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J. Bernier, O. Carisse and T.C. Paulitz

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Article abstract

Venturia inaequalis, the causal agent of apple scab, overwinters in apple (*Malus pumila*) leaves on the orchard floor by producing pseudothecia. The objectives of this survey were to make a collection of fungi to be subsequently tested for their potential as psychrophile biocontrol agents against *V. inaequalis* and to acquire knowledge on the diversity of the microflora of dead apple leaves. Fungi were recovered from dead apple leaves collected in the spring and fall of 1993. A total of 345 isolates from 49 genera were identified. Fifteen gene were not previously recorded as colonizers of apple leaves in North America.

Communication brève / Short Communication

Fungal communities isolated from dead apple leaves from orchards in Quebec

Julie Bernier[1], Odile Carisse[2], and Timothy C. Paulitz[1]

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Venturia inaequalis, the causal agent of apple scab, overwinters in apple (*Malus pumila*) leaves on the orchard floor by producing pseudothecia. The objectives of this survey were to make a collection of fungi to be subsequently tested for their potential as psychrophile biocontrol agents against *V. inaequalis* and to acquire knowledge on the diversity of the microflora of dead apple leaves. Fungi were recovered from dead apple leaves collected in the spring and fall of 1993. A total of 345 isolates from 49 genera were identified. Fifteen genera were not previously recorded as colonizers of apple leaves in North America.

Bernier, J., O. Carisse et T. C. Paulitz 1996. Communauté fongique isolée des feuilles mortes de pommiers dans des vergers du Québec. PHYTOPROTECTION 77 : 129-134.

Le champignon causant la tavelure du pommier, *Venturia inaequalis*, hiverne dans les feuilles mortes de pommier (*Malus pumila*) sous forme de pseudothèces. Les objectifs de cette étude étaient de monter une collection de champignons afin de vérifier subséquemment leur résistance au froid et leur potentiel antagoniste contre *V. inaequalis* et d'acquérir des connaissances sur la microflore des feuilles mortes de pommiers. Des champignons ont été isolés sur des feuilles mortes de pommiers récoltées au printemps et à l'automne de 1993. Au total, 345 isolats fongiques provenant de 49 genres ont été identifiés. Quinze genres sont rapportés pour la première fois comme colonisateurs des feuilles de pommiers en Amérique du Nord.

In Quebec, apple scab caused by the fungus *Venturia inaequalis* (Cke.) Wint., is the most important disease in apple (*Malus pumila* Mill.) production. Effective control of apple scab requires from 6 to 16 fungicide applications every season (Jones and Aldwinckle 1990). On top of the environmental problems that result from fungicide applications, the pathogen is becoming increasingly resistant to fungicides, particularly to benomyl [methyl-N-(1-butylcarbamoyl)-2-benzimidazole carbamate], dodine [n-dodecyl guanidine acetate] and fenarimol [2,4'-dichlorophenyl-alpha-pyrimidin-5-pirimidinemethanol] (Carisse and Pelletier 1994; Jones 1981). Therefore, there is an increasing interest in alternative ways to

1. Macdonald Campus of McGill University, 21 111 Lakeshore Road, Sainte-Anne-de-Bellevue, Quebec, Canada H9X 3V9
2. Agriculture and Agri-Food Canada, 430 Gouin Blvd., Saint-Jean-sur-Richelieu, Quebec, Canada J3B 3E6. Author to whom correspondence should be addressed

control this disease including biological control.

Because *V. inaequalis* grows as a saprophyte when it overwinters in the apple leaf litter, researchers have looked for possible organisms colonizing dead apple leaves. Cinq-Mars (1949) and Ross (1953) recovered apple leaf microflora to find potential natural antagonists of *V. inaequalis*. This approach was also pursued by Andrews and Kenerley (1979), Andrews *et al.* (1983), Heye (1982) and Simard *et al.* (1957). From all these studies, one interesting potential antagonist, *Athelia bombacina* (Heye 1982), was found in Wisconsin but has never been commercialized. Since the work of Andrews and Kenerley (1979), Andrews *et al.* (1983) and Heye (1982), this strategy has not been pursued. In addition, no work has focused on organisms adapted to the cold climatic conditions of eastern Canada. Rather than testing biocontrol agents developed in warmer climates, we proposed to search for indigenous organisms in Quebec.

We hypothesized that potential antagonists are present on apple leaves and that sampling of several orchards should provide a large diversity of microbial genera and increase the chance of finding potential biocontrol agents. Knowledge of the diversity of apple leaf microflora would contribute to develop a biological control agent against apple scab, particularly for orchard testing and for studies on fitness and adaptability of the biocontrol agent.

