Prevalence of *Botrytis* Cryptic Species in Strawberry Nursery Transplants and Strawberry and Blueberry Commercial Fields in the Eastern United States

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Abstract

Botrytis isolates from strawberry transplants originating from Canada and the northern United States as well as isolates collected from strawberry and blueberry commercial fields in the southeastern United States were investigated for the frequency of *Botrytis cinerea*, other cryptic *Botrytis* spp. reported recently, and the transposable elements (TE) using six genetic markers. *B. cinerea* sensu stricto was predominant (94%) in strawberry and blueberry in all surveyed regions. *Botrytis* group S, a newly reported clade on strawberry from Germany, was found at low frequencies (6%) in strawberry in the United States and Canada and on blueberry isolates from Florida. Neither *B. caroliniana* nor *B. pseudocinerea* were detected in the U.S. or Canadian populations. *Transposa* isolates containing the TE *boty* and *flipper* accounted for 74% of 410 isolates studied herein. Isolates containing *boty* only or *flipper* only elements were found

at 21 and 2%, respectively. However, *boty* isolates were predominant in the blueberry population with more than 50%. The TE were found in *B. cinerea* and *Botrytis* group S at similar frequencies, except that *flipper* was more frequent (10.7%) in *Botrytis* group S, compared with 1.6% in *B. cinerea*. The sensitivity of 256 *Botrytis* isolates from the different genetic groups described above was evaluated to seven fungicides registered to control gray mold in commercial fields. Results indicate that *B. cinerea* and *transposa* isolates have higher resistance frequencies to almost all fungicides tested compared with the other *Botrytis* genotypes or species, whereas the TE *flipper* may be related to resistance to fludioxonil. Similarities observed between nursery and commercial field populations and their impact on gray mold development and management are discussed.

The genus *Botrytis* was separated from *Sclerotinia* in the early 19th century (Smith, 1900) and its relation to the Botryotinia family was established (Groves and Loveland 1953). Since then, its speciation, and species within, has been continuously redefined. Two decades ago, Beever and Weeds (2004) recognized at least 20 different species in the genus *Botrytis* and 10 additional species have been identified in the last decade (Walker 2016). The species *Botrytis cinerea* (teleomorph *Botryotinia fuckeliana* de Bary) remains one of the most studied species and is known for its unusual genetic variability. The species' variability has been linked to the mode of reproduction, including asexual and heterokaryotic reproduction (less often sexual reproduction); high genetic diversity, defined by its ability to undergo several mutational stages; the presence of multiple vegetative compatibility groups (Beever and Parkes 2003); and extrachromosomal genetic elements, including linear plasmids (Hiratsuka et al. 1987), mycoviruses (Castro et al. 2003; Kecskeméti et al. 2014), and transposable genetic elements (TE) (Diolez et al. 1995; Giraud et al. 1999). In recent years, studies revealed other *Botrytis* spp., coexisting with *Botrytis cinerea*, capable of causing gray mold on multiple crops. For example, *B. pseudocinerea* was differentiated from *B. cinerea* based on the vegetative incompatibility locus and was reported on grape, strawberry, tomato, and blueberry (Fournier et al. 2003; Johnston et al. 2014; Leroch et al. 2013; Saito et al. 2014). Other region-specific species such as *B. caroliniana* have been reported at very low frequencies on strawberry and blackberry in the Carolinas (Li et al. 2012). A new clade named *Botrytis* group S and a new species, *B. fragariae*, were reported to be widespread in German strawberry fields (Leroch et al. 2013; Rupp et al. 2017). Initially thought to be host specific, *Botrytis* group S was reported on other hosts recently (Kanetis et al. 2017; Konstantinou et al. 2015). The genetic variability within the *Botrytis* spp. has previously been defined based on the presence of TE. *Boty* and *flipper*, classified into class I (RNA-TE) and class II (DNA-TE)-type TE, respectively (Kidwell and Linch 2001), have

been largely investigated in *B. cinerea* and used to characterize potential subgroups such as *transposa* and *vacuma* types (Giraud et al. 1999).

