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New Isolates of Rhizoctonia Diseases of Turfgrasses

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ABSTRACT

Among the major pathogens of a broad range of turfgrass species, *Rhizoctonia* spp. are known as causal agents of important turf diseases. Recently, several new *Rhizoctonia* diseases of turfgrasses have been found. These have included "brown ring patch" on cool-season turfgrasses, "Waitea reddish-brown patch" on cool-season turfgrasses and "spring rot" on warm-season turfgrasses. Causal pathogens of these diseases were identified and that *W. circinata* var. *circinata*, *Rhizoctonia* sp. closely related to *W. circinata* and the binucleate *Rhizoctonia* AG-D subgroup III are the casual agents, respectively. Furthermore, causal pathogens of the already known *Rhizoctonia* diseases such as "large patch" and "Rhizoctonia patch (elephant foot print)" on warm season turfgrasses have been reconsidered and now identified as *R. solani* AG 2-2 LP and binucleate *Rhizoctonia* AG-D, subgroup II, respectively. Identification of new groups of *Rhizoctonia* as pathogens is mainly possible due to the introduction of the molecular techniques for grouping of this genus. In this paper, these recently found *Rhizoctonia* diseases of turfgrasses are introduced and taxonomical aspects of *Rhizoctonia* spp. are discussed.

Keywords: causal pathogen, molecular techniques for identification, new diseases

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INTRODUCTION

Many types of turfgrasses are used on golf courses and sports fields. The major turfgrasses are the bermudagrasses (*Cynodon* species) and zoysiagrasses (*Zoysia* species) among the perennial, warm-season turfgrasses, and the bluegrass (*Poa* species), perennial ryegrass (*Lolium* perenne) and the bentgrass (*Agrostis* species) among the perennial, cool-season turfgrasses. These turfgrasses used on golf courses and sports fields are subjected to dense cultivation and excessive mowing to present a fine spectacle and playing quality. For example, we cannot get a high quality of a green in golf courses if turfgrasses are not grown with dense cultivation and excessive mowing every day. However, such treatments lead to inappropriate irrigation or stagnation of air, and turf becomes prone to stresses and diseases. About 50 pathogenic fungi are known to be turfgrass pathogens, and 8 among the 50 pathogenic fungi cause severe damage as the major pathogens on zoysiagrasses and 15 pathogenic fungi on the bentgrasses (Tani and Beard 1997). Among the major pathogens, *Rhizoctonia* spp. are known as causal agents of important turf diseases.

Although the causal pathogens of the *Rhizoctonia* genus attack a broad range of turfgrass species, the *Rhizoctonia* causal pathogens exhibit some species specialization. So far several diseases caused by *Rhizoctonia* spp. were reported and the causal pathogens are grouped as *Rhizoctonia* spp. which attack warm-season and cool-season turfgrasses. Some *Rhizoctonia* could attack both warm-season and cool-season turfgrasses.

Large patch disease, which is thought as most important disease for warm-season turfgrasses, usually appears on Japanese zoysiagrass (*Zoysia japonica* Steud) and Manila zoysiagrass (*Z. matrella* Err) in fairways, roughs, sport fields and lawns. Formerly *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] anastomosis group 2, subgroup 2, cultural type IV (AG-2-2 IV) was thought as a causal pathogen of this disease, but now *R. solani* AG-2-2 LP is considered as a causal pathogen (Hyakumachi *et al*. 1998). Rhizoctonia spring dead spot disease and Rhizoctonia patch (known as elephant footprint in Japan) disease for warm-season turfgrasses occur on fairways and roughs of golf courses, and are both caused by *Rhizoctonia cerealis* van der Hoeven [teleomorph: *Ceratobasidium cerealis* Murray and Burpee], a binucleate *Rhizoctonia* AG-D. Isolates of AG-D were further divided into subgroups I and II based on pathogenicity, morphology in culture, and molecular techniques (Tanaka *et al*. 1994; Toda

Received: 8 Juy, 2008. Accepted: 5 September, 2008.

Invited Review

et al. 1999b). AG-D subgroup I (AG-DI) causes a Rhizoctonia spring dead spot disease that produces circular or irregular patches less than 1 m in diameter with whitish color during early and mid spring. Affected leaves in the patch exhibit whitish lesions. AG-D subgroup II (AG-DII) causes Rhizoctonia patch disease, appearing as circular patches with a whitish color from summer through early autumn (Toda *et al*. 1999b).

The disease on cool-season turfgrasses caused by *Rhizoctonia* during mid summer in many parts of the world is primarily brown patch disease caused by *R. solani* AG-1 IA, AG-1 IB and AG-2-2 IIIB (Burpee and Martin 1992; Hayakawa and Hyakumachi 2007). Rhizoctonia brown patch initially appears as circular-shaped patches with a diameter of 25 to 125 mm. The patches develop quickly up to 600 mm in diameter and fade to a light-brown color. The patches may coalesce within ten days to form irregular shapes of larger patches. Another disease of cool-season turfgrasses caused by *Rhizoctonia* is yellow patch (Burpee 1980; Burpee and Martin 1992; Toda *et al*. 1999b). Yellow patch, caused by binucleate *Rhizoctonia* AG-D I, the same pathogen causing Rhizoctonia spring dead spot disease for warm-season turfgrasses, develops yellow to straw-colored patches during cool and warm periods. They appear in round-shaped patches of 200 to 500 mm in diameter. The color of the patches tends to become brown, and thus it also is called winter patch and winter brown patch.

This paper focuses on reconsidered pathogens among Rhizoctonia diseases and on recently reported diseases of turfgrasses caused by *Rhizoctonia* spp. and their taxonomical aspects.

RECONSIDERED PATHOGENS AMONG RHIZOCTONIA DISEASES

Rhizoctonia solani AG-2-2 LP as a causal pathogen of large patch disease for warm-season turfgrasses

Large-patch disease on warm-season turfgrasses (**Fig. 1**) is one of the most important turfgrass diseases in Japan and southern North America. The main hosts of this disease are St. Augustine grass (*Stenotaphrum secundatum*) and zoysia grass (*Zoysia* species) and a minor host is the hybrid Bermuda grass (*Cynodon dactylon* X *C. transvaalensis*) (Tani and Beard 1997). Large-patch disease commonly occurs in the spring and fall, showing sheath blight with reddish brown color. This disease typically occurs as small patches that are initially about 10 cm in diameter and that rapidly expand to 5 or 10 m during the season. In some cases, the turf in the center part of the patch recovers as the patch size increases.

The pathogen causing large-patch disease has been identified as *R. solani*. *R. solani* consists of 14 anastomosis groups (AG 1 to 13 and BI), which have been divided based on hyphal anastomosis behavior, cultural morphology, host range, pathogenicity and other characters (Ogoshi 1976; Carling *et al*. 1994, 1999, 2002). *R. solani* AG 2 has been divided into three subgroups, AG 2-1, AG 2-2 and AG 2-3, based on anastomosis behavior and thiamin requirement

Fig. 1 Typical patches of large patch disease on a fairway of zoysiagrass.

