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HDC Project M 22

Examination of the efficacy of two novel fungicides against *Dactylium dendroides*

by

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PRACTICAL SECTION FOR GROWERS

Objectives and background

Cobweb disease has been a significant, and sometimes serious, problem for many growers during the last few years. Many of the *Dactylium* isolates collected from farms around the UK are demonstrating some degree of resistance to the fungicides being used to control this pathogen. It is prudent, therefore, to be on the lookout for new chemicals as existing chemicals become less effective for one reason or another.

This project (M 22) was commissioned to examine the efficacy of two 'new' chemicals in controlling *Dactylium*. Some epidemiological information was required before such efficacy testing could be done and this work was also carried out.

Summary of Results

Symptoms of *Dactylium* infection, as either cobweb growth on the casing surface or spotting on mushroom caps, were produced in a manageable way using either mycelium or spore inoculum. Mycelium inoculum incorporated into casing produced abundant cobweb growth while a high concentration of spores (10^6-10^7 spores/ml) sprayed onto casing produced abundant spotting symptoms. A lower concentration of spores produced only a low incidence of spotting symptoms. Spore inoculum also led to the development of cobweb symptoms but usually at a later stage in the crop cycle compared with mycelium inoculum (Figures 1 and 6).

Symptom expression was related to time of inoculation and inoculum concentration. When a higher concentration of inoculum was applied at pinning, symptoms developed during the first flush; when applied after the first flush, symptoms developed during the second flush. When a low concentration of spores was applied to casing after the first flush a low incidence of spotting symptoms developed in the second flush and a small amount of cobweb growth occurred in the third flush.

Two isolates of *Dactylium* (isolate 192B1 and isolate 202A), which represent two different *Cladobotryum* species, behaved similarly in terms of symptom expression although there was some

some evidence to suggest that spores of isolate 202A may not be as effective as those of isolate 192B1 in producing cobweb growth on the casing.

By salting all visible areas of cobweb growth at the end of a flush, prior to applying water, the spread of *Dactylium* was largely prevented. Thus, inoculation experiments can effectively measure the development of *Dactylium* symptoms in response to various treatments such as different rates of fungicide use.

Both of the chemicals which were tested for their ability to control *Dactylium* in a mushroom crop succeeded in reducing the incidence of cobweb growth on the casing. The most effective rates of Chemical A (0.395-1.0 gram a.i./m^2) had significant phytotoxic effects on the mushroom crop itself reducing yields by 30-50% over 3 flushes. This would probably preclude its viability as a commercial product. The most effective rates of Chemical D (0.7-1.5 gram a.i./m^2), were less phytotoxic with only the higher rate causing a significant yield reduction of 14%. This was still a better yield compared with that from a diseased crop in the absence of any fungicide (Figure 11).

The growth of cobweb on the casing surface was inhibited by both chemicals at the highest rate of chemical used. Some restricted growth onto casing occurred, from infected mushrooms or pins, particularly with Chemical D in the third flush following inoculation with a concentrated spore suspension. It should be remembered that this treatment was an extreme one but it indicates that if the inoculum level is very high some restricted growth on casing can occur which, if not detected, could result in raising inoculum levels further. This hypothesis was not tested in these experiments. All patches of *Dactylium* were identified and salted before the next watering operation. It may be useful to obtain further information regarding the spread of *Dactylium* within a crop treated with Chemical D where small patches of cobweb growth are deliberately watered over. The occurrence of restricted growth may reflect a decreasing concentration of the active ingredient in the upper layer of casing and this is another area where additional information would be useful.

Figure 1.

Yield of clean and spotted mushrooms following three types of casing inoculation at pinning using isolates 192B1 and 202A.

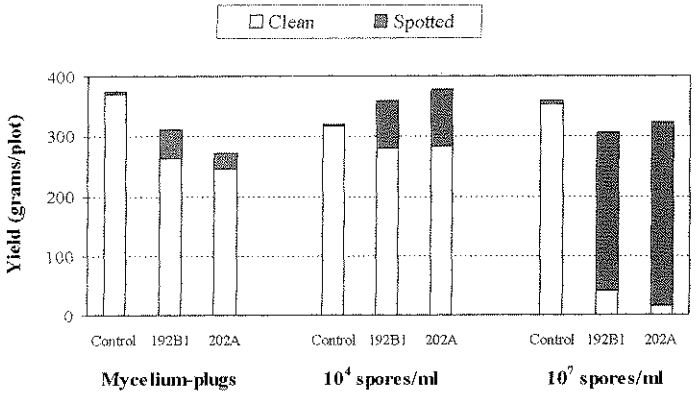


Figure 6.

Growth of *Dactylium* on casing following inoculation between 1st and 2nd flush with isolate 192B1 using spores or mycelium.

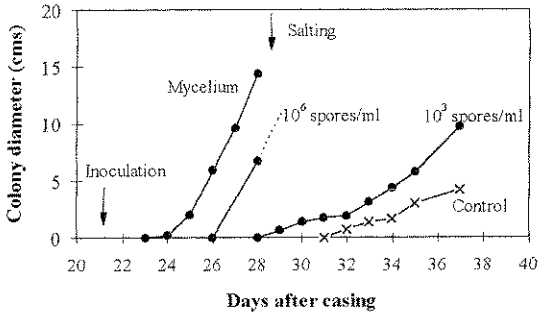
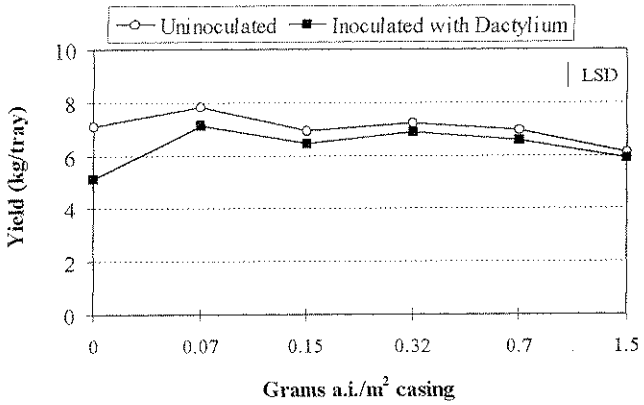


Figure 11. Yield of clean mushrooms harvested from crops treated with different rates of **Chemical D**. Least significant difference (LSD) at $P = 0.05$ covers all comparisons.



Action points for growers

- A high *Dactylium* spore load from pinning onwards will result in substantial cap spotting in the developing crop from as early as the first flush.

- Removal of cobweb growth by careful salting prior to watering can significantly reduce the incidence of spotting in successive flushes.

- A new chemical has been identified which looks promising for the control of *Dactylium*.

Practical and financial benefits from study

This work has identified the relationship between types of inoculum and the expression of *Dactylium* symptoms and that significant control of symptoms can be obtained by good hygiene practices which rigorously identify and treat areas of cobweb prior to watering events.

A new chemical has been identified which looks promising for the control of *Dactylium* and which, if approved for use, should benefit the mushroom industry through improved yields in the place of losses due to cobweb.

A. GENERAL INTRODUCTION

During the last few years Cobweb disease, caused by species of *Cladobotryum* (generally known as *Dactylium*) has become both endemic, and occasionally epidemic, within the mushroom industry. The reasons for the sudden increases over recent years are unknown, but it is likely that changes in growing practices and the development of fungicide resistance may be important. The problems caused by this disease have been widely discussed in the Mushroom Journal and it appears that many growers are experiencing serious difficulties.

Work already commissioned by the HDC (Project M14a) has shown that existing pesticides are increasingly unable to control the disease and new products are urgently required. Initial screening work has been carried out by HRI with the support from a pro-active spawn company and this has resulted in the identification of two promising chemicals. Further evaluation is now required and the objective of this project is to assess the two chemicals for their ability to control *Dactylium* introduced into a mushroom crop by inoculation. In order to demonstrate effectively that a given chemical can or cannot control *Dactylium* it is important to be able to produce disease symptoms in a controlled manner. There is little substantial information in the literature concerning successful inoculation methods for *Dactylium* so, prior to embarking upon the testing of the two chemicals mentioned above, it was decided to evaluate different inoculation methods first. This would also provide useful information on the epidemiology of *Dactylium* infection.

This disease has demonstrated its ability to cause considerable financial loss due to direct crop loss (Cobweb), cap spotting, spoilage in the market chain, foreshortened crops, 'diseasing' and ineffectual pesticide usage. In some situations, the final flush of mushrooms has been lost to the disease resulting in major financial losses to growers and it is estimated that the total loss for the growers to date is probably in the region of several millions of pounds.

The provision of pesticides more effective than those currently available would have obvious and considerable positive financial benefit for the UK mushroom industry.

B. PART I - Development of an effective method of *Dactylium* inoculation

Introduction

In order to demonstrate that any fungicide can effectively control a given disease it is important to be able to produce a controlled outbreak of the disease by inoculation. A review of the literature concerning inoculation of mushroom crops with *Dactylium* indicated that responses using spore inoculum could be variable depending on the time of inoculation (Dar and Seth 1992). Some reports (Van Zaayen & Van Andrichem 1982; Fletcher, pers comm) also implied that spore inoculum only produced symptoms in later flushes while Van Zaayen & Van Andrichem (1982) produced symptoms in earlier flushes using mycelium inoculum. Preliminary inoculation experiments at HRI also indicated that inoculated plots, if left unchecked, could completely contaminate control plots very quickly. It was decided therefore to take a closer look at inoculation methods prior to evaluating the efficacy of two novel chemicals in controlling *Dactylium* as an inoculation method/methods was required which allowed *Dactylium* to establish in a manageable fashion and which, if necessary, could be contained using standard non-fungicide disease control methods.

Materials and Methods

Inoculum

Two isolates were used in this study: Isolate 192B1 (*Cladobotryum dendroides*) and isolate 202A (*Cladobotryum mycophilum*). For convenience both are discussed using the term *'Dactylium'* which is the name used by the mushroom industry to identify this group of organisms. Two types of inoculum were investigated: *Dactylium* mycelium and *Dactylium* spores. Mycelium inoculum consisted of plugs (8 mm diameter) taken from the growing margin of a mycelial culture of *Dactylium* grown on mushroom extract agar (Appendix 1). Spore inoculum was prepared by flushing sporulating cultures of *Dactylium* with sterile water. The resultant suspension was then filtered through sterile cotton wool to remove mycelial debris. The concentration of spores was calculated using a haemocytometer and the

suspension was diluted to give a stock spore suspension in the region of 1 x 10^7 spores/ml. A second spore suspension was also prepared by a 1/1000 dilution of the stock to give a spore concentration of about 1 x 10^4 spores/ml. A 10 ml aliquot of spore suspension was used to inoculate plots. The spore suspensions prepared for the 'inoculation between first and second flush' experiment were of a concentration of 1 x 10^6 and 1 x 10^3 spores/ml due to fewer sporulating culture plates being available.

Inoculation timing

Inoculation of mushroom crops was done on two separate occasions either (i) at pinning or (ii) between the first and second flush. The plots which were inoculated between the first and second flush were kept in a separate chamber during case run and the first flush so as to prevent any contamination from plots inoculated at pinning.

Crop details

Spawn run compost produced by the HRI Mushroom Unit was used in this experiment (compost no. 3/96; A15 spawn). Pots measuring 25 cm diameter were filled with 3.5 kg of spawn-run compost and cased to 4-5 cm with a black peat/sugar beet lime casing. The pots were then divided into two lots and case-run in two separate chambers awaiting inoculation.

Inoculation at pinning

On Day 11 after casing when pins were beginning to form on the casing surface, inoculation was carried out. For mycelium-inoculated plots, a small quantity of casing was removed from the centre of each pot and 3 x 8 mm diameter plugs of inoculum were put into place. The plugs were then covered with casing.

The two spore suspension inocula were applied using a 'Hozelok Spraymist' sprayer holding 500 ml. Spraying technique was calibrated to enable a 10 ml aliquot of suspension to be

sprayed onto the casing surface of each pot. Each isolate was applied separately using separate sterilised sprayer units. Control plots received 10 ml of sterile water.

No water was applied to the casing on days 11, 12 or 13 but the equivalent of approximately 1.5 litres/m^2 was applied between days 14 and 15. *Dactylium* expression was recorded as growth on the casing and the presence of spotting. The crop was terminated after the first flush.

Inoculation between the first and second flush

A second series of pots in a separate chamber were inoculated between the first and second flush. The equivalent of 1.5 litres/m^2 of water was applied to the pots after the first flush was picked off. On day 21, 2 days after the last pick of the first flush, the pots were inoculated in an identical manner to that described above. Once developing pins had reached pea-size a further 1.3 litres/m^2 of water were applied over two days as casing was looking somewhat dry.

Dactylium expression was recorded over the following days. Mushrooms were harvested and separated into healthy and spotted. At the end of the second flush visible patches of *Dactylium* growth were salted and diseased mushrooms were removed using rubber gloves and discarded in a concentrated (2%) solution of 'Environ'. This crop was then further watered with 3.5 litres/m^2 over 3 days and cropped for a third flush.

