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Reprinted from "Phytopathologia Mediterranea„
Vol. XX, 1981 - pag. 89-95

UNIONE FITOPATOLOGICA MEDITERRANEA - ITALIA

Phytopath. medit. 1981, *20*, 89-95

Pyricularia oryzae Cav. and *Nakataea sigmoidea* Hara, pathogens of Rice in southern Spain.

JUAN P. MARIN-SÁNCHEZ and RAFAEL M. JIMÉNEZ-DIAZ

Departamento de Patologia Vegetal, Escuela Tecnica Superior de Ingenieros Agronomos, Universidad de Cordoba, Spain.

Summary - Infection of Rice by *Pyricularia oryzae* and *Sclerotium oryzae* were found to be widespread during disease surveys carried out in Las Marismas (Sevilla, southern Spain) in 1977 and 1978. All cultivars used in the area are susceptible to both pathogens.

P. oryzae most frequently caused necrosis in the uppermost culm nodes and rachis, originating severe yield losses. Infections by *P. oryzae* occurred in all fields sampled and conidia were captured from the air through the entire crop season. Conidia from sporulating lesions were divided into four types, according to size and morphology, and differences in virulence were found among them in artificial inoculations.

Prevalence and incidence of infections by *S. oryzae* varied among the eight sampled zones in the area. *Nakataea sigmoidea*, the conidial stage of the pathogen, was isolated from necrotic spots in leaf blades and proved to be pathogenic in artificial inoculations. Leaf spots were only found in one of the sampled zones, with low incidence and severity values. Conidia of the pathogen were trapped from the air in low number at the end of the crop season, in the first weeks of September.

Resumen - *Pyricularia oryzae* CAV. Y *Nakataea sigmoidea* HARA, PATÓGENOS DEL ARROZ EN EL SUR DE ESPAÑA.

Durante prospecciones de enfermedades de Arroz llevadas a cabo en Las Marismas (Sevilla, Sur de España) en 1977 y 1978, se encontraron infecciones por *Pyricularia oryzae* y *Nakataea sigmoidea* ampliamente distribuídas en el área. Todos los cultivares utilizados en el área son susceptibles a ambos patógenos.

P. oryzae causó más frecuentemente necrosis en los nudos superiores del tallo y en el raquis, originando pérdidas de rendimiento severas. Las infecciones por *P. oryzae* se detectaron en todos los campos muestreados y se capturaron conidias en el aire durante toda la estación de cultivo. Las conidias obtenidas de lesiones esporulantes se clasificaron en cuatro tipos según su tamaño y morfología, que mostraron diferencias en virulencia en inoculaciones artificiales.

La prevalencia e incidencia de las infecciones por *Sclerotium oryzae* variaron entre las ocho zonas muestreadas en el área. *Nakataea sigmoidea*, el estado conidial del patógeno, se aisló de manchas necróticas en láminas foliares y fué patógeno en inoculaciones artificiales. Las manchas foliares se encontraron sólo en una de las zonas muestreadas, y con escasa incidencia y severidad. En trampas de adherencia se capturaron del aire conidias del patógeno en número reducido al final de la estación de cultivo, en las primeras semanas de Septiembre.

