LABORATORY ASSAYS FOR EVALUATION OF PRODUCTS FOR CONTROL OF POLE ROT OF TOBACCO CAUSED BY RHIZOPUS ARRHIZUS

Richard D. Reeleder[1]

Procedures for the rapid screening of chemical products for control of pole rot of tobacco were developed. A leaf disk assay was developed using disks cut from mature leaves selected from greenhouse-grown plants ('Delgold'). Leaf disks were placed in a glass Petri dish humid chamber. Leaf disks were first sprayed with the candidate product, and then they were sprayed with a suspension of *Rhizopus arrhizus* Fischer sporangiospores, the causal agent of pole rot. After three days of incubation at 30°C, leaf disks were rated for the degree of rot. A similar assay, using sections of wooden sticks to simulate curing kiln sticks and the kiln structure, also was developed. Chemicals providing a high level of protection in both assays were Acticide CS (octylinone) and Bravo (chlorothalonil). Several products (Bayleton, Botran, Dithane M-45, Dyrene, Mystox WFA, and Triton X-100) provided a high level of control in the disk assay, but they had intermediate or poor control in the stick assay. The surfactants Agral 90, Nonidet P-40 and Renex 36, the sucker control product Razor, and Pool Algicide also controlled rot of leaf disks by *R. arrhizus*. When potato dextrose agar was amended with these products and used in radial growth assays, the resulting ED_{50} values generally reflected results of disk assays. When effects of products on sporangiospore germination were assessed, products effective in controlling rot in disk assays varied in their ability to inhibit germination and in their toxicity to spores.

Additional key words: *Nicotiana tabacum*, barn rot chlorothalonil, nonylphenoxypolyethoxyethanol, triadimefon, mancozeb, anilazine, dicloran, orthophenylphenate chlorthal dimethyl, octylphenolethylene, benzylammonium chloride, polyoxyethylene tridecyl alcohol, polyoxyethylene-mono-octylphenyl ether.

INTRODUCTION

Pole rot, caused by *Rhizopus arrhizus* Fischer, is the most serious disease of flue-cured tobacco grown in Ontario, Canada (3). Yearly losses are between 1-2% of the cured crop. The disease occurs after harvest while tobacco is being cured. Temperatures and humidities prevalent during the early stages of flue curing promote disease development (2,3,5). Similar diseases caused by *Botrytis cinerea* and *Erwinia* spp. (2) are reported from other tobacco growing regions, but *R. arrhizus* is the most important incitant in Ontario.

Dicloran (Botran), applied to tying twine or leaf butts, has been used to control the disease effectively elsewhere, but residues of this fungicide in cured leaves have precluded its use in Ontario (4,9). Recommended control measures in Ontario include sanitation of the kiln area to remove tobacco debris and treatment of kilns with formaldehyde. Several growers now routinely treat kiln sticks with formaldehyde before use. These measures reduce the incidence of pole rot on some farms, however, the hazardous nature of formaldehyde and its apparent failure to provide sufficient control on some farms have necessitated a search for alternative control measures. In addition, some growers and extension specialists believe that rainfall or irrigation shortly before harvest reduces the incidence of pole rot. This suggests that inoculum of *R. arrhizus* may be carried into the kilns on leaves, although other explanations for this phenomenon are possible. Thus, in addition to treatment of harvested leaves and kilns with chemical products, treatments applied to leaves before harvest may also be of value in management of pole rot. Products applied to leaves might be useful in reducing inoculum levels on leaf tissue and, where sufficient residues persist, may also provide protection to harvested leaves from inoculum present on kiln sticks. Laboratory assays to rapidly screen products for their potential as leaf or kiln treatments are required to select the most promising candidates for field-scale tests.

I report here on the development and use of laboratory assays for screening chemical products for their effectiveness in reducing *R. arrhizus* inoculum in the kiln area and for their potential as treatments applied to leaves before curing.

[1] Plant Pathologist, Agriculture Canada Research Station, P. O. Box 186, Delhi, Ontario, N4B 2W9

Contribution received July 10, 1992, Tob. Sci. 37:34-38, 1993.