Statistical design

Six replicate pots were prepared for each treatment. A total of 18 treatments in all were prepared consisting of three inoculum types (1 mycelium and 2 spore concentrations) and three isolate categories (isolates 192B1, 202A, None), giving 3 x 3 = 9 treatments for each of two inoculation timings (at pinning and between first and second flush). The plots for each inoculation timing were housed in separate chambers. Plots were positioned in two blocks arranged in a 3 x 3 Latin square with respect to inoculum type. Each main plot was split for isolate-category to give a replicated Latin square split-plot design.

Results

Inoculation at pinning

Yield

The total yield for uninoculated plots was on average 351 grams/3.5 kg spawn run compost. This was equivalent to 100 kg/tonne for the first flush only. There was generally no difference between the two *Dactylium* isolates in terms of yield or in the proportion of spotted mushrooms recorded but the method of inoculation had a dramatic effect on the yield of clean mushrooms (Figure 1). Almost all harvested mushrooms were spotted from plots inoculated with a spore suspension of 10^7 spores/ml whereas only 20-25% of mushrooms were spotted when inoculated with a spore suspension of 10^4 spores/ml. Fewer mushrooms were spotted when mycelium inoculum was used, 9-16%, but total yields were significantly lower in these plots as a result of *Dactylium* growth (Cobweb) suppressing pins, and enveloping mushrooms.

Spotting

The emergence of spotted mushrooms with time following inoculation was similar for both *Dactylium* isolates so the results are only presented for isolate 192B1 (Figure 2). Spotting symptoms were more frequent when a spore suspension was used as inoculum. When the spore concentration was relatively high (10^7 spores/ml) 87-95% of mushrooms developed spotting symptoms. Some maturing pins were showing symptoms by the third and fourth day after inoculation but there was a steady emergence of spotting symptoms right up until the last pick of the first flush on day 19 - eight days after inoculation. By contrast, fewer maturing pins developed spotting symptoms following inoculation with a lower concentration of spores (10^4 spores/ml). In addition spotting symptoms did not emerge until towards the end of the first flush. An average of about 2 spotted mushrooms per plot developed on mycelium-inoculated plots, mostly at the end of the first flush, although in general a number of mushrooms were also engulfed by *Dactylium* mycelium.

Figure 1. Yield of clean and spotted mushrooms following inoculation of casing at pinning with three inoculum types and two *Dactylium* isolates - 192B1 and 202A.

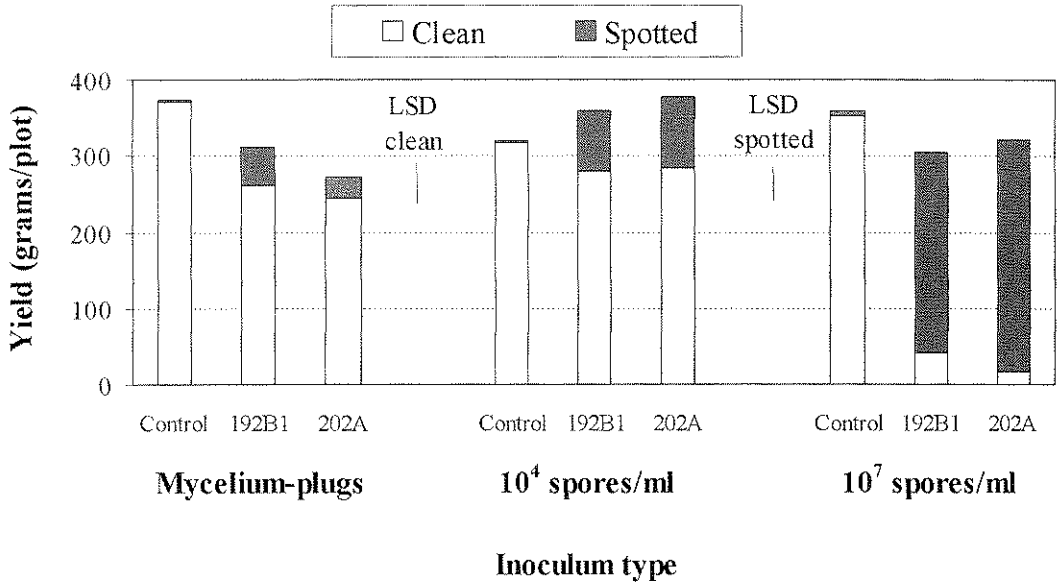
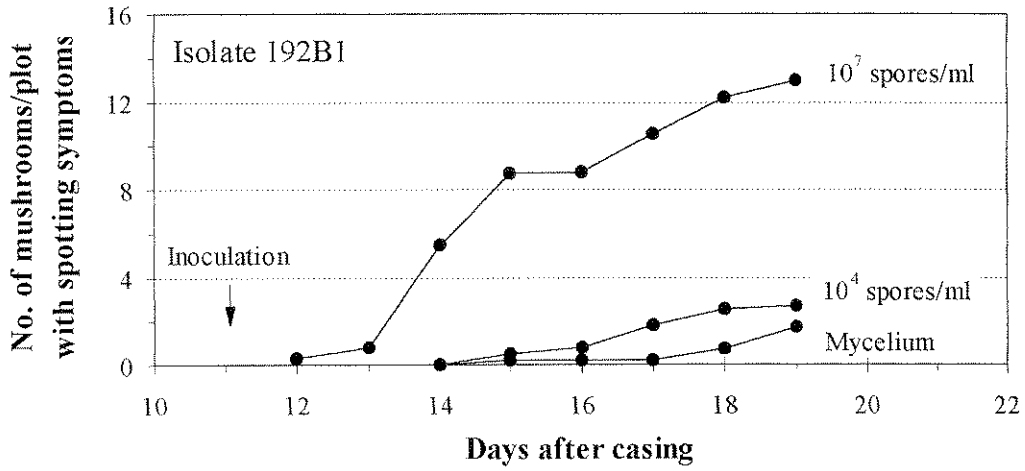


Figure 2. Emergence of spotting symptoms on mushrooms following inoculation of casing at pinning with spores or mycelium.



Cobweb symptoms

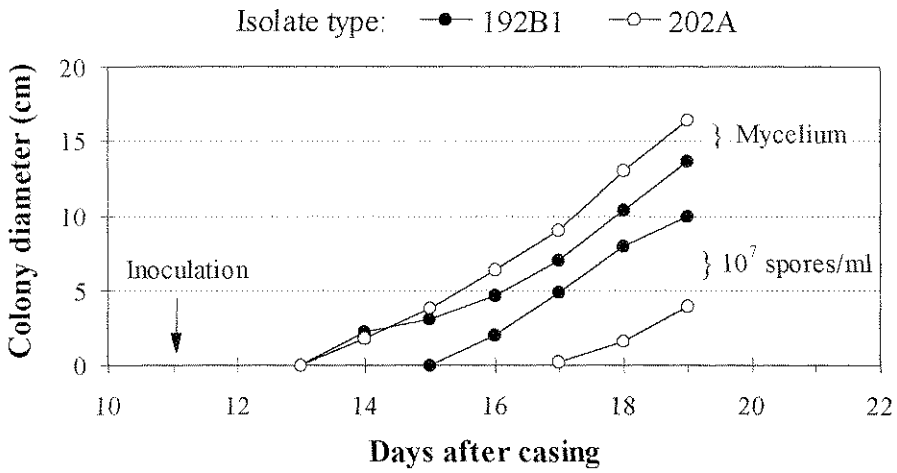
Following inoculation with mycelial plugs of *Dactylium*, both isolates began to grow outward in a circular pattern achieving colony diameters in the region of 13-16 cm by the end of the first flush (Figure 3). Mycelial growth on the casing was observed 3 days after inoculation and consisted of a thin network of hyphae extending radially from the inoculation point. The daily radial growth rate increased over time to reach approximately 1.67 cm/day for isolate hyphae 192B1 and 1.85 cm/day for isolate 202A. It is not obvious from the data if there is a significant difference between these two isolates in their growth on casing. Isolate 202A appeared to be unaffected by the watering events on days 14 and 15 whereas the rate of growth of isolate 192B1 was reduced.

Following inoculation with a high concentration of spores (10^7 spores/ml), patches of *Dactylium* occurred on the casing surface during the first flush (Table 1). From 2 to 5 patches per plot occurred when isolate 192B1 was used with an average final diameter of coalesced patches of 10 cm. Patches formed less frequently with isolate 202A with 0 to 2 patches per plot being recorded. These also developed later so that by the end of the first flush their average diameter was only 3.9 cm. No patches of *Dactylium* developed on casing which was uninoculated or which was inoculated with a lower concentration of spores.

Table 1. Number and diameter of *Dactylium* patches occurring on casing at the end of 1st flush following inoculation at pinning

Inoculum Source	Number of *Dactylium* patches/plot		Patch Diameter (cms)	
	192B1	202A	192B1	202A
Mycelium plugs	1	1	13.7	16.4
10^4 spores/ml	0	0	-	-
10^7 spores/ml	2-5	0-2	10.0	3.9

Figure 3. Growth of *Dactylium* (Cobweb symptoms) on casing following inoculation at pinning with spores or mycelium.



Inoculation between first and second flush

Yield

The average yield of clean mushrooms for uninoculated plots over three flushes was 1.06 kg/plot which was equivalent to 303 kg/tonne of spawn-run compost. In the second flush, the total yield of clean and spotted mushrooms did not vary significantly with inoculation method apart from plots inoculated with mycelium of isolate 192B1 (Figure 4). There was a significant drop in yield in the third flush for treatments inoculated with mycelium and 10^6 spores/ml due to a reduction of cropping area as a result of cobweb growth on the casing.

Spotting

No spotted mushrooms were recorded in the first flush. Following inoculation at the end of the first flush, spotted mushrooms occurred predominantly on plots inoculated with a high concentration of spores (Figure 4). Up to 72% of the mushroom yield harvested from these treatments developed spotting. Spotting symptoms were observed 4 to 7 days after inoculation (Figure 5). Very few spotted mushrooms (<3%) developed on plots inoculated by other methods. After carefully treating any visible areas of *Dactylium* growth at the end of the second flush or removing heavily diseased pots, no spotted mushrooms were recorded in the third flush.

Cobweb symptoms

Cobweb-growth was recorded on all plots inoculated with mycelial plugs of isolate 192B1 four days after inoculation (Figure 6). Growth from the plugs progressed rapidly with a hyphal extension rate of between 1.9 and 2.4 cm/day. Only three of the six plots inoculated with mycelial plugs of isolate 202A developed cobweb-growth during the second flush (data not shown). Growth in these plots proceeded rapidly at a similar rate as for isolate 192B1. All colonised plots were salted and removed at the end of the second flush but uncolonised plots were left in position to see what would happen in the third flush. A further two out of the three remaining 202A inoculated plots developed cobweb growth which also grew rapidly at hyphal extension rates of about 1.4 to 1.8 cm/day. One plot remained uncolonised and cropped normally.

Figure 4. Yield of clean and spotted mushrooms from three flushes following inoculation of casing after the 1st flush with three inoculum types and two *Dactylium* isolates - 192B1 and 202A.

