and Site 2 (503 isolates) were tested in a simplified method based on relative mycelial growth on media amended with discriminating doses of fungicides. Thirteen isolates from Site 1 and Site 2 included 9 isolates sensitive to six tested fungicides and 4 isolates resistant to dicarboximide or to both dicarboximide and benzimidazole fungicides. Sensitivity profiles of isolates recovered from Site1 and Site 2 are shown in Table 4.

[b] Among 60 isolates of different origin, 23% of isolates were sensitive to six fungicides tested, and the rest of isolates showed resistance to at least one of the fungicides.

disc, expressed as the percentage of the mean diameter of the untreated control. The resistance factor (RF), was estimated as the ratio of the mean EC_{50} of the resistant phenotype to the mean EC_{50} of the sensitive phenotypes (Leroux et al. 1999). An additional 503 isolates from diseased grape berries and from the air were tested using media amended with the DDs of the fungicides. The RG values of mycelial colonies at the DD were used as a simplified sensitivity test. EC_{50} values for these isolates were established based on the relationships between the EC_{50} values of the isolates, whose growth was evaluated on media containing several concentrations of the fungicides, and the RG scores of these isolates on CDA amended with the DDs of those same fungicides ($EC_{50\ (D)}$) (Jo et al. 2006; Köller et al. 1997).

Fitness of isolates in vitro, pathogenicity, and response to fungicides

Data on colony diameter of isolates on unamended CDA (recorded when testing sensitivity of isolates to fungicides) were used to assess the fitness of isolates. The isolates sensitive to all six tested fungicides were regarded as fungicide-sensitive or wild-type (WT), and were compared with the resistant isolates. *B. cinerea* strains were also compared for their pathogenicity potential, expressed as the size of the lesions formed on detached bean leaves (*Phaseolus vulgaris* cv. Hilda) that had been inoculated with conidial suspensions. The oldest leaves were cut from 2- to 3-week-old plants (branching stage), and placed on 0.8% agar amended with chloramphenicol at 30 mg l^{-1} in 30-cm-diam. Petri dishes, 3 leaves per plate. To promote infection, the inoculum suspension was supplemented with half-strength potato dextrose broth (PDB; Difco Laboratory, Detroit, MI). Non-inoculated control leaves were also supplemented with half-strength PDB. Each leaf was inoculated with three 10-µl drops of the suspension, containing 10^6 conidia ml^{-1} of an appropriate *B. cinerea* strain, and three leaves were used for each strain. The dishes were covered to maintain high humidity, and kept in a growth chamber under 12 h/day light at 22–24°C.

Lesion diameter was measured 72 h after inoculation. The experiment was repeated twice.

The virulence of isolates sensitive and resistant to fungicides and the response to fungicide treatment was compared on bean plants (cv. Hilda) in greenhouse experiments. Seeds were planted in 1-litre pots that contained a 3:7 (vol./vol.) mixture of peat and volcanic gravel. Plants were irrigated every 1–3 days, and treated with 5 $g l^{-1}$ 20:20:20 NPK fertilizer once a week. Two to three-week-old plants were sprayed to run-off with the formulated fungicides in aqueous suspensions at the recommended commercial concentrations: 0.1 mg l^{-1} Benlate 50 WP (50% a.i.), 0.1 mg l^{-1} Celest SC 230 (23% a.i.), 0.25 mg l^{-1} Mythos SL 300 (30% a.i.), 0.15 mg l^{-1} Ohayo SC 500 (50% a.i.), 0.15 mg l^{-1} Rovral 50 WP (50% a.i.), and 0.15 mg l^{-1} Teldor SC 500 (50% a.i.). The control plants were sprayed with water. After 2–3 h, one 10-μl drop of conidial suspension supplemented with half-strength PDB (10^6 conidia ml^{-1}) was placed on the upper side of a leaf. Altogether, 10 oldest leaves of each plant were inoculated, and 3 plants were inoculated with each isolate. Non-inoculated control leaves were also supplemented with the 10-μl drops of half-strength PDB. The pots with inoculated plants were covered with two transparent polypropylene bags and kept in a greenhouse with mean day/night temperatures of 25/20°C, respectively. The relative humidity inside the bags was close to 100%. The diameters of the developing lesions were measured 10 days after inoculation. The experiment was repeated once.

Data analysis

An EC_{50} value for each of the fungicides was calculated by regressing the relative inhibition of growth against $\log_{10}$ fungicide concentration using the inverse prediction function of JMP 5 software. Data on colony diameter or lesion size were analyzed by analysis of variance (ANOVA) and, when a significant F value was observed, treatments were compared using Fisher's protected least significance difference (LSD) test. For field experiment, the area under disease progress curve (AUDPC) was calculated by trapezoidal integration of the disease percentage values taken at different time points, as described previously (Campbell and Madden 1990). Analysis of percentage data were based on arcsine-square root-transformed percentage values. AUDPC data were subjected to analysis of variance and, when a significant F value was observed, treatments were compared using Fisher's protected least significance difference (LSD) test. AUDPC data for different fungicide treatments were compared with the AUDPC data for control using Duncan's multiple range test ($P \leq 0.05$). The reduction in the level of the disease in plots treated with the fungicides was calculated as follows: 100—(100×mean severity of treated grapes or mean incidence of treated grapes/ mean severity of control or mean incidence of control) (Baroffio et al. 2003). All tests were performed at $P=0.05$ using JMP 5.0 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA).

Results

Comparison of RG and EC_{50} values

Sensitivities of 73 isolates to six fungicides were quantified as EC_{50} values. The relative mycelial growth (RG) values of mycelial colonies of an additional 503 isolates grown on media containing a single DD of fungicide were evaluated in a simplified sensitivity test. To compare both sensitivity measures, regressions of EC_{50} values and corresponding RG values were derived for the 73 isolates, which were representative of a wide range of sensitivities (data not shown). Both measures were highly correlated for benomyl, fenhexamid, fluazinam, fludioxonil, iprodione and pyrimethanil ($r=0.99$; 0.92; 0.77; 0.79; 0.88; and 0.92, respectively; $P<0.0001$). This correlation confirmed that the RG values were accurate representations of the isolates' sensitivities. These regression models were used to predict an EC_{50} value for each isolate, using RG values on CDA amended with the DD of a particular fungicide ($EC_{50(D)}$). There was a high correlation between the $EC_{50(D)}$ and the EC_{50} for fenhexamid, fluazinam, fludioxonil and iprodione ($r=0.92$, 0.75, 0.90, and 0.88; respectively, $P<0.0001$). $EC_{50(D)}$ values for benomyl and pyrimethanil were qualitatively estimated based on the growth or no growth at all on CDA amended with the DDs of these fungicides.

Sensitivities of *B. cinerea* isolates to the fungicides

Benomyl The group of 73 isolates was characterized by a bimodal frequency distribution of EC_{50} values, which varied from 0.024 to more than 10 μg ml^{-1}. Among these isolates, 53 isolates showed a roughly normal distribution of EC_{50} values, which varied from 0.02 to 0.04 μg ml^{-1}, with a mean value of 0.03 μg ml^{-1} (Fig. 1 "Benomyl"). These isolates did not grow on media supplemented with benomyl concentrations of 0.1 μg ml^{-1} or more. Their growth was significantly reduced at benomyl concentrations of 0.025 and 0.05 μg ml^{-1}, but not at the lower concentrations of 0.001 and 0.01 μg ml^{-1}. The other 20 isolates grew normally on all of the benomyl-amended media and had EC_{50} values greater than 10 μg ml^{-1}. The first group was classified as sensitive to benomyl (BenS) and the second one as resistant to benomyl (BenR). Resistant isolates were separated from sensitive isolates by a RF of more than 300 (Table 2). Based on RG on CDA amended with benomyl at 0.1 μg ml^{-1} (DD), 516 *B. cinerea* isolates from vineyards were separated into two significantly different groups ($P<0.0001$): those that did not grow on this medium ($EC_{50}<0.1$ μg ml^{-1}; 422 isolates) and those that did grow on this medium (EC_{50} >> 0.1 μg ml^{-1}; 94 isolates).