MATERIALS AND METHODS

Plant Production

Tobacco ('Delgold') seeds were germinated on moist filter paper in Petri plates then transplanted into clay pots (25 cm diam) containing steamed muck soil. Plants were produced in the greenhouse at temperatures ranging from 25-35°C during the day and 15-25°C at night. Plants were watered as required. At flowering, inflorescences were removed from the tops of the plants. Mature leaves removed from the plants were used as a source of leaf disks for screening assays. A color chart (ISCC-NBS Color-Name Charts Illustrated with Centroid Colors; Supplement to NBS Circular 553; Inter-Society Color Council, National Bureau of Standards, Washington, DC) was used to aid in selection of mature leaves. In preliminary tests, leaf disks taken from leaves with a color corresponding to #116 (brilliant yellow green) yielded consistently high levels of rot when inoculated with *R. arrhizus* in a disk assay. Thus, leaves with this color were used in the leaf disk assays described below.

Production of Inoculum of *R. arrhizus*

Three-day-old cultures were produced on Difco potato dextrose agar (PDA) under ambient laboratory conditions (24 ± 2°C). Sporangiospores were removed from the culture by adding approximately 5 mL of sterile 0.1 M sodium phosphate buffer (pH 6.8) and rubbing the surface of the culture gently with a bent glass rod. The suspension was poured off and adjusted to 1×10^6 spores per mL in sterile buffer.

Leaf Disk Assay

A procedure was developed to select products inhibitory to *R. arrhizus* which could be applied to tobacco plants either before or after harvest. Selected leaves were rinsed with 1% sodium hypochlorite, then rinsed three times with sterile water before air drying in a laminar flow hood. Leaf disks (2-cm diam) were cut from interveinal areas using a sterile cork borer. Three disks were placed on a 6.8 x 6.8 cm nylon mesh square contained in a 9-cm diam sterile glass Petri dish lined with moist filter paper. A second nylon square was placed over the disks. Four such replicate dishes were prepared for each treatment. Disks then were treated with one of 23 candidate products (**Table 1**) by spraying disks with a suspension (1000 mg a.i. L^{-1}) of the candidate product for 7 seconds. Spraying was carried out using an air brush

Model 350, Badger Air-Brush Co., Franklin Park, IL) operated at 69 kPa. Approximately 1 mL was applied to the disks in each Petri dish. Inoculum of *R. arrhizus* was then applied similarly. The covers of the dishes were put in place and the dishes were placed in a 30°C dark incubator (Conviron G 30) at 95% RH. For each series of products tested, additional dishes were prepared as checks on virulence of inoculum (no product applied) and on contamination of leaves by *R. arrhizus* (no product or inoculum applied). After 3 days, each disk was rated for percentage of rot, and a mean value was calculated for each of the four replicate dishes. Each product was evaluated twice. The General Linear Model of SAS (11) and Duncan's Multiple Range Test (11) were used to compare treatments.

Stick Assay

A procedure was developed to select products (**Table 1**) which could replace formaldehyde as disinfectant treatments for kiln sticks. Wooden coffee-stirring sticks (11 cm x 1 cm x 2 mm) were used to simulate kiln sticks. In preliminary tests, soaking sticks in Difco potato dextrose broth (PDB) provided superior and more consistent levels of colonization by *R. arrhizus* than soaking sticks in distilled water; thus PDB was used throughout. Sections (5.5 cm in length) of these sticks were soaked in PDB for 2 hr followed by autoclaving for 1 hr at 103 kPa and 120°C. Sticks then were soaked in a suspension (1000 mg a.i. L^{-1}) of the test product for 1 hr. Four sticks were placed in a sterile glass petri dish lined with moistened filter paper. Four replicate dishes were prepared for each treatment. Sticks then were inoculated and incubated as described previously for leaf disks. Colonization by *R. arrhizus* of the sticks in each dish was estimated after 3 days using a 1-6 scale, where 1 indicated no visible growth and 6 indicated dense hyphal growth completely covering the sticks. Each product was evaluated twice. Data were analyzed as above.

