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Integrated approach to controlling *Diaporthe* canker of deciduous fruit in South Africa

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ABSTRACT

Diaporthe canker is a newly recognized fungal disease affecting the longevity and productivity of deciduous fruit orchards in the Cape Province of South Africa. A challenge to the South African fruit growing industry is to establish an alternative, non-chemical approach to control stem canker fungi. Such an assignment deals with *Diaporthe ambigua* as a canker pathogen of pome and stone fruit trees in South Africa. Special emphasis is given to the possible development of a biological control strategy. Endeavors included the search for hypovirulence, a reduced virulence associated with the presence of double-stranded RNA (dsRNA).

The introductory section of this chapter presents a comprehensive review of the literature pertaining to those *Diaporthe* spp. directly or indirectly implicated with canker of pome, stone, and woody small fruit trees. Special emphasis is given to the disease situation in South Africa. Over-reliance on host association has led to the description of a plethora of *Diaporthe* spp. on a wide range of hosts. *D. ambigua* is regarded as one of several small-spored species associated with *Diaporthe* canker of pome, stone, and woody small fruit trees, and their rootstocks worldwide.

Results presented in a subsequent section attribute a newly recognized canker disease of apple, pear, and plum rootstocks in South Africa to a species of *Diaporthe*. Supported by morphological data and random amplified polymorphic DNA analysis of South African and reference strains of *Diaporthe*, our research has shown that *D. ambigua* is the most appropriate name for the fungus. Nursery rootstocks infected with *D. ambigua* are readily killed by this fungus. This is in contrast to mature rootstocks which are normally killed over an extended period of time. All deciduous fruit cultivars that we have tested were susceptible to *D. ambigua* infection. A significant strain x rootstock interaction was found, indicating that strains of *D. ambigua* react differently on various rootstocks.

The population of *D. ambigua* in apple, pear, and plum orchards in South Africa represents a large number of vegetative compatibility

groups (VCGs) that occur in close proximity to one another. Results suggest that ascospores act as the primary propagules of infection. Increasing numbers of VCGs in populations lacking predominance of one VCG, as found in *D. ambigua,* may reduce the effectiveness of dsRNA transfer and thus the effectiveness of biological control. However, all dsRNA-containing strains of *D. ambigua* originated from ascospores representative of a spectrum of VCGs, suggesting transfer of dsRNA to sexual progeny. Based on its association with ascospores, it is possible that the relatively small dsRNA element in *D. ambigua* is associated with mitochondria.

Interest in the viral dsRNA associated with hypovirulence of *D. ambigua* stemmed primarily from its potential as a biological control agent. Strains of this fungus harboring the single dsRNA segment, although able to initiate an infection, generally cause only limited invasion. Results presented in a following section demonstrate the conversion of vegetatively compatible virulent strains of *D. ambigua* to the hypovirulence phenotype following transmission of the hypovirulence-associated dsRNA via anastomosis. In addition to reduced virulence, the dsRNA-containing strains (original and converted) were also shown to display various hypovirulence-associated traits. These traits included reduced laccase activity, reduced gallic acid oxidation, diminished oxalate accumulation, and suppressed sporulation.

Hybridization among single dsRNA segments from hypovirulent *D. ambigua* strains indicates a high degree of homology. The presence of *D. ambigua* in a localized area carrying a single dsRNA segment of similar size and homology suggests that natural spread of dsRNA within the *D. ambigua* population has occurred. Hypovirulent strains of *D. ambigua* appear to occur naturally in the South African population of the pathogen. These strains are less virulent than dsRNA-free strains. Furthermore, hypovirulence can apparently be transferred easily to dsRNA-free strains. An ideal opportunity, therefore appears to exist for biological control of *D. ambigua* using hypovirulence. Moreover, it could provide insight into our understanding of engineering of hypovirulence in the future.

INTRODUCTION

Sporadic outbreaks of *Diaporthe* canker on fruit trees, attributed to a variety of species of the fungus, have been reported worldwide (12,19,20,36,48,113,127). Typical lesions generally appear only two years after infection (36,90). This explains unsuccessful inoculations attempts (48) and the 'absence' of disease in current and biennial shoots (36). A lack of knowledge with respect to dissemination of the pathogen, infection biology, and disease development has contributed to an underestimation of the importance of *Diaporthe* canker in the past (36).

Persistent research of *Diaporthe* canker in Japan, where it is also known as European pear canker, has led to acknowledgement as the most serious disease of *Pyrus* L. (pear) trees. *Diaporthe* canker on *Malus* Borkh. (apple) in Japan is known as apple blight (59).

Symptoms of *Diaporthe* canker are often confused with other well-known, serious canker diseases such as fire blight caused by *Erwinia amylovora* (Burr.) Winslow *et al.*

(134). Current apple and pear shoots are particularly susceptible to infection (59). When *Diaporthe* canker first appeared on *Pyrus*, there was concern that fire blight had spread to Japan. Subsequent studies on the etiology of the disease, however, showed that fire blight was not involved in that outbreak (112).

The severity of *Diaporthe* canker in South Africa on hosts other than fruit trees became evident with the destruction of entire *Aspalathus linearis* (Burm. f.) R. Dahlg. (rooibos tea) plantations in the Cape Province (116). *A. linearis* is an indigenous, perennial, leguminous plant and used for the production of a herbal tea. Nortier, the only *A. linearis* cultivar grown commercially, is highly susceptible to *Diaporthe* canker.

In an attempt to identify the cause of the disease of *A. linearis*, Smit & Knox-Davies (117) compared *Diaporthe* strains isolated from *A. linearis* with *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. f. sp. *caulivora* Ath. & Cald. strains originating from *Glycine max* (L.) Merr. (soybean) in the southeastern United States of America. Both *A. linearis* and *G. max* belong to the Fabaceae (Leguminosae) (102), justifying this comparison. Anamorph and teleomorph characters of the fungus causing canker of *A. linearis* were identical to those of *D. phaseolorum* f. sp. *caulivora* (117). Moreover, the *Diaporthe* strains from *A. linearis* caused typical stem canker symptoms when inoculated into soybean (W.A. Smit, *unpublished data*). All the strains causing canker of *A. linearis* were thus considered to belong to the single species, *D. phaseolorum* (*sensu lato*). No *formae speciales* or varieties were designated for these strains (117).

Diaporthe canker on pome fruit trees was first detected in South Africa in 1989 when a fungus tentatively identified as *D. phaseolorum* was isolated from 3-year-old *Pyrus* rootstocks. The first *Diaporthe*-infected *Malus* rootstocks were found in a nursery during 1990. *Diaporthe* canker, apparently to a lesser extent, also occurs on stone fruit rootstocks such as Marianna (*Prunus* L.) (118,119).

Morphological similarity between South African strains of *Diaporthe* isolated from *A. linearis*, *Malus*, *Pyrus* and *Prunus* suggests that they represent a single species. Differences in their host range, however, has necessitated a more thorough comparison (119). One aim of this review is to provide background information to the taxonomy of *Diaporthe* spp. Special emphasis is also placed on the intensively studied *D. phaseolorum*, known for its complex nomenclatural history.

