Article

Global Distributions of *Clarireedia* Species and Their In Vitro Sensitivity Profiles to Fungicides

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Abstract: Dollar spot is reported to be caused by multiple *Clarireedia* species and is a serious problem on many turfgrasses around the world. To our knowledge, the distribution of different *Clarireedia* species and their sensitivity profiles to fungicides remains unknown. In this study, a total of 275 isolates were characterized by ITS sequence. Amounts of 124, 59 and 75 isolates were identified as *C. jacksonii*, *C. monteithiana* and *C. paspali*, respectively, while each species of *C. homoeocarpa* and *C. bennettii* had only five isolates. Four and three isolates were identified as two potential new species, which remained to be further characterized. *C. jacksonii* and *C. monteithiana* were distributed worldwide, while *C. paspali* was restricted to China. Of the isolates with host information, 81% (93/115) and 19% (22/115) of *C. jacksonii* isolates were collected from C3 and C4 plants, respectively, 97% (56/58) of the *C. monteithiana* isolates were collected from C4 plants and all *C. paspali* isolates were collected from C4 plants. The coexistence of different *Clarireedia* species on the same C4 host type in the same locales was found in Shanghai (*Paspalum vaginatum*), Jiangsu (*Paspalum vaginatum*) and Florida (*Cynodon dactylon*). The study revealed that differential fungicide sensitivity patterns were observed in different species in *Clarireedia* for the first time. Similar differential sensitivity profiles were also found in the locales with coexistence of at least two species. The findings from this study suggest that the adjacent coexistence of different *Clarireedia* species and the differential fungicide sensitivity profiles of different species will complicate dollar spot disease control.

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1. Introduction

Dollar spot, caused by the *Clarireedia* species (formerly *Sclerotinia homoeocarpa*), is one of the most economically important diseases of turfgrass worldwide [1,2]. A total of five distinct pathogenic species causing dollar spot within the genus *Clarireedia* have been documented [1,2], and it is speculated that additional species of *Clarireedia* may exist, possibly based on geographical distributions [2,3]. The five species in *Clarireedia* include the type species for the genus, *C. homoeocarpa* and four recently characterized species, *C. bennettii*, *C. jacksonii*, *C. monteithiana* and *C. paspali*.

Among the five species of *Clarireedia* that have been identified, *C. homoeocarpa* has only been found in the United Kingdom, whereas *C. bennettii* is primarily found in the United Kingdom and the United States [4]. The two species appear to be historical and represent a minority of the isolates which cause dollar spot. The modern species with worldwide distribution are *C. jacksonii* and *C. monteithiana*, which are also widely distributed in China [1]. *C. paspali* is an emerging species which frequently recovered from seashore *Paspalum* (*Paspalum vaginatum*). It has not been reported in regions or countries other than eastern and southern China [1]. However, the collection of more isolates from diverse regions around the world is needed to understand if the *C. paspali* is geographically

restricted. *C. monteithiana* and *C. paspali* isolates are predominantly collected from C4 turfgrass [1,2], suggesting a host preference in the two species. However, in contrast to a previous finding [2], *C. jacksonii* is not restricted to C3 turfgrass hosts and could be recovered from C4 turfgrass at a high frequency in China [1]. Different species of *Clarireedia* have been found on the same host type, indicating that they are host adapted; complicating dollar spot management. It is imperative to understand the role of geography and host type for the distributions of different *Clarireedia* species in order to most effectively manage dollar spot.