Toxicity of Products

Preliminary tests indicated that certain surfactants were effective in preventing *R. arrhizus* from colonizing leaf disks. To determine if these effects were fungistatic or fungicidal, *in vitro* tests were carried out that assessed the effects of selected products (surfactants and fungicides) on spore germination and hyphal elongation. For spore germination studies, sporangiospore suspensions (1 x 10^6 spores per mL) were prepared in 1.5% PDB amended with the product (1000 mg a.i. L^{-1}). After 16 hr incubation on a shaker at 200 rpm and a temperature of 24 ± 1°C, sporangiospore germination was determined by removing an aliquot from each of four replicate flasks and observing at least 100 sporangiospores per aliquot under the microscope. Sporangiospores were considered to have germinated when the length of the germ tube was equal to or greater than the diameter of the spore. To determine if products were inhibitory or lethal, spores were separated from the liquid products by vacuum filtration, using a Buchner funnel and glass fibre filter paper (Whatman 934-AH). Then they were washed three times with sterile distilled water before being resuspended in PDB. Wettable powder fungicides could not be separated from the sporangiospores in this manner. Therefore, in order to reduce the effect of the fungicide, these suspensions were diluted 1:100 in 1.5% PDB and not filtered. After an additional 6 hr shaking at 200 rpm, sporangiospore germination was again determined. Germination data were calculated as the proportion of spore germination in PDB amended with a product to the germination in unamended PDB for each time period. Resulting values were analyzed as above.

Effects of products on hyphal elongation were assessed by measuring radial growth of *R. arrhizus* colonies growing on PDA amended with the product. PDA was amended by adding a known amount of the product to sterile, molten PDA held at 55°C. Amounts were selected to provide a range of concentrations (0 to 2000 ppm, at increments of 500 ppm) for each product. Molten, amended PDA was poured into sterile plastic petri dishes (100 x 15 mm) and allowed to solidify. A 5-mm agar disk from a one-day-old culture of *R. arrhizus* was placed on the cooled agar surface in the middle of the Petri dish. Cultures were incubated in the dark at 22 ± 1°C and colony radii were determined after 24 hr. Four replicate dishes were prepared for each concentration of the product. Linear regression analyses (11) were performed on log-transformed data to determine the effect of concentration on growth rate. The resulting regression equation was used to determine the concentration of active ingredient required to reduce growth by 50% (ED_{50}).

RESULTS

Several chemicals were effective in reducing leaf disk colonization by *R. arrhizus* (**Table 2**). Some provided complete control of the fungus while others were either ineffective or gave degrees of rot which were intermediate between the inoculated check and the uninoculated control treatments. The fungicides Bayleton, Botran, Bravo, Dyrene, and Dithane M-45; the disinfectants Mystox WFA, Acticide CS, and Pool Algicide; the sucker control product Razor; and the surfactants Agral 90, Nonidet P-40, Triton X-100, and Rennex 36 were all highly effective, and they were not significantly different from the noninoculated control treatment (**Table 2**). Other products were either less effective or not significantly different from the inoculated check.

Fewer products were effective in eliminating *R. arrhizus* inoculum from the wooden stick sections. Bravo, Copac E, Acticide CS, and Acticide SPX all yielded results which were not significantly different from the noninoculated check (**Table 2**). Other products were either less effective or not significantly different from the inoculated check. Each product was not tested in both the stick and leaf disk assay, because some products (e.g., sucker control treatments) were thought to have potential for one application but not the other.

Generally, hyphal elongation and spore germination data reflected results from leaf disk and stick assays. Products with very high ED_{50} values for hyphal elongation (e.g., Kumulus, Easout, Tween 20) were ineffective in both disk and stick assays. The surfactant Agral 90 and the sucker control product Razor were effective in reducing hyphal elongation, but they did not inhibit spore germination. Kocide 101, which had a relatively low ED_{50} and was highly inhibitory to spores, was only moderately effective in the disk assay, and it was ineffective in the stick assay. Fungicides which had low ED_{50} values were generally also inhibitory to spore germination. In these cases, dilution of the suspension failed to reduce inhibition. The fungicide Botran delayed spore germination, but, by the termination of the experiment, most spores had germinated.

DISCUSSION

The epidemiology of pole rot of tobacco is poorly understood. *R. arrhizus* is recovered readily from plant debris in or around tobacco kilns and soil. Kiln sticks become contaminated when tobacco debris remaining on the sticks becomes colonized by *R. arrhizus*. Observations by growers and extension personnel suggest that, while reduction of inoculum through treatment of kilns with formaldehyde reduces the incidence of pole rot, *R. arrhizus* may also be imported into the curing kilns on tobacco leaves. This may be due to the presence of spores of *R. arrhizus* on the leaves or to colonization of injured tobacco by *R. arrhizus* before or at harvest (2,3,4). This would indicate that both kiln and leaf treatments will be necessary before high levels of control of

pole rot can be achieved.

