Stackburn, seedling blight, leaf spot of rice - *Alternaria padwickii*

*Alternaria padwickii* is an asexually reproducing fungus that infects seeds of rice. It is one of several fungi responsible for seed discoloration, seed rot and seedling blight, but has also been detected as a sheath-rotting pathogen (Naeimi et al., 2003). It occurs in southern Asia and in countries on other continents worldwide, but its presence in mainland North America is not confirmed. Transport to and transmission in new areas may be prevented by use of tested clean seed. Where the pathogen is already present, application of seed treatments should reduce disease incidence, but the fungus has an undetermined ability to survive as sclerotia in plant debris and soil.

*Alternaria padwickii* (Ganguly) M.B. Ellis 1971 (Ascomycetes, Pleosporales)

Colonies on PDA spreading, grayish, sporulating. Reverse often deep pink or purple. Conidiophores solitary, unbranched, smooth, 100-180 x 3-4 µm. Apices often swollen to 5-6 µm, minutely echinulate, bearing one monotretic conidiogenous cell. Conidia single, fusiform to obclavate, with filamentous true beak, 95-170 (including beak) x 11-20 µm. Body hyaline to straw- or golden-brown, with 3-5, commonly 4, transverse septa, often constricted at septa, smooth or minutely echinulate. Beak hyaline, 0-1 or more septate, half to more than half the length of spore body. Sclerotia spherical, black, multicellular, walls reticulate, 50-200 µm diam.

For further details, see Padwick (1950); Ellis (1971); Ellis and Holiday (1972); Jain (1975).

**Distribution:** Africa, Asia, Oceania, South America (Surinam).

**Hosts:** On coleoptiles, fruit, leaves, roots, seeds of *Oryza sativa*, *Saccharum* sp. (Poaceae).

**Notes:** Ganguly (1947) described a new species *T. padwickii* based on isolations from rice in India. It was transferred to *Alternaria* by Ellis (1971) who noted that the conidiogenous cells in *Trichoconis* differ, producing multiple colorless conidia, each on a long denticle.

In his monograph on *Alternaria*, Simmons (2007) does not consider *A. padwickii* other than to designate a lectotype. Ganguly (1947) did not identify a type specimen in his original material. Dugan and Peever (2002) examined a different IMI Herbarium specimen and a non-sporulating live strain from Texas (Kulik, 1975) and included the species, under the name *Trichoconiella padwickii* (Ganguly) Jain, in their key to *Alternaria* species on grasses. Jain (1975) created the new genus *Trichoconiella* for *T. padwickii*, disagreeing with its placement in *Alternaria* because no longitudinal or oblique septa were observed in the conidia, and the conidiophores are straight, little differentiated from the hyphae, and non-proliferating, with a single conidiogenous site, unlike those typical of *Alternaria*.

**DISTRIBUTION**

*Alternaria padwickii* has been recorded on rice worldwide, primarily in tropical regions (CMI, 1984). The scattered countries where it is recorded beyond southern Asia suggests that introduction occurred on imported seed.

Tisdale (1922) and Tullis (1936) reported on a rice-infecting and sclerotium-producing *Alternaria* in the USA as *Trichoconis caudata* (Appel & Strunk) Clem. before *A. padwickii* was named. Despite the possibility that the fungus was introduced in seed during the early development of rice cultivation in North America and the assumption that the "stackburn" pathogen described by Tisdale (1922) and Tullis (1936) was *A. padwickii* as suggested by Padwick, 1950; of Kulik (1975, 1977; Groth, 1992), this species is not generally recorded as occurring in the United States (Farr et al., 1996; CMI, 1984; USDA/SMML, 2010). Ou (1985) indicated that records of *Trichoconis caudata* on rice in the southern USA (Tullis, 1936) are misidentifications of *A. padwickii*, but this has not been confirmed.

*Alternaria padwickii* is also reported from rice in Cuba (Arnold, 1986), Panama (Ferrer et al., 1980), Surinam (CMI, 1984) and Venezuela (Rodriguez and Nass, 1990) in the Caribbean basin. In Costa Rica, it is recorded on the grass *Axonopus compressus* (CMI, 1984), which is native to the western hemisphere but widely naturalized in other tropical regions (USDA-ARS, 2010).

