USDA - NE IPM Center

Final Research Project Report

A. Grant Data

Title: Inadequate Control of Trichoderma Green Mold on Mushrooms

Type: Critical & Emerging Issues

Lead Investigator: Daniel J. Royse, Professor of Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Team Member: C. Peter Romaine, Professor of Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

States Involved: Delaware, Maryland, and Pennsylvania

Funding Year: 1 May 2005 to 30 June 2006

Funding Amount: $23,000 (requested $41,620)

B. Nontechnical Summary

We provide suggestive evidence that the recurrence of Trichoderma green mold on cultivated mushrooms, a disease that reached epidemic proportion in the 1990s in the northeastern U.S., is due to the emergence of formidable resistance in the pathogen to the only fungicide available for disease management. We also present data that proposes a direct relationship between disease incidence and on-farm sources of pathogen contamination. Our project findings were published in the major mushroom industry trade journal and presented in a series of grower meetings in which farm hygiene was emphasized as the first and foremost defense in an IPM program for green mold disease. With a reduced fungicide efficacy, farm hygiene now forms the principal tactic for disease control for the mushroom industry in the Northeast.

C. Introduction

Last year, mushroom growers in the northeastern U.S. produced more than 567 million pounds (67% of the U.S. total) of Agaric mushrooms valued at more than $501 million. The U.S. mushroom industry is experiencing a resurgence of the destructive green mold epidemic that prevailed during the early to mid 1990s (Fig. 1). The epidemic form of the disease is caused by a newly recognized and highly aggressive genotype of the pathogen, *Trichoderma aggressivum* f. *aggressivum* (*Ta2*). In the recent past, growers have effectively managed the disease primarily through the treatment of the spawn (mushroom seed) with benomyl (Benlate) and, subsequently, thiophanate-methyl (Topsin M).

It was unclear if the resurgence of the green mold epidemic was due to the development of benzimidazole resistance within the pathogen population, technical problems associated with treating the spawn with the fungicide, inadequate farm hygiene, or some combination of these factors. We proposed to identify the underlying reason(s) for the dramatic increase in the incidence and severity of Trichoderma green mold on commercial mushrooms in the Northeast. Because alternative fungicides are not available, we proposed to work with growers to reduce selection pressure on the pathogen by emphasizing sanitation for disease control.

Fungicide resistance is the most critical research priority identified by the "Pest Management Strategic Plan for Mushrooms in the Northeast", and green mold disease is identified in "Crop Profiles" as one of the most serious diseases on the cultivated mushrooms.

D. Objectives

1. Survey the *Ta2* population on mushroom operations for sensitivity to thiophanate-methyl.
2. Determine if cases of high disease incidence are related to improper application of the fungicide to the spawn.
3. Survey commercial mushroom operations with high rates of green mold for widespread on-site sources of infection.
4. Work with growers to emphasize sanitation as the primary method for disease management.

E. Approach

Specimens of *Trichoderma* spp. were collected from commercial mushroom operations located in Pennsylvania and Delaware. Green mold isolates were grown in pure culture and biotyped by *Ta2*-specific PCR analysis.

For a thiophanate-methyl and benomyl resistance ratings, each *Ta2* isolate was grown in triplicate on a potato dextrose yeast agar (PDYA) amended with from 0 to 500 ppm active ingredient (a.i.) of the fungicide. The EC_{50}, which is the concentration of the fungicide in ppm a.i. resulting in a 50% reduction in mycelial growth, was determined for each isolate. An isolate of *Ta2* that was collected in the 1990s was included as a reference for the baseline level of fungicide resistance.

Performance of thiophanate-methyl in a spawn protection test against fungicide-sensitive and -resistant Trichoderma green mold was conducted as a part of this study. Here, a lawn of *Ta2* spores was overlaid on a PDYA medium and then spawn grains that were treated with fungicide in the laboratory were placed on the medium. After a one-week incubation, variation in the size of the clear zones surrounding the grains ("halos") where *Ta2* spore germination and growth were inhibited by the fungicide were observed for the collected isolates.

Replicate Organism Detection and Counting (RODAC) plates containing PDYA (50-mm diameter) were used to survey a commercial mushroom farm for sources of T*a2* contamination. The plates were exposed to a variety of animate and inanimate objects throughout the farm by briefly touching the PDYA medium to the surface of the object. Plates were incubated at 22-24˚C for 7-10 days, and green mold colonies growing on the plates were subjected to PCR-based analysis for *Ta2*.

