European Journal of Plant Pathology **106:** 449–454, 2000.

Mucor species in orchard soil – population dynamics and pathogenicity on pear fruit

Marta Mari, Tiziano Cembali, Lucia Casalini and Gian Carlo Pratella
CRIOF, University of Bologna, Via Filippo Re, 8, 40126, Bologna, Italy (Phone: +39051766563; Fax: +39051765049; E-mail: mari@agrsci.unibo.it)

Accepted 6 March 2000

Key words: zygomycetes, packinghouse, dump-tank waters, propagules

Abstract

Strains of *Mucor piriformis* were isolated from orchard soils and from packinghouse dump-tank waters. Pathogen propagules were not found in fruit sample washings. *M. piriformis* was the most prevalent of the *Mucor* isolates, only one of 67 isolates was a different *Mucor* sp., possibly *M. racemosus*. The population of the pathogen propagules fluctuated in an annual cyclic pattern, declining in warm months and increasing after harvest. The viability of sporangiospores was markedly affected by rain. There was a good correlation ($r = 0.88$) between the number of recovered propagules in the soil and the amount of rainfall. *M. piriformis* isolates caused decay on pear at 0 °C after 14 days.

Introduction

Mucor species are postharvest pathogens that infect pears and apples (Bertrand and Saulie-Carter, 1980), strawberries (Edney, 1964), peaches and nectarines (Smith et al., 1979) and less frequently tomatoes (Moline and Kuti, 1984). The etiology of *Mucor* decay includes of several species. The most important are *M. piriformis* A. Fisher (Colhoun, 1938; Lopatecki and Peters, 1972), *M. mucedo* P. Mich. ex Saint-Amans (Dennis, 1975; Moline and Kuti, 1984; Reyes, 1990), *M. hiemalis* Wehmer (Kunimoto et al., 1977), *M. strictus* Hagem (Edney, 1965), *M. racemosus* Fresen. (Colhoun, 1938; Lunn, 1978) and *M. circinelloides* (Smith et al., 1979).

In Italy, *M. piriformis* was generally considered to be a pathogen of minor importance on pome fruits as it occurred only sporadically. In the last decade the pathogen has caused substantial losses more frequently than in the past (Caccioni and Guizzardi, 1992). *M. piriformis* is a wound parasite, associated on pears and apples with macro- and micro-wounds caused mainly at harvest or during handling before storage. The pathogen reaches packinghouse dump-tank water carried in soil on the bottoms of bins.

Links have been established between *M. piriformis* populations in dump-tank waters and the decay of pear fruit (Spotts, 1986). The fungus survives in the top 5 cm of soil as sporangiospores and population densities are higher in September, 2 months after harvest than at harvest, due to pear fruit left on the ground which are colonised by *M. piriformis* (Michailides and Spotts, 1986).

The aim of this research was to detect *Mucor* species in pear orchard soil and in packinghouse dump-tank waters and to study their pathogenicity on pears.

Materials and methods

Pathogen recovery: sampling from dump-tank waters

Nine water samples of 1000 ml each were collected from dump-tanks of two packinghouses in Emilia Romagna (Italy) before, during, and at the end of

treatment before storage. Subsamples (0.5 ml) were diluted with 4.5 ml of sterile distilled water containing 0.05% (v/v) Tween 80. Aliquots (0.1 ml) from these subsamples were spread on Petri dishes of potato dextrose agar, acidified (pH 3.5) with 40 ml of 10% lactic acid per litre (APDA). Plates were incubated at 0 °C for 60 days and then checked for *Mucor* spp. Colonies of *Mucor* spp. were at first white, with sporangiophores emerging singly from the mycelium. A single sporangiospore culture was obtained from each isolate and stored at −20 °C.

Pathogen recovery: sampling from soil

Samples (0.5 g) of soil were collected at a depth of 5 cm with a soil-tube sampler in a pear orchard a few days before harvest. Samples were collected from 60 trees chosen at random (Spotts and Cervantes, 1986). Each sample was dried at 40 °C for 4 h, and then kept in desiccator for 4 days. Later, samples were ground in a mortar and 0.5 g of soil was mixed with 4.5 ml of sterile distilled water containing 0.05% (v/v) Tween 80. Aliquots (0.5 ml) of this suspension were spread on Petri dishes as described above.