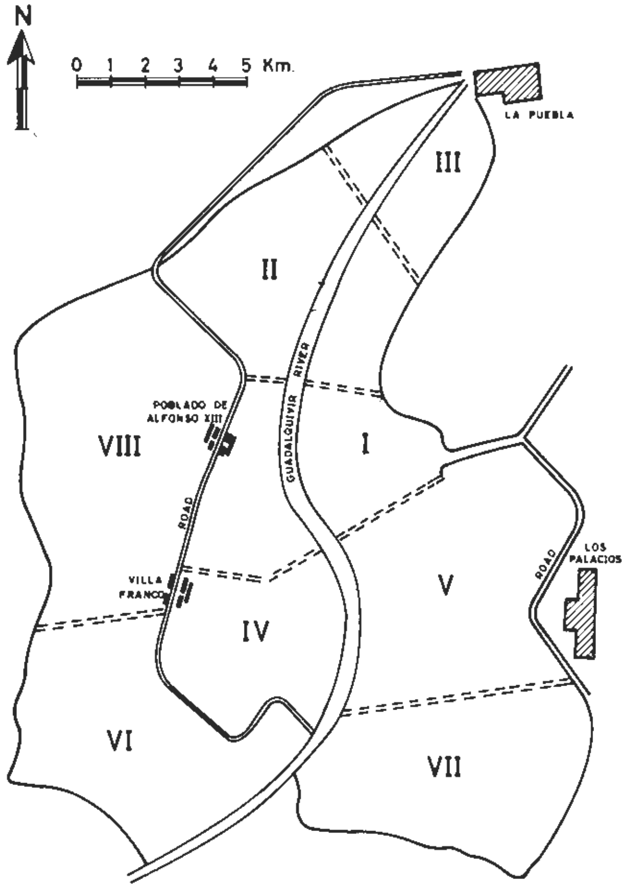
Riassunto - *Pyricularia oryzae* CAV. E *Nakataea sigmoidea* HARA, PATOGENI DEL RISO NELLA SPAGNA MERIDIONALE. Diffuse infezioni di *Pyricularia oryzae* e di *Sclerotium oryzae* sono state riscontrate nel corso di un'indagine sulle malattie del Riso condotta a Las Marismas (Siviglia, Spagna meridionale) nel 1977 e nel 1978. Tutte le varietà coltivate nella zona hanno mostrato suscettibilità ad entrambi i patogeni.

P. oryzae che frequentissimamente induceva necrosi dei nodi più alti dei culmi e dei rachidi, causava gravi perdite di produzione. Tale patogeno era presente in tutti i campi oggetto dell'indagine e i suoi conidi sono stati catturati durante tutto l'arco di coltivazione del Riso. I conidi ottenuti dalle lesioni sporificate hanno potuto essere ripartiti in 4 gruppi sulla base della forma e delle dimensioni. Infezioni artificiali hanno messo in risalto che fra essi gruppi di conidi esisteva una differente virulenza nei confronti dell'ospite.

La prevalenza e l'incidenza delle infezioni di *S. oryzae* hanno invece variato entro le 8 zone in cui l'area oggetto dell'indagine era stata ripartita. *N. sigmoidea*, la forma conidica del fungo, isolata da macchie necrotiche presenti sulla lamina fogliare, ha mostrato di essere patogena a seguito di infezioni artificiali. Le macchie fogliari riscontrate in una soltanto delle 8 zone visitate, hanno avuto bassa incidenza e modesta severità. I conidi di *N. sigmoidea* sono stati catturati in modesto numero verso la fine del ciclo vegetativo del Riso, nella prima settimana di settembre.

Introduction

About 25,000 ha of Rice (*Oryza sativa* L.) are grown in Las Marismas del Guadalquivir (Sevilla, southern Spain), an area quite uniform in climatic and edaphic conditions. The crop is sown by the end of April and is harvested from the beginning to the end of September depending upon cultivars. According to farmers' claims yield losses due to diseases are severe, yet very little attention has been paid to diseases affecting the crop in that region. Consequently, a research program was established aimed at gaining knowledge on the etiology and importance of fungal diseases affecting Rice in Las Marismas (Marín-Sánchez, 1979). This