Fenhexamid The EC_{50} values for 73 isolates varied from 0.003 to 0.069 μg ml^{-1}. These values had a roughly normal distribution, with a mean EC_{50} of 0.01 μg ml^{-1} (Fig. 1 "Fenhexamid"). No isolates

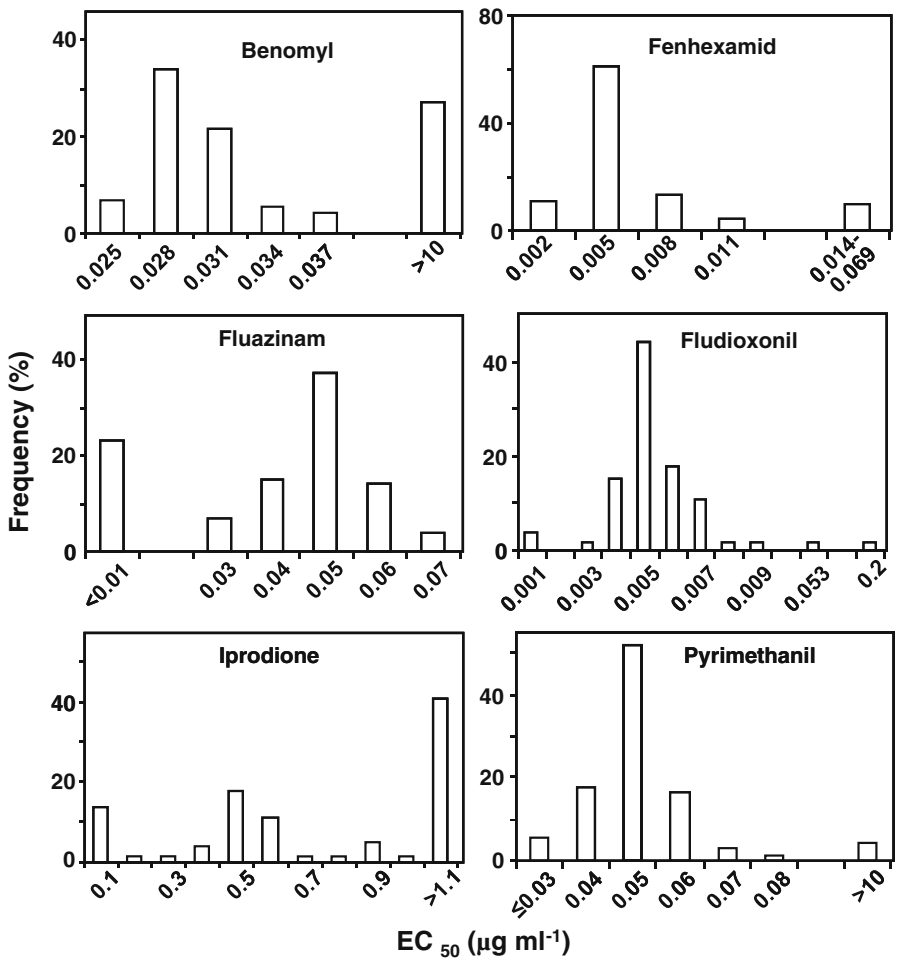

Fig. 1 Frequency distribution of EC_{50} values for six fungicides among 73 *Botrytis cinerea* isolates

Table 2 Toxicity of fungicides to *Botrytis cinerea* defined using a mycelial growth test

Active ingredient	Trade name	Phenotype[a]	EC_{50}[b] (μg ml^{-1})	RF [c]	DD[d] (μg ml^{-1})
Benomyl	Benlate 50 WP	BenS	0.03		
		BenR	>10	>300	0.1
Fenhexamid	Teldor SC 500	HydS	0.01		
		HydR	0.12	12	0.1
Fluazinam	Ohayo SC 500	PyrS	0.008		
		PyrLS	0.056	7	0.1
Fludioxonil	Celest SC 230	PhenS	0.005		
		PhenR	0.1	20	0.1
Iprodione	Rovral 50 WP	DicS	0.2		
		DicR	2.2	≥10	1.0
Pyrimethanil	Mythos SL 300	AniS	0.05		
		AniR	>10	>200	0.1

[a] S=sensitive, R=resistant; LS=less sensitive; Ben=benzimidazole, Hyd=hydroxyanilide, Pyr=phenylpyridinamine, Phen=phenylpyrrole, Dic=dicarboximide, Ani=anilinopyrimidine.

[b] Mean concentration of a.i. associated with 50% inhibition of mycelial growth.

[c] Resistance Factor=average EC_{50} of resistant or less sensitive isolates / average EC_{50} of sensitive isolates.

[d] Discriminatory concentration (μg of a.i. ml^{-1}).

grew on the media amended with 1 and 10 μg ml^{-1} of fenhexamid, although a few isolates did form resistant sectors during their exposure to these fungicide concentrations. On CDA amended with 0.1 μg ml^{-1} of fenhexamid (DD), 68 isolates exhibited zero to less than 20% RG, and 5 isolates exhibited 20 to 35% RG. Overall, among the 516 field isolates, 4 isolates had RG levels of at least 50% and were classified as resistant to fenhexamid (HydR). These resistant isolates had a mean $EC_{50(D)}$ of 0.12 μg ml^{-1} and were separated from sensitive isolates by a RF of 12 (Table 2). The rest of isolates were regarded sensitive (HydS). Among sensitive isolates, 8 isolates showed 20 to 45% growth on DD with a mean $EC_{50(D)}$ of 0.05, and other isolates (504) exhibited zero to less than 20% RG with a mean $EC_{50(D)}$ of 0.008 μg/ml (Table 2).

Fluazinam EC_{50} values of 73 isolates varied in a bimodal way, ranging from 0.005 to 0.075 μg ml^{-1}, with a mean EC_{50} of 0.04 μg ml^{-1}. EC_{50} values for most isolates had a roughly normal distribution, ranging from 0.03 to 0.075 μg ml^{-1}. Other isolates were more sensitive, with EC_{50} values less than 0.01 μg ml^{-1} (Fig. 1 "Fluazinam"). No isolates grew on the media amended with 1 and 10 μg ml^{-1} of fluazinam. On the medium with 0.1 μg ml^{-1} of fluazinam (DD), 18 isolates did not grow (EC_{50} values from 0.005 to 0.01 μg ml^{-1}), whereas the rest of the isolates, whose EC_{50} values varied from 0.026 to 0.075, displayed limited growth up to 35% of RG. Similarly, about 10% of isolates from vineyards at Sites 1 and 2 had RG levels between 5 and 35% (with a mean RG value of about 10%) and were regarded as less sensitive to fluazinam (PyrLS). These isolates usually showed some level of reduced sensitivity to fenhexamid, iprodione and pyrimethanil, which was evident by the comparison of WT and PyrLS isolates for RG on media amended with DDs of the corresponding fungicides (Table 3). Generally, the PyrLS isolates grew more slowly on the unamended medium, and there was a correlation between this growth retardation and their RG values on DD (r= 0.99). That is, larger RG values on DD were associated with the slower growth of mycelia on unamended media. Isolates resistant to fluazinam were not found.

