Sensitivity of *Botrytis squamosa* to Different Classes of Fungicides

D. M. Tremblay, Agriculture and Agri-Food Canada, St. Jean sur Richelieu, QC, Canada; B. G. Talbot, Department of Biology, Sherbrooke University, Sherbrooke, QC, Canada; and O. Carisse, Agriculture and Agri-Food Canada, St. Jean sur Richelieu, QC, Canada

ABSTRACT

Tremblay, D. M., Talbot, B. G., and Carisse, O. 2003. Sensitivity of *Botrytis squamosa* to different classes of fungicides. Plant Dis. 87:573-578.

An automated quantitative (AQ) assay was compared with radial growth on solid media and with dry weight in liquid culture for assaying fungicide sensitivity in *Botrytis squamosa*, the causal agent of onion leaf blight. Five isolates of *B. squamosa* were assayed for sensitivity to mancozeb (Dithane DG) and iprodione (Rovral) at five concentrations (0.5, 1.0, 5.0, 10.0, and 50 ppm). For mancozeb, the correlations between 50% effective concentration (EC_{50}) values obtained with the three assays were not significant; however for iprodione, correlations between EC_{50} values for AQ and radial growth and for AQ and dry weight were significant ($r = 0.98$ and 0.99 , respectively). The AQ method was less time consuming and more reliable than the two standard assays. The AQ method was used to evaluate the sensitivity of 35 field isolates of *B. squamosa* to mancozeb (Dithane DG), iprodione (Rovral), vinclozolin (Ronilan DF), and chlorothalonil (Bravo 500). All isolates were sensitive to mancozeb (EC_{50} ranged from 3.36 to 12.97) and chlorothalonil ($EC_{50} < 1.5 \mu\text{g/ml}$), but four isolates were insensitive to both iprodione ($EC_{50} \geq 3.98 \mu\text{g/ml}$) and vinclozolin ($EC_{50} \geq 17.49 \mu\text{g/ml}$). The ratio of the EC_{50} values of the least-sensitive and the most-sensitive isolates of *B. squamosa* was 1.08, 3.86, 6.98, and 37.59 for chlorothalonil, mancozeb, iprodione, and vinclozolin, respectively. Cross-resistance was observed for the two dicarboximide fungicides, iprodione and vinclozolin, with a significant correlation ($r = 0.94$) in the sensitivity of the 35 isolates to these two fungicides.

Onion (*Allium cepa* L.) is one of the most important vegetable crops produced on muck soils in North America, with an annual production of approximately 182,000 and 3,253,000 metric tons in Canada and the United States, respectively (29,33). In several areas of onion production, the most destructive disease is Botrytis leaf blight, caused by *Botrytis squamosa* J. C. Walker (16,19). On muck soils in Canada, New York, and Michigan, the disease is endemic and can threaten commercial onion production during moist and warm summer conditions (5,34). Conidia are the main source of inoculum produced on either over-wintered sclerotia present on the soil surface or onion debris in cull piles located near production fields. During the growing season, conidia are produced at night when onion leaves are wet and temperatures are between 8 and 22°C (31,32). During the leaf-spotting

phase of the disease, the leaves must be wet for at least 6 h for infection to occur at temperatures between 15 and 20°C (2,10). Lesions produced in response to infection are whitish in color, 1 to 5 mm in length, and surrounded by a white halo. Later in the season, the leaf-spotting phase progress to a dieback phase, during which *B. squamosa* colonizes senescent leaves, and sporulates on dead leaf tips. Under severe disease pressure, onion yield is reduced because of early defoliation, especially when defoliation occurs at the time of bulb initiation that corresponds to approximately 5 weeks before harvest (1,3).

Resistance to leaf blight has been identified in Japanese bunching onion (*A. fistulosum*; 11). However, there are currently no commercially available onion cultivars resistant to Botrytis leaf blight, although some cultivars are less susceptible than others. Consequently, most growers manage the disease by spraying protectant fungicides or combinations of systemic and protectant fungicides (8,22). In most areas of onion production in northeastern North America, protectant fungicides (mainly ethylene bisdithiocarbamates [EBDCs]) are applied on a 7- to 10-day schedule from mid-June until harvest. Under high disease pressure, spray intervals may be shortened or EBDC fungicides may be replaced with dicarboximide fungicides, such as iprodione and vinclozolin. Depending on the year, as many as 6 to 14 fungicide sprays may be

applied per season. Furthermore, when onions are produced in muck soils, there are limited possibilities for crop rotation due to restricted acreages in northeastern America and to a limited number of crops that can be grown economically on these soils. Consequently, populations of *B. squamosa* can be exposed to fungicide selection pressure over several years.