In Florida, gray mold is the major threat to strawberry production because of highly conducive weather conditions (i.e., adequate temperature and extended wetness) during the growing season from September to March. In the southeastern United States, strawberry is grown as an annual crop from transplants shipped annually from nurseries across Canada and the northern United States. These transplants have been shown to carry latent infections of *Botrytis* spp. and other pathogens (Oliveira et al. 2013, 2017; Rahman et al. 2015) and, therefore, are a major primary inoculum source in commercial fields. It is likely that different subpopulations of *Botrytis*, selected in the nurseries under different weather and fungicide spray conditions, are introduced annually to the commercial strawberry fields. Moreover, the selection of new southern highbush varieties of blueberry, with few hours of chill requirement, has made Florida among the top leading producing states. The crop is spread throughout northern and central Florida (i.e., the main strawberry production region near the Gulf-coast area). In blueberry, especially on highbush cultivars, *Botrytis* spp. can cause severe blossom blight and significant yield reduction. Blueberry is a perennial crop on which introduction of exogenous *Botrytis* populations is expected to be lower compared with strawberry. However, depending on the cultivar, blueberry flowering occurs between February and May, which coincides for many early cultivars with the peak bloom period on strawberry. It is possible that a substantial amount of *Botrytis* inoculum released from strawberry fields is disseminated to neighboring blueberry fields. In addition, fungicide input is considerably lower compared with strawberry. The exact impact of such practices on the *Botrytis* population structure and the fungicide resistance phenotypes in blueberry fields is unknown. Therefore, this study aimed to investigate (i) the prevalence and distribution of different *Botrytis* spp. and genetic TE populations collected from strawberry in eastern North America and in blueberry Florida fields and (ii) assess their respective sensitivities to seven fungicides commonly used to control gray mold or blossom blight in strawberry and blueberry fields.

Materials and Methods

Collection and storage of *Botrytis* isolates. In total, 410 *Botrytis* isolates collected from strawberry (330) and blueberry (80) were used in this study. Isolates from strawberry included 20, 57, 243, 50, and 40 isolates collected in 2010, 2011, 2012, 2013, and 2014,

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respectively. Geographically, 340 and 70 strawberry isolates were collected from different states and provinces in the United States and Canada, respectively. In the United States, isolates were collected from California, North Carolina, South Carolina, and Florida, and Canadian isolates originated from nurseries in Nova Scotia, Ontario, and Quebec (Table 1). The 80 isolates from blueberry were collected from different blueberry fields in central Florida from March to June 2013. The 410 isolates included 65, 90, and 255 from flowers, leaves or stems, and fruit, respectively. For isolates from fruit, a small plug was taken from the inside lesions and transferred onto a general isolation medium (GI; 19 g of potato dextrose agar, 15 g of agar, 125 mg of ampicillin, and 200 mg of streptomycin per liter) and incubated for 4 days at 22°C, and a 6-mm mycelial plug taken from the margin of GI plates was transferred to malt yeast extract agar (MYA) plates. Flowers and leaves were surface sterilized in 0.8% sodium hypochlorite for 2 min, rinsed with sterile water, frozen at -20°C overnight, incubated at 22°C for 5 days, and checked for the presence of *Botrytis* spp. using a stereoscope. Spores on *Botrytis*-like conidiophores were aseptically transferred to GI plates and grown as explained above. All isolates were grown on MYA until profuse sporulation was observed, single spored as described previously (Amiri et al. 2013), and stored as mycelial plugs in 20% glycerol at -80°C.

Genomic DNA extraction for *Botrytis* population structure. Isolates from -80°C were freshly regrown on MYA for 7 to 10 days or until profuse sporulation was observed. Sporulating mycelia were harvested from half 60-mm MYA plates and transferred to lysing tubes and DNA was extracted using the FastDNA kit (MP Biomedical, Solon, OH) following the manufacturer's protocol. The concentration and quality of DNA was checked using a spectrophotometer. DNA was diluted to 20 ng/μl and stored at -20°C until used.

