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Host, mating type and fertility of *Magnaporthe grisea* in Santiago Island, Cape Verde archipelago

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Summary. A severe leaf spot and blast disease caused by the fungus *Magnaporthe grisea* was detected on *Panicum maximum* in Santiago Island, Cape Verde. In a limited survey to determine the hosts of this fungus throughout the sub-humid and humid areas of this island, isolates of *M. grisea* were obtained from *Brachiaria lata* ssp. *caboverdeana*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Digitaria ciliaris*, *Digitaria nuda*, *Eleusine indica* ssp. *indica*, *Rottboellia cochinchinensis*, *Setaria verticillata* and *Stenotaphrum secundatum*. The mating types (MATs) of 27 Capeverdean isolates were investigated by crossing them with four standard hermaphroditic testers. The MATs of 13 isolates (48%) were determined, of which six were MAT1-1 and seven MAT1-2. Mature ascospores were produced by three MAT1-2 isolates from *E. indica* ssp. *indica*. Attempts to mate hermaphrodite MAT1-2 Capeverdean isolates with the MAT1-1 Capeverdean isolates were unsuccessful.

Key words: *Magnaporthe grisea,* Cape Verde, host range, *Brachiaria lata* ssp. *caboverdeana*, mating type.

Introduction

In September 2000, a severe leaf-spot and blast disease was observed on *Panicum maximum* Jacq. in grassland and forest areas of São Jorge dos Órgãos and Longueira in the Santa Cruz district of Santiago, the most important island of the Cape Verde archipelago. The conidial stage of *Magnaporthe grisea* (Herbert) Barr, *Pyricularia grisea* Sacc., was consistently found when infected leaves were placed on moist filter paper in Petri dishes. As *M. grisea* is one of the most important pathogens of gramineous plants in tropical and subtropical regions, a survey of limited scale was undertaken to determine the hosts of this fungus in the Pico da Antónia and Malagueta massifs and the Assomada plateau (altitude 400–600 m).

Cape Verde is an archipelago lying 600 km west of Dakar, Senegal, the westernmost point of continental Africa. It is part of the Macaronesian region (East Atlantic Islands), which also includes the Azores, Canaries, Madeira and the Selvagens (Beyhl *et al*., 1995). The climate is tropical with two seasons: a cool dry season from December to June, and a warm season from July to November. Rainfall is low and unreliable, most of it occurring in August and September. The climate of the particular area studied is sub-humid to humid, with an annual precipitation in São Jorge dos Órgãos of about 400 mm and temperatures ranging from 15 to 23°C in January and February and from 22 to 28°C in October (Silva, 1996). Relative humidity is fairly high throughout the year. From 1 July to

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24 September 2000, precipitation was 263 mm in 38 rainy days (INIDA, unpublished data).

The ability of *M. grisea* isolates to produce perithecia has been investigated and extensive mating experiments have shown two mating types, MAT1-1 and MAT1-2, in *M. grisea* populations on various hosts. Fertility in field isolates of this fungus ranged from total sterility, through female sterility, to full fertility (Notteghem and Silué, 1992). Silué and Notteghem (1990) and Hayashi *et al.* (1997) reported that co-inoculation of rice with compatible isolates under controlled conditions of humidity and temperature resulted in the production of perithecia on dead tissue kept in a humid chamber and on dead tissue of standing plants in a moist chamber.

The objectives of the present study were to identify the hosts of *M. grisea* in Santiago Island, to determine the mating type of each isolate obtained when mated with two MAT1-1 and two MAT1-2 standard testers, and to assess isolate fertility in crosses with standard testers and in inter-crosses between Capeverdean isolates of opposite mating types.

Materials and methods

Hosts and symptomatology

During the survey gramineous plants showing leaf spot and blast symptoms were photographed *in situ* and the symptoms recorded.