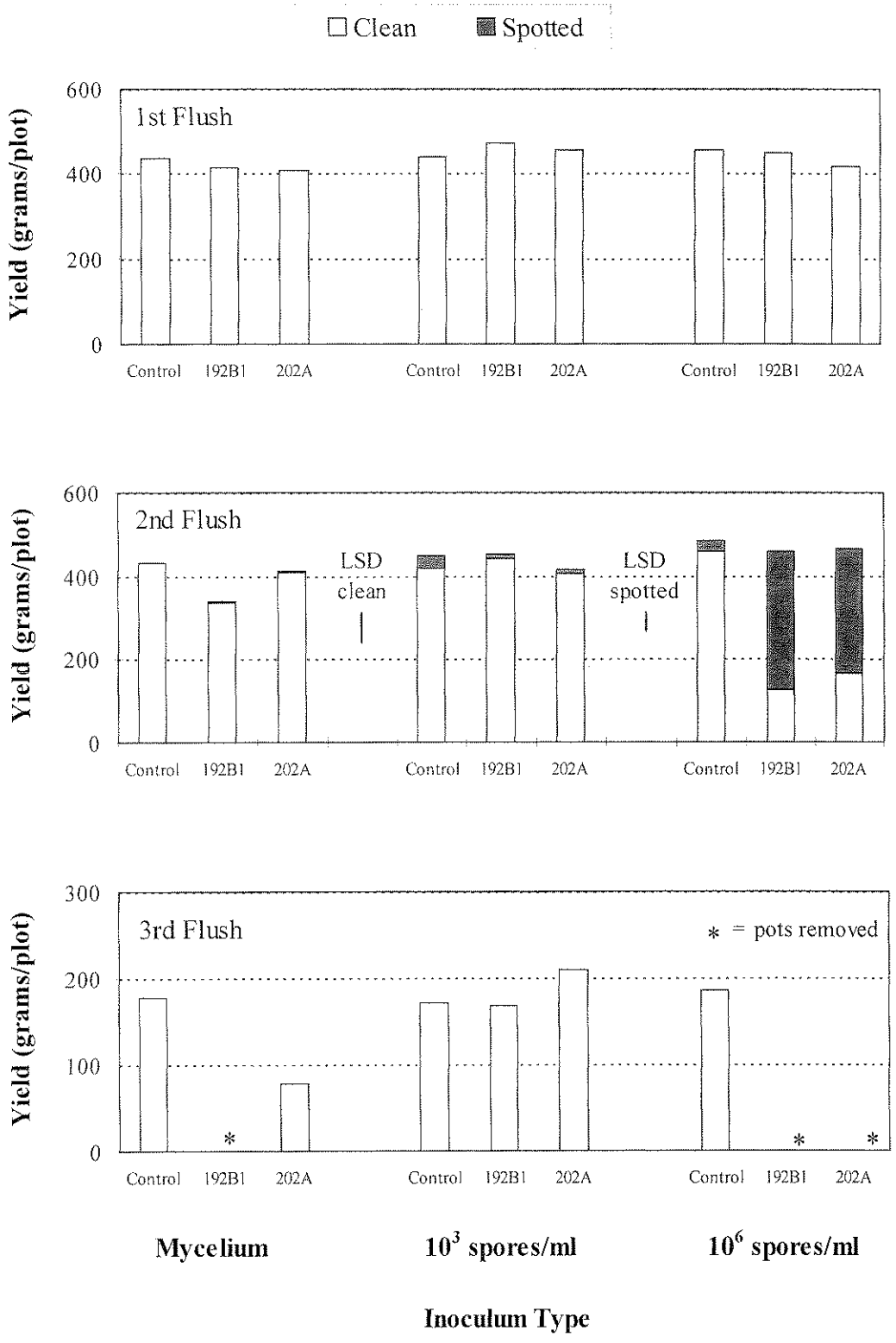


Figure 5. Emergence of spotting symptoms on mushrooms following inoculation of casing after the first flush with spores or mycelium.

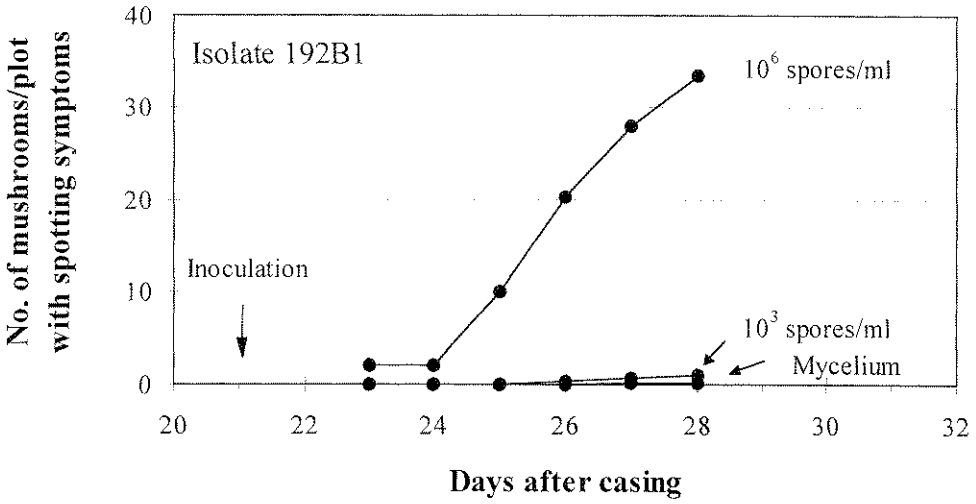
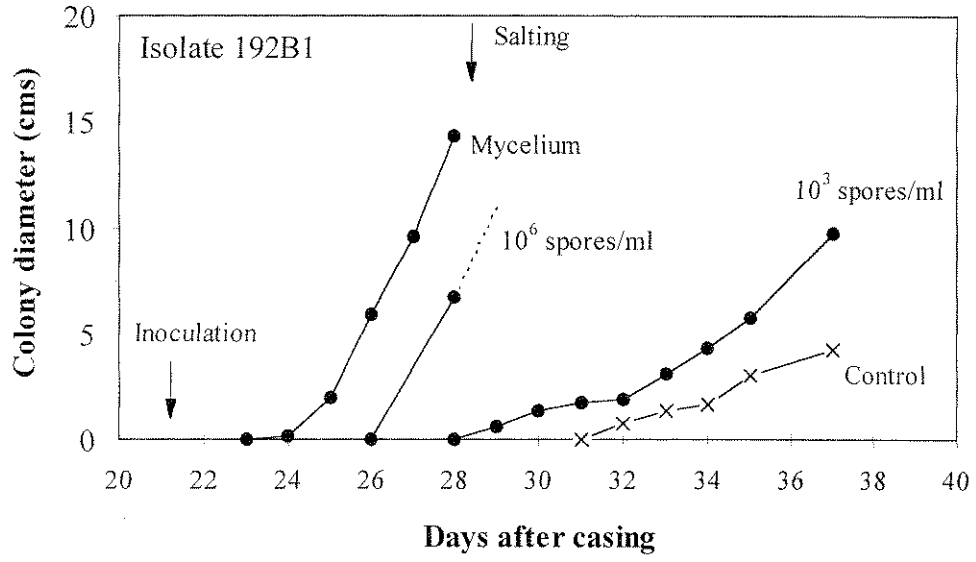


Figure 6. Growth of *Dactylium* (Cobweb symptoms) on casing following inoculation after the first flush with spores or mycelium.



At the end of the second flush between one and four patches of *Dactylium* growth had occurred on all plots inoculated with a high concentration of spores which proceeded to grow rapidly (Figure 6). No *Dactylium* was present during the second flush on plots inoculated with a low concentration of spores or on uninoculated controls and these were kept for a third flush to see if anything would develop. Four out of the six plots inoculated with a low concentration of 192B1 spores went on to develop *Dactylium* patches in the third flush which then grew rapidly (Figure 6) but none of the plots inoculated with a low concentration of 202A spores developed any *Dactylium*. Some control plots had developed *Dactylium* by the third flush but usually only one or two of the six replicates.

Discussion

Symptoms of *Dactylium* infection as either cobweb growth on the casing surface or spotting on mushroom caps were produced in a manageable way using either mycelium or spore inoculum. Mycelium inoculum incorporated into casing abundant cobweb growth while a high concentration of spores (10^6-10^7 spores/ml) sprayed onto casing produced abundant spotting symptoms. A lower concentration of spores produced only a low incidence of spotting symptoms. Spore inoculum also led to the development of cobweb symptoms but usually at a later stage in the crop cycle compared with mycelium inoculum.

Symptom expression was related to both type of inoculum and time of inoculation. When mycelium or concentrated spore inoculum was applied at pinning symptoms developed during the first flush. When it was applied after the first flush symptoms developed during the second flush. When a low concentration of spores was applied to casing either at pinning or after the first flush some spotting symptoms emerged in the developing flush but not in subsequent ones. A small amount of cobweb growth did occur in the third flush following inoculation between the first and second flush implying that small levels of inoculum early in a crop can give rise to *Dactylium* in later flushes.

Two isolates of *Dactylium* (isolate 192B1 and isolate 202A), which represent two different

Cladobotryum species, behaved fairly similarly in terms of symptom expression although there was some evidence to suggest that spores of isolate 202A may not be as effective as those of isolate 192B1 in producing cobweb growth on the casing. This may explain why isolates similar to 192B1 were more commonly found in a recent HDC survey (project M14a) than isolates similar to 202A.

By salting all visible areas of cobweb growth at the end of a flush, prior to applying water, the spread of *Dactylium* was largely controlled. Thus inoculation experiments can effectively measure the development of *Dactylium* symptoms in response to various treatments such as different rates of fungicide use.

Conclusions

1. Mycelium inoculum incorporated into casing at pinning, or after the first flush, will lead to cobweb-growth on the casing surface after 3 to 4 days.

2. A concentrated spore suspension of 10^6-10^7 spores/ml applied to casing at pinning or after the first flush will lead to 70-95% of harvested mushrooms developing spotting symptoms. Some cobweb growth will also occur 5-6 days after inoculation.

3. A dilute spore suspension of 10^3-10^4 spores/ml will result in less than 25% of harvested mushrooms developing spotting symptoms. Cobweb growth on the casing will tend to be infrequent and delayed until a later flush.

B. PART II - Efficacy of two chemicals in controlling *Dactylium*

Introduction

During the severe outbreaks of *Dactylium* experienced by many growers in 1995, HRI in conjunction with a pro-active spawn company screened a number of new chemicals for their potential to inhibit the growth of *Dactylium* under laboratory conditions. This work identified two chemicals, referred to as Chemical A and Chemical D, which looked promising and a research proposal was put to the HDC to test the efficacy of these products *in vivo*, the results of which are presented in the following pages. Each chemical was tested out on a separate crop which had been inoculated with *Dactylium* in such a way as to give symptoms in both the first and second flush.

Materials and Methods

Compost and crop management

HRI Formula III compost was used for both crops, using A12 spawn from Sylvan. Fifty kg of compost was filled into wooden trays and spawn-run, cased and case run according to standard procedures at HRI. Crops were watered according to standard practices by mushroom unit staff until inoculation with *Dactylium*. After inoculation, any watering required was done by scientific staff to prevent exposure of mushroom unit staff to contamination with *Dactylium*.

All picking was done by personnel dedicated to the inoculated crops. Control trays within an inoculated crop were picked by dedicated personnel to prevent contamination of the controls by pickers. Inoculated crops were picked by a second set of dedicated personnel who picked from least affected to worse affected crops to minimise cross-contamination. At the end of each flush all visible areas of *Dactylium* were salted and all diseased mushrooms were picked off and discarded gently in a bucket of 0.5% Environ using rubber gloves.

Once all *Dactylium* sources had been identified and treated, the crop was watered in preparation for the next flush. Three flushes were harvested in this way.

Fungicide treatment

Both chemicals were applied to casing at different rates of active ingredient/m^2 to determine both phytotoxic effects and efficacy in controlling *Dactylium*. The rates used reflected the range of concentrations over which it was felt that meaningful results might be obtained and they are listed in Table 2.

Table 2. Grams of active ingredient/m^2 used in Experiments

	Chemical A	Chemical D
Rate 1	0	0
Rate 2	0.0125	0.07
Rate 3	0.034	0.15
Rate 4	0.125	0.32
Rate 5	0.34	0.70
Rate 6	1.0	1.5
Target volume	2 l/m^2	2 l/m^2
Actual volume	1.9 l m/^{2}	2.2 l/m^2

Both fungicides were made up in volumes of water equivalent to 200 litres for 100 m^2 of bed area, and they were applied as the first watering after casing. Each fungicide rate was prepared separately in a holding tank beginning with the least concentrated, and was drenched onto the casing using a hand held lance with a Number 2/3 rose attached to a 0.5 Horse power self-priming electric pump. A target volume of 2 l/m^2 of bed area was aimed for with an average of 1.9 l/m^2 of Chemical A being applied and 2.2 l/m^2 of Chemical D.

Inoculation

Each crop was inoculated on two separate occasions so as to give symptoms in both the first and the second flush. Each tray was divided into 2 sub-plots, one of which was inoculated, and the other was not. The isolate 192B1 was used in this study.

Dactylium in First Flush.

A plug of *Dactylium* culture was placed centrally into three replicate sub-plots for each rate of fungicide. A small amount of casing was removed from the surface prior to the placement of the *Dactylium* plug, and then replaced. Growth of *Dactylium* was recorded at the end of each flush as the number of *Dactylium* patches present on the casing and the area that they occupied. Prior to re-watering in preparation for the next flush, all patches of *Dactylium* were treated with salt as described above.

Dactylium in Second Flush.

A second set of trays in the same cropping house was used to monitor the efficacy of the fungicides to control *Dactylium* in the second flush. On this occasion a second inoculation method was used whereby a concentrated suspension of *Dactylium* spores was prepared (10^6 spores/ml) of which 10 ml was sprayed onto one half of a cased tray just after the first flush. Three replicate sub-plots were prepared for each rate of fungicide used. *Dactylium* symptoms were recorded as the weight of spotted mushrooms harvested, and the number and area of *Dactylium* patches emerging following inoculation. At the end of the second flush, all visible *Dactylium* patches were salted as described earlier and the crop re-watered in preparation for the third flush.

Statistical design and analyses

A separate cropping chamber was allocated to each of the two fungicides tested. Within each house 'chemical rate' treatments were arranged as a trojan square with alternate rates assigned to different 'alphabets'. Each chemical rate plot was split for inoculation level (ie. inoculated or not inoculated). Inoculation timing treatments (ie. first or second

flush symptoms) were randomly assigned to chemical rate plots to allow comparison between them.