(Ogoshi 1976; Naito and Kanematsu 1994; Kanematsu and Naito 1995). Two cultural types within AG2-2 were distinguished by pathogenicity and cultural morphology, and placed in separate intraspecific groups (ISGs) named IIIB and IV (Ogoshi 1987). The main criterion for differentiating these cultural types is their relative growth in response to high temperature; IIIB isolates can grow at 35°C, while IV isolates cannot (Kuninaga and Yokosawa 1982; Ogoshi 1987; Sneh *et al*. 1991). Isolates obtained from turf with large-patch symptoms (LP isolates) were reported previously as *R. solani* AG2-2 type IV (Oniki *et al*. 1986a). In our study, it was apparent that LP isolates belonged to AG2-2 because of a high frequency of hyphal anastomoses with isolates of AG2-2 type IIIB and type IV, and because they are thiamin auxotrophic. However, they differed from isolates of type IV in their cultural characteristics, including formation of sclerotia, zonation, colour of mycelia and pigment deposition (**Fig. 2**), optimum temperature for growth, and pathogenicity against radish and zoysia grass. Furthermore, LP isolates failed to grow at 35°C, unlike type IIIB (Sneh *et al*. 1991). Our results revealed that the isolates taken from areas with large-patch disease in warm-season turfgrasses differed from known types IIIB and IV of AG2-2. In the RFLP analyses of genomic DNA and of the rDNA-ITS region, LP isolates were also different from those of type IIIB and type IV, and so *R. solani* AG2-2 can now be divided into three cultural types: IIIB, IV and LP. Genetic analysis may also provide additional significant information to determine the relatedness among isolates within cultural types of AG2-2. From of our results, it is concluded that large-patch disease is primarily caused by a new cultural type (LP) of *R. solani* AG2-2. Isolates of both the groups ISG2D (Liu and Sinclair 1992) and AG2-2 turf (Johnk and Jones 1993), which were distinguished from IIIB and IV types by isozyme analysis, RFLPs of rDNA-ITS region, and analysis of cellular fatty acids, were obtained from St. Augustine grass, which is a warm-season turfgrass. ISG2D and AG2-2 turf are now thought to be in the same group as the new cultural type LP, because isolates obtained from St. Au-

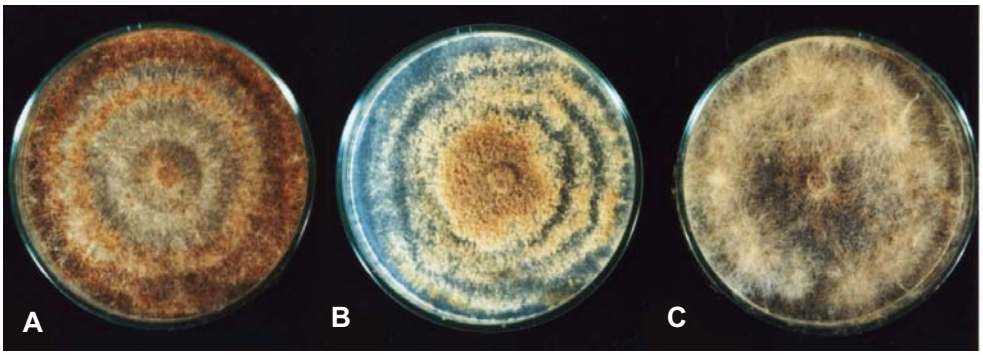

Fig. 2 Cultural morphology of IIIB (A), IV (B) and LP (C) isolates of *Rhizoctonia solani* AG-2-2.

gustine grass with large-patch symptoms also belonged to the new cultural type.

Binucleate *Rhizoctonia* AG-D subgroup II (AG-D II) as a causal pathogen of Rhizoctonia patch (elephant footprint) disease for warm-season turfgrasses

Binucleate *Rhizoctonia* consists of 21 anastomosis groups (AG-A to AG-U) (Ogoshi *et al*. 1979, 1983; Sneh *et al*. 1991; Hyakumachi *et al*. 2005). Fungi in binucleate *Rhizoctonia* AG-D are soilborne fungal pathogens that cause foot-rot, root-rot, and damping-off diseases in several crop plants (Boerema and Verhoeven 1977; Ogoshi *et al*. 1979; Burpee 1980; Burpee *et al*. 1980; Lipps and Herr 1982; Ogoshi *et al*. 1983; Uchino *et al*. 1983; Oniki *et al*. 1986b; Kataria and Hoffmann 1988; Cubeta *et al*. 1991; Damaj *et al*. 1993). These pathogens also cause Rhizoctonia-patch disease (**Fig. 3**) and elephant-footprint disease (**Fig. 4**) of Japanese and Manila zoysiagrasses, and winterpatch disease (**Fig. 5**) of bentgrass (*Agrostis* spp.) (Oniki *et al*. 1986b; Tani 1989; Tanpo *et al*. 1990). These three turfgrass diseases cause aesthetic damage to turfgrasses on golf courses. Rhizoctonia-patch and winter-patch diseases commonly occur during the cool season in Japan, which is from December to May. On the other hand, elephant-footprint disease occurs during the hot season which is from June to September. Tanaka *et al*. (1994) suggested that isolates of AG-D obtained from turfgrasses exhibiting symptoms of these three diseases could be divided into two subgroups based on cultural characteristics and pathogenicity. Tanaka *et al*. (1994) proposed that isolates from the Rhizoctonia-patch and winter-patch diseases belong to subgroup AG-D I, while the fungus causing elephant footprint disease belongs to subgroup AG-D II. From their cultural characteristics, isolates obtained from wheat with foot-rot disease and from mat rush with winter-stem-rot disease also belong to subgroup AG-D I. To test whether these two subgroups are genetically distinct, we compared the genetic structures of the two subgroups of *Rhizoctonia* AG-D by RFLP analysis of the ITS region from rDNA and by RAPD analysis. As a result, we could show that the two subgroups of AG-D are clearly distinguishable from each other. Restriction digest patterns of the ITS region from rDNA by the enzymes *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, and *Mbo*I clearly distinguished two subgroups of AG-D, which were different from other binucleate groups except AG-Q. Hyphae of AG-Q isolates could anastomose with those of AG-D isolates, and they are now thought to belong to subgroup AG-D I (Toda *et al*. 1999b). A dendrogram constructed from the RAPD data also showed each of the two subgroups made an individual cluster with mean similarity coefficients of 0.62 for AG-D I and 0.94 for AG-D II. The values of these two coefficients clearly show that the AG-D I group consists of variant isolates, while the AG-D II group consists of similar isolates. Although the isolates were acquired from various areas in Japan, the data of RFLP analysis of the ITS region from rDNA and of RAPD analysis obtained from subgroups AG-D I and AG-D II indicate that there are no geographical relationships among the isolates in these groups. Based on RFLP analysis of the ITS region from rDNA and on RAPD analysis, isolates obtained from wheat exhibiting symptoms of foot-rot disease, and from mat rush with winter-stem-rot disease, belong to subgroup AG-D I. These disease symptoms were found during the cool season in Japan (Ikata and Yoshida 1940; Takamatsu 1989), and the cultural characteristics and pathogenicity of these fungi are very similar to those of AG-D I isolates. Thus, DNA analyses support the data for the season of occurrence, cultural characteristics, and pathogenicity for these isolates. Thus, we propose that isolates of foot rot and winter-stem-rot diseases belong to subgroup AG-D I. In addition, CAG-1 isolates, causal agent of sharpeye-spot of cereals, and of other diseases of several crop plants, were obtained during the cool season and their cultural characteristics were almost identical to subgroup AG-D I (Burpee 1980; Lipps and Herr 1982). Therefore, we also propose that the known CAG-1 isolates possibly belong to subgroup AG-D I. Subgroup AG-D II isolates causing elephant footprint disease were clearly distinguished from AG-D I based on cultural characteristics, pathogenicity, and DNA analysis. Hyphae of all AG-D II isolates anastomosed with all AG-D I isolates. The results of RAPD analysis showed that the cluster composed of AG-D II isolates had high similarity, and that little variability was observed among the banding patterns of AG-D II isolates.

Fig. 3 Typical patches of Rhizoctonia patch disease on a fairway of zoysiagrass.

Fig. 4 Typical patches of elephant-footprint disease on a fairway of zoysiagrass.

Fig. 5 Typical patches of winter patch disease on a green of creeping bentgrass.