Pathogen recovery: sampling from fruit

Three separate samples consisting of five fruits were collected 3 days before harvest, from each of three pear trees at 40, 100 and 200 cm above soil level. After collection the samples were washed in 100 ml of distilled water containing 0.05% Tween 80 on a rotary shaker for 1 h at 150 rpm. Then 0.1 ml aliquots of washing water were plated on APDA and incubated at 0 °C for 1 month.

The resulting colonies of *Mucor* spp. were isolated and cultured on malt extract agar (MEA). After 3 days at 20 °C they were grouped in classes by morphological characteristics, and by light microscope observation using keys for the Mucorales (Zycha et al., 1969; Hanlin, 1973). In addition, five representative isolates were sent to the International Mycological Institute (UK) to confirm the preliminary identification.

Population dynamics of Mucor *spp. in orchard soil*

A non-irrigated pear orchard in the Emilia Romagna region was used in this study. For a year, three samples were collected monthly from the top 5 cm of soil at 50 cm (fall area) from 20 labelled pear trees. Soil samples, from around each tree, were combined and mixed (20 g), then suspended in 100 ml of sterile water, blended for 1 min and left to settle for 15 s. Then 0.5 ml aliquots of the suspension were spread on APDA (5 replicates for each monthly sample). Plates were incubated at 4 °C for 10 days and the number of *Mucor* spp. colonies were counted by observing plates through transmitted light. Rainfall was recorded every month.

Pathogenicity of Mucor *strains*

Four *M. piriformis* (2AL, 410, 420 and 1010) and one *Mucor* sp. (1AL) were used. Strains were grown on MEA at 20 °C for 7–10 days. Sporangiospore suspensions were prepared by washing the colonies grown on MEA in sterile water and Tween 80 (0.05%, v/v). Suspension concentrations were adjusted to 10^4 sporangiospores ml^{-1} by means of a haemacytometer.

Conference pears selected for uniform ripeness and size were disinfected superficially with 1% sodium hypochloride and rinsed in water. Fruits were wounded with a scalpel and cubes ($3 \times 3 \times 3$ mm) of tissue removed. Two wounds were made on each fruit at the equator and the fruits inoculated by dipping (1 min) in the *Mucor* suspension. After 1 h dried fruits were placed on trays, wrapped in polythene bags and incubated at 0, 2, 5, 10 and 20 °C for 14 days. For each temperature there were 3 replicates of 20 fruits each giving a total of 120 wounds. Lesion diameters (mm) and infected wounds (%) were recorded weekly. The experiment was repeated once.

Statistical analysis

Statistical analysis was performed using the SYSTAT 5.2 software package (Wilkinson, 1990). All data on percentage of infected fruits was analysed for variance (one way ANOVA). Mean separation was performed using the least significant difference (LSD) test at $P = 0.05$. A complete randomised block design was used in each test.

Results

Pathogen recovery

Eight of the samples from the dump-tank waters contained *Mucor* propagules. Isolated strains showed noticeable differences in both microscopic and macroscopic features and were divided into two groups.

The first group included 2 isolates (1AL and 5AL), identified as *Mucor* sp. close to *Mucor racemosus*. The strains developed on MEA as white colonies, becoming brownish-grey with age. Sporangiophores were short, up to 22 μm diameter, and sporangia were hyaline, becoming brownish to grey with age. Columellas were ellipsoidal, obovoid with a truncate base, chlamydospores were numerous in sporangiophores, and sometimes in columellas. The second group included 6 strains (2AL, 3AL, 4AL, 7AL, 9AL), identified as *Mucor piriformis*. Strains of this group grew fast on MEA, producing colonies after 3 days at 20 °C, white at first then brownish or grey. Sporangiophores were colourless, often sympodially branched, emerging singly from the mycelium with a few side branches terminating in sporangia. Sporangia were up to 250–270 μm in diameter with walls that disintegrate rapidly on contact with water. Colourless columellas were generally pear-shaped, with traces of a basal collarette. Hyaline ellipsoid sporangiospores ranged in size from 4–6 to 8–12 μm.

Fifty-nine of the 240 samples of soil collected from the orchard contained propagules of *Mucor piriformis*. *Mucor* spp. was not found in washings from fruit samples collected from the trees at harvest.

