Sources of Inoculum and Management for *Rhizoctonia solani* Damping-off on Tobacco Transplants under Greenhouse Conditions

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ABSTRACT

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Damping-off and target spot are important diseases of tobacco transplants produced under greenhouse conditions. Identification of sources of inoculum for these diseases caused by *Rhizoctonia solani* is an important first step in disease management. Control strategies based on sanitation and the eradication of primary inoculum were studied. Potting mix and Styrofoam trays used in transplant production were assayed to determine if they were sources of primary inoculum. Eleven sources of potting mix were sampled over a 2-year period. None of the mixes contained viable inoculum of *R. solani*. *R. solani* was isolated from previously used trays after 1 year of storage by removing and plating pieces of Styrofoam from individual tray cells on alkaline water agar (AWA). Sclerotia and melanized hyphae of *R. solani* were observed in the cracks present in the cells of the trays. Dry heat (70 to 80°C for 2 h) and chemical (sodium hypochlorite and sodium chloride) treatments reduced the levels of inoculum on trays up to 45% compared to controls, but only methyl bromide and steam treatments (80°C for 0.5 to 2.0 h) eradicated inoculum of *R. solani* from trays. Elimination of primary inoculum from previously used trays effectively controlled target spot and stem rot diseases caused by *R. solani*.

Growing healthy transplants is an essential first step in tobacco production. Traditionally, tobacco transplants have been produced solely in seed (plant) beds in the field (8), but since the late 1980s an increasing number of growers have produced their transplants in greenhouses. For example, approximately 60% of all transplants in North Carolina were produced in greenhouses in 1995, with 85% of these produced in float systems (22).

In the float system, cells of Styrofoam trays are filled with a soilless (1:1 peat:vermiculite) medium, seeded with pelletized seed, and floated on a shallow water reservoir. The cells are perforated on the bottom to allow for water and nutrient uptake. Trays are typically 65 × 34 cm with 200 to 348 cells per tray, which allows the production of more transplants of uniform size in a smaller area compared to seed beds. The high plant density and high moisture conditions present in greenhouses provide extended periods of leaf wetness and promote succulent plant

tissues that favor the development and spread of seedling diseases.

One of the most common diseases of tobacco seedlings in the greenhouse is damping-off, caused by *Rhizoctonia solani* Kühn (13,19). Estimated losses to this pathogen in the production of tobacco seedlings in North Carolina were about 0.5% in 1993 and 1994 (15). In addition to damping-off, some isolates of *R. solani* cause target spot (20), a leaf disease initiated by basidiospores of *Thanatephorus cucumeris* (A.B. Frank) Donk. (teleomorph of *R. solani*). Basidiospores of *T. cucumeris* are produced on hymenia which are formed on the soil surface, on infected stems, and on leaves during periods of high relative humidity, prolonged leaf wetness, and moderate temperatures (20,21). Isolates of *R. solani* associated with non-foliar symptoms on tobacco (root rot, stem rot or sore shin, and damping-off) were characterized as AG-1, AG-2-2 and AG-4, whereas isolates of *R. solani* associated with foliar symptoms (target spot) were AG-3 (23).

Because environmental conditions present in greenhouses are highly conducive for development and spread of diseases caused by *R. solani*, elimination of initial inoculum of this pathogen is critical for production of healthy plants. Propagules (hyphae and sclerotia) of *R. solani* survive free in soil (3,17), and in association with organic matter and host tissue (16,19). In greenhouse systems, potential sources of

inoculum include infested potting media and germination trays as well as windborne basidiospores. Hyphae or spores produced from these survival structures can infect stems or leaves of seedlings and initiate disease foci.

Current control measures for tobacco seedling diseases caused by *R. solani* include sanitation, primarily with NaOCl rinses, and the use of fungicides (outside of US) (6,18). Some biocontrol organisms for target spot have been identified (2,21), but are not used commercially. The objectives of this study were to identify sources of initial inoculum of *R. solani* for greenhouse-produced tobacco transplants and to evaluate the effects of various control methods for eradication of the primary inoculum.

MATERIAL AND METHODS

Identification of inoculum sources. In the first year of the study, four different brands of commercial potting medium were purchased from a local commercial source. In the second year of the study, unopened bags of five different brands of potting medium were collected from commercial tobacco growers. A 1 kg sample of potting medium was taken from each bag of medium for assay. With first year samples, a sub-sample (10 g dry weight) was taken from each source and plated on five petri dishes containing Flower's selective medium for *Rhizoctonia* (9), and on five dishes containing an alkaline water agar (AWA) plus antibiotics (100 µg/ml streptomycin sulfate and 100 µg/ml penicillin-G sodium salt) medium. The alkaline water agar medium contained 15 g of agar (Bacto, Difco Laboratories, Detroit) and was adjusted to pH 8.5 with approximately 0.8 ml/liter of 1.0 N NaOH (Sigma Chemical Co., St. Louis). In the second year, each sub-sample was placed only on 10 dishes of AWA. Potting medium was placed on the agar media using the protocol described by Henis et al. (10), and fungal colonies were evaluated after incubation for 48 h in the dark at room temperature (21 to 25°C).