The objectives of this survey were to make a collection of fungi to be subsequently tested for their potential as biocontrol agents and to acquire knowledge on the diversity of the microflora of dead apple leaves.

The sampling was done in six apple (*M. pumila* cv. McIntosh) orchards representing different apple growing regions in the province of Quebec, Canada. The orchards were situated at Covey Hill (lat. 45°01′ N, long. 73° 48′ W), Deschambault (lat. 46°39′ N, long. 71°56′ W), Frelighsburg (lat. 45°03′ N, long. 72°50′ W), Île d'Orléans (lat. 46°55′ N, long. 70°58′ W), Mont Saint-Hilaire (lat. 45°33′ N, long. 73°10′ W), and Saint-Joseph-du-Lac (lat. 45°32′ N, long. 74°00′ W). These orchards had been abandoned for more than 5 yr. It is very unlikely that fungicide treatment or residues could have affected the natural fungal microflora. Dead apple leaves lying on the ground were collected arbitrarily twice in 1993. The first collection was done in the spring after snow melt between 20 April and 23 April. The second collection was carried out just before the first snow fall in the fall, between 9 November and 15 November. The leaves were stored in paper bags and refrigerated at 4°C until processed (not more than 4 wk later).

Two isolation methods were employed to recover the largest number of organisms possible. In the first method, for each orchard, arbitrarily chosen leaves were placed in glass petri dishes of 9-cm diam containing a Whatman filter paper saturated with distilled water to provide a moist environment. The petri dishes (four plates for each temperature level) were incubated at each of eight different temperatures (-2, 0, 2, 4, 6, 10, 18, and 24°C), to favor growth of mesophilic and psychrophilic fungi, for 2-3 wk. The leaves were observed under a dissecting microscope and each mass of spores, fruiting bodies or mycelia was transferred to a half-strength V8 agar media (Dhingra and Sinclair 1985) amended with 100 $\mu g\ mL^{-1}$ of chlorotetracycline and 200 $\mu g\ mL^{-1}$ of streptomycin.

For the second method, 10 g of non-rinsed leaves were homogenized in a blender with 100 mL distilled water. The homogenates were diluted from 10^{-1} to 10^{-3} with four replicates per dilution. Aliquots of 0.5 mL of the suspensions were spread on V8 agar medium (half strength, with antibiotics as described above) and on potato agar made from an infusion of 200 g of unpeeled potatoes boiled for 0.5 h. The latter medium (used to favor the basidiomycetes) was also amended with 15 $\mu g\ mL^{-1}$ of benomyl (diluted in 95% alcohol). Antibiotics and the fungicide were added after autoclaving. In this method, there were four replicate plates for each medium for each dilution. There were 144 plates (6 orchards x 2 media x 3 dilutions x 4 replicates) incubated at each of the eight temperature levels from -2 to 24°C for 3-4 wk.

Colonies with different morphologies were transferred to V8 medium. Isolated fungi were identified according to the morphology of their structures (Barnett and Hunter 1987; Hanlin 1990).

From the spring collection, 189 different isolates were obtained and 71% of them were identified. They belonged to 38 different genera (Table 1). The most common genera were: *Alternaria, Cladosporium, Coniothyrium, Penicillium, Phoma, Trichoderma* and members of the order Mucorales. *Aspergillus, Brachysporium, Curvularia, Geotrichum, Monilia, Mycogone, Tuberculariа,* and yeasts were isolated only from homogenized leaves. The genera *Arthrobotrys, Cephalosporium, Chaetomium, Chaetophoma, Chalara, Diplodia, Hendersonia, Humicola, Hyalodendron, Paecilomyces, Pestalotia, Pyrenochaeta, Rhinotrichum, Rhizoctonia, Selenophoma, Sphaeropsis, Varicosporium* and *Verticillium* were isolated only by the intact leaf method. From the autumn collection, 156 different isolates were obtained and 69% were identified, for a total of 26 genera (Table 1). *Alternaria, Candida, Cladosporium, Coniothyrium, Epicoccum, Trichoderma,* members of the Mucorales and yeasts were the most common. *Bactrodesmium, Ceratosporella, Chaetomium, Cylindrocarpon, Gilmaniella, Gliomastix, Monilia, Papularia, Phoma* and various Mucorales were isolated only from diluted leaf homogenates. *Melanconium, Sclerotinia, Trichoderma* and *Trichothecium* were isolated only by the intact leaf method. When the two collections were compared (Table 1), 23 genera were found only in the spring, conversely 11 genera appeared only in the fall collection.