Sample collection, isolation and culture

Symptomatic plants were collected from grassland and forest areas of the Santa Cruz district and from the Serra da Malagueta, along the road between Assomada and Tarrafal. Diseased leaves were also collected from *Stenotaphrum secundatum* in turf grass plots located in the Santa Cruz district, and in Tarrafal at sea level (Fig. 1). The plant material were allowed to air dry for three to five days before being stored in paper bags. When the material was brought to the laboratory, selected infected samples were placed on moist filter paper in Petri dishes at 25°C to induce sporulation. Conidia were transferred with a glass micro-roll pin to a water-agar medium and monoconidial isolates were picked up and grown separately as pure culture. Long-term storage was on sterile filter paper disks at -20°C (Notteghem and Silué, 1992).

Determination of mating type and ascospore germination

The mating types of the *M. grisea* isolates were determined by pairing each isolate three times with four standard hermaphroditic testers kindly supplied by J.L. Notteghem. The testers of mating type MAT1-1, KA 3 and KA 7 (OG 2 and IN 1) isolated from finger millet [*E. coracana* (L.) Gaertn.], and those of mating type MAT1-2, KA 9 (OG 5) and GUY 11, from finger millet and rice respectively, produced numerous perithecia when paired with isolates of the opposite mating type (Notteghem and Silué, 1992; Lima, 1998).

For mating-type evaluation, crosses were made by pairing agar blocks cut from the margin of growing colonies of two standard isolates of each mating type with two agar blocks cut from the margins of growing colonies of each isolate to be tested, on rice-flour agar plates (rice flour, 14 g; yeast extract, 2.5 g; dextrose, 5 g; agar, 15 g; water, 1 litre). The agar plates were sealed, incubated at 28ºC for three days, and then placed under continuous fluorescent lighting at 20–22°C. After four weeks the junctions between mated individuals were examined under a stereomicroscope for perithecial formation.

To measure fertility in the Capeverdean *M. grisea* isolates, mature perithecia from the margin on the growth side of each isolate were removed from the agar surface, transferred to 4% water agar medium and crushed. After an hour, lysis of the asci walls allowed the ascospores to disperse. Individual ascospores were placed on potato dextrose agar and observed for germination.

Assessing fertility in crosses between Capeverdean *M. grisea* isolates of opposite mating types

Female fertile Capeverdean isolates were paired with all the opposite mating type Capeverdean isolates by pairing agar blocks cut from the margins of growing colonies on rice flour agar plates. Each cross was repeated three times.

Results

Hosts and symptomatology

Symptomatic samples of some plant species failed to produce *M. grisea* conidia. Nonetheless, a total of 27 monoconidial isolates of *M. grisea* were obtained from samples, with distinct leaf spot and