Fludioxonil The EC_{50} values for 73 isolates varied from 0.001 to 0.2 μg ml^{-1} with a roughly normal distribution (Fig. 1 "Fludioxonil"). Seventy of the 73 *B. cinerea* isolates were highly sensitive to fludiox-

Table 3 Sensitivities of the wild-type isolates and of isolates less sensitive to fluazinam, to other fungicides

Fungicide	RG[a], %	
	WT[b]	PyrLS[c]
Benomyl	0.0	0.0
Fenhexamid	1.0 B[d]	8.0A
Fluazinam	0.4B	7.5A
Fludioxonil	0.0	0.0
Iprodione	1.9B	3.4A
Pyrimethanil	3.8B	8.7A

[a] Relative mycelial growth on media amended with discriminatory concentrations of the fungicide expressed as percentage of the growth on unamended media.

[b] WT=wild-type isolates sensitive to the six fungicides listed in the column "Fungicide". Mean data for 350 WT isolates.

[c] PyrLS=isolates less sensitive to fluazinam. Mean data for 30 PyrLS isolates. PyrLS isolates used here were sensitive to all other fungicides.

[d] In each row, values followed by the same letter do not differ significantly according to Fisher's protected least significance difference (LSD) test ($P \leq 0.05$).

onil, with EC_{50} values varying from 0.001 to 0.009 μg ml^{-1}. These isolates did not grow on medium amended with 0.1 μg ml^{-1} of fludioxonil (DD), and were regarded sensitive to fludioxonil (PhenS) (mean EC_{50}=0.005 μg ml^{-1}). Two isolates (originally isolated from non-grape hosts) grew on DD, forming colonies that were about 20% the size of those on the unamended medium (mean EC_{50} value of 0.065 μg ml^{-1}), but did not grow on the media with 1 and 10 μg ml^{-1}. These two isolates were regarded less sensitive to fludioxonil. A third isolate (isolated at Site 1) did grow partially on the media containing 1 and 10 μg ml^{-1} of fludioxonil and had an EC_{50} value of 0.1 μg ml^{-1}, and was classified as resistant (PhenR, Table 2). The resistant isolate was separated from sensitive isolates by a RF of 20. All three isolates were simultaneously resistant to iprodione, and one isolate was also resistant to benomyl and pyrimethanil. No additional PhenR isolates were found among the 516 isolates tested.

Iprodione The EC_{50} values of the 73 *B. cinerea* isolates had a multimodal distribution (Fig. 1 "Iprodione"), and ranged from 0.08 to 8.19 μg ml^{-1}. All these isolates did not show any significant growth retardation on CDA amended with 0.001 and 0.01 μg ml^{-1} of iprodione. A concentration of 0.1 μg ml^{-1} caused growth retardation of up to 65% in 15% of the isolates, although all of the isolates continued to exhibit at least some growth. On media containing 1 μg ml^{-1} of iprodione, 31 isolates stopped growing or showed limited growth ≤ 20% RG (mean EC_{50}=0.32 μg ml^{-1}), 15 isolates showed growth of 20 to 49% (mean EC_{50}=1.46 μg ml^{-1}) and 27 isolates had RG values between 50 and 100% (mean EC_{50}=2.38 μg ml^{-1}). According to previous data, sensitive isolates of *B. cinerea* have a mean EC_{50} value of 0.1–0.3 μg ml^{-1} and resistant isolates have EC_{50} values ranging from 0.8 to 3.0, or >10 μg ml^{-1} (Leroux et al. 1999; Beever et al. 1989). Based on these data, isolates with $EC_{50} \geq 0.8$ μg ml^{-1} (with corresponding RG values ≥ 15% on media amended with 1 μg/ml of iprodione (DD)) were regarded as resistant. Six of the resistant isolates showed limited growth (RG values from 20 to 40%) on medium amended with 10 μg ml^{-1} of iprodione. Our isolates were classified into two groups: sensitive to iprodione (DicS) isolates, which showed less than 15% growth on DD and had EC_{50} values ranging from 0.1 to 0.6 μg ml^{-1} (mean EC_{50}= 0.2 μg ml^{-1}), and resistant isolates (DicR), which exhibited at least 15% RG on DD and had EC_{50} values of at least 0.8 μg ml^{-1} (mean EC_{50}=2.2 μg ml^{-1}). Among the 516 isolates from the vineyards, 85 isolates (16.5%) had RG values of at least 15% on DD and were regarded as resistant. Resistant isolates were separated from sensitive isolates by a RF of ≥ 10 (Table 2).

Pyrimethanil Similar to the responses to benomyl, the group of 73 isolates had a bimodal frequency distribution of pyrimethanil EC_{50} values, which varied from 0.01 to > 10 μg ml^{-1} (Fig. 1 "Pyrimethanil"). A group of 70 of these isolates showed a roughly normal distribution of EC_{50} values, which varied from 0.01 to 0.08, with a mean value 0.05 μg ml^{-1}. Most of these isolates either did not grow on the media amended with 0.1 μg ml^{-1} of pyrimethanil (DD) or exhibited about 10% RG; whereas 6 isolates showed 20–40% RG. The EC_{50} values of these less sensitive isolates varied from 0.06 to 0.08 μg ml^{-1}. Three of the 73 isolates were resistant to pyrimethanil (AniR). These isolates grew on media amended with pyrimethanil at concentrations of up to 1 μg ml^{-1}, without any significant reduction in colony diameter, and showed 20 to 40% colony retardation on medium amended with 10 μg ml^{-1} of pyrimethanil.

EC_{50} values for these isolates were greater than 10 μg ml^{-1}, and resistant isolates were separated from sensitive isolates by a RF of more than 200 (Table 2). Among the 516 vineyard isolates, 16 isolates (3.1%) were resistant (RG on DD of 60–95% and $EC_{50(D)}$>10 μg ml^{-1}) and 23 (4.5%) were less sensitive (RG values of 22–45%).

Phenotypes and multiple resistances among the tested isolates

Seventy-four percent of the 516 isolates from the vineyards were sensitive to the six tested fungicides, and the other 26% of the isolates were classified into 10 phenotypes characterized by resistance to one or more fungicides. The most frequently occurring phenotypes were resistant to benomyl (BenR), resistant to iprodione (DicR), and resistant to both (BenR DicR) (10, 6 and 7%, respectively). About 3% of the isolates were resistant to anilinopyrimidines with a half of these isolates resistant to other fungicides simultaneously. Five isolates (about 1%) were resistant to fenhexamid, four of which were also resistant to pyrimethanil. One isolate was resistant to both fludioxonil and iprodione (Table 4). We did not find any isolates that were resistant to fluazinam, although about 10% of the isolates showed reduced sensitivity to fluazinam.

Table 4 *Botrytis cinerea* phenotypes recovered from vineyards in the Golan Heights, 2006–2008

Phenotype[a]	Isolates	
	Number	% of total
AniR	8	1.6
AniR BenR	1	0.2
AniR BenR DicR	3	0.6
AniR DicR	1	0.2
AniR HydR	3	0.6
BenR	51	9.9
BenR DicR	37	7.2
DicR	30	5.8
DicR PhenR	1	0.2
HydR	1	0.2
Wild-type	380	73.6
Total	516	100

[a] R=resistant; Ani=anilinopyrimidine, Ben=benzimidazole, Dic=dicarboximide, Hyd=hydroxyanilide, Phen=phenylpyrrole, Pyr=phenylpyridinamine.

Fitness of resistant isolates in vitro

As a group, 131 resistant vineyard isolates (isolates resistant to one or more fungicides) had a mean colony diameter of 40.4 mm, which was significantly less (P<0.0001) than the average colony diameter for the group of 367 WT isolates sensitive to all six fungicides tested, which was 45.8 mm (a 12% difference in mean colony diameter). Isolates resistant to pyrimethanil, benomyl and fenhexamid grew significantly more slowly than the WT isolates. In contrast, the growth of the isolates resistant to iprodione did not differ from that of the WT isolates. Resistant isolates were often simultaneously resistant to two or more fungicides. In order to determine which of the resistances was responsible for the observed growth retardation, the groups of isolates with defined phenotypes were compared with the WT isolates. The isolates resistant to benomyl only had significantly slower mycelial growth than the WT isolates. However, the growth of the dicarboximide-resistant isolates and that of the isolates resistant to both benomyl and iprodione did not differ from that of the WT isolates. The growth of the isolates resistant to pyrimethanil only did not differ from that of the WT isolates. This implies that the reduced growth of the isolates with multiple resistances including resistance to pyrimethanil was caused by other resistances present in the isolates. Isolates less sensitive to fluazinam showed significant growth retardation. There was a correlation between colony diameter on the agar media and lesion diameter on detached bean leaves (r=0.84 at P< 0.0001). Isolates growing slowly on agar media formed smaller lesions on leaves (Table 5).

