Two New *Fusarium* Species Infecting Rice in Southern Spain

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ABSTRACT

Marín-Sánchez, J. P., and Jiménez-Díaz, R. M. 1982. Two new *Fusarium* species infecting rice in southern Spain. Plant Disease 66:332-334.

Fusarium culmorum, F. moniliforme, F. equiseti, and *F. semitectum* were found infecting rice (*Oryza sativa*) in Las Marismas (Sevilla) in 1977 and 1978. These species differed in distribution, incidence of infections, and symptomatology induced in affected rice plants, with *F. moniliforme* being the most important. As far as we know, this is the first report of *F. equiseti* and *F. semitectum* as pathogens of rice. *F. semitectum* was isolated from necrotic lesions in evolving sheaths of panicles and glumes and from vascular tissues of culms showing brown discoloration. *F. equiseti* was isolated from adult plants showing a discoloration in vascular tissues of the culm.

In southern Spain, rice (*Oryza sativa* L.) is grown over about 25,000 ha in Las Marismas del Guadalquivir, an area quite uniform in climatic and edaphic conditions. Little attention has been paid to the diseases affecting the crop in that region, although farmers claim that yield losses due to diseases are severe. Consequently, we carried out investigations on etiology, incidence, and severity of fungal diseases affecting rice in Las Marismas (6). Here we report the part of our results concerning *Fusarium* spp.

A variety of symptoms in rice have been reported in association with *Fusarium* spp. infections. *F. moniliforme* Sheldon induces blight or stem elongation of seedlings and induces necrosis of stems, leaves, or kernels or stem elongation in adult plants (8,12). *F. nivale* (Fr.) Ces. causes necrosis of leaves (7) or panicles (11). Both *F. semitectum* Berk. & Rav. and *F. moniliforme* have been reported in chalky dry-rot kernels (10). However, infections by these species do not seem to be associated with severe losses, except perhaps by *F. moniliforme,* which has been reported to cause up to 20% reduction in yield (9). Among the other species, *F. culmorum* (W. G. Smith) Sacc. has been recovered from rice straw and has been considered as a saprophyte or weak parasite (8); *F. semitectum* and *F. moniliforme* have been reported infecting kernels, but nothing has been given on their pathogenicity (10).

MATERIALS AND METHODS

Disease surveys were carried out in the rice-growing area of Las Marismas throughout the crop season in 1977 and 1978. For sampling purposes, eight zones uniformly distributed in the area were defined and labeled I through VIII. In each zone, all fields were samples within an average area per field of 10 ha. The samples included 16–17% of the total rice acreage in Las Marismas. Disease incidence was assessed in a 1-m^2 frame thrown at random at least 20 times in each field sampled. Overall losses of seed yield for each cultivar were estimated by comparing the average yield in unaffected fields with the average yield in fields having a given incidence of the disease. Those losses should be considered only as an indication of the effect the diseases may have had on yield, because other factors (ie, grower practices, soil types) might also be involved.

Isolations from plants were attempted from every type of symptom found. Small pieces of the affected tissues were surface disinfested in 0.5% free-chlorine solution (10% Neoclor) for 1–2 min and plated onto potato-dextrose agar. All cultures were incubated at 25 ± 1 C and alternating 12-hr periods of fluorescent light (about 2,500 lux) and darkness. Identification of *Fusarium* spp. was based on Booth (1,2) and at least five monoconidial cultures. Color and growth rate of colonies were determined from cultures grown on potato-sucrose agar at pH 6.67 in petri plates. Perfect states were obtained following the method described by Hsieh et al (4).

Pathogenicity of isolates was tested on seedlings at the four-leaf stage of cultivars Bahia, Girona, Ribello, and Sequial. Seedlings were grown in a greenhouse in 12-cm-diameter plastic pots containing a sterile soil mixture or in a growth chamber in similar pots with perlite watered with a half-strength nutritive solution (3), and supplied with a 14 hr/day photoperiod of fluorescent light of about 15,000 lux. Temperature and relative humidity ranged from 20 to 28 C and 70 to 90% in the greenhouse and from 20 to 22 C and 70 to 90% in the growth chamber. Inoculum was obtained by washing conidia from 7- to 14-day-old cultures grown on potato-dextrose or potato-sucrose agar in petri plates as described. The suspension of conidia (10^5 macroconidia per milliliter) or conidia and mycelium in sterile distilled water that was obtained (13) was sprayed onto the foliage of rice seedlings with an atomizer. In some experiments, 0.5% Universal peptone M66 (E. Merck Darmstadt, Western Germany) was added to the conidial suspension just before inoculation (13). Inoculation was also performed by dipping rice seedlings with wounded roots in an inoculum suspension or by adding the inoculum to the soil and then wounding the root system (5). After inoculation, the seedlings were covered with plastic bags for variable periods of time and placed in the greenhouse or in the growth chamber. Seedlings used as controls were treated similarly except without inoculum. At least three replicates of 10 potted seedlings were used for each isolate, and the experiments were repeated several times with similar results.