Fungicides registered for control of Botrytis leaf blight in Canada belong to one of three classes: benzonitriles, dicarboximides, and EBDCs (10). Failure to control this disease when using EBDCs has been reported and has motivated the use of dicarboximide fungicides (20,22). Insensitivity to EBDCs generally is not a problem, whereas there have been a number of reports of repeated applications of dicarboximides or benzonitriles resulting in selection of highly resistant populations of *B. cinerea* (6,17). Despite these reports and the intensive use of dicarboximide- and benzonitrile-based fungicides, there are few reports on the sensitivity of *B. squamosa* populations to these fungicides.

Most studies on the sensitivity of *Botrytis* spp. to fungicides have been carried out with *B. cinerea*, a species closely related to *B. squamosa*. Until recently, these experiments were conducted using standard methods of assaying for fungicide sensitivity, such as mycelial growth inhibition on solid or liquid media (15,30). In 1995, Raposo et al. (27) described an automated quantitative (AQ) assay, which proved to be reliable for screening for fungicide sensitivity in populations of *B. cinerea* (27). Automated quantitative assays were developed to determine fungal growth inhibition using microplate wells (7,23). Growth was measured by spectrophotometry and 50% effective concentration (EC_{50}) values were defined as the concentration of fungicide that reduced absorbance by half (27). This AQ assay was less cumbersome and time-consuming than the conventional fungicide sensitivity assays.

The objectives of this study were to (i) evaluate the AQ assay for fungicide sensitivity in *B. squamosa* compared with standard methods of screening for fungicide sensitivity and (ii) evaluate the sensitivity of *B. squamosa* to different classes of fungicides currently registered for control of Botrytis leaf blight of onion in Canada.

MATERIALS AND METHODS

Single-conidium isolates of *Botrytis squamosa*. During the summer of 2000,

Corresponding author: O. Carisse
E-mail: carisseo@em.agr.ca

This work was financially supported by a grant from the Fond pour la Formation de Chercheurs et l'Aide à la Recherche Action concertée FCAR-IRDA and by a Matching Investment Initiative between Agriculture and Agri-Food Canada and la Fédération des Producteurs Maraîchers du Québec.

Accepted for publication 28 December 2002.

Publication no. D-2003-0317-04R
© 2003 The American Phytopathological Society

onion leaves with typical symptoms of Botrytis leaf blight were collected in 32 onion fields located 30 to 50 km southwest of Montreal, Québec. A total of 48 samples were brought to the laboratory and each sample consisted of at least 10 blighted onion leaves. To enhance sporulation, blighted onion leaves were cut into pieces approximately 0.5 cm long, placed in a 90-mm-diameter petri dish on a moistened filter paper, and incubated at room temperature (20 to 25°C) for 2 to 10 days. Small pieces of leaves, each bearing a single sporulating lesion, were cut out of each leaf sample and placed in a 1.5-ml sterile Eppendorf tube (one per tube) containing 300 µl of a sterile glycerol solution (5%) amended with novobiocin (100 µg/ml; Sigma-Aldrich, St. Louis) and tetracycline (50 µg/ml, Sigma-Aldrich) to avoid bacterial contamination. After 15 s of agitation using a vortex, the spore suspension was poured onto a 90-mm-diameter water agar plate (agar at 20 g/liter) amended with novobiocin (100 µg/ml) and tetracycline (50 µg/ml), and incubated at room temperature for 6 h. Pieces of agar containing only one spore were removed from the water agar plates and placed on potato dextrose agar (PDA; Difco Laboratories, Detroit) plates (one per plate) amended with the antibiotics as described for the water agar. The plates were incubated in the dark at 18.5°C until mature sclerotia were produced (1 to 3 weeks). A total of 35 single-conidium isolates of *B. squamosa* (at least one isolate per field) were obtained and maintained on PDA plates until required for the experiment.

Comparison of assays to evaluate fungicide sensitivity. To compare the different assays and assess the reliability of the AQ assay (27) for measuring fungicide sensitivity in *B. squamosa*, 5 single-conidium isolates of *B. squamosa* were arbitrarily chosen from the 35 isolates. This technique was compared with two standard methods of assaying for fungicide sensitivity (15) using the fungicides Dithane DG (mancozeb, active ingredient [a.i.] 75%; Dow AgroSciences, Indianapolis, IN) and Rovral (iprodione, a.i. 50%; Aventis Group, Research Triangle Park, NC) at concentrations of 0.5, 1.0, 5.0, 10.0, and 50 ppm a.i.