Pathogenicity and responses to fungicides in the greenhouse experiment

Resistance to benzimidazoles was accompanied by the complete or significant failure of benomyl to provide disease control, and resistance to fludioxonil and pyrimethanil caused significant reductions in the efficacies of these fungicides. Resistance to iprodione resulted in a 10–20% reduction in the efficacy of this fungicide. The observed reduced sensitivities to

Table 5 Colony and lesion size of isolates of *Botrytis cinerea* with fungicide-resistant and fungicide-sensitive phenotypes

Phenotype[a]	Number of isolates tested on agar media/bean leaves	Colony diameter (mm)[b]	Lesion diameter (mm)[c]
AniR	8/1	43.8a	26.4a
AniR HydR	4/1	26.8d	16.8bc
BenR	48/1	37.4c	16.6bc
BenR DicR	35/1	42.3ab	NT
DicR	30/2	45.2ab	19.1b
PyrLS	10/3	40.2b	15.7c
Wild-type	233/3	45.8a	24.1a

[a] R=resistant, LS=less sensitive; Ani=anilinopyrimidine, Ben=benzimidazole, Dic=dicarboximide, Hyd=hydroxyanilide, Phen=phenylpyrrole, Pyr=phenylpyridinamine.

[b] Each strain was incubated on defined minimal media (Edwards and Seddon 2001) in 9-cm Petri plates at 20°C. The diameter of each colony was measured 3 days after 5-mm-diam. agar plugs that had been colonized by mycelia of the appropriate strain were placed at three points on each plate. Mean values with different letters in each column are significantly different according to Fisher's protected least significance difference (LSD) test ($P \leq 0.05$).

[c] Leaves from 2- to 3-week-old bean plants were placed on 0.8% agar in 30-cm-diam. Petri plates and inoculated with 10-µl drops of a suspension containing 10^6 conidia/ml of the appropriate *B. cinerea* strain. The dishes were covered and kept in a growth chamber with 12 h of light: 12 h of dark, at 22–24°C. Lesion diameter was measured 72 h after inoculation.

fenhexamid and fluazinam had no practical effects on their performance (Table 6). In comparison to sensitive isolates, the isolates with lower sensitivity to fluazinam and the isolate resistant to fludioxonil formed smaller lesions on bean leaves. Isolates resistant to other fungicides appeared to be equally fit.

Disease incidence, efficacy of treatments and dynamics of resistance in the vineyards

The level of disease differed at the two vineyard sites. In the control plots, mean disease incidence at the end of August ranged from 4.2 to 32.5% at Site 1 and from 36.7 to 70.8% at Site 2. Mean disease severity ranged from 0.3 to 3.3% and from 8.6 to 34.0% at Site 1 and Site 2, respectively. The efficacy of the fungicide treatments also varied from vineyard to vineyard and from year to year. However, the mean relative efficacies of the fungicides over the 3 years of the study were similar in the two vineyards (Table 7, Fig. 2). Disease incidence at Site 1 in 2008 was very low, and no *B. cinerea* isolates were recovered and tested from that site in that year.

Benzimidazole fungicides Carbendazim was ineffective in controlling bunch rot at Sites 1 and 2 (Table 7). The incidence of disease in the treated plots did not differ significantly from the incidence in the untreated plots, although there was a trend toward lower incidence in the treated plots at Site 2 (Fig. 3 "Delsen"). Resistance to benzimidazole fungicides was found with frequencies of 8 and 9% at Site 1 in 2006 and 2007, respectively; and of 7% at Site 2 in 2006. The frequency of resistance increased at Site 2 in 2007 and 2008, reaching 25 and 22%, respectively (Fig. 3 "Benzimidazoles"). Resistant isolates were recovered both from plots treated with carbendazim and from untreated plots. The frequency of resistance in the treated plots was not always higher than the frequency in the untreated ones.

Dicarboximide fungicides Iprodione was ineffective or weakly effective in controlling bunch rot at Sites 1 and 2 during 2006 and 2007 with the incidence of disease in the treated plots not different significantly from the incidence in the untreated plots. Across all plots and years, iprodione applications provided 28% disease control, but the difference between the iprodione-treated plots and the control plots was mostly not significant. In 2008, against the background of very low disease incidence, iprodione provided significant disease control ($P \leq 0.05$) (Table 7). The frequencies of resistance to dicarboximide fungicides were 21 and 11% at Site 1 in 2006 and 2007, respectively. The frequencies of resistance at Site 2 were 25, 20 and 10% in 2006, 2007 and 2008, respectively (Fig. 3

Table 6 Effectiveness of fungicide treatments against sensitive and resistant isolates of *Botrytis cinerea* in a greenhouse experiment

Treatment	Isolate	Phenotype[a]	Lesion size[b], %
Benomyl	B127	BenR	67.9a
	B401	BenR DicR	100.8a
	B152	AniR BenR DicR	54.8a
	B254	Wild-type	1.3c
Fenhexamid	B995	AniR HydLS	4.3c
	B254	Wild-type	1.8c
Fluazinam	B310	PyrLS	4.8c
	B254	Wild-type	2.0c
Fludioxonil	B463	DicR PhenR	52.1a
	B254	Wild-type	0.0c
Iprodione	B165	AniR BenR DicR	18.1b
	B339	BenR DicR	12.5b
	B152	AniR BenR DicR	21.7b
	B254	Wild-type	2.2c
Pyrimethanil	B152	AniR BenR DicR	68.8a
	B143	AniR BenR DicR	86.8a
	B254	Wild-type	1.3c

[a] R=resistant, LS=less sensitive; Ani=anilinopyrimidine, Ben=benzimidazole, Dic=dicarboximide, Hyd=hydroxyanilide, Phen=phenylpyrrole, and Pyr=phenylpyridinamine.

[b] Bean plants (cv. Hilda) were inoculated with 10-µl drops of conidial suspensions (10^6 conidia/ml), covered with transparent polypropylene bags and incubated in the greenhouse for 10 days. Lesion diameter was expressed as the percentage of the average diameter of the lesions on the untreated control plants that were infected with a wild-type isolate. The disease level in the control plants was considered to be 100%. Mean values with different letters are significantly different according to Fisher's protected least significance difference (LSD) test ($P \leq 0.05$).

"Dicarboximides"). Resistant isolates were recovered from both iprodione-treated and untreated plots.

Anilinopyrimidine fungicides Two anilinopyrimidine fungicides, pyrimethanil and cyprodinil, the latter in combination with fludioxonil, were used at Sites 1 and 2. Pyrimethanil effectively reduced bunch rot at both sites, with a mean 52% reduction in disease across all plots and years (Table 7, Fig. 3 "Mythos"). The frequency of resistance to pyrimethanil was about 2% during 2006 and 2007 at Sites 1 and 2. In 2008, there was an increase in the frequency of resistance at Site 2: 10% of the tested isolates at this site were resistant (Fig. 3 "Anilinopyrimidines").