In contrast to other tobacco-producing areas, field application of fungicides is not practiced in Ontario. The subsequent lack of fungicide residues is important in the marketing of the crop. Thus, field application of fungicides is not likely to be supported by the tobacco industry unless cured tobacco can be shown to be free of fungicide residues. Dicloran, although effective, has been rejected for use in Canada because of unacceptably high residues. Some of the products which proved effective as leaf disk treatments in this study may be useful as field treatments before harvest or as treatments applied to butt ends of leaves tied onto kiln sticks. However, they will be acceptable only if tobacco quality is maintained and residues are within acceptable values. Larger-scale testing of these products is necessary to evaluate these factors. The active ingredient (chlorthal

Table 1. Products evaluated in assays.

Product Name	Common Chemical Name of Active Ingredient	Supplier/Manufacturer
Acticide CS	octylinone	THOR Chemicals (UK) Limited Cowley House, Earl Road Cheadle Hulme, Chesire United Kingdom SK8 6QP
Acticide SPX	chloromethyliso-thiozolin	THOR Chemicals (UK) Limited Cowley House, Earl Road Cheadle Hulme, Chesire United Kingdom SK8 6QP
Agral 90	nonylphenoxypoly-ethoxyethanol	ICI Chipman 400 Jones Road Box 9910 Stoney Creek, Ontario L8G 3Z1
Bayleton	triadimefon	Mobay Corporation Agricultural Chemicals Division Box 4913 Kansas City, MO 64120
Benlate	benomyl	DuPont Canada Inc. Box 2300, Streetsville Mississauga, Ontario L5M 2J4
Botran	dichloran	Nor-Am Chemical Company 3509 Silverside Road P.O. Box 7495 Wilmington, DE 19803
Bravo 500 F	chlorothalonil	ISK Biotech Ltd. 931 Commissioners Rd. E., Suite 102 London, Ontario L5Z 3H9
Chiptak	n-decanol	ICI Chipman 400 Jones Road Box 9910 Stoney Creek, Ontario L8G 3Z1
Copac E	ammoniacal copper sulphate	BASF Canada Inc. Agricultural Chemicals Division 345 Carlingview Drive Toronto, Ontario N9W 1K1
Delete	n-decanol	Cochran Corporation 2227 Deadrick Ave. Memphis, TN 38114
Dithane M-45	mancozeb	Rohm and Haas Company 2 Manse Road West Hill, Ontario M1E 3T9
Dyrene	anilazine	Chemagro Limited 77 Belfield Road Etobicoke, Ontario M9W 1G6
Easout	thiophanate-methyl	Ciba-Geigy Canada Ltd. Agrochemicals Division 6860 Century Avenue Mississauga, Ontario L5N 2W5
Emtrol	n-decanol + n-octanol	Henkel Canada 2290 Argentia Road Mississauga, Ontario L5N 6H9
Emtrol Ten	n-decanol	Henkel Canada 2290 Argentia Road Mississauga, Ontario L5N 6H9
Kocide 101	cupric hydroxide	Kocide Chemical Corporation P.O. Box 45539 12701 Almeda Road Houston, TX 77045
Kumulus S	sulphur lignosulphonate	BASF Canada Inc. Agricultural Chemicals Division 345 Carlingview Drive Toronto, Ontario N9W 1K1
Mystox LB	alkyl amine salt and non-halogenated phenol	Catomance Limited P.O. Box 18, 96 Bridge Road Welwyn Garden City, Herts United Kingdom AL7 1JW
Mystox WFA	sodium orthophenyl-phenate	Catomance Limited P.O. Box 18, 96 Bridge Road Welwyn Garden City, Herts United Kingdom AL7 1JW
Nonidet P-40	octylphenolethylene	Sigma Chemical Company P.O. Box 14508 St. Louis, MO 63178
Pool Algicide	benzylammonium chloride	Canadian Tire Corporation Ltd. Toronto, Ontario M4P 2V8
Razor	chlorthal dimethyl	ISK Biotech 5966 Heisley Road Mentor, OH 44061-8000
Renex 36	polyoxyethylene tridecyl alcohol	ICI Specialty Chemicals Atkemix Inc. Brantford, Ontario N3T 5T2
Ridomil EC 240	metalaxyl	Ciba-Geigy Canada Ltd. Agricultural Division 6860 Century Avenue Mississauga, Ontario L5N 2W5
Tenn Copp 5E	copper tallate	Tennessee Chemical Company 3400 Peachtree Road, NE Suite 401 Atlanta, GA 30326
Tilt EC 250	propiconazole	Ciba-Geigy Canada Ltd. Agricultural Division 6860 Century Avenue Mississauga, Ontario L5N 2W5
Triton X-100	polyoxyethylene mono-octylphenyl ether	Sigma Chemical Company P.O. Box 14508 St. Louis, MO 63178
Tween 20	polyoxyethylene sorbitan monolaurate	J.T. Baker Inc. 222 Red School Lane Phillipsburg, NJ 08865
Tween 80	polyoxyethylene sorbitan monoleate	J.T. Baker Inc. 222 Red School Lane Phillipsburg, NJ 08865