This chapter also discusses recent progress on three major fronts. The first focuses on the impact of a new and serious canker disease caused by *Diaporthe ambigua* Nits. The clonal nature of the majority of *Pyrus* rootstocks cultivated in South Africa underlies the particular importance of the disease. The second deals with the determination of vegetative compatibility among strains of *D. ambigua*. The efficacy of a potential biological control strategy against *Diaporthe* canker is largely dependent on the phenomenon of vegetative compatibility as shown in various ascomycetous fungi. The implications of vegetative compatibility are discussed with reference to the sexuality of the fungus and the transmission of double-stranded RNA (dsRNA)-mediated hypovirulence. The third involves advances in the development of dsRNA (mycovirus) biology and the extended experimental and practical utility provided by

these advances.

TAXONOMY OF *DIAPORTHE*

Diaporthe Nits. is the type genus of the Diaporthaceae. The genus constitutes the largest group in the family, although many species have been segregated from it to establish related new genera. Genera have also been transferred to *Diaporthe* following critical study (86,110,129,137,139).

Considerable difficulty has been experienced by mycologists in identifying *Diaporthe* spp., because many different species have been described on a wide range of different hosts (42,61,65,131,135,137). Wehmeyer (137) monographed *Diaporthe* and related genera and synonymised many species. Thus the total number of the *Diaporthe* spp. was reduced to seventy. In Wehmeyer's study, species with small ascospores had a large number of synonyms and a wide host range, whereas the species concept for the large-spored species was relatively narrow, with a limited host range. Wehmeyer (137) as well as Munk (87) suggested that the number of *Diaporthe* spp. could decrease further after critical comparative studies, including the teleomorph and anamorph states of the large-spored species.

In his monograph, Wehmeyer (137) recognized two sections for *Diaporthe*, based on entrostromatic development without reference to the host. Section *Effusae* was defined by those species with dorsal blackened zones of scattered or clustered perithecia, within an entostromatic area that does not penetrate into bark tissues. Section *Pustulatae* accommodated species with dorsal blackened zones that dip into the bark between perithecial groups. Munk (87) and Gilman *et al.* (42), also followed the lead of Wehmeyer (137) and grouped the species of *Diaporthe* on the basis of stromatic configuration. For primary separation of groups of *Diaporthe* spp., Kobayashi (65) and Barr (13) chose to emphasize differences in ascospore morphology. This was due to the fact that stromal type and presence of blackened zones is not always obvious in individual collections (13).

In recent years, re-evaluation of the status of the *Diaporthe* spp., and the basis of recognition of strains at varietal level, has been conducted (67,84,85). A number of characteristics contribute to difficulties in designing a satisfactory classification of *Diaporthe* spp., particularly at infraspecific level. Among the more significant of these are the plurivorous nature of *D. phaseolorum* and the fact that it is highly variable both in morphology and in its ability to cause disease (84,85). Recent investigations also suggested that pathogenicity may, at least in part, be affected by cytoplasmic agents, possibly dsRNA. Furthermore, the use of terms such as variety, *forma specialis*, biotype and race has not always been consistent. Difficulty in properly defining such entities remains a serious problem.

DIAPORTHE SPECIES ASSOCIATED WITH FRUIT TREES

Several *Diaporthe* spp., directly or in association with synonymous species, have been implicated in canker and die-back diseases of pome fruit (*Malus*; *Pyrus*), stone fruit (*Prunus*), and woody small fruit (*Morus* L. [mulberry]) trees, and their rootstocks

Table I. Host range of *Diaporthe* species implicated in canker and die-back diseases of pome, stone, and woody small fruit trees, and their rootstocks

Diaporthe species	Host[a]	Reference
D. ambigua	Ma	96
	Py	45,111,131,137
D. crataegi	C	31,131
D. eres	C	42,137
	Ma	31,137
	Pr	31,42,53,137
	Py	31,42,137
	S	31,137
D. impulsa	Py	30,136
	S	135,136,137
D. medusaea	Mo	65
	Py	37
D. melanocarpa	A	42
	Py	137
D. nomurai	Mo	65, 114
D. padi	Ma	65
	Pr	41,42,137
	S	137
D. perniciosa	Ma	17,29,89,95
	Pr	17,20,26,31,48,49,70,95,108,128
	Py	19,95
D. phaseolorum	Ma	119
	Pr	119
	Py	119
D. pruni	Pr	41,42,135
D. prunicola	Pr	41,42,65
D. rudis	C	31
D. tanakae	Ma	36
	Py	66,88,90,91,113,127,130
D. tuberculosa	A	30,42

[a] A= *Amelanchier* spp.; C= *Crataegus* spp.; Ma= *Malus* spp.; Mo= *Morus* spp.; Pr= *Prunus* spp.; Py= *Pyrus* spp.; S= *Sorbus* spp.

(Table I). *Prunus* refers to one or more cultivar(s) included in the sub-genera *Prunophora* Focke (apricot; plum), *Amygdalus* (L.) Focke (almond; peach), and *Cerasus* Pers. (sweet and sour cherry) (50,103,138). Rootstocks of importance are the genera *Sorbus* L. (mountain ash), *Amelanchier* L. (service berry) and *Crataegus* L. (hawthorn)

A1: Ascospore with appendages
- *D. pruni*

A2: Ascospore without appendages
- B1: Ascospore less than 15 µm in length
  - C1: Ascospore less than 2.5 µm in diameter
    - *D. prunicola*
  - C2: Ascospore 2.5 µm or more in diameter
    - *D. ambigua*
    - *D. arctii*
    - *D. conorum*
    - *D. eres*
    - *D. medusaea*
    - *D. nomurai*
    - *D. perniciosa*
    - *D. phaseolorum*
- B2: Ascospore 12-18 µm (average about 15 µm) in length
  - C1: Ascospore less than 4 µm in diameter
    - *D. padi*
    - *D. rudis*
  - C2: Ascospore 4 µm or more in diameter
    - *D. impulsa*
    - *D. tanakae*
    - *D. tuberculosa*
- B3: Ascospore more than 15 µm in length
  - C1: Ascospore less than 5 µm in diameter
    - *D. melanocarpa*
  - C2: Ascospore 5 µm or more in diameter
    - *D. crataegi*

Fig. 1. Combined key to the species of *Diaporthe*, directly or indirectly associated with pome stone, and woody small fruit trees and their rootstocks, based on ascospore dimensions (references: Table I).

(138).

In the following section, we deal with *Diaporthe* spp. associated with pome, stone, and woody small fruit trees and their rootstocks in alphabetical order. These are also treated in two sections i.e. those with small ascospores (average less than 15 µm in length), and those species with intermediate to large ascospores (average 15 µm or more in length). A key to these species of *Diaporthe*, based on ascospore dimensions, is also provided (Fig. 1).

Species with small ascospores

Of particular importance in this section is the species *D. ambigua*. Arguments for and against synonymy with *Diaporthe eres* Nits. are provided.

D. ambigua (*Diaporthe perniciosa* Marchal). Marchal (according to Cayley, 20) described *D. perniciosa* in 1921 while working on rots of stored fruits in Belgium. Cayley (20) reported *D. perniciosa* to be responsible for a die-back disease of stone fruit trees in Britain. Britton-Jones (19) confirmed her findings and stated that the diseases known as 'bark canker' and 'die-back' in fruit trees, including *Pyrus*, were caused by the same fungus, i.e. *D. perniciosa*. An unusual and serious outbreak of die-back on *Prunus* in the United Kingdom in the summer of 1983 (49) resembled the die-back disease reported by Cayley, earlier this Century (48).

