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A Rapid Seedling Based Screening Technique to Assay Tobacco for Resistance to *Phytophthora nicotianae*

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Abstract

Black shank caused by *Phytophthora nicotianae* is a serious root and stem disease of cultivated tobacco worldwide. In this study, a rapid seedling-based screening technique was developed to evaluate tobacco cultivars for resistance to *P. nicotianae*. This technique was compared with a stem inoculation technique commonly used on adult plants. The overall aim was to develop an improved and rapid technique that could also be used to characterize races of *P. nicotianae*. A strong positive correlation was found between results of the seedling assay and adult plant trials for all isolates and cultivars tested. Furthermore, *P. nicotianae* isolates could be characterized as race 0 or 1 using both stem inoculation and the rapid seedling assay. This assay will facilitate rapid and large-scale screening for black shank resistance and therefore has the potential to reduce yield losses because of this disease in the field.

Introduction

Phytophthora nicotianae is an important root and stem pathogen in South Africa where it causes a disease known as black shank on tobacco (Prinsloo, 1994; Van Jaarsveld, 1995). Symptoms of black shank include wilting and stunting of plants, and also characteristic black lesions at the bases of the stems (Lucas, 1975; Shew, 1991). For effective black shank control, growers use a combination of crop rotation, cultivar resistance and fungicide applications (Melton, 1998). Fungicides such as metalaxyl, are however expensive and growers tend to use lower than optimum dosages (Csinos and Bertrand, 1994), which may contribute to the development of metalaxyl resistant *P. nicotianae* populations in South Africa, Korea and the USA (Shew, 1985; Kim and Kang, 1997; Van Jaarsveld et al., 2002a).

Breeding and the deployment of resistant cultivars are the most economical approaches to controlling *P. nicotianae*. Modern flue-cured cultivars are derived from five main sources of resistance to races 0 and 1 of the pathogen. They include cultivars Florida 301 and Beinhart 1000-1 in *Nicotianae tabacum* with partial resistance to race 1 and 0 (Tisdale, 1922; Silber and Heggestad, 1963); Coker 371-Gold with resistance to race 0 (Carlson et al., 1997), as well as *N. longiflora* (Burley cultivar L8) and *N. plumbaginifolia* with single dominant gene resistant to race 0 (Valleau et al., 1960; Chaplin, 1962).

The selection of cultivars and breeding lines for resistance to *P. nicotianae* has traditionally been conducted in fields where the pathogen is known to occur. Field trials are, however, labour intensive, expensive and time-consuming. Furthermore, the inoculum is commonly not evenly distributed and results can thus be equivocal. More than one race of *P. nicotianae* may also occur simultaneously in a tobacco field as reported in South Africa and the USA, which results in further confusion (Melton, 1998; Van Jaarsveld et al., 2002b).

A variety of techniques have been developed to screen tobacco germplasm for resistance to *P. nicotianae*. These include root, stem and leaf inoculations (Apple, 1957; Hendrix and Apple, 1967; Litton et al., 1970; Rufty et al., 1987; Tedford et al., 1990). Root and stem inoculation trials may be limited by the availability of space in greenhouses. Leaf inoculations are non-destructive but they are limited in the detection of resistance expression (Tedford et al., 1990). Maia et al. (1995) developed two seedling-screening

techniques. The first of these utilized young plants sprayed with zoospores at the cotyledon stage. The second technique relied on inoculating individual seedlings at the roots with mycelium discs placed in 1.5 ml Eppendorf tubes filled with water. These two techniques have shown that both specific and non-specific resistance can be detected on very young plants (Maia et al., 1995). The aim of the present study was to develop an improved rapid screening technique based on the method of Maia et al. (1995). Results using this technique were then compared with those from stem inoculations on adult plants in the greenhouse. The potential to use this screening technique for race characterization was also considered.

Materials and Methods

Fungal isolates and tobacco cultivars

Four differential and two commercial tobacco cultivars were used in this study. The differential cultivars, KY 14, KY14 × L8, Coker 371-Gold and Beinhart 1000-1 were selected based on their specific resistance genes and respective susceptibility to races 0 and 1 of *P. nicotianae* (Nielsen, 1995). KY 14 is highly susceptible while Beinhart 1000-1 is the most resistant cultivar to both races (Silber and Heggestad, 1963; Chaplin, 1966). KY14 × L8 and Coker 371-Gold are resistant to races 0 but susceptible to race 1 (Carlson et al., 1997; Csinos, 1999). The two flue-cured commercial cultivars used, were LK33/60 and Vuma/3/46.