Management of dollar spot is the top priority in the turfgrass industry and requires high inputs of chemical fungicides. Multiple fungicides from four different chemical classes, including benzimidazole carbamate (MBC), dicarboximide, sterol demethylation inhibitor (DMI) and succinate dehydrogenase inhibitor (SDHI) fungicides, have been developed and marketed for dollar spot control [5]. However, repeated fungicidal applications select for isolates of *Clarireedia* species with reduced sensitivity to fungicides with differing modes of actions (MOAs) [5–11]. It is necessary to monitor the development of fungicide sensitivity in *Clarireedia* species, which is important for subsequent fungicide applications for dollar spot control. To our knowledge, all existing studies on fungicide sensitivity in *Clarireedia* species have considered the isolates as a single species (formerly *S. homoeocarpa*), and comparative analysis in fungicide sensitivity has mostly focused on isolates collected from different locales (e.g., golf courses) rather than those from different species [5,12,13]. Meanwhile, it has been demonstrated that there are morphological, vegetative, pathogenic and mating-type differences between *C. jacksonii* and *C. monteithiana* [4,14]. These findings warrant us an investigation into whether different *Clarireedia* species show differential sensitivity profiles to fungicides or different patterns in developing fungicide resistance.

The objectives of this study were to (i) identify dollar spot isolates into specific species based on a partial internal transcribed spacer (ITS) sequence, and characterize their global distributions, (ii) determine the sensitivity of different *Clarireedia* species in vitro to thiophanate-methyl (TM), iprodione, propiconazole and boscalid, and (iii) analyze if different *Clarireedia* species show differential sensitivity patterns to these fungicides.

2. Materials and Methods

2.1. Medium and Fungicides

Minimum medium (MM) consisted of 10 g glucose, 1.5 g K₂HPO₄, 2 g KH₂PO₄, 1 g (NH₄)₂SO₄, 0.5 g MgSO₄·7H₂O, 2 g yeast extract, and 12.5 g agar per liter of distilled water. Technical grade thiophanate-methyl (TM, 95% active ingredient), iprodione (98% active ingredient), propiconazole (96.5% active ingredient), and boscalid (98.5% active ingredient) were purchased from Zhejiang Heben Pesticide & Chemicals Co., Ltd. (Wenzhou, China) and used for in vitro assays in this study. The fungicides were dissolved in acetone to provide stock solutions at 100 mg mL⁻¹. Solutions were sealed with Parafilm, stored at 4 °C and diluted as required.

2.2. Sample Collection and Pathogen Isolation

Dollar spot samples were collected from 13 golf courses from May 2008 to September 2016. For dollar spot control, all golf courses were documented to have a history of benzimidazole and DMI fungicides applications, five golf courses (BA, BHA, BHU, LFT, LKS) had utilized dicarboximide fungicides, and three golf course (LFT, LKS, QHB) had utilized SDHI fungicides. Samples were collected from different host types, including *Agrostis stolonifera*, *Poa pratensis*, *Cynodon dactylon*, and *Paspalum vaginatum* (Table S1).

The *Clarireedia* species was isolated from leaf tissue with symptoms of dollar spot using the method described previously [6]. Identification of the *Clarireedia* species was based on cultural morphology. Five plugs (5 mm in diameter) of each isolate were put into a 2-mL microtube, immersed by 20% glycerin and stored long-term at 10 °C.

2.3. Sequencing of Internal Transcribed Spacer Regions

Clarireedia species cultures were incubated on MM at 25 °C for four days to produce enough mycelium for DNA extraction. Mycelial mats of each isolate were scraped from MM using a sterilized scalpel and transferred into a 2-mL microcentrifuge tube. Genomic DNA was extracted using the CTAB method described previously [15].

The internal transcribed spacer (ITS) region of ribosomal RNA gene proved useful to differentiate different species in the genus *Clarireedia* in previous studies [1,2,4], and the phylogenetic tree constructed from ITS produced a topology similar to the combined multi-locus dataset [1,2]. Therefore, we used the ITS sequence for species identification instead of multi-locus molecular markers. To molecularly characterize fungal isolates, the partial ITS sequence was amplified using the ITS4 and ITS5 primer pairs [16].