RECENTLY REPORTED DISEASES CAUSED BY *RHIZOCTONIA* SPP.

Disease caused by *Waitea circinata* var. *circinata*

A new disease (**Fig. 6**) was found in Japan on creeping bentgrass (*Agrostis stolonifera* var. *palustris* Huds.) in 1994 (Kitabayashi *et al*. 1994; Mushika *et al*. 1995) and in the United States on annual bluegrass (*Poa annua* L.) in 2003 (de la Cerda *et al*. 2007). This disease was found on bentgrass used for greens on golf courses throughout Japan and occurred during rainy periods. Symptoms included circular or irregular small patches of tan to yellow-brown color ranging from 10 to 50 cm in diameter. The symptoms of this disease were found on plant tissues above the soil surface. The affected turf eventually developed brownish rings, but the turf in the center of the rings recovered. In some cases, the turf recovered completely. Isolates of a *Rhizoctonia* sp. obtained from plants affected by this new disease, were multinucleate and anastomosed with tester isolates of *Rhizoctonia oryzae* Ryker & Gooch and *R. zeae* Voorhees (Kitabayashi *et al*. 1994). However, the cultural morphology of these isolates was different from these species. *Waitea circinata* Warcup & Talbot was classified into three varieties, *W. circinata* var. *circinata*, *W. circinata* var. *oryzae*, and *W. circinata* var. *zeae*, based on differences in the colony morphology of the vegetative state (Gunnell 1986). Although the anamorphic name of *W. circinata* var. *circinata* has not been assigned, *R. oryzae* and *R. zeae* were represented under the anamorphic names of *W. circinata* var. *oryzae* and *W. circinata* var. *zeae*, respectively (Gunnell 1986). *W. circinata* var. *circinata* forms orange to dark brown, globose sclerotia up to 2 mm in diameter; *W. circinata* var. *oryzae* forms orange to salmon, irregularly shaped sclerotia; and *W. circinata* var. *zeae* forms orange to brown, regularly shaped sclerotia up to 1 mm in diameter (Leiner and Carling 1994). *R. oryzae* and *R. zeae* were assigned to *Waitea* anastomosis groups WAG-O and WAG-Z, respectively (Oniki *et al*. 1985). Hyphal fusion was frequent among isolates belonging to each group, but was rare between the two groups (Oniki *et al*. 1985). *R. oryzae* has been isolated from wheat, barley (Martin and Lucas 1983; Ogoshi *et al*. 1990; Mazzola *et al*. 1996), and rice (Ryker and Gooch 1938), while *R. zeae* has been isolated from corn (Sumner and Bell 1982), tall fescue (Martin and Lucas 1983), and rice (Oniki *et al*. 1985). *W. circinata* var. *circinata* obtained from agricultural soil in Alaska was pathogenic to barley (Leiner and Carling 1994), although no diseases caused by *W. circinata* var. *circinata* have been named. Both *R. oryzae* and *R. zeae* are causal agents of turfgrass diseases in North America (Burpee and Martin 1992; Smiley *et al*. 1992). Diseases of turfgrass caused by these pathogens occur most frequently during the warm and humid season, at temperatures between 28 and 36°C, inciting leaf and sheath spot (Burpee and Martin 1992; Smiley *et al*. 1992). The turfgrass disease caused by *R. oryzae* in Japan was reported as a white patch disease on bentgrass in 1990 (Tanpo *et al*. 1990). However, the symptoms of white patch disease (Tanpo *et al*. 1990) are quite similar to the new disease described here. According to Tanpo *et al*. (1990), the initial symptom of white patch disease also appears as an irregular small patch ranging from 10 to 60 cm in diameter with a light-whitish color. As with the new disease, the symptoms of white patch were found on plant tissues above the soil surface, and healthy leaves remain on several areas of turf inside the rings throughout the season. The symptoms of white patch disease can disappear completely at the end of the season. These characteristic symptoms of white patch disease (Tanpo *et al*. 1990) were very similar to symptoms of the new disease. Other diseases of bentgrass caused by *Rhizoctonia* spp. are yellow patch and brown patch (Burpee 1980; Burpee and Martin 1992; Toda *et al*. 1999b). Yellow patch, caused by a binucleate *Rhizoctonia* sp. in AG-D, develops yellow to straw-colored patches during cool and warm periods. Brown patch disease, caused by *R. solani* AG 2-2 IIIB, develops during the warm and humid weather in summer. Although the new disease occurs during the same season as brown patch disease, it causes less damage than brown patch and shows a yellow brown color on creeping bentgrass leaves, while brown patch symptoms include gray-purple or gray-brown lesions on leaves.

Fig. 6 A new disease (brown ring patch disease) caused by *Waitea circinata* var. *circinata* on a green of creeping bentgrass.

A new disease on bentgrass caused by a *Rhizoctonia* sp., typified by NP isolates, was reported in Japan in 1994 (Kitabayashi *et al*. 1994). It was apparent that these isolates belonged to the *Waitea* anastomosis group (WAG) because they anastomosed with isolates of WAG-O (*W. circinata* var. *oryzae*) and WAG-Z (*W. circinata* var. *zeae*) (Oniki *et al*. 1985). We clarified that NP isolates were different from isolates of *W. circinata* var. *oryzae* and *W. circinata* var. *zeae*, based on cultural characteristics, including color and size of sclerotia, color of mycelia, pigment deposition on media, optimum temperature for growth, and pathogenicity. The colony color of NP isolates was similar to that of isolates of *R. oryzae* that were reported as the causal agent of white patch disease (RW isolates) (Tanpo *et al*. 1990) on creeping bentgrass and some isolates of *W. circinata* var. *circinata*, and the mycelia growth rate of NP isolates was the same as that of RW isolates and *W. circinata* var. *circinata*. Mycelia of NP isolates, RW isolates, and *W. circinata* var. *circinata* grew faster at temperatures below 16°C than *W. circinata* var. *oryzae* and *W. circinata* var. *zeae*, which corresponds with the report of Leiner and Carling (Leiner and Carling 1994). The pathogenicity test also showed that the symptoms observed on creeping bentgrass inoculated by NP isolates were similar to those caused by RW isolates and *W. circinata* var. *circinata*. The results of this study indicate that NP isolates causing a new disease of bentgrass and RW isolates causing white patch disease of bentgrass have characteristics similar to *W. circinata* var. *circinata*. Based on RAPD-PCR analysis and RFLP of the rDNA-ITS region, NP isolates could be distinguished from *W. circinata* var. *oryzae* and *W. circinata* var. *zeae*, but could not be distinguished from RW isolates and *W. circinata* var. *circinata*. RAPD primers produced many fragments with NP isolates, RW isolates, three varieties of *R. circinata*, and *R. solani* AG 2-2 IIIB, and there was a high level of variation among the isolates. On the basis of fragment patterns, a phylogenetic tree based on neighbor-joining indicated three clusters and one outgroup. Isolates of *W. circinata* var. *oryzae* and *W. circinata* var. *zeae* each made an individual cluster, while NP isolates, RW isolates, and *W. circinata* var. *circinata* made one cluster. Therefore, we can consider that NP isolates, RW isolates, and *W. circinata* var. *circinata* belong to the same RAPD group. The length of the rDNA-ITS regions and restriction enzyme digest patterns with three enzymes could not distinguish isolates of NP, RW, and *W. cir-*