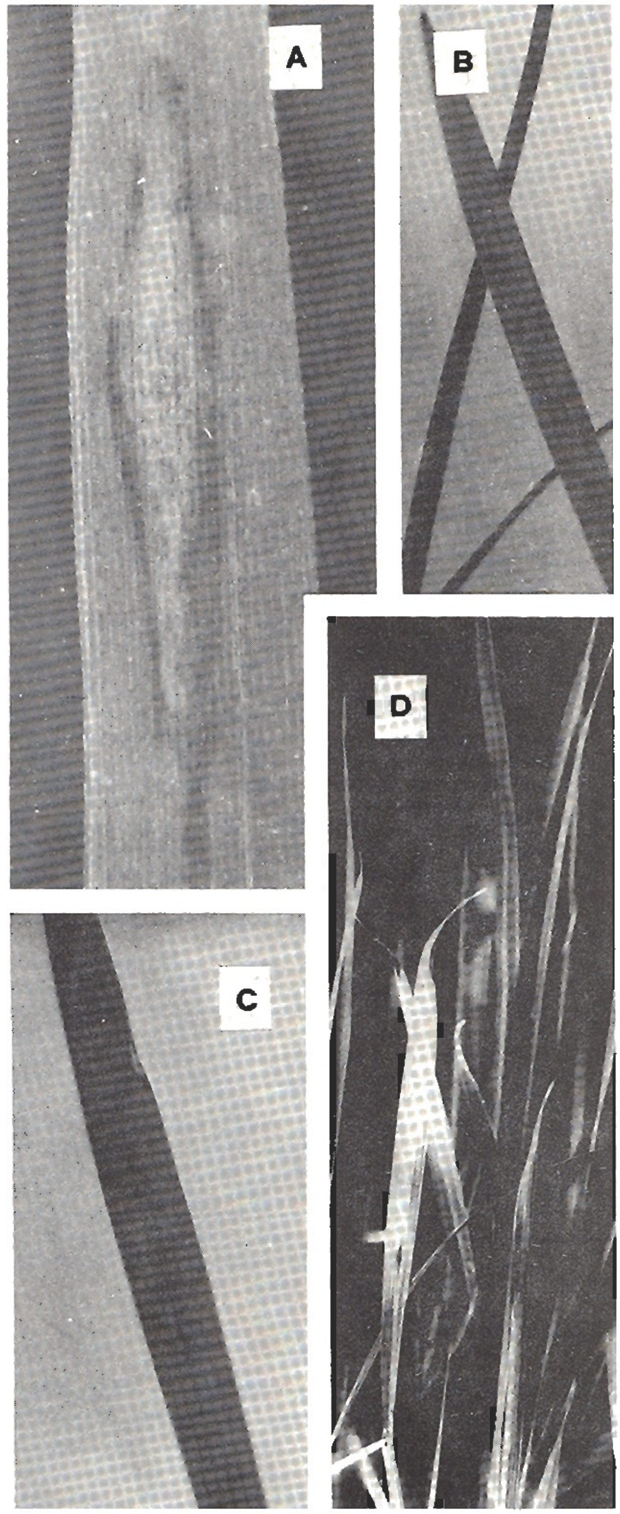


Fig. 1. - Rice-growing area in Las Marismas (Sevilla) where disease surveys were carried out in 1977 and 1978. For sampling purposes it was divided in eight zones, labeled I-VIII.

Fig. 1. - Area di coltivazione del Riso a Las Marismas (Siviglia), ove è stata condotta l'indagine nel 1977 e nel 1978. Per la raccolta dei campioni l'area è stata divisa nelle zone da I a VIII.

Fig. 2. - Symptoms in rice plants infected by *Pyricularia oryzae* (A-C) and *Nakataea sigmoidea* (D). (A) Large elliptic necrotic spot with gray center. (B) Chlorotic specks induced by cultures of conidial type 1 in artificial inoculation. (C) Elliptic necrotic spot induced by cultures of conidial type 4 in artificial inoculation. (D) Necrotic spot developed in artificial inoculation.

Fig. 2. - Sintomi su piante di Riso infette da P. oryzae *(A-C) e da* N. sigmoidea *(D). A, macchia necrotica di forma ellittica di tonalità grigia al centro; B, piccole lesioni clorotiche indotte artificialmente da conidi tipo 1; C, macchia necrotica di forma ellittica indotta artificialmente da conidi tipo 4; D, macchia necrotica indotta da infezione artificiale.*

report presents that part of our results dealing with diseases induced by *Pyricularia oryzae* Cav. and *Nakataea sigmoidea* Hara.

Blast, induced by *P. oryzae*, is the most important disease of Rice and occurs in all major Rice-growing regions of the world (Ou, 1972, 1980). The occurrence of the disease in Spain has been mentioned (Anonymous, 1968; Benlloch, 1975), but knowledge of the importance of the disease is lacking. The pathogen infects all aboveground plant parts causing a variety of symptoms, the most conspicuous being leaf spot, whose number, size and type depend upon cultivar susceptibility (Ou, 1972, 1980). Also noteworthy is the infection of the panicle base («neck blast» or «neck rot») which results in devastating attacks (Ou, 1972, 1980).