Phytophthora nicotianae isolates previously characterized as races 0 and 1 (Van Jaarsveld et al., 2002b) were selected for this study. They included: three isolates from the Lowveld (Mpumalanga), one from PTK (Northern Province) and two from the MKTV (North-West Province) tobacco trading areas in South Africa (Table 1). The cultures used for inoculation have been deposited in the culture collections of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, RSA and the Cooperative Research Centre for Tropical Plant Protection, University of Queensland, Australia.

Inoculum production

Phytophthora nicotianae cultures were grown for 5 days on potato-dextrose agar at 27°C in the dark. Four 6 mm discs taken from the actively growing margins of fresh cultures were placed in each of five Petri dishes (65 mm diameter) containing 12 ml sterile Pea broth solution (Ribeiro, 1978) amended with 0.01 g β-sistosterol (Sigma AnalaR) and incubated at 28°C for 2 days with continuous light. After incubation, mycelial mats were washed once with sterile Petri's salt solution (Ribeiro, 1978). The Petri's salt solution [0.4 g $Ca(NO_3)_2{\cdot}4H_2O$, 0.15 g $MgSO_4{\cdot}7H_2O$, 0.15 g KH_2PO_4 and 0.06 g KCl in 1000 ml water] were amended with 0.002 g EDTA Disodium salt (Holpro Lovasz, Midrand, South Africa) and 0.002 g $MnSO_4$ (ACE, Reuven, South Africa). Mycelial mats were incubated at 28°C under continuous light in Petri's salt solution (12 ml Petri's salt solution in each Petri dish). After 48 h, the mycelial mats were harvested and cultures were examined using a light microscope to confirm the presence of sporangia and the production of zoospores.

Table 1
Phytophthora nicotianae isolates used in this study

Ascession number	Mating type	*P. nicotianae* race	Tobacco trading areas in South Africa
CMW 6921	A1	0	PTK
CMW 6916	A2	0	MKTV
CMW 6922	A1	0	Lowveld
CMW 6923	A2	1	MKTV
CMW 6924	A1	1	Lowveld
CMW 6925	A1	1	Lowveld

Seedling inoculations

Tobacco seeds were germinated on Hygrotech seedling medium consisting of sphagnum peat moss, fertilizer and medium grade vermiculite (pH 7) (Hygrotech Seed Ltd, Pretoria North, South Africa). After approximately 3 weeks, seedlings at the four-leaf stage were selected. Seedlings of each cultivar were removed from the seedbeds and gently rinsed with dH_2O to remove potting mixture from the roots. Individual seedlings were transferred into polystyrene trays (20 × 20 cm) with small cavities (3 mm diameter). Each seedling was individually placed in a cavity in the polystyrene trays, with the leaves of the seedling arranged above the tray surface and the roots hanging through the drainage hole below the tray. Trays containing seedlings were then transferred to 3 l plastic containers (22.5 × 22.5 × 7 cm). Each plastic container contained a suspension of 100 ml sterile Petri salt solution, 100 ml sterile dH_2O and 60 ml *P. nicotianae* inoculum (7.2×10^4 zoospores/ml). The polystyrene trays with seedlings floated on the suspension in the plastic containers. Thereafter, seedlings were sprayed lightly with dH_2O and the plastic containers containing the polystyrene trays with seedlings were placed in clear plastic bags in order to maintain a high level of humidity. The plastic containers with seedlings and inoculum were maintained at room temperature (28–30°C) for 3 days.

Ten seedlings of each tobacco cultivar (Coker 371-Gold, KY14, KY14 × L8, Beinhart 1000-1, Vuma/3/46 and LK33/60) were placed in a polystyrene tray. Two polystyrene trays containing all cultivars were inoculated with each of the six *P. nicotianae* isolates. Seedlings of tobacco cultivars were placed in a completely randomized block design in the trays. In each case the seedlings were examined for symptoms using a scale that reflects different levels of infection (Table 2). This disease rating scale is based on Maia et al. (1995) disease index for seedlings. For controls, a separate plastic container of seedlings were floated on distilled water free of *P. nicotianae* inoculum. Re-isolations from infected plants were performed on selective Pimaricin, Ampicillin, Rifampicin, PCNB, Hymexosal (PARPH) media (Tsao and Guy, 1977) to verify that symptoms were the result of the effect of the inocula-