PCR amplifications were accomplished in 30 µL reactions containing 1 × TSINGKE master mix (Tsingke Biotech Co. Ltd., Nanjing, China), 200 nM of each primer and 50 ng of genomic DNA. The PCR cycling consisted of an initial preheating step at 94 °C for 2 min, followed by 30 cycles of 94 °C for 15 s, 57 °C for 30 s and 72 °C for 1 min and a final extension step at 72 °C for 10 min. PCR products were detected by gel electrophoresis using a 1% agarose gel. The DNA fragment was excised from the agarose gel and purified using a TianGEN gel purification kit (DP209-03, TianGEN Biotech Co. Ltd., Beijing, China). The PCR products were sent to Sangon Biotech Co. Ltd. (Shanghai, China) for sequencing. Some ITS sequences (62 isolates) have been submitted to GenBank in a previous study [1], and more ITS sequences (51 isolates) were submitted in this study (Table S1).

2.4. Species Identification

The ITS fragments for all isolates were sequenced prior to assembly and then aligned using the ClustalW algorithm implemented in MEGA v7 [17]. ITS sequences of 162 *Clarireedia* isolates were also retrieved from GenBank for alignment and were used to construct a phylogenetic tree. Alignments were manually checked with characteristics weighted equally. Gaps were completely deleted. Maximum likelihood trees were generated using the Tamura 3-parameter model in MEGA v7 and tested with 1000 bootstrap replicates [17]. A 50% majority-rule consensus tree was estimated. The aligned sequences were analyzed together with the ITS sequences of *C. paspali* (MH392087), *C. homoeocarpa* (MF964322), *C. bennettii* (MF964321), *C. jacksonii* (MF964320), *C. monteithiana* (KF545306) type strain obtained from GenBank. The ITS sequences of *Rutstroemia firma* (MH861930) and *R. bolaris* (LT158432) were included as outgroup members. Isolates grouped together with the type strains were defined to be the same species as the type strains. Otherwise, they were defined as *Clarireedia* sp.

2.5. In Vitro Assessment of Fungicide Sensitivity

A subset of isolates identified as different *Clarireedia* species were tested for fungicide sensitivity. Sensitivities of *Clarireedia* isolates to TM, iprodione, propiconazole, and boscalid were determined in vitro by using mycelia growth assays [5]. Agar plugs (5 mm in diameter) containing actively growing mycelium were transferred to MM and MM amended with propiconazole, iprodione and boscalid at 0.1, 1 and 10 µg mL⁻¹, respectively. These concentrations have been reported to be effective discriminatory doses in our recent paper and other previous studies [5,18,19]. Each isolate was tested on three replicates of each fungicide concentration and the experiment was performed twice. Owing to the qualitative nature of *Clarireedia* isolates resistance to TM [19], a single discriminatory concentration of 10 µg mL⁻¹ was used for in vitro sensitivity screening. Resistance to TM was scored 5 d after incubation at 20 °C for the presence of mycelium growth on TM-amended MM [18,19]. Mean radial mycelial growth for propiconazole, iprodione and boscalid was scored 3 d after incubation at 25 °C. Relative mycelia growth (RMG) value was calculated using the formula: % RMG = (mean fungicide amended MM/mean nonamended MM) × 100. RMG values for individual isolates from different species were compared and separated at $p < 0.05$ according to Fisher's least significant difference test implemented in Data Process-

ing System software (version 7.05, Hangzhou RuiFeng Information Technology Co. Ltd., Hangzhou, China)

3. Results

3.1. Species Identification

A total of 275 isolates were characterized by ITS sequences and all isolates fell into seven distinct groups. The three main groups included 124, 59 and 75 isolates which were identified as *C. jacksonii*, *C. monteithiana* and *C. paspali*, respectively. Two groups with five isolates in each were identified as *C. homoeocarpa* and *C. bennettii*. Four and three isolates in the remaining two groups were identified as the potential new species in *Clarireedia* (*Clarireedia* sp. 1 and *Clarireedia* sp. 2) (Table 1 and Table S1).