The collected data comprised healthy yield, spotted yield, number of *Dactylium* patches emerging and area of *Dactylium* patches emerging. Data were analyzed by analysis of variance and the statistics are presented in Appendix II (Chemical A) and Appendix III (Chemical D).

Results

Chemical A

Phytotoxic effect on yield

Chemical A applied to casing had a slight stimulating effect on the yield of healthy mushrooms when used at a rate of up to 0.0395 grams a.i./m^2 but significant yield reduction occurred at rates of 0.125 grams a.i./m^2 or above (Figure 7). A yield of 108% was recorded from a crop treated with 0.0395 grams a.i./m^2 compared with 84% at a rate of 0.125 g a.i./m^2. Casing colonisation by *Agaricus* was poor in casing treated at the higher rates but an improvement with time occurred only at the rate of 0.125 g a.i./m^2. At this rate first and second flush yields were down on untreated controls but they had picked up by the third flush. When the crop was inoculated with *Dactylium*, Chemical A was effective in terms of yield recovery at a rate of 0.125 g a.i./m^2. At lower rates of use the yield of mushrooms was reduced due to the presence of the disease.

Spotting symptoms

Spotted mushrooms were harvested predominantly from inoculated treatments with only a small number of mushrooms from uninoculated treatments developing spotting symptoms (Figure 8). Spotted mushrooms occurred over the range of fungicide concentrations tested but significantly more were harvested from inoculated crops receiving no fungicide, or fungicide at the lowest rate of 0.0125 g a.i./m^2 (12-16% of total yield). Lower yields of spotted mushrooms were harvested from the intermediate fungicide treatments (5-8% of total yields) while fewer spotted mushrooms were harvested from inoculated crops which had received the highest rate of fungicide (3.5% of total yield).

Emergence of *Dactylium* patches

Dactylium patches developed on the casing of inoculated crops during the experiment but these were largely confined to casing which had received either no fungicide treatment or

Figure 7. Yield of clean mushrooms harvested from crops treated with different rates of **Chemical A**. Least significant difference (LSD) at P = 0.05 covers all comparisons.

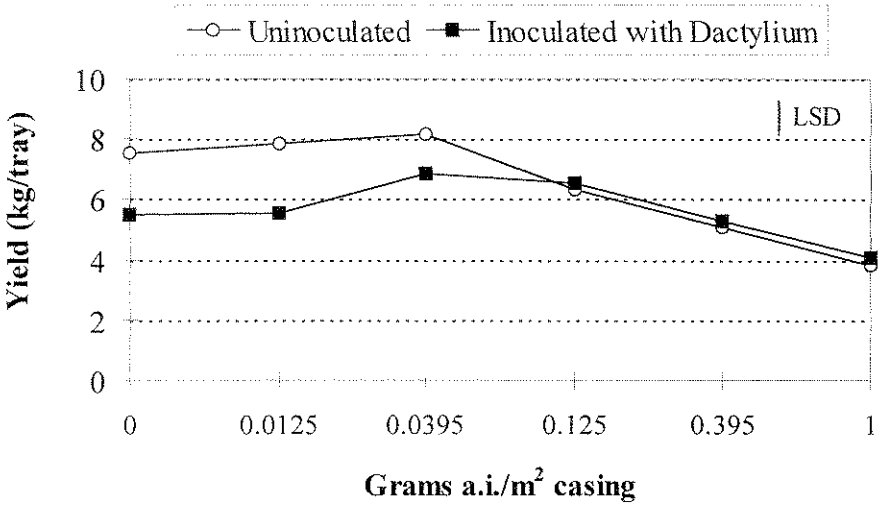
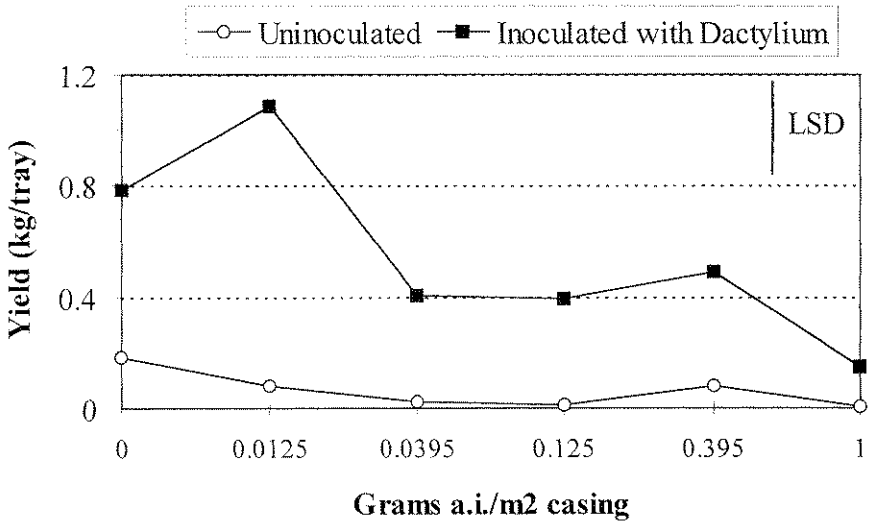


Figure 8. Yield of spotted mushrooms from crops treated with different rates of **Chemical A.** Least significant difference (LSD) at P = 0.05 covers all comparisons.



fungicide at the two lowest rates tested (Figure 9). There was a significant reduction in the number of *Dactylium* patches observed on casing treated with Chemical A particularly at rates of 0.125 g a.i./m^2 upwards.

The area of casing covered by *Dactylium* growth was calculated at the end of each flush for all treatments to give an estimate of the potential for each recorded patch to grow further. Chemical A at a rate of 0.125 g a.i./m^2 reduced the area of *Dactylium* in inoculated treatments to an average of less than 2% cover compared with over 15% cover in the absence of the fungicide (Figure 10). However, one patch which occurred on this treatment in the third flush achieved a diameter of 12 cm. At the 0.395 rate of fungicide use *Dactylium* growth was very much inhibited and was generally confined to mushrooms rather than casing. At the highest rate of use no cobweb growth was recorded but the statistical analyses predicted the development of a small area of *Dactylium* based on data from combined means.

Discussion

Chemical A gave significant control of the mushroom pathogen *Dactylium dendroides* at a concentration of active ingredient of 0.125 g/m^2 but some growth on casing was still possible at this concentration. This rate had some phytotoxic effect on yield by slowing down casing colonisation in the first flush resulting in a 13-16% yield reduction over 3 flushes. *Dactylium* expression was controlled more effectively at the higher rates of use (0.395 - 1.0 grams a.i./m^2) but these rates were also more phytotoxic with yields being reduced by 30-50% over 3 flushes. Chemical A could be a useful control chemical against *Dactylium*.

Conclusion

Chemical A is effective at controlling the emergence of *Dactylium* on casing when used at rates of 0.395 grams of active ingredient/m^2 or higher. However, these rates of use dramatically reduce yields due to poor casing colonisation by *Agaricus* and therefore may not be acceptable to growers.

A rate of use equal to 0.125 grams a.i./m^2 gave significant control in conjunction with only

Figure 9. Number of *Dactylium* (Cobweb) patches emerging on casing treated with different rates of **Chemical A**. Least significant difference (LSD) at $P = 0.05$ covers all comparisons.

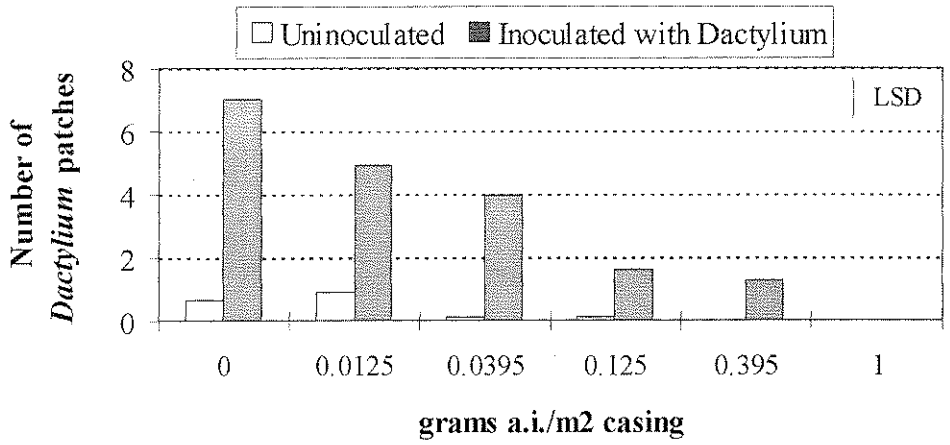
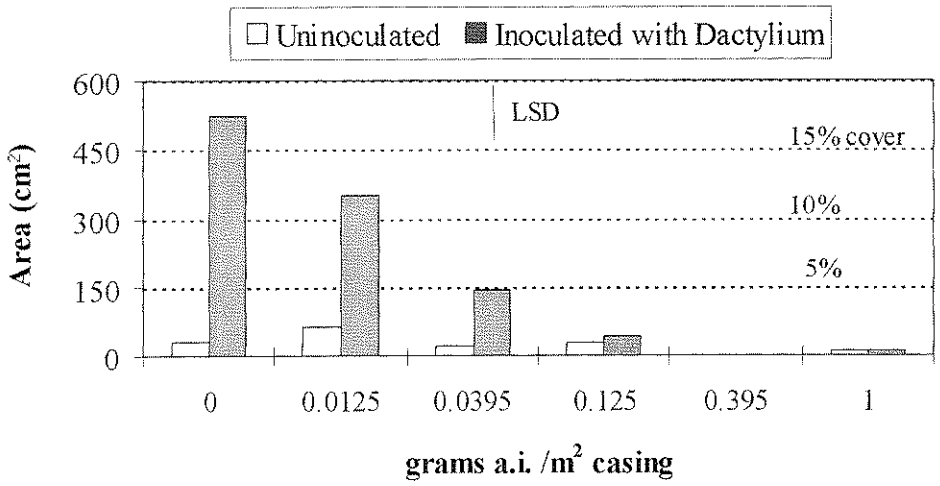


Figure 10. Area of *Dactylium* (Cobweb) patches on casing treated with different rates of **Chemical A.** Least significant difference (LSD) at $P = 0.05$ covers all comparisons.



moderate phytotoxic effects. When *Dactylium* succeeded in establishing however growth on the casing did occur with this rate of use. Nonetheless, this rate of use could prove to be an effective control treatment for *Dactylium* in conjunction with effective disease monitoring, particularly in the third flush, so that any emerging *Dactylium* patches could be treated before they grew too big.

Results

Chemical D

Phytotoxic effect on yield

Chemical D applied to casing had a stimulating effect on the yield of healthy mushrooms when used at a low rate of 0.07 grams a.i./m^2. No phytotoxic effect on yield was recorded at rates of between 0.15 and 0.7 grams a.i./m^2, but significant yield reduction to 86% of control occurred at a rate of 1.5 grams a.i./m^2 (Figure 11). Casing colonisation by *Agaricus* was good in casing treated at all rates of use. When the crop was inoculated with *Dactylium*, Chemical D significantly improved yield over the range of concentrations tested compared with the inoculated control with no fungicide treatment. Healthy yield from inoculated plots lagged behind uninoculated plots however due to significant numbers of spotted mushrooms occurring over the range of concentrations tested.

Spotting symptoms

There was no significant relationship between the rate of Chemical D used and the yield of spotted mushrooms although a greater proportion, 18% of total yield, was recorded for inoculated plots receiving no chemical (Figure 12). The development of spotting symptoms was determined by whether plots were inoculated or not and also by the type of inoculum used. Some of the spotting symptoms recorded were due to *Trichoderma harzianum* but these were relatively few and not confined to any single treatment.

Emergence of *Dactylium* patches

Patches of *Dactylium* emerged on all casings treated with Chemical D but significantly fewer occurred at rates of 0.32 grams a.i./m^2 and above (Figure 13). Mycelium-inoculated plots developed an average of about 6 patches per plot on casing not treated with fungicide but this dropped significantly with increasing concentrations of Chemical D.

Figure 11. Yield of clean mushrooms harvested from crops treated with different rates of **Chemical D**. Least significant difference (LSD) at P = 0.05 covers all comparisons.