cinata var. *circinata*. However, RFLP analysis with *Hap*II of the rDNA-ITS region was useful to separate isolates of NP, RW, and *W. circinata* var. *circinata* from *W. circinata* var. *oryzae* and *W. circinata* var. *zeae*. Furthermore, restriction patterns by *Mbo*I and *Hin*fI clearly distinguished *W. circinata* var. *oryzae* from *W. circinata* var. *zeae*. The restriction sites of rDNA-ITS regions in this study did not corresponded with previously reported sequences of *R. oryzae* (Mazzola *et al*. 1996). Isolates of *W. circinata* var. *oryzae* had a high level of variation based on RAPD analysis and a different response of hyphal growth to high temperatures (Ogoshi *et al*. 1990; Leiner and Carling 1994). There might be several subgroups in this variety. The results obtained from DNA analyses, showing that NP isolates could not be distinguished from RW isolates and *W. circinata* var. *circinata* but could be distinguished from *W. circinata* var. *oryzae* and *W. circinata* var. *zeae*, were consistent with their cultural characteristics. Tanpo *et al*. (1990) reported that *R. oryzae* (RW isolates) was the causal agent of white patch disease on bentgrass in Japan. The disease symptoms caused by *R. oryzae* were very similar to the new disease caused by NP isolates in this report except for whitish color. We used isolates of Tanpo *et al*. (1990), which showed almost the same cultural characteristics and pathogenicity as NP isolates and *W. circinata* var. *circinata* but not *W. circinata* var. *oryzae*. Moreover, RW isolates were placed into the same groups as *W. circinata* var. *circinata* based on RAPD-PCR and RFLP analysis of rDNA-ITS region. Therefore, the identification of RW isolates as *R. oryzae* proposed by Tanpo *et al*. (1990) should be reconsidered as *W. circinata* var. *circinata*. Based on the results of this study, we recognized that the NP isolates causing the new disease and the RW isolates, which had been considered to be *R. oryzae*, cause of white patch disease, both belong to *W. circinata* var. *circinata*. However, the name "white patch" cannot be used for this turf disease, because the same name has already been used by another fungal disease for tall fescue (*Festuca arundinacea* Schreb) caused by *Melanotus phillipsii* Singer (Tani and Beard 1997). As Tanpo *et al*. (1990) noted, the affected turf develops rings and shows a clear patch with a brownish color including yellow or white. From these symptoms, we propose that the name of the new disease is "brown ring patch".

Disease caused by *Rhizoctonia* sp. closely related to *Waitea circinata*

A severe disease, which had not been recognized before, was noted on creeping bentagass [*Agrostis stolonifera* L. var. *palustris* (Huds.) Farw] and Kentucky bluegrass (*Poa pratensis* L.) during mid summer from 1999 to present in golf courses in Aichi and Chiba prefectures, Japan (**Fig. 7**) (Toda *et al*. 2007). We isolated many unknown *Rhizoctonia* sp. (UR isolates) from diseased leaves of the two grasses. In general, the disease on creeping bentgrass caused by *Rhizoctonia* in mid summer is primarily brown patch disease caused by *R. solani* AG 1-IA and AG 2-2 IIIB (Burpee and Martin 1992). The younger mycelia of the UR isolates, however, differed from the light yellow mycelia of *R. solani*. However, the UR isolates were similar to those of the white vegetative state of *W. circinata*. UR isolates could also anastomose with all the tester isolates of the three varieties of *W. circinata* (*W. c.* var. *oryzae*, var. *zeae* and var. *circinata*). But the variety names have not yet been proposed properly and are invalid. Mature colonies of UR isolates, however, apparently differed from those of known varieties of *W. circinata*. *W. circinata* is known as the teleomorph of the cereal pathogens *R. oryzae* Ryker & Gooch and *R. zeae* Voorhees (Oniki *et al*. 1985; Gunnell 1986). This fungus has been classified into three varieties, *W. c.* var. *oryzae*, *W. c.* var. *zeae* and *W. c.* var. *circinata*, based on differences in colony morphology of their vegetative state (Gunnell 1986; Leiner and Carling 1994). *R. oryzae* and *R. zeae* had been assigned the anamorphic names of *W. c.* var. *oryzae* and *W. c.* var. *zeae*, respectively. The anamorphic name of *W. c.* var. *circinata* has not been assigned yet. The classification of these three varieties has been supported by molecular analysis (Toda *et al*. 2005) and by whole cell fatty acid analysis (Priyatmojo *et al*. 2002). *W. c.* var. *circinata* obtained from agricultural soil in Alaska is pathogenic to barley (Leiner and Carling 1994). *W. c.* var. *oryzae* is pathogenic to rice, wheat and barley (Oniki *et al*. 1985; Ogoshi *et al*. 1990; Mazzola *et al*. 1996; Paulitz *et al*. 2003). *W. c.* var. *zeae* is pathogenic to rice, corn, onion, sugarbeet and tall fescue (Sumner and Bell 1982; Martin and Lucas 1983; Oniki *et al*. 1985; Ogoshi *et al*. 1990; Kuznia and Windels 1994; Erper *et al*. 2006). All varieties of *W. circinata* can also cause turfgrass diseases during the warm season. *W. c.* var. *circinata* causes yellow-brown patches in bentgrass, referred to as brown ring patch, in spring through autumn (Toda *et al*. 2005). *W. c.* var. *oryzae* infects Bermuda grass and St. Augustine grass, causing foliar lesions during the summer (Burpee and Martin 1992). *W. c.* var. *zeae* also causes foliar lesions on Bermuda grass, St. Augustine grass, creeping bentgrass and annual bluegrass (Burpee and Martin 1992; Hsiang and Dean 2001) during mid summer.

Fig. 7 A new disease (Waitea reddish-brown patch disease) caused by a new *Rhizoctonia* sp. closely related to *Waitea circinata* on a green of creeping bentgrass.

For identifying varieties within *W. circinata*, it is important to observe the differences in cultural morphology of their vegetative state (Gunnell 1986; Leiner and Carling 1994) and to analyze molecular profile data of the tester isolates (Toda *et al*. 2005). Using these methods and the pathogenicity test, we classified the UR isolates as a new *Rhizoctonia* sp. closely related to *W. circinata* and distinctly different from the three known varieties of *W. circinata* because the perfect state of the UR isolates has never been observed in nature or produced in vitro. *Rhizoctonia* sp. used with the UR isolates was clearly included in the *Waitea* anastomosis group (WAG) because they anastomosed with tester isolates of *W. circinata* varieties and the hyphal morphology was also similar to that of *W. circinata*. Oniki *et al*. (1985) reported anastomosis between WAG-O and WAG-Z at low frequencies (less than 33%). In this study, however, more than 33% anastomosis was sometimes observed between isolates of WAG-O and WAG-Z and 18-39% anastomosis among all isolates of UR and *W. circinata*. Thus, the varieties of *W. circinata* cannot be clearly distinguished based on anastomosis frequency alone. UR isolates were consistently distinguished from the other three varieties of *W. circinata* by colony color and sequences of the rDNA-ITS region. The comparison of colony color and sclerotia shape has been considered to be important for distinguishing the three varieties of *W. circinata* (var. *circinata*, var. *oryzae* and var. *zeae*) (Leiner and Carling 1994; Toda *et al*. 2005) (**Fig. 8**). From the results of our study, comparison of their colony color is rather important, because no differences in the shape of sclerotia were observed between UR isolates and *W. c.* var. *circinata*. We emphasize that

Table 1 Morphological characteristics of UR isolates, *Waitea circinata* var. *circinata*, var. *oryzae* and var. *zeae*.

Variety [a]	Nuclei per cell	Hyphal diameter (µm)	Colony color [b]	Sclerotia [b]		
				Diameter (mm)	Shape	Color
UR	3 – 10	4.7 – 7.8	Light yellow	1 – 3	Irregular	Dark brown
circinata	3 – 9	4.8 – 7.7	Dark brown	1 – 3	Irregular	Dark brown
oryzae	3 – 8	4.8 – 7.5	White to salmon pink	1 – 3	Irregular	Salmon pink
zeae	3 – 10	4.5 – 8.0	Orange	0.5 – 1	Subspheroid	Orange

[a] UR: UR isolates ; *circinata*: isolates of *W. c.* var. *circinata*; *oryzae*: isolates of *W. c.* var. *oryzae*; *zeae*: isolates of *W. c.* var. *zeae*
[b] On PDA after 30 days incubation.