Table 1. The information of *Clarireedia* species identified by partial internal transcribed spacer (ITS) sequence in this study.

Species	No. of Isolates	History	Host Type			Distribution ^b
			C3	C4	N/A ^a	
<i>Clarireedia homoeocarpa</i>	5	1937–2008	4	0	1	United Kingdom
<i>Clarireedia bennettii</i>	5	1937–1973	0	0	5	The Netherlands, United Kingdom, USA
<i>Clarireedia jacksonii</i>	124	1972–2016	93	22	9	Canada, China (5), Ireland, Italy, Japan (4), The Netherlands, Spain, United Kingdom, USA (16)
<i>Clarireedia monteithiana</i>	59	2001–2016	2	56	1	Canada, China (5), Dominican Republic, Japan (5), USA (7)
<i>Clarireedia paspali</i>	75	2012–2016	0	75	0	China (3)
<i>Clarireedia</i> sp. 1	4	2015	0	4	0	China (1)
<i>Clarireedia</i> sp. 2	3	2005–2013	3	0	0	Japan (2), Norway
Total	275		102	157	16	-

^a: N/A represents isolates with no known host information; ^b: number of states or provinces in a country reported with *Clarireedia* species. was shown in the bracket.

3.2. Global Distributions of *Clarireedia* Species

C. homoeocarpa was found to be restricted to the United Kingdom from 1937 to 2008, and *C. bennettii* could be found in the Netherlands, United Kingdom and the United States historically (from 1937 to 1973). *C. jacksonii* and *C. monteithiana* were found to be distributed worldwide (Figure 1 and Table 1), and they were first documented in 1972 and 2001 respectively. When taking USA and China as examples, *C. monteithiana* was mainly distributed in the transitional zones and southern regions, while *C. jacksonii* could be found in both northern and southern regions (Figure 1). *C. paspali* was only found in eastern and southern China and was first reported in 2012 (Figure 1 and Table S1). One potential new species (*Clarireedia* sp. 1) was found in only one locale in Hainan, China. The other potential new species (*Clarireedia* sp. 2) had three isolates, which were collected from Kyoto and Shizuoka in Japan, and from Scandinavia in Norway (Table S1).

Figure 1. Global distributions of different *Clarireedia* species.

3.3. Host Preference of *Clarireedia* Species

Of the 115 *C. jacksonii* isolates with host information, 81% (93/115) and 19% (22/115) were collected from C3 and C4 plants, respectively (Table 1). A proportion of 97% (56/58) of the *C. monteithiana* isolates were collected from C4 plants (Table 1). Two isolation events were found on C3 plants in Florida (Accession No. PFH0479) and Hyogo in Japan (Accession No. AB647342) (Table S1). One of the two events was found in the locale in Florida with C3 and C4 turfgrass growing adjacently [14]. All of the *C. paspali* isolates were collected from C4 plants (Table 1). *Clarireedia* sp. 1 and *Clarireedia* sp. 2 were solely recovered from C4 and C3 respectively (Table 1). The coexistence of different *Clarireedia* species on the same C4 host type in the same locales was found in Shanghai (*Paspalum vaginatum*), Jiangsu (*Paspalum vaginatum*) and Florida (*Cynodon dactylon*) (Table S1).

3.4. In Vitro Sensitivity Profiles of *Clarireedia* Species

Of the 88 *Clarireedia* species isolates that were tested, 24 were identified to be resistant to TM, 7 of the 24 TM-resistant isolates from six golf courses were *C. paspali*, and the remaining 17 TM-resistant isolates from nine golf courses were *C. jacksonii*. Of the 22 *C. jacksonii* isolates, 17 were resistant to TM and 7 of the 56 *C. paspali* isolates were resistant to TM, while none of the 10 *C. monteithiana* isolates from three golf courses were resistant to TM (Tables 2 and S2).