F. Progress

Considering the 45% reduction in the proposed budget, much of the work that had been planned could not be executed. Nonetheless, we successfully identified the probable cause for the increase in the occurrence of Trichoderma green mold on commercial mushroom crops in the Northeast, and recommended a course-of-action to the industry to minimize the economic impact of this disease.

G. Results

We previously showed that the *Ta2* pathogen population in Pennsylvania underwent an approximate three-fold increase in resistance to benomyl (EC_{50} of 0.4 to 1.4 ppm) between 1994 and 2002 (Fig. 2). In an effort to explain the more recent dramatic rise in the incidence of green mold disease on farms in the Northeast, we again surveyed the *Ta2* population in April-May 2005 for sensitivity to benomyl. As in the earlier survey, PCR analysis was carried out on the green mold isolates, so as to restrict the survey to the *Ta2* genotype (Fig. 3). Our survey results revealed the pathogen population had developed formidable resistance in the three intervening years to both benomyl and thiophanate-methyl (Figs. 4-6). Of 15 *Ta2* isolates collected at commercial operations in Pennsylvania and Delaware, 14 (93%) grew on a medium amended with saturating levels (50 or 500 ppm) of benomyl or thiophanate-methyl. By comparison, the growth of a 1996 isolate of *Ta2* was completely inhibited at these levels of the fungicides.

In a spawn protection test, up to 8 lbs of Topsin M (mixed with 100 lb of gypsum as carrier) per 1600 units of spawn, which is four times the allowable application rate, failed to produce clear zones of green mold inhibition (halos) surrounding the treated spawn grains with a 2005 isolate of *Ta2*, whereas pronounced halos were observed using a 1996 isolate at all experimental rates of the fungicide (Fig. 7). Presumably, this same lack of protection against the resistant form of the pathogen is taking place today with spawn in the compost.

Despite the emergence of extraordinarily high-level resistance to benomyl and thiophanate-methyl, the pathogen population remained largely sensitive to a third benzimidazole, thiabendazole (Table 1).

A survey of a commercial mushroom operation in Pennsylvania that was continually struggling with a high incidence of green mold disease disclosed extensive on-site *Ta2* contamination. Of 96 RODAC sampling plate, five scored positively for *Ta2*, including an electric light cord in a growing room, bathroom doorknob, time card, office door, and mushroom picking basket (Fig. 8). Additionally, a sixth sample of a green mold growing in a production bed was identified as *Ta2*.

H. Impact

Northeast growers produce more than 70% of the total U.S. mushroom crop. Green mold caused by the highly aggressive genotype, *Ta2*, is a significant disease that threatens the welfare of this industry. In the past, green mold has been effectively managed through the use of benzimidazole fungicides. However, based on the findings of the study herein, it can be concluded that the pathogen population has developed formidable resistance to both benomyl and its replacement thiophanate-methyl. While we have disclosed an unprecedented level of field resistance in the *Ta2* population, it remains unclear how this impacts the efficacy of thiophanate-methyl for disease control. However, given the magnitude of the increase in resistance and the prevalence of the resistance variants, the resurgence of the disease now occurring on farms in the Northeast is mostly probably explained by a significant decline in the field performance of the fungicide.

As our survey was limited to the major mushroom-growing region in the Northeast, we do not know how strong of a foothold the fungicide-resistant *Ta2* has on the U.S.

mushroom industry at large. Needless to say, the continued use of thiophanate-methyl will undoubtedly favor the predominance of resistant forms of the pathogen throughout North America.

The discovery of pathogen resistance to the only fungicide available for disease control has serious ramifications. As the mushroom is a fungus, few fungicides with efficacy for green mold are also compatible with the crop, and so no alternatives are currently available to the industry.

Our research findings were published in *Mushroom News*, a monthly periodical that reaches more than 80% of the growers, and presented in a series of six grower conferences and meetings held during 2005 and 2006, each with an attendance of between 40 and 120 participants. While we reported on the discovery of high-level fungicide resistance, we utilized the findings of the farm monitoring study for *Ta2* contamination to underscore how the disease is best managed through the use of sanitary practices aimed at excluding the pathogen from the mushroom production area. Growers acknowledged the primary importance of hygiene in a disease control program, and therefore, our objectives were achieved.