After re-examining of the type species of *D. ambigua*, Wehmeyer (137) included *D. ambigua* on *Pyrus* (described 1867), together with *D. perniciosa* (described in 1921), as synonyms of *D. eres* (described in 1867). More recent reports, however, acknowledge *D. perniciosa* as the cause of canker and die-back of *Malus* (17,89,95), *Pyrus* (95), and *Prunus* (17,26,31,95,128).

Diaporthe eres. This species was treated by Nitschke as the type species of the genus. *D. eres* was retained by Wehmeyer (137) for the large species complex of hosts forms which it represents. Like *Diaporthe arctii* (Lasch) Nits., this species occurs on a large number of hosts and is extremely variable.

Kobayashi (65) identified *D. eres* on the basis of few perithecia constituting a loose group, occurring in the bark of broad-leaved trees, an enclosed black zone enveloping several perithecial groups, loose hyphal elements rather than compact stromatic tissue around the perithecial necks and perithecia, as well as on the basis of ascus and ascospore size. According to Wehmeyer (137) and Kobayashi (65), the perithecial necks of *D. eres* usually do not protrude from the woody bark surface, but elongate slightly under moist conditions. They also do not form clusters such as those found in *Diaporthe medusaea* Nits.

D. arctii, which is similar to *D. eres* on woody stems, is found on a large number of herbaceous plants (137). *D. arctii* is barely distinguishable from *D. eres*, due to the presence of perithecia that occur singly and scattered, and its somewhat larger asci. Perithecial necks also do not protrude from the bark surface. The *Phomopsis* state of *D. arctii* cannot be distinguished from that of *D. eres*, based on morphology (65).

Diaporthe conorum (Desm.) Niessl has a wide host range on coniferous plants and is morphologically very similar to *D. eres*. Wehmeyer (137) treated *D. conorum* as a synonym of *D. eres*. Kobayashi (64,65) has,

however, shown differences in the pathogenicity, physiology, and morphology between *D. conorum* and *D. eres*. Kobayashi (65) chose to maintain *D. conorum* as a distinct species because he felt that insufficient evidence exists to synonymise these taxa.

Diaporthe medusaea. This fungus is distinguished from *D. eres* by its long clustered perithecial necks protruding from the bark surface and perithecia in groups. Dimensions of the teleomorph and anamorph states in *D. medusaea* seem to be quite similar to those of *D. eres* (65,137). Wehmeyer (137) believed that these two species should be synonymised in the future. Consequently, Kobayashi (65), identified *Diaporthe* strains from *Morus* tentatively as *D. medusaea*. Fukutomi *et al.* (37), however, recognized *D. medusaea* as the cause of *Diaporthe* canker on *Pyrus* in Japan.

Brayford (18) found that perithecial neck length is controlled by environmental factors especially humidity and light, and is therefore, not a reliable taxonomic character. This casts doubt on the distinction of *D. medusaea* from the similar *D. eres* (18).

Diaporthe nomurai Hara. The teleomorph of *D. nomurai*, the cause of mulberry canker in Japan and Korea, is similar to that of *D. medusaea* and *D. eres*. Alpha conidia in the *Phomopsis* state of *D. nomurai* are, however, considerably larger than those of *D. eres* or *D. medusaea* (47). Kobayashi (65) was unable to examine material from fresh cankers with either conidial or perithecial states. He, therefore, choose to retain *D. nomurai* as a distinct species, based on its large alpha conidia. Matsuno & Nishina (80) described conditions of conidial germination in *D. nomurai* and supported the maintenance of this species.

Diaporthe phaseolorum. This well-known pathogen of herbacious plants, has had a complex nomenclatural history. Thus, a number of binomials have been established for what is currently considered to be a single species. *D. phaseolorum* and *Phomopsis phaseoli* (Desm.) Sacc. are recognized, respectively, as the valid binomials for the teleomorph and anamorph of the species complex. Smit & Knox-Davies (116) identified a canker pathogen on woody *A. linearis* plants as *D. phaseolorum*. Moreover, a fungus that was tentatively identified as *D. phaseolorum*, was isolated from *Malus*, *Pyrus*, and *Prunus* in South Africa (119).

Kulik (67) addressed the question of the advisability of distinguishing different varieties of *D. phaseolorum*. Since variation in morphology within the varieties is at least equal to the variation amongst them, it was concluded that varieties should not be recognized. Because of the apparent absence of clearly distinct differences within the complex, particularly morphologically, the adoption of the special form ["*forma specialis*"] for infraspecific categorization was suggested (83,85). Based on molecular data, Zhang *et al.* (141) regarded *D. phaseolorum* f. sp. *caulivora* as a variety of *D. phaseolorum*.

Wehmeyer (137) noted that *D. phaseolorum* is very similar to *D. arctii*. It, however, differs from *D. phaseolorum* in having smaller ascospores, asci, and perithecia. *D. arctii* perithecia also have more filiform necks than *D. phaseolorum*.

Diaporthe prunicola (Peck) Wehm. When compared with other small-spored species in

this section, *D. prunicola* is morphologically distinct. This is due to a small ascospore diameter ranging from 1.5-2 µm. The length of its ascospores, however, correspond well with those of *D. eres* and the other small-spored species in this section (42).

Species with intermediate to large ascospores

Species included in this section are: *Diaporthe crataegi* Nits., *Diaporthe impulsa* (Cke. & Pk.) Sacc., *Diaporthe melanocarpa* Dearn., *Diaporthe padi* Otth., *Diaporthe pruni* Ell. & Ev., *Diaporthe rudis* (Fr.) Nits., *Diaporthe stictostoma* (Ell.) Sacc., *Diaporthe tanakae* Kobayashi & Sakuma, and *Diaporthe tuberculosa* (Ell.) Sacc. The only species with appendages is *D. pruni*. Of particular interest in this section, however, is the species *D. tanakae*.

Diaporthe tanakae. Nakashima & Takimoto (88) reported a new canker disease of *Pyrus* from Korea and tentatively assigned the pathogen to *Phomopsis*. The same disease was also noted from Japan two years later (130). In 1920, European pear trees were severely damaged in Japan by a canker caused by a species of *Diaporthe*. Tanaka (127) attributed this loss to the European pear canker pathogen, *D. ambigua*. However, as noted by Kobayashi (65), the European pear canker pathogen had been misidentified by Tanaka (127). *D. ambigua* had already been treated as a synonym of *D. eres* by Wehmeyer (137) after a critical examination of the type specimen of *D. ambigua*. However, the *Diaporthe* discussed by Tanaka (127) is different from *D. eres* [=*D. ambigua*] (66).

Sakuma & Miyagawa (113) reported a canker and die-back disease of *Pyrus* prevalent throughout Tohoku district in the northern part of Honshu, Japan. The *Diaporthe* state, as well as the *Phomopsis* state were found on both cankered and dead twigs. The general morphology of this European pear die-back fungus was identical to that of "*D. ambigua*" *sensu* Tanaka (113). There is no other species morphologically similar to this fungus and a new species, *D. tanakae* was proposed for it (66). Furthermore, Fujita *et al.* (36) identified the apple blight fungus in the Tohoku district of Japan as *D. tanakae*, which was the first record of the fungus on *Malus* in Japan.