Because the leaves used for dilution plating were not surface sterilized, some of the organisms recovered may have come from the phylloplane, and may not be endophytes. Genera found only by the dilution plating method may not be common on or in the leaves, so they would be difficult to isolate from intact leaves because of competition from faster growing fungi. In fact, several fungi were isolated only once. On the other hand, it was demonstrated (Petrini 1991) that some epiphytes may, under appropriate conditions, colonize the interior of the host tissues. In the dead leaves used in the present study, it was assumed that there was no host specificity required for the endophytes to colonize the tissue due to the absence of host defenses present in living plants. Some epiphytic fungi may switch to an endophytic life style, first to decompose the leaf, and second to protect themselves against adverse conditions (snow cover) and also to reduce antagonistic activities by competitive microorganisms.

According to Bessey (1950) and Barnett and Hunter (1987), 18 of the 49 genera obtained are recorded as saprophytes, 25 as both saprophytes and parasites, and 6 as parasites only. More specifically, 11 of these potentially parasitic genera are recorded as apple tree pathogens in QSPP (1992) including *Alternaria, Cladosporium, Coniothyrium,* and *Fusarium.* Since many isolates were not identified to the species level, we do not know whether the specific isolates found in the orchards are pathogenic to apple.

The fungi were classified into three groups, depending on the range of temperatures at which they were isolated. Fungal genera isolated at cold temperatures (-2 to 10°C) were *Aspergillus, Cylindrocarpon, Diplodia, Geotrichum, Hendersonia, Selenophoma, Sphaeropsis, Tuberculariа, Varicosporium,* and *Verticillium.* Genera of warm temperatures (18 and 24°C) were *Arthrobotrys, Brachysporium, Chaetophoma, Chalara, Curvularia, Gilmaniella, Gliomastix, Humicola, Hyalodendron, Melanconium, Monilia, Mycogone, Pestalotia, Rhinotrichum, Sclerotinia,* and *Trichothecium.* The following genera, were isolated from cold as well as from warm temperatures and constitute the last group, *Alternaria, Aureobasidium, Bactrodesmium, Botrytis, Candida, Cephalosporium, Ceratosporella, Chaetomium, Cladosporium, Coniothyrium, Epicoccum, Fusarium, Mortierella,* order Mucorales, *Paecilomyces, Papularia, Penicillium, Phoma, Pyrenochaeta, Rhizoctonia, Trichoderma, Ulocladium,* and yeasts.

The fungi isolated solely at cold temperatures were infrequent, so we cannot determine *per se* whether more samples would have revealed their presence at

Table 1. Number of isolates of 49 fungal genera recovered from dead apple (*M. pumila*) leaves in Quebec orchards in spring and fall 1993[a]

Genera	Spring		Fall	
	Method 1 (no. isolates)	Method 2 (no. isolates)	Method 1 (no. isolates)	Method 2 (no. isolates)
Alternaria	19	2	6	7
Arthrobotrys	1	-	-	-
Aspergillus	-	1	-	-
Aureobasidium	3	1	2	4
Bactrodesmium	-	-	-	2
Botrytis	2	1	-	-
Brachysporium	-	1	-	-
Candida	5	2	1	10
Cephalosporium	3	-	-	-
Ceratosporella	-	-	-	6
Chaetomium	1	-	-	1
Chaetophoma	1	-	-	-
Chalara	1	-	-	-
Cladosporium	21	9	12	30
Coniothyrium	12	2	1	14
Curvularia	-	1	-	-
Cylindrocarpon	-	-	-	1
Diplodia	1	-	-	-
Epicoccum	1	2	7	15
Fusarium	6	5	4	4
Geotrichum	-	2	-	-
Gilmaniella	-	-	-	1
Gliomastix	-	-	-	1
Hendersonia	1	-	-	-
Humicola	1	-	-	-
Hyalodendron	1	-	-	-
Melanconium	-	-	1	-
Monilia	-	1	-	1
Mortierella	-	-	2	1
Order Mucorales	1	12	-	11
Mycogone	-	3	-	-
Paecilomyces	2	-	-	-
Papularia	-	-	-	2
Penicillium	14	3	1	8
Pestalotia	1	-	-	-
Phoma	10	1	-	8
Pyrenochaeta	2	-	-	-
Rhinotrichum	1	-	-	-
Rhizoctonia	1	-	1	1
Sclerotinia	-	-	1	-
Selenophoma	1	-	-	-
Sphaeropsis	1	-	-	-
Trichoderma	11	9	10	-
Trichothecium	-	-	1	-
Tubercularia	-	1	-	-
Ulocladium	-	-	1	1
Varicosporium	1	-	-	-
Verticillium	1	-	-	-
Yeasts	-	5	1	14

[a] Two isolation methods were employed : method 1 refers to isolation under binocular and method 2 refers to isolation from a leaf decoction plating.

higher temperatures. The most common genera were isolated relatively equally over the whole temperature range. Further testing would be needed to determine whether the isolates are true psychrophiles. Nevertheless, using a range of isolation temperatures has increased the diversity of isolates.