Table 2
Disease rating scale for symptoms on seedlings after infection by *Phytophthora nicotianae*

Disease scale	Symptoms[a]
1	Healthy green seedlings
2	Bottom leaves turn light yellow
3	Bottom and middle leaves are yellow
4	Seedlings are damping off, all leaves are yellow and stems are brown
5	Seedlings dead

[a]Symptoms evaluated 3 days after inoculation of *P. nicotianae*.

ted pathogen. The entire experiment was repeated once and results were compared with those from stem inoculations on adult plants in the greenhouse.

Adult plant inoculations

The six *P. nicotianae* isolates selected for this study were grown on potato dexstrose agar (Biolab, Midrand, South Africa) at 27°C in the dark for 5 days. Agar discks (5 mm in diameter), taken from the actively growing edges of these cultures were used as inoculum for pathogenicity tests. Eight weeks after planting, tobacco plants were inoculated by removing the cambium from the stems of the test plants using a corkborer (5 mm diameter), 2 cm above ground level. A disc of agar, colonized with *P. nicotianae* was placed into the wound, mycelium side downwards and sealed with parafilm.

Eight plants of each tobacco cultivar (Coker 371-Gold, KY14, KY14 × L8, Beinhart 1000-1, Vuma/3/46 and LK33/60) were inoculated with each of the six *P. nicotianae* isolates. An equal number of control plants were inoculated with sterile discs of agar. A completely randomized block design was used in the trial and the entire trial was repeated once. In each case the plants were examined for lesion length, 7 days after inoculation. Re-isolations were made from the lesions on PARPH medium (Tsao and Guy, 1977) to verify that the inoculated pathogen had caused the lesion.

Validation of the seedling assay

Data obtained from the seedling and adult plant inoculations were statistically analysed for variances and differences among isolates and tobacco cultivars. Mean values were tested for significance according to Tukey's procedure (Steel and Torrie, 1980). Pearson's correlation coefficient was calculated among results of the stem inoculation and the seedling assays using the SAS CORR Procedure (SAS Institute Inc., Cary, NC, USA).

Results

Seedling inoculations

Symptoms developed on susceptible seedlings 48 h after inoculation with *P. nicotianae*. For example, KY14 seedlings inoculated with isolate CMW 6924 exhibited yellow leaves, brown stems and root rot symptoms (Figs 1–2). Nine of the latter 10 KY14 seedlings were dead after 3 days and had disease rating scale values of 5 (Table 3). By contrast, seedlings with resistance to *P. nicotianae*, such as the 10 Beinhart 1000-1 seedlings inoculated with isolate CMW 6922 were all green and healthy after 3 days and had disease rating scale values of 1–2 (Table 3).

Significant differences (LSD $0.05 = 0.85$) were found for disease ratings associated with the various *P. nicotianae* isolates on the differential and commercial cultivars tested (Table 3). The seedling assay allowed us to effectively distinguish between susceptible seedlings of KY14 where disease ratings ranged from 3.10 to 4.95 and for resistant seedlings of Beinhart 1000-1 the values ranged from 1.00 to 2.00. All seedlings of Beinhart 1000-1 inoculated with either race of the pathogen survived. The commercial cultivars LK33/60 and Vuma/3/46 were more susceptible to race 1 where disease ratings ranged from 3.10 to 5.00 than race 0 where disease ratings ranged from 1.10 to 3.45.