For propiconazole, the RMG of individual isolates ranged from 17.02 to 78.87, 14.29 to 39.71, and 4.29 to 45.00 for *C. jacksonii*, *C. monteithiana* and *C. paspali*, respectively. A significantly higher mean RMG was observed in *C. jacksonii* isolates against propiconazole ($p < 0.001$) (Tables 2 and S2).

For iprodione, the RMG of individual isolates ranged from 8.77 to 71.79, 31.94 to 44.12, and 0.00 to 62.79 for *C. jacksonii*, *C. monteithiana* and *C. paspali*, respectively. No significant difference was observed in the mean RMG for different *Clarireedia* species against iprodione ($p > 0.05$) (Tables 2 and S2).

Table 2. Sensitivity profiles of the three *Clariireedia* species to thiophanate-methyl, propiconazole, iprodione, and boscalid.

Species	No. of Locations	No. of Isolates	No. of Isolates Resistant to Thiophanate-Methyl	Relative Mycelial Growth (%)					
				Propiconazole		Iprodione		Boscalid	
				Range	Mean ^a	Range	Mean	Range	Mean
<i>Clariireedia jacksonii</i>	9	22	17	17.02–78.87	48.67 a	8.77–71.79	34.67 a	23.46–67.57	42.85 a
<i>Clariireedia monteithiana</i>	3	10	0	14.29–39.71	28.32 b	31.94–44.12	36.97 a	35.71–60.87	46.51 a
<i>Clariireedia paspali</i>	6	56	7	4.29–45.00	27.75 b	0.00–62.79	34.11 a	0.00–23.73	14.01 b

^a Means followed by different letters in the same column are significantly different according to Fisher's least significant difference test at $p = 0.05$.

For boscalid, the RMG of individual isolates ranged from 23.46 to 67.57, 35.71 to 60.87, and 0.00 to 23.73 for *C. jacksonii*, *C. monteithiana* and *C. paspali*, respectively. A significantly lower mean RMG for *C. paspali* against boscalid was observed ($p < 0.001$) (Tables 2 and S2).

3.5. In Vitro Sensitivity Profiles at Locales with *Clariireedia* Species Coexistence

In the four golf courses with *Clariireedia* species co-existing, similar in vitro sensitivity profiles to the four fungicides were observed (Table 3). TM-resistance was found in *C. jacksonii* and *C. paspali* isolates, but not in *C. monteithiana* isolates. The RMG of *C. jacksonii* isolates to propiconazole was higher than that of *C. paspali* and/or *C. monteithiana* isolates at each golf course. No apparent differentiation in RMG for iprodione was observed among different species in the same locales. The RMG for boscalid was lower in all *C. paspali* isolates when compared to that of the *C. jacksonii* or *C. monteithiana* isolates co-existing in the same locales (Table 3).

Table 3. Fungicide sensitivity in *Clariireedia* isolates from *Paspalum vaginatum* in the four locales with at least two species coexistence.