USE AND LIMITATIONS OF TRANSMISSIBLE HYPOVIRULENCE

Transmissible hypovirulence is a novel form of biological control in which virulence of a fungal pathogen is attenuated by an endogenous RNA virus (21). In *Cryphonectria parasitica* (Murr.) Barr, dsRNA-containing hypovirulent strains have been shown to serve as biocontrol agents by virtue of their ability to convert compatible virulent, virus-free strains to hypovirulence following anastomosis (4,77,93,132). Although mycoviruses and related dsRNA genetic elements are commonly associated with fungi (73,94), nothing was known of hypovirulence in *D. ambigua*.

Lindberg (75) provided the first report of a transmissible disease of a plant-pathogenic fungus (*Helminthosporium victoriae* Meehan & Murphy) that resulted in reduced virulence of the pathogen (76). Such conversions are correlated with the transmission of viral dsRNAs (6,39,40,133). Grente & Berthelay-Sauret (44) subsequently coined the term 'hypovirulence' to describe the strains of *C. parasitica* isolated from European chestnut

trees in Italy, that had recovered from chestnut blight.

Higley & Tachibana (54) suggested that variation in disease response to *D. phaseolorum* may be due to genetic diversity within cultivars. Kulik (68) demonstrated variation in pathogenicity among single-ascospore strains of *D. phaseolorum*. The strains not only varied in their pathogenicity to soybean seedlings, but a correlation was also found between their pathogenicity and growth rate *in vitro*. Kulik (68) thus speculated that the mechanism involved in reduction or increase in pathogenicity may be similar to that reported in *C. parasitica* (33).

Lee *et al.* (72) found dsRNA segments in strains of *D. phaseolorum*. However, no association was detected between their presence and virulence, toxin production, growth rate or the activity of phenol oxidase. Although recent studies have dealt with varieties of *D. phaseolorum* at the molecular level (35,141), there is still much to learn about variation in this fungus. A fully satisfactory explanation of the peculiarities of *D. phaseolorum* will clearly require further investigations.

DNA-mediated transformation studies employing full-length and partial cDNA clones of the large viral dsRNA (L-dsRNA) isolated from the hypovirulent *C. parasitica* strain EP713, provided the first direct evidence that viral RNA is responsible for the hypovirulence phenotype (24,25). In addition, Chen *et al.* (21) demonstrated mitotic stability and nuclear inheritance of integrated viral cDNA in the engineered hypovirulent strains of *C. parasitica*. Furthermore, Chen *et al.* (22) successfully used synthetic infectious hypovirus transcripts corresponding to a full-length *C. parasitica* hypovirus RNA coding strand, to extend virus-mediated virulence-attenuation to a new fungal taxonomic family. It is, therefore, theoretically possible to introduce and establish *C. parasitica*-originated hypovirus infections into spheroplasts of *D. ambigua*, or even fungal species not previously reported to harbor viruses.

Vegetative incompatibility, the inherent inability of fungal strains to fuse and exchange nuclear material, has been used to study population dynamics of fungi (43,74). Identification of vegetative compatibility groups (VCGs) has been useful in determining the source of races, new to a particular geographical area. Increasing numbers of VCGs are considered an indicator of genetic diversity within a population (43,74).

The transmission of cytoplasmic hypovirulence determinants by hyphal anastomosis is determined to a large degree by the vegetative compatibility of the donor and recipient strains (3,4). Increasing numbers of VCGs in populations lacking predominance of one VCG, may reduce the effectiveness of dsRNA transfer and thus biological control in a hypovirulent system (7). Although vegetative incompatibility may be a significant barrier to successful interaction of virulent and hypovirulent strains, some hypovirulent strains successfully transmit the agents of hypovirulence to virulent strains from a variety of VCGs (77).

The proliferation of VCGs is the probable factor limiting establishment of biological control of *C. parasitica* in North America (4,7,8,77). This is in contrast to Europe where

biological control through hypovirulence is successful and where there is also little diversity in the pathogen (7,52,82). Ploetz & Shokes (97,98) reported the predominance of one VCG among several vegetative incompatibility groups in *D. phaseolorum*.

SPECIES IDENTIFICATION

The causal agent of the canker disease of apple, pear, and plum rootstocks in South Africa is a species of *Diaporthe* (118,122). Several researchers have reported *D. perniciosa* in association with die-back diseases of apple, pear, plum and peach elsewhere (20,48,92). Random amplified polymorphic DNA (RAPD) analysis of *Diaporthe* isolates, using different primers of arbitrary sequence, is supportive of Wehmeyer's grouping of *D. ambigua*, *D. perniciosa*, and *D. eres* (118,122). The taxonomy of the *Diaporthe* spp. on pome and stone fruit trees in South Africa needs further investigation, but this must await a thorough treatment of the genus *Diaporthe*. Over-reliance on host association has led to the establishment of a plethora of *Diaporthe* spp. (104). We foresee that sequencing studies will synonymise many of the *Diaporthe* spp. described on different hosts worldwide. We believe that the most appropriate name to use for the fungus on apple, pear, and plum in South Africa is *D. ambigua*. The identity of the fungus is supported by morphological characteristics of South African isolates of *Diaporthe* (118,122).

ETIOLOGY AND SYMPTOMS

Nursery rootstocks infected by *D. ambigua* are readily killed (118,122). This is in contrast to mature rootstocks which are normally killed over an extended period. Although they did not monitor nursery infections, Nakatani *et al.* (90) and Fujita *et al.* (36) reported similar results for mature apple and pear trees inoculated with *D. tanakae* in Japan. Their results indicated that typical lesions generally appeared only 2 years after infection, explaining the absence of symptoms on current and biennial shoots in the field.

Characteristic symptoms of *D. ambigua* infections include sunken, pointed lesions with marginal longitudinal cracks (118,122). Similar symptom expression was reported for apple and pear cultivars infected by *D. tanakae* in Japan (36,66,91,112,127), and plum cultivars infected by *D. perniciosa* in Britain (20,48).

A significant ($P<0.001$) isolate x rootstock interaction has been found, indicating that isolates of *D. ambigua* react differently on various rootstocks (118,122). However, all deciduous fruit cultivars tested were susceptible to *D. ambigua* infection (118,122). This is in contrast to results of Fujita *et al.* (36) where *D. tanakae* isolates from apple infected cuttings of both apple and European pear cultivars through wounds, whereas their isolates from pear cultivars infected only European pear shoots. Furthermore, their inoculations of unwounded shoots in the field indicated that *D. tanakae* isolates from apple infect apple cultivars, and those from European pear, infected pear cultivars.

The canker pathogen on rooibos tea, previously identified as *D. phaseolorum* (116), is morphologically identical to the *Diaporthe* sp. isolated from pome and stone fruit rootstocks (118). Furthermore, all isolates including those from rooibos tea clustered together in RAPD

analyses. The *Diaporthe* sp. from rooibos tea was also pathogenic to all apple, pear, and plum cultivars tested (118,122). Rooibos tea is indigenous to South Africa, and geographically separated from pome and stone fruit cultivation. We consider the *Diaporthe* sp. on rooibos tea to be *D. ambigua*. We also believe the *D. ambigua* that infects fruit trees in South Africa is native in the country and that it probably originated from native woody plants such as rooibos tea. Population studies, particularly at the molecular level, are required to clarify this hypothesis.