Other fungicides Fenhexamid and the mixture of fludioxonil and cyprodinil were both highly effective in reducing bunch rot, with mean (across all plots and years) reductions in disease incidence of 65 and 60%, respectively (Table 7). A few fenhexamid-resistant isolates were recovered at Site 2 in 2007 and 2008, and one fludioxonil-resistant isolate was found at Site 1 in 2006. In our experiment, fluazinam was not effective against bunch rot. This treatment reduced the incidence of disease by 31% (similar to iprodione), but the difference between the fluazinam-treated plots and the control plots was not significant ($P<0.05$; Table 7). We did not find any isolates that were strongly resistant to fluazinam, but we did find more isolates less sensitive to fluazinam in plots treated with fluazinam than in untreated plots (21 vs. 9%, respectively). The mean RG on the media containing the DD of fluazinam for isolates recovered from the fluazinam-treated plots was significantly higher than the mean RG of the isolates recovered from the other plots ($P=0.01$). Also, as a group, the isolates recovered from the fluazinam-treated plots showed mean growth retardation of about 7% on CDA, as compared with isolates from the untreated plots ($P \leq 0.05$).

Resistant isolates in the air in the vineyards

During August and September of 2007 and 2008, about 1,800 Petri dishes (300 dishes of each of five selective media and plain BSTM) were exposed in the vineyards at Sites 1 and 2. The mean number of *B. cinerea* CFU trapped on unamended BSTM varied between dates and vineyards, but there were always more CFU in the air at Site 2 than at Site 1, which correlates with the higher level of disease observed at Site 2. We collected an average 0.5 CFU/dish at Site 1 and 2.0 CFU/dish at Site 2. We were then able to select resistant isolates (on fungicide-supplemented media) that occurred with frequencies of 0.5 to 2% or higher. However, we could not necessarily collect rarer phenotypes. The large amount of CFU in the open air decreased the selectivity of the media because of the growth of some non-*Botrytis* fungi, which deposited their metabolites into the medium, decreasing its toxicity, which allowed WT *Botrytis* to grow on BSTM amended with fungicides. This issue was particularly important for the BSTM with

Table 7 Grey mould levels in untreated plots and reduction of disease in treated plots at two sites, Golan Heights

Vineyard	Year	Disease in untreated plots (AUDPC)[a]		Reduction of disease incidence (AUDPC, %)[b] in plots treated with:					
		Incidence	Severity	Carbendazim	Fenhexamid	Fluazinam	Fludioxonil+cyprodinil	Iprodione	Pyrimethanil
Site 1	2006	25.0a	1.5a	18.9	75.3*	55.1	60.3*	33.3	65.7*
	2007	32.5a	3.3a	−22.5	52.8	6.4	54.3	26.8	36.0
	2008	4.2b	0.3b	NT[c]	NT	NT	NT	NT	NT
Site 2	2006	70.8a	34.0a	16.6	45.5	40.5	NT	11.1	61.5*
	2007	63.3a	17.0b	38.8	61.2*	39.5	60.5*	11.8	62.5*
	2008	36.7b	8.6b	37.0	67.6*	13.2	83.5*	59.1*	33.2
Mean				15.5	60.1*	31.4	64.7*	27.6	52.1*

[a] Disease incidence and severity were evaluated three times each season during three seasons by examining 30 clusters from each plot each time. Disease incidence was calculated as the percentage of clusters showing any disease symptoms. Disease severity was assessed as the percentage of symptomatic berries per cluster. Disease ratings were plotted over time to obtain disease progress curves and to calculate AUDPC (Campbell and Madden 1990). Numbers in the columns followed by the same letter are not significantly different according to Fisher's protected least significance difference (LSD) test ($P \leq 0.05$).

[b] AUDPC were expressed as percentage to AUDPC in control plots, and the reduction in the level of the disease in plots treated with the fungicides was calculated (Baroffio et al. 2003). Means followed by asterisks are significantly different from the control according to Duncan's multiple range test ($P \leq 0.05$).

[c] NT=not tested.

pyrimethanil. Only about 30% of *B. cinerea* isolates recovered from this medium were resistant to pyrimethanil, despite the good selectivity of the medium in the laboratory tests. Media with benomyl and, to a lesser extent, media with iprodione showed a good level of selectivity. All of the isolates recovered from BSTM amended with benomyl and most of the isolates from BSTM amended with iprodione were resistant to the corresponding fungicide. Resistance to benzimidazoles was more common among the isolates recovered from the air than among the isolates recovered from plants ($P \leq 0.05$). Isolates resistant to dicarboximides were found with similar frequency among isolates from plants and from the air.

Discussion

Until the middle of the 1990s, chemical control of grey mould was mainly based on the use of benzimidazole and dicarboximide fungicides, and the intensive use of these site-specific fungicides and

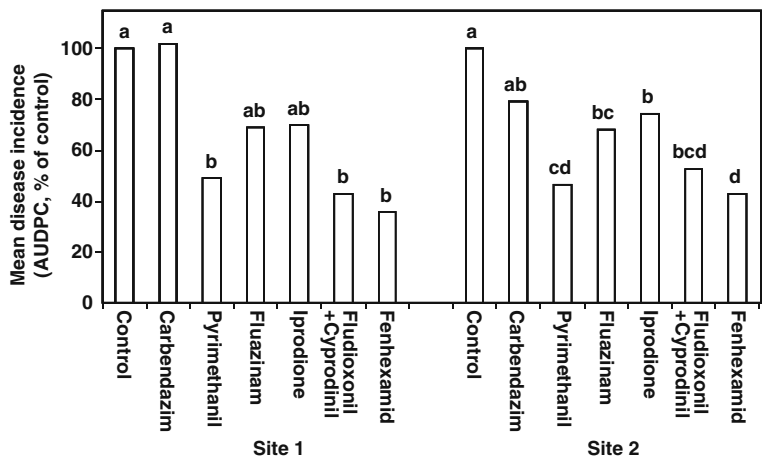

Fig. 2 Mean relative incidence of botrytis bunch rot for 3 years in treated and untreated plots of grapevine at two sites. Different letters above bars indicate significant differences with the control according to Duncan's multiple range test ($P \leq 0.05$)

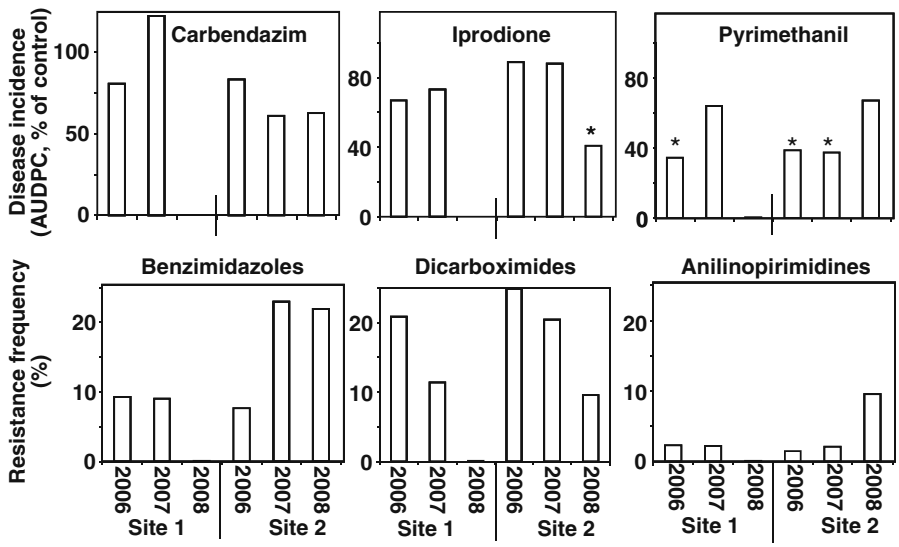

Fig. 3 Grey mould incidence in plots treated with carbendazim, iprodione and pyrimethanil and frequency of *B. cinerea* resistance to these fungicides in these plots. The mean level of disease in the control plots was considered to be 100%. Asterisks above bars indicate significant differences with the control according to Duncan's multiple range test ($P \leq 0.05$)

the high-risk character of *B. cinerea* for development of resistance have led to the rapid selection of strains resistant to one or both of these groups of fungicides (Leroux 2004). Despite the widespread incidence of resistance, benzimidazole and dicarboximide fungicides are still widely used (Morton and Staub 2008), and up-to-date study of the current frequency of resistance to and effectiveness of these fungicides would be valuable.