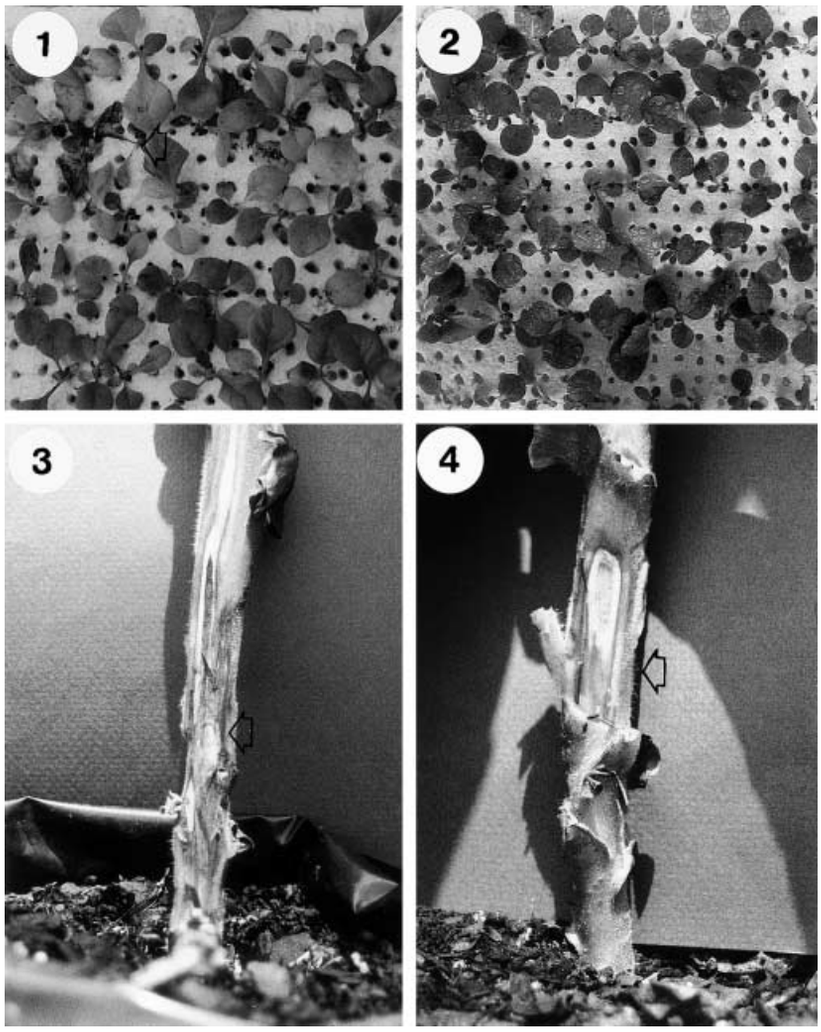
Phytophthora nicotianae isolates could be distinguished as races 0 and 1 based on the response of the cultivars KY14 × L8 and Coker 371-Gold to infection. Disease ratings of KY14 × L8 and Coker 371-Gold seedlings inoculated with race 1 isolates ranged from 3.85 to 5.00 and did not differ significantly (LSD $0.05 = 0.85$) from the susceptible cultivar KY14, where values ranged from 3.10 to 4.95. However, when seedlings of KY14 × L8 and Coker 371-Gold were inoculated with race 0 isolates, disease ratings ranged from 1.65 to 2.60 and differed significantly (LSD $0.05 = 0.85$) from KY14 where values ranged from 3.00 to 4.45. The disease ratings of the cultivars KY14 × L8 and Coker 371-Gold infected with race 0 and 1 isolates thus reflected the well-established resistance of these cultivars to race 0 of *P. nicotianae*.

Adult plant inoculations

In stem inoculation trials, symptoms on susceptible adult plants included wilting and rapid collapse of the stem tissue. This resulted in black lesions at the bases of stems, which is characteristic of black shank (Fig. 3). Adult plants with resistance to *P. nicotianae* remained green and healthy with only small lesions at the points of inoculation (Fig. 4).

Significant differences (LSD $0.05 = 31.36$) were found in lesion lengths associated with the various *P. nicotianae* isolates on differential and commercial cultivars (Table 4). Lesion lengths on the resistant cultivar Beinhart 1000-1 were significantly (LSD $0.05 = 31.36$) smaller (28.1–45.8 mm) than those on susceptible cultivar KY14. All adult Beinhart 1000-1 plants inoculated with races 0 and 1 survived. Lesion lengths on the commercial cultivars LK33/60 and Vuma/3/46 showed that they have higher levels of resistance to race 0 with lesions ranging from 51.1 to 64.9 mm compared with those associated with race 1 ranging from 58.8 to 114.5 mm.