EXPLOITING HYPOVIRULENCE

The population of *D. ambigua* in apple, pear and plum orchards in South Africa contains strains belonging to a large number of VCGs (124,125). Given the ubiquitous presence of perithecia throughout the area of disease occurrence, this is perhaps not surprising. Strains from adjacent trees usually differed in VCG (124,125). One interpretation for the large number of VCGs in close proximity to one another, suggests that ascospores are effective primary propagules. In general, a sexually reproducing population would be expected to have a high level of VCG diversity (74). It is possible, however, that nursery seedlings possess latent infections caused by many VCGs of *D. ambigua*, which then serve as foci of conidial infection. Similar conclusions were made in a study of Cytospora canker of peach caused by *Leucostoma persoonii* (Nits.) Höhn (1).

Analysis of VCGs in populations of filamentous Ascomycetes has been used to assess whether a pathogen has been recently introduced into an area or whether it has been present for an extended period of time (43). In the case of *D. ambigua*, it would be difficult to draw such conclusions without knowledge regarding the regulation of the sexual cycle. The formation of the sexual state of *D. ambigua* by individual single ascospores in culture, is indicative of homothallism (124,125). Furthermore, fertile perithecia occur abundantly on sampled rootstocks (124,125). The presence of more than one VCG in progeny from individual perithecia collected from various M793 and Granny Smith apple rootstocks, however, is indicative of outcrossing. Apparently *D. ambigua* is a homothallic fungus that has the capacity to outcross. This is similar to the situation with *C. parasitica* (2). The basis of sexuality in *D. ambigua* clearly needs further study.

Strains of *D. ambigua* from different orchards in the Cape Province of South Africa generally are from different VCGs (124,125). In this study, samples originated mainly from newly established nursery orchards, each with a different source (and sometimes mixed sources) of seedling and budding material. Most studies of VCGs in other Ascomycetes have been conducted in well established orchards (1,101). In such orchards, a strain of a specific VCG could have spread the disease to eventually produce an aggregation of trees with cankers representative of a common VCG. However, a clustered pattern of VCGs within a well established orchard could have originated from nursery seedlings possessing latent infections caused by many VCGs.

Predominance of one or a few VCGs among *D. ambigua* strains was not observed in a study of vegetative compatibility (124,125). This is in contrast to *D. phaseolorum* where one VCG dominates in strains collected from small areas

(97). Our data are consistent with the hypothesis that VCG and fitness are not correlated.

If no VCG dominates the population, then as the number of VCGs increases the efficacy of dsRNA transfer and thus the success of biological control appears to be reduced. Thus the high number of VCGs probably limits the use of dsRNAs as a biological control of *C. parasitica* in North America (4,7,8,77). This proliferation is in contrast to the situation in Europe, where biological control through hypovirulence is successful, and where there is less diversity in the pathogen population (7,52). Interaction between vegetative incompatibility genes affects horizontal (intermycelial) dsRNA transmission in *C. parasitica* (58). However, dsRNA in *C. parasitica* stops reassortment of vegetative compatibility genes by suppressing mating-type-specific gene expression (140).

All sampled dsRNA-containing strains of *D. ambigua* from apple orchards in South Africa originated from sexual spores (124,125). Polashock & Hillman (99,100) reported a small dsRNA element associated with the mitochondria of a *C. parasitica* strain. This small mitochondrially-associated element can be transmitted into compatible strains by hyphal anastomosis such as in the case of the more common larger size dsRNA viruses of *C. parasitica* which are members of the Hypoviridae. Transmission of virus-like elements to ascospore progeny appears to be blocked in the Hypoviridae (3,4). In contrast to the Hypoviridae, however, the small mitochondrially associated element can be transferred to sexual progeny (99). Since it is present in ascospores, it is possible that the relatively small dsRNA element in *D. ambigua* (120,123) is mitochondrially associated. All dsRNA-containing strains of *D. ambigua* need to be analyzed in the future to determine whether transfer of dsRNA by anastomosis is accompanied by mitochondrial recombination, in a recipient strain.

HYPOVIRULENCE-ASSOCIATED TRAITS

Smit *et al.* (120,123) demonstrated the occurrence of hypovirulence for the first time in *D. ambigua*. This was associated with the presence of a single dsRNA segment. In addition to reduced virulence, dsRNA-containing strains of *D. ambigua* also exhibited a number of hypovirulence-associated traits including reduced phenol oxidase activity, reduced gallic acid oxidation, diminished oxalate accumulation, and suppressed sporulation. These distinguishing characteristics are consistent with data previously published for the related *C. parasitica* (4,5,32,34,51,55,105). The presence of dsRNA in fungi is not, however, always associated with reduced virulence and hypovirulence-associated traits (94).

DsRNA-containing hypovirulent strains of *D. ambigua* were able to convert compatible virulent, virus-free strains of the same VCG to hypovirulence after anastomosis (120,123). Moreover, dsRNA-free strains of *D. ambigua* converted to hypovirulence, exhibited the same composition of hypovirulence-associated traits as displayed by the original dsRNA-containing hypovirulent strains. These results provide clear evidence that the dsRNA confers hypovirulence on strains of *D. ambigua* in South Africa. Furthermore, the characteristics of this hypovirulence are also similar to those

found in *C. parasitica* (4,6,77,93,132,133). DsRNA associated with the hypovirulence phenotype have also been reported for a number of other plant pathogenic fungi such as *L. persoonii* (46), *Ophiostoma ulmi* (Buisman) Nannf. (94), and *Sclerotinia sclerotiorum* (Lib.) de Bary (16). Conversion of dsRNA-free strains to the hypovirulence phenotype is coincident with transmission of dsRNAs during anastomosis with compatible hypovirulent strains, providing the basis for biological disease control in *D. ambigua*.

Using the Bavendamm's tests for phenol oxidase reaction and gallic acid oxidation, Smit *et al.* (120,123) found clear differences in oxidation between dsRNA-containing strains and dsRNA-free strains of *D. ambigua*. Bavendamm (14,15) pointed out differences between fungi with respect to their oxidative enzymes. According to Davidson *et al.* (27), most fungi that oxidize tannic acid also oxidize gallic acid. Although Bavendamm's tests have mainly been applied in Basidiomycete fungi (60), they were recently used to determine phenol oxidation in dsRNA-free and dsRNA-containing strains of *C. parasitica* (105) and are evidently also applicable in the case of *D. ambigua*.

The enzyme responsible for the color reaction in Bavendamm's test for phenol oxidase in the study of Smit *et al.* (120,123) was identified as phenol oxidase of the laccase type. In fungi, laccase activity has been suggested to be involved in degradation of lignin (9,63), pathogenesis (38,78,81), and sporulation (71). It was, therefore, not surprising that we found a reduction of laccase activity in hypovirulent dsRNA-containing strains of *D. ambigua*. Similar results have been found were found in the related fungus, *C. parasitica* (23,55,105,106).