The genetic structure of *Botrytis* spp. was investigated based on six different markers (Table 2). The presence of TE *boty* and *flipper*, which have been used to distinguish the *transposa* and *vacuina*

sympatric species of *B. cinerea*, was investigated as described previously (Levis et al. 1997; Ma and Michailides 2005). Both *boty* and *flipper* elements are present in *transposa* but absent in *vacuina* species (Diolez et al. 1995; Levis et al. 1997). The presence of *B. pseudocinerea* was detected by the mutation at codon 367 of the *hch* gene (Fournier et al. 2003), whereas the presence and *Botrytis* group S was verified by the presence of a 21-bp insertion in the *mrr1* gene (Leroch et al. 2013). The presence of the *B. caroliniana*, previously reported in the Carolinas, was investigated using specific markers amplifying different fragment sizes of the G3PDH gene (Li et al. 2012). The intergenic spacer (IGS) patterns were investigated using an IGS polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP), as described by (Kretschmer and Hahn 2008; Giraud et al. 1997). Restrictions were initially done with *MspI* and *HinfI* enzymes but the later was selected to conduct the study because it produced higher and consistent restriction profiles. All PCR amplifications and enzyme restrictions were conducted following the conditions described in Table 2.

Fungicide sensitivity tests. In all, 262 *Botrytis* isolates collected from Florida strawberry fields were tested for sensitivity to pyraclostrobin (Pyra), boscalid (Bosc), penthiopyrad (Penth), fluopyram (Flup), fludioxonil (Flud), fenhexamid (Fenh), and pyrimethanil (Pyri). Sensitivity was evaluated using a germ tube elongation inhibition assay on agar media amended with fungicides at doses reported previously to discriminate sensitive from resistant isolates. Sensitivities to Pyra, Fenh, and Flud were assessed on malt extract agar amended with 10, 50, and 0.1 and 10 μg/ml, respectively (Weber and Hahn 2011). For Bosc, Penth, and Flup, sensitivity was assessed on yeast bacto acetate agar amended with 5, 3, and 2.5 μg/ml, respectively. The 5 μg/ml for Bosc was adjusted from Leroch et al. (2013) whereas the discriminatory doses for Penth and Flup were determined in this study after isolates with different mutations in the *SdhB* subunit had been tested on multiple doses (Amiri et al. 2014, 2017). Sensitivity to Pyri was assessed

Table 1. Origin and number of *Botrytis* sp. isolates collected from strawberry and blueberry

Country	State or province	Host	Total	Number of isolates				
				Year of isolation				
				2010	2011	2012	2013	2014
Canada	Ontario	Strawberry	12	...	...	6	6	...
	Quebec	Strawberry	30	...	8	10	15	...
	Nova Scotia	Strawberry	27	...	8	8	9	...
United States	North Carolina	Strawberry	13	...	5	8	...	...
	South Carolina	Strawberry	18	...	8	10	...	...
	Florida	Blueberry	80	...	...	80	...	...
	California	Strawberry	10	...	...	1	9	...
	Florida	Strawberry	220	20	28	120	11	40
Total	...	...	410	20	57	243	50	40

Table 2. Primer sequences and polymerase chain reaction conditions used to amplify multiple loci in this study

Marker	Name	Primer Sequence (5'-3')	Amplification (°C-sec) ^a				Final Ext (°C-min)	RFLP ^b	Reference
			Den	Ann	Ext	Cyc			
<i>Boty</i>	F BotyF4	CAGCTGCAGTATACTCGGGGA	94-40	68-40	72-60	40	72-10	...	Ma and Michailides 2005
	R BotyR4	GGTGCTCAAAGTGTTACGGGAG							
<i>Flipper</i>	F F300	GCACAAAACCTACAGAAGA	94-40	60-40	72-60	40	72-10	...	Levis et al. 1997
	R F1550	ATTCGTTTCTTGACTGTA							
<i>Mrr1</i>	F Mrr1-spez-f	TATCGGTCTTGCAGTCCGC	94-40	58-50	72-60	35	72-5	...	Leroch et al. 2013
	R Mrr1-spez-R	TTCCGTACCCCGATCTTCGGAA							
<i>IGS</i>	F IGS1a	TCCCGGTGAGCCTTTTA	95-60	60-60	72-150	30	72-10	<i>MspI</i>	Giraud et al. 1997
	R IGS1b	CCATCGGCCAGTAATCCA						<i>HinfI</i>	
<i>G3PH</i>	F G3PDH-F1	CGACCCGAGCTAATTTATGTCACGT	94-40	56-30	72-60	32	72-5	...	Levis et al. 1997
	F G3PDH-F2	GGGTGTCAACAACGAGACCTACACT							
	R G3PDH-R	ACCGGTGTCCGATGGGATGAT							
<i>Hch</i>	F hch-262	AAGCCCTTCGATGTCTTGGA	94-30	55-90	72-60	30	72-10	<i>HhaI</i>	Fournier et al. 2003
	R hch-520L	ACGGATTCCGAACAAAGTAA							