Phytophthora nicotianae isolates could be distinguished as races 0 and 1 based on the lesion lengths on cultivars KY14 × L8 and Coker 371-Gold. Lesions



Figs. 1–4 Seedling and adult tobacco plants inoculated with *P. nicotianae*. Fig. 1 The rapid seedling-based screening technique exhibiting susceptible seedlings with yellow leaves, brown stems and root rot symptoms after 3 days. Fig. 2 The rapid seedling-based screening technique exhibiting healthy green seedlings that serve as the control. Fig. 3 Adult tobacco plant showing the characteristic black lesion after inoculation with *P. nicotianae*. Fig. 4 Adult tobacco plants with resistance to *P. nicotianae* remained green and healthy with only small lesions at the point of inoculation

on KY14 × L8 and Coker 371-Gold inoculated with race 1 isolates ranged from 71.6 to 119.0 mm and did not differ significantly (LSD 0.05 = 31.36) from those on the susceptible cultivar KY14, where lesions ranged from 147.4 to 189.5 mm long. However, when adult plants of KY14 × L8 and Coker 371-Gold were inoculated with race 0 isolates, lesion lengths ranged from 40.5 to 64.1 mm and differed significantly (LSD 0.05 = 31.36) from KY14 where the values ranged from 120.3 to 126.1 mm. Lesion lengths on KY14 × L8 and Coker 371-Gold plants inoculated with race 1 were significantly larger than those caused by race 0 thus reflecting the established resistance of these cultivars to race 0.

Validation of the seedling assay

A Pearson's correlation coefficient value of 0.61887 (Probability > |r| under HO: < 0.0001) was calcu-

Table 3
Disease rating scale values of six tobacco cultivars after inoculation with six *P. nicotianae* isolates in the seedling assay[a]

	Race 0			Race 1		
Cultivars	CMW 6921	CMW 6916	CMW 6922	CMW 6923	CMW 6924	CMW 6925
Differential cultivars						
KY14	4.45e[b]	3.00cd	3.10d	3.10b	4.95b	3.65b
KY14×L8	2.00ab	1.80a	2.05c	4.00c	4.90b	5.00d
Coker 371-Gold	2.60a–d	2.40a–c	1.65a–c	3.85bc	4.65b	4.10b–c
Beinhart 1000-1	1.80a	2.00ab	1.00a	1.75a	1.80a	2.00a
Commercial cultivars						
LK33/60	2.95cd	2.90cd	1.95bc	3.70bc	5.00b	4.60c–d
Vuma/3/46	2.10ab	3.45d	1.10ab	3.10b	4.50b	4.20b–d

[a]Each value represents the mean of 10 replicates;
[b]Within each column, values followed by the same letter are not significantly different. Tukey LSD (0.05) = 0.85.

Table 4
Lesion lengths on six tobacco cultivars after inoculation with six *P. nicotianae* isolates in the adult plant assay[a]

	Race 0			Race 1		
Cultivars	CMW 6921	CMW 6916	CMW 6922	CMW 6923	CMW 6924	CMW 6925
Differential cultivars						
KY 14	123.1b[b]	126.1b	120.3b	189.5e	181.5e	147.4e
KY 14 × L8	63.4a	49.8a	64.1a	96.8b–d	97.0b–d	119.0de
Coker 371-Gold	50.1a	40.5a	46.0a	71.6a–b	96.3bc	82.6b–d
Beinhart 1000-1	36.3a	39.0a	41.5a	45.8a	33.3a	28.1a
Commercial cultivars						
LK33/60	64.9a	57.8a	51.1a	114.5d	105.6b–d	77.0bc
Vuma/3/46	58.8a	62.9a	59.9a	73.1a–c	71.3ab	58.8ab

[a]Each value represents the mean of eight replicates, lesion lengths are measured in the cambium (mm).
[b]Within each column, values followed by the same letter are not significantly different Tukey LSD (0.05) = 31.36.

lated among results of the stem inoculation and the seedling assays, indicating a corresponding relationship between the two resistance screening techniques. Thus plants with resistance to *P. nicotianae* could be reliably identified using the seedling assay and the stem inoculation technique.

The difference between *P. nicotianae* isolates belonging to race 0 or 1 could be identified as using both the stem inoculation technique and the rapid seedling assay. Disease ratings on seedlings and average lesion lengths (Tables 3 and 4) for isolates CMW 6921, CMW 6916 and CMW 6922 were significantly lower ($P < 0.05$) in the cultivars KY14 × L8 and Coker 371-Gold, which are known to have resistance to race 0 than the susceptible cultivar KY14. Isolates CMW 6921, CMW 6916 and CMW 6922 were thus characterized as race 0 of *P. nicotianae*. Isolates CMW 6923, CMW 6924 and CMW 6925 were likewise characterized as race 1 as these isolates had high disease ratings and longer lesions on Coker 371-Gold and KY14 × L8.