The laccase activity in dsRNA-free strains of *D. ambigua* was found at the advancing edges of colonies (120,123). That is an important requirement for an enzyme suggested to play a role in the infection process (105). In *C. parasitica*, however, laccase activity is extracellular as well as intracellular (107). According to these authors, the extracellular laccase activity in *C. parasitica* temporally precedes the intracellular laccase, and is encoded by different genes. They also observed both laccases to be down-regulated by the dsRNA in a hypovirulent strain of that fungus, suggesting common regulatory factors for the separate laccase genes (106,107). Moreover, a third type of laccase has been discovered in *C. parasitica* (62). In *D. ambigua*, however, there is still much to learn concerning the basis of dsRNA-mediated regulation of laccase gene(s).

Smit *et al.* (120,123) found a reduction of oxalate accumulation in dsRNA-containing strains of *D. ambigua*, when compared to dsRNA-free strains of this fungus. Oxalate accumulation, potentially involved in pathogenesis, has been reported to be suppressed in hypovirulent strains of *C. parasitica* (51,55,105). Variations in pathogenicity have also been associated with oxalate in other fungi (79).

The combined results obtained from dsRNA-containing strains of *D. ambigua* are suggestive of a specific viral coding domain, controlling several hypovirulence-associated traits. This would be the same as that found in *C. parasitica*. To understand the mechanism by which dsRNA perturbs fungal gene expression, knowledge of the host genes that are affected

and their roles in fungal development and virulence is important. In addition, information concerning the molecular structure and coding potential of the dsRNA itself is required.

RELATEDNESS OF dsRNA

A number of different *D. ambigua* strains from South Africa were found to contain hypovirulence-associated dsRNA (120,123). In all cases a single dsRNA segment, approximately 4.3 kb was observed in these strains (120,123). Single dsRNA segments associated with hypovirulence have also been found in fungi such as *C. parasitica* (56,105) and *S. sclerotiorum* (16). This is in contrast to most fungal strains, including well-studied strains of *C. parasitica*, where a complexity of dsRNA banding patterns have been observed (6,28,34,46,57,69,109). In *C. parasitica* strain EP713, combined results suggest that all genetic information of the hypovirulence-associated virus resides within the single largest dsRNA (115). Furthermore, Shapira *et al.* (115) demonstrated that most of the complexity observed for dsRNAs present in hypovirulent *C. parasitica* strains is a result of the generation and maintenance of internally deleted defective forms of the single largest dsRNA.

Hybridization amongst the different single dsRNA segments from *D. ambigua* strains showed a high degree of homology (121,126). The presence of *D. ambigua* in a localized area carrying a single dsRNA segment of similar size and homology suggests that natural spread of dsRNA within the *D. ambigua* population has occurred. These results also indicate establishment of a single size dsRNA population in the region, excluding cross-hybridization with other potential dsRNA populations (121,126).

OUTLOOK

Interest in the viral dsRNA associated with hypovirulence of *D. ambigua* stemmed primarily from its potential as a biological control agent. The presence of *D. ambigua* in a localized geographic area, carrying a single dsRNA segment of similar size and homology, suggests that natural spread of dsRNA within the *D. ambigua* population has occurred. Hypovirulent strains of *D. ambigua* appear to occur naturally in the South African population of the pathogen. These strains are less virulent than dsRNA-free strains. Furthermore, hypovirulence can apparently be transferred easily to dsRNA-free strains. An ideal opportunity, therefore, appears to exist for biological control of *D. ambigua* using hypovirulence. Moreover, results of these studies could provide insight into our understanding of engineering of hypovirulence in the future.

Several studies designed to identify the underlying basis for host susceptibility versus resistance and hypovirus-mediated hypovirulence have focused on the role of fungus-encoded plant cell wall-degrading enzymes. Meaningful progress has also been made with development of transgenic apple and pear plants expressing marker and indicator genes (10). This advancement paves the way for introducing a beneficial gene (e.g. genes that encode enhanced resistance against *Diaporthe* and other stem canker fungi) into existing superior commercial varieties. A potent antifungal PGIP gene was cloned and is now to be inserted into existing fruit varieties

(10). When inserted, the transformed cultivars will secrete the polygalacturonase inhibiting protein. Furthermore, improved disease management practices and research on the identification of molecular markers for disease resistance (11) hold promise for controlling *Diaporthe* canker in South Africa.

LITERATURE CITED

1. Adams, G., Hammar, S. & Proffer, T. 1990. Phytopathology 80, 287-291.
2. Anagnostakis, S.L. 1977. Exp. Mycol. 1, 306-316.
3. Anagnostakis, S.L. 1982. Genetics 102, 25-28.
4. Anagnostakis, S.L. 1982. Science 215, 466-471.
5. Anagnostakis, S.L. 1984. In: Jennings, D.H. & Rayner, A.D.M. (Editors), The Ecology and Physiology of the Fungal Mycelium, Pages 353-366, Cambridge University Press, Cambridge.
6. Anagnostakis, S.L. & Day, P.R. 1979. Phytopathology 69, 1226-1229.
7. Anagnostakis, S.L., Hau, B. & Kranz, J. 1986. Plant Dis. 70, 536-538.
8. Anagnostakis, S.L. & Kranz, J. 1987. Phytopathology 77, 751-754.
9. Ander, P. & Eriksson, K.E. 1976. Arch. Microbiol. 109, 118.
10. Anonymous, 1996. Infruitec Research Review, compiled by the Agricultural Research Council, South Africa.
11. Anonymous, 1997. Infruitec Research Review, compiled by the Agricultural Research Council, South Africa.
12. Atkinson, J.D. 1971. Diseases of tree fruits in New Zealand, Government Printer, Wellington, New Zealand.
13. Barr, M.E. 1978. Mycologia Memoir No. 7, J. Cramer Publisher, Lehre, Germany.
14. Bavendamm, W. 1928. Z. Pflanzenkr. Pflanzenschutz 38, 257-276.
15. Bavendamm, W. 1928. Zentralbl. Bakteriol. Parasitenkd. 76, 172-226.
16. Boland, G.J. 1992. Can. J. Plant Pathol. 14, 10-17.
17. Brautigam, S., Gille, A. & Bar, T. 1988. Gartenbau 35, 27-29.
18. Brayford, D. 1990. Mycol. Res. 94, 691-697.
19. Briton-Jones, H.R. 1925. J. Pomol. 4, 162-183.
20. Cayley, D.M. 1923. J. Genet. 13, 353-370.
21. Chen, B., Choi, G.H. & Nuss, D.L. 1993. EMBO J. 12, 2991-2998.
22. Chen, B., Choi, G.H. & Nuss, D.L. 1994. Science 264, 1762-1764.
23. Choi, G.H., Larson, T.G. & Nuss, D.L. 1992. Mol. Plant-Microbe Interact. 5, 119-128.
24. Choi, G.H. & Nuss, D.L. 1992. EMBO J. 11, 473-477.
25. Choi, G.H. & Nuss, D.L. 1992. Science 257, 800-803.
26. Ciurea, A. 1987. Stud. Cercet. Biol. Ser. Biol. Veg. 39, 73-75.
27. Davidson, R.W., Campbell, W.A. & Blaisdell, D.J. 1938. J. Agric. Res. 57, 683-695.
28. Dodds, J.A. 1980. Virology 107, 1-12.
29. Dunegan, J.C. 1932. Phytopathology 22, 922-924.
30. Ellis, J.B. & Everhart, B.M. 1892. The North American Pyrenomycetes, Publ. by the authors, Newfield, New Jersey.
31. Ellis, M.B. & Ellis, J.P. 1985. Microfungi on Land Plants, MacMillan Publ. Co., New York.
32. Elliston, J.E. 1978. In: MacDonald, W.L., Cech, F.C., Luchok, J. & Smith, H.C. (Editors), proceedings of the American