dimethyl) in the sucker control chemical Razor is also marketed as a herbicide (Dacthal). If other plant pathogens are inhibited by chlorthal dimethyl as markedly as *R. arrhizus*, then the use of this product on other crops as a herbicide may also contribute to disease control. Marks & Cerra (7) showed that applications of chlorthal dimethyl decreased the incidence of *Phytophthora* root rot of pine. Other herbicides have shown similar suppressive effects on plant pathogenic fungi (1,8,12). Compounds with surfactant activity are also known to inhibit fungal growth (6,10).

In these studies, there generally was a good relationship between the results of disk and stick assays and the effects on hyphal elongation and spore germination. Kocide 101 was more effective *in vitro* than would have been predicted from the disk and stick assays. Easout was less effective *in vitro* than expected. The ability of the various products to remain available to *R. arrhizus* after application to disks or sticks may have affected the results in some cases. In others, inhibitory effects during certain assays may have been due to breakdown products of the active ingredient that were not present in other procedures. Although most products with low ED_{50} values also appeared to be relatively lethal to spores, it is possible that washing or dilution did not remove a sufficient amount of the product. In these cases the observed toxicity could have been a fungistatic effect. Overall, however, these studies indicate that disk and stick assays reflect the ability of the products to inhibit *R. arrhizus*. These assays are less costly than *in vitro* tests on spore germination or hyphal elongation. Furthermore, the use of germination assays alone would not have detected potentially useful products such as Agral 90 and Razor. However, the ability of leaf disk and stick assays to predict performance of products under farm conditions is as yet undetermined. The leaf disk procedure in particular may also have value in detection of microbes useful in the biological control of pole rot.

The health hazards associated with use of formaldehyde suggest that the development of effective, less hazardous treatments of kiln sticks should be a priority. Several products appear promising in this regard. However, they need to be evaluated with respect to residues remaining on sticks after treatment and for possible movement of these residues into the cured leaf. High residues may preclude the use of compounds if such residues pose a health hazard or adversely affect tobacco quality. As indicated previously, large scale testing is needed to fully evaluate the potential of these products as kiln or kiln stick disinfectants. The products which showed promise in these studies for control of *R. arrhizus* also may be of use for other postharvest rots.

LITERATURE CITED

1. Cerkauskas, R.F., P.R. Verma, and D.L. McKenzie. Effects of herbicides on *in vitro* growth and carpogenic germination of *Sclerotinia sclerotiorum*. **Can. J. Plant Pathol.** 8:161-166. 1986.

2. Gayed, S.K. *Tobacco Diseases*. Canada Department of Agriculture, Ottawa. Publ. 1641. 56 pp. 1978.

3. Gayed, S.K., and M.C. Watson. Diseases of flue-cured tobacco in Ontario and estimates of disease losses, 1972-73.

Table 2. Effectiveness of products in leaf disk, stick, hyphal elongation, and sporangiospore germination assays.