Population dynamics of Mucor *spp. in orchard soil*

In the late spring and summer, population densities were lowest, with less than 10^2 propagules g^{-1} of orchard soil (Figure 1). In October, about 2 months after harvest, population densities increased, registering the highest annual level with 9.3×10^2 propagules g^{-1} of soil. Later, in winter, *Mucor* propagules declined, falling to just 26 propagules g^{-1} of soil in February.

Population densities were also positively correlated (0.88) with the monthly rainfall (Figure 2). Low population densities of propagules in June, July and February (50, 50 and 26 propagules g^{-1} of soil) were associated with low precipitation (16, 15.8, 9.6 mm) in these months, whilst the highest population density in October (930 propagules g^{-1} of soil) was associated with the highest precipitation (138.4 mm). The low level of *Mucor* propagules in November (14 propagules g^{-1} of soil) might be due, also, to low rainfall (31.2 mm).

Pathogenicity of Mucor *strains*

Three of the four *M. piriformis* isolates tested caused decay on pears stored at 0 °C. *M. piriformis* isolate 1010 did not infect the fruits at 0 °C or at 20 °C (Table 1). The decay on fruits infected with isolates 2AL and 410 of *M. piriformis* became measurable 14 days after inoculation, at 0 °C (Table 1) while isolate 1AL (*Mucor* sp.) infected the 22.5% of fruits only after 21 days (data not shown in Table 1). Isolates 2AL and 410 caused a significantly higher incidence ($P < 0.05$) of infection than the other isolates at 0 and 20 °C. Sporangiophores and grey to black sporangia appeared on fruits 14 days after inoculation. *M. piriformis* isolates 2AL and 410, were also pathogenic at 5 and 10°C causing an incidence of more than 70%, of infected

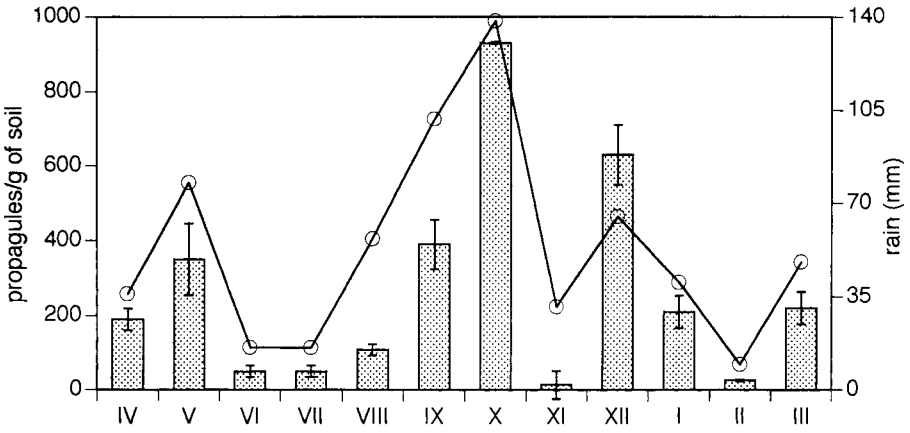

Figure 1. Population density of propagules of *Mucor* spp. in the upper 5 cm of soil in a pear orchard over a 12 month period. Roman numerals on the *X*-axis refer to months, beginning with April. Each column is the average of three values, ± standard error and each point is the monthly rainfall.

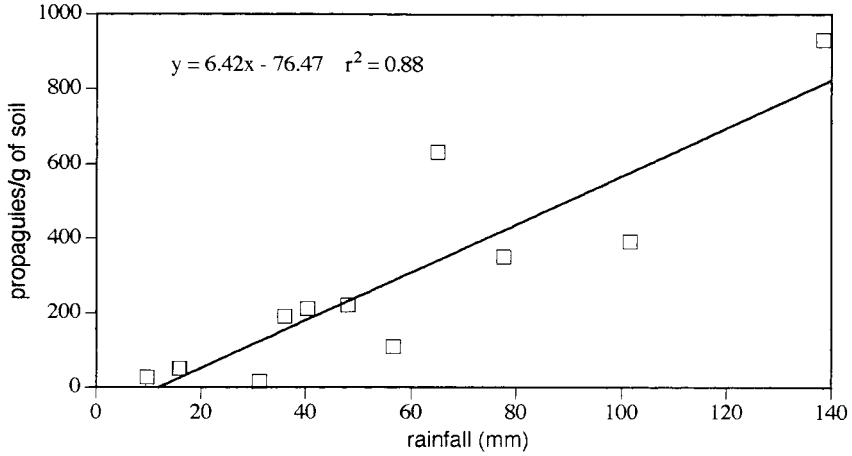

Figure 2. Regression line representing the effect of rainfall on *Mucor* spp. propagule density referring to the same period as in Figure 1. The correlation coefficient (r) is significant at $P \leq 0.05$. Each point is the average of three values.

