Resistance of *Botryosphaeria dothidea* from Pistachio to Iprodione

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ABSTRACT

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Out of 79 field-collected isolates of *Botryosphaeria dothidea*, causal agent of panicle and shoot blight of California pistachio, 1 had low resistance to the dicarboximide fungicide iprodione (effective fungicide concentration to inhibit 50% of mycelial growth [EC₅₀] = 2.726 µg/ml), whereas all the other isolates were sensitive (EC₅₀ < 1 µg/ml). *B. dothidea* isolates readily developed resistance to iprodione in vitro. Furthermore, these in vitro-derived iprodione-resistant (IR) isolates retained high virulence on pistachio. The EC₅₀ values for these IR isolates were greater than 25 µg/ml, whereas the EC₅₀ values for the original wild-type counterparts were less than 1 µg/ml. Iprodione resistance significantly declined when these IR isolates were propagated on pistachio leaves in the absence of the fungicide. IR isolates also were resistant to vinclozolin, another dicarboximide fungicide, but sensitive to tebuconazole and benomyl. IR isolates showed an increase in osmotic sensitivity on media amended with NaCl. Applications of iprodione at 500 µg/ml, which is effective against naturally sensitive isolates, failed to control disease caused by IR isolates in both the laboratory and greenhouse. The results indicate that, although naturally occurring IR isolates of *B. dothidea* may be rare in California pistachio orchards, the fungus readily develops resistance to iprodione in vitro and, more importantly, retains high levels of virulence on pistachio.

Additional keywords: *Dothiorella* sp., fungicide resistance, Rovral

Botryosphaeria panicle and shoot blight, caused by *Botryosphaeria dothidea* (imperfect stage *Dothiorella* sp.), has recently become an important disease of pistachio (*Pistacia vera* L.) throughout California. Initially, only pistachios grown in the Sacramento Valley were affected by this pathogen; however, by the late 1990s, severe disease outbreaks significantly reduced both yield and fruit quality in orchards of the Central San Joaquin Valley (19). Recently, samples of blighted pistachio shoots and clusters collected from the southernmost part of Kern County were also found to have pycnidia of *B. dothidea* (20). Due to the rapid spread of this pathogen throughout California, *Botryosphaeria* panicle and shoot blight is now considered the single greatest threat to the California pistachio industry.

Control of *Botryosphaeria* blight becomes difficult once this disease has been established in orchards. A number of fungicides have been tested and proved to provide only marginal control. The dicarboximide fungicide iprodione (Rovral; Rhône Poulenc Ag. Co., Lyon, France), however, is effective for controlling

Botryosphaeria panicle and shoot blight of pistachio (16,17). Isolates resistant to iprodione were rarely observed in nature after the introduction of this fungicide in the mid 1970s. However, by the early 1980s, resistant isolates of *Botrytis cinerea* were reported from several crops (13). Loss of efficacy of dicarboximides due to resistance has been reported for *Botrytis cinerea* from vineyards in New Zealand (21), greenhouse-grown cucumbers in Israel (27), and strawberry fields in England (11). Furthermore, many fungal plant pathogens, such as *Alternaria alternata* (2,15), *Sclerotinia minor* (3,10,23), and *S. homeocarpa* (5), were reported to readily develop iprodione-resistant (IR) variants in the laboratory. After several years of intensive use of dicarboximide fungicides, IR fungal isolates have also been reported from the field (4,5,24).

The fitness of dicarboximide-resistant fungal isolates varies significantly (25). Some dicarboximide-resistant isolates have been reported to show lower virulence (5,8,14,26) and higher osmotic sensitivity (1,7,12) than their wild-type counterparts. However, high fitness in dicarboximide-resistant isolates has also been reported (3,8,14). A study of the fitness of IR *B. dothidea* isolates might provide information useful in prolonging the efficacy of iprodione in controlling *Botryosphaeria* blight on California pistachio. The objectives of this study were to (i) survey naturally occurring *B. dothidea* isolates from

California pistachio orchards for sensitivity to iprodione; (ii) select IR isolates of *B. dothidea* in the laboratory and test the stability of resistance, osmotic sensitivity, and virulence for these isolates; (iii) test the efficacy of iprodione for controlling *Botryosphaeria* blight caused by IR isolates; and (iv) test for cross-resistance with vinclozolin, tebuconazole, and benomyl.

