Mycoscience 37: 471–475, 1996

Short Communication

Fungi associated with roots and fruits of black peppers in the Dominican Republic

Tsuneo Watanabe[1], Juan de D. Moya[2], José L. González[2] and Akira Matsuda[3]

[1]Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Science City, Ibaraki 305, Japan
[2]Centro Nordeste de Desarrollo Tecnologico Agropecuario (CENDETECA), San Francisco de Macoris, Dominican Republic
[3]Specialist, Japan International Cooperation Agency (JICA)

Accepted for publication 11 November 1996

Several soilborne diseases have occurred in black pepper (*Piper nigrum*) plantations in the Dominican Republic since 1987. To understand environments in which the disease occurs fungal floras associated with them were studied. A total of 1,119 isolates, 603 from root segments of 12 vines, and 516 from fruit samples of 30 vines were identified as belonging to a total of 39 genera, namely, 31 from roots and 26 from fruits, excluding unsporulated fungi. The most dominant fungi were *Fusarium* in both root and fruit samples, followed by *Rhizoctonia*, *Diplodia*, and *Pythium* from roots, and *Diplodia*, *Colletotrichum* and *Pestalotia* from the fruits. Preliminary pathogenicity tests towards leaves or roots of black pepper were conducted.

Key Words——fungus flora; identification; isolation; pathogenicity.

Since the start of a governmental project between the Dominican Republic and Japan on the culture development of black pepper (*Piper nigrum* L.) in the Dominican Republic in 1987, black peppers have been planted every year, and concomitantly several soilborne diseases have occurred, including Phytophthora foot rot, and Fusarium root disease (Matsuda et al., 1994).

To understand the biological environment in black pepper plantations in relation to disease occurrence, it is essential to know the fungi associated with the vines. However, there are scarce data on the fungi related to black peppers to our knowledge.

This is a preliminary report on the fungal floras associated with roots and fruits of black peppers in the Dominican Republic.

Materials were collected from four locations at Exhibition Farms of La Majagua (abbreviated as LM), Tojin (T), and Sierra Prieta (SP) and CENDETECA (Centro Nordeste de Desarrollo Tecnologico Agropecuario at San Francisco de Macoris) (K) on four different occasions in May 1996. A total of 12 vines including at least one healthy and one diseased vine from each location, aged 2 to 5 yr, were selected for sampling at four locations.

Main and adventitious roots were collected at random and cut into small segments (approximately 5×5×5 mm). A total of 762 root segments were prepared, consisting of 90 from LM, 196 from K, 240 from T and 236 from SP.

A total of 655 fruit samples were collected, consisting of 150 fruits from 6 vines in LM, 212 from 11 in K, 168 from 8 in T and 125 from 5 in SP.

Root segments and fruit samples were washed and air-dried, then placed without surface sterilization on water agar (WA) in 9-cm sterile plastic Petri dishes (4 root segments or fruits/plate). Pure cultures were obtained by hyphal tippings to potato-dextrose agar (PDA).

Most of the fungal isolates studied were deposited both dry and alive in CENDETECA in the Dominican Republic, and some living cultures were also deposited in American Type Culture Collection (ATCC).

Inoculation studies were conducted by placing 4-mm agar culture discs of test fungi onto excised leaves of black pepper (2 discs/leaf) in a moist condition. The leaves were collected from at least 200 branches at K, washed and air-dried prior to inoculation. Agar culture discs (4-mm diam) removed from 10-d-old PDA cultures were used as inocula. Two different discs were placed centrally on the upper side of each leaf. Uninoculated PDA discs were used as controls. The inoculated leaves were placed in aluminum trays and kept moist by covering the trays with cling film (Saran Wrap, Asahi-kasei, Tokyo, Japan), and the leaves were scored for infection after 5 d. Experiments were conducted using a total of eight leaves (four leaves/isolate or control) per experiment, and each experiment was repeated one to two times separately under room conditions at 25–30°C.

Two-mo-old (six per pot) or six-mo-old potted seedlings (two per pot) were inoculated by burying inocula (10-d-old PDA cultures in 9-cm plastic Petri dishes) of six isolates of *Pythium* species at a depth of 2 cm near the stems. The seedlings were grown in 15 cm diam polyethylene pots with 1,000 ml of soil. As controls, PDA

(one plate/pot) was used. This experiment was conducted under greenhouse conditions at temperatures between 20 and 37°C.