**RISK OF INTRODUCTION**

Because *Alternaria padwickii* is commonly seed-borne and is carried at high levels, it is likely to be introduced on imported seed unless efforts are made to exclude it. The likelihood of establishment depends on the technological level of rice cultivation in the area, and whether the costs of seed testing, seed cleaning, and/or seed treatment can be supported. The impact of the pathogen where it is not controlled can vary depending on the place of rice in the local economy - whether is a subsistence crop,
one of several food crops, or primarily a cash crop. Loss of seedling stand and vigor and quality of the harvest can be tolerated at differing levels under these different systems.

**SIMILARITIES TO OTHER SPECIES**

Other *Alternaria* species reported on rice by Agarwal et al. (1975), *A. longissima* (= *Prathoda longissima* fide Simmons (2007)) and *A. tenuis* (= *A. alternata* fide Ellis, 1971), produce much longer, narrow conidia and much smaller, beakless conidia (Ellis, 1971; Mathur and Kongsdal, 2003).

No other clearly defined species of *Alternaria* occurs on rice (Simmons, 2007). The species reported on *Sorghum vulgare*, *A. sorghicola* E.G. Simmons, produces conidia from branched conidiophores, conidia in chains that have both transverse and longitudinal septa, with or without long beaks that terminate in cells that are secondary conidiophores (Simmons, 2007).

A number of other fungi cause seed rot and seedling blight (Ou, 1985; Rush, 1992), sheath rot (Naeimi et al., 2003), and grain discoloration (Tullis, 1936; Padwick, 1950; Saini, 1985; Lee, 1992b) of rice. Among these is the brown spot pathogen, *Cochliobolus miyabeanus*, which also produces small spots on leaves. The lesions are pale brown to gray with a reddish-brown margin. The conidia are fusiform to almost cylindrical, curved, pale to golden brown, 63-153 x 14-22 µm, beakless, with up to three times as many apparent transverse septa as occur in *A padwickii* (Lee, 1992a).

**DETECTION AND INSPECTION METHODS**

Above-ground plant tissues at the seedling stage or on leaves of older plants show oval to circular spots, 3-10 mm diam. Lesions are tan, later becoming grey to white with a narrow, dark-brown border. Sclerotia appearing as small, black dots are produced in the centre of older lesions on all infected parts.

Seeds may have brown-black spots or blotches. Severe infection can result in shriveled, discoloured and brittle grain. Several other fungi can cause similar discoloration (Tullis, 1936; Saini, 1985).

**DIAGNOSTIC METHODS**

These are primarily developed for detection of seed or grain infection (see Seedborne Aspects of Disease, Seed Health Tests).

In a moist chamber, leaf spots caused by *A. padwickii* become covered within several days by a dense white cottony mycelium, distinguishing them from those of other leaf-spotting fungi (Padwick, 1950).

Sequences for the ITS regions of rDNA for six Indian isolates were recently made available for comparison in GenBank (NCBI, 2010).

**NOTES ON CROPS/OTHER PLANTS AFFECTED**

Padwick (1950) refers to *A. padwickii* causing spots on the leaves of an unspecified wild grass in rice paddy fields. In Costa Rica, it is recorded on the grass *Axonopus compressus* (CMI, 1984), which is native to the western hemisphere but widely naturalized in other tropical regions (USDA-ARS, 2010). This fungus has also been reported in India on seeds of millet (*Pennisetum typhoides*) in Uttar Pradesh (Mathur et al., 1973), on seeds of *Sorghum halepense* (Mathur and Prakash, 1972), on eucalyptus in Kerala (Thankamma and Nair, 1989), and on the invasive weed *Marsilea quadrifolia* (Sarbhoy et al., 1971). In Brazil, it is recorded on the grass *Brachiaria decumbens* (= *Urochloa decumbens*) as well as on rice (Mendes et al., 1998).