I. Appendix

Figures:

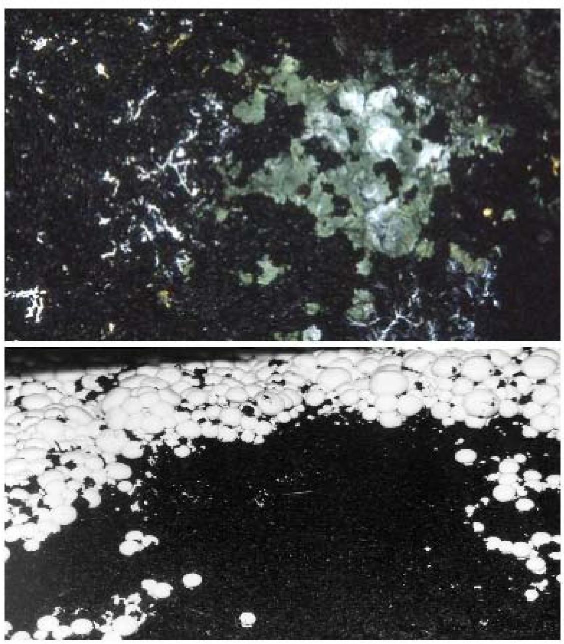

Figure 1. Trichoderma green mold on *Agaricus bisporus*. **Upper**: *Trichoderma aggressivum* f. *aggressivum* (*Ta2*) growing on the surface of the casing layer in the production bed. **Lower**: an area of the bed involving subsurface colonization of the compost by *Ta2*. Both examples illustrate the inhibitory effect of *Ta2* on the development of mushrooms.

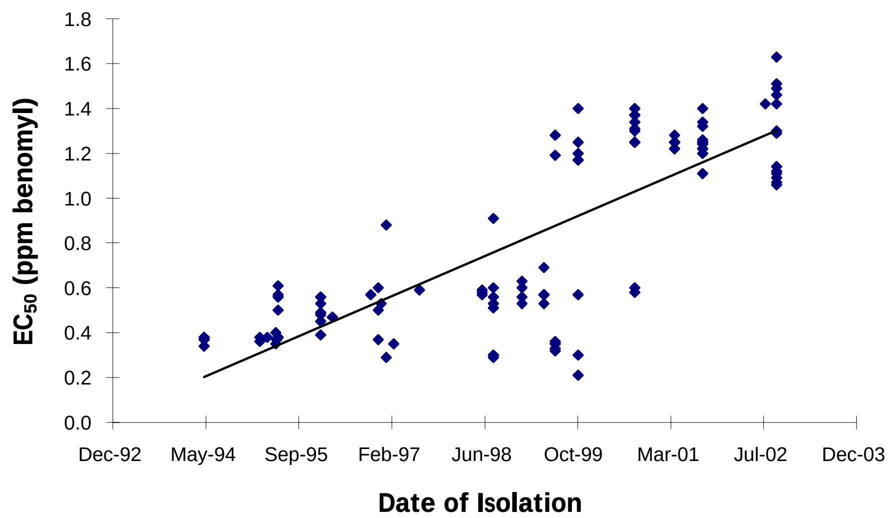

Figure 2. Scatter plot depicting the change in benomyl (Benlate) resistance between 1994 and 2002 within the Pennsylvania *Trichoderma aggressivum* f. *aggressivum* (*Ta2*) population. Resistance is expressed as the EC_{50}, which is the concentration of benomyl in parts per million (ppm) affecting a 50% inhibition in the mycelial growth of a *Ta2* isolate. Data are based on 122 isolates of *Ta2*.

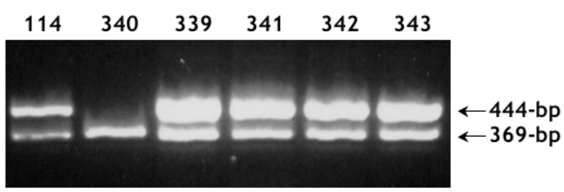

Figure 3. Multiplex PCR analysis of the *Trichoderma aggressivum* f. *aggressivum* (*Ta2*) genotype. Based on the amplification of a 444-bp DNA (444-bp), green mold isolates 339 and 341-343 were identified as *Ta2*. Isolate 340 was identified as a non-Ta2 genotype, owing to the absence of the 444-bp DNA and presence of the 369-bp *Trichoderma* spp.-specific DNA (369-bp). Isolate 114 (114) is a Ta2 control.