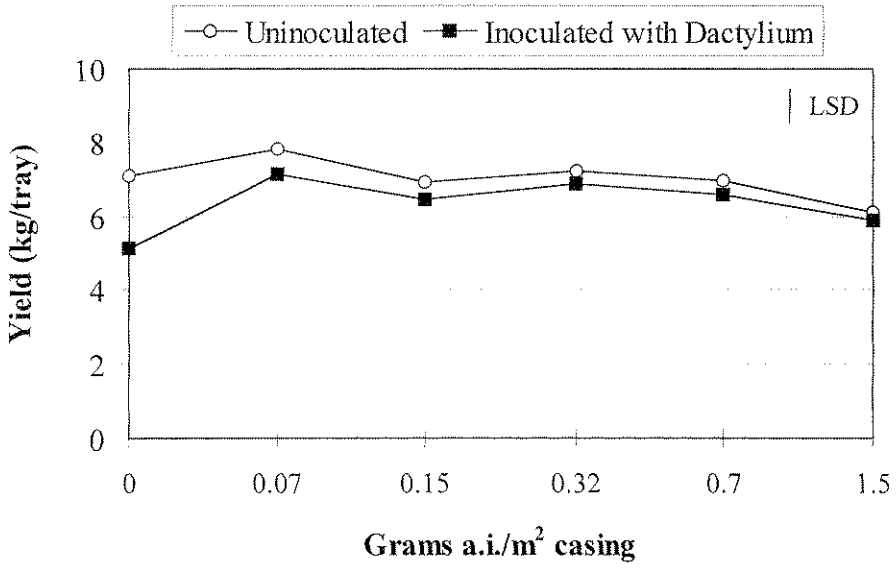
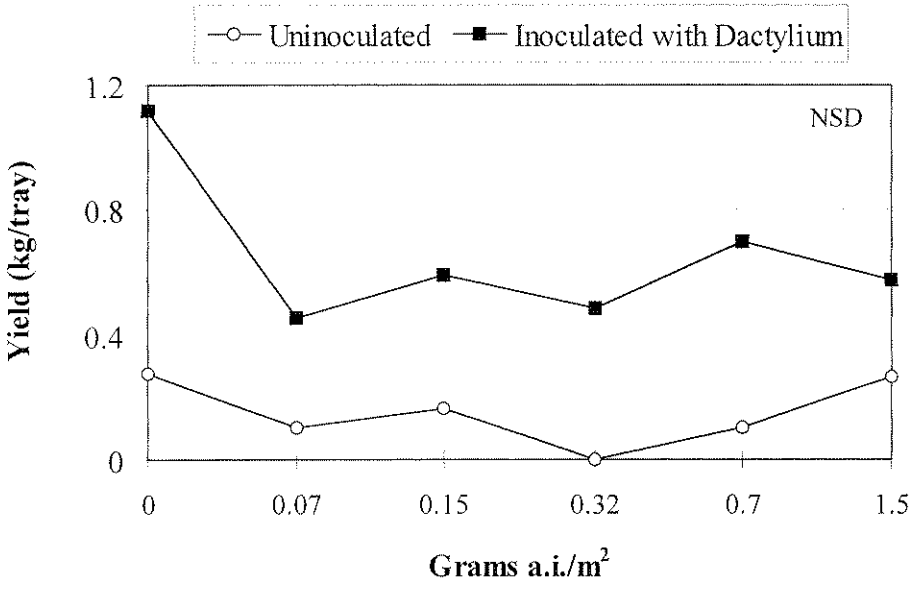


Figure 12. Yield of spotted mushrooms from crops treated with different rates of **Chemical D**. No significant difference (NSD) at P = 0.05 recorded with respect to rate of chemical.



The number of *Dactylium* patches emerging from casing inoculated with a spore suspension was higher than for mycelium-inoculated casing with around 10 patches occurring on casing with no fungicide applied (Figure 13). A high number of *Dactylium* patches also occurred on casing treated with Chemical D at the lower rates but fewer patches were recorded on casing treated at the higher rates. Invariably such patches grew out from infected pins (Plate 3, Appendix IV).

The area of casing covered by *Dactylium* growth was calculated at the end of each flush to give an estimate of the potential for each recorded patch to grow further. Chemical D at a rate of 0.32 g a.i./m^2 reduced the area of *Dactylium* in inoculated treatments to an average of less than 2.5% cover compared with over 15-40% cover in the absence of the fungicide (Figure 14). At a rate of 0.7 grams a.i./m^2 *Dactylium* growth still occurred on the casing but it was largely restricted, and confined to spore-inoculated plots in the third flush. At the highest rate of fungicide used, *Dactylium* growth was very much inhibited and was generally confined to mushrooms rather than casing.

Discussion

Chemical D significantly reduced the expression of the mushroom pathogen *Dactylium dendroides* at concentrations of active ingredient of 0.32 g a.i./m^2 and above but it was capable of restricted growth on casing at concentrations of 0.32, and to a lesser extent 0.7 grams a.i./m^2. Only the highest rate of Chemical D used, 1.5 grams a.i./m^2, had a significant phytotoxic effect so the most effective concentration in terms of disease control and minimum phytotoxic yield reduction lies between 0.7 and 1.5 grams a.i./m^2. At these rates there was a dramatic reduction in both the number of *Dactylium* patches emerging after inoculation, and the further growth of those patches, although *Dactylium* patches of up to 5 cm diameter were recorded in the third flush on casing treated with 0.7 grams a.i./m^2.

Spotting of mushrooms was not prevented as a result of the use of Chemical D. This is probably not to be expected since the mushrooms themselves would have only minimal concentrations of the chemical and therefore be vulnerable to spotting by spores landing on pins and sporophores.

Figure 13. Number of *Dactylium* (Cobweb) patches emerging on casing treated with different rates of **Chemical D.** Least significant difference (LSD) at $P = 0.05$ covers all comparisons.

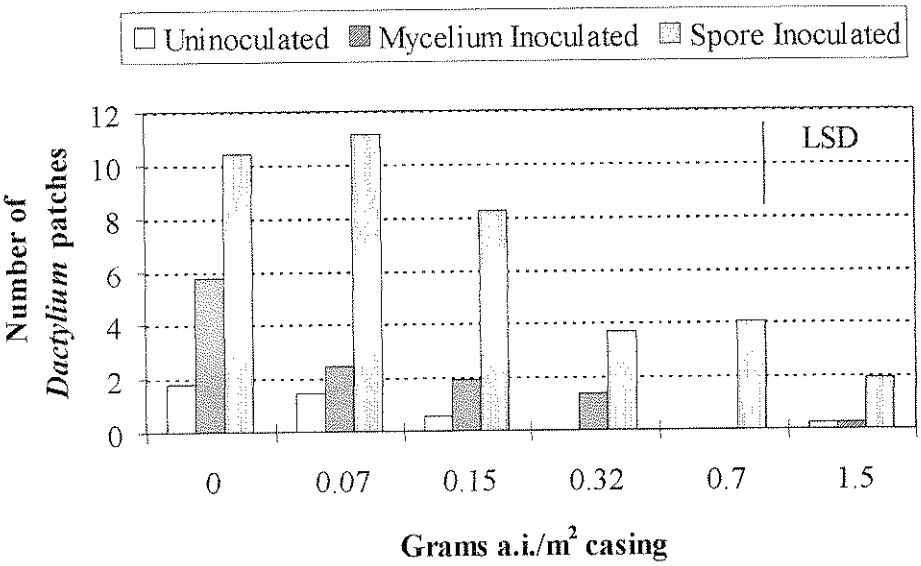
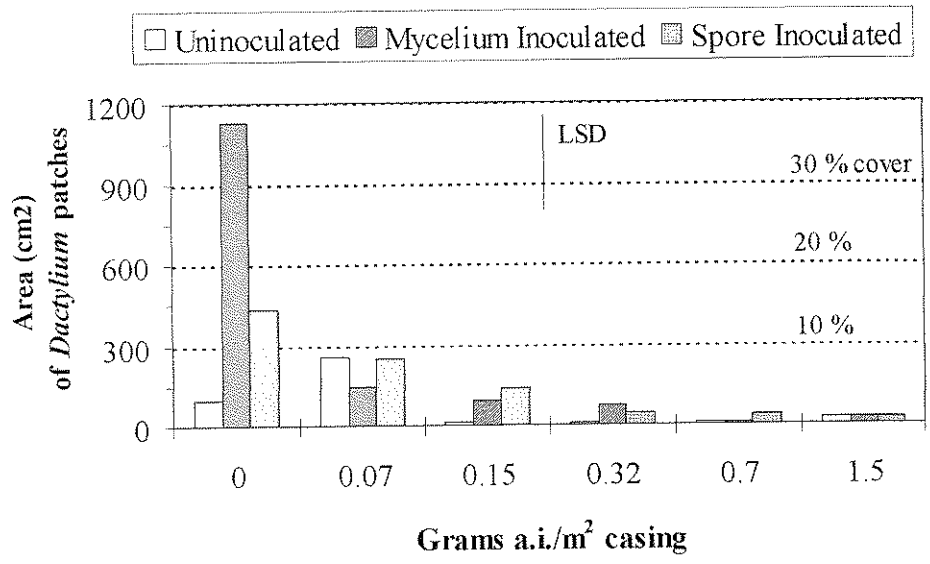


Figure 14. Area of *Dactylium* (Cobweb) patches on casing treated with different rates of **Chemical D.** Least significant difference (LSD) at $P = 0.05$ covers all comparisons.



Conclusion

Chemical D appears to be effective in significantly controlling the emergence and growth of *Dactylium* on casing when used at rates of between 0.7 and 1.5 grams of active ingredient/m^2 of casing. Yield reductions due to phytotoxicity of the chemical toward *Agaricus* were recorded at the highest rate of use but not at the 0.7 g a.i./m^2 rate. In view of the ability of *Dactylium* to grow on casing treated with 0.7 g a.i./m^2 of Chemical D, albeit restrictedly, implies that the most effective concentration probably lies between 0.7 and 1.5 g a.i./m^2.

Chemical D did not prevent mushrooms from developing spotting symptoms due to *Dactylium*.

C. OVERALL DISCUSSION

Both of the chemicals which were tested for their ability to control *Dactylium* in a mushroom crop succeeded in reducing the incidence of cobweb growth on the casing. The most effective rates of Chemical A (0.395-1.0 gram a.i./m^2) had significant phytotoxic effects on the mushroom crop itself reducing yields by 30-50% over 3 flushes. This would probably preclude its viability as a commercial product. The most effective rates of Chemical D (0.7-1.5 gram a.i./m^2) were less phytotoxic with only the higher rate causing a significant yield reduction of 14%. This was still a better yield compared with that from a diseased crop in the absence of any fungicide.

The growth of cobweb on the casing surface was inhibited by both chemicals at the highest rate of chemical used. Some restricted growth onto casing occurred, from infected mushrooms or pins, particularly with Chemical D in the third flush following inoculation with a concentrated spore suspension. It should be remembered that this treatment was an extreme one but it indicates that if the inoculum level is very high some restricted growth on casing can occur which, if not detected, could result in raising inoculum levels further. This hypothesis was not tested in these experiments. All patches of *Dactylium* were identified and salted before the next watering operation. It may be useful to obtain further information regarding the spread of *Dactylium* within a crop treated with Chemical D where small patches of cobweb growth are deliberately watered over. The occurrence of restricted growth may reflect a decreasing concentration of the active ingredient in the upper layer of casing over time and this is another area where additional information would be useful.

Preliminary fungicide resistance tests on agar media indicate that the ED50 value for two contrasting *Dactylium* isolates 192B1 and 202A are in the region of 0.05 ppm for Chemical D. A similar ED50 value was recorded for isolate 192B1 with respect to Chemical A but the data (not shown) were more variable for isolate 202A. It is important to monitor any changes in the ED50 values so as to pick up any tendency for resistant isolates to emerge.

No residue data was obtained from mushrooms harvested from crops treated with either Chemical A or D. This information is necessary in order to determine whether or not any

residues are present and whether any levels detected are within government guidelines for these products.

In order to obtain approval for use on mushrooms additional information regarding residues is obligatory. More information on efficacy would also be desirable by concentrating on the most effective rates and exploring some of the questions posed above. The results presented above suggest that Chemical D at a rate of between 0.7 and 1.5 grams a.i./m^2 of casing bed area is likely to have potential as a new control chemical for *Dactylium*. It would, however, be useful to compare its performance with other fungicides used by the mushroom industry today.

D. OVERALL CONCLUSIONS

1. Heavy contamination of casing during cropping with spores of *Dactylium* will lead to massive cap spotting and numerous patches of cobweb growth developing from as early as four days after the contamination event.

2. Chemical A inhibited the development of cobweb growth on casing but effective concentrations were phytotoxic to *Agaricus* reducing yields by up to 50%.

3. Chemical D inhibited the development of cobweb growth on casing at rates of 0.7-1.5 grams a.i./m^2 of bed area but small restricted areas of cobweb grew out from infected mushrooms during the third flush. This would probably only occur during the height of an epidemic and may be tolerated. At such times good hygiene practices should identify and treat any areas of visible *Dactylium*, preventing them from being watered over.

E. RECOMMENDATIONS FOR FUTURE WORK

1. Chemical D should be considered as a potential new chemical for the control of *Dactylium*. A repeat inoculation trial concentrating on one or two rates of use should be carried out to verify its ability to control *Dactylium* under a heavy inoculation load. This work should also look at the spread of *Dactylium* within a treated crop where areas of cobweb growth are not treated prior to watering to determine whether the treatment is still effective under these conditions.