In this work, carbendazim and iprodione were ineffective or weakly effective in controlling bunch rot, and the presence of resistant isolates could partially explain this low level of efficacy. Strongly benzimidazole-resistant isolates and low-to-moderate dicarboximide-resistant isolates were found in the test vineyards with a frequency of 10 to 25%. The shifts in frequency of resistance did not correspond with changes in disease incidence in plots treated with the corresponding fungicides. A previous study also failed to document a clear correlation between the proportion of dicarboximide-resistant strains in a vineyard and the performance of these fungicides: a positive correlation between disease incidence and resistance frequency was found only at frequencies of resistance of more than 15%; whereas the high incidence of disease in blocks where resistance was less than 15% was attributed to poor fungicide application (Beever et al. 1989). Dicarboximides appeared to be able to control resistant strains when disease was not severe (Beever et al. 1989; Löchner et al. 1987), and we also observed the relatively high efficacy of iprodione at site 2 in 2008, when incidence of bunch rot was low.

Reduced fitness of the resistant fungi (Raposo et al. 2000) and instability of the resistance to dicarboximides (Yourman et al. 2001) could be partially responsible for the lack of correlation between the frequency of resistance and the performance of the fungicides. Previous studies supported the theory of either reduced or unaffected fitness of dicarboximide-resistant isolates of *B. cinere*a, whereas benzimidazole-resistant strains have generally been found to be as fit as benzimidazole-sensitive strains (Raposo et al. 1996, 2000). In our tests, we differentiated isolates resistant to each class of fungicides from those resistant to both classes. Isolates resistant solely to dicarboximides or to both benzimidazoles and dicarboximides exhibited similar levels of fitness in the examinations of mycelial growth on agar medium or lesion size on bean leaves. In contrast, isolates resistant only to benzimidazoles showed significantly slower growth on agar media and lesions on bean leaves and are possibly less fit than the isolates resistant to both groups of fungicides.

The anilinopyrimidines (APs) constitute a new class of mainly protective fungicides with a broad spectrum of activity against different pathogens. The APs do not affect the germination of *B. cinerea* conidia, but do inhibit its germ tube elongation and initial mycelial growth (Leroux 2004). Several cases of accumulated field resistance have been described (Leroux 2004; Myresiotis et al. 2007). When an AP fungicide was used alone, resistance often but not

always (Hilber and Hilber-Bodmer 1998) developed and treatment performance was reduced after 2–6 years. This was not the case when an AP fungicide was applied in combination with fludioxonil (Baroffio et al. 2003). In our experiments, treatments with APs did not cause the sharp increase in the frequency of resistant isolates. The maximum observed was 10% in one of the two vineyards, following 3 years of treatments with pyrimethanil. We also observed a tendency toward reduced efficacy of pyrimethanil, but no similar decrease in the efficacy of the cyprodinil + fludioxonil treatment.

Three AP-resistant phenotypes (AniR1, AniR2 and AniR3) were detected in field populations of *B. cinerea* (Chapeland et al. 1999). AniR1 strains are moderately to highly resistant to APs and they respond like WT isolates to other fungicides; the specific resistance in AniR1 might be related to a change at the target site. Low-level resistance in AniR2 and AniR3 was mainly noted at the germ-tube elongation stage, and this resistance extended to several other fungicide classes (multi-drug resistant phenotypes) (Kretschmer et al. 2009). EC_{50} values for isolates sensitive to pyrimethanil ranged from 0.03 to 0.08 μg ml^{-1} in the present work and from 0.03 to 0.5 μg ml^{-1} in the previous studies (Chapeland et al. 1999; Myresiotis et al. 2007) with resistance factors of 10 to 200 for AniR1, and below 10 for AniR2 or AniR3 (Leroux 2004). Strongly pyrimethanil-resistant isolates recovered from vineyards in the present work most probably possess the AniR1 phenotype; whereas less sensitive isolates could possess the AniR2 or AniR3 multi-drug resistance phenotypes.

High variability in fitness parameters among both AP-resistant and AP-sensitive isolates was observed in a previous study, and, as a group, resistant isolates showed reduced mycelial growth and virulence (Bardas et al. 2008). In the present work, AP-resistant isolates that were also resistant to other fungicides grew significantly more slowly than the WT isolates and formed smaller lesions on bean leaves. However, the isolates that were resistant to pyrimethanil only were no different from the WT isolates.

An inhibitor of sterol biosynthesis, the hydroxyanilide fenhexamid is a novel botryticide whose primary target site is the 3-keto reductase involved in sterol C-4 demethylation (Leroux 2004). *B. cinerea* responds with varying sensitivity to *in vitro* treatments with fenhexamid at various stages of its development. The EC_{50} value for conidial germination is greater than 10 μg ml^{-1}; whereas the corresponding value for the inhibition of subsequent germ-tube elongation and mycelial growth is less than 0.1 μg ml^{-1} (Hänßler and Pontzen 1999). In several survey studies, EC_{50} values for fenhexamid-sensitive isolates, as defined using a mycelial growth test, ranged from less than 0.01 to about 0.1 μg ml^{-1} (Esterio et al. 2007; Förster et al. 2007; Leroux et al. 1999; Ma and Michailides 2005; Myresiotis et al. 2007), which is similar to our data. A discriminatory dose of 0.1 μg ml^{-1} of fenhexamid was used earlier to distinguish between sensitive and resistant isolates, with isolates showing EC_{50} values of at least 0.1 μg ml^{-1} (Baroffio et al. 2003) or more than 0.1 μg ml^{-1} (Esterio et al. 2007) being regarded as resistant. We used the same DD and divided the isolates into two groups: sensitive (99% of isolates tested) and resistant. However, there was a gradient from sensitivity to resistance on this DD with about 1% of isolates showing 20–45% RG, which were regarded as less sensitive (EC_{50} values about 0.07 μg ml^{-1}). Similarly, isolates less sensitive to fenhexamid with EC_{50} values between 0.084 and 0.1 μg ml^{-1} were described in Chile (Esterio et al. 2007). The subdividing of isolates on resistant and sensitive categories based on RG on DD is helpful in monitoring programs, but may not always correspond to the described resistant phenotypes. To date, four fenhexamid-resistant phenotypes have been recognized: multi-drug-resistant AniR3 (MDR2); "naturally" resistant HydR1 (recognized recently as *B. pseudocinerea*); HydR2, whose resistance seemed to be based on p450-mediated detoxification; and HydR3, whose resistance is based on reduced sensitivity of the target site (Fillinger et al. 2008; Kretschmer et al. 2009). In the present study, four of the five isolates with low-level resistance or less sensitivity to fenhexamid were simultaneously resistant to anilinopyrimidines, and probably possess the multi-drug resistance phenotype MDR2 or MDR3 (Kretschmer et al. 2009). The fifth strain was also low-level resistant, but was sensitive to other tested fungicides, and could be of low-to-moderately resistant HydR3 phenotype (Fillinger et al. 2008). No highly fenhexamid-resistant isolates were found in this work. In a French study, HydR3 isolates were detected at frequencies of up to 50%, and the fenhexamid treatments were still effective, suggesting that the fitness of these resistant isolates may be

reduced (Fillinger et al. 2008). Isolates with low level resistance to fenhexamid found in this work showed low fitness and were controlled with fenhexamid similar to the sensitive isolates.