The radial growth (RG) method of essaying for fungicide sensitivity was conducted using a solid agar medium. The commercially formulated fungicides were diluted in sterile water to prepare stock solutions of 10,000 and 1,000 µg a.i./ml (ppm a.i.). Fungicides were prepared by appropriate dilution of the stock solutions and added to PDA to obtain final concentrations of 0.5, 1.0, 5.0, 10.0, and 50.0 ppm a.i.. The fungicide-amended medium was inoculated with a 4-mm-diameter mycelial plug cut from the edge of a 3-week-old, actively growing culture of *B. squamosa*.

The cultures then were incubated in the dark at 18.5°C for 72 h. For both fungicides, each of the five isolates was inoculated onto three replicate plates of each of the five concentrations of fungicide. The entire experiment was conducted twice, each time in a completely randomized experimental design. Colony diameter (mm) was measured using a caliper after 72 h of incubation.

The dry weight (DW) method of essaying for fungicide sensitivity was conducted using a liquid medium. Fungicide concentrations were prepared as described above and the suspension added to potato dextrose broth (PDB; Difco Laboratories) to obtain the same final concentrations as for the solid media. The amended liquid medium was poured into 125-ml Erlenmeyer flasks (50 ml per Erlenmeyer) and inoculated with a mycelial plug as described for the RG method. Each of the five isolates was inoculated into three replicate flasks for each of the five concentrations of both fungicides. The entire experiment was conducted twice as a completely randomized design. After 8 days of incubation in the dark at room temperature (20 to 25°C) under constant agitation (125 rpm) on an orbital Shaker (Model 4530; Thermo Forma, Marietta, OH), the liquid culture was filtered through filter paper (Whatman #541, Whatman International Ltd., Banbury, UK) using a porcelain Büchner funnel. Mycelia were oven dried at 80°C for 48 h and DW was measured in grams.

For the AQ assay, conidia of the five single-conidium isolates were produced from sclerotia. Mature sclerotia were collected from 3- to 4-week-old cultures of *B. squamosa* and placed on sterile filter paper (Whatman #2) on top of a thin layer of cotton at the bottom of 90-mm-diameter plastic petri dishes. To provide high levels of humidity without soaking the sclerotia, each filter paper was moistened with 20 ml of double-distilled water. The sclerotia were incubated at 15°C in a incubator with a 12-h photoperiod provided by ultraviolet lights (Sylvania F20T12/BLB, 310 to 420 nm; Osram Sylvania Ltd., Mississauga, Canada) placed 18 cm above the plates (3,4). When the sclerotia were covered with conidia (after approximately 10 days), sclerotia were removed one at a time and held over the top of a sterile Eppendorf tube with forceps. Conidia then were gently scraped from each sclerotium using a scalpel and collected in the Eppendorf tube. The dry spores were stored at 4°C until required for the fungicide assays.

Solutions of PDB, each amended with a different concentration of fungicide, were prepared as described previously. Each well of a sterile 96-wells enzyme-linked immunosorbent assay (ELISA) microplate (MicroWell Plates, Nalge Nunc International, Rochester, NY) was filled with 50 µl of fungicide-amended PDB. Dry spores of *B. squamosa* were suspended in PDB

and the concentration of conidia adjusted to 20,000 conidia/ml using a hemacytometer. This conidial suspension (50 µl) was added to each well of the ELISA plate to obtain a final concentration of 1,000 conidia per 100 µl in each well. Each well was considered a replication, and each treatment was replicated eight times. Absorbance of the suspension at 400 nm was measured using a microplate reader (µ-Quant, Bio-Tek Instruments, Winooski, VT) 30 min after filling the wells, and after 72 h of incubation in the dark at 20°C and 90% relative humidity in a controlled environment chamber (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) (7). This high level of humidity was maintained in order to prevent evaporation of the solution from the wells. Absorbance was used as a measure of fungal growth and was calculated by subtracting the initial absorbance value from the value obtained again after 72 h of incubation.

Sensitivity of isolates of *B. squamosa* to fungicides. Each of the 35 single-conidium isolates of *B. squamosa* was tested in vitro for sensitivity to four fungicides commonly used for management of Botrytis leaf blight. The fungicides tested were, for the EBDC class, Dithane DG (Dow AgroSciences, Indianapolis, IN); for the benzonitrile class, Bravo 500 (Syngenta Crop Protection, Greensboro, NC); and two dicarboximide fungicides, Rovral (Aventis Group) and Ronilan DF (BASF Agricultural Chemical, Inc., Research Triangle Park, NC). Sensitivity of the isolates was tested at each of seven concentrations of fungicide: 0.00, 1.78, 3.16, 5.62, 10.00, 17.78, 31.62, and 56.23 ppm a.i., which corresponded to 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, and 1.75 log (fungicide concentration) in ppm a.i. To assay sensitivity of the 35 single-conidial isolates to each fungicide, conidia were collected in glycerol instead of using dry spores, mainly because of the number of isolates and the possibility of storing the spore suspensions. Sporulating sclerotia were soaked in 1 ml of sterile water containing 5% glycerol (Sigma-Aldrich). The spore suspension was agitated for 30 s using a vortex, the sclerotium was removed, and the suspension was adjusted to 2×10^5 conidia/ml using a hemacytometer. The spore suspensions were stored at -20°C for up to 2 months in sterile 1.5-ml Eppendorf tubes. Preliminary tests showed that storage of the spores in this manner did not affect spore germination. The AQ assay was conducted as described above, except that 12 replicate wells were inoculated instead of 8. Percent inhibition of each isolate at each fungicide concentration was calculated by dividing absorbance of the wells for each treatment by the mean absorbance of the control treatment (no fungicides) for that isolate. The EC₅₀ value was defined as the fungicide concentration (ppm) that suppressed fungal growth to one