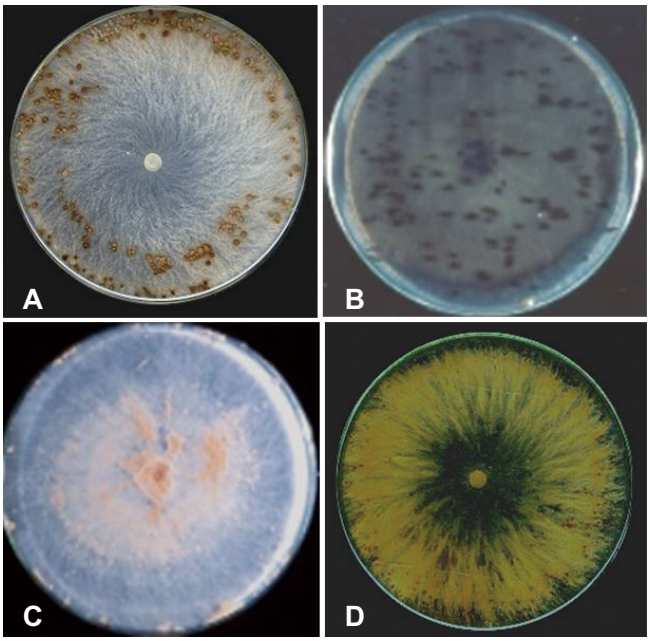

Fig. 8 Cultural morphology of a new *Rhizoctonia* sp. (A) closely related to *Waitea circinata* (B) and var. *oryzae* (C), var. *zeae* (D) and *circinata* of *Waitea circinata* after 30 days incubation on PDA. Reprinted from **Toda T, Hayakawa T, Maghalu JM, Yaguchi S, Hyakumachi M** (2007) A new *Rhizoctonia* sp. closely related to *Waitea circinata* causes a new disease of creeping bentgrass. *Journal of General Plant Pathology* **73**, 379-387, with kind permission of The Phytopathological Society of Japan and Springer Science+Business Media, ©2007.

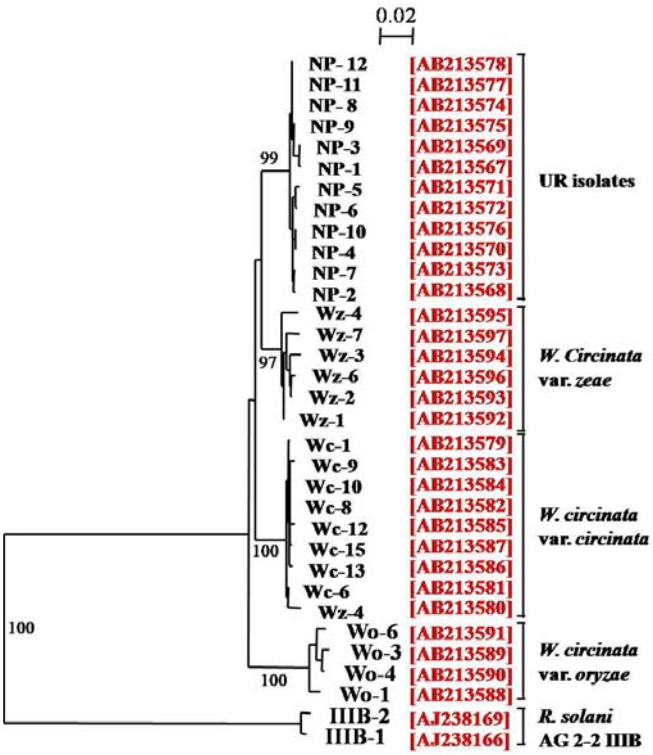

Fig. 9 Phylogenetic distance tree, constructed by the neighborjoining method, to compare the rDNA-ITS region sequence of UR isolates with three varieties of *Waitea circinata*. Two isolates of *Rhizoctonia solani* AG 2-2 IIIB were used as the outgroup. The accession numbers in the GenBank database are given after each isolate number. The numbers on the branches are confidence values obtained for 1,000 bootstrap replicates (only values above 90% are shown). The variety or anastomosis group of each isolate is in the right column. Bar represents a phylogenetic distance of 2%. Reprinted from **Toda T, Hayakawa T, Maghalu JM, Yaguchi S, Hyakumachi M** (2007) A new *Rhizoctonia* sp. closely related to *Waitea circinata* causes a new disease of creeping bentgrass. *Journal of General Plant Pathology* **73**, 379-387, with kind permission of The Phytopathological Society of Japan and Springer Science+Business Media, ©2007.

more than 30 days of incubation is necessary to compare colony colors; UR isolates maintained a light yellow color after 30 days, while the colony color of *W. c.* var. *circinata*, var. *oryzae* and var. *zeae* gradually became dark brown, white to salmon pink, and orange, respectively (**Table 1**). Numerous studies have demonstrated the usefulness of sequence analysis of the rDNA-ITS region for classifying and identifying intragroups within *Rhizoctonia* sp. (Boysen *et al*. 1996; Kuninaga *et al*. 1997; Johanson *et al*. 1998; Salazar *et al*. 2000a, 2000b; Gonzalez *et al*. 2001; Hsiang and Dean 2001). In our study, sequences of rDNA-ITS region contributed to the successful classification of *W. circinata* varieties; similarities within each variety were very close (97.2-100%), but similarities were significantly lower between varieties (87.8-94.6%). The phylogenetic tree obtained from the ITS sequence consistently separated the isolates of *W. circinata* into the appropriate variety, with bootstrap values of nearly 100% (**Fig. 9**). The results of pathogenicity tests provided important data to identify the causal agent of the new disease caused by the UR isolates on creeping bentgrass; the reddish brown lesions on creeping bentgrass leaves induced by UR isolates differed from those induced by the other varieties, and the disease severity was significantly higher than that caused by the isolates of the other three varieties of *W. circinata* (**Table 2, Fig. 10**). Symptoms on creeping bentgrass induced by UR isolates in our test were very similar to those appearing on golf courses. Although Kentucky bluegrass was not used for the pathogenicity tests, the symptoms caused by UR isolates on Kentucky bluegrass in golf courses were similar to those on creeping bentgrass. Therefore, Kentucky bluegrass would likely develop reddish brown lesions with high severity, the same as creeping bentgrass, after inoculation with UR isolates. *W. c.* var. *circinata* is moderately pathogenic to creeping bentgrass causing yellow brown symptoms, referred to as brown ring patch disease (Toda *et al*. 2005). *W. c.* var. *zeae* is moderately pathogenic to creeping bentgrass with light brown symptoms (Martin and Lucas 1983; Burpee and Martin 1992). Isolates of *W. c.* var. *oryzae* have not been isolated from either creeping bentgrass or Kentucky bluegrass. In the pathogenicity tests in our study, the symptoms on creeping bentgrass induced by three *W. circinata* varieties were almost similar to those in previous reports (Martin and Lucas 1983; Burpee and Martin 1992; Toda *et al*. 2005). Because the disease has never been reported in the world, we propose the disease name *Waitea* reddish brown patch disease of creeping bentgrass caused by a new *Rhizoctonia* sp. Our results also show that the new *Rhizoctonia* sp. is

Table 2 Disease severity of UR isolates, *Waitea circinata* var. *circinata*, var. *oryzae* and var. *zeae*on creeping bentgrass.