Discussion

In this study, a rapid seedling-based technique was developed to identify and characterize plants with resistance to black shank. This can now be effectively used in screening F1 progeny from breeding trials. Resistance identified using this seedling assay was positively correlated with that identified with the more commonly used adult plant inoculation technique (Hendrix and Apple, 1967; Wills, 1971; Wills and Moore, 1971).

Using the rapid screening technique developed in this study, the commercial cultivar Vuma/3/46 was rated as more susceptible to race 1 than with the stem inoculation technique. Here, it must be recognized that the two techniques are fundamentally different from the one linked to natural root infection and the other to stem wound infection. When this difference is considered, the result is not unusual and other authors (Hendrix and Apple, 1967; Csinos, 1999) have also reported differences between stem and root resistance to *P. nicotianae*. Thus the cultivar Vuma/3/46 exhibits high levels of stem resistance in adult plants while it displays a level of susceptibility in seedling roots.

The rapid screening assay, in combination with the differential cultivars, was effective in characterizing *P. nicotianae* isolates as belonging to races 0 and 1. This technique could potentially be used to identify *P. nicotianae* races on commercial tobacco farms. Knowledge of the races of *P. nicotianae* present on commercial farms is valuable to extension officers who must recommend cultivars with appropriate resistance to growers.

The seedling assay presented in this study has been used successfully in the characterization of large numbers of disease-resistant F1 progeny in breeding trials at the Lowveld Tobacco Growers Association (Mpumalanga) in South Africa. The technique provides rapid results and is inexpensive. Using a zoospore suspension as inoculum has reduced the incubation period for seedlings to 3 days. This is in contrast to the technique of Maia et al. (1995) where seedlings were inoculated with mycelium discs and incubated for 7 days. The use of polystyrene trays and plastic containers is also less tedious than transferring seedlings, mycelium discs and water to individual Eppendorf tubes as described by Maia et al. (1995).

The limitation of the seedling assay described in this study is that it is difficult to select seedlings with low or moderate resistance to infection. Maia et al. (1995) emphasized the importance of choosing isolates of *P. nicotianae* with intermediate levels of pathogenicity that will kill all susceptible cultivars, but not those with low levels of resistance. Adult plant screening of wild germplasm and parental material should not be discarded from breeding programmes as not all resistance may express itself at the seedling stage.

The ability to screen large numbers of tobacco plants rapidly at the seedling stage is an essential component of effective black shank resistant breeding programmes. It allows for the testing of large germplasm resources in a systematic manner and under standard conditions. Ultimately, this will promote the availability of new black shank-resistant cultivars for commercial deployment. Financially, growers will benefit from improved yields and higher quality plants as well as lower production costs.

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References

Apple, J. L. (1957): Pathogenic, cultural, and physiological variation within *Phytophthora parasitica* var. *nicotianae*. Phytopathology **47,** 733–740.

Carlson, S. R., M. F. Wolff, H. D. Shew, E. A. Wernsman (1997): Inheritance of resistance to race 0 of *Phytophthora parasitica* var. *nicotianae* from the flue-cured tobacco cultivar Coker 371-Gold. Plant Dis. **81,** 1269–1274.

Chaplin, J. F. (1962): Transfer of black shank resistance from *Nicotiana plumbaginifolia* to flue-cured *N. tabacum*. Tob. Sci. **6,** 184–189.

Chaplin, J. F. (1966): Comparison of tobacco black shank resistance from four sources. Tob. Sci. **10,** 55–58.

Csinos, A. S. (1999): Stem and root resistance to tobacco black shank. Plant Dis. **83,** 777–780.

Csinos, A. S., P. F. Bertrand (1994): Distribution of *Phytophthora parasitica* var. *nicotianae* races and their sensitivity to Metalaxyl in Georgia. Plant Dis. **78,** 471–474.

Hendrix, J. W., J. L. Apple (1967): Stem resistance to *Phytophthora parasitica* var. *nicotianae* in tobacco derived from *Nicotianae longiflora* and *N. plumbaginifolia*.. Tob. Sci. **11,** 148–150.