3. The efficacy of Chemical D should be compared with that of an existing chemical used to control *Dactylium*.

4. Casing treated with Chemical D should be analyzed during the cropping period to determine changes in the level of active ingredient in the casing profile over time.

F. ACKNOWLEDGEMENTS

We would like to thank the HRI Mushroom Unit staff for their input in setting up the crops; Bruce Adie for his help during the execution of this research and Andrew Mead for his analysis of the data.

G. REFERENCES

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H. APPENDICES

Appendix I

Mushroom Extract Agar

Mushroom Extract

Fresh mushrooms	140 grams
Distilled water	1 litre

Slice mushrooms and put in a blender. Add water and macerate for one minute. Warm the mixture in a saucepan taking care not to let it boil. After 5 minutes filter through four layers of muslin. Dispense into 250 ml quantities and freeze.

Mushroom Extract Agar

Mushroom extract	250 mls
Distilled water	250 mls
Agar technical no. 3	6 grams

Place all ingredients in a 500 ml Duran flask and autoclave for 15 minutes at 121°C,

APPENDIX II

CHEMICAL A-1

***** Analysis of variance *****

Variate: tothyld | Total healthy yield - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	1.260E+06	6.301E+05		
block stratum	2	9.450E+05	4.725E+05		
layer.block stratum					
ChemRate	4	4.419E+07	1.105E+07		
layer.block.plot stratum					
ChemRate	5	5.645E+07	1.129E+07	17.59	0.008
Residual	4	2.567E+06	6.417E+05	2.72	
layer.block.plot.tray stratum					
InocTime	1	2.008E+05	2.008E+05	0.85	0.375
ChemRate.InocTime	5	2.942E+06	5.884E+05	2.49	0.091
Residual	12	2.836E+06	2.363E+05	0.35	
layer.block.plot.tray.half stratum					
InocLev	1	1.235E+07	1.235E+07	18.22	<.001
InocLev.ChemRate	5	2.161E+07	4.323E+06	6.38	<.001
InocLev.InocTime	1	1.236E+06	1.236E+06	1.82	0.190
InocLev.ChemRate.InocTime	5	3.799E+06	7.599E+05	1.12	0.376
Residual	24	1.627E+07	6.780E+05		
Total	71	1.667E+08			

***** Tables of combined means *****

Variate: tothyld Total healthy yield - all flushes

InocLev	Control	192B1
	6476.	5647.

ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	6539.	6713.	7519.	6453.	5182.	3963.

InocTime	First	Second
	6114.	6009.

InocLev	ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25 (1.00)
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		7561.	7855.	8184.	6341.	5087.	3826.
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		5516.	5571.	6854.	6565.	5276.	4100.

InocLev	InocTime	First	Second
Control		6397.	6554.
192B1		5831.	5463.

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		6540.	6537.
0.0125	letter	1	1
	alphabet	2	2
		6455.	6972.
0.0395	letter	2	2
	alphabet	1	1
		7538.	7500.
0.125	letter	2	2
	alphabet	2	2

A-2

		6886.	6020.	
0.395	letter	3	3	
	alphabet	1	1	
		5244.	5119.	
1.25	letter	3	3	
	alphabet	2	2	
	.	4023.	3903.	

InocLev	ChemRate	InocTime	First	Second
Control	Control	letter	1	1
		alphabet	1	1
			7537.	7584.
	0.0125	letter	1	1
		alphabet	2	2
			7698.	8011.
	0.0395	letter	2	2
		alphabet	1	1
			7756.	8611.
	0.125	letter	2	2
		alphabet	2	2
			6565.	6116.
	0.395	letter	3	3
		alphabet	1	1
			5314.	4860.
	1.25	letter	3	3
		alphabet	2	2
			3513.	4139.
192B1	Control	letter	1	1
		alphabet	1	1
			5543.	5490.
	0.0125	letter	1	1
		alphabet	2	2
			5211.	5932.
	0.0395	letter	2	2
		alphabet	1	1
			7319.	6389.
	0.125	letter	2	2
		alphabet	2	2
			7206.	5924.
	0.395	letter	3	3
		alphabet	1	1
			5174.	5378.
	1.25	letter	3	3
		alphabet	2	2
			4532.	3667.

*** Standard errors of differences of combined means ***

$t(5\%, 10) = 2.228$

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	194.1	176.0	114.6	379.4
Except when comparing means with the same level(s) of				
alphabet		6.7		336.2
letter.alphabet				475.4
InocLev.alphabet				336.2
d.f.	24	4	12	10

L.S.D.(5%) = 475.4 × 2.228 = 1059

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	225.4	265.2	544.4
Except when comparing means with the same level(s) of			
alphabet		198.6	515.2
letter.alphabet		280.7	552.1
InocTime	274.5		
InocLev.alphabet			515.2
InocLev.letter.alphabet			552.1
alphabet.InocTime		198.6	515.2
letter.alphabet.InocTime			672.3
InocLev.alphabet.InocTime			515.2
d.f.	35.11	5.87	19.29

A-3

***** Analysis of variance *****

Variate: totsyld | Total spotted yield - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	80846.	40423.		
block stratum	2	461720.	230860.		
layer.block stratum					
ChemRate	4	1018897.	254724.		
layer.block.plot stratum					
ChemRate	5	685615.	137123.	1.61	0.333
Residual	4	341276.	85319.	1.07	
layer.block.plot.tray stratum					
InocTime	1	216263.	216263.	2.70	0.126
ChemRate.InocTime	5	223140.	44628.	0.56	0.730
Residual	12	959845.	79987.	1.17	
layer.block.plot.tray.half stratum					
InocLev	1	3920000.	3920000.	57.16	<.001
InocLev.ChemRate	5	1396715.	279343.	4.07	0.008
InocLev.InocTime	1	421668.	421668.	6.15	0.021
InocLev.ChemRate.InocTime	5	59013.	11803.	0.17	0.970
Residual	24	1645867.	68578.		
Total	71	11430864.			

***** Tables of combined means *****

Variate: totsyld Total spotted yield - all flushes

InocLev	Control	192B1
	83.	550.

ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	483.	582.	213.	261.	283.	78.

InocTime	First	Second
	262.	372.

InocLev	ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25 (1.00)
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		183.	80.	23.	129.	79.	7.
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		784.	1084.	403.	393.	487.	148.

InocLev	InocTime	First	Second
Control		105.	62.
192B1		419.	681.

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		477.	490.
0.0125	letter	1	1
	alphabet	2	2
		532.	632.
0.0395	letter	2	2
	alphabet	1	1
		46.	381.
0.125	letter	2	2
	alphabet	2	2

A-4

			255.	267.
0.395		letter	3	3
		alphabet	1	1
			257.	309.
1.25		letter	3	3
		alphabet	2	2
			5.	150.

InocLev	ChemRate	InocTime	First	Second
Control	Control	letter	1	1
		alphabet	1	1
			268.	98.
	0.0125	letter	1	1
		alphabet	2	2
			95.	65.
	0.0395	letter	2	2
		alphabet	1	1
			-17.	63.
	0.125	letter	2	2
		alphabet	2	2
			199.	59.
	0.395	letter	3	3
		alphabet	1	1
			86.	72.
	1.25	letter	3	3
		alphabet	2	2
			1.	14.
192B1	Control	letter	1	1
		alphabet	1	1
			687.	882.
	0.0125	letter	1	1
		alphabet	2	2
			970.	1199.
	0.0395	letter	2	2
		alphabet	1	1
			108.	698.
	0.125	letter	2	2
		alphabet	2	2
			311.	475.
	0.395	letter	3	3
		alphabet	1	1
			427.	547.
	1.25	letter	3	3
		alphabet	2	2
			9.	287.

*** Standard errors of differences of combined means ***

$t(5\%, 8) = 2.306$

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	61.7	60.0	66.7	122.6
Except when comparing means with the same level(s) of				
alphabet		1.2		106.9
letter.alphabet				151.2
InocLev.alphabet				106.9
d.f.	24	4	12	8.436

LSD(5%)

151.2 × 2.306 = 349

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	90.8	130.1	199.5
Except when comparing means with the same level(s) of			
alphabet		115.5	190.2
letter.alphabet		163.3	222.5
InocTime	87.3		
InocLev.alphabet			190.2
InocLev.letter.alphabet			222.5
alphabet.InocTime		115.5	190.2
letter.alphabet.InocTime			213.8
InocLev.alphabet.InocTime			190.2
d.f.	30.27	8.83	20.26

***** Analysis of variance *****

Variate: tpd | Number of dactillium patches - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	1.444	0.722		
block stratum	2	1.694	0.847		
layer.block stratum					
ChemRate	4	65.139	16.285		
layer.block.plot stratum					
ChemRate	5	64.861	12.972	9.20	0.026
Residual	4	5.639	1.410	0.91	
layer.block.plot.tray stratum					
InocTime	1	24.500	24.500	15.75	0.002
ChemRate.InocTime	5	10.333	2.067	1.33	0.317
Residual	12	18.667	1.556	0.87	
layer.block.plot.tray.half stratum					
InocLev	1	144.500	144.500	81.28	<.001
InocLev.ChemRate	5	80.000	16.000	9.00	<.001
InocLev.InocTime	1	46.722	46.722	26.28	<.001
InocLev.ChemRate.InocTime	5	21.111	4.222	2.37	0.069
Residual	24	42.667	1.778		
Total	71	527.278			

***** Tables of combined means *****

Variate: tpd Number of dactillium patches - all flushes

InocLev	Control	192B1
	0.28	3.11

ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	3.81	2.88	2.02	0.86	0.59	0.01

InocTime	First	Second
	1.11	2.28

InocLev	ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		0.65	0.88	0.10	0.11	-0.08	0.01
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		6.98	4.88	3.93	1.61	1.25	0.01

InocLev	InocTime	First	Second
Control		0.50	0.06
192B1		1.72	4.50

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		2.81	4.81
0.0125	letter	1	1
	alphabet	2	2
		2.21	3.55
0.0395	letter	2	2
	alphabet	1	1
		0.93	3.10
0.125	letter	2	2
	alphabet	2	2

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		0.45	1.28	
0.395	letter	3	3	
	alphabet	1	1	
		0.25	0.92	
1.25	letter	3	3	
	alphabet	2	2	
		0.01	0.01	

InocLev	ChemRate	InocTime	First	Second
Control	Control	letter	1	1
		alphabet	1	1
			0.98	0.31
	0.0125	letter	1	1
		alphabet	2	2
			1.71	0.05
	0.0395	letter	2	2
		alphabet	1	1
			0.10	0.10
	0.125	letter	2	2
		alphabet	2	2
			0.28	-0.05
	0.395	letter	3	3
		alphabet	1	1
			-0.08	-0.08
	1.25	letter	3	3
		alphabet	2	2
			0.01	0.01
192B1	Control	letter	1	1
		alphabet	1	1
			4.65	9.31
	0.0125	letter	1	1
		alphabet	2	2
			2.71	7.05
	0.0395	letter	2	2
		alphabet	1	1
			1.77	6.10
	0.125	letter	2	2
		alphabet	2	2
			0.61	2.61
	0.395	letter	3	3
		alphabet	1	1
			0.59	1.92
	1.25	letter	3	3
		alphabet	2	2
			0.01	0.01

*** Standard errors of differences of combined means ***

$t(5\%, 11) = 2.201$

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	0.314	0.445	0.294	0.703
Except when comparing means with the same level(s) of				
alphabet		0.423		0.689
letter.alphabet				0.770
InocLev.alphabet				0.689
d.f.	24	4	12	11.27

LSD(5%)

$0.770 \times 2.201 = 1.69$

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	0.430	0.676	1.024
Except when comparing means with the same level(s) of			
alphabet		0.662	1.015
letter.alphabet		0.720	1.054
InocTime	0.444		
InocLev.alphabet			1.015
InocLev.letter.alphabet			1.054
alphabet.InocTime		0.662	1.015
letter.alphabet.InocTime			1.089
InocLev.alphabet.InocTime			1.015
d.f.	33.33	9.64	26.40

***** Analysis of variance *****

Variate: tad | Area of dactillium patches - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	18356.	9178.		
block stratum	2	14248.	7124.		
layer.block stratum					
ChemRate	4	373145.	93286.		
layer.block.plot stratum					
ChemRate	5	565921.	113184.	7.22	0.039
Residual	4	62705.	15676.	8.11	
layer.block.plot.tray stratum					
InocTime	1	3085.	3085.	1.60	0.230
ChemRate.InocTime	5	97885.	19577.	10.13	<.001
Residual	12	23185.	1932.	0.18	
layer.block.plot.tray.half stratum					
InocLev	1	433551.	433551.	41.26	<.001
InocLev.ChemRate	5	590398.	118080.	11.24	<.001
InocLev.InocTime	1	6074.	6074.	0.58	0.455
InocLev.ChemRate.InocTime	5	7079.	1416.	0.13	0.983
Residual	24	252216.	10509.		
Total	71	2447848.			