In preliminary tests, incidence of damping-off was higher in previously used Styrofoam trays than new trays. To determine if used trays were a potential source of inoculum of *R. solani*, Styrofoam trays that had contained plants infected with *R. solani* were assayed for presence of the pathogen. Trays were washed vigorously

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under running tap water and dried for 24 h prior to sampling. Each tray consisted of 72 cells (12 × 6 cells) and was made by cutting a standard 252-cell tray into three smaller trays. Small (3 to 5 mm in diameter) pieces of Styrofoam were removed from the inside of each of 45 cells of each tray by pinching the surface of the trays with forceps. Samples were taken at 1 and 4 cm depth from the top of the tray. These samples (90 per tray) were placed on the two assay media described above, incubated for 48 h in the dark at room temperature, and the number of colonies of *R. solani* counted. A sample of 54 *R. solani* isolates were used to determine the AG groups of isolates present in the trays. Each isolate was paired in a water agar medium along with known testers of AG-3 and AG-4 (courtesy of Mark Cubeta, North Carolina State University).

Growers typically store trays in greenhouses from one growing season to the next. Fifty-six trays, which had been used in a damping-off experiment, were washed thoroughly under running tap water and stored for 1 year on a greenhouse bench. Trays were kept dry during the entire storage period, and temperatures ranged from 16 to 38°C in the greenhouse. After 1 year, 26 of the trays were immersed in 1.3% NaOCl solution for 5 min, rinsed under running tap water, and dried for 48 h. Six of the 26 trays also were steamed at 80°C for 30 min. Trays then were filled with a potting mix (inoculum free), seeded with pelletized flue-cured tobacco seed, and placed into an individual float system in a

greenhouse. Trays were observed weekly for presence of damping-off, and final disease incidence was determined 45 days after seeding (plants approximately transplant size).

Inoculum density and disease incidence. To determine if the level of initial inoculum present in trays affected the incidence of damping-off, the remaining 30 trays that had been stored for 1 year were assayed for the presence of *R. solani*. Forty-five samples were taken as before from each tray at a 1 cm depth, and the Styrofoam pieces were placed onto five petri dishes of AWA plus antibiotics (nine pieces/dish). After incubation for 48 h in the dark at room temperature, the number of *R. solani* colonies were determined for each tray. Based on results of the assay, four different infested treatments were established along with two control treatments. The six treatments were: i) new trays; ii) new trays with a single rice grain infested with *R. solani* in each of four cells per tray; iii) old trays with 1 to 5 (average 2.3) infested cells per tray; iv) old trays with 5 to 10 (average 7.3) infested cells per tray; v) old trays with 11 to 20 (average of 18) infested cells per tray; and vi) old trays steamed at 80°C for 30 min (average of 23 infested cells per tray prior to steaming). Trays then were seeded with flue-cured tobacco, and each tray was placed into an individual float system in a greenhouse. Trays were observed weekly for presence of damping-off and final disease incidence was determined 45 days after seeding.

Tray disinfestation. Eight treatments, used to varying degrees by growers, were tested for efficacy in the disinfestation of seeding trays: i) immersion in 0.5% NaOCl for 10 min.; ii) dry heat (oven) at 70°C for 2 h; iii) dry heat at 80°C for 2 h; iv) steam at 80°C for 30 min.; v) steam at 80°C for 1 h; vi) steam at 80°C for 2 h; vii) fumigation with methyl bromide 98% (24.3 g/m³); and viii) immersion in 10% solution of B-10 (Bioxy Incorporated, Raleigh, NC) for 1 h. Efficiency of each treatment was determined by sampling each tray before and after treatment, and was based on reduction in the number of cells which yielded *R. solani*. In each tray, 45 of the 72 cells were sampled at a depth of 1 cm and plated on 5 petri dishes (9 pieces/dish) containing AWA. Number of *R. solani* colonies in each dish was evaluated after 48 h of incubation in the dark at room temperature.

Data analysis. All experiments were conducted in a randomized block design at least twice with at least three replicates per treatment. Data were analyzed by the ANOVA or GLM procedures of SAS (SAS Institute, Cary, NC) and significant differences among treatments were determined by the Waller-Duncan *k*-ratio test.

RESULTS AND DISCUSSION

Sources of inoculum. *R. solani* was not recovered from any of the sources of potting media assayed. The AWA medium allowed growth and subsequent identification of many other microorganisms present in the potting media, including species of

Fig. 1. Sclerotia and melanized hyphae of *Rhizoctonia solani* (arrows) in crevices from a piece of Styrofoam tray.

Table 1. Effect of infestation level of Styrofoam trays by *Rhizoctonia solani* on the development of damping-off on tobacco seedlings

Treatment	Cells infested ^x	Percent damping-off ^y
< 5 infested cells/tray	1	52.6 a ^z
New, inoculated trays (rice grains)	4	42.1 a
5-10 infested cells/tray	7.3	32.9 a
11-20 infested cells/tray	17.5	34.7 a
New tray (control)	0	0 b
Steamed trays (80°C/30 min)	0	0 b

^x Average number of cells infested with *R. solani* per tray.