^a Temperature and cycle duration used for polymerase chain reaction (PCR) amplifications: denaturation (Den), annealing (Ann), Extension (Ext), and number of cycles (Cyc).

^b Restriction fragment length polymorphism (RFLP) digestion of indicated PCR products was achieved following the manufacturers' protocols.

on 0.5% sucrose agar with discriminatory doses of 1 and 25 µg/ml (Weber and Hahn 2011). Assays were conducted on 150-mm plates, which allowed testing for 42 isolates simultaneously. A transparent 42-section cellophane grid was attached to the bottom of the plates and 7 µl of spore suspension of approximately 10⁶ spores/ml was placed in each section. Germination and germ tube elongation were evaluated after 18 to 24 h of incubation at 22°C. Isolates were considered resistant if the germ tube length was at least twice the conidium diameter.

Data analysis. Percent values of resistance frequencies in each genetic group containing *boty*, *flipper*, or both were log transformed and subjected to χ^2 analyses. Resistance frequencies to different fungicides in different genetic groups were log transformed before analysis of variance and a mean separation was performed using Student's *t* test. All analyses were conducted in JMP software (JMP statistical software, version 12.1.0; SAS Institute Inc., Cary, NC).

Results

Prevalence of *Botrytis* spp. and TE. Among the 330 isolates from strawberry, 93.6 and 6.4% were *B. cinerea* and *Botrytis* group S, respectively (Fig. 1). Geographically, the frequency of *Botrytis* group S was 6.1 and 7.4% in the United States and Canadian populations, respectively. *Botrytis* group S was found in the blueberry population from Florida at a frequency of 3.8%, versus 96.2% of *B. cinerea* (Fig. 1). Neither *B. pseudocinerea* nor *B. caroliniana* were found among the strawberry or blueberry isolates tested in this study, regardless of the geographic origin.

The TEs *boty* and *flipper* were found in combination (*transposa*) or separately in a large majority of *B. cinerea* isolates. Thus, *transposa* isolates (*flipper* + *boty*) made up to 74.4% of strawberry populations followed by *boty*-only, *vacuma*, and *flipper*-only isolates at 21.7, 2.2, and 1.7%, respectively (Fig. 2A). Geographically, the *transposa* group was predominant in all regions with somewhat similar frequencies, ranging from 64.7% in Canada to 79.4% in the United States. The frequency of *boty* isolates was the highest in Canada (33.8%), two times (15.3%) higher than in the United States (Fig. 2A). Isolates containing *flipper* element only were found in the U.S. strawberry population only at a low frequency of 4.1% (Fig. 2A). The *vacuma* group was found in the United States and Canada at frequencies of 1.2 and 1.5%, respectively. Interestingly, the blueberry population from Florida was dominated by the *boty* isolates with 56.3% of isolates, whereas 43.8% were *transposa* (Fig. 2A). *Flipper* or *vacuma* types were not found in the blueberry population. The structure of *Botrytis* group S showed similar patterns compared with *B. cinerea* (Fig. 2B), with a predominance of *transposa* and *boty* isolates, even though the frequencies of *flipper* and *vacuma* groups were higher in group S compared with *B. cinerea* (Fig. 2B).