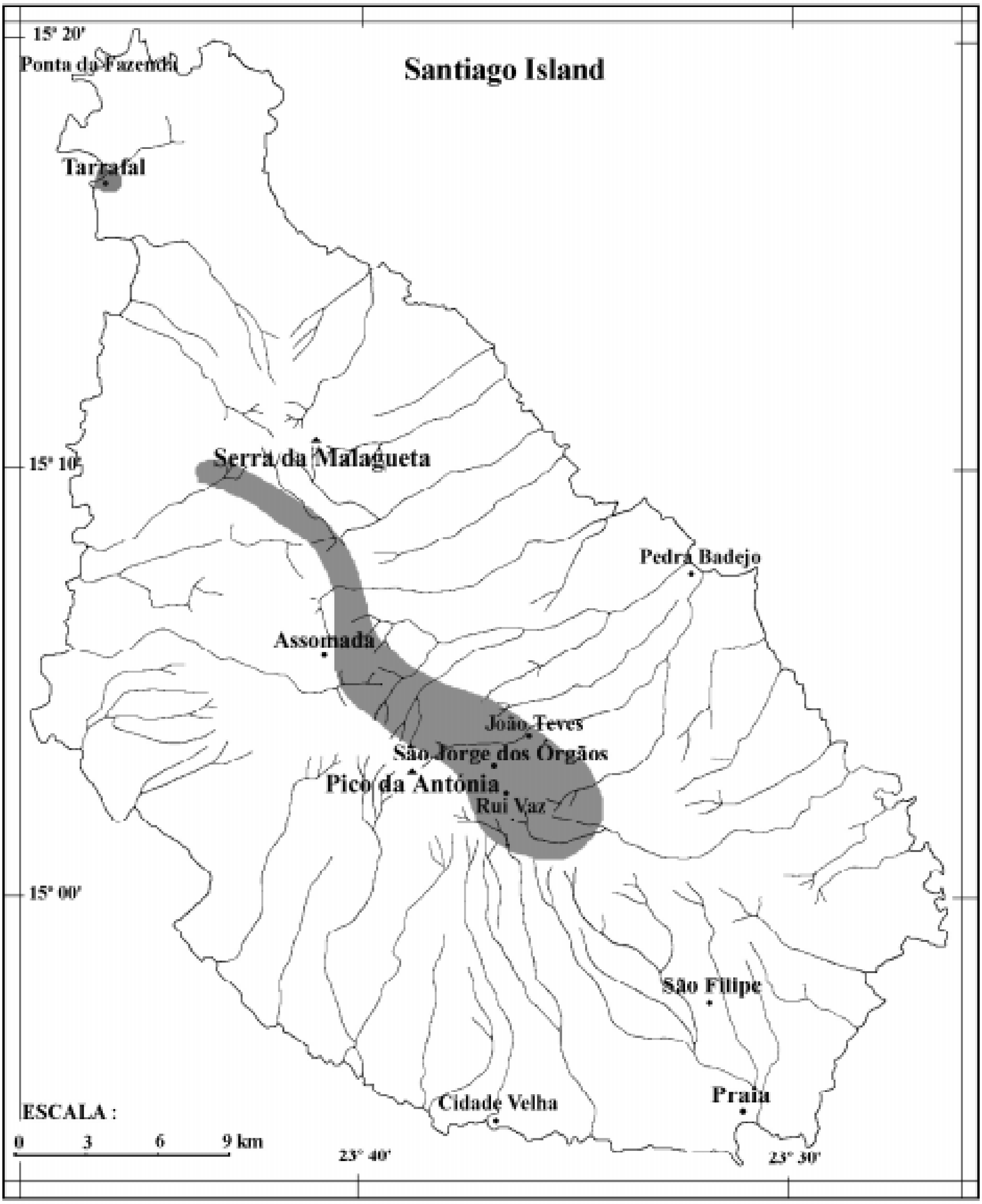

Fig. 1. Location of sites sampled for *Magnaporthe grisea* hosts in Santiago Island, Cape Verde.

blast symptoms, of *Brachiaria lata* (Schumach.) C.E. Hubb. ssp. *caboverdeana* Conert & Ch. Köhler, *Cynodon dactylon* (L.) Pers., *Dactyloctenium aegyptium* (L.) Willd., *Digitaria ciliaris* (Retz.) Koeler, *Digitaria nuda* Schumach, *Eleusine indica* (L.) Gaertn. ssp. *indica*, *Panicum maximum*, *Rottboellia cochinchinensis* (Lour.) Clayton, *Setaria verticillata* (L.) P. Beauv. and *Stenotaphrum secundatum* (Walter) Kuntze (Table 1).

Symptoms observed on different hosts differed greatly. On *P. maximum* and *S. verticillata* they consisted of numerous (several hundred) small brown spots and necrotic lesions. On *P. maximum* the lesions were round, oval to spindle-shaped, with whitish to brown centres and dark-brown to reddish-purple margins. Fully developed lesions measured 2–6 mm in length and 1–2 mm in breadth. Lesions frequently coalesced to form extended necrotic areas, which caused partial or complete blighting of the leaf blade. On *S. verticillata* the

Table 1. Host, mating type and fertility for *Magnaporthe grisea* isolates from Santiago Island, Cape Verde.