Product	Disk[a]	Stick[b]	ED_{50}[c]	Proportion of Spore Germination	
				Inhibition[d]	Toxicity[e]
Acticide CS	0.00 c[f]	1.00 f[f]	11.01	0.00 e[f]	0.00 d[f]
Acticide SPX	ND[g]	1.00 f	2.04	ND	ND
Agral 90	0.03 c	ND	9.93	0.94 b	0.96 b
Bayleton	0.28 c	4.88 abc	6.97	0.00 e	0.00 d
Benlate	ND	4.50 cde	16.93	ND	ND
Botran	0.00 c	4.00 cde	16.92	0.05 e	0.93 b
Bravo 500 F	0.00 c	1.62 f	97.00	0.00 e	0.09 d
Chiptak	0.94 a	ND	ND	ND	ND
Copac E	ND	1.00 f	340.68	ND	ND
Delete	0.96 a	ND	ND	ND	ND
Dithane M-45	0.07 c	3.75 cde	217.57	0.00 e	0.00 d
Dyrene	0.09 c	3.38 de	23.04	0.00 e	0.00 d
Easout	0.62 b	4.62 bcd	>2000.00	0.54 d	1.00 a
Emtrol	0.96 a	ND	ND	ND	ND
Emtrol Ten	1.00 a	ND	ND	ND	ND
Kocide 101	0.64 b	4.20 cde	4.88	0.00 e	0.00 d
Kumulus S	ND	5.62 abc	>2000.00	ND	ND
Mystox LB	ND	3.25 e	7.56	ND	ND
Mystox WFA	0.06 c	4.00 cde	3.82	0.00 e	0.00 d
Nonidet P-40	0.00 c	ND	8.63	ND	ND
Pool Algicide	0.00 c	ND	31.78	0.00 e	0.10 d
Razor	0.05 c	ND	54.08	0.98 ab	0.99 ab
Renex 36	0.00 c	ND	14.32	ND	ND
Ridomil EC 240	ND	6.00 a	110.78	0.99 a	1.00 a
Tenn Copp 5E	ND	4.20 cde	4.25	0.69 c	0.80 c
Tilt EC 250	ND	5.75 ab	ND	ND	ND
Triton X-100	0.00 c	4.88 abc	20.83	ND	ND
Tween 20	1.00 a	4.25 cde	>2000.00	ND	ND
Tween 80	0.87 a	ND	ND	ND	ND
Check[h]	0.93 a	5.94 a	—	—	—
Control[i]	0.00 c	1.08 f	—	—	—

[a] Mean proportion of leaf disks rotted by *R. arrhizus*. Comparisons were made using pooled data from two trials.
[b] Mean coverage of sticks by *R. arrhizus*; where 1 indicates no mycelial growth visible and 6 indicates complete and dense coverage of stick by mycelium. Comparisons were made using pooled data from two trials.
[c] ED_{50} = effective dose (mg/liter) of active ingredient required to reduce radial growth by 50%.
[d] Proportion of sporangiospores germinated (in comparison with germination in unamended PDB) after 16 hr exposure to product.
[e] Proportion of sporangiospores germinated (in comparison with germination in unamended PDB) after removal of product by rinsing or dilution.
[f] Values in the column followed by the same letter are not significantly different (Duncan's Multiple Range Test, alpha = 0.05).
[g] ND = not done.
[h] No product applied.
[i] No product or inoculum applied.

Can. Plant Dis. Surv. 55:31-35. 1975.

4. Gayed, S.K., and E.K. Walker. The effect of dichloran on pole rot and certain leaf properties of flue-cured tobacco. **Tob. Sci.** 18:60-62. 1974.

5. Gayed, S.K. *Rhizopus arrhizus* causing pole rot of flue-cured tobacco in Ontario. **Can. J. Plant Sci.** 52:103-106. 1972.

6. Madelin, T.M. The effect of a surfactant in media for the enumeration, growth and identification of airborne fungi. **J. Appl. Bacteriol.** 63:47-52. 1987.

7. Marks, G.C., and R. Cerra. Effects of propazine and chlorthal dimethyl on *Phytophthora cinnamomi* root disease of *Pinus radiata* seedlings and associated microflora. **Soil Biol. Biochem.** 23:157-164. 1991.

8. Michailides, T.J., and R.A. Spotts. Effects of certain herbicides on the fate of sporangiospores of *Mucor piriformis* and conidia of *Botrytis cinerea* and *Penicillium expansum*. **Pestic. Sci.** 33:11-22. 1991.

9. Paddick, R.G., and P.L. Turner. Control of *Rhizopus* barn rot of flue-cured tobacco using dicloran. **Aust. J. Exp. Agric. Anim. Husb.** 13:612-615. 1973.

10. Papavizas, G.C., and R.D. Lumsden. Improved medium for isolation of *Trichoderma* spp. from soil. **Plant Dis.** 66:1019-1020. 1982.

11. SAS Institute Inc. *SAS/STAT Guide for Personal Computers*, Version 6 Edition. SAS Institute Inc., Cary, N.C. 378 pp. 1985.

12. Teo, B.K., P.R. Verma, and R.A.A. Morrall. The effects of herbicides and mycoparasites at different moisture levels on carpogenic germination in *Sclerotinia sclerotiorum*. **Plant Soil.** 1391:99-107. 1992.