Fenhexamid-resistant field isolates have also been recovered in California, Chile, Greece, Switzerland and other regions (Baroffio et al. 2003; Esterio et al. 2007; Leroux 2004; Ma and Michailides 2005; Myresiotis et al. 2007). No resistance was found in vineyards in Switzerland in the first 2 years, but there was a steady increase in the size of the resistant subpopulation over the next 3 years. In one of the vineyards, only resistant isolates were found in the fifth year, and there was a reduction in the efficacy of fenhexamid in that vineyard (Baroffio et al. 2003). In other cited works and in our study, the proportion of resistant isolates in examined populations did not exceed 1–3%, confirming that baseline populations of *B. cinerea* often contain a small proportion of isolates with reduced sensitivity to fenhexamid and reduced fitness (Suty et al. 1999). In our work, fenhexamid was highly effective in reducing bunch rot, with the mean reduction of disease incidence of 52%. Three years of fenhexamid treatments did not cause the development of resistance.

Fluazinam is a phenylpyridinamine derivative that is highly toxic to the conidia and mycelia of *B. cinerea*. Fluazinam inhibits the germination of *Botrytis* conidia, as well as its germ tube elongation and mycelial growth (Leroux 2004). EC_{50} values for fluazinam-sensitive isolates, as defined using the mycelial growth test, have been shown to range from 0.04 to 0.07 μg ml^{-1} (Kalamarakis et al. 2000) or from 0.005 to 0.020 μg ml^{-1} (Leroux et al. 1999), depending on the technical specifications of the tests used and/or the population tested. In our work, the EC_{50} values of 73 isolates had a bimodal distribution and ranged from 0.005 to 0.075 μg ml^{-1}, with a mean EC_{50} value of 0.04 μg ml^{-1}. In Japan, sensitive *B. cinerea* isolates from field sites that had never been treated with fluazinam exhibited EC_{50} values of about 0.003 μg ml^{-1}; whereas strains exhibiting low (about 10) or high (about 10,000) resistance factors were recovered from treated crops (Leroux 2004). The bimodal distribution of EC_{50} values observed in this study confirms the presence of two groups of isolates with differential sensitivity: sensitive and less sensitive. The isolates less sensitive to fluazinam were simultaneously less sensitive to fenhexamid, iprodione and pyrimethanil and showed signs of decreased fitness. These growth patterns are characteristic of multi-drug resistance (MDR) (Kretschmer et al. 2009). Most probably, the isolates in this work found to be less sensitive to fluazinam are MDR strains.

In this work, fluazinam was not very effective against bunch rot. It reduced the incidence of disease by about 30%, but the difference between the level of disease in these plots and the level of disease in the control plots was not significant. Treatments with fluazinam during three growing seasons did not cause the development of strong resistance. Similarly, a monitoring program in vineyards in Champagne did not find any shift in the sensitivity of *B. cinerea* towards fluazinam (Leroux 2004). However, we did recover isolates with reduced sensitivity to fluazinam (presumably of MDR character) more often from plots treated with fluazinam than we did from plots not treated with this fungicide. The rising prevalence of MDR populations of *B. cinerea* was documented recently in France and Germany (Kretschmer et al. 2009). In our greenhouse experiments, isolates that are less sensitive to fluazinam were controlled with fluazinam similar to the WT isolates which implies that the presence of less sensitive isolates in the pathogen population was not the reason for the relatively low efficacy of fluazinam in the vineyards.

The phenylpyrrole fludioxonil is a derivative of pyrrolnitrin, a natural antifungal compound present in several *Pseudomonas* species. Fludioxonil inhibits both conidial germination and mycelial growth, but the latter process is more sensitive. Similar to dicarboximides, fludioxonil induces swelling, abnormal branching and cell-bursting in germ tubes. Fludioxonil appears to be 30–40 times more toxic than dicarboximides in terms of its effect on hyphal growth *in vitro*. However, under field conditions, the recommended application rates for both fludioxonil and dicarboximides are similar (Leroux 2004).

EC_{50} values for fludioxonil-sensitive isolates, as defined by the mycelial growth test, ranged from 0.001 to 0.016 μg ml^{-1} (Chapeland et al. 1999; Förster et al. 2007; Hilber et al. 1995; Leroux et al. 1999; Myresiotis et al. 2007; Vignutelli et al. 2002; Ziogas et al. 2005). In our tests, EC_{50} values for *B. cinerea* isolates were distributed roughly normally ranging from 0.001 to 0.009 μg ml^{-1}, with a mean EC_{50} value of 0.005 μg ml^{-1}. Three isolates (one

from the vineyard in the Golan Heights and two from greenhouses) were less sensitive to fludioxonil, with EC_{50} values between 0.05 and 0.1 μg ml^{-1}. High level resistance to fludioxonil was not found among field isolates in this work, as it was not found in a number previous studies (Baroffio et al. 2003; Förster and Staub 1996; Leroux et al. 1999; Vignutelli et al. 2002), although *B. cinerea* mutants sensitive to osmotic stress and highly resistant to phenylpyrroles, dicarboximides and aromatic hydrocarbons can be easily produced in the laboratory (Leroux 2004). To our knowledge, two *B. cinerea* field isolates with reduced sensitivity to phenylpyrroles, as well as moderate resistance to dicarboximides, have been described: strain CH73.92 from France, which has an EC_{50} value of 0.035 μg ml^{-1} (Vignutelli et al. 2002); and strain Bc682 from Japan, which has an EC_{50} value of 0.05 μg ml^{-1} (Oshima et al. 2002). Similarly, three isolates less sensitive to fludioxonil found in this work were simultaneously resistant to iprodione, and most probably have MDR2 or MDR3 phenotype (Kretschmer et al. 2009).

The mixture of fludioxonil and cyprodinil effectively controlled bunch rot in the two vineyards in the present study, with mean reductions of disease incidence of 54–84%. Only one isolate with low level resistance to fludioxonil was found among the 516 isolates tested. This finding indicates that 3 years of treatments with fludioxonil and cyprodinil did not cause the development of resistance, which, together with the generally good performance of this fungicide, underscores the value of this chemical for the control of bunch rot. Similarly, no resistance to fludioxonil was observed in long-term monitoring studies conducted in vineyards in France and Switzerland (Baroffio et al. 2003; Förster and Staub 1996). However, the increasing occurrence of *B. cinerea* MDR strains with less sensitivity to fludioxonil and other fungicides was described recently in commercial vineyards in France and Germany. The authors concluded that a massive appearance of MDR populations is expected to be a major threat for chemical control of grey mould disease in the near future (Kretschmer et al. 2009).

In summary, the monitoring data obtained in the current study demonstrate that the *B. cinerea* populations in the two vineyards did not shift toward strong resistance to fluazinam, fludioxonil or fenhexamid after 3 years of treatment with these fungicides. In contrast, some selection for anilinopyrimidine resistance was observed. Resistance to the older benzimidazole and dicarboximide fungicides most probably existed in the tested populations before we began our study, and we observed the increased frequency of benzimidazole resistance, but not dicarboximide resistance, following our 3 years of fungicide applications. The rising prevalence of isolates less sensitive to fluazinam, presumably possessing the MDR traits, was observed in plots treated with this fungicide. High performance of fludioxonil and fenhexamid together with low risk of the development of resistance makes these fungicides a valuable tool in preventing grey mold in vineyards in Golan Heights.

Acknowledgments This research was partially supported 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. The authors acknowledge Ruth Harpaz for her assistance with the disease evaluations and thank Suleiman Farhat for spraying the experiments. Contribution No 521/09 from the ARO, Volcani Center, Institute of Plant Protection.

References

Bardas, G. A., Myresiotis, C. K., & Karaoglandis, G. S. (2008). Stability and fitness of anilinopyrimidine-resistant strains of *Botrytis cinerea*. *Phytopathology, 98*, 443–450.