It was also noticed that some genera were found only in specific orchards (Fig. 1). For example, in the spring collection, nine genera were found only in the Mont-Saint-Hilaire orchard. Sampling only one orchard would have resulted in a reduced number of genera recovered.

Our collection contained 24 new genera not previously reported from apple leaves. The genera *Bactrodesmium, Brachysporium, Cephalosporium, Ceratosporella, Chaetophoma, Chalara, Curvularia, Cylindrocarpon, Gilmaniella, Hendersonia, Humicola, Melanconium, Monilia, Mortierella, Mycogone, Papularia, Pyrenochaeta, Rhinotrichum, Rhizoctonia, Selenophoma, Sphaeropsis, Tubercularia, Ulocladium, Varicosporium* have not been isolated from green leaves or dead leaves by previous workers (Andrews and Kenerley 1978, 1979, 1980; Ross 1953; Simard *et al.* 1957).

Our collection also contains 9 genera that have not been reported on *Malus* spp. : *Cephalosporium, Cylindrocarpon, Hendersonia, Monilia, Pyrenochaeta, Selenophoma, Sphaeropsis, Tubercularia,* and *Ulocladium* (Farr *et al.* 1989; Jones and Aldwinckle 1990).

This survey clearly demonstrates the large diversity of fungal species living in or on dead apple leaves. These fungi

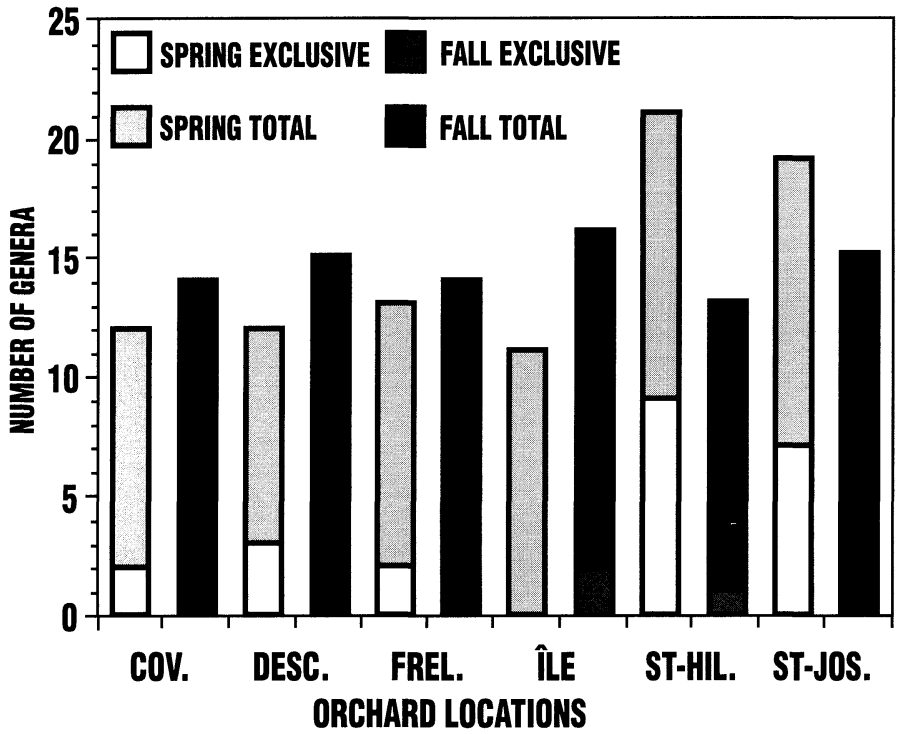

Figure 1. Number of genera isolated from dead apple leaves *(M. pumila)* collected in orchards at each of six locations in Quebec in the spring and fall of 1993. Total : total number of genera isolated in a given orchard; exclusive : number of genera found exclusively in the given orchard; COV. : Covey Hill; DESC. : Deschambault; FREL. : Frelighsburg; ÎLE : Île d'Orléans; ST-HIL. : Mont Saint-Hilaire; ST-JOS. : Saint-Joseph-du-Lac

may be phylloplane inhabitants of living leaves, airborne colonizers of senescent leaves, or soil inhabitants. Several sampling sites were necessary to provide a large diversity of genera since the composition of the microbial community for each orchard was very different. The large diversity of fungal isolates recovered should increase the chances of finding antagonists that interfere with the overwintering of *Venturia inaequalis*. Sampling in several orchards, during two seasons and using two isolation methods probably helped in obtaining a high number of genera.

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