Chestnut Symposium Proceedings, Pages 95-100, West Virginia University Press, Morgantown, WV.
33. Elliston, J.E. 1982. In: Ingram, D.S. & Williams, P.H. (Editors), Advances in Plant Pathology, Vol. 1, Pages 1-33, Academic Press, London.
34. Elliston, J.E. 1985. Phytopathology 75, 151-158.
35. Fernández, F.A. & Hanlin, R.T. 1996. Mycologia 88, 425-440.
36. Fujita, K., Sugiki, T. & Matsunaka, K. 1988. (In Japanese; English summary). Bull. Aomori Field Crops Hortic. Exp. Stn. 6, 17-35.
37. Fukutomi, M., Matsushiro, H. & Tachimoto, M. 1991. Bull. Res. Inst. Agric. Res. (Ishikawa) 2, 27-32.
38. Geiger, J.-P., Nicole, M., Nandris, D. & Rio, B. 1986. Eur. J. For. Pathol. 16, 22-37.
39. Ghabriel, S.A. 1980. Annu. Rev. Phytopathol. 18, 441-461.
40. Ghabriel, S.A., Sanderlin, R.S. & Calvert, L.A. 1979. Phytopathology 69, 312-315.
41. Gilman, J.C. & Archer, W.A. 1929. Iowa State Coll. J. Sci. 3, 299-507.
42. Gilman, J.C., Tiffany, L.H. & Lewis, R.M. 1959. Iowa State Coll. J. Sci. 33, 325-393.
43. Glass, N.L. & Kuldau, G.A. 1992. Annu. Rev. Phytopathol. 30, 201-224.
44. Grente, J.R. & Berthelay-Sauret, S. 1978. In: MacDonald, W.L., Cech, F.C., Luchoc, J. & Smith, H.C. (Editors), proceedings of the American Chestnut Symposium, Pages 30-34, Virginia Univ. Press, Morgantown, WV.
45. Grove, W.B. 1917. Bull. Misc. Inf., R. Bot. Gard. 2, 49-73.
46. Hammar, S., Fulbright, D.W. & Adams, G.C. 1989. Phytopathology 79. 568-572.
47. Hara, K. 1925. Diseases of Cultivated Plants (Jitsuyô-Sakumotsu-Byôri-Gaku), Tokyo.
48. Harris, D.C. 1988. Plant Pathol. 37, 604-606.
49. Harris, D.C. & Cardon, J.A. 1984. Report of East Malling Research Station for 1983, 103.
50. Hartmann, H.T., Kester, D.E. & Davies, F.T. Jr. 1990. Plant Propagation: Principles and Practices, 5th edition, Prentice-Hall Inc., New Jersey.
51. Havir, E.A. & Anagnostakis, S.L. 1983. Physiol. Plant Pathol. 23, 369-376.
52. Heiniger, U. & Rigling, D. 1994. Annu. Rev. Phytopathol. 32, 581-600.
53. Hemmi, T. 1942. J. Plant Prot. 29, 4-6.
54. Higley, P.M. & Tachibana, H. 1987. Plant Dis. 71, 815-817.
55. Hillman, B.I., Shapira, R. & Nuss, D.L. 1990. Phytopathology 80, 950-956.
56. Hillman, B.I., Tian, Y., Bedker, P.J. & Brown, M.P. 1992. J. Gen. Virol. 73, 681-686.
57. Hoch, J.G., Tavantzis, S.M., Campana, R.J. & Anagnostakis, S.L. 1985. Can. J. Bot. 63, 297-300.
58. Huber, D.H. & Fulbright D.W. 1994, (Abstr.). In: proceedings of the Fifth International Mycological Congress, Page 95, Vancouver, British Columbia, Canada.
59. Jones, A.L. & Aldwinckle, H.S. 1990. Compendium of Apple and Pear Diseases. APS Press, Minnesota.
60. Käärik, A. 1965. Stud. For. Suec. 31, 1-80.
61. Kendrick, B. & DiCosmo, F. 1979. In: Kendrick, B. (Editor), The Whole Fungus, Vol. 1, Pages 283-302, National Museum of Natural Science, Ottawa.
62. Kim, D.H., Rigling, D., Zhang, L. & Van Alfen, N.K. 1995. Mol. Plant-Microbe

Interact. 8, 259-266.
63. Kirk, T.K. & Shimada, M. 1985. In: Higuchi, T. (Editor), Biosynthesis and Biodegradation of Wood Components, Academic Press, San Diego, CA.
64. Kobayashi, T. 1958. Bull. Gov. For. Exp. Sta. 107, 17-25.
65. Kobayashi, T. 1970. Bull. Gov. For. Exp. Stn. 226, 1-242.
66. Kobayashi, T. & Sakuma, T. 1982. Trans. Mycol. Soc. Jpn. 23, 37-40.
67. Kulik, M.M. 1984. Mycologia 76, 274-291.
68. Kulik, M.M. 1989. Mycologia 81, 549-553.
69. Lakshman, D.K. & Tavantzis, S.M. 1994. Phytopathology 84, 633-639.
70. Latham, A.J., Morgan-Jones, G. & Campbell, H.L. 1992, (Abstr.). Plant Dis. 76, 426.
71. Leatham, G.F. & Stahmann, M.A. 1981. J. Gen. Microbiol. 125, 147-157.
72. Lee, Y.H., Snow, J.P., Berggren, G.T. & Valverde, R.A. 1990, (Abstr.). Phytopathology 80, 966.
73. Lemke, P.A. 1979. Viruses and Plasmids in Fungi, Marcel Dekker, New York.
74. Leslie, J.F. 1993. Annu. Rev. Phytopathol. 31, 127-151.
75. Lindberg, G.D. 1959. Phytopathology 49, 29-32.
76. Lindberg, G.D. 1960. Phytopathology 50, 457-460.
77. MacDonald, W.L. & Fulbright, D.W. 1991. Plant Dis. 75, 656-661.
78. Marbach, I., Harel, E. & Mayer, A.M. 1985. Phytochemistry 24, 2559-2561.
79. Marciano, P., Di Lenna, P. & Magro, P. 1983. Physiol. Plant Pathol. 22, 339-345.
80. Matsuno, M. & Nishina, S. 1986. J. Seric. Sci. Jpn. 55, 415-420.
81. Mayer, A.M. 1987. Phytochemistry 26, 11-20.
82. Mittempergher, L. 1978. In: MacDonald, W.L., Cech, F.C., Luchok, J. & Smith, H.C. (Editors), proceedings of the American Chestnut Symposium, Pages 34-37, West Virginia Univ. Books, Morgantown, WV.
83. Morgan-Jones, G. 1985. In: proceedings of the Conference on *Diaporthe / Phomopsis* disease complex of soybean (1984), Pages 1-7, U.S. Dep. Agric.
84. Morgan-Jones, G. 1989. In: Pascale, A. (Editor), proceedings of the World Soybean Research Conference IV, Pages 1699-1706, Buenos Aires.
85. Morgan-Jones, G. 1992. Fitopatol. Bras. 17, 359-367.
86. Müller, E. & von Arx, J.A. 1962. Beitr. Kryptogamenflora Schweiz 11, 1-922.
87. Munk, A. 1957. Dan. Bot. Ark. 17, 1-491.
88. Nakashima, T. & Takimoto, S. 1924. J. Plant Prot. 11, 415-421.
89. Nakatani, F. & Hiraragi, T. 1987. Annu. Rep. Plant Prot. North Jpn. 38, 68-71.
90. Nakatani, F., Hiraragi, T. & Sekizawa, H. 1981. Annu. Rep. Plant Prot. North Jpn. 32, 141-143.
91. Nakatani, F., Hiraragi, T. & Sekizawa, H. 1984. Bull. Iwate Hortic. Exp. Stn. 5, 47-70.
92. Nawawi Bin Hoji Ayub, A. & Swinburne, T.R. 1970. Ann. Appl. Biol. 66, 245-255.
93. Nuss, D.L. 1992. Microbiol. Rev. 56, 561-576.
94. Nuss, D.L. & Koltin, Y. 1990. Annu. Rev. Phytopathol. 28, 37-58.
95. Ostasheva, N.A. 1983. Byull. Gl. Bot. Sada 127, 91-95.
96. Petrak, F. & Sydow, H. 1936. Ann. Mycol. 34, 237-251.
97. Ploetz, R.C. & Shokes, F.M. 1986. Can. J. Bot. 64, 2197-2200.