Baroffio, C. A., Siegfried, W., & Hilber, U. W. (2003). Long-term monitoring for resistance of *Botryotinia fuckeliana* to anilinopyrimidine, phenylpyrrole, and hydroxyanilide fungicides in Switzerland. *Plant Disease, 87*, 662–666.

Beever, R. E., Laracy, E. P., & Pak, H. A. (1989). Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. *Plant Pathololgy, 38*, 427–437.

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

Chapeland, F., Fritz, R., Lanen, C., Gredt, M., & Lerouz, P. (1999). Inheritance and mechanisms of resistance to anilinopyrimidine fungicides in *Botrytis cinerea* (*Botryotinia fuckeliana*). *Pesticide Biochemistry and Physiology, 64*, 85–100.

Edwards, S. G., & Seddon, B. (2001). Selective media for the specific isolation and enumeration of *Botrytis cinerea* conidia. *Letters in Applied Microbiology, 32*, 63–66.

Elad, Y., Yunis, H., & Katan, T. (1992). Multiple resistance to benzimidazoles, dicarboximides and diethophencarb in field isolates of *Botrytis cinerea* in Israel. *Plant Pathology, 41*, 41–46.

Elmer, P., & Michailides, T. (2004). Epidemiology of *Botrytis cinerea* in orchard and vine crops. In Y. Elad, P. Williamson, P. Tudzinski, & N. Delen (Eds.), *Botrytis:*

Biology, pathology and control (pp. 243–272). Dordrecht: Kluwer Academic.

Esterio, M., Auger, J., & Garcia, H. (2007). First report of fenhexamid resistant isolates of *Botrytis cinerea* on grapevine in Chile. (Abstr.). *Plant Disease, 91*, 768.

Fillinger, S., Leroux, P., Auclair, C., Barreau, C., Al Hajj, C., & Debieu, D. (2008). Genetic analysis of fenhexamid-resistant field isolates of the phytopathogenic fungus *Botrytis cinerea*. *Antimicrobial Agents and Chemotherapy, 52*, 3933–3940.

Fitt, B. D. L., Creighton, N. F., & Brainbridge, A. (1985). Role of wind and rain in dispersal of Botrytis fabae conidia. *Transactions British Mycological Society, 85*, 307–312.

Förster, H., Driever, G. F., Thompson, D. C., & Adaskaveg, J. E. (2007). Postharvest decay management for stone fruit crops in California using the "reduced-risk" fungicides fludioxonil and fenhexamid. *Plant Disease, 91*, 209–215.

Förster, B., & Staub, T. (1996). Basis for use strategies of anilinopyrimidine and phenylpyrrole fungicides against *Botrytis cinerea*. *Crop Protection, 15*, 529–537.

Hänßler, G., & Pontzen, R. (1999). Effect of fenhexamid on the development of *Botrytis cinerea*. *Pflanzenschutz-Nachrichten Bayer, 52*, 158–176.

Hilber, U. W., & Hilber-Bodmer, M. (1998). Genetic basis and monitoring of resistance of *Botryotinia fuckeliana* to anilinopyrimidines. *Plant Disease, 82*, 496–500.

Hilber, U. W., Schwinn, F. J., & Schuepp, H. (1995). Comparative resistance patterns of fludioxonil and vinclozolin in *Botryotinia fuckeliana*. *Journal of Phytopathology, 143*, 423–428.

Jo, Y.-K., Niver, A. L., Rimelspach, J. W., & Boehm, M. J. (2006). Fungicide sensitivity of *Sclerotinia homoeocarpa* from golf courses in Ohio. *Plant Disease, 90*, 807–813.

Kalamarakis, A. E., Petsikos-Panagiotarou, N., & Ziogas, B. N. (2000). Activity of fluazinam against strains of *Botrytis cinerea* resistant to benzimidazoles and/or dicarboximides and to a benzimidazole-phenylcarbamate mixture. *Journal of Phytopathology, 148*, 449–455.

Köller, W., Wilcox, W., Barnard, J., & Braun, P. (1997). Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. *Phytopathology, 87*, 184–190.

Korolev, N., Mamiev, M., & Elad, Y. (2009). Resistance to fungicides among *Botrytis cinerea* isolates from tomato and other hosts in Israel. *Acta Horticulturae, 808*, 367–375.

Kretschmer, M., Leroch, M., Mosbach, A., Walker, A.-S., Fillinger, S., Mernke, D., et al. (2009). Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLoS Pathogens, 5*, Article e1000696. Retrieved June 15, 2010, from http://www.plospathogens.org

Leroux, P. (2004). Chemical control of *Botrytis* and its resistance to chemical fungicides. In Y. Elad, P. Williamson, P. Tudzinski, & N. Delen (Eds.), *Botrytis: Biology, pathology and control* (pp. 195–222). Dordrecht: Kluwer Academic.

Leroux, P., Chapeland, F., Desbrosses, D., & Gredt, M. (1999). Patterns of cross-resistance to fungicides in *Botryotinia fuckeliana* (*Botrytis cinerea*) isolates from French vineyards. *Crop Protection, 18*, 687–697.

Löchner, F. J., Lorenz, G., & Beetz, K.-J. (1987). Resistance management strategies for dicarboximide fungicides in grapes: results of six years' trial work. *Crop Protection, 6*, 139–147.

Ma, Z., & Michailides, T. J. (2005). Genetic structure of *Botrytis cinerea* populations from different host plants in California. *Plant Disease, 89*, 1083–1089.

Morton, V., & Staub, T. (2008). A short history of fungicides. *APSnet Feature, March 2008.* Retrieved from http://apsnet.org/online/feature/fungi

Myresiotis, C. K., Karaoglandis, G. S., & Tzavella-Klonari, K. (2007). Resistance of *Botrytis cinerea* isolates from vegetable crops to anilidopyrimidine, phenylpyrrole, hydroxyanilide, benzimidazole, and dicarboximide fungicides. *Plant Disease, 91*, 407–413.

Oshima, M., Fujimura, M., Banno, S., Hashimoto, C., Motoyama, T., Ichiishi, A., et al. (2002). A point mutation in the two-component histidine kinase *BcOS-1* gene confers dicarboximide resistance in field isolates of *Botrytis cinerea*. *Phytopathology, 92*, 75–80.

Raposo, R., Delcan, J., Gomez, V., & Melgarejo, P. (1996). Distribution and fitness of isolates of *Botrytis cinerea* with multiple fungicide resistance in Spanish greenhouses. *Plant Pathology, 45*, 497–505.

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

Savary, S., Delbac, L., Rochas, A., Taisant, G., & Willocquet, L. (2009). Analysis of nonlinear relationships in dual epidemics, and its application to the management of grapevine downy and powdery mildews. *Phytopathology, 99*, 930–942.

Suty, A., Pontzen, R., & Stenzel, K. (1999). Fenhexamid—sensitivity of *Botrytis cinerea*: determination of baseline sensitivity and assessment of the risk of resistance. *Pflanzenschutz-Nachrichten Bayer, 52*, 145–157.

Vignutelli, A., Hilber-Bodmer, M., & Hilber, U. W. (2002). Genetic analysis of resistance to the phenylpyrrole fludioxonil and the dicarboximide vinclozolin in *Botryotinia fuckeliana* (*Botrytis cinerea*). *Mycological Research, 106*, 329–335.

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

Yourman, L. F., Jeffers, S. N., & Dean, R. A. (2001). Phenotype instability in *Botrytis cinerea* in the absence of benzimidazole and dicarboximide fungicides. *Phytopathology, 91*, 307–315.

Ziogas, B. N., Markoglou, A. N., & Spyropoulou, V. (2005). Effect of phenylpyrrole-resistance mutations on ecological fitness of *Botrytis cinerea* and their genetic basis in *Ustilago maydis*. *European Journal of Plant Pathology, 113*, 83–100.