The organ from which the isolates were collected had some impact on the presence of the different *Botrytis* genotypes. Frequencies of *B. cinerea* and *Botrytis* group S were similar on leaves, fruit, or flowers but *transposa* isolates were significantly more frequent (80%) on fruit compared with 46% on flowers (Table 3). On the other hand, *boty* isolates were significantly lower (12 to 26%) on fruit compared

with 53.2% on flowers, whereas *vacuma* isolates were not found on flowers and *flipper* isolates were only found on fruit (Table 3). The IGS-RFLP assay based on *Hinf*1I (*Hinf*I) digestion of IGS fragments yielded six different electrophoretic patterns (data not shown). Although two patterns were predominant in strawberry and blueberry isolates, there was not a clear correlation between a given IGS pattern and the different *Botrytis* groups.

Fungicide sensitivities of *B. cinerea* and *Botrytis* group S with different TE types. The frequency of isolates sensitive to all fungicides tested was significantly higher in *Botrytis* group S compared with *B. cinerea* (Fig. 3A). Resistance frequencies to Pyra and Bosc were significantly higher in *B. cinerea* and numerically higher for Fenh and Penth compared with *Botrytis* group S (Fig. 3A). Resistance frequencies for Pyri and reduced sensitivity to Flud were higher in *Botrytis* group S but not significantly different from *B. cinerea*.

There was some variability in resistance frequencies between isolates with different TE. The four *vacuma* isolates tested in this study were all resistant to Pyra, Bosc, and Pyri but sensitive to Fenh (data not shown). The *transposa* and *boty* isolates exhibited significantly higher resistance frequencies for Pyra, Bosc, Pyri, and Fenh compared with *flipper* isolates (Fig. 3B). Interestingly, reduced sensitivity to Flud was the highest (12.5%) in *flipper* isolates compared with 6.6% in *boty* isolates and very low (0.5%) in the *transposa* group (Fig. 3B). Resistance to the succinate dehydrogenase inhibitor Flup was observed in none of the 262 isolates tested.

Overall, *transposa* and *boty* isolates had similar frequencies of isolates from similar multifungicide resistance (MFR) phenotypes (Fig. 3C). Interestingly, *boty* isolates had a higher frequency (8.2%) of isolates resistant to five fungicides (MFR5) compared with *transposa* (1.6%) isolates (Fig. 3C), whereas only MFR1 and MFR2 phenotypes were detected in *flipper* isolates.

Discussion

The *Botrytis* population from strawberry in eastern North America and from blueberry in Florida is largely dominated by *B. cinerea* sensu stricto in all surveyed regions. *Botrytis* group S, a newly recognized clade from strawberry in Germany (Leroch et al. 2013), was found to coexist with *B. cinerea* on strawberry and blueberry in almost all regions surveyed. Our findings, together with recent reports from grape in New Zealand (Johnston et al. 2014), tomato in Greece (Konstantinou et al. 2015), and blackberry from the Carolinas (Li et al. 2014), present evidence that *Botrytis* group S is not host specific, as was initially believed (Leroch et al. 2013). In recent studies, a new species named *B. fragariae* was reported in strawberry fields in Germany and the Carolinas on the U.S. east coast (Dowling et al. 2017; Rupp et al. 2017). The occurrence of *B. fragariae* in other crops and its relationship with *Botrytis* group S were not investigated by the authors and, therefore, *Botrytis* group S should be distinguished from *B. fragariae*. The above studies do not provide strong evidence for a clear host- or region-specific distribution of *B. cinerea* and *Botrytis* group S populations. Thus, *B. cinerea* was overwhelmingly predominant on both strawberry and blueberry in the Americas in this study and on strawberry in Greece (Konstantinou et al. 2015) but was less frequent than *Botrytis* group S in German strawberry fields (Leroch et al. 2013). Moreover, *Botrytis* group S was seldom found in German vineyards (Leroch et al. 2013) whereas it was common in New Zealand vineyards (Johnston et al. 2014). One hypothesis to explain the predominance of *Botrytis* group S in Germany strawberry fields may be related to the predominance of the MDRh1 sensitivity phenotype group in this region (Leroch et al. 2013). However, the reasons for the MDRh1 predominance in this region are not fully understood, given that spray regimes and types are not expected to be significantly different from other regions where strawberry are grown. To what extent different fitness abilities between the two *Botrytis* groups, different production systems (i.e., annual versus perennial), and weather conditions can explain such geographic variability remains unclear. Neither *B. pseudocinerea* nor *B. caroliniana* were found in the Americas, reinforcing their minor ecological status in the *Botrytis* genus (Isenegger et al. 2008; Kanetis et al. 2017; Samuel et al. 2012; Váczy et al. 2008).