According to Matsuda et al. (1994), *Phytophthora capsici* Leonian was isolated from 60% of 72 black peppers with foot rot in Sierra Prieta in 1992. This pathogen was trapped using a specific isolation medium. In the present study, in order to isolate as many species as possible unsterilized well-washed samples were plated on WA, and fungi were isolated from the incubated samples by single hyphal tippings.

A total of 603 fungal isolates were obtained from 762 root segments of 12 vines, comprising 42 isolates from LM, 197 from K, 172 from T, and 192 from SP; and 516 isolates were obtained from 655 fruit samples of 30 vines, comprising 101 from LM, 164 from K, 196 from T and 55 from SP.

Representative isolates separated based on colony morphology were assigned code and isolation numbers for further studies. Fungi on water agar in the original isolation plates were also identified by observing them microscopically for more than 10 d to gain the additional information.

The fungal genera collected from roots and fruits in four locations are listed alphabetically with the number of isolates in Table 1.

A total of 39 genera, including 31 from roots and 26 from fruits, were obtained, excluding unsporulated fungi. Although the number of isolates from the respective locations is different, 8 to 20 genera were obtained from the root samples of the respective locations, and 9 to 19 from the fruit samples.

No particular differences were found between healthy root and diseased root isolates, but more numerous genera were obtained from healthy root isolates than diseased root isolates. For example, 9 and 11 genera were obtained from the healthy root samples from T and SP, respectively, whereas 6 genera each were obtained from the diseased samples in these locations.

The most dominant fungi were *Fusarium* in both root and fruit samples, followed by *Rhizoctonia*, *Diplodia*, and *Pythium* from roots, and *Diplodia*, *Colletotrichum* and *Pestalotia* from the fruits.

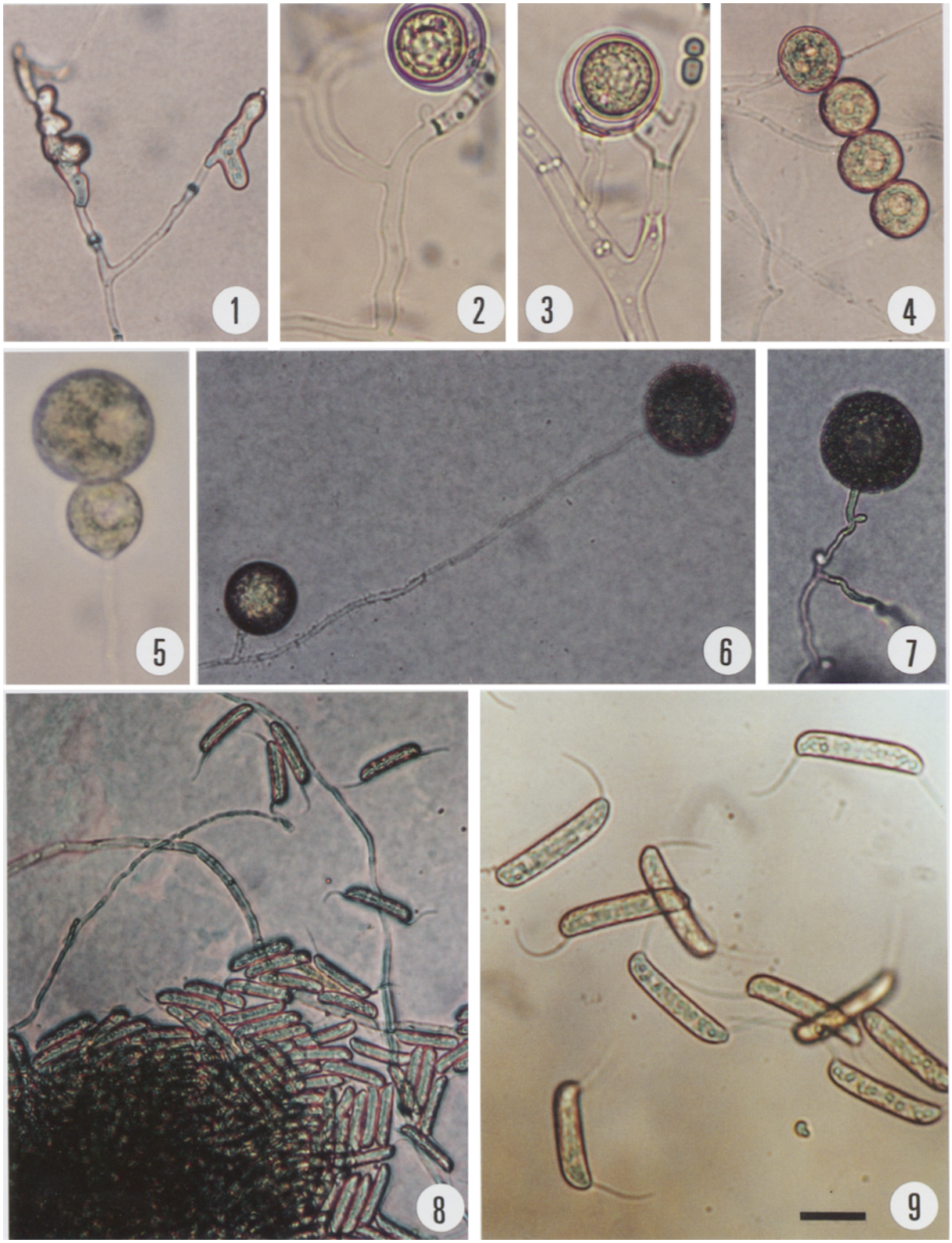
The 454 *Fusarium* isolates from the roots accounted for 75.3% of all of the root isolates, and among them, *F. solani* (Mart.) App. & Wr. emend. Snyder & Hansen was predominant (63.8% of 603 isolates). The 170 *Fusarium* isolates from the fruits accounted for 32.9% of the 516 fruit isolates, and *F. moniliforme* (Sheldon) emend. Snyder & Hansen was predominant (20.7%). *Rhizoctonia* was frequently isolated from the roots, but it was detected only once on the fruits from SP samples. The 44 isolates included three types of *R. solani* on the basis of colony morphology, and an unidentified species (Isolate SP24) (=ATCC 200568) with blackish colony and black sclerotia. The 134 isolates of *Diplodia frumenti* Ellis & Everh. were obtained from roots and fruits at all locations. The 12 isolates of *Pythium* were identified into three species, *P. deliense* Meurs, *P. intermedium* de Bary, and *P. splendens* Braun, and unidentified species. In addition, *P. acanthophoron* Sideris and *P. sylvaticum* Campbell & Hendrix were detected on the isolation media during this study.