**SYMPTOMS**

This seed-borne fungus causes pre- and post-emergence seed rot (Srinivasaiah et al., 1984). After emergence, small dark brown lesions may occur on the roots, the coleoptile or the early leaves. The parts of the seedling above lesions are blighted or the whole seedling dies (Padwick, 1950; Rush, 1992). Spots on later leaves, only occasionally seen, are oval to circular, 3-10 mm diam and tan, later becoming grey to white with a narrow, dark-brown border (Padwick, 1950). Sheath rot in rice caused by *A. padwickii*, among other fungi, was reported for the first time by Naeimi et al. (2003) in northern Iran. In the "stackburn" phase of the disease, spots on glumes are pale brown to white or faintly pink or reddish-brown, usually with a darker border (Groth, 1992). Infected grain is dark colored, chalky, brittle, and/or shriveled, with reduced viability (Lee, 1992b; Groth 1992). The small black sclerotia appear in the center of lesions on all infected parts (Ou, 1985) and may be numerous in infected grains (Padwick, 1950).

**BIOLOGY AND ECOLOGY**

The recovery of *A. padwickii* conidia from the air in a rice field (Sreeramulu and Vittal, 1966) supports the suggestion of Tisdale (1922) that the fungus survives in soil or crop debris, presumably as sclerotia and mycelium that are embedded within host tissues. Wild grasses have been found to be infected (Padwick, 1950; CMI, 1984; Mendes et al., 1998) and may be another source of inoculum for the ripening rice.
Numbers of airborne conidia released were highest between 6.00 and 12.00 h and, on a seasonal basis in India, greatest conidal densities occurred at the time when ears were ripening (Sreeramulu and Vittal, 1966). Maximum growth in culture occurs at 26-28°C (Chuaiprasit, 1976), but there are no reported data on the conditions that stimulate sporulation in culture or in the field.

Rotem (1992) noted that, although microsclerotia have been reported from a few species in the genus, even those records are rare and most data on survival of Alternaria species relate to conidia and mycelium. The role of sclerotia in the epidemiology of this pathogen may be unique.

Tullis (1936) indicated that the pathogen gains entry to seeds through the glumes and attacks the kernel before the rice is mature. The fungus can be isolated from the glumes, endosperm and embryo of infected seed of susceptible varieties (Srinivasaiah et al., 1984). Seedborne incidence was favoured by high rainfall and by small fluctuations in temperature and relative humidity (Abdul-Kair et al., 1988).

This pathogen is also extensively seedborne and its transmission to seedlings has been demonstrated under laboratory test conditions (Mathur et al., 1972).

ASSOCIATIONS

Alternaria padwickii is one of a number of fungi found associated with the panicle rice mite, Steneotarsonemus spinki Smiley, which damages rice grains worldwide and has recently been rediscovered in the rice-growing states of the southern USA (Hummel et al., 2009).

Feeding by rice bugs such as Leptocorisa oratorius was considered to enhance infection of the grain by fungi including A. padwickii, in the Philippines (Lee et al., 1986). A "loose vector relationship" between the rice stink bug, Oebalus pugnax (F.) and fungi causing grain discoloration, such as A. padwickii, that is introduced during feeding (Lee et al., 1993).

MOVEMENT AND DISPERSAL

Natural dispersal: Conidia of A. padwickii are airborne and most abundant at the time of heading and grain ripening (Sreeramulu and Vittal, 1966; Groth 1992). Large numbers of conidia may become airborne during the harvest (Atluri and Murthy, 2002). Sclerotia and mycelium could be carried in plant debris (Ellis and Hollidat, 1972) with or without soil.

Vector transmission: Not reported.

Accidental introduction: Because the fungus is one of many infecting rice seed, often at high levels (Mathur et al., 1972), it is likely to have been introduced to new areas of rice cultivation in untreated imported seed. It has been detected in seed of exotic germplasm imported to India (Agarwal et al., 2006).

SEEDBORNE ASPECTS OF DISEASE

Incidence

Very high levels of seed infection (39-80%) have been recorded (Vir et al., 1971; Mathur et al., 1972; Ou, 1985). Infection rates of 28.9% were detected in a seed lots in Iran (Zad and Khosravi, 2000). Up to 25% of seeds harvested from naturally infected paddy cv. Pusa 33 exhibited the disease in Karnal, India, in 1996 (Dharam Singh et al., 2001).