Fig. 1. Overall frequency of *Botrytis cinerea* and *Botrytis* group S in a population of 410 *Botrytis* isolates from strawberry (330) and blueberry (80) fields in North America.

Studies of *Botrytis* genetic structure in North America are rare compared with studies of the European populations. Thus, only two reports were found in the literature, including one study from California that did not include small fruit (Ma and Michailides 2005) and another on 11 isolates from blueberry in Chile (Muñoz et al. 2002). Herein, *Botrytis* populations containing or not containing the TE *boty* and *flipper* were widespread across the Americas but at variable frequencies depending on the organ of collection. *Transpos* isolates, carrying both *flipper* and *boty*, were predominant on strawberry in North America, whereas *boty* isolates were slightly predominant (56%) in Florida blueberry fields. Our findings agree with previous reports on the predominance of *transpos* isolates in almost all regions and crops investigated (i.e., grape, kiwi, tomato, and strawberry) (Giraud et al. 1999; Johnston et al. 2014; Kretschmer and Hahn 2008; Ma and Michailides 2005; Muñoz et al. 2002; Samuel et al. 2012; Váczy et al. 2008). We also present some evidence for seasonal variability in TE preponderance in relation to the phenological growth stage. Thus, *boty* isolates were predominant in populations collected early in the season from strawberry leaves (37 to 80%) and blueberry flowers (53%) whereas *transpos*-type isolates were overwhelmingly predominant (>73 to 94%) in isolates collected from fruit later in the season regardless of the growing region. The dominance of *transpos* isolates on fruit was related to their higher aggressiveness on fruit, whereas *vacuma* isolates were suggested to be more saprophytic and fit to survive on leaves and other grape organs (Giraud et al. 1999; Martinez et al. 2003). The predominance of *boty* over *vacuma* isolates observed in small fruit in this study has been reported in other crops

and regions (Johnston et al. 2014; Kretschmer and Hahn 2008; Ma and Michailides 2005; Milicevic et al. 2006; Váczy et al. 2008). Although different fitness abilities between *transpos* and *boty* isolates have not been investigated, one can speculate that *boty* isolates may act similarly to *vacuma* in term of aggressiveness to fruit, which could explain their relative lower frequency compared with *transpos* on fruit but higher on leaves and flowers. The geographic distribution of *transpos* and *boty* isolates seen in eastern North

Table 3. Overall frequency of different genotype groups of *Botrytis* from leaves, flowers, and fruit collected in Canada and the United States

Genotype	Group	Frequency (%) ^a			
		Canada (n = 70)	United States		
			Flowers ^b (n = 65)	Leaves (n = 10)	Fruit (n = 235)
<i>Botrytis</i> group	<i>cinerea</i>	90.0	95.2	100.0	93.6
	Group S	10.0	4.8	0.0	6.4
Transposable	<i>transpos</i>	61.4	46.8	20.0	82.1
	<i>boty</i>	37.1	53.2	80.0	12.3
	<i>flipper</i>	0.0	0.0	0.0	4.3
	<i>Vacuma</i>	1.4	0.0	0.0	1.3

^a Overall frequency of different genetic groups found on each organ and country relative to the total number of isolates.

^b Isolates from flowers are collected from strawberry and blueberry fields in Florida.

Fig. 2. Overall frequency of transposable elements (TE) **A**, by country and host and **B**, among *Botrytis cinerea* and *Botrytis* group S in a population of 410 *Botrytis* isolates from strawberry (330) and blueberry (80) fields. *Transpos* isolates contain both *boty* and *flipper* elements, *vacuma* isolates lack both TE, and *boty* and *flipper* isolates contain only the *boty* or *flipper* element, respectively.