Variety	Disease severity [a]	Re-isolation frequency (%)
UR	3.84 c [b]	83.0
circinata	2.06 b	93.3
oryzae	0.52 a	40.0
zeae	2.32 b	76.3
Control	0.00 a	0.0

[a] 0 = symptomless; 1 = small lesions; 2 = lesions covering < 10 % of leaf; 3 = lesions covering 10 to 50 % of the leaf; 4 = lesions covering > 50 % of a wilted leaf; and 5 = leaf was completely withered. Average of disease severity was estimated using 5 UR isolates, 4 isolates of *W.c.* var. *circinata*, 4 isolates of *W.c.* var. *oryzae* and 4 isolates of *W.c.* var.*zeae*.

[b] Values followed by the same letter do not differ significantly ($p < 0.01$) according to Kruskal-Wallis test.

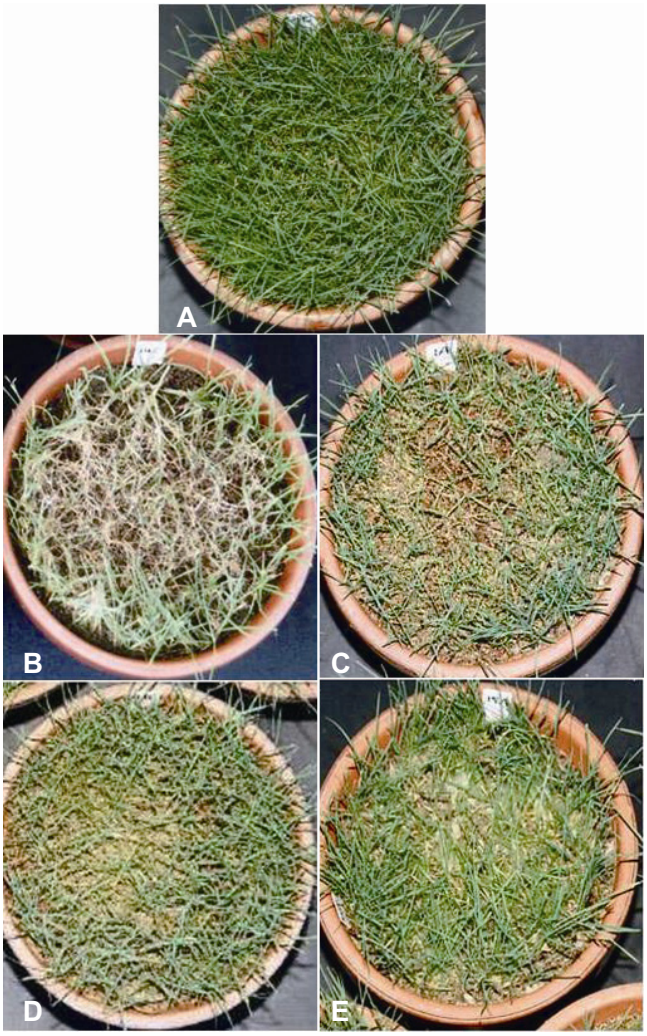

Fig. 10 Creeping bentgrass with symptoms induced after artificial inoculation with UR isolates (B) and three varieties (C-E) of *Waitea circinata*. Uninoculated creeping bentgrass is shown as the control (A). Reprinted and modified from **Toda T, Hayakawa T, Maghalu JM, Yaguchi S, Hyakumachi M** (2007) A new *Rhizoctonia* sp. closely related to *Waitea circinata* causes a new disease of creeping bentgrass. *Journal of General Plant Pathology* **73**, 379-387, with kind permission of The Phytopathological Society of Japan and Springer Science+Business Media, ©2007.

very close to *W. circinata*. In the future, if the perfect state of the new *Rhizoctonia* sp. is observed or produced, the fungus should be described as a new variety of *W. circinata*.

Disease caused by a binucleate *Rhizoctonia* AG-DIII

A new Rhizoctonia disease on Japanese zoysia grass (*Zoysia japonica* Steud) was found on fairways and roughs of golf courses in Shizuoka, Japan, from mid through late

Fig. 11 A new disease (spring rot disease) caused by binucleate *Rhizoctonia* AG-D III on a fairway of zoysiagrass.

spring 2002 (**Fig. 11**) (Hayakawa *et al*. 2006). This disease formed irregular reddish-brown patches approximately 1 m in diameter. The affected turf showed symptoms of water-soaked sheath rot and reddish-brown foliar discoloration, but did not show any symptoms in the stolons and roots. Hyphae of *Rhizoctonia* sp. were frequently isolated from affected leaf sheaths. Unknown (UN) isolates had two nuclei per cell and anastomosed with tester isolates of binucleate *Rhizoctonia* anastomosis group (AG)-D. Binucleate *Rhizoctonia* AG-D is the causal pathogen of foot rot, root rot, and damping-off disease in several crops (Burpee 1980; Lipps and Herr 1982; Oniki *et al*. 1986b). Based on anastomosis reactions, binucleate *Rhizoctonia* spp. have been assigned to AGs, of which 21 groups have been designated as AG-A through AG-U (Ogoshi *et al*. 1979, 1983; Sneh *et al*. 1991; Hyakumachi *et al*. 2005) in Japan. Also, *Ceratobasidium* anastomosis groups (CAGs) have been established as CAG 1 through 7 (Burpee *et al*. 1980), and AG-D was clarified to be identical to CAG-1 (Ogoshi *et al*. 1983). These patterns were supported using molecular techniques (Cubeta *et al*. 1991; Damaji *et al*. 1993). As described before, isolates of AG-D were further divided into subgroups I and II based on pathogenicity, morphology in culture, and molecular techniques (Tanaka *et al*. 1994; Toda *et al*. 1999b). AG-D subgroup I (AG-D I) causes a Rhizoctonia patch disease and AG-D subgroup II (AG-D II) causes elephant footprint disease. From the results of comparison study of the UN isolates obtained from Japanese zoysia grasses with tester isolates of *Rhizoctonia* AG-D I and II, based on cultural morphology, hyphal growth rate at different temperatures, pathogenicity on Japanese zoysia grass, and sequence analysis of the internal transcribed spacer (ITS) region on ribosomal DNA (rDNA-ITS region), UN isolates were assigned as a new subgroup within *Rhizoctonia* AG-D. Therefore, we propose that the UN isolates form a new subgroup of *Rhizoctonia* AG-D, subgroup III, and that the name of a new disease caused by AG-D subgroup III is “spring rot” disease on Japanese zoysia grass.

The UN isolates were categorized as members of *Rhizoctonia* AG-D because they anastomosed with tester isolates of AG-D but not with other binucleate isolates. Anastomosis frequencies ranged from 30 to 50% without differences between subgroups. Therefore, AG-D subgroups including UN isolates could not be distinguished based on anastomosis frequencies. The standard cultural morphology of AG-D III was defined briefly by comparison with other subgroups of AG-D (**Fig. 12**). Occasionally, several AG-D III isolates did not form sclerotia. On the other hand, colonies of some AG-D I isolates remained light yellow in color. Therefore, we have to be cautious when determining subgroups based on morphological characterizations alone. For conventional identification of AG-D III, comparison of hyphal growth rate among AG-D subgroups seems to be

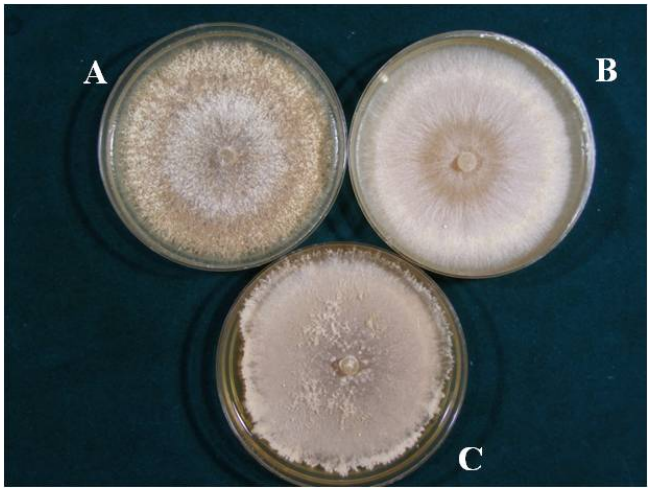

Fig. 12 Cultural morphology of binucleate *Rhizoctonia* AG-D I (A), AG-D II (B) and AG-D III (C) isolates.