***** Tables of combined means *****

Variate: tad Area of dactillium patches - all flushes

InocLev	Control	192B1
	23.	178.

ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	278.	206.	81.	35.	-7.	11.

InocTime	First	Second
	107.	94.

InocLev	ChemRate	Control	0.0125	0.0395	0.125	0.395	1.25
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		31.	63.	21.	29.	-16.	11.
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		525.	350.	142.	41.	1.	11.

InocLev	InocTime	First	Second
Control		39.	7.
192B1		176.	181.

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		251.	305.
0.0125	letter	1	1
	alphabet	2	2
		292.	121.
0.0395	letter	2	2
	alphabet	1	1
		60.	102.
0.125	letter	2	2
	alphabet	2	2

			36.	34.
	0.395	letter	3	3
		alphabet	1	1
			-6.	-9.
	1.25	letter	3	3
		alphabet	2	2
		.	11.	11.

InocLev	ChemRate	InocTime	First	Second
Control	Control	letter	1	1
		alphabet	1	1
			22.	40.
	0.0125	letter	1	1
		alphabet	2	2
			147.	-21.
	0.0395	letter	2	2
		alphabet	1	1
			21.	21.
	0.125	letter	2	2
		alphabet	2	2
			48.	10.
	0.395	letter	3	3
		alphabet	1	1
			-16.	-16.
	1.25	letter	3	3
		alphabet	2	2
			11.	11.
192B1	Control	letter	1	1
		alphabet	1	1
			479.	570.
	0.0125	letter	1	1
		alphabet	2	2
			436.	264.
	0.0395	letter	2	2
		alphabet	1	1
			100.	183.
	0.125	letter	2	2
		alphabet	2	2
			24.	58.
	0.395	letter	3	3
		alphabet	1	1
			4.	-1.
	1.25	letter	3	3
		alphabet	2	2
			11.	11.

*** Standard errors of differences of combined means ***

$t(5\%, 8) = 2.306$

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	24.2	38.0	10.4	56.6
Except when comparing means with the same level(s) of				
alphabet		29.4		51.2
letter.alphabet				59.2
InocLev.alphabet				51.2
d.f.	24	4	12	7.66

LSD(5%)

$\times 2.306 = 136.5$

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	26.3	42.1	72.6
Except when comparing means with the same level(s) of			
alphabet		34.5	68.5
letter.alphabet		25.4	64.4
InocTime	34.2		
InocLev.alphabet			68.5
InocLev.letter.alphabet			64.4
alphabet.InocTime		34.5	68.5
letter.alphabet.InocTime			83.7
InocLev.alphabet.InocTime			68.5
d.f.	35.51	4.61	12.72

APPENDIX III

CHEMICAL D -1

***** Analysis of variance *****

Variate: tothyld | Total healthy yield - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	47470.	23735.		
block stratum	2	1278583.	639291.		
layer.block stratum					
ChemRate	4	5335257.	1333814.		
layer.block.plot stratum					
ChemRate	5	10659990.	2131998.	54.56	<.001
Residual	4	156308.	39077.	0.14	
layer.block.plot.tray stratum					
InocTime	1	685816.	685816.	2.49	0.140
ChemRate.InocTime	5	20046947.	4009389.	14.57	<.001
Residual	12	3303253.	275271.	0.62	
layer.block.plot.tray.half stratum					
InocLev	1	8352466.	8352466.	18.83	<.001
InocLev.ChemRate	5	6281562.	1256312.	2.83	0.038
InocLev.InocTime	1	8646.	8646.	0.02	0.890
InocLev.ChemRate.InocTime	5	1480845.	296169.	0.67	0.652
Residual	24	10645117.	443547.		
Total	71	68282259.			

***** Tables of combined means *****

Variate: tothyld Total healthy yield - all flushes

InocLev	Control	192B1
	7019.	6338.

ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	6092.	7471.	6677.	7047.	6773.	6011.

InocTime	First	Second
	6581.	6776.

InocLev	ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		7074.	7809.	6912.	7232.	6969.	6116.
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		5109.	7132.	6442.	6861.	6576.	5905.

InocLev	InocTime	First	Second
Control		6910.	7127.
192B1		6251.	6424.

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		4896.	7287.
0.0696	letter	1	1
	alphabet	2	2
		7351.	7591.
0.15	letter	2	2
	alphabet	1	1
		6531.	6823.
0.323	letter	2	2
	alphabet	2	2

			7321.	6773.
	0.696	letter	3	3
		alphabet	1	1
			7124.	6421.
	1.5	letter	3	3
		alphabet	2	2
			6262.	5759.

InocLev	ChemRate	InocTime	First	Second
Control	Control	letter	1	1
		alphabet	1	1
			6068.	8081.
	0.0696	letter	1	1
		alphabet	2	2
			7617.	8002.
	0.15	letter	2	2
		alphabet	1	1
			6829.	6996.
	0.323	letter	2	2
		alphabet	2	2
			7370.	7094.
	0.696	letter	3	3
		alphabet	1	1
			7100.	6838.
	1.5	letter	3	3
		alphabet	2	2
			6478.	5754.
192B1	Control	letter	1	1
		alphabet	1	1
			3724.	6494.
	0.0696	letter	1	1
		alphabet	2	2
			7084.	7180.
	0.15	letter	2	2
		alphabet	1	1
			6233.	6651.
	0.323	letter	2	2
		alphabet	2	2
			7271.	6452.
	0.696	letter	3	3
		alphabet	1	1
			7148.	6005.
	1.5	letter	3	3
		alphabet	2	2
			6047.	5763.

*** Standard errors of differences of combined means ***

$t(5\%, 28) = 2.048$

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	157.0	102.2	123.7	290.5
Except when comparing means with the same level(s) of				
alphabet		111.4		293.8
letter.alphabet				384.5
InocLev.alphabet				293.8
d.f	24	4	12	27.95

LSD 5%

$\times 2.048 = 787$

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	199.8	237.3	451.9
Except when comparing means with the same level(s) of			
alphabet		241.4	454.0
letter.alphabet		302.9	489.5
InocTime	222.0		
InocLev.alphabet			454.0
InocLev.letter.alphabet			489.5
alphabet.InocTime		241.4	454.0
letter.alphabet.InocTime			543.8
InocLev.alphabet.InocTime			454.0
df	35.61	15.71	37.51

***** Analysis of variance *****

Variate: totsyld | Total spotted yield - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	675452.	337726.		
block stratum	2	154390.	77195.		
layer.block stratum					
ChemRate	4	487639.	121910.		
layer.block.plot stratum					
ChemRate	5	1228552.	245710.	1.27	0.421
Residual	4	775641.	193910.	2.56	
layer.block.plot.tray stratum					
InocTime	1	1078981.	1078981.	14.22	0.003
ChemRate.InocTime	5	199155.	39831.	0.53	0.753
Residual	12	910250.	75854.	0.89	
layer.block.plot.tray.half stratum					
InocLev	1	4546118.	4546118.	53.19	<.001
InocLev.ChemRate	5	561260.	112252.	1.31	0.292
InocLev.InocTime	1	2511040.	2511040.	29.38	<.001
InocLev.ChemRate.InocTime	5	138907.	27781.	0.33	0.893
Residual	24	2051207.	85467.		
Total	71	15318592.			

***** Tables of combined means *****

Variate: totsyld Total spotted yield - all flushes

InocLev	Control	192B1
	152.	654.

ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	696.	279.	377.	241.	401.	422.

InocTime	First	Second
	280.	525.

InocLev	ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		277.	101.	164.	-2.	102.	267.
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		1116.	457.	590.	485.	701.	576.

InocLev	InocTime	First	Second
Control		216.	87.
192B1		345.	963.

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		564.	828.
0.0696	letter	1	1
	alphabet	2	2
		203.	354.
0.15	letter	2	2
	alphabet	1	1
		195.	560.
0.323	letter	2	2
	alphabet	2	2

			47.	436.
0.696		letter	3	3
		alphabet	1	1
			305.	498.
1.5		letter	3	3
		alphabet	2	2
			368.	475.

InocLev	ChemRate	InocTime	First	Second
Control	Control	letter	1	1
		alphabet	1	1
			420.	134.
	0.0696	letter	1	1
		alphabet	2	2
			168.	33.
	0.15	letter	2	2
		alphabet	1	1
			184.	144.
	0.323	letter	2	2
		alphabet	2	2
			-24.	20.
	0.696	letter	3	3
		alphabet	1	1
			155.	49.
	1.5	letter	3	3
		alphabet	2	2
			392.	143.
192B1	Control	letter	1	1
		alphabet	1	1
			709.	1522.
	0.0696	letter	1	1
		alphabet	2	2
			239.	676.
	0.15	letter	2	2
		alphabet	1	1
			206.	975.
	0.323	letter	2	2
		alphabet	2	2
			117.	853.
	0.696	letter	3	3
		alphabet	1	1
			455.	946.
	1.5	letter	3	3
		alphabet	2	2
			344.	808.

*** Standard errors of differences of combined means ***

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	68.9	83.6	64.9	145.7
Except when comparing means with the same level(s) of				
alphabet		0.8		119.4
letter.alphabet				168.8
InocLev.alphabet				119.4
d.f.	24	4	12	6.32

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	94.7	140.1	219.4
Except when comparing means with the same level(s) of			
alphabet		112.4	202.8
letter.alphabet		159.0	231.9
InocTime	97.4		
InocLev.alphabet			202.8
InocLev.letter.alphabet			231.9
alphabet.InocTime		112.4	202.8
letter.alphabet.InocTime			238.7
InocLev.alphabet.InocTime			202.8
d.f.	33.20	599	11.68

***** Analysis of variance *****

Variate: tpd | Number of dactillium patches - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	1.444	0.722		
block stratum	2	2.028	1.014		
layer.block stratum					
ChemRate	4	64.222	16.056		
layer.block.plot stratum					
ChemRate	5	135.792	27.158	9.80	0.023
Residual	4	11.083	2.771	0.74	
layer.block.plot.tray stratum					
InocTime	1	78.125	78.125	20.99	<.001
ChemRate.InocTime	5	17.458	3.492	0.94	0.491
Residual	12	44.667	3.722	1.76	
layer.block.plot.tray.half stratum					
InocLev	1	260.681	260.681	123.48	<.001
InocLev.ChemRate	5	85.569	17.114	8.11	<.001
InocLev.InocTime	1	115.014	115.014	54.48	<.001
InocLev.ChemRate.InocTime	5	35.569	7.114	3.37	0.019
Residual	24	50.667	2.111		
Total	71	902.319			

***** Tables of combined means *****

Variate: tpd Number of dactillium patches - all flushes

InocLev	Control	192B1
	0.44	4.25

ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	4.67	3.75	2.73	1.27	1.02	0.64

InocTime	First	Second
	1.31	3.39

InocLev	ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		1.25	0.75	0.39	0.02	0.02	0.23
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		8.09	6.75	5.06	2.52	2.02	1.06

InocLev	InocTime	First	Second
Control		0.67	0.22
192B1		1.94	6.56

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		3.75	5.59
0.0696	letter	1	1
	alphabet	2	2
		1.92	5.59
0.15	letter	2	2
	alphabet	1	1
		1.23	4.23
0.323	letter	2	2
	alphabet	2	2

		0.68	1.85	
0.696	letter	3	3	
	alphabet	1	1	
		0.02	2.02	
1.5	letter	3	3	
	alphabet	2	2	
		0.23	1.06	

InocLev Control	ChemRate Control	InocTime	First	Second
		letter	1	1
		alphabet	1	1
			1.75	0.75
	0.0696	letter	1	1
		alphabet	2	2
			1.42	0.09
	0.15	letter	2	2
		alphabet	1	1
			0.56	0.23
	0.323	letter	2	2
		alphabet	2	2
			0.02	0.02
	0.696	letter	3	3
		alphabet	1	1
			0.02	0.02
	1.5	letter	3	3
		alphabet	2	2
			0.23	0.23
192B1	Control	letter	1	1
		alphabet	1	1
			5.75	10.42
	0.0696	letter	1	1
		alphabet	2	2
			2.42	11.09
	0.15	letter	2	2
		alphabet	1	1
			1.89	8.23
	0.323	letter	2	2
		alphabet	2	2
			1.35	3.68
	0.696	letter	3	3
		alphabet	1	1
			0.02	4.02
	1.5	letter	3	3
		alphabet	2	2
			0.23	1.89

First = mycelium inoculated
Second = spore inoculated.