half of that of the same isolate of *B. squamosa* on unamended media. EC₅₀ values were calculated for each isolate and each fungicide by regression analysis of the percent inhibition of fungal growth versus the log of the fungicide concentration (ppm).

Data analysis. To compare these methods for screening for fungicide sensitivity, growth and DW measurements each were normalized as percentage of the growth or DW, respectively, on unamended media (0 ppm). Linear regression of the percent inhibition of fungal growth as a function of the log of the concentration of fungicide was used to determine EC₅₀ values. Linear regression was carried out using the general linear modeling (GLM) procedure in SAS (version 8.02; SAS Institute Inc., Cary, NC). Tests of homogeneity of variances (*F* test) were conducted to determine if data from the two experimental runs could be pooled and to ensure the underlying assumptions of the statistical models. Analysis of variance was used to test the significance of the assay on the percent inhibition of fungal growth and on the coefficient of determination of the regression of percent inhibition on log of the concentration of fungicide. Multiple comparison tests (least significant difference) were used to detect significant differences among means for both percent inhibition and coefficient of determination at the 0.05 confidence level. The variation factor, defined as the ratio between the EC₅₀ value of the least- and most-sensitive isolates, was used to compare the sensitivity of the isolates to the four fungicides. Correlation analysis was used to determine the relationship of EC₅₀ values obtained for the 35 isolates and the four fungicides.

RESULTS

Comparison of assays to evaluate fungicide sensitivity. The homogeneity of variance test indicated that data from the two experimental runs could be pooled (*P* > 0.05); hence, analysis was conducted on pooled data. For mancozeb, the assay used to evaluate fungicide sensitivity had a significant effect on percent inhibition of fungal growth (*P* < 0.0001) and the percent inhibition was significantly higher when the DW assay was used (Table 1). For iprodione, the assay had a significant effect on percent inhibition of fungal growth (*P* < 0.0069) and highest percent inhibition was obtained with the RG assay (Table 1). For mancozeb, the mean EC₅₀ values for the five single-conidium isolates of *B. squamosa* used to compare the three assays for screening for fungicide sensitivity ranged from 5.33 to 28.91 ppm for the RG assay, 0.00 to 0.24 ppm for the DW assay, and 3.32 to 4.91 ppm for the AQ assay (Fig. 1A). For iprodione, the EC₅₀ values ranged from 0.12 to 1.21 ppm for the RG assay, 0.24 to 1.80 ppm for the DW assay, and 0.51 to 3.53 ppm for the AQ assay (Fig. 1B). The EC₅₀ values obtained for the

five isolates with the DW assay were lower than the EC₅₀ values obtained with the RG and AQ assays. The mean EC₅₀ values for iprodione and for the five isolates obtained using all three assays ranged between 0.12 and 3.53 ppm. All three assays identified

isolate C as the least sensitive of the five isolates to iprodione. Overall, the three assays provided similar ranking in terms of level of sensitivity to iprodione, which was not the case for mancozeb. For example, the mean EC₅₀ value obtained for manco-

Table 1. Percent inhibition of fungal growth and coefficient of determination of the regression of percent inhibition on log of the concentration of fungicide for different assays to measure fungicide sensitivity of *Botrytis squamosa*

Assay ^z	Fungicide ^y	
	Dithane	Rovral
Percent inhibition of fungal growth		
RG	32.82 b	73.02 a
DW	83.42 a	68.06 ab
AQ	24.44 b	62.08 b
LSD	11.71	7.63
Coefficient of determination		
RG	0.67 a	0.91 a
DW	0.21 b	0.70 b
AQ	0.82 a	0.81 ab
LSD	0.16	0.14

^y Values within the same column with the same letter are not significantly different according to the least significant difference (LSD) test (*P* < 0.05).

^z RG = radial grown, DW = dry weight, AQ = automated quantitative, and LSD = least significant difference.