Isolate No.	Site	Host	Mating type	Fertility
CV 15	Santa Cruz - Chão de Vaca	*Brachiaria lata* subsp. *caboverdeana*	unknown	
CV 11	Santa Cruz - Ribeirão Galinha	*Cynodon dactylon*	unknown	
CV 5	Santa Cruz - Chão de Vaca	*Dactyloctenium aegyptium*	unknown	
CV 21	Santa Cruz - Ribeirão Galinha	*Digitaria ciliaris*	MAT1-2	Female sterile
CV 14	Santa Catarina - Serra da Malagueta	*Digitaria nuda*	MAT1-1	Female sterile
CV 20	Santa Cruz - Ribeirão Galinha	*Digitaria nuda*	unknown	
CV 2	Santa Cruz - Longueira	*Eleusine indica* ssp. *indica*	MAT1-2	Hermaphrodite
CV 6	Santa Cruz - Chão de Vaca	*Eleusine indica* ssp. *indica*	MAT1-2	Hermaphrodite
CV 7	Santa Catarina - Assomada	*Eleusine indica* ssp. *indica*	unknown	
CV 8	Santa Cruz -São Jorge dos Órgãos	*Eleusine indica* ssp. *indica*	MAT1-2	Female sterile
CV 16	Santa Cruz - Longueira	*Eleusine indica* ssp. *indica*	MAT1-2	Hermaphrodite
CV 17	Santa Cruz -São Jorge dos Órgãos	*Eleusine indica* ssp. *indica*	MAT1-2	Female sterile
CV 18	Santa Cruz -São Jorge dos Órgãos	*Eleusine indica* ssp. *indica*	unknown	
CV 22	Santa Cruz - João Teves	*Eleusine indica* ssp. *indica*	unknown	
CV 23	Santa Cruz - Ribeirão Galinha	*Eleusine indica* ssp. *indica*	unknown	
CV 1	Santa Catarina - Serra da Malagueta	*Panicum maximum*	MAT1-1	Female sterile
CV 10	Santa Cruz - Chão de Vaca	*Panicum maximum*	unknown	
CV 19	Santa Cruz - João Teves	*Panicum maximum*	unknown	
CV 25	Santa Cruz - Chão de Vaca	*Panicum maximum*	unknown	
CV 3	Santa Cruz -São Jorge dos Órgãos	*Rottboellia cochinchinensis*	MAT1-1	Female sterile
CV 9	Santa Cruz - Serra da Malagueta	*Setaria verticillata*	unknown	
CV 13	Santa Cruz - João Guerra	*Setaria verticillata*	MAT1-1	Female sterile
CV 26	Santa Catarina - Serra da Malagueta	*Setaria verticillata*	MAT1-1	Female sterile
CV 27	Santa Catarina - Assomada	*Setaria verticillata*	unknown	
CV 4	Tarrafal - Tarrafal	*Stenotaphrum secundatum*	MAT 1-1	Female sterile
CV 12	Santa Cruz - São Jorge dos Órgãos	*Stenotaphrum secundatum*	unknown	
CV 24	Santa Cruz -São Jorge dos Órgãos	*Stenotaphrum secundatum*	MAT1-2	Female sterile

spots were round, 0.5–1 mm in diameter, with reddish-purple or brown centres, and dark-brown to reddish-purple margins. On the other plants, *B. lata* ssp. *caboverdeana*, *C. dactylon*, *D. aegyptium*, *D. ciliaris*, *D. nuda*, *E. indica* ssp. *indica*, *R. cochinchinensis* and *S. secundatum*, there were fewer lesions. In spite of differences in the size, lesions on all these plants were always elliptical with more or less pointed ends. The centre of the spots was whitish to grey and the margins were brown, dark-brown or reddish-purple, sometimes with a chlorotic border. On *R. cochinchinensis* spots were 1–3 cm long and up to about 0.6 cm wide.