RESULTS

Four species of *Fusarium*—*F. culmorum, F. equiseti* (Corda) Sacc., *F. moniliforme,* and *F. semitectum*—were isolated from affected plants in zones I–IV (Table 1). No affected plants were found in zones V–VIII. The *Fusarium* spp. differed in distribution in the zones samples, and severity, type, and location of symptoms varied among affected plants. Representative cultures of our isolates have been deposited in the Commonwealth Mycological Institute, Kew, Surrey, England, under IMI 236866 and 236867 (*F. culmorum*); IMI 236873 (*F. equiseti*); IMI 236869 (*F. moniliforme*); and IMI 236868, 236870, 236872, 236874, and 236875 (*F. semitectum*).

F. culmorum was isolated from brown necrotic spots and necrotic tips in leaf blades of seedlings. These ranged from minute and irregular in shape to rectangular spots, measuring 1–2 × 4–6 mm and covering 3–5% of the leaf blade surface. This species did not infect adult plants and caused only negligible yield losses, if any. It was found only in zone I (Fig. 1, Table 1), where it affected all fields of cultivars Girona and Sequial with incidences ranging from 15 to 25% and 10 to 15%, respectively. Fields of

Accepted for publication 8 June 1981.

0191-2917/82/04033203/$03.00/0

Bahia and Ribello were not affected. Affected fields included 44.0% of the acreage of zone I and 5.5% of the acreage in the entire area (Table 1).

F. moniliforme was isolated from plants killed at the boot stage. Dead plants had dark brown to black roots and bore erect panicles with no kernels. The culms showed a black discoloration from the lowest internode up to the fourth one or at about three-fourths of the total height of the culm. Adventitious roots that developed in the nodes of the discolored portion of the culms were covered with abundant white mycelium formed inside as well as outside of the nodes by *F. moniliforme.* In some plants, the crown tissues were so rotted that the root system could be separated easily from the culm. Infections of plants occurred in zones I–III (Fig. 1, Table 1), where only the cultivar Ribello was affected. Infections were found in 90% of the fields of Ribello. They included 6.0% of the acreage in three of the zones sampled and 2.3% of the total acreage sampled. Incidence of attacks varied among sampled zones from 5–18% to 50–70%, as did estimated yield losses, which amounted to 18–23% in zone I, the most severely affected zone (Table 1).

F. equiseti was isolated from adult plants showing a discoloration in vascular tissues of the culm. The discoloration developed acropetally from the culm base and reached the third node in some cases. Infected culms were reddish at the beginning, later becoming brown to black in the most severe infections. Affected plants occurred only in zone I and in the cultivars Ribello and Sequial but not in Bahia and Girona (Fig. 1, Table 1). Average incidence was 5% in Sequial and 35% in Ribello. Yield losses were negligible in the former cultivar but averaged 10% in the latter (Table 1).

F. semitectum was isolated from adult plants in zones I, II, and IV (Fig. 1, Table 1). The number of affected fields varied among zones and cultivars (Table 1). Although all cultivars grown were affected in zone I, only Ribello but not the others (Bahia, Ital-Patna, Sequial) were affected in zone III, and Frances but not Bahia was affected in zone IV. Affected fields included 13.3% of the acreage in the three zones and 5.2% of the acreage sampled in the entire area. *F. semitectum* was isolated from affected panicles and culms. In the panicles, irregular lesions—at first yellowish and later dark brown—were observed in the evolving sheath at the boot stage. By that time, necrosis had developed that totally or partially covered the surface of the kernels. Similar necrosis continued to develop from flowering to ripening. Infected culms showed a reddish to brown vascular discoloration that developed from the culm base and in some plants reached the fourth internode. The discoloration occasionally extended from the vascular tissues to adjacent ones, resulting in a sectorial pattern. Average incidences of infections were 7.5, 25, or 40% with culms and panicles and 10% with panicles only (Table 1). Yield losses were estimated in fields having infected culms. Losses averaged 10% in fields with 25% incidence and 15% in fields with 40% incidence.