Table 1. Pathogenicity of *Mucor* strains on pear fruits[a]

Pathogen	Strains	0 °C		20 °C	
		Lesion diameter (mm)	Infected wound (%)	Lesion diameter (mm)	Infected wound (%)
M. piriformis	2AL	8 ± 0.61*	15 b**	52 ± 1.58	100 a
M. piriformis	410	10 ± 0.79	32.5 a	46.2 ± 42	92.5 a
M. piriformis	420	0 ± 0	0 c	22 ± 1.2	77.5 b
M. piriformis	1010	0 ± 0	0 c	0 ± 0	0 d
Mucor sp.	1AL	0 ± 0	0 c	43 ± 8.26	25 c

[a]Wounded fruits were inoculated by dipping for 1 min in a spore suspension (10^4spore ml^{-1}). Fruits were stored for 14 days. Single test group consisted of 3 replicates of 20 fruits for a total of 120 wounds per group.

*± Standard error.

**In each column, numbers followed by the same letter are not significantly different at $P = 0.05$ according to LSD test.

wounds after 14 days (Figure 3) while *M. piriformis* isolate 420 in the same conditions did not produce any infection.

Discussion

Our results revealed the presence of *Mucor* propagules in dump-tank waters, in eight out of nine samples tested. Factors such as the amount of fruit, bin contamination, the volume of fruit processed, can increase the pathogen population (Spotts and Cervantes, 1986).

The serial dilution plating technique on APDA is very efficient for recovering propagules from infested soil or debris (Michailides and Spotts, 1990). With this simple technique 240 soil samples were tested and 59 strains of *Mucor* spp. isolated. No pathogen propagules were found in washings of fruit samples taken from the trees at harvest. This could be expected, as *M. piriformis* was not recovered from the surface of leaves or fruit on the trees, or from samples of orchard air in a previous study (Michailides and Spotts, 1986).

All isolates from soil samples were identified as *M. piriformis*, but morphological differences among these isolates were noted. Tuft height, sporangiophore diameter, ratio of tall/short sporangiophores may vary in strains of *M. piriformis* (Schipper, 1975).

Pathogen propagule populations fluctuate in an annual cyclic pattern. In Italy they decline during

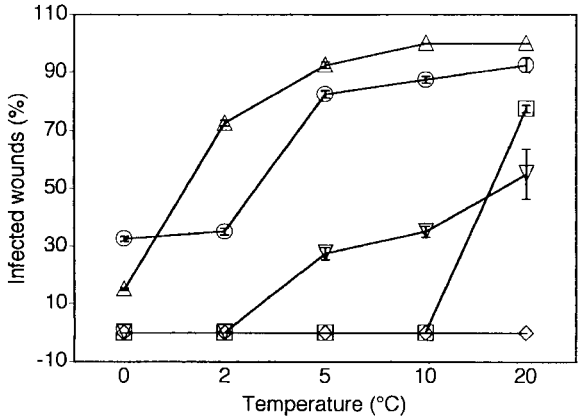

Figure 3. Effect of temperature on *Mucor* infection of Conference pears during an incubation period of 14 days. Each point is the average of 120 wounds, ± standard error. 2AL (Δ) 410 (○) 420 (□) 1010 (◇) 1AL (▽).

the warmest months: June, July and August, while increasing in September and October when fruits fall to the ground before and during harvest and become colonised by *Mucor* sp. Although optimum temperature for growth of *M. piriformis* is 20 °C, the low density of pathogen propagules recovered during the warm months suggests that *M. piriformis* competes poorly with other soil saprophytes (Michailides and Ogawa, 1987). The ability of sporangiospores to tolerate low temperature is not surprising in view of their ability to germinate and grow in cold months at 0 °C or below (Dennis and Cohen, 1976). Our results show that the viability of sporangiospores is markedly affected by rainfall and subsequent humidity in the soil. Indeed there is a good correlation ($r = 0.88$) between recovered propagules in the soil and amount of rainfall. However even during a dry summer, when many irrigation systems are in operation, soil moisture can reach levels highly favourable to *M. piriformis* and a consistent increase in populations can occur.