Code	Species ^a	TM Sensitivity ^b	Relative Mycelium Growth (%) ^b			Collection Year	Site	Location	Province
			P	Y	D				
BH15-5	CM	S	14.29	36.11	40.82	September 2016	Fairway	Binhai Golf Club	Shanghai
BH17-8	CM	S	30.43	36.76	60.87	September 2016	Fairway	Binhai Golf Club	Shanghai
BH18-2	CM	S	34.38	34.68	59.38	September 2016	Fairway	Binhai Golf Club	Shanghai
BH18-4	CM	S	39.66	31.94	42.11	September 2016	Fairway	Binhai Golf Club	Shanghai
BH18-4	CM	S	39.71	37.14	51.47	September 2016	Fairway	Binhai Golf Club	Shanghai
BH6-4	CM	S	20.59	38.24	42.65	September 2016	Fairway	Binhai Golf Club	Shanghai
BH17-6	CJ	R	78.87	52.17	33.80	September 2016	Fairway	Binhai Golf Club	Shanghai
LKS1-1	CJ	R	73.56	17.81	36.49	September 2016	Fairway	Links Golf Club	Shanghai
LKS11-2	CJ	R	67.05	16.95	31.17	September 2016	Fairway	Links Golf Club	Shanghai
LKS1-2	CJ	R	77.38	58.73	28.38	September 2016	Fairway	Links Golf Club	Shanghai
LKS13-6	CJ	R	73.97	22.58	49.33	September 2016	Fairway	Links Golf Club	Shanghai
LKS11-3	CP	R	21.21	27.45	18.57	September 2016	Fairway	Links Golf Club	Shanghai
TC5-6	CJ	R	51.72	71.79	42.53	September 2016	Fairway	Xingdonghai Golf Club	Jiangsu
TC8-3	CP	S	10.71	36.21	20.43	September 2016	Fairway	Xingdonghai Golf Club	Jiangsu
TC8-5	CP	S	5.26	45.07	15.79	September 2016	Fairway	Xingdonghai Golf Club	Jiangsu
TC12-4	CP	R	4.29	24.39	5.06	September 2016	Fairway	Xingdonghai Golf Club	Jiangsu
TC10-4	CM	S	30.43	44.12	40.58	September 2016	Fairway	Xingdonghai Golf Club	Jiangsu
TC5-8	CM	S	22.08	32.35	41.56	September 2016	Fairway	Xingdonghai Golf Club	Jiangsu
TC8-6	CM	S	27.14	35.48	35.71	September 2016	Fairway	Xingdonghai Golf Club	Jiangsu
XTH18F-4	CP	R	19.12	18.92	20.59	September 2016	Fairway	Xingtianhong Golf Club	Shanghai
XTH18T-5	CP	R	16.28	18.00	10.00	September 2016	Fairway	Xingtianhong Golf Club	Shanghai
XTHA10-5	CP	R	23.29	14.52	5.56	September 2016	Fairway	Xingtianhong Golf Club	Shanghai
XTHA15T-7	CP	R	19.35	23.88	16.49	September 2016	Fairway	Xingtianhong Golf Club	Shanghai
XTH27G-2	CJ	R	60.34	48.65	37.50	September 2016	Fairway	Xingtianhong Golf Club	Shanghai
XTH27G-3	CJ	R	56.45	37.84	23.46	September 2016	Fairway	Xingtianhong Golf Club	Shanghai
XTH27G-3	CJ	R	59.74	47.14	29.31	September 2016	Fairway	Xingtianhong Golf Club	Shanghai
XTH27G-5	CJ	R	47.27	22.22	30.91	September 2016	Fairway	Xingtianhong Golf Club	Shanghai

^a CM = *Clariireedia monteithiana*, CJ = *Clariireedia jacksonii*, CP = *Clariireedia paspali*. ^b TM= Thiophanate-methyl, S = Sensitive, R = Resistant. ^c Relative mycelial growth of *Clariireedia* isolates for propiconazole, iprodione and boscalid were estimated on the discriminatory concentrations of 0.1, 1 and 10 µg/mL respectively. P = Propiconazole, I = Iprodione, and B = Boscalid.

4. Discussion

This study reported on the distribution of *Clariireedia* species around the world, and the differential sensitivity patterns of *C. jacksonii*, *C. monteithiana*, and *C. paspali* populations to TM, propiconazole and boscalid, but not to iprodione. The information presented here

will have important implications for the management of dollar spot disease and future monitoring for the development of fungicide resistance in dollar spot pathogens.