In artificial inoculations of seedlings with wounded roots, *F. culmorum, F. moniliforme,* and *F. semitectum* caused generalized necrosis of roots, culms, and sheaths of the lower leaves in all cultivars tested. Symptoms developed by 20 days after inoculation, and all inoculated seedlings died 5 days later. Inoculations with *F. equiseti* caused symptoms similar to those induced by the other *Fusarium* spp., or localized necrosis in culms and sheaths that resulted in 40–75% of the seedlings killed. Neither *F. equiseti* nor *F. semitectum* induced vascular discoloration, probably because the seedlings were so severely affected soon after inoculation. Foliar inoculations of seedlings with macroconidia of *F. culmorum* yielded subrectangular, necrotic lesions or necrotic tips but only when peptone was added to the inoculum suspension. However, these symptoms resembled those observed in the field attributed to *F. culmorum.*

DISCUSSION

Our results showed that *F. moniliforme* is the most important *Fusarium* sp. infecting rice in southern Spain, with the estimated yield losses caused by the pathogen similar to those reported in other parts of the world (8,9). *F. semitectum, F. equiseti,* and particularly *F. culmorum* seem to cause yield losses to a lesser extent, although the first two species may reduce the yield substantially in some fields. Symptoms associated with *F. moniliforme* were in general similar to those described by Ou (8). We did not, however, observe elongation of the culms in affected seedlings or adult plants. Infections by *F. moniliforme* occurred only in late plantings of the short-cycle cultivar Ribello in zones I–III. With the practice of late planting (approximately 30 days later than normal), the plants experienced higher temperatures during early growth. Our observations are thus consistent with Ou's suggestion (8) that infections by *F. moniliforme* are favored by high temperatures at early stages of plant development.

Although *F. culmorum* infected the culm of seedlings with wounded roots, in nature the pathogen was only recovered from necrotic leaf tissue of seedlings. Leaf blade infections might occur in the field

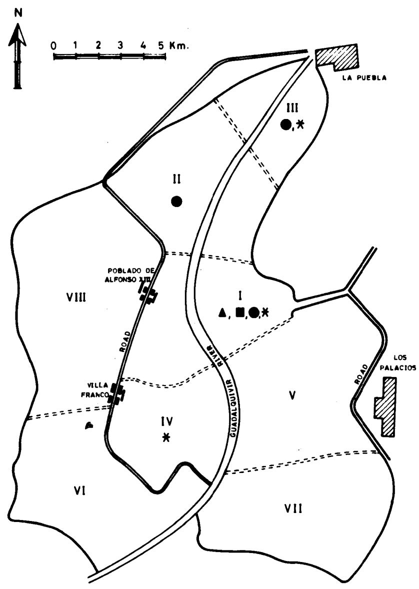

Fig. 1. Distribution of *Fusarium culmorum* (▲), *F. equiseti* (■), *F. moniliforme* (●), and *F. semitectum* (✱) infecting rice in the rice-growing area surveyed in Las Marismas, Sevilla, in southern Spain. The surveyed area was divided for sampling purposes into eight zones, I–VIII.

Table 1. Importance and distribution of *Fusarium* spp. infecting rice in southern Spain[a]

Fusarium spp.	Zone with affected fields[b]	Cultivar[c]	Fields affected/ sampled (no.)	Acreage of affected fields in zone (%)	Acreage of affected fields in entire area (%)	Incidence of infection (%)	Estimated yield loss (%)
F. culmorum	I	Girona	15/15	30.0	3.7	15–25	...
		Sequial	7/7	14.0	1.8	10–15	...
F. moniliforme	I	Ribello	2/3	4.0	0.5	50–70	18–23
	II	Ribello	5/5	10.0	1.3	5–18	5
	III	Ribello	2/2	4.0	0.5	15–25	5–8
F. equiseti	I	Ribello	1/3	2.0	0.2	35	10
		Sequial	3/7	6.0	0.8	3–7	...
F. semitectum	I	Bahia	5/25	10.0	1.3	5–10	...
		Girona	7/15	14.0	1.8	10–14	...
		Ribello	1/3	2.0	0.3	40	15
		Sequial	4/7	8.0	1.0	6–10	...
	III	Ribello	1/2	2.0	0.3	25	10
	IV	Frances	2/10	4.0	0.5	10	...

[a] Disease surveys were carried out in Las Marismas (Sevilla) in 1977 and 1978.
[b] Eight zones (I–VIII) were defined in the rice-growing area. They included 16–17% of the total rice acreage in the area. No affected plants were found in zones V–VIII.
[c] All cultivars and fields were sampled in each zone.

because the leaves are in continuous contact with floodwater. This possibility is suggested by the development of necrosis of leaves in artificial inoculations only following a prolonged period of coverage with plastic bags.

To the best of our knowledge, the symptoms we have observed associated with infections of rice by *F. equiseti* and *F. semitectum* have not been previously described. These infections may be unique; we did not observe symptoms of chalky dry-rot of kernels that have been associated with infections by *F. semitectum* and *F. moniliforme* (10).

ACKNOWLEDGMENTS

We thank C. Booth and C. Butterfiel, Commonwealth Mycological Institute, Kew, Surrey, England, who confirmed or determined the identity of our *Fusarium* isolates.

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