Fig. 1. Effective concentration (EC₅₀) of fungicide A, mancozeb and B, iprodione required to reduce mycelial growth by 50% for five isolates of *Botrytis squamosa* obtained from onion fields in southwest Montreal in 2000. EC₅₀ values were obtained using radial growth (RG), dry weight (DW), and automated quantitative (AQ) assays. The assays were conducted with fungicides at concentrations of 0.5, 1.0, 5.0, 10.0, and 50.0 ppm active ingredient.

zeb for isolate B was 28.910, 0.001, and 4.348 for the RG, DW, and AQ, assays, respectively. For mancozeb, there were no significant correlations between EC_{50} values obtained for AQ and RG ($r = 0.13$, $P = 0.8346$) and for AQ and DW ($r = -0.31$, $P = 0.6083$). For iprodione, there was a significant correlation between EC_{50} values for AQ and RG ($r = 0.99$, $P = 0.0002$) and for AQ and DW ($r = 0.98$, $P = 0.0019$). The different fungicide sensitivity assays were compared based on the coefficient of determination (R^2) of the regressions of percent inhibition on log fungicide concentration. For both fungicides, there was a significant effect of the assay used on the coefficient of determination, which was significantly higher for the AQ and RG assays (Table 1). Based on pooled data, for mancozeb, the R^2 values ranged from 27 to 68% for the RG assay, from 1 to 15% for the DW assay, and from 75 to 98% for the AQ assay (Fig. 2). The R^2 values were higher for iprodione than for

mancozeb, except for two values below 60% for the DW assay. Except for isolates C and E assayed with iprodione, R^2 values were higher for the AQ assay than for the other two assays and, hence, this assay was selected to evaluate a larger set of isolates of *B. squamosa* for sensitivity to fungicides.

Sensitivity of isolates of *B. squamosa* to fungicides. The EC_{50} values for the 35 isolates of *B. squamosa* assayed for fungicide sensitivity to mancozeb ranged from 3.36 to 12.97 ppm, and 34 of the isolates had EC_{50} values between 3.36 and 8.05 ppm. Isolate 2 was by far the least sensitive to mancozeb of all the isolates tested, with an EC_{50} value of 12.97 ppm. The variation factor for mancozeb was 3.86 (Fig. 3A). The EC_{50} values for iprodione ranged from 1.33 to 9.29 ppm, with a variation factor of 6.98 (Fig. 3C). Thirty-one isolates had an EC_{50} value between 1.33 and 1.36 ppm and four isolates showed some level of insensitivity to iprodione, with EC_{50} values of 3.98, 5.14,

6.24, and 9.29 ppm (Fig. 3C). The EC_{50} values for vinclozolin ranged from 1.33 to >50 ppm, with a variation factor of 37.59 (Fig. 3A). Thirty-one isolates had an EC_{50} value between 1.33 and 1.35 ppm (Fig. 3A) and four isolates showed some insensitivity to vinclozolin, with EC_{50} values from 17.49 to >50 ppm. All 35 isolates were sensitive to chlorothalonil, with EC_{50} values ranging from 1.31 to 1.41 ppm and with a variation factor of 1.08 (Fig. 3D).

Of the 35 single-conidium isolates of *B. squamosa* tested, the same four isolates showed insensitivity to both iprodione and vinclozolin. There was a significant correlation between the EC_{50} values for iprodione and the EC_{50} values for vinclozolin ($r = 0.948$, $P < 0.0001$), but the correlation between the EC_{50} values for chlorothalonil and mancozeb was not significant ($r = -0.177$, $P = 0.310$).

DISCUSSION

In this study, we compared an AQ assay with standard methods for the evaluation of fungicide sensitivity in *B. squamosa*. Sensitivity to four currently registered fungicides for control of Botrytis leaf blight of onion in Canada was evaluated for 35 single-conidium isolates. Overall, the AQ assay was more reliable than the standard methods of measuring fungicide sensitivity based on RG and DW as measured by a higher coefficient of determination (R^2) of the regression analysis used to calculate the EC_{50} values. For mancozeb, there was no correlation between EC_{50} values obtained with the three assays. However, for iprodione, the correlations between EC_{50} values for AQ and RG and for AQ and DW were positive ($r = 0.98$ and 0.99 , respectively). This is in accordance with the results reported by Raposo et al. (27) on the sensitivity of *B. cinerea* to iprodione and to a mixture of carbendazim and diethofencarb. Similarly, Pijls et al. (24) used light absorbance readings to measure the sensitivity of *Septoria tritici* to flutriafol, a sterol biosynthesis inhibitor. The AQ assay has several advantages over the standard methods of measuring fungicide sensitivity, especially when evaluating a large number of fungal isolates or fungicides. Raposo et al. (27) reported that the AQ assay used 100 times less growth medium than assays based on measuring radial growth of fungi on agar plates. Despite the advantages of the AQ assay, which also include lower costs and time savings, the production of spores could be a limiting factor for fungi, which do not sporulate readily in culture (3). For example, to obtain spores of *B. squamosa*, sclerotia must be produced, which means extra steps compared with assays that require only mycelium.