In this study, 97% of the *C. monteithiana* isolates, all *C. homoeocarpa*, *C. paspali* and two potential new species isolates were exclusively collected from either C3 or C4 turfgrass, strongly indicating a host preference. Meanwhile, all isolates in Europe and the northern regions of the USA and China were *C. jacksonii*, which also indicates a strong geographic distribution (Figure 1). We propose that both host type and geography are likely to influence the distribution of *Clarireedia* species. Previous studies also revealed that *C. jacksonii* isolates were different from *C. monteithiana* in many aspects, including pathogenicity and mycelial melanization processes [4,14]. Different pathogenicity may cause host preference, while melanization may help isolates resist environmental stress factors present at different geographic locations [14]. It is possible that different *Clarireedia* species have undergone some degree of host specialization, evolving different host specific virulence factors [20]. When spread to multiple regions around the world, some *Clarireedia* species (e.g., *C. jacksonii*) could be better adapted to the cold climate while others preferred warm climates [21]. Further comparative genomics and competition studies among different *Clarireedia* species will aid in understand if they are selected by different host types or environmental factors.

It is still not clear how *Clarireedia* species have spread worldwide. Long distance dissemination by spores seems unlikely for *Clarireedia* species, because spore production was only rarely reported in *C. homoeocarpa* collected from fescues (*Festuca* sp.) in the United Kingdom [22]. Seeds or vegetative materials of turfgrass are the possible vectors for the long distance dissemination, which has been speculated in relevant studies [21,22]. In this study, isolates of the *Clarireedia* species from China were incorporated with world isolates for the analysis. Similarly, *C. jacksonii* and *C. monteithiana* were widely distributed in China, moreover, a new species (*C. paspali*) was also found to be distributed widely in eastern and southern China. It should be noted that turfgrass seeds or vegetative materials are mainly imported in China, which provides an optimal way for the long distance dissemination of *Clarireedia* species. One potential new species, found in Japan and Norway, further supports the possibility of human-mediated dissemination over long distance via seeds or vegetative materials of turfgrass. It is not known how the new species *C. paspali* emerged in China. It may have evolved through a recombination event in China, or may have been spread from other regions around the world via human-mediated dissemination, although it has not been reported in the places other than China. However, some unknown species have been reported at many regions around the world [2,21], suggesting the possibility of other origins of *C. paspali*. Further study, including on isolates of unknown species from worldwide collections, will aid in better understanding of how *C. paspali* emerged in China.

Differential sensitivities in *Clarireedia* species populations against multiple fungicides have been frequently reported at locations worldwide [6,8,12,13,18,23]. However, none of these studies reported if differential sensitivity patterns existed among different *Clarireedia* species. There are two main reasons which may explain why no such studies have been reported. Firstly, dollar spot was not characterized as being caused by different species until 2018. Formerly, it was reported to be caused by the fungus *Sclerotinia homoeocarpa* [2]; Secondly, most studies on fungicide sensitivity in *Clarireedia* species populations were restricted in certain regions or by the same host type, where only one species may exist. However, two or more *Clarireedia* species co-existed on the same host type at multiple locales in China and the United States, indicating that it is necessary to investigate the fungicide sensitivity patterns among different species in *Clarireedia* in these places.

No *C. monteithiana* isolates were resistant to TM, which may indicate that it is difficult to develop TM-resistance in *C. monteithiana* populations. A similar phenomenon was also indicated in a previous study, in which none of the F-type isolates were found to be resistant to benzimidazole fungicides [14]. F-type isolates were confirmed to be *C. monteithiana* in our study (Figure S1). Similar sensitivity patterns among different species were also observed in the fungicide propiconazole, the least mean RMG of *C. monteithiana*, suggesting that *C. monteithiana* may have difficulty developing resistance to propiconazole compared

to *C. jacksonii* or *C. paspali*. Unlike TM and propiconazole, sensitivity of different *Clarireedia* species to boscalid seems inherently different, because almost all *C. paspali* isolates showed less RMG than that of *C. jacksonii* and *C. monteithiana* isolates, and most golf courses had no known history of SDHI fungicide application. The differential sensitivity patterns among the species observed in this study was further indicated by analyzing the locales with at least two *Clarireedia* species from the same turfgrass host. However, further studies are still needed to better understand the sensitivity patterns observed in this study, by collecting larger samples representing different species of *Clarireedia* from the same host in the same locales.