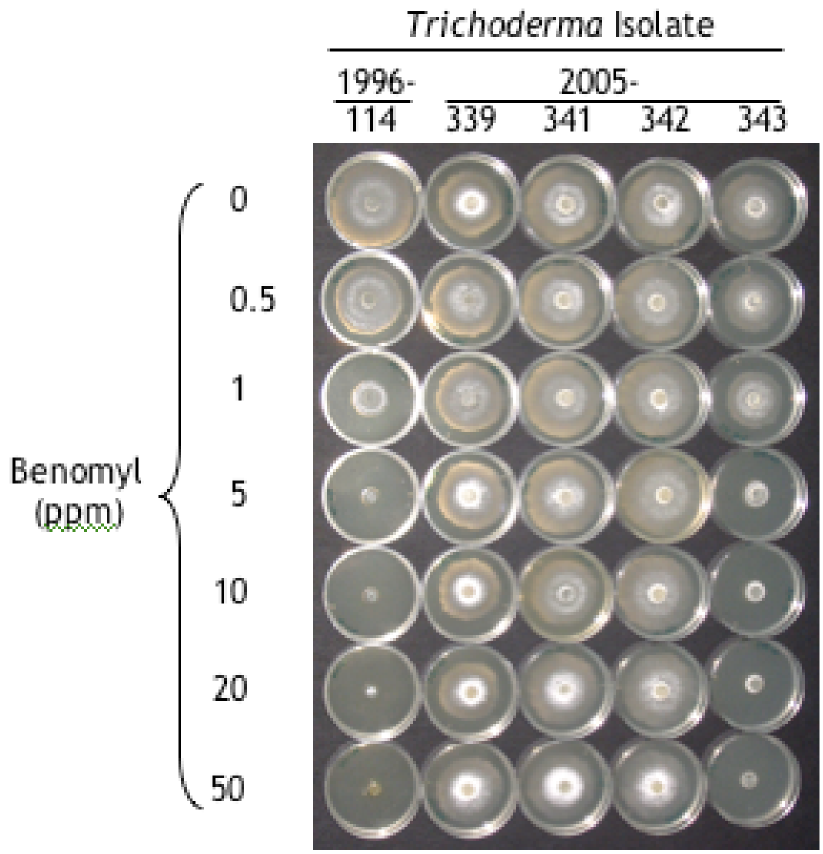

Figure 4. Benlate (benomyl) resistance among 2005 isolates of Trichoderma green mold. Of four isolates of *Trichoderma aggressivum* f. *aggressivum* (339 and 341-343), three grew unabated on a medium amended with up to 50 ppm of benomyl. By comparison, the growth of a 1996 isolate (114) was completely inhibited between 1 and 5 ppm.