America suggests that these genotypes are fit to survive under various weather conditions such as the ones occurring in Canada, the northern United States, or Florida.

Differences in resistance frequencies to fungicides commonly used to control gray mold in strawberry and blueberry fields were observed between *B. cinerea* sensu stricto and *Botrytis* group S. More isolates from the latter were sensitive than *B. cinerea* to almost all fungicides tested, except for Pyri and Flud. Although other studies reported sensitivity phenotypes similar to ours to a certain extent in

strawberry isolates from greenhouses in Greece and Cyprus (Kanetis et al. 2017; Konstantinou et al. 2015), different phenotypes were found in German strawberry fields (Leroch et al. 2013). We found more *Botrytis* group S isolates sensitive to all fungicides, whereas resistance frequencies to Pyra, Bosc, Fenh, and Penth were higher in *B. cinerea* sensu stricto. On the other hand, the fact that reduced sensitivity to Flud and resistance to Pyri were higher in *Botrytis* group S indicates that these isolates may present some similarities with MDR1h phenotypes, which showed higher minimum inhibitory concentrations values

Fig. 3. A, Resistance frequencies of *Botrytis cinerea* sensu stricto and *Botrytis* group S; B, resistance frequencies in *transposa*, *boty*, and *flipper* isolates to six fungicides; and C, frequency of multifungicide resistance (MFR) phenotypes in *Botrytis* isolates ($n = 268$) from small fruit in Florida. S, MFR1, MFR2, MFR3, MFR4, and MFR5 indicate isolates sensitive or resistant to one, two, three, four, or five fungicides, respectively; asterisks (*) indicate significant differences.

to Flud and cyprodinil compared with other phenotypes (Leroux et al. 2013). This suggests that multidrug resistance mechanisms may be causing resistance observed in strawberry and blueberry fields in Florida but at a significantly lower frequency than the ones reported in German populations. Moreover, despite differences in spray regimes between strawberry and blueberry fields in terms of spray frequency and fungicide types, we did not notice significant differences in resistance frequencies between *B. cinerea* and *Botrytis* group S from both hosts for all fungicides tested. In two recent studies, significant differences were observed between strawberry and tomato populations from greenhouses but not for all fungicides (Kanetis et al. 2017; Konstantinou et al. 2015). Whether population shifts and exposure to fungicides in open field populations, as in our study, are different from what may occur in greenhouse populations remains to be determined.

Transposa and *boty* isolates showed higher resistance frequencies than *flipper* isolates for all fungicides except Flud, for which *flipper* and *boty* isolates had higher resistance frequencies. Previous studies from grape focused on comparing resistance profiles in *transposa* and *vacuma* isolates to benzimidazole and dicarboxamides fungicides (Giraud et al. 1999; Martinez et al. 2005). These studies reported higher resistance frequencies in *transposa* compared with *vacuma* isolates. In the present study, we focused on fungicides commonly used in strawberry and blueberry and our findings indicate that the two major populations, *transposa* and *boty*, may possess a similar ability to develop resistance to most fungicides and that their management could be achieved by similar spray management programs. The low number of *flipper* isolates found in our study may not be enough to make major conclusions. However, it is interesting that more *boty* and *flipper* isolates have a reduced sensitivity to Flud than do *transposa* isolates. These findings may explain the low frequency of Flud-resistant isolates in strawberry and blueberry fields and may also entail a fitness penalty in *flipper* isolates similar to what was observed in field isolates resistant to Flud (Leroux et al. 2002). The role of these two TE in causing reduced sensitivity to Flud, specifically, requires further investigation.

The genetic structure of *Botrytis* spp. in the growing regions of strawberry may be highly defined and affected by continuous population migrations through nursery transplants. This high diversity together with the widespread resistance to multiple current fungicides may require drastic changes in fungicide usage, including phasing out of those with high resistance frequencies, developing new molecules with different modes of action, and using some fungicides exclusively in the nurseries. Further studies focusing on the genetic populations and potential population drift are warranted to better understand and evaluate the risks.

Acknowledgments

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