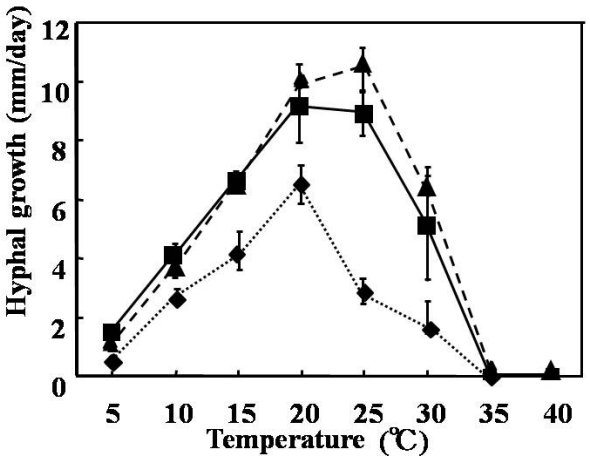

Fig. 13 Mycelial growth rate of unkown (UN) isolates and isolates of two subgroups of binucleate *Rhizoctonia* anastomosis group (AG)-D. Bars indicate standard deviation. Four UN isolates (◆, dotted line), two isolates of AG-D I (■, solid line), and two isolates of AG-D II (▲, dashed line) were represented by each line. Reprinted from **Hayakawa T, Toda T, Ping Q, Mghalu JM, Yaguchi S, Hyakumachi M** (2006) A new subgroup of *Rhizoctonia* AG-D, AG-D III, obtained from Japanese Zoysia grass exhibiting symptoms of a new disease. *Plant Disease* **90**, 1389-1394, with kind permission of The American Phytopathological Society, ©2006.

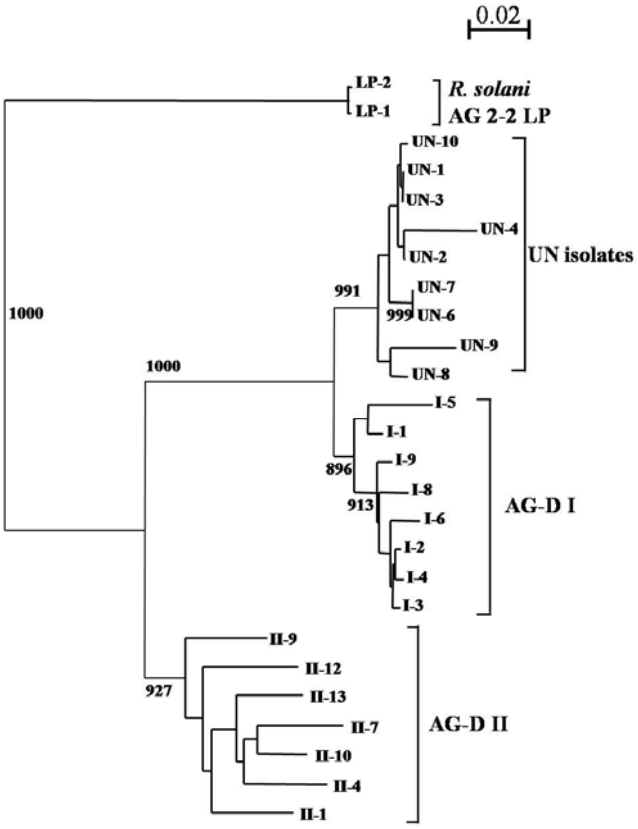

Fig. 14 Phylogenetic distance tree, constructed by the neighbor-joining method, to compare the nearly complete internal transcribed spacer (ITS) region of ribosomal DNA genes sequence of unknown (UN) isolates with *Rhizoctonia* anastomosis group (AG)-D I and II. Two isolates of *Rhizoctonia solani* AG 2-2 LP were used as an outgroup. The numbers on the branches are confidence values obtained for 1,000 bootstrap replicates (only values above 70% are shown). AGs and subgroups of each isolate are shown in right column. Bar represents a phylogenetic distance of 2%. Reprinted from **Hayakawa T, Toda T, Ping Q, Mghalu JM, Yaguchi S, Hyakumachi M** (2006) A new subgroup of *Rhizoctonia* AG-D, AG-D III, obtained from Japanese Zoysia grass exhibiting symptoms of a new disease. *Plant Disease* **90**, 1389-1394, with kind permission of The American Phytopathological Society, ©2006.

easier than observation of cultural morphology, especially at 25°C (**Fig. 13**). Sequence data of the rDNA-ITS region are more efficient for identifying each AGD subgroup. Sequence homology within each AG-D subgroup was very high, but significantly lower between subgroups. Moreover, phylogenetic analysis using the sequence data separated into three clearly defined clusters corresponding to the three AG-D subgroups (**Fig. 14**).

The spring rot disease on Japanese zoysia grass caused by AG-D subgroup III initially was considered to be Rhizoctonia patch disease caused by AG-D subgroup I. The major diseases on zoysia grasses in spring are gray snow mold caused by *Typhula* spp. (Smith *et al*. 1989) and pink snow mold caused by *Microdochium nivale* (Smith *et al*. 1989). In turfgrass, the symptoms of spring rot are clearly different from those of gray and pink snow molds but similar to those of Rhizoctonia patch. When we carefully observed the symptoms of spring rot, several differences were found; discoloration was reddish brown, the shape of the patch was more irregular than Rhizoctonia patch disease, and severe symptoms persisted later into the season. Based on results of the greenhouse experiment, the symptoms induced by AG-D III isolates were easily distinguishable from those induced by AG-D I and II. AG-D III isolates were significantly less virulent than those of other AG-D subgroups (**Table 3**). Therefore, the "sheath rot" disease caused by AG-D III could be recognized as a new disease on Japanese zoysia grass. However, because spring rot and Rhizoctonia patch diseases can occur in the same season, we should

Table 3 Disease severity of UN isolates, binucleate *Rhizoctonia* AG-D I and AG-D II on Japanese zoysia grass.

Isolates	Disease severity [a]	Re-isolation (%)[b]
Experiment 1		
UN [c]	48.8 b [d]	77.5
AG-D I	97.5 c	77.5
AG-D II	98.8 c	77.5
Control	0.00 a	0.0
Experiment 2		
UN	52.5 b	83.3
AG-D I	87.5 c	85.0
AG-D II	100.0 c	92.5
Control	0.0 a	0.0

[a] Disease severity ranging : 0 % = leaf did not show symptom; 5 % = showing lesions covering less than 10 % of leaf area; 30 % = showing lesions covering from 10 to 50 % of the leaf area; 75 % = showing lesions covering more than 50 % of leaf area; and 100 % = leaf completely withered.
[b] Reisolation (%) = binucleate *Rhizoctonia* appearance / 20 plants ×100
[c] UN: UN isolates obtained from sheath rot symptoms; AG-D I: isolates of *Rhizoctonia* AG-D subgroup I; AG-D II: isolates of *Rhizoctonia* AG-DII. Six UN isolates, 2 isolates of AG-D I and 2 isolates of AG-D II were represented, respectively.
[d] Values followed by the same letter do not differ significantly (P = 0.05) according to Kraskal-Wallis test.

diagnose the disease symptoms carefully. Under natural conditions, it is very difficult to distinguish between the diseases based on symptoms alone. Therefore, rapid and accurate methods such as molecular techniques are required for diagnosis of these turfgrass pathogens.