The 94 *Colletotrichum* isolates from the fruits were

Table 1. List of fungi with number of isolates associated with roots and fruits of black peppers collected from four locations at Exhibition Farms of La Majagua (LM), Tojin (T), and Sierra Prieta (SP), and CENDETECA (K) in the Dominican Republic.

Genus	Roots				Fruits			
	LM	K	T	SP	LM	K	T	SP
Acremonium	+[a]		7	+		1	1	
Aspergillus				3				+
Botryodiplodia	1							
Candida								+
Chaetomium					+	+		
Chalara				+			1	
Cladosporium		+			1	+		
Colletotrichum				1	40	21	25	8
Cunninghamella		1						
Curvularia					3		1	1
Cylindrocarpon		+						
Cylindrocladium	+	+	2			+	+	
Dactylaria		+				+		
Dematophora				1				
Diplodia	6	2	5	4	33	9	74	1
Fusarium	27	151	143	133	20	57	75	18
Gliocladium			2			+		
Gongronella			1	+				
Humicola			1					
Mortierella	2	5	1	1	+	2		
Mucor			+	2		1	18	5
Mycoleptodiscus				5				
Nigrospora								+
Oidiodendron		1		1		+		
Paecilomyces							+	+
Penicillium			+	1				
Pestalotia				1	3	37	1	13
Phoma						6		
Phomopsis						9		3
Pyrenochaeta								5
Pythium	1	8		3				
Ramichloridium			+			+		
Rhizoctonia	5	23	8	8				+
Sarcopodium		+		+				
Stachybotrys			+					
? Stilbum				3				
Trichoderma			1	+		+		+
Verticillium			+		+	+	+	
Volutella		5		+				
Unidentified		1	1	25	1	21		1
Total	42	197	172	192	101	164	196	55

a) Detected on the isolation plates but not studied further.