Determination of mating type

Mature perithecia were observed on the four standard hermaphroditic *M. grisea* testers about a month after they were inter-crossed. Among the 27 Capeverdean isolates, the mating type of 13 (48%) was determined: six (22%) were MAT1-1 and seven (26%) MAT1-2 (Table 1). MAT1-1 isolates occurred on a greater number of hosts and in a greater number of locations. They were from the grassland and forest areas of Pico da Antónia (São Jorge dos Órgãos and João Guerra), from the Malagueta massifs and from Tarrafal. All MAT1-2 isolates were from the grassland and forest areas of Pico da Antónia. None of the six Capeverdean MAT1-1 isolates reacted as female fertile. Perithecial formation with viable ascospores was observed in three of the seven MAT1-2 isolates, and these hermaphrodite isolates were all from *E. indica* ssp. *indica*.

Fertility in crosses between Capeverdean *M. grisea* isolates of opposite mating types

Attempts to mate Capeverdean hermaphrodite MAT1-2 isolates with Capeverdean MAT1-1 isolates were unsuccessful, indicating that they were incompatible.

Discussion

On Santiago Island *M. grisea* is apparently pandemic throughout the sub-humid and humid areas of Pico da Antónia, the Malagueta massifs and the Assomada plateau. Despite the failure to isolate it from some symptomatic grasses reported to be their hosts in other parts of the world, we observed severe attacks of this fungus on *P. maximum*, and isolates were obtained from a further nine grasses. Some of these, *D. aegyptium*, *D. ciliaris*, *E. indica* ssp. *indica*, *P. maximum*, *R. cochinchinensis* and *S. verticillata*, appeared with high frequency in the vegetational communities described by Gomes (1994) and Duarte (1998) for the sub-humid and humid areas of Santiago Island. Owing to the severe water shortage in Cape Verde, many farmers depend on natural grasses to feed their livestock. Because some of these grasses represent the most important forage in the climatic areas in question (Marcarian *et al*., 1990), the occurrence of *M. grisea* has important economic consequences.

M. grisea is reported to cause infection in many monocotyledons (Asuyama, 1965; Ou, 1985; Mackill and Bonman, 1986; Urashima *et al*., 1993). However, for some of the hosts identified in this survey it is difficult to decide whether they are new reports owing to the lack of information about them. Although Cape Verde is part of the Macaronesian region, which also includes the Azores, Canaries, Madeira and the Selvagens, the grasses found there bear a closer resemblance to those in Africa than to those on the other Macaronesian Islands (Beyhl *et al*., 1995). The level of endemism in the gramineae in Cape Verde is very low and among the hosts of *M. grisea* on Santiago Island only *B. lata* ssp. *caboverdeana* is endemic (Duarte, 1998). So, of the 10 gramineae identified in the survey, only one is probably a newly reported host of *M. grisea*.

Our results showed that both mating types occur in the *M. grisea* populations of Santiago Island. Crosses with the four standard testers revealed that 22% of the isolates were MAT1-1, 26% MAT1-2 and 52% failed to mate with any of the testers. These ratios are similar to those reported in other studies (Notteghem and Silué, 1992; Viji *et al*., 1998; Mekwatanakarn *et al*., 1999).

Variation in fertility was observed in isolates from different hosts. Female sterility dominated among fertile isolates and occurred in isolates from *D. ciliaris*, *D. nuda*, *E. indica* ssp. *indica*, *R. cochinchinensis, S. secundatum* and *S. verticillata*. All the hermaphrodite isolates came from *E. indica* ssp. *indica* and both mating types were found in the *Eleusine* isolates. Attempts to mate hermaphrodite isolates from *E. indica* ssp. *indica* with Capeverdean isolates of opposite mating types were unsuccessful, suggesting that they were inter-sterile. The perfect state of *M. grisea* has never been observed in nature. However, the occurrence of hermaphroditic isolates suggests that the cross is possible if conditions are similar to those for perithecia production *in vitro* (Silué and Notteghem, 1990; Hayashi *et al.*, 1997). Despite a fairly high relative humidity at medium altitude on Santiago Island, temperatures in January and February range from 15 to 23ºC. The fact that both mating types occur on Santiago Island and that worldwide many *Eleusine* isolates are hermaphrodite and highly fertile (Kato, 1977; Notteghem and Silué, 1992) makes the fertility of the *M. grisea* population of Santiago Island an interesting subject for further study.

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