Latent and Saprophytic Fungal Infections of Grapefruit in South Texas

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ABSTRACT

Colletotrichum sp. and *Alternaria* sp. were the most common fungi isolated and identified from apparently healthy leaves from grapefruit trees in the lower Rio Grande Valley of Texas. These fungi were also the most abundant fungi isolated from incubated detached leaves. *Colletotrichum* sp. was commonly isolated from healthy twigs while *Diplodia* sp. and *Phomopsis* sp. were the most abundant colonizers of dead twigs. Decayed leaves from the orchard floor yielded *Phyllostictina* sp., the first time this fungus has been documented from Texas citrus. Few fungi were isolated from asymptomatic fruit.

INTRODUCTION

Latent fungal infections are invasions of host tissue without the expression of symptoms by the host plant or the appearance of fungal fruiting bodies. Some latent infections are important in the commercial production of citrus since they may become pathological under environmental conditions conducive to their development. For example, latent infections by **Diplodia natalensis** P. Evans under the button of fruit often result in a post-harvest decay of the fruit. Latent infections of leaves and twigs by **Colletotrichum gloeosporioides** Penz. can result in a stem disease called withertip if the climate is conducive to fungal development (Fawcett, 1936). One study indicated that latent infections by **Guignardia citricarpa** Kiely, the cause of citrus black spot, are capable of persisting within plant tissues for years before manifesting themselves in the form of disease (Kotze, 1981). Thus, the study of latent infections can be important in the epidemiology of citrus diseases. The purpose of this study was to identify fungal infections of asymptomatic grapefruit tissue from South Texas by isolation and culture of recoverable fungi and by microscopic examination of grapefruit tissue.

MATERIALS AND METHODS

Leaf samples were collected from 5 grapefruit trees in various locations within a 4-acre 25 year-old orchard at the Citrus Center in Weslaco. In the last week of June 1986, five asymptomatic leaves from the March and June 1986 flushes of growth and from the previous fall growth flush were picked from each tree. Three sections of leaf tissue approximately ¼" in diameter were cut at random from each leaf, surface sterilized for 5 minutes in a 10% sodium hypochlorite solution, and aseptically transferred onto potato-dextrose agar plus streptomycin. Subsequent cultures were grown on V-8 juice agar. At the same time, 5 leaves from the same flushes and trees as above were surface sterilized and placed in a cheesecloth bag in a mist chamber. After drying for 5 days, the leaves were sprayed with a fine water mist for approximately 3 minutes per day for a period of 5 weeks. One leaf disk approximately ¼" in diameter was then cut from each leaf and plated on V-8 juice agar. Five fruit from each tree were also harvested from the test trees. Small pieces of the fruit rind were surface sterilized and plated in a manner similar to the leaves, except that the epidermis of the rind was removed.

To identify fungi from healthy or dead twigs, isolations were made from 220 chips of wood from either asymptomatic live twigs or dead twigs collected in an 8 acre block of 22 year-old grapefruit trees at the Citrus Center. The wood chips were surface sterilized, aseptically transferred to petri dishes of V-8 agar, and incubated 4 to 10 days at room temperature.

Fungal infections were also examined microscopically on fallen leaves on the orchard floor. In addition, single disks were cut from 30 fallen leaves, surface sterilized, and plated as above.

RESULTS AND DISCUSSION

The most common latent infection in healthy fresh grapefruit leaves from all flushes was caused by **Colletotrichum** sp. (Table 1). The number of **Colletotrichum** infections increased as the age of the leaves increased. Similarly, the number of infections by **Alternaria** sp. was greatest in the oldest leaves. The incidence of infection by other fungi was relatively low in all flushes. Only 16% of the leaf disks were apparently not infected by a fungus.

Table 1. Occurrence of latent fungal infections in fresh, asymptomatic grapefruit leaves.[z]

Fungus	Leaf flush		
	Fall 1985	March 1986	June 1986
Colletotrichum sp.	62	56	46[y]
Alternaria sp.	22	4	8
Helminthosporium sp.	0	1	5
Nigrospora sp.	0	7	2
Cladosporium sp.	1	0	0
Unidentified ascomycete	1	0	0
None	9	10	17

[z]Leaf samples collected June 24, 1986.

[y]Number of isolations in 75 leaf disks approximately ¼" in diameter.