MATERIALS AND METHODS

Survey of *B. dothidea* sensitivity to iprodione. Seventy-nine isolates, collected from eight California pistachio orchards from 1997 to 1999, were used to assess the sensitivity of *B. dothidea* to iprodione. Tissue plugs (0.5 by 0.5 cm) cut from diseased leaves and fruit were surface sterilized by soaking in 0.05% sodium hypochlorite (10% commercial bleach; Western Family Foods, Inc., Portland, OR) for 3 min and rinsed three times with sterilized deionized water. To isolate the fungus, tissue plugs were placed on acidified (2.5 ml of 25% [vol/vol] lactic acid per liter) Difco potato dextrose agar (APDA). Isolation of *B. dothidea* was confirmed after the petri dishes were incubated at 30°C for 5 days. Isolates collected in 1997 and 1998 were routinely stored on grade 40 silica gel (Davison Chemical, Baltimore) at 4°C, whereas isolates collected in 1999 were stored in APDA slants at 9°C.

Technical iprodione (a.i. 95%) was dissolved in acetone to the concentration of 10 µg/µl and added to the APDA medium to produce five standard concentrations: 0, 0.25, 0.5, 1.0, and 2.0 µg of iprodione per milliliter of medium. A 5-mm mycelial plug was cut from the edge of a 3-day-old colony and placed on the center of an APDA dish amended with each of the five iprodione concentrations. Two replicates for each concentration were used. The colony diameter was measured with the original mycelial plug diameter (5 mm) subtracted from this measurement after the dishes were incubated at 30°C for 3 days. For each isolate, a linear regression of percent inhibition related to control of mycelial growth versus the log₁₀ transformation for each of the five iprodione concentrations was obtained. The effective concentration to inhibit 50% of mycelial growth (EC₅₀) of each isolate was calculated using a linear equation. Frequencies of EC₅₀ for the 79 tested isolates were calculated using the procedure FREQ of SAS (version 7.0; SAS Institute, Cary, NC) and the distribution of EC₅₀ for these isolates was determined.

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Induction of iprodione-resistant (IR) isolates in vitro. IR isolates were selected after growing wild-type isolates on APDA amended with iprodione at 50, 400, and 1,000 µg/ml of medium from the first through the third generation, respectively. For each generation, dishes were incubated at 30°C for 20 days. The EC₅₀ value for each isolate of the third generation was determined on APDA amended with iprodione at 0, 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml of medium. After three generations, six IR isolates, designated 1R, 3R, 4R, 9R, 11R, and 15R, were obtained from nine wild-type isolates, each of which originated from a corresponding wild-type iprodione-sensitive (IS) isolate 1S, 3S, 4S, 9S, 11S, and 15S. These six IR isolates and their corresponding IS isolates were used in the following studies.

Detection of cross-resistance. Technical grade vinclozolin (a.i. 98.8%; BASF Agricultural Chemical, Inc., Research Triangle Park, NC) and benomyl (a.i. 95%; Du Pont, Inc., Wilmington, DE) were dissolved in acetone and adjusted to 80 and 10 µg/µl, respectively. Technical tebuconazole (a.i. 97.5%; Bayer AG, Inc., Bayerwerk, Germany) was dissolved in ethanol and adjusted to 10 µg/µl. APDA media were amended with each of the fungicides at the following concentrations: vinclozolin at 0, 0.125, 0.25, 0.5, 1.0, 12.5, 25, 50, and 100 µg/ml; benomyl at 0, 0.1, 0.2, 0.4, and 0.8 µg/ml; and tebuconazole at 0, 0.125, 0.25, 0.5, and 1.0 µg/ml. Two replicates of each fungicide concentration were used. A 5-mm mycelial plug for each IR or IS isolate of *B. dothidea* was taken from the edge of a 3-day-old colony and transferred onto the APDA dish amended with each concentration of the above fungicides. Petri dishes were incubated at 30°C for 3 days and the diameter for each colony was measured. The experiment was performed twice. The EC₅₀ was calculated as described above and, because the two experiments did not differ statistically ($P >$

0.05), the average EC₅₀ values from two experiments for each isolate were used in the data analysis. In order to compare the cross-resistance between fungicides, the EC₅₀ for IR and IS isolates to iprodione were plotted versus the EC₅₀ of these isolates to vinclozolin, tebuconazole, or benomyl, and correlation was tested using the procedure CORR of SAS (version 7.0, SAS Institute).