The results of this study showed that most isolates of *B. squamosa* isolated from onion fields in southwest Montreal in 2000 were sensitive to mancozeb, iprodione, vinclozolin, and chlorothalonil. All 35

Fig. 2. Coefficient of determination of the regression of percent inhibition of growth of *Botrytis squamosa* against the log of fungicide concentration for **A**, mancozeb and **B**, iprodione. Regressions were conducted on percent inhibition obtained using radial growth (RG), dry weight (DW), and automated quantitative (AQ) assays. The assays were conducted with fungicides at concentrations of 0.5, 1.0, 5.0, 10.0, and 50.0 ppm active ingredient.

isolates assayed were sensitive to mancozeb, with EC_{50} values ranging from 3.36 to 12.97 ppm. Although all were sensitive, there was a continuum in EC_{50} values obtained. This is the first report on sensitivity of *B. squamosa* to EBDCs and the results are based on only 35 isolates; therefore, it is difficult to establish the concentration of fungicide at which an isolate could be considered insensitive. In most studies on fungicide sensitivity in *B. cinerea* populations, EBDCs were not included (6,11,12,14,17,26,30). However, Delen et al (13) tested the sensitivity to mancozeb of 27 isolates of *B. cinerea* isolated from greenhouses crops in Turkey. All isolates grew on culture media amended with man-

cozeb at <3 ppm and 7, 26, 41, and 26% of the isolates grew on media amended with 3 to 10, 10 to 30, 30 to 100, and >100 ppm, respectively. Fungicide insensitivity generally is not a concern with protectant fungicides such as EBDCs because of their multisite mode of action. Accordingly, insensitivity to an EBDC fungicide has not been reported for *B. squamosa*, although Lorbeer and Ellerbrock reported decline in disease control with such fungicide in 1976 (20). Of the 35 field isolates of *B. squamosa* tested in this study, four isolates showed insensitivity to iprodione and vinclozolin, with EC_{50} values >3.98 ppm. In their study, Stehmann and De Waard (26) suggested that isolates of *B. cinerea* capa-

ble of growing on culture media amended with benomyl or vinclozolin at 5 ppm were highly resistant, whereas isolates inhibited at 5 ppm but not at 1 ppm were moderately resistant, and those inhibited at 1 ppm were sensitive to these fungicides. Insensitivity of isolates of *B. cinerea* and *B. squamosa* to dicarboximide fungicides was first reported more than 20 years ago (9,25). Insensitive strains of both *B. cinerea* and *B. squamosa* were produced readily in vitro by growing the isolates on culture media amended with iprodione at up to 550 ppm or exposing the isolates to ultraviolet light (25). Insensitivity of these strains was stable and these isolates showed cross-tolerance to other dicarboximide fungicides.

Fig. 3. EC_{50} values for 35 single-conidium isolates of *Botrytis squamosa* from onion crops in southwest Montreal assayed for sensitivity to **A**, vinclozolin, **B**, mancozeb, **C**, iprodione, and **D**, chlorothalonil. The automated quantitative (AQ) assay was used to test fungicide sensitivity at concentrations of 0.00, 1.78, 3.16, 5.62, 10.00, 17.78, 31.62, and 56.23 ppm active ingredient.

However, Presley and Maude (26) reported that applications of iprodione controlled iprodione-insensitive isolates of *B. squamosa* when inoculated on onion leaves. In their study, iprodione-insensitive isolates readily infected onion leaves but spore production from lesions was lower than for the sensitive isolates (26). In our study, insensitive isolates of *B. squamosa* were not induced in vitro and the isolates were cultured on unamended medium prior to being tested for fungicide sensitivity; therefore, these isolates represented components of *B. squamosa* populations in onion fields. As reported in similar studies (14,28), cross-insensitivity was observed for the two dicarboximide fungicides assayed because all isolates resistant to vinclozolin also were resistant to iprodione. All isolates tested were sensitive to chlorothalonil, with EC₅₀ values below 1.5 ppm. Fungicide scheme was similar among growers; most growers sprayed with mancozeb alone or mixed with iprodione. Based on the growers' reports, the most widely used fungicide was mancozeb, with five to nine applications per season, followed by iprodione, with one to four applications, and chlorothalonil, with one to three applications. There was no relationship between the frequency of iprodione and chlorothalonil applications and the fungicide sensitivity measured with the AQ assay.