Culm rot, induced by *Sclerotium oryzae* Catt., has been recorded in almost every place where Rice is grown (Ellis, 1971; Ou, 1972). Its occurrence in Spain has been mentioned without further consideration (Benlloch, 1975). Attacks by the pathogen occur at final stages of plant development, first in the sheaths, then later in the culm base. They are characterized by brown to black necrotic lesions, variable in shape, which result in the culm rot, and by numerous sclerotia which develop on affected tissues (Hashioka, 1970; Ou, 1972). The conidial stage of the fungus, *N. sigmoidea* (*Helminthosporium sigmoideum* Cav.), develops in pure cultures of *S. oryzae* as well as on Rice seedlings inoculated with conidia (Ou, 1972). Although the ability of conidia to infect Rice under natural conditions is unknown, it has been suggested that the conidia may be wind dispersed and thus contribute to the spread of the pathogen (Ferreira and Webster, 1975).

Materials and methods

Disease surveys, including distribution and intensity of infections, were carried out in the Rice-growing area of Las Marismas in 1977 and 1978. For sampling purposes the area was divided in eight zones, labeled I-VIII (Fig. 1). Within each zone about 500 ha were sampled. Sampled fields in the area included 16-17% of the total Rice acreage. Disease incidence was assessed in a 1 m² frame thrown at random at least 20 times in each sampled field. Severity of infections was assessed by the leaf area covered by lesions of *P. oryzae* (Padmanabhan, 1963) and *N. sigmoidea* (Aluko, 1970) for 50-100 affected plants taken from five spots in each field. Overall losses of seed yield for each cultivar were estimated by comparing the average yield in non-affected fields with the average yield in fields having a given incidence of the disease. Although non-affected fields were roughly in the same conditions than affected ones, those losses should be considered only as an indication of the effect the disease may have had on yield, since other factors (i.e. grower practices, soil types, etc.) might also be involved.

In 1978, a trial was carried out in zone I (see figure 1) to test the main cultivars grown in Las Marismas, namely 'Bahia', 'Frances', 'Girona', 'Ribello' and 'Sequial', for susceptibility to *P. oryzae* under field conditions and natural infections. Cultivars were sown on May 5 in six-row plots, 2 m long, 15 cm apart, replicated twice in a randomized complete block design. The plots were fertilized with $(NH_4)_2SO_4$ (21%) at rate 762 kg/ha before sowing. Disease reaction was rated according to Padmanabhan (1963) at the boot stage, on July 20. The trial was repeated at the same place with cultivars being sown on July 30 and disease rating made on September 30.

The content of conidia of *P. oryzae* and *N. sigmoidea* in the air was sampled by means of glass slides covered with glycerin jelly (Ono, 1963) and placed horizontally 5 cm and 1 m above the ground near plots in the susceptibility trial. Spore counts were made at 2-day intervals from about 1 month before sowing to harvest time. Weather was monitored with a hygrothermograph in a wood shelter at a level of 1 m above the ground and with a rain gauge, both being placed in the proximity of the slides.

Isolations were made on malt-extract agar (MA) and rice-polish agar (RPA), (Tuite, 1969). Small pieces of the affected tissues were surface disinfested in a 1:9 (v/v) solution of a household bleach (5.25% sodium hypochlorite) and plated onto the media. Pieces of the affected tissues were also placed in moist chambers. Pure cultures and moist chambers were incubated at 25 ± 1°C and alternating 12 h periods of fluorescent and NUV light of about 2,500 lux. Monoconidial cultures of *P. oryzae* and *N. sigmoidea* were obtained from sporulating leaf spots in moist chambers and from pure cultures.

Pathogenicity of isolates was tested on seedlings at the three to four-leaf stage of cultivars 'Bahia', 'Girona', 'Ribello' and 'Sequial', in a greenhouse o a growth chamber. In the greenhouse seedlings grew in pots containing a sterile soil mixture and were managed as recommended by Laterell *et al.* (1963). In the growth chamber seedlings grew in pots with perlite watered with a half strengh nutritive solution (Hoagland and Arnon, 1950), and were supplied with a 14 h/day photoperiod of fluorescent light of about 15,000 lux. Temperature and relative humidity (RH) ranged 20-28°C and 70-90% in the greenhouse, and 20-24°C and 70-90% in the growth chamber.

