

EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR *PHYTOPHTHORA PARASITICA* VAR. *NICOTIANAE* IN TOBACCO



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Diseased tobacco (*Nicotiana tabacum* L.) samples collected from a black shank nursery or commercial fields in 1988 and 1989 were used to compare enzyme-linked immunosorbent assay (ELISA) *Phytophthora* kits to standard culture plating identification techniques (CID) for *Phytophthora parasitica* var. *nicotianae*. Each of the samples tested was tentatively identified as black shank based on symptoms and field history. The immunoassays were double antibody sandwich ELISAs. A "dipstick" ELISA test was evaluated in 1988, and the Alert™, "D" On-Site Assay, ELISA was used in 1989. Stronger ELISA reactions were obtained from pith tissue in stem lesions than from rhizosphere soil or infected roots. In 1988, all of the 39 samples tested positive for *Phytophthora* with the ELISA "dipstick," whereas the CID technique only detected

Phytophthora in 76.9% of the samples. Three of the 39 samples were identified as *Phytophthora* by CID, but were negative by ELISA, and 9 of 39 samples were identified for *Phytophthora* by ELISA but did not yield cultures. In 1989, 29 of 55 samples evaluated were diagnosed as black shank by both Alert™ *Phytophthora* Assay ELISA test kit and by the CID technique. Two of the 55 samples were identified as *Phytophthora* by CID techniques but were negative by ELISA, and 8 of 55 samples were positive by ELISA but negative by the CID technique. The Alert™ test was quick and very comparable in diagnoses to CID techniques.

Additional key words: Black Shank, Disease Detection, ELISA, *Nicotiana tabacum*.

INTRODUCTION

Black shank of tobacco (*Nicotiana tabacum* L.) incited by the pathogen *Phytophthora parasitica* Dast. var. *nicotianae* (B. de Haan) Tucker is a serious soilborne disease in the tobacco production regions of the Eastern U.S.A. Typically those conditions most favorable for rapid growth of tobacco are also conducive to rapid propagule generation and pathogen dissemination (3,4,5). Since the pathogen is persistent in the soil, several control methods are used to manage the disease, including rotations, cultivar resistance, and chemicals (1,2).

Diagnosis of field problems requires time-consuming culturing and expertise in microscopic observation and identification. The application of ELISA for the detection of plant pathogenic fungi has increased in the last decade, at least in part as a result of the development and use of monoclonal antibodies (9). As early as 1969 Merz et. al (8) compared species of *Phytophthora* serologically. Monoclonal antibodies reactive with a number of species of *Phytophthora* have been produced and employed in ELISA (7,10,12) or with other detection methods (6). Agri-Diagnostics Associates, Cinnaminson, NJ, has developed field-usable immunoassays incorporating monoclonal antibodies that react with different species of *Phytophthora*. However, *P. parasitica* var. *nicotianae* is the only *Phytophthora* reported to cause disease on tobacco; therefore, positives from other *Phytophthora* should not be a problem. An initial "dipstick" format (11) was replaced in 1989 by a more sensitive, 10-minute "flow-through" ELISA (10). The same monoclonal antibody was incorporated into both assays. This study evaluates both assays for rapid diagnosis of *P. parasitica* var. *nicotianae* and compares them to standard culture and identification techniques.

MATERIALS AND METHODS

Eighty-nine samples were obtained from diseased tobacco plants in the black shank nursery located at the Coastal Plain Experiment Station (CPES) in Tift County, GA, and five diseased specimens were received by the CPES or the Cooperative Extension Service Diagnostic Laboratory at the Rural Development Center in Tifton, GA. All samples were believed to be black shank based on field history and symptoms.

The immunoassays used in this study were double antibody sandwich ELISAs developed by Agri-Diagnostics Associates. All instructions and materials required to conduct the tests were provided in the kit. In all cases, unless otherwise specified, roots and stems of plants were washed gently in running tap water. Diseased tissue was aseptically removed from the pith at or near the leading edge of the lesion. Rhizosphere soil was collected by gently removing soil from infected roots.

Isolations of *P. parasitica* var. *nicotianae* were made by placing bits of infected pith into sterilized tap water. After 48-72 hr at ca. 20°C, water cultures were microscopically observed for characteristic mycelium and ovoid, papillate sporangia. The combination of symptoms and the presence of the characteristic sporangia were considered positive identification for *Phytophthora parasitica* var. *nicotianae*.