To our knowledge, this is the first report of dicarboximide insensitivity in *B. squamosa* in North America. Although such insensitivity or failure to control disease has not been reported in onion fields, our results demonstrate that there is a potential for the development of dicarboximide insensitivity in populations of *B. squamosa*. There is no report on the occurrence of the perfect stage of *B. squamosa* (*Botryotinia squamosa* (Vien-Bourg.)) in muck soils of Québec; however, it has been reported in the area of New York (21). Accordingly, antiresistance strategies should be implemented in order to delay insensitivity development. Mixing or alternating applications of dicarboximides with broad-spectrum contact fungicides such as chlorothalonil should help delay the proliferation of dicarboximide-resistant populations of *B. squamosa*. In 1990, Lorbeer and Vincelli (22) reported that, although some level of disease control was achieved with applications of dicarboximide fungicides for Botrytis leaf blight, control was improved significantly when these fungicides were mixed with a contact fungicide such as chlorothalonil. The use of fungicide mixtures and rotation of fungicide classes may be more expensive than the use of dicarboximides alone. Because cost-effectiveness for fungicides is a major consideration for onion producers, chlorothalonil or dicarboximide fungicides may be used only under high disease pressure (18).

In order to determine whether the range of sensitivity to dicarboximides fungicides observed in this study accounts for reduced field performance of the fungicides towards *B. squamosa*, the ability of iprodione- and vinclozolin-insensitive isolates to grow, infect, and sporulate on onion leaves should be studied (12). In addition, it will be important to establish the ability of these isolates to cause leaf blight in onion fields routinely sprayed with these fungicides.

ACKNOWLEDGMENTS

We thank B. Mimee for his technical assistance.