Difficulty in developing fungicide resistance has been reported in various studies [24–29]. Banno et al. identified isolates of *B. cinerea* from Japan that had two distinct *cytb* profiles; the first had a group I intron immediately after position 143, while the second one did not possess the intron. Isolates without the intron developed the G143A substitution and, consequently, were found to be highly resistant to the QoI fungicides; while isolates with the intron did not develop a high resistance to QoI fungicides [24]. An intron adjacent to position 143 in *cytb* has also been observed in other plant-pathogenic fungi, including *Alternaria solani*, *Monilinia fructicola*, *Phyllosticta ampellicida*, *Fusarium effusum*, *Bipolaris oryzae*, *Guignardia citricarpa* and *Magnaporthe poae* [25–30], and none of these showed a high resistance to QoI fungicides. Preliminary analysis showed a high frequency of introns gain or loss in the genomes of different *Clarireedia* species (unpublished data), suggesting insertions and deletions may play an important role in the differential sensitivity patterns of *Clarireedia* species. Further studies are needed to compare the targeted genes of TM and propiconazole among different *Clarireedia* species and investigate the role of insertions and deletions in key regions for fungicide sensitivity.

The inherent differential sensitivity among different species in the same genus or different isolates within the same species has also been reported [31–34]. Two possible mechanisms might be responsible for the differential sensitivity. Ueyama et al. reported that isolates of *Rhizoctonia* AG 4 are able to metabolize pencycuron and are less sensitive, whereas AG3 isolates cannot metabolize this fungicide and are sensitive [35]. Amino acid variations in the targeted genes of fungicides were reported as the other possible mechanism which may cause the differential sensitivity in *Colletotrichum* spp. and *B. cinerea* [31,33]. Wild-type isolates of *B. cinerea* in Florida [36], Germany [37], and China were frequently characterized as highly sensitive (HS) to boscalid, compared to other sensitive isolates, and amino acid polymorphisms in *SdhC* are responsible for the HS isolates. By genetic transformation analyses, Shao et al. revealed that four different types of mutations in *SdhC* could cause increased the sensitivity of wild-type *B. cinerea* isolates to boscalid and fluopyram, which included R-G1 (G85A, I93V, M158V and V168I), R-G2 (G37S), R-G3 (I93V), and R-G4 (I79V, G85A and L151I) [33]. Phylogeny of genes for species identification and the potential targeted genes (*SdhB*, *SdhC* and *SdhD*) of boscalid, as well as metabolism analysis, may be useful for better understanding if the differential boscalid sensitivity observed in *Clarireedia* species is inherent.

5. Conclusions

This study revealed the global distribution of different *Clarireedia* species. Both host type and geography are likely to influence the distribution of *Clarireedia* species. The coexistence of different *Clarireedia* species on the same C4 host type in the same locales was found in different regions. No *C. monteithiana* isolates were resistant to thiophanate-methyl or propiconazole. Differential sensitivity among different species was observed in terms of propiconazole and boscalid ($p < 0.0001$), but not in iprodione. The study will have important implications for dollar spot management, especially in the regions with the coexistence of different *Clarireedia* species.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11102036/s1>, Figure S1: Phylogenetic tree from maximum likelihood analysis of the internal transcribed spacer sequences from global *Clarireedia* isolates. Numbers at each branch

indicate percentage of occurrences of that branch in 1000 bootstrap replications. Isolates grouped together with the type strains were defined to be the same species as the type strains. Otherwise, they were defined as *Clarireedia* sp.; Table S1: Summary information of 275 global *Clarireedia* isolates.; Table S2: In vitro fungicide sensitivity profiles of the three *Clarireedia* species from different regions.

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