When fresh asymptomatic grapefruit leaves were collected and allowed to dry and decay in a mist chamber, **Colletotrichum** sp. and **Alternaria** sp. were the most commonly isolated fungi, although the incidence of their recovery was lower than recovery from fresh leaves (data not presented). However, these leaves did yield three fungi, **Diplodia, Phomopsis,** and **Macrophoma** sp. not isolated from fresh leaves collected and processed directly from the field. Fungal infections were found in only 2 of the 25 pieces of fruit. In healthy grapefruit twigs, **Colletotrichum** sp. was the most commonly encountered fungus (Table 2). **Diplodia** and

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Table 2. Fungi isolated from healthy and dead grapefruit twigs.

Fungus	Number of isolations in 220 pieces of wood	
	Healthy wood	Dead wood
Colletotrichum sp.	185	32
Phomopsis sp.	15	124
Diplodia sp.	12	176
Fusarium sp.	5	9
Nigrospora sp.	2	18

Phomopsis sp. were commonly isolated from dead twigs, whereas **Colletotrichum** was more commonly isolated from healthy twigs. The fungi on the fallen leaves from the orchard floor, identified either directly by microscopic examination of fruiting bodies or indirectly by examination of fungi recovered in culture from leaf disks, are listed in Table 3.

Table 3. Fungi directly and indirectly identified from fallen and decayed grapefruit leaves.

Direct microscopic examination	Indirect identification via culture of leaf disks[z]
Alternaria sp.	**Alternaria** sp.
Ascochyta sp.	**Cladosporium** sp.
Cladosporium sp.	**Colletotrichum** sp.
Colletotrichum sp.	**Diplodia** sp.
Diplodia sp.	**Fusarium** sp.
Fusarium sp.	**Helminthosporium** sp.
Helminthosporium sp.	Unidentified ascomycete
Nigrospora sp.	
Phyllostictina sp.	

[z]30 leaf disks approximately ¼" in diameter were plated on V-8 juice agar.

Phyllostictina sp. and **Ascochyta** sp. were the only fungi identified directly on the leaves from the orchard which were not also identified in cultures of these leaves or in cultures of the leaves taken fresh off the trees. The reason for this anomaly was unknown.

The fungus **Colletotrichum** sp. was the most common organism causing latent infections in Texas grapefruit leaves. Baker (1938) found that this fungus also caused frequent latent infections in fruit from grapefruit trees. Adam **et. al.** (1949) came to a similar conclusion in their studies with oranges. In Texas citrus **Colletotrichum** sp. is commonly associated with damaged citrus tissue but in periods of prolonged rainfall accompanied by warm temperatures a wilt and decay of very young shoots can result from infection. Our results indicate that this fungus is extremely common in citrus tissue but apparently becomes pathogenic only in rare cases.

Latent infections of **Alternaria** sp. are also relatively common in Texas grapefruit. **Alternaria** sp. is often associated with necrotic spots on oranges and grapefruit leaves in Texas, but it is probably a secondary invader of weakened tissue and not a primary causal agent of the leaf spots.

Dead wood from grapefruit twigs was commonly colonized by **Diplodia** and **Phomopsis** sp., fungi present but not common in h ealthy tissue. **Diplodia** sp. has been implicated as a causal agent in Rio Grande gummosis, a disease of citrus in Texas always associated with dead wood (Davis, 1980). **Phomopsis** sp. is the cause of melanose, an important rind blemishing disease of citrus (Fawcett, 1936). Apparently, these fungi quickly colonize dead wood and have the ability, at least to a small degree, to form latent infections in live citrus twigs.

Leaves on the orchard floor were colonized by **Phyllostictina** sp., the imperfect state of **Guignardia. Guignardia citricarpa** is the cause of citrus black spot, a serious disease of citrus in South Africa and Asia. We assume that the organism found in our study is an avirulent **Phyllostictina** sp. since citrus black spot is not present in North America. McOnie (1964a) suggested that **Guignardia citricarpa (Phyllostictina citricarpa)**, the pathogenic species, is not found in localities where citrus black spot symptoms are not evident. However, **Phyllostictina** sp. is a commonly occurring avirulent organism in a wide range of plant species. McOnie clarified this issue by showing that although the pathogenic species and the nonpathogenic species are morphologically indistinguishable, they are physiologically and pathogenically different (McOnie 1964b). An avirulent **Phyllostictina** sp. is known to exist latently in citrus in Florida (J.O. Whiteside, personal communication), but this is the first time this organism has been identified in Texas.

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