Effect on Seed Quality

Infection in rice causes black discoloration beneath the husk on the tip and beyond, even up to half of the length of the paddy seed, depending on the infection intensity (Dharam Singh et al., 2001). Grain discoloration caused by A. padwickii alone or in combination with other fungi can influence market value (Saini, 1985; Lee et al., 1986). High levels of seed infection and associated seed rotting can have a serious impact on stand establishment in nursery beds and in the field (Mathur et al., 1972; Ou, 1985).

Pathogen Transmission

Transmission of A. padwickii from seed to seedlings was demonstrated under laboratory test conditions (Mathur et al., 1972). The fungus can be isolated at high levels from the glumes, endosperm and embryo of infected seed of susceptible varieties (Srinivasaiah et al., 1984). Although high mortality of seedlings occurs when infected seeds are planted (Mathur et al., 1972), it is likely that infected seeds are a source of inoculum for the planted crop.

Seed Treatment

Several seed-treatment fungicides are reported to give control of seed infection, including edifenphos, iprobenfos and benomyl (Rajan and Nair, 1979), N-ethylmercurio-4-toluenesulfonanilide (Aleshin et al., 1980) and mancozeb (Vir et al., 1971). Seed treatment with hot water or mancozeb appears to be essential to control seedborne infection in high-yielding cultivars (Rath, 1974).
**Seed Health Tests**

Blotter (Misra et al., 1994).

1. Use 9.5 cm Petri plates made of Pyrex glass or clear plastic to allow NUV light to penetrate. The plates should contain two to three layers of good-quality white or coloured blotter paper moistened with distilled water.

2. Place seeds from the working sample (with or without pre-treatment) equidistant on the Petri plates at 25 seeds/plate.

3. Incubate seeds at 22°C under a 12-h light and 12-h dark cycle with NUV light for 6-8 days.

4. Examine plates for characteristic colonies of *A. padwickii*. Express results as a percentage of the number of total seeds.

**Culture plate**

1. Pre-treat 400 seeds with 1% sodium hypochlorite for 10 min.

2. Drain off excess liquid. Place seeds (10 seeds per agar plate) on either malt extract agar or potato dextrose agar in 9.5-cm Petri dishes.

3. Incubate at 22°C for 5-8 days either under alternate cycles of NUV light and darkness or total darkness.

4. Examine plates for characteristic colonies of *A. padwickii* from the third through the eighth day of incubation. Express results as a percentage of seeds infected.

**Notes on methods**

Research on blotter and culture plate methods has been ongoing since the early 1970s (Kulik, 1975; Mathur and Neergaard, 1970; Shetty and Shetty, 1988). Mathur et al. (1972) stressed the importance of the light source in optimizing test results. Cheeran and Raj (1972) report having detected *A. padwickii* in extracted embryos of rice. Shetty and Shetty (1988) preferred the use of rice extract agar rather than PDA due to easier identification of the pathogen. Mathur and Kongsdal (2003) note that a pink-purple color often surrounds the infected seed on paper and that colonies of this species on PDA are white above and almost black below with few conidia.

**IMPACTS**

**Economic impact:** *Alternaria padwickii* is widespread in many major rice-growing regions throughout the world. Grain discoloration caused by this pathogen alone or in combination with other fungi can reduce market value (Saini, 1985; Lee et al., 1986). Leaf spots usually do not cause much damage. High levels of seed infection and associated seed rotting can have a serious impact on stand establishment in nursery beds and in the field, but the importance of the disease is "commonly underestimated" (Ou, 1985). In the USA Kulik (1977) found a low correlation between seed infection by *A. padwickii* and reduction of seed germination.

**MANAGEMENT**

**SPS measures**

The testing and certification of rice seed is necessary to prevent the importation of this seedborne pathogen into new areas or the increase of inoculum in already infested areas. The fungus has been detected in seed of exotic germplasm imported to India (Agarwal et al., 2006) as well as in inert matter contaminating local seed lots in Bangladesh (Khokon et al., 2005). In New Zealand, *A. padwickii* was detected only in seed lots of foreign origin, not in the native ones (Lau and Sheridan, 1975).