Measurement of osmotic sensitivity. For each IR or IS isolate of *B. dothidea*, a 5-mm mycelial plug was taken from an actively growing colony and transferred to an APDA dish amended with 1, 2, 4, 6, or 8% (wt/vol) NaCl, respectively. APDA dishes without NaCl were used as controls. Two replicates for each NaCl concentration were used. Dishes were incubated at 30°C for 3 days, and the colony diameter was measured for each dish. The percentage of the mycelial radial growth inhibition (RGI) was calculated using the formula $RGI = (C - N)/C$, where C is colony diameter of non-NaCl control and N is that for an NaCl treatment. The experiment was performed twice and the data were analyzed using analysis of variance (ANOVA) of SAS (version 7.0, SAS Institute).

Resistance stability of IR isolates following mycelial propagation. Pistachio leaves in the greenhouse were wounded and inoculated with mycelial plugs taken from each of the six IR isolates. Four leaves per IR isolate were used. Two weeks after inoculation, a small (5-by-5-mm) diseased tissue plug was removed from the lesion edge for each inoculated leaf and surface sterilized in 0.05% sodium hypochlorite for 3 min. Four diseased tissue plugs for each isolate were placed in a single dish and incubated at 30°C for 5 days. A mycelium plug, 5 mm in diameter, taken from the center of each dish, was then transferred into a fresh dish and incubated at 30°C for 3 days. Four mycelial plugs were taken from the edge of each colony for inoculation onto four pistachio

leaves in the greenhouse again, and the dish was stored at 9°C for detection of EC₅₀ of each IR isolate generation. After four successive generations of inoculation using the same method as described above, the EC₅₀ to iprodione for each generation was determined on APDA media amended with iprodione at 0, 1.56, 3.125, 6.25, 12.5, 25.0, 50.0, and 100 µg/ml. The least significant difference (LSD) of SAS (version 7.0, SAS Institute) was used to compare EC₅₀ among each generation for each IR isolate.

Resistance stability of IR isolate 9R following spore propagation. Leaves detached from greenhouse-grown pistachio trees were surface sterilized as described above. Each leaf was inoculated with a mycelial plug of IR isolate 9R. The inoculated leaves were incubated at room temperature ($25 \pm 2^\circ\text{C}$) in a humidified plastic container for 20 days. Nine single-conidial isolates were obtained from pycnidia produced on these leaves. The EC₅₀ value for each single-conidial isolate to iprodione was determined using the method described above and the EC₅₀ values for single-conidial isolates were compared with the EC₅₀ for the original isolate 9R using the LSD of SAS.

Efficacy of iprodione in controlling disease caused by IR and IS isolates. In laboratory tests, fully expanded detached pistachio leaves were surface sterilized as previously described and sprayed with iprodione at 500 µg/ml (the manufacturer's recommended field dosage) with a hand-held sprayer. Leaves sprayed with water were used as the nontreatment controls. After drying, each leaf was inoculated with a 3-mm mycelial plug from each IR isolate (1R, 3R, 4R, 9R, 11R, and 15R) and each IS isolate (1S, 3S, 4S, 9S, 11S, and 15S). Two replicates (10 leaves/replicate) for each isolate were used, and the leaves incubated at 30°C in humidified plastic containers for 4 days. The lesion length for each leaf was recorded. The efficacy of iprodione (EI) in controlling disease was calculated using the following formula: $EI = (LC - LT)/LC$, where LC is the average lesion length (in centimeters) from 10 nontreated control leaves and LT is that from 10 iprodione-treated leaves. The experiment was performed twice and data analyzed using ANOVA of SAS to determine the significant differences in EI between IR and IS sensitive isolates.

In greenhouse tests, 1 day prior to greenhouse inoculations, potted pistachio trees, about 2 m in height, were each sprayed with 100 ml of iprodione at 500 µg/ml using a hand sprayer. Trees sprayed with water alone were used as the noniprodione control. Each of three trees was inoculated with the IR isolates (1R, 3R, 4R, 9R, 11R, and 15R), the low resistance (LR) isolate L21, or the IS isolates (1S, 3S, 4S, 9S, 11S, and 15S). For fungal inoculation, mycelial fragments were washed from 7-

Fig. 1. Frequency distribution of effective concentration (EC₅₀) of iprodione to inhibit 50% of mycelial growth for 79 isolates of *Botryosphaeria dothidea* collected from eight different pistachio orchards throughout California during 1997 through 1999.

day-old colonies, ground in a blender, and filtered through two layers of sterile cheesecloth. The suspension was adjusted to 20,000 mycelial fragments per milliliter by measuring concentrations with a hemacytometer and used directly for inoculations. Each inoculated tree was covered with a plastic bag for 12 h to maintain high humidity, then placed in the greenhouse ($25 \pm 4^\circ\text{C}$) for 15 days and assessed for disease severity. The following system was used for severity assessment: 0 = leaves without lesions, 1 = lesion area less than one-fourth of the leaf area, 2 = lesion area between one-fourth and one-half of the leaf area, 3 = lesion area between one-half and three-fourths of the leaf area, and 4 = lesion area > three-fourths of the leaf area. The disease index (*DI*) for each tree was calculated using the formula

$$DI = \left(\sum_{i=0}^4 N_i * i \right) / \sum_{i=0}^4 N_i$$

where *i* is severity (0 to 4) and *N_i* is the number of leaves with the severity of *i*.