^y Average percent disease incidence per tray.

^z Numbers followed by the same letter are not significantly different according to Waller-Duncan *k*-ratio test (*P* = 0.05).

Table 2. Recovery of *Rhizoctonia solani* from Styrofoam trays before and after disinfestation treatments

Treatment	Initial Level	Final Level	Percent Control
Inoculated control	9 ^x	9	0 a ^y
Dry heat 82°C/2 h	6	6	0 a
Dry heat 71°C/2 h	7	6	14.3 a
Dip in 0.5% NaOCl/10 min	9	5	44.5 b
Dip in 10% B-10/1 h	6	2	66.7 b
Steam 80°C/2 h	6	0	100 c
Steam 80°C/1 h	6	0	100 c
Steam 80°C/30 min.	6	0	100 c
Methyl bromide	7	0	100 c

^x Average number of *Rhizoctonia solani* colonies per tray isolated on alkaline water agar plus antibiotics.

^y Numbers followed by the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test (*P* = 0.05).

Penicillium, *Aspergillus*, *Trichoderma*, *Paecilomyces*, *Sporothrix*, *Cladosporium*, and *Fusarium*. Since some species of these genera are antagonists of *R. solani* (7,12,14) it may be possible to establish known biocontrol agents in potting media prior to seeding. Species of *Pythium* pathogenic on tobacco have been isolated from commercial potting media (5), but no isolates of *Pythium* were recovered in this study.

R. solani was recovered from previously used trays in which damping-off had occurred. Viable inoculum of *R. solani* was recovered from 84% of the previously infested trays (average of 14% of cells infested per tray) at 1 cm depth, but from only 5% of the trays (average of 3% of cells infested per tray) at 4 cm depth. Differences in recovery may reflect the level of activity of *R. solani* in the different areas of the cells; the isolates primarily colonized stem tissue and did not cause root rot. The textured surfaces of the Styrofoam trays provided numerous small cracks that allowed growth of fungal hyphae. Sclerotia and melanized hyphae (resting hyphae) of *R. solani* were easily observed in the walls of the trays (Fig. 1). These structures serve as survival propagules of *R. solani* (1,4,19). Of isolates obtained from trays, 45 of 54 were AG-4 and 9 were AG-3, which indicates isolates from both anastomosis groups can survive in the Styrofoam trays, and both target spot and stem rot may develop from inoculum on old trays.

Damping-off of tobacco seedlings occurred in *R. solani*-infested trays that had been stored for 1 year in the greenhouse. Upon seeding, levels of damping-off ranged from 0 to 90% (75% of infested trays had more than 40% of infested cells per tray), but no disease developed in infested trays that were steamed before seeding. These results indicate that *R. solani* can survive for a long period of time on dry trays in storage, even after being washed with a 1.3% NaOCl solution. It appears that the surface disinfectant was unable to penetrate into the cracks in the walls of the trays where inoculum of *R. solani* was located, while steaming treatments penetrated the crevices of the Styrofoam, killing both sclerotia and resting hyphae of *R. solani*.

Inoculum of *R. solani* was recovered from 63% of the trays stored for 1 year on a greenhouse bench. As much as 55% of the cells in a given tray contained viable *R. solani*, and overall, 18% of the cells were infested with *R. solani*. Similar levels of disease developed in infested trays, regardless of level of initial inoculum, which indicates that the threshold level for development of disease caused by *R. solani* may

be as low as a single infested cell (Table 1). Because trays are reused many times, management of diseases caused by *R. solani* requires the use of treatments that can eradicate survival structures of *R. solani* within the cracks of the walls of the trays.

Tray disinfestation. Significant differences were observed among disinfestation treatments. Complete control of *R. solani* was observed only with steam and methyl bromide treatments (Table 2). The use of dry heat at 70 and 80°C did not eliminate inoculum from the trays, and none of the chemical treatments used gave acceptable levels of control. Similar results have been reported previously for vegetables (11). The rough surface and hydrophobic nature of the Styrofoam may prevent penetration of liquid disinfectants into cracks in the tray walls and subsequent contact with fungal propagules. Inoculum levels were reduced between 14 and 45% by the NaOCl and B-10 treatments (Table 2), and this reduction is most likely due to the elimination of inoculum present on the surface of the tray walls. This level of inoculum reduction is not adequate for disease control. The effectiveness of the methyl bromide and steam treatments is probably due to their ability to penetrate into cracks in the tray walls. Because methyl bromide may not be available in the future, other treatments should be developed for sterilization of trays. Steam treatments successfully eradicated inoculum from trays, but if too much heat is used reduction in tray size may result, so that trays soon become unusable with automated vacuum seeders. After 1 or 2 h of steaming, trays were on average 2.0 cm shorter; after 30 min this reduction was only 0.5 cm. Current recommendations are for the use of steam at 72 to 80°C for 30 min, or methyl bromide at 24.3 g/m³ (15), but additional tests may improve methods for use by growers.

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