Figs. 1–9. Three *Pythium* species (1–7), and *Mycoleptodiscus terrestris* (8, 9).
1–3. *P. deliense* (Isolate K6). Immature lobate sporangia (1) and sexual organs bearing single oospores (2, 3). Note the oogoniumphore bending toward the antheridium (2). 4, 5. *P. intermedium* (K5). Catenulate hyphal swellings (4, 5). Note basipetal development (5). 6, 7. *P. splendens* (LM50). Large terminal hyphal swellings (6, 7). 8, 9. *M. terrestris* (SP22). Sporodochium (8) and detached conidia (8, 9). Scale bar: Figs. 1, 4, 6–8 = 30 μm; 2, 3, 5, 9 = 15 μm.

not readily separable on the basis of colony characteristics. Isolates forming salmon-pink sporodochia often without setae and having hyaline cylindrical conidia with rounded ends, 10–20 × 4.5–5 μm, were tentatively identified as *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz., isolates forming sporodochia with a few setae and elliptical conidia, mostly 10–14 × 5.5–7.5 μm, were identified as *C. acutatum* J. H. Simmonds, following the previous reports (Gunnel and Gubler, 1992; Sutton, 1980), but isolates (e. g., LM21) forming sporodochia with setae and hyaline, rather shorter cylindrical conidia, measuring 5–6.3 × 2.5–3.5 μm could not be identified. Only one isolate of *Colletotrichum* was obtained from the roots in this study. The 55 isolates of *Pestalotia* were also varied in colony morphology, but no further work on speciation was conducted.

The total of 1,119 isolates from samples of root segments and fruits on WA belonged to at least 39 genera (species name and isolate number for representative isolates are given in parentheses): *Acremonium* (*Acremomium* spp., T4, T10, T27, T32; LM2; K45), *Aspergillus* (*Aspergillus* sp., sect. *Clavati*, SP2, *Aspergillus* sp. SP21), *Botryodiplodia* (*Botryodiplodia* sp., LM4, LM15), *Chalara* (*Chalara* sp., T36), *Cladosporium* (*C. cladosporioides* (Fresn.) de Vries, LM17), *Colletotrichum* (*C. acutatum*, LM11, LM25; K24, *C. gloeosporioides*, LM22, LM23; K25; T37, T38, *Colletotrichum* spp., LM2, LM21; SP4, SP48-SP51, SP53), *Cunninghamella* (*C. echinulata* (Thaxter) Thaxter, K7), *Cylindrocladium* (*C. tenue* (Bugnicourt) T. Watanabe, LM51; T16 (=ATCC 200586)), *Curvularia* (*C. affinis* Boedijn, LM18; *C. lunata* (Wakker) Boedijn, T35; SP45), *Diplodia* (*D. frumenti* Ellis & Everh., LM10, LM20; K23, K26; T7, T34; SP23), *Fusarium* (*F. lateritium* Nees: Fr., K32; T23, T30, *F. oxysporum* Schlechtend.: Fr., LM7, LM17; K17; T1; SP13, SP38, *F. solani*, LM13; K16; T3, T5; SP1, *F. moniliforme*, LM14, LM16; K39; T28, T39, *F. roseum* (Lk.) Snyder & Hansen, K40; T8; SP18, *Fusarium* sp., K11, K47; T19), *Gliocladium* (*G. catenulatum* Gilman & Abbott, T11, T24), *Gongronella* (*G. butleri* (Lendn.) Peyronel & Dal Vesco, T26), *Humicola* (*H. fuscoatra* Traaen, T17), *Mortierella* (*Mortierella* spp., LM15; K8-K10; T18; SP6), *Mucor* (*Mucor* spp., K51; T29; SP15, SP39), *Mycoleptodiscus* (*M. terrestris* (Gerd.) Ostazeski, SP3, SP22 (=ATCC 200587)) (Figs. 8, 9), *Oidiodendron* (*Oidiodendron* sp., K14; SP32), *Penicillium* (*Penicillium* sp., SP30), *Pestalotia* (*Pestalotia* spp., LM3; K29, K36; T31; SP10, SP19, SP33-SP37, SP41), *Phoma* (*Phoma* sp., K27), *Phomopsis* (*Phomopsis* sp., K31, K38; SP44), *Pyrenochaeta* (*Pyrenochaeta* sp., SP43, SP52, SP54). *Pythium* (*P. deliense*, K6 (=ATCC 200559), *P. intermedium*, K5 (=ATCC 200569), *P. splendens*, LM50; K4 (=ATCC 200560); SP7, *Pythium* sp., SP5) (Figs. 1–7), *Rhizoctonia* (*R. solani* Kuehn, LM12; K12, K13; T6, T14, *Rhizoctonia* sp., SP24 (=ATCC 200568)), *Stilbum* (*Stilbum* sp., SP20), *Trichoderma* (*Trichoderma* sp., T15), *Volutella* (*Volutella* sp., K51), and unidentified fungi including one basidiomycetous fungus from the fruit with clamped hyphae (K43). Eight of these genera, i.e., *Botryodiplodia*, *Cunninghamella*, *Dematophora*, *Mycoleptodiscus*, *Penicillium*, *Pythium*, *Stilbum* and *Volutella* were isolated only from the root samples, whereas 4 genera, i.e., *Curvularia*, *Phoma*, *Phomopsis*, and *Pyrenochaeta* were obtained only from the fruit samples. In addition, 9 genera, i.e., *Candida*, *Chaetomium*, *Cylindrocarpon*, *Dactylaria*, *Nigrospora*, *Paecilomyces*, *Ramichloridium*, *Sarcopodium*, *Stachybotrys* and *Verticillium* were identified on WA but not further studied.