The EI was calculated using the following formula: $EI = (DIC - DIT)/DIC$, where *DIC* is *DI* of noniprodione control and *DIT* is *DI* of an iprodione treatment. The experiment was performed twice and results were averaged because the two experiments did not differ statistically ($P > 0.05$).

RESULTS

Survey for iprodione resistance.

Among 79 field-collected *B. dothidea* isolates tested for sensitivity to the dicarboximide fungicide iprodione, only the L21 isolate showed low resistance to iprodione. The EC_{50} of 78 California pistachio isolates of *B. dothidea* ranged from 0.20 to 0.90 $\mu\text{g/ml}$, whereas the EC_{50} for isolate L21 was 2.726 $\mu\text{g/ml}$ (Fig. 1).

Induction of IR isolates. After 5 days of incubation, none of nine isolates grew on APDA amended with iprodione at 50 $\mu\text{g/ml}$. However, six of nine *B. dothidea* isolates showed some degree of resistance to the fungicide after 20 days of incubation. After successive selection for three generations on iprodione-amended media, these six isolates grew slowly on APDA amended with iprodione at 1,000 $\mu\text{g/ml}$. The EC_{50} values for these six isolates were >25 $\mu\text{g/ml}$ and were considered IR isolates, whereas the EC_{50} values for wild-type isolates 1S, 3S, 4S, 9S, 11S, and 15S were <1 $\mu\text{g/ml}$ (Table 1). The IR isolates were designated 1R, 3R, 4R, 9R, 11R, and 15R.

Cross-resistance. All six IR isolates also showed resistance to another dicarboximide fungicide, vinclozolin. The EC_{50} values for these IR isolates to vinclozolin were >50 $\mu\text{g/ml}$, compared with the original wild-type isolates that had an EC_{50} <1 $\mu\text{g/ml}$ (Table 1). The IR isolates were still sensitive to tebuconazole and benomyl (Table 1). The EC_{50} to iprodione correlated significantly with the EC_{50} to vinclozolin

($r = 0.866$, $P = 0.0003$), but not with the EC_{50} to tebuconazole or benomyl.

Osmotic sensitivity. All IR isolates grew slower on APDA dishes amended with NaCl than their wild-type parent

counterparts (IS isolates; Fig. 2). Most IR isolates reached the maximum inhibition of mycelium growth at about 2% NaCl, whereas a higher concentration of NaCl was needed to inhibit the

Table 1. Values for effective concentration (EC_{50}) of iprodione, vinclozolin, tebuconazole, and benomyl to inhibit 50% of mycelial growth of *Botryosphaeria dothidea* isolates from California pistachio

Isolate ^a	EC_{50} ($\mu\text{g/ml}$)			
	Iprodione	Vinclozolin	Tebuconazole	Benomyl
1S	0.730	0.224	0.081	0.056
3S	0.568	0.220	0.057	0.053
4S	0.606	0.205	0.038	0.038
9S	0.502	0.191	0.142	0.075
11S	0.594	0.168	0.066	0.086
15S	0.541	0.230	0.021	0.071
1R	43.544	>50	0.049	0.047
3R	78.215	>50	0.058	0.039
4R	71.411	>50	0.055	0.052
9R	34.169	>50	0.115	0.054
11R	25.831	>50	0.191	0.078
15R	84.617	>50	0.045	0.039

^a Isolates 1R, 3R, 4R, 9R, 11R, and 15R were derived from wild-type isolates 1S, 3S, 4S, 9S, 11S and 15S, respectively, after three generations of selection on media amended with iprodione.

Fig. 2. Osmotic sensitivity of *Botryosphaeria dothidea* isolates from pistachio. The inhibition of mycelial growth on acidified potato dextrose agar media amended with different NaCl concentrations was tested for six iprodione-sensitive isolates (●) and six iprodione-resistant isolates (○). Bars denote standard error.