Inoculations were performed by spraying a suspension of conidia in sterile distilled water (10^4 conidia/ml) onto the foliage with an atomizer. Inoculum was obtained from 15-day-old cultures of *P. oryzae* grown on RPA, and from 20-day-old cultures of

N. sigmoidea grown on water agar (Luttrell, 1963). Tween-20 (polyoxyethylene sorbitan monolaurate) was added to the inoculum suspension at the rate of 1 drop/100 ml just before inoculation. Inoculation was also performed with sclerotia of *S. oryzae*. They were obtained from 25-day-old cultures and were placed in numbers of 30 sclerotia/seedling on the surface of the pots. After inoculation the seedlings were covered with plastic bags for 48-72 h. Seedlings which served as controls were treated as inoculated seedlings except for the absence of inoculum. At least three replicates of 10 potted seedlings were used in the experiments, which were repeated several times.

Results

SYMPTOMATOLOGY, DISTRIBUTION AND INTENSITY OF INFECTIONS BY *P. oryzae*.

P. oryzae was isolated from generalized necrosis which developed most frequently in the first and second uppermost culm nodes and rachis, and from restricted necrotic lesions in the leaves. Necrosis in the nodes and rachis were first observed by the end of August, at the beginning of flowering. They were associated with kernels failing to develop totally or partially. Necrosis in leaves ranged from brown specks to round and brown small spots, and large elliptic spots with a brown margin and gray center (Fig. 2A). Leaf spots were first observed by the end of May in zone I, covering 1-5% of the leaf blade surface of 'Bahia' seedlings and in zone V, covering 1-5% and 0.5-2% of leaf blade surface in seedlings of 'Girona' and 'Sequial', respectively. Later in the season the severity of leaf infections did not vary substantially among zones, but it did among cultivars, ranging 10-75% for 'Girona', 20-25% for 'Ribello', 10-25% for 'Bahia', 5-20% for 'Frances' and 6-15% for 'Sequial'.

Infections by *P. oryzae* were widespread. All sampled fields and cultivars were affected, with the incidence of infections varying widely among cultivars within zones, and among zones for a given cultivar. Higher incidence occurred in cultivars 'Girona' and 'Ribello', for which estimated losses of seed yield ranged 20-90% and 30-75%, respectively (Table I).

Our observations during the disease surveys were confirmed in the susceptibility trial, with all cultivars tested being susceptible to *P. oryzae* under natural conditions. Disease reactions for each cultivar did not vary among replicates, but they decreased in severity from the first to the second plantings. Thus, infections of 'Girona' and 'Ribello' were very severe in the earlier planting and severe in the later planting; and infections of 'Bahia', 'Frances' and 'Sequial' were severe when sown in May and moderately severe when planted in July.