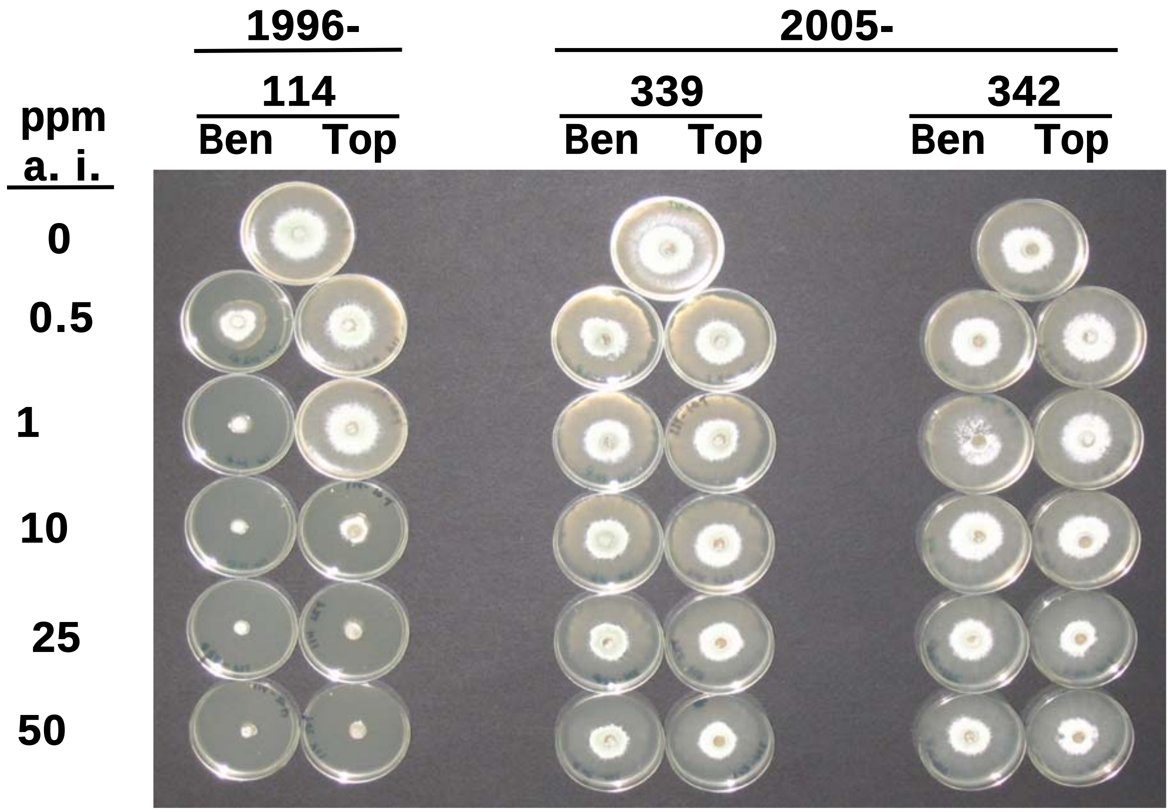

Figure 5. Comparison of Benlate (benomyl) and Topsin M (thiophanate-methyl) resistance among 2005 isolates of Trichoderma green mold. Shown are two representative 2005 isolates of *Trichoderma aggressivum* f. *aggressivum* (339 and 342) growing unabated on a medium amended with up to 50 ppm of benomyl (Ben) and thiophanate-methyl (Top). By comparison, the growth of a 1996 isolate (114) was completely inhibited between 0.5 and 10 ppm.

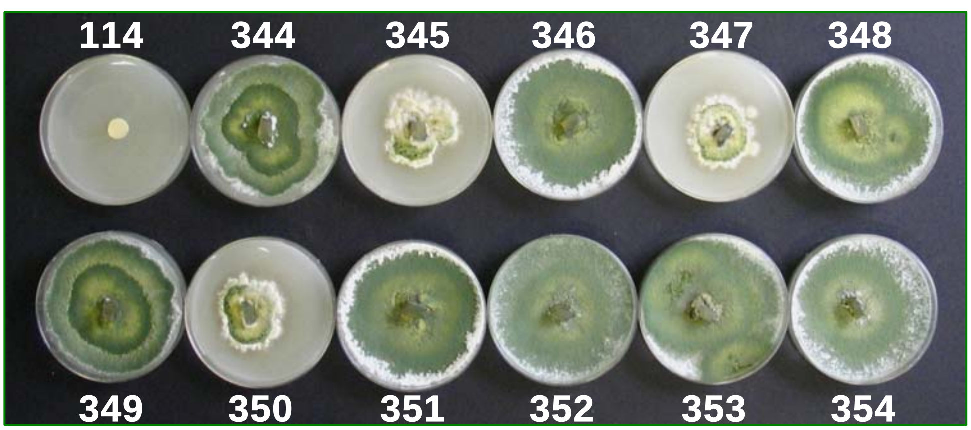

Figure 6. Thiophanate-methyl resistance among 2005 isolates of Trichoderma green mold. Shown are 11 isolates of *Trichoderma aggressivum* f. *aggressivum* (Ta2) (344-354) from commercial mushroom farms in Pennsylvania and Delaware growing on a medium amended with a saturating level (500 ppm) of thiophanate-methyl. By comparison, the growth of a 1996 isolate (114) was completely inhibited.