Table 2. Values for effective concentration (EC₅₀) of iprodione to inhibit 50% of mycelial growth of iprodione-resistant isolates of *Botryosphaeria dothidea* in different generations following mycelial propagation in greenhouse inoculation tests

Generation	EC ₅₀ (µg/ml) ^z					
	1R	3R	4R	9R	11R	15R
0	43.544 a	78.215 a	71.411 a	34.169 a	25.831 a	84.617 a
1	42.299 a	78.539 a	71.456 a	19.622 b	11.292 b	76.804 a
2	40.325 a	57.530 b	NA	17.610 b	10.245 c	68.364 ab
3	42.110 a	42.004 c	75.359 a	10.344 c	5.156 d	56.289 bc
4	24.194 b	24.152 d	45.956 b	6.650 c	2.3186 e	45.348 c

^z Values in columns followed by the same letter are not significantly different according to least significant difference of SAS test at $P = 0.05$. NA = stock culture of second generation for isolate 4R was contaminated.

Fig. 3. Comparisons of effective concentration (EC₅₀) of iprodione to inhibit 50% of mycelial growth between an iprodione-resistant isolate (9R) of *Botryosphaeria dothidea* and nine single-conidial isolates derived from isolate 9R. Each bar represents a mean of two experiments. Comparisons were conducted with least significant difference of SAS and values with the same letter are not significantly different at $P = 0.05$.

mycelium growth of the IS isolates (Fig. 2).

Resistance stability of IR isolates. After successive inoculation of pistachio leaves with each IR isolate, the EC₅₀ values for the fourth generation of all IR isolates were significantly lower than those for the first generation (Table 2). Comparisons between the 9R isolate and the nine single-conidial isolates derived from the 9R isolate demonstrated that the EC₅₀ values for the six single-conidial isolates were significantly lower ($P = 0.05$) than that for 9R, but three others showed no difference at $P = 0.05$ (Fig. 3).

Efficacy of iprodione in controlling disease caused by IR and IS isolates and the LR isolate. Laboratory tests demonstrated that, when iprodione was not applied, IR and IS isolates caused similar lesion lengths (no statistical difference) on detached pistachio leaves (Fig. 4A). However, when iprodione at 500 µg/ml was applied, all six IS isolates caused significantly smaller lesions than all six IR isolates (Fig. 4B). The efficacy of iprodione in controlling disease caused by any of the IR isolates was also significantly lower than that caused by the IS isolates (Fig. 4C).

Greenhouse tests also showed that, when mixtures of the six IR or six IS isolates

were separately used to inoculate trees not treated with iprodione, no difference in disease index was found (Fig. 5A). However, when iprodione at 500 µg/ml was applied, the disease index caused by the IR isolates was significantly higher than that caused by IS isolates at $P = 0.05$ (Fig. 5B). Additionally, disease indices caused by LR isolate L21 in both iprodione and control treatments were significantly lower than those caused by both IR and IS isolates (Fig. 5A and B). Efficacy of iprodione in controlling disease caused by the IR isolates was significantly lower than that caused by IS isolates or LR isolate L21 at $P = 0.05$ (Fig. 5C).

DISCUSSION

Our results show that most *B. dothidea* wild-type isolates encountered from California pistachio orchards are sensitive to iprodione. Only one (L21) out of 48 *B. dothidea* isolates collected from a commercial orchard in Glenn County that was treated with iprodione in 1995 and 1996 showed low resistance to iprodione. Interestingly, this isolate had low virulence to pistachio. No other naturally occurring IR isolates were found in seven other California pistachio orchards where iprodione had never been used. These results suggest that

naturally occurring IR isolates of *B. dothidea* are rare to absent in California pistachio orchards, especially in those orchards without a prior history of iprodione usage.

The development of fungal resistance to iprodione has been well documented (4,10,15,22,25). The results presented here for California pistachio isolates of *B. dothidea* also concur with these findings. Naturally occurring IR *B. dothidea* isolates are not commonly encountered in the field; however, this fungal species also easily develops resistance in culture under iprodione selection pressure. When subjected to media amended with iprodione, six out of nine tested isolates developed resistance readily. The EC₅₀ values for these isolates to iprodione were >25 µg/ml, whereas the EC₅₀ for the original wild-type counterparts were <1 µg/ml.