Kim, J. H., Y. K. Kang (1997): Screening alternative fungicides to control metalaxyl resistant *Phytophthora parasitica* var. *nicotianae* in Korea. Coresta Information Bull. **2,** 128.

Litton, C., P. D. Legg, G. B. Collins (1970): A greenhouse technique for screening tobacco seedlings for black shank resistance. Tob. Sci. **14,** 124–125.

Lucas, G. B. (1975): Diseases of Tobacco. Harold E. Parker and Sons Printers, Fuquay-Varina, North Carolina.

Maia, N., R. Delon, F. Trentin, P. Bonnet, N. Dubelski, J. P. Lutz, P. Ricci (1995): Two techniques for assaying resistance to *Phytophthora parasitica* var. *nicotianae* on young tobacco plants. Tob. Sci. **39,** 43–48.

Melton, T. A. (1998): Disease Management. Flue-Cured Tobacco Information, North Carolina Cooperative Extension Service, North Carolina State University, College of Agriculture and Life Science, pp. 92–112.

Nielsen, M. T. (1995): Coresta black shank collaborative study. Coresta Information Bull. **1,** 31–34.

Prinsloo, G. C. (1994): Black shank of tobacco in the Republic of South Africa. (Abstr.) p. 89. In: Coresta Congress 1994, Harare, Zimbabwe.

Ribeiro, O. K. (1978): A Source Book of the Genus *Phytophthora*. Cramer, Vaduz, Liechtenstein. 417 pp.

Rufty, R. C., E. A. Wernsman, G. V. Jr. Gooding (1987): Use of detached leaves to evaluate tobacco haploids and double haploids for resistance to tobacco mosaic virus, *Meloidogyne incognita*, and *Pseudomonas syringae* pv. *tabaci*. Phytopathology **77,** 60–62.

Shew, H. D. (1985): Response of *Phytophthora parasitica* var. *nicotianae* to metalaxyl exposure. Plant Dis. **69,** 559–562.

Shew, H. D. (1991): Root and stem diseases caused by fungi. In: Shew, H.D. and G.B. Lucas (eds), Compedium of Tobacco Diseases, pp. 17–29. American Phytopathological Society, St. Paul, Minnesota, USA.

Silber, G., H. E. Heggestad (1963): Comparative black shank resistance of Beinhart 1000 (Quindiaz), N.C. 5346, and Rg in F1 generation involving various tobacco types. Tob. Sci. **7,** 144–147.

Steel, R. G. D., J. H. Torrie (1980): Principles and Procedures for Statistics, 2nd edn. McGraw-Hill, New York.

Tedford, E. C., T. L. Miller, M. T. Nielsen (1990): A detached-leaf technique for detecting resistance to *Phytophthora parasitica* var. *nicotianae* in tobacco. Plant. Dis. **74,** 313–316.

Tisdale, W. B. (1922): Tobacco diseases in Gadsden County in 1922. Florida Agric. Exp. Sta. Bull. **166,** 77–118.

Tsao, P. H., S. O. Guy (1977): Inhibition of *Mortierella* and *Pythium* in a *Phytophthora* isolation medium containing hymexazol. Phytopathology **67,** 796–801.

Valleau, W. D., G. W. Stokes, E. M. Johnson (1960): Nine years experience with the *Nicotianae longiflora* factor for resistance to *Phytophthora parasitica* var. *nicotianae* in the control of black shank. Tob. Sci. **4,** 92–94.

Van Jaarsveld, E. (1995): Black Shank. Kaleidoscope of Tobacco, a publication of the Lowveld Tobacco Growers Association **1,** 20–28.

Van Jaarsveld, E., M. J. Wingfield, A. Drenth (2002a): Effect of metalaxyl sensitivity and cultivar resistance on control of *Phytophthora nicotianae* in tobacco. Plant Dis. **86,** 362–366.

Van Jaarsveld, E., M. J. Wingfield, A. Drenth (2002b): Evaluation of tobacco cultivars for resistance to races of *Phytophthora nicotianae* in South Africa. J. Phytopathol. **150,** 456–462.

Wills, W. H. (1971): A comparison of black shank resistance of tobacco measured in inoculated whole plants and excised leaf tissue. Tob. Sci. **15,** 47–50.

Wills, W. H., L. D. Moore (1971): Response of some cultivars and lines of tobacco to stem inoculation with *Phytophthora parasitica* var *nicotianae*. Tob. Sci. **15,** 51–53.