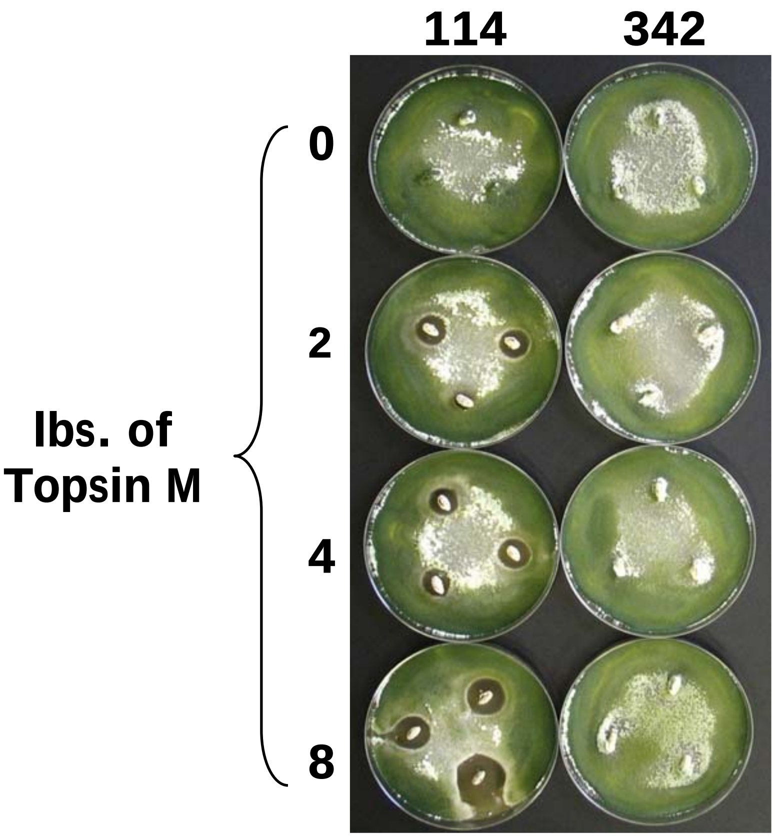

Figure 7. Performance of thiophanate-methyl (Topsin M) in a spawn protection test against fungicide-sensitive and -resistant Trichoderma green mold. A 1996 fungicide-sensitive isolate (114) of *Trichoderma aggressivum* f. *aggressivum* (*Ta2*) developed clear zones of spore inhibition (halos) surrounding the spawn grains treated with 2, 4, and 8 lbs. of Topsin M per 1600 units of spawn, whereas visible halos were absent using a

Table 1. Thiabendazole sensitivity of *Trichoderma aggressivum* f. *aggressivum (Ta2).*

Ta2 Isolate	EC_{50} (ppm a.i. thiabendazole)
114	9.9
347	4.9
350	6.8
345	7.9
351	9.6
349	11.0
354	13.0
346	15.5
352	16.0
353	20.0
348	20.8

EC_{50} = fungicide concentration (ppm) affecting a 50% inhibition of growth of a *Ta2* isolate. Isolate 114 was collected in 1996 and all other isolates in 2005.

Figure 8. Monitoring for on-farm sources of *Trichoderma aggressivum* f. *aggressivum* (*Ta2*). Of 95 RODAC sampling plates, six were positive for *Ta2*. These samples were taken from the electric cord of the pick light in room 23 (19), doorknob of men's bathroom (44), worker's time card (52), door edge in plant office (83), cleaned picking basket (85), and a live green mold sample from a production bed (93).

<u>PowerPoint Presentations</u>:

1. Romaine, C. P. and D. J. Royse. 2005. A re-evaluation of green mold and La France disease in hybrid mushrooms. 18^{th} North American Conference. Ottawa, CN.
2. Romaine, C. P. and D. J. Royse. 2005. Principles of disease control in mushrooms. Creekside Mushroom Co., Butler. PA.
3. Romaine, C. P. and D. J. Royse. 2005. Disinfectants: types, efficacy, and use patterns. Mushroom Growers Meeting on Pest Control. Sylvan America Inc., Hartfield Golf Club, Hockessin, DE.
4. Romaine, C. P. and D. J. Royse. 2005. Seeing green? Topsin-resistant *Trichoderma* discovered. Mushroom Growers Meeting on Pest Control. Sylvan America Inc., Hartfield Golf Club, Hockessin, DE.
5. Romaine, C. P. and D. J. Royse. 2005. First report of thiophanate-methyl-resistant *Trichoderma*. IPM Committee Meeting. 48^{th} Annual Penn State Mushroom Industry Conference, University Park, PA.
6. Royse, D. J. and C. P. Romaine. 2006. Progress on imazalil research for the control of Trichoderma green mold. IPM Committee Meeting. 49^{th} Annual Penn State Mushroom Industry Conference, University Park, PA.

<u>Publications</u>:

1. Romaine, C. P., D. J. Royse and C. Schlagnhaufer. 2005. Superpathogenic *Trichoderma* resistant to Topsin M found in Pennsylvania and Delaware. Mush. News 53:6-9.