In 1988, eight tobacco cultivars (McNair 944, K-326, Coker 371 Gold, Speight G-70, NC 2326, Speight G-28, Coker 48, NC 95) and TI 1071 were used to evaluate the sensitivity of the *Phytophthora* dipstick ELISA kit (11). This test required 3.0 hr per sample to complete. In 1989, 55 samples of 12 tobacco cultivars (Speight G-70, Speight G-28, K-326, NC 95, McNair 944, Coker 48, KK-370, VA 116, NC 2326, K-340, NC 37NF, K-399) and TI 1071 were used to evaluate the sensitivity of an improved ELISA technique, the Alert™ *Phytophthora* "D" On-Site Assay. Only about 10 min were required per sample with the Alert™ kit (10). In both years, the ELISA assays were compared to an isolation identification technique.

RESULTS AND DISCUSSION

Extensive preliminary studies with the ELISA kits indicated that noninfected tobacco plants of several cultivars did not yield false positive reactions. When compared to roots and rhizosphere soil, infected pith tissue gave the strongest reactions. In 1988, all 39 plants with symptoms of black shank tested positive for *Phytophthora* with the dipstick ELISA, whereas the culture-identification technique (CID) only detected *Phytophthora* in 76.9% of the plants. Nine of the 39 samples were negative in the CID technique but were positive for black shank when tested with the dipstick ELISA (Table 1). The two assays were in agreement with respect to diagnosis 76.9% of the time.

The levels of resistance to *P. parasitica* var. *nicotianae* in modern tobacco cultivars varies from very low to very high (1). The cultivars McNair 944, Speight G-28, Speight G-70, Coker 48, and K-399 all have moderate resistance, and Coker

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Contribution received February 6, 1991. Tob. Sci. 36: 6-7, 1992.

371 Gold has high resistance to the fungus under Georgia conditions. The breeding line 1071 is immune to race 0 but is susceptible to race 1 of the pathogen. In these tests the ELISA test detected both races of the pathogen equally well.

In 1989, 55 diseased plant samples, for which black shank was diagnosed on the basis of symptoms and field history, were tested. The Alert™ *Phytophthora* "D" On-Site Assay uses a flow-through format which has very abbreviated steps requiring only 10 min per sample for identification (10). Of the 55 samples that were tested, 29 were judged positive by both of the methods, and 16 were negative by both of the methods that resulted in 81.8% agreement between the two assays (Table 1). Eight were positive by ELISA, but negative by CID, and two were negative by ELISA but positive by CID. ELISA gave a positive reaction for 67.3% of the samples but only 56.4% of the 55 plants yielded cultures. Negative results in both tests for 16 samples most probably indicated failure of both methods to detect *P. parasitica* var. *nicotianae*. Since most of the samples came from a black shank disease nursery, misdiagnosis is highly unlikely. Not all positive reactions with the ELISA were unequivocal. Color intensity varied from sample to sample, as did abundance of sporangia in water cultures.

Discrepancies between the immunoassay and culture isolation methods may be the result of a number of factors, including sampling error, presence of very low levels of *P. parasitica* var. *nicotianae* in the tissue, differential detection of non-viable pathogen, and/or the condition of the sample. In a study of *Phytophthora* detection in woody ornamental plants, most of the discrepancy observed between immunoassay and culture plating results was attributed to sampling error (7).

One characteristic of most ELISAs, including these immunoassays for *Phytophthora*, is that they can detect both viable and nonviable propagules. Samples that are brought or mailed to a laboratory for diagnosis may be in poor physical condition and may be severely dehydrated or overrun with secondary pathogens and/or saprophytes. Such samples may be extremely poor candidates for isolation of *Phytophthora* spp. by standard isolation methods. However, as long as the antigen with which the antibodies react has not degraded and remains present in sufficient concentration, an appropriate diagnosis can be made by immunoassay.

There are other advantages of ELISA over traditional culture methods for *P. parasitica* var. *nicotianae* detection. The first is speed and ease of diagnosis by ELISA, particularly by the newer version (Alert™ On-Site immunoassay), compared to culture identification. The production of water cultures is relatively time consuming (24-48 hr required for formation of sporangia) and often yields variable results. Water quality, condition of the sample, and incubation conditions affect fungal growth and development of sporangia. In addition, the On-Site assay can be carried out in the field, if necessary, by persons without training in fungal identification or access to a laboratory.

ACKNOWLEDGEMENT

The authors wish to thank Wanda Tillery and Lewis Mullis for technical assistance, Agri-Diagnostics Associates for providing test kits, and State and Hatch for funds provided to the College of Agriculture, University of Georgia.

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Table 1. Comparison of immunoassay technique (ELISA) with culture-microscopic identification for diagnosis of tobacco black shank

Year	Samples Evaluated	Positive Diagnosis		Agreement
		ELISA	Culture-Identification (CID)	
	No.	-----%	-----%	%
1988 ^a	39	100.0	76.9	76.9
1989 ^b	55	67.3	56.4	81.8

^a Thirty-six samples were from the black shank nursery in Tifton, and three were from growers' fields.

^b Fifty-three samples were from the black shank nursery in Tifton, and two samples were from growers' fields.