Some fungi were mycologically noteworthy. For example, isolate K5 was tentatively identified as *Pythium intermedium* on the basis of chains of hyphal swellings basipetally developed (Figs. 4, 5), and non-formation of sexual organs, but the hyphal swellings were larger (up to 30 μm), as compared with the previous reports (Plaats-Niterink, 1981; Watanabe, 1983, 1994), and non-deciduous. The optimum temperature for growth of this fungus was 28°C, versus 23–25°C for other isolates in the previous reports. Five root isolates (SP3, SP22) from SP formed sporodochia with conidia bearing two filiform appendages (Figs. 8, 9) in fresh cultures within 10 d after inoculation. Conidia were hyaline, aseptate or 1-septate, mostly 30 × 5 μm, with two filiform appendages from both ends laterally. In further cultural works, irregular-shaped black sclerotia (up to 1 mm) or stroma were formed abundantly on agar cultures, but sporulation was observed very rarely; these isolates were identified as *Mycoleptodiscus terrestris*. They grew in the range of 7 to 37°C with the optimum temperature of 22°C.

More than 60 isolates from petioles, although not included in Table 1, were also studied during this study and identified as belonging to eight genera, i.e., *Acremonium*, *Botryodiplodia*, *Chaetomium*, *Colletotrichum*, *Diplodia*, *Fusarium*, *Pestalotia*, and *Trichoderma*.

Nine isolates were pathogenic toward excised black pepper leaves among 174 fungal isolates tested; others were very weakly pathogenic or non-pathogenic. Brown lesions (up to 18 mm in diam) often with a halo or yellowing spots developed around the point of inoculation on the inoculated upper leaves with 3 isolates of *Phomopsis* (K31, K38, K53). Smaller lesions (3–7 mm in diam) developed with 1 isolate of *Pestalotia* (SP34). In addition, tiny lesions (less than 2 mm in diam) developed with 3 isolates of *Colletotrichum* (T33, SP50, SP53), and 1 isolate each of *Diplodia* (T34) and *Pestalotia* (SP33). All of these isolates were obtained from the fruits.

The seedlings inoculated with *P. deliense* (Isolate K6) and *P. splendens* (LM50, K4, SP7) in soil were diseased within 10 d after inoculation. None of the controls were diseased (Matsuda et al., 1996, unpublished).

In these pathogenicity tests, three isolates of *Phomopsis*, and one of *Pestalotia* were definitely pathogenic toward leaves, and two *Pythium* species were also pathogenic toward roots. Further work on the pathogenicity of these fungi is in progress.

Acknowledgements——The senior author wishes to thank Japan International Cooperation Agency (JICA), Tokyo, Japan for the financial support for his trip to The Dominican Republic from April to June, 1996.

Literature cited

Gunnel, P. S. and Gubler, W. D. 1992. Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. Mycologia **84**: 157–165.

Matsuda, A., Hamada, M. and González, J. L. 1994. Outbreak and control of foot rot on black peppers in Dominican Republic. Agrochemicals Japan, No. **64**: 23–26.

Plaats-Niterink, A. J. van der. 1981. Monograph of the genus *Pythium*. Stud. Mycol. **21**: 1–242.

Sutton, B. C. 1980. The Coelomycetes, Fungi imperfecti with pycnidia, aceruvuli and stromata. Commonwealth Mycol. Inst., Kew, England.

Watanabe, T. 1983. Formation and deciduousness of sporangia of *Pythium intermedium*. Trans. Mycol. Soc. Japan **24**: 25–33.

Watanabe, T. 1994. Pictorial atlas of soil and seed fungi. Lewis Publ., Boca Raton, Fl., U.S.A.