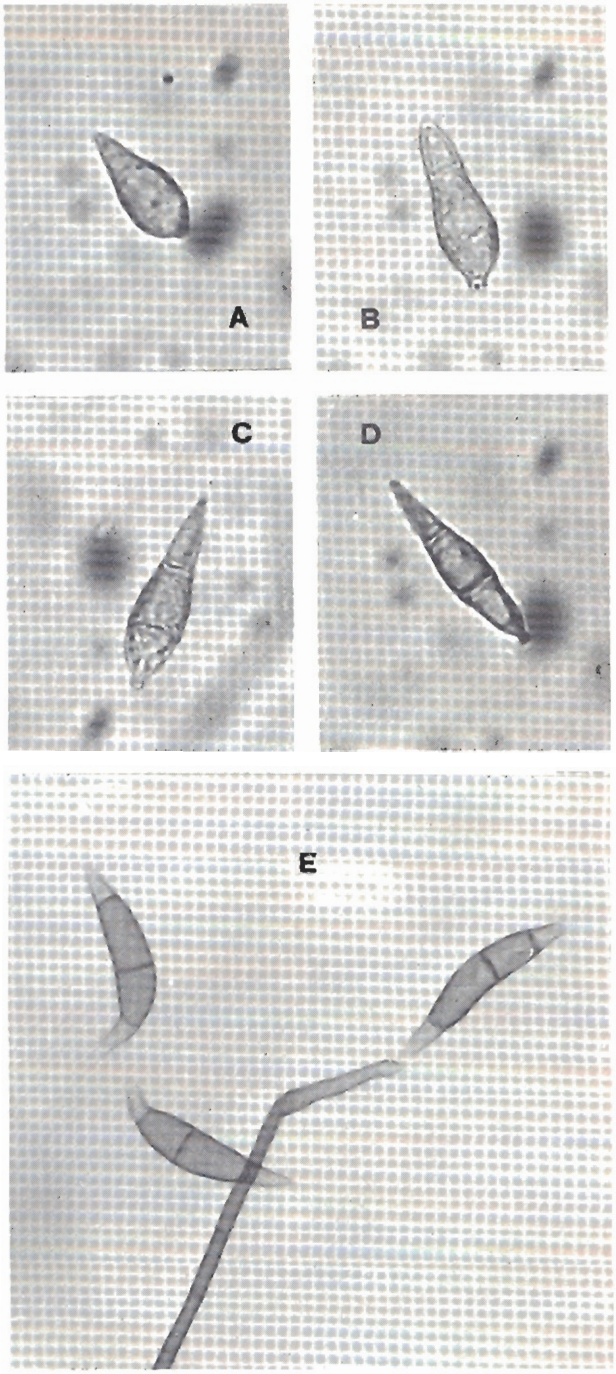


Fig. 3. - Conidia of *Pyricularia oryzae* (A-D) and *Nakataea sigmoidea* (E). (A) Conidial type 1. (B) Conidial type 2. (C) Conidial type 3. (D) Conidial type 4.
Fig. 3. - Conidi di P. oryzae *(A-D) e di* N. sigmoidea *(E). A, tipo conidico 1; B, tipo conidico 2; C, tipo conidico 3; D, tipo conidico 4.*

TABLE I. - Distribution and intensity of infections of Rice by *Pyricularia oryzae* in Las Marismas (Sevilla) (*a*).
TABELLA I. - *Distribuzione e intensità delle infezioni su Riso di* P. oryzae *a Las Marismas (Siviglia)* (a).

Sampled zone (*b*)	Cultivar	Acreage of affected fields (%)	Range of incidence (%)	Higher estimated losses of seed yield (%)
I		100		
	'Bahia'	50	5-35	—
	'Girona'	30	10-70	35
	'Sequial'	14	6-50	18
	'Ribello'	6	7-80	30
II		100		
	'Bahia'	60	5-25	—
	'Girona'	30	8-50	20
	'Ribello'	10	12-45	—
III		100		
	'Bahia'	80	5-40	—
	'Sequial'	10	16-55	—
	'Ribello'	10	18-90	75
IV		100		
	'Bahia'	80	3-25	—
	'Frances'	20	8-60	—
V		100		
	'Girona'	50	10-50	—
	'Sequial'	50	3-25	—
VI		100		
	'Bahia'	80	3-18	—
	'Ribello'	20	5-30	—
VII	'Girona'	100	11-65	25
VIII		100		
	'Bahia'	60	4-22	—
	'Girona'	20	12-99	90
	'Ribello'	20	11-80	—

(*a*) - Disease surveys were carried out in 1977 and 1978.
(*b*) - For sampling purposes, eight zoned were defined in the rice-growing area and labelled I-VIII (Fig. 1). About 500 ha within each zone were sampled. They included 16-17% of the total rice acreage in Las Marismas.

TABLE II. - Distribution and intensity of infections of Rice by *Nakataea sigmoidea* in Las Marismas (Sevilla) (*a*)
TABELLA II. - *Distribuzione e intensità delle infezioni su Riso di* N. sigmoidea *a Las Marismas (Siviglia)* (a).

Sampled zone (*b*)	Cultivar	Affected fields (No)/Sampled fields (No)	Acreage of affected fields (%)	Range of incidence (%)
I			58	
	'Bahia'	18/25	36	25-60
	'Girona'	6/15	12	10-30
	'Sequial'	4/7	8	20-55
	'Ribello'	1/3	2	8-30
II			36	
	'Bahia'	12/30	24	15-45
	'Girona'	5/15	10	5-25
	'Ribello'	1/5	2	6-27
III			38	
	'Bahia'	17/40	34	18-50
	'Sequial'	1/5	2	16-55
	'Ribello'	1/5	2	3-24
IV			56	
	'Bahia'	21/40	42	14-50
	'Frances'	7/10	14	18-47
V			36	
	'Girona'	11/25	22	15-35
	'Sequial'	7/25	14	25-65
VI			44	
	'Bahia'	19/40	38	6-25
	'Ribello'	3/10	6	3-17
VII	'Girona'	13/50	26	13-23
VIII			72	
	'Bahia'	23/30	46	23-57
	'Girona'	7/10	14	11-28
	'Ribello'	6/10	12	7-27

(*a*) - Disease surveys were carried out in 1977 and 1978.
(*b*) - For sampling purposes, eight zoned were defined in the rice-growing area and labelled I-VIII (Fig. 1). About 500 ha within each zone were sampled. They included 16-17% of the total rice acreage in Las Marismas.