MOLECULAR TECHNIQUES FOR IDENTIFICATION AND CLASSIFICATION OF *RHIZOCTONIA* SPP.

The conventional methods of identification of the causal pathogen of turfgrass diseases are mainly based on the observation of their cultural morphology, on pathogenicity testing and on anastomosis behavior. However, these methods are laborious and time-consuming and give variable results. This makes it difficult to determine an appropriate control measure against turfgrass diseases. The use of molecular markers in plant pathology has been recognized as a rapid tool in disease diagnosis. Especially, polymerase chain reaction (PCR) using specific primers designed from the ribosomal DNA internal transcribed spacer (rDNA-ITS) regions is frequently performed for identifying molecular markers useful for disease diagnosis (Poupard *et al*. 1993; Mazzora *et al*. 1996; Johanson *et al*. 1998).

Ribosomal RNA genes are known to be conserved, and so sequence component analyses of these genes are phylogenetically and taxonomically informative (Bruns *et al*. 1991). Analysis of ribosomal DNA has been used for classification of *Rhizoctonia* species (Jabaji-Hare *et al*. 1990; Vilgalys and Gonzalez 1990; Liu and Sinclair 1992, 1993; Liu *et al*. 1993, 1995; Balali *et al*. 1996). Also, the delimitation of binucleate *Rhizoctonia* AGs has been supported by DNA analyses (Cubeta *et al*. 1991; Damaj *et al*. 1993). In addition, some reports have indicated that RFLP analysis of the ITS region from rDNA and RAPD analysis could be used for inter- or intra-group differentiation of *Rhizoctonia* spp. (Liu and Sinclair 1992; Duncan *et al*. 1993; Liu and Sinclair 1993; Boysen *et al*. 1996; Keijer *et al*. 1996; Yang *et al*. 1996; Schneider *et al*. 1997; Justesen *et al*. 2003). Although restriction fragment length polymorphisms (RFLPs) used with rDNA probes are thought to indicate divergent variations among isolates of *R. solani*, no differences between AG2-2 IIIB and AG2-2 IV were found using this method (Jabaji-Hare *et al*. 1990; Vilgalys and Gonzalez 1990). Random amplified polymorphic DNA (RAPD) assay has frequently been used for the identification of several fungal species (Nicholson and Razanoor 1994; Nicholson *et al*. 1996; Parry and Nicholson 1996; Nicholson *et al*. 1997). RAPD is also recognized as a useful method for grouping different AGs of *R. solani* (Duncan *et al*. 1993; Yang *et al*. 1995), identifying and differentiating among *Rhizoctonia* species (Lilja *et al*. 1996; Yang *et al*. 1996; Toda *et al*. 1999a; Pascual *et al*. 2000). Moreover, primers designed from RAPD-PCR products could also be used as specific primers for the detection of *Rhizoctonia* (Nicholson and Parry 1996). However, in the case of *R. solani* AG 2-2, PCR using primers designed from rDNA-ITS regions could specifically amplify AG 2-2, but could not distinguish its three cultural types (Salazar *et al*. 2000a). On the other hand, by designing specific primers from specific RAPD-PCR products, we could differentite the three cultural types of AG 2-2 (Toda *et al*. 2004b). The specific primers could amplify a single product which was obtained only from AG 2-2 LP isolates and leaf sheaths exhibiting large-patch symptom but not from other isolates of *R. solani*, other turfgrass pathogens or healthy zoysia grass leaf sheaths.

Among the various molecular classification methods used for classification of *Rhizoctonia* spp., the rDNA-ITS sequence analysis seems to be the currently most appropriate one (Salazar *et al*. 2000; Justesen *et al*. 2003; Toda *et al*. 2004a; Sharon *et al*. 2006, 2008). A comprehensive approach for the identification and classification of *Rhizoctonia* spp. isolates could be obtained based on rDNA-ITS sequence alignment analysis, complemented with detailed percent sequence similarity within and among AGs and subgroups.

CONCLUSIONS

A summary of the Rhizoctonia diseases, causal pathogens, and host turfgrasses is placed in **Table 4.** So far, isolates belonging to *R. solani* AG-2-2 LP and binucleate *Rhizoctonia* AG-D II and AG-D III and isolates of *W. circinata* var. *circinata* and *Rhizoctonia* sp. closely related to *Waitea circinata* were not obtained from plants other than warm-season and cool-season turfgrasses, respectively. It would be interesting to know whether these isolates can be obtained only from these turfgrasses, or from various plant species. Isolates belonging to *R. solani* AG-2-2 LP, AG-2-2 IIIB and binucleate *Rhizoctonia* AG-D I are distributed widely both in Japan and North America. Isolates of *W. circinata* var. *circinata* had been reported from Japan only, but very recently, they were also reported from North America, while isolates of *Rhizoctonia* sp. closely related to *Waitea circinata*, binucleate *Rhizoctonia* AG-D II and AG-D III were only reported from Japan. On the other hand, *Waitea circinata* var. oryzae and var. zeae were reported as pathogens of turfgrass diseases from North America but not from Japan. Isolates of binucleate *Rhizoctonia* AG-D III were distributed in a small location and obtained only from three golf courses in Shizuoka prefecture, Japan, that are 4 to 5 km apart from each other. The temperature of all three golf courses ranges from approximately 11 to 20°C during mid-through late spring. Even though the reported occurrence of sheath rot diseases caused by AGD III is limited to golf courses in close proximity, many golf courses all over the world share a similar climate. Therefore the sheath rot disease caused by AG-D III may not be a local disease and may occur on other golf courses as well. Meanwhile, as the isolates of *Rhizoctonia* sp. closely related to *Waitea circinata* which causes Waitea reddish-brown path disease on cool-season turfgrasses are distributed widely in Japan and their pathogenicity is very high, there is a high possibility that this disease will bring a serious problem in golf courses and sports fields in the near future.

Table 4 A summary of the Rhizoctonia diseases, causal pathogens, and host turfgrasses.

Pathogens	Diseases	Host turfgrasses	Occurrence [a]	
			Japan	Other
R. solani AG-2-2 LP	Large patch	Warm-season turfgrasses	○	○
R. solani AG-2-2 IIIB	Brown patch	Cool-season turfgrasses	○	○
R. solani AG-1 (IA, IB)	Brown patch	Cool-season turfgrasses	○	○
W. circinata var. *oryzae*	–	Warm-season turfgrasses	–	○
W. circinata var. *zeae*	–	Warm-season turfgrasses	–	○
	–	Cool-season turfgrasses	–	○
W. circinata var. *circinata*	Brown ring patch	Cool-season turfgrasses	○	○
Rhizoctonia sp. (closely related to *W. circinata*)	Waitea reddish-brown patch	Cool-season turfgrasses	○	–
Binucleate *Rhizoctonia* AG-DI	Rhizoctonia patch (Rhizoctonia spring dead spot)	Warm-season turfgrasses	○	–
	Winter patch (Yellow patch)	Cool-season turfgrasses	○	○
Binucleate *Rhizoctonia* AG-DII	Rhizoctonia patch (Elephant footprint)	Warm-season turfgrasses	○	–
Binucleate *Rhizoctonia* AG-DIII	Spring rot	Warm-season turfgrasses	○	–

[a] ○: reported , –: not reported

Identification of new groups of *Rhizoctonia* as pathogens is mainly possible due to the introduction of the molecular techniques for grouping of this genus, and so far, among the various molecular classification methods, the rDNA-ITS sequence analysis seems to be most appropriate.

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