Altered colony morphology of IR fungi growing on fungicide-amended medium has been reported for some fungal plant pathogens. For instances, cultures of *Alternaria alternata* pv. *citri*, when grown on media amended with iprodione, readily develop resistance sectors (24). In this study of *B. dothidea*, however, after a period of incubation on iprodione-amended media, some isolates began to grow at varying rates. Although colonies were sometimes irregular in appearance, particularly in their colony margins, it appeared, in general, that the whole colony grew rather than individual sectors. Similar findings were reported for *S. minor* (10). Additionally, in vitro-derived IR isolates of *B. dothidea* showed an increase in osmotic sensitivity (Fig. 2). Similar findings were also reported for laboratory and field dicarboximide-resistant isolates of *B. cinerea* (1,7,12), *Aspergillus nidulans* (1), *Penicillium expansum* (1), and *Neurospora crassa* (9). The osmotic sensitivity may be associated with a change in cell wall composition, thereby causing a decrease in fitness of the resistant isolates in the field (6,9).

The fitness of dicarboximide-resistant fungal isolates, especially those induced in vitro, vary significantly (25). For instance, some dicarboximide-resistant fungal isolates have been reported to show lower fitness (i.e., virulence) than their wild-type counterparts (5,8,14,22,26). However, high fitness in fungicide-resistant isolates has also been reported (2,3,8,14). In this study, the *B. dothidea* isolate L21, a naturally occurring iprodione LR isolate, showed low virulence to pistachio. Conversely, laboratory-induced IR isolates of *B. dothidea* retained the original high virulence levels associated with wild-type IS isolates. Thus, although fungicide resistance levels in the IR isolates of *B. dothidea* did decline following successive propagation on pistachio leaves in the absence of fungicide (Table 2), high levels of virulence to pistachio were maintained for both sets of isolates.

Fig. 4. Comparisons in lesion length between iprodione-sensitive (□) and iprodione-resistant (■) isolates of *Botryosphaeria dothidea*. Data were collected from pistachio leaves **A**, not sprayed with iprodione, **B**, sprayed with iprodione at 500 µg/ml, and **C**, comparisons of iprodione efficacy in controlling *Botryosphaeria* panicle and shoot blight among isolates. Each bar represents a mean of two experiments; bars in a graph with the same letter are not significantly different at $P = 0.05$.

The efficacy of iprodione in controlling IR fungal isolates can be variable (25). In vitro-derived (3) and in vivo-derived (22) fungicide-resistant isolates of *S. minor* from peanut were virulent, but control by dicarboximides in the field was equal to that achieved against sensitive isolates. Conversely, iprodione failed to reduce lettuce drop caused by a virulent IR isolate of *S. minor*, but significantly reduced lettuce drop incited by wild-type sensitive isolates (10). In the present study, iprodione at 500 µg/ml showed good efficacy in controlling pistachio *Botryosphaeria* blight caused by the LR isolate L21 and by the wild-type IS isolates. In contrast, iprodione at the same concentration had a little effect in controlling disease caused by IR isolates both in laboratory and greenhouse tests (Fig. 5).

Isolates of *B. dothidea* readily developed resistance to iprodione on media amended with this fungicide, indicating that this fungus has a great propensity to overcome sensitivity to this fungicide. Applications of iprodione at up to 500 µg/ml failed to control the disease by these IR isolates in

the greenhouse tests. Therefore, although IR isolates are rarely encountered in the fields, *B. dothidea* isolates may develop substantial tolerances to this fungicide fairly quickly, over only several generations. The retention of full virulence in these IR isolates of *B. dothidea* should pose a significant threat to commercial plantings of California pistachio. Thus, an appropriate strategy to control *Botryosphaeria* panicle and shoot blight in California would be a series of applications, which involve the sequential rotation of fungicides without cross-resistance. For example, tebuconazole shows good efficacy in controlling this disease (18) and does not display cross-resistance with iprodione (Table 1). Furthermore, iprodione resistance levels in the IR isolates of *B. dothidea* did decline following successive propagation on pistachio leaves in the absence of this fungicide. Therefore, alternating applications of iprodione, tebuconazole, and benomyl may delay resistance development of iprodione in California pistachio orchards.

Fig. 5. Comparisons in disease index between iprodione-sensitive isolates (S), iprodione-resistant isolates (R), and an isolate with low iprodione resistance (LR) of *Botryosphaeria dothidea* **A**, without iprodione application, **B**, with iprodione application at 500 µg/ml, and **C**, comparisons of iprodione efficacy in controlling *Botryosphaeria* panicle and shoot blight among isolates. Each bar represents a mean of two experiments; bars in a graph with the same letter are not significantly different at $P = 0.05$.

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