VARIATION ON MORPHOLOGY AND VIRULENCE AMONG CULTURES OF *P. oryzae*.

The conidia which developed on infected tissues in moist chambers could be divided into four types, 1-4, according to spore length and morphology. Moist chambers were incubated for 12 days at 25 ± 1°C and alternating 12 h periods of fluorescent and NUV light of about 2,500 lux. Type 1 conidia were the shortest, measuring 20.8×8.3 μm. They had a subspherical basal cell and an apical cell without constriction at the septum (Fig. 3A). Type 2 conidia measured 24.9×8.3 μm, and were characterized by a subspherical basal cell and a blunt apical cell (Fig. 3B). Conidia of type 3 differed from those of type 2 only in having the apical cell tapering to the end (Fig. 3C), and conidia of type 4 were the longest, measuring 29.1×8.3 μm, with the basal cell campanulate and the apical cell narrow and tapering to the end (Fig. 3D).

Isolates of *P. oryzae* were virulent to all cultivars inoculated under artificial conditions. Seedlings of 'Girona', the most susceptible cultivar, were inoculated with monoconidial cultures of each of the conidial types described above. Disease reactions varied depending upon the conidial type. Cultures with type 1 conidia induced abundant chlorotic specks and very few if any necrotic ones (Fig. 2B), and cultures of conidial types 2 and 3 caused necrotic spots 1-2 mm in diameter. Cultures of type 4 were the most virulent, inducing necrotic flecks, small round necrotic spots, and elliptic necrotic spots up to 5 mm in length (Fig. 2C).

SYMPTOMATOLOGY, DISTRIBUTION AND INTENSITY OF INFECTIONS BY *N. sigmoidea*.

N. sigmoidea was isolated from necrotic spots in the leaf blades, which were first observed in plants at the boot stage by the end of July. Those spots were purplish-blue, round to elliptic, about 2 mm in length. They were found in 7 of 12 sampled fields of cultivar 'Sequial' in zone V, with incidence ranging 0.3-0.5% and severity less than 1% of the leaf blade surface. Isolations yielded conidial cultures first, and sclerotia developed in subcultures upon routine transfers. The conidia (Fig. 3E) were curved, bearing three septa most frequently, with the cells at the center darker than those at the ends, and measuring 58.2-66.5 (60.0) $\mu m \times 10.4$-12.5 (11.0) μm. They formed on olivaceous conidiophores up to 218 μm long and 4.2-6.3 (5.5) μm wide. Monoconidial cultures did not show variation in colony or spore morphology. In artificial inoculations they first induced the same type of symptoms observed in the field, but later the necrotic spots grew in length to form irregular lesions (Fig. 2D).

Infections were also found in the sheaths and culm bases of plants at the boot stage, with symptoms resembling those described by Hashioka (1970) and Ou (1972), and bearing numerous sclerotia of *S. oryzae*. Isolations from the affected tissues gave fungal cultures on which sclerotia developed profusely but no conidia were found. In artificial inoculations, sclerotia obtained from isolations and those formed in monoconidial cultures caused restricted or generalized necrosis in the sheath and culm base of seedlings.

Culm rot occurred in all sampled zones with variable intensity, the acreage of affected fields ranging from 72% in zone VIII and 52% in zone I, to 26% in zone VII (Table II). Also, all sampled cultivars were affected. The incidence of infections varied widely among cultivars within a zone, as well as among zones for a given cultivar, the higher values occurring consistently in 'Bahia' and 'Sequial' (Table II).

CONIDIA CONTENT IN THE AIR.

CONIDIA OF *P. oryzae* were trapped throughout the crop season. On the contrary those of *N. sigmoidea* were detected only in the first weeks of September, at the end of the crop cycle. The mean number of conidia trapped in glass slides every 4 days, the mean temperature, RH and the precipitation, are represented in Figure 4. Counts of conidia of *P. oryzae* reached a maximum of 8 conidia per slide on June 3, with plants at the seedling stage. Counts decreased afterward to a lower level with a second maximum (4 conidia per slide) at the end of September, with kernels at milky-ripe stage. Apparently, the number of conidia trapped bore no relationship to precipitation during the crop, but to higher values of RH.