Two isolates (2AL and 410) of *Mucor piriformis* taken from dump-tank water and orchard soil, respectively, caused decay in pear fruit at 0 °C within a relatively short time (14 days). The low incidence of infected wounds (15% and 32.5%, respectively) appears to be compensated by secondary infections, spread by the dissemination of sporangiospores in exuded juice dripping from infected fruit (Michailides and Spotts, 1990). At 20 °C the isolate of *Mucor* sp. close to *M. racemosus* (1AL) caused larger decay lesions than the isolates of *M. piriformis* (420), although with the first isolate the intensity of disease was lower (25% against 77.5%). Thus, *M. piriformis* appears to be not only the most prevalent of *Mucor* isolates but also the most virulent.

References

Bertrand P and Saulie-Carter J (1980) *Mucor* rot of pears and apples. Oregon State University Agriculture, Exp Special Report: 568

Colhoun J (1938) Fungi causing rots of apple fruits in storage in Northern Ireland. Annals of Applied Biology 25: 88–89

Caccioni D and Guizzardi M (1992) *Mucor piriformis* Fisher su pomacee in post-raccolta. Informatore Fitopatologico 9: 59–62

Dennis C (1975) Effect of preharvest fungicides on spoilage of soft fruit after harvest. Annals of Applied Biology 80: 237–242

Dennis C and Cohen E (1976) The effect of temperature on strains of soft fruit spoilage fungi. Annals of Applied Biology 82: 51–56

Edney K (1964) Postharvest rotting of strawberries. Plant Pathology 13: 87–89

Edney K (1965) A rot of Conference pears caused by *Mucor strictus* (Hagem). Plant Pathology 14: 189–190

Hanlin TR (1973) Keys to the Families, Genera and Species of the Mucorales. Verlag von J Cramer Lehre Germany

Kunimoto RK, Ito PG and Ko NH (1977) Mucor rot of guava fruits caused by *Mucor hiemalis*. Tropical Agriculture Trinidad 54: 185–187

Lopatecki LE and Peters W (1972) A rot of pears in cold storage caused by *Mucor piriformis*. Canadian Journal of Plant Science 52: 875–879

Lunn JA (1978) *Mucor racemosus* Fresenius. No. 529. In: Descriptions of Pathogenic Fungi and Bacteria. Commonw Mycol Inst Assoc Appl Biol Kew Surrey England

Michailides TJ and Spotts RA (1986) Factors affecting dispersal of *Mucor piriformis* in pear orchards and into the packinghouse. Plant Disease 70: 1060–1063

Michailides TJ and Ogawa JM (1987) Colonization sporulation and persistence of *Mucor piriformis* in unamended and amended orchard soils. Phytopathology 77: 257–261

Michailides TJ and Spotts RA (1990) Postharvest diseases of pome and stone fruits caused by *Mucor piriformis* in the Pacific Northwest and California. Plant Disease 74: 537–543

Moline HE and Kuti JO (1984) Comparative studies of two *Mucor* species causing postharvest decay of tomato and their control. Plant Disease 68: 524–526

Reyes AA (1990) Pathogenicity, growth, and sporulation of *Mucor mucedo* and *Botrytis cinerea* in cold or CA storage. HortScience 25: 549–552

Schipper MAA (1975) On *Mucor mucedo, Mucor flavus* and related species. Studies in Mycology 10: 1–33

Smith Jr WL, Moline HE and Johnson KS (1979) Studies with *Mucor* species causing postharvest decay of fresh produce. Phytopathology 69: 865–869

Spotts RA (1986) Relationships between inoculum concentrations of three decay fungi and pear fruit decay. Plant Disease 70: 386–389

Spotts RA and Cervantes LA (1986) Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouses. Plant Disease 70: 106–108

Wilkinson L (1990) SYSTAT: The System for Statistics. Evanston, IL: SYSTAT, Inc.

Zycha H, Siepmann R and Linnemann G (1969) *Mucorales*, Journal of Cramer Lehre Germany 355 pp