Only data for "First" control presented in text

*** Standard errors of differences of combined means ***

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	0.342	0.517	0.455	0.787
Except when comparing means with the same level(s) of				
alphabet		0.412		0.722
letter.alphabet				0.839
InocLev.alphabet				0.722
df	24	4	12	8.21

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	0.569	0.942	1.261
Except when comparing means with the same level(s) of			
alphabet		0.889	1.222
letter.alphabet		1.114	1.394
InocTime	0.484		
InocLev.alphabet			1.222
InocLev.letter.alphabet			1.394
alphabet.InocTime		0.889	1.222
letter.alphabet.InocTime			1.186
InocLev.alphabet.InocTime			1.222
d.f.			25.39

$t(5\%, 25) = 2.060$

LSD(5%)

$\times 2.060 = 2.87$

D-7

***** Analysis of variance *****

Variate: tad | Area of dactillium patches - all flushes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
layer stratum	2	125846.	62923.		
block stratum	2	69234.	34617.		
layer.block stratum					
ChemRate	4	1185965.	296491.		
layer.block.plot stratum					
ChemRate	5	847823.	169565.	10.57	0.020
Residual	4	64179.	16045.	0.59	
layer.block.plot.tray stratum					
InocTime	1	73089.	73089.	2.69	0.127
ChemRate.InocTime	5	255542.	51108.	1.88	0.172
Residual	12	326327.	27194.	0.78	
layer.block.plot.tray.half stratum					
InocLev	1	409814.	409814.	11.77	0.002
InocLev.ChemRate	5	942862.	188572.	5.41	0.002
InocLev.InocTime	1	12641.	12641.	0.36	0.553
InocLev.ChemRate.InocTime	5	517269.	103454.	2.97	0.032
Residual	24	835894.	34829.		
Total	71	5666484.			

***** Tables of combined means *****

Variate: tad Area of dactillium patches - all flushes

InocLev	Control	192B1
	47.	198.

ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
letter	1	1	2	2	3	3
alphabet	1	2	1	2	1	2
	451.	157.	60.	30.	11.	23.

InocTime	First	Second
	154.	90.

InocLev	ChemRate	Control	0.0696	0.15	0.323	0.696	1.5
Control	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		123.	118.	8.	6.	4.	23.
192B1	letter	1	1	2	2	3	3
	alphabet	1	2	1	2	1	2
		779.	196.	113.	54.	19.	24.

InocLev	InocTime	First	Second
Control		65.	28.
192B1		243.	153.

ChemRate	InocTime	First	Second
Control	letter	1	1
	alphabet	1	1
		610.	292.
0.0696	letter	1	1
	alphabet	2	2
		200.	114.
0.15	letter	2	2
	alphabet	1	1
		50.	71.
0.323	letter	2	2
	alphabet	2	2

		38.	23.	
0.696	letter	3	3	
	alphabet	1	1	
		4.	19.	
1.5	letter	3	3	
	alphabet	2	2	
	.	23.	24.	

InocLev Control	ChemRate Control	InocTime	First	Second
		letter	1	1
		alphabet	1	1
			95.	151.
	0.0696	letter	1	1
		alphabet	2	2
			255.	-20.
	0.15	letter	2	2
		alphabet	1	1
			10.	5.
	0.323	letter	2	2
		alphabet	2	2
			6.	6.
	0.696	letter	3	3
		alphabet	1	1
			4.	4.
	1.5	letter	3	3
		alphabet	2	2
			23.	23.
192B1	Control	letter	1	1
		alphabet	1	1
			1126.	433.
	0.0696	letter	1	1
		alphabet	2	2
			145.	248.
	0.15	letter	2	2
		alphabet	1	1
			90.	136.
	0.323	letter	2	2
		alphabet	2	2
			69.	39.
	0.696	letter	3	3
		alphabet	1	1
			4.	34.
	1.5	letter	3	3
		alphabet	2	2
			23.	25.

First = mycelium inoculated
Second = Spore Inoculated.

Only data for "First" Control presented in text.

*** Standard errors of differences of combined means ***

Table	InocLev	ChemRate	InocTime	InocLev ChemRate
rep.	36	12	36	6
s.e.d.	44.0	58.4	38.9	96.0
Except when comparing means with the same level(s) of				
alphabet		61.4		97.9
letter.alphabet				107.7
InocLev.alphabet				97.9
d.f.	24	4	12	16.53

Table	InocLev InocTime	ChemRate InocTime	InocLev ChemRate InocTime
rep.	18	6	3
s.e.d.	58.7	89.1	139.8
Except when comparing means with the same level(s) of			
alphabet		91.1	141.1
letter.alphabet		95.2 (*)	143.8
InocTime	62.2		
InocLev.alphabet			141.1
InocLev.letter.alphabet			143.8
alphabet.InocTime		91.1	141.1
letter.alphabet.InocTime			152.4 (**)
InocLev.alphabet.InocTime			141.1
d.f.	24	11.02	(**) 24

(*) 12

$t(5\%, 24) = 2.064$

$\times 2.064 = 315$

Appendix IV

Plate 1

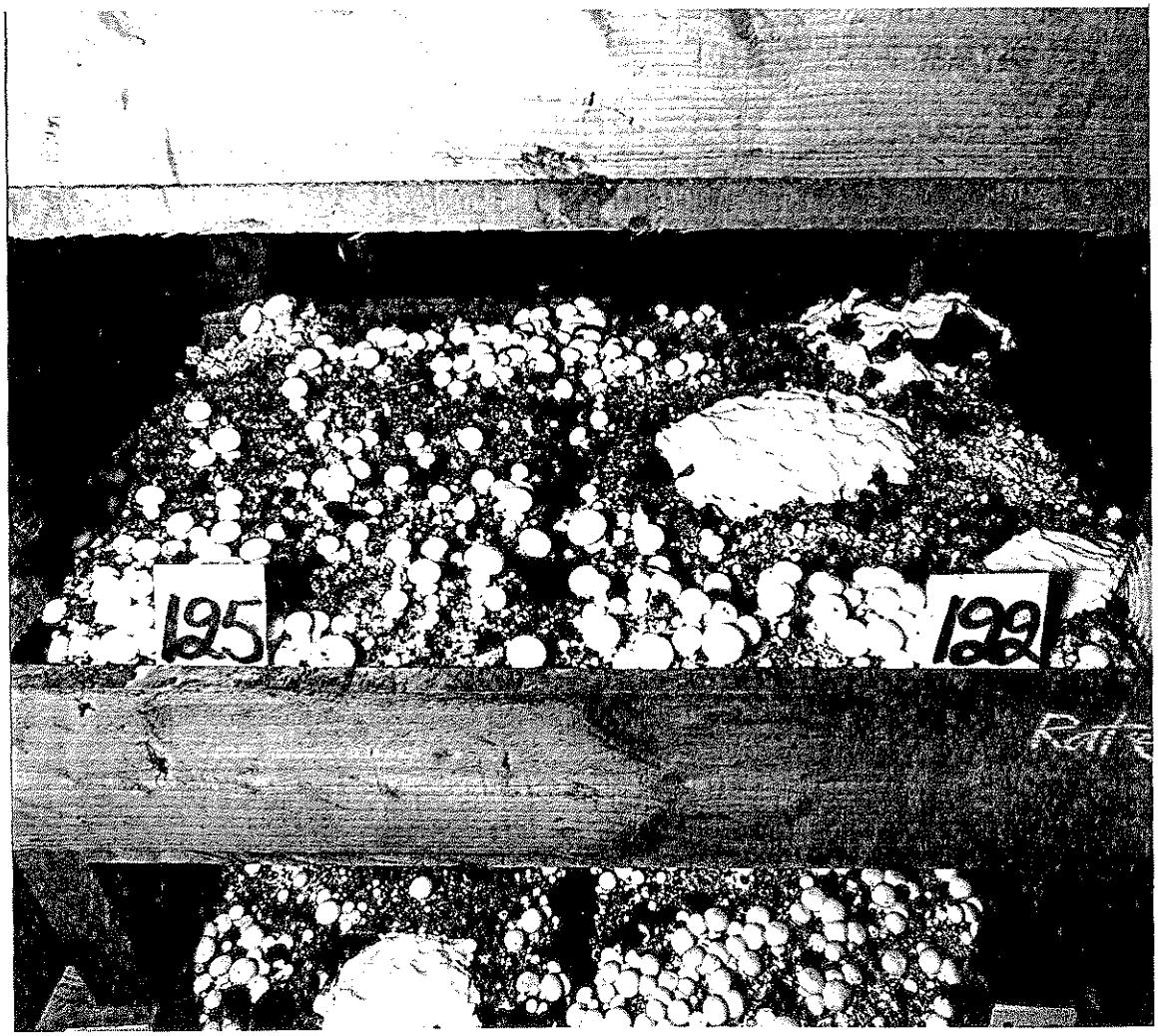

Plate 1: Third flush on untreated casing. Right hand side inoculated with a concentrated spore suspension after the first flush; left hand side uninoculated. Note cobweb growth despite salting, also contamination of left hand side control plot in the far corner.

Appendix IV

Plate 2

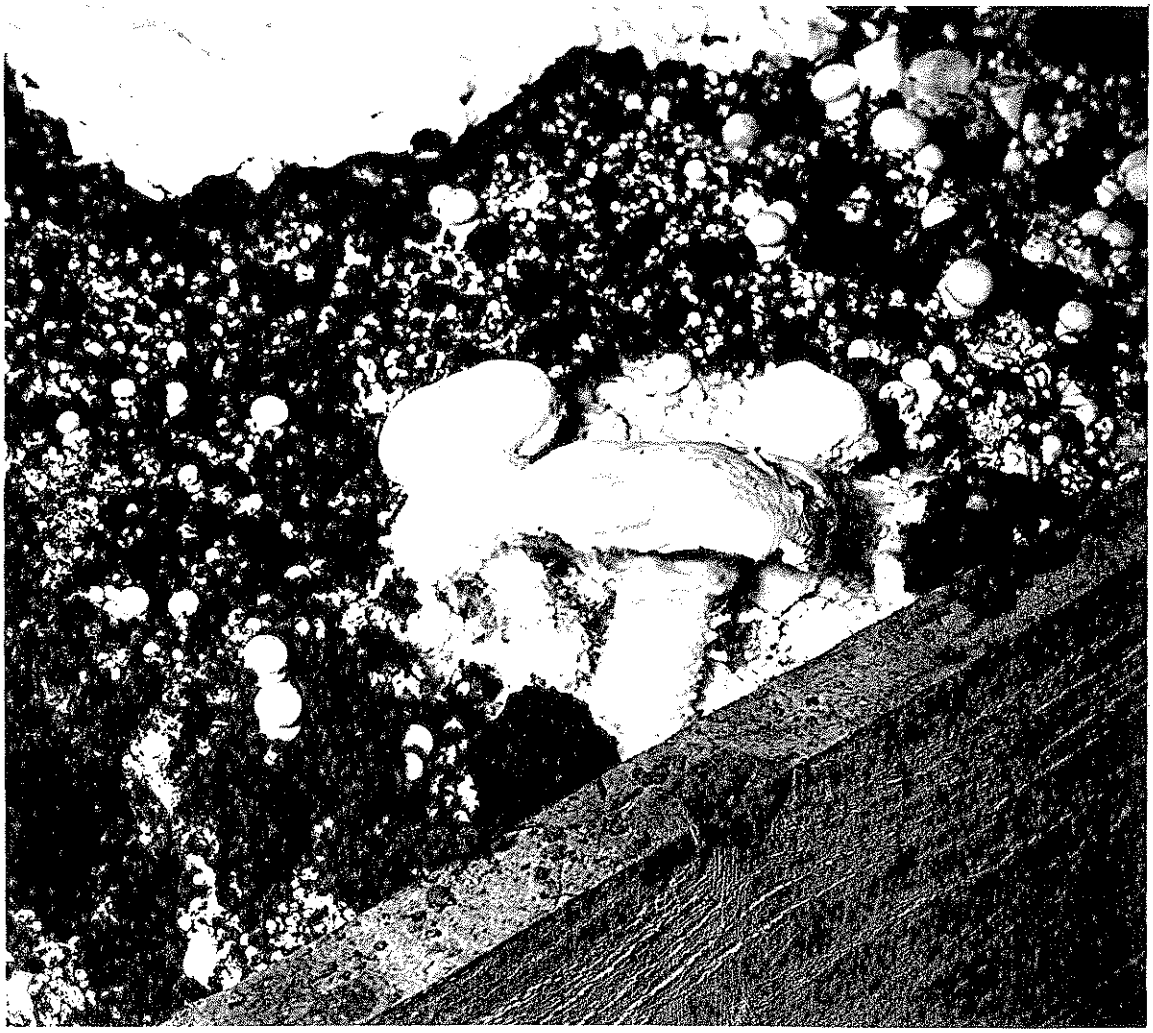

Plate 2: Third flush on casing treated with Chemical D at a rate of 0.15 g a.i./m^2 and inoculated with concentrated spore suspension after the first flush. Note growth of cobweb onto casing from infected mushrooms.

Appendix IV

Plate 3