98. Ploetz, R.C. & Shokes, F.M. 1989. Can. J. Bot. 67, 2751-2755.
99. Polashock, J.J. & Hillman B.I. 1994, (Abstr.). Phytopathology 84, 1063.
100. Polashock, J.J. & Hillman, B.I. 1994. In: proceedings of the National Academy of Sciences 91, Pages 8680-8684, USA.
101. Proffer, T.J. & Jones, A.L. 1989. Plant Dis. 73, 508-514.
102. Radford, A.E., Dickison, W.C., Massey, J.R. & Bell, C.R. 1974. Vascular Plant Systematics, Harper and Row Publishers, Inc., New York.
103. Rehder, A. 1954. Manual of Cultivated Trees and Shrubs Hardy in North America, Macmillan, New York.
104. Rehner, S.A. & Uecker, F.A. 1994. Can. J. Bot. 72, 1666-1674.
105. Rigling, D., Heiniger, U. & Hohl, H.R. 1989. Phytopathology 79, 219-223.
106. Rigling, D. & Van Alfen, N.K. 1991. J. Bacteriol. 173, 8000-8003.
107. Rigling, D. & Van Alfen, N.K. 1993. Appl. Environ. Microbiol. 59, 3634-3639.
108. Roberts, J.W. 1942. Phytopathology 32, 335-336.
109. Romaine, C.P., Schlagnhaufer, B. & Goodin, M.M. 1994. Curr. Genet. 25, 128-134.
110. Saccardo, P.A. 1882. Sylloge Fungorum I, 606-694.
111. Saccardo, P.A. 1915. Nuovo G. Bot. Ital. (Nuova Ser.) 22, 48.
112. Sakuma, T., Batra, L.R., Nakatani, F. & Sawamura, K. 1982. Bull. Fruit Tree Res. Stn. Ser. C (Morioka) 9, 79-89.
113. Sakuma, T. & Miyagawa, H. 1981. Bull. Fruit Tree Res. Stn. Ser. C (Morioka) 8, 67-77.
114. Sawada, K. 1952. Bull. Gov. For. Exp. Stn. 53, 135-194.
115. Shapira, R., Choi, G.H. & Nuss, D.L. 1991. EMBO J. 10, 731-740.
116. Smit, W.A. & Knox-Davies, P.S. 1989. Phytophylactica 21, 183-188.
117. Smit, W.A. & Knox-Davies, P.S. 1989. Phytophylactica 21, 301-306.
118. Smit, W.A., Viljoen, C.D., Wingfield, B.D., Wingfield, M.J. & Calitz, F.J. 1996. Plant Dis. 80, 1331-1335.
119. Smit, W.A. & Wingfield, M.J. 1991, (Abstr.). Phytopathology 81, 1181.
120. Smit, W.A., Wingfield, B.D. & Wingfield, M.J. 1994, (Abstr.). In: proceedings of the fifth International Mycological Congress, Vancouver, British Columbia, Canada, Page 202.
121. Smit, W.A., Wingfield, B.D. & Wingfield, M.J. 1994, (Abstr.). In: proceedings of the fifth International Mycological Congress, Vancouver, B.C., Canada, Page 202.
122. Smit, W.A., Wingfield, B.D. & Wingfield, M.J. 1994, (Abstr.). Phytopathology 84, 1140.
123. Smit, W.A., Wingfield, B.D. & Wingfield, M.J. 1996. Phytopathology 86, 1311-1316.
124. Smit, W.A., Wingfield, B.D. & Wingfield, M.J. 1997. Phytopathology 87, S91.
125. Smit, W.A., Wingfield, B.D. & Wingfield, M.J. 1997. Plant Pathol. 46, 366-372.
126. Smit, W.A., Wingfield, B.D. & Wingfield, M.J. 1997. Trends in Plant Pathology, Vol. 1.
127. Tanaka, I. 1934. (In Japanese). Hokkaido Natl. Agric. Exp. Stn. Rep. 31, 85-122.
128. Tomaz, I.L. & Rego, M.C.N.F. 1989. Publ. Lab. Patol. Veg. Veri Almeida 54, 1-6.
129. Traverso, J.B. 1906. Flora Italica Cryptogama 1, 1-352.
130. Tsuji, Y. 1926. (in Japanese). Rep. Plant Quar. 1, 1-124.
131. Uecker, F.A. 1988. Mycologia Memoir

No. 13, J. Cramer Publishers, Berlin.
132. Van Alfen, N.K. 1986. In: Buck, K.W. (Editor), Fungal Virology, Pages 143-162, CRC Press, Inc., Boca Raton, FL.
133. Van Alfen, N.K., Jaynes, R.A., Anagnostakis, S.L. & Day, P.R. 1975. Science 189, 890-891.
134. Van der Zwet, T. & Beer, S.V. 1991. U.S. Dep. Agric. Agric. Inf. Bull. 631, 1-83.
135. Wehmeyer, L.E. 1926. Pap. Mich. Acad. Sci. 6, 377-396.
136. Wehmeyer, L.E. 1929. Pap. Mich. Acad. Sci. 9, 477-494.
137. Wehmeyer, L.E. 1933. Univ. Mich. Stud., Ann Arbor, Sci. Ser. 9, 1-349.
138. Westwood, M.N. 1978. Temperate-Zone Pomology, second edition, W.H. Freeman and Company, San Francisco.
139. Winter, G. 1887. Gymnoasceen und Pyrenomyceten. Rabenhorst's Kryptogamenflora II.
140. Zhang L. & Van Alfen N.K. 1994, (Abstr.). Phytopathology 84, 1075.
141. Zhang, A.W., Riccioni, L., Pedersen, W.L., Chen, W.D. & Hartman, G.L. 1997. Phytopathology 87, S108.