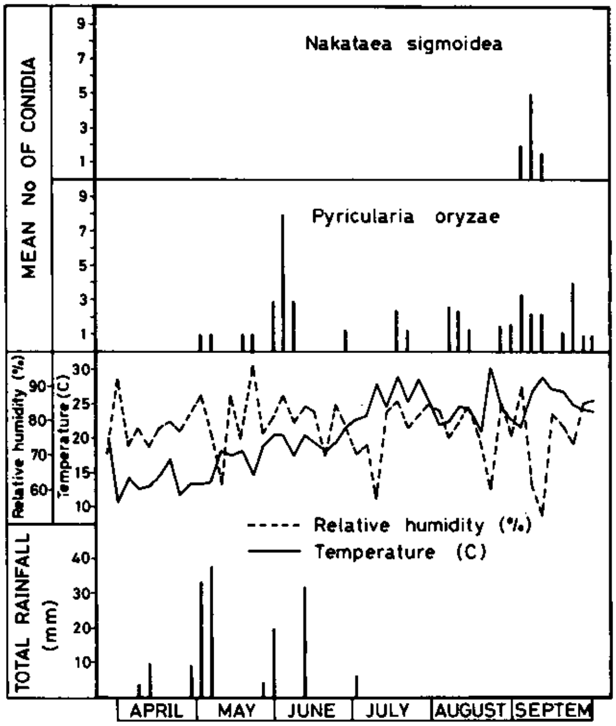


Fig. 4. - Mean numbers of conidia of *Pyricularia oryzae* and *Nakataea sigmoidea* trapped from the air, rainfall, temperature and relative humidity at Las Marismas, from April 1 to September 30, 1978.

Fig. 4. - Numero medio di conidi di P. oryzae *captati su vetrino, pioggia, temperatura e umidità relativa occorse da aprile a settembre 1978 a Las Marismas.*

Discussion

Both blast and culm rot severely affect rice in Las Marismas del Guadalquivir. *P. oryzae* is widespread in the sampled area, and conidia are available for infections through the entire crop season (Fig. 4). Although attacks occurred with variable incidence, they caused important losses (Table I). Severe losses were particularly associated with necrosis of the uppermost culm nodes and rachis, as has been reported (Ou, 1972, 1980). Ou (1980) pointed out that resistant cultivars would be the ideal way to control Rice blast. Our results indicate that all cultivars used in Las Marismas are susceptible to the pathogen population prevalent in that area, and they should be replaced by resistant cultivar in order to reduce yield losses in the crop.

The extreme variability of *P. oryzae* in pathogenicity and spore morphology has been recognized by a number of researchers (Ou, 1972, 1980). The variation in spore size and morphology which we found agrees with that reported by Tochinai and Shimamura (Ou, 1972), our conidial types 1 and 4 corresponding to their short and long types, respectively. Also as reported by those authors, we observed that affected nodes gave most frequently short conidia and that long conidia were collected from lesions in the rachis and leaves. The different disease reactions that we obtained in artificial inoculations with each of the conidial types suggest that the morphology of conidia might be related to virulence. That was also suggested by the variation in morphology of conidia obtained from acute or chronic leaf spots (Ou, 1972), and by the fact that isolates from one type of lesion may induce the same type or modify the lesion type induced by other isolates when a mixture of both is used as inoculum (Yoshikatsu and Suzuki, 1978). Our monoconidial cultures proved to be unstable, as they changed in conidial morphology upon successive transfers and/or isolations. Thus, our results are largely preliminary and more research is needed on the subject.

Culm rot of Rice occurred with variable prevalence and incidence in all sampled zones of Las Marismas. Also, all cultivars seem to be susceptible to the disease. The isolation of the conidial stage of *N. sigmoidea* from foliar lesions, the production of virulent conidial cultures from them and the presence of conidia in the air, indicate that the conidial stage may contribute to the dissemination of the pathogen as suggested by Ferreira and Webster (1975). However, the fact that conidia were captured in low numbers and at the end of the crop season suggest that they must not play a major role in the severity of the disease.

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