**CONTROL**

**Cultural control and sanitary measures**

Padwick (1950) recommended destruction of rice stubble and straw by burning. A row spacing trial (15, 20, and 25 cm wide) showed that seedborne infections were highest with the closest spacing. Seed infection also increased proportionally as nitrogenous fertilizer applications were increased from 0 to 200 kg/ha (Agarwal et al., 1975).

**Physical/mechanical control**

Hand cleaning of farmers seed was found effective in improving seed germination and seedling vigor in Bangladesh (Mathur et al., 2004). The hot water treatment of seed is an alternative to cleaning and use of chemicals but may reduce the level of germination. Tisdale (1922) found that, after a presoaking period
of 16 hours in tepid water, soaking seed for 15 min in water at 54°C gave the best results for germination and disinfection. In India, Suryanaryana et al. (1963) recommended use of 50°C water for 10 min, but germination was reduced to 62%. Alternative variations gave improved germination but did not completely eliminate infection.

The proper drying of the grain before storage should reduce later development of infection (Lee, 1992b).

**BIOLOGICAL CONTROL**

A formulation of rice rhizosphere-inhabiting bacteria *Pseudomonas fluorescens* in powdered talc was effective in reducing seedling infection by *A. padwickii* and *Bipolaris oryzae* when applied to seeds at the rates of 5 and 10 g per kg (Praveen Kumar et al., 2001).

**CHEMICAL CONTROL**

Several fungicide sprays gave satisfactory control of grain discoloration, including: chlorothalonil, mancozeb, carboxin, and fenapanil (Ferrer et al., 1980); polyoxin (Arunyanart et al., 1981); edifenphos, iprobenfos and benomyl (Rajan and Nair, 1979); and iprodione (Rodriguez et al., 1988).

Despite the deep-seated nature of seed infection, several seed-treatment fungicides are reported as giving control of seed infection, including edifenphos, iprobenfos, and benomyl (Rajan and Nair, 1979); N-ethylmercurio-4-toluenesulfonanilide (Aleshin et al., 1980); and mancozeb (Vir et al., 1971). Seed treatment with hot water or mancozeb appears to be essential to control seedborne infection in high-yielding rice cultivars (Rath, 1974).

Chemicals from natural sources may provide control that is less toxic to mammals and less polluting in the environment. Shanmugam (2004) tested leaf extracts of twenty plant species and found that a 10% concentration of the extract of *Prosopis juliflora* (mesquite) had an inhibitory effect equivalent to that of a fungicide on mycelial growth and spore germination of seed-borne fungi of rice, including *A. padwickii*. The essential oils from the leaves and rhizomes of *Curcuma longa* (turmeric) are also effective against the fungus, but at higher concentrations (Behura et al., 2000). Seeking a treatment available to small farmers in Cameroon, Nguefack et al. (2008) tested the essential oils of three locally grown plants applied as a slurry to rice seed. Those of *Ocimum gratissimum* (African basil) and *Thymus vulgaris* (thyme) had an effect similar to that of mancozeb in reducing transmission of fungi from seeds to seedlings. A natural chemical, 2-hydroxy-4-methoxybenzaldehyde, isolated from the Indian plant *Decalepis hamiltonii* was almost as effective as thiram as a treatment against *A. padwickii* and other seed-borne fungi (Devihalli Chikkaiah et al., 2009).

**HOST RESISTANCE**

Although Rath (1974) reported no resistance to *A. padwickii* in high-yielding rice cultivars, some breeding lines were found resistant in nursery tests in Java, Indonesia (Soepriaman et al., 1976).

**GAPS IN KNOWLEDGE/RESEARCH NEEDS**

Survival of the fungus in soil and plant debris under various conditions of cultivation (flooding, fallow, crop rotation, eradication of alternative wild hosts) should be investigated.

The placement and relationships of this species within the genus *Alternaria* and/or another genus should be clarified by morphological and molecular methods. With the identity established, the host range, particularly among wild grasses and other weeds of rice fields, should be determined. The role of any alternative hosts as sources of late-season inoculum may then be apparent.

The distribution of this species in regions of rice cultivation, particularly whether it occurs within the USA, should be clarified.

**References**