LITERATURE CITED

1. Alderman, S. C., Biernbaum, J. A., Hoffhines, M., Timberlake, D. M., Welch, D. P., Lacy, M. L., and Barr, R. O. 1987. A simulation model of the spread of Botrytis leaf blight of onion and its effect on onion yields. *Agric. Ecosyst. Environ.* 19:55-70.
2. Alderman, S. C., and Lacy, M. L. 1983. Influence of dew period and temperature on infection of onion leaves by dry conidia of *Botrytis squamosa*. *Phytopathology* 73:1020-1023.
3. Balis, F. J., and Lorbeer, J. W. 1992. The physiology of conidiation in *Botrytis squamosa*. Pages 123-126 in: *Recent Advances in Botrytis Research*. Proc. 10th Int. Botrytis Symp. Heraklion, Crete, Greece. K. Verhoeff, N. E. Malathrakakis, and B. Williamson, eds. Pudoc Scientific Publishers, Wageningen, The Netherlands.
4. Bergquist, R. R., and Lorbeer, J. W. 1972. Apothecial production, compatibility and sex in *Botryotinia squamosa*. *Mycologia* 64:1270-1281.
5. Boivin, G., and Sauriol, P. 1984. Dispersion statistics and sequential sampling plan for leaf blight caused by *Botrytis squamosa* in onions. *Phytopathology* 74:1385-1387.
6. Bollen, G. J., and Scholten, C. 1971. Acquired resistance to benomyl and some other systemic fungicides in a strain of *Botrytis cinerea* in cyclamen. *Neth. J. Plant Pathol.* 77:83-90.
7. Broekard, W. F., Terras, F. R. G., Cammue, B. P. A., and Vanderlyden, J. 1990. An automated quantitative assay for fungal growth inhibition. *FEMS Microbiol. Lett.* 69:55-60.
8. Centre de Référence en Agriculture et Agroalimentaire du Québec. Répertoire des traitements de protection des cultures. 2001-2002. CRAAQ, Ste-Foy, Quebec, Canada.
9. Channon, A. G., and Thompson, M. C. 1977. Studies on the fungicidal control of stem lesions caused by *Botrytis cinerea* on glasshouse tomatoes. *Ann. Appl. Biol.* 85:359-368.
10. Clark, C. A., and Lorbeer, J. W. 1976. Comparative histopathology of *Botrytis squamosa* and *B. cinerea* on onion leaves. *Phytopathology* 66:1279-1289.
11. Currah, L., and Maude, R. B. 1984. Laboratory tests for leaf resistance to *Botrytis squamosa* in onions. *Ann. Appl. Biol.* 105:277-283.
12. Davis, R. P., and Dennis, C. 1981. Studies on the survival and infective ability of dicarboximide-resistant strains of *Botrytis cinerea*. *Ann. Appl. Biol.* 98:395-402.
13. Delen, N., Yildiz, M., and Maraite, H. 1984. Benzimidazole and dithiocarbamate resistance of *Botrytis cinerea* on greenhouses crops in Turkey. *Meded. Fac. Landbouwwet. Rijksuniv. Gent* 49:153-161.
14. Elad, Y., Yunis, H., and Katan, T. 1992. Multiple resistance to benzimidazoles, dicarboximides and diethofencarb in field isolates of *Botrytis cinerea* in Israel. *Plant Pathol.* 41:41-46.
15. Georgopoulos, S. G. 1982. Detection and measurement of fungicide resistance. Pages 24-31 in: *Fungicide Resistance in Crop Protection*. J. Dekker and S. G. Georgopoulos, eds. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
16. Hancock, J. G., and Lorbeer, J. W. 1963. Pathogenesis of *Botrytis cinerea*, *B. squamosa*, and *B. allii* on onion leaves. *Phytopathology* 53:669-673.
17. Leroux, P., Lafon, R., and Gredt, M. 1982. La résistance du *Botrytis cinerea* aux benzimidazoles et aux imides cycliques: Situations dans les vignobles Alsaciens, Bordelais et Champenais. *OEPP/EPPO Bull.* 12:137-143.
18. Levy, Y., Levi, R., and Cohen Y. 1983. Buildup of a pathogen subpopulation resistant to a systemic fungicide under various control strategies: A flexible simulation model. *Phytopathology* 73:1475-1480.
19. Lorbeer, J. W. 1992. Botrytis leaf blight of onion. Pages 186-211 in: *Plant Diseases of International Importance*. H. S. Chaube, U. S. Singh, A. N. Mukhopadhyay, and J. Kumar, eds. Prentice Hall, Englewood Cliffs, NJ.
20. Lorbeer, J. W., and Ellerbrock, L. A. 1976. Failure of ethylene bisdithiocarbamates to control Botrytis leaf blight of onion. *Proc. Am. Phytopathol. Soc.* 3:75-84.
21. Lorbeer, J. W., and Ellerbrock, L. A. 1977. Sources of primary inoculum of *Botrytis squamosa*. *Phytopathology* 67:363-372.
22. Lorbeer, J. W., and Vincelli, P. C. 1990. Efficacy of dicarboximides fungicides and fungicide combinations for control of Botrytis leaf blight of onion in New York. *Plant Dis.* 74:235-237.
23. Ludwig, A., and Boller, T. 1990. A method for the study of fungal growth inhibition by plant proteins. *FEMS Microbiol. Lett.* 69:61-66.
24. Pijls, C. F. N., Shaw, M. W., and Parker, A. 1994. A rapid test to evaluate in vitro sensitivity of *Septoria tritici* to flutriafol, using a microtiter plate reader. *Plant Pathol.* 43:726-732.
25. Presley, A. H., and Maude, R. B. 1980. Control of *Botrytis cinerea* and *Botrytis squamosa* in overwintered salad onion by fungicides sprays. *Ann. Appl. Biol.* 94:197-204.
26. Presley, A. H., and Maude, R. B. 1982. Tolerance in *Botrytis squamosa* to iprodione. *Ann. Appl. Biol.* 100:117-127.
27. Raposo, R., Colgan, R., Delcan, J., and Melgarejo, P. 1995. Application of an automated quantitative method to determine fungicide resistance in *Botrytis cinerea*. *Plant Dis.* 79:294-296.
28. Raposo, R., Delcan, J., Gomez, V., and Melgarejo, P. 1996. Distribution and fitness of isolates of *Botrytis cinerea* with multiple fungicide resistance in Spanish greenhouses. *Plant Pathol.* 45:497-505.
29. Statistique Canada, Division de l'Agriculture, Sous-Division de l'Horticulture. Productions de fruits et légumes. June 2001. Statistique Canada, Ottawa, Canada.
30. Stehmann, C., and De Waard, M. A. 1996. Sensitivity of populations of *Botrytis cinerea* to triazoles, benomyl and vinclozolin. *Eur. J. Plant Pathol.* 102:171-180.
31. Sutton, J. C., James, T. D. W., and Rowell, P. M. 1983. Relation of weather and host factors to an epidemic of Botrytis leaf blight in onions. *Can. J. Plant Pathol.* 5:256-265.
32. Sutton, J. C., Swanton, C. J., and Gillespie, T. J. 1978. Relation of weather variables and host factors to incidence of airborne spores of *Botrytis squamosa*. *Can. J. Bot.* 56:2460-2469.
33. United States Department of Agriculture. 2002. Vegetables 2001 Summary. National Agricultural Statistics Service, Washington, DC.
34. Vincelli, P. C., and Lorbeer, J. W. 1988. Relationship of precipitation probability to infection potential of *Botrytis squamosa* on onion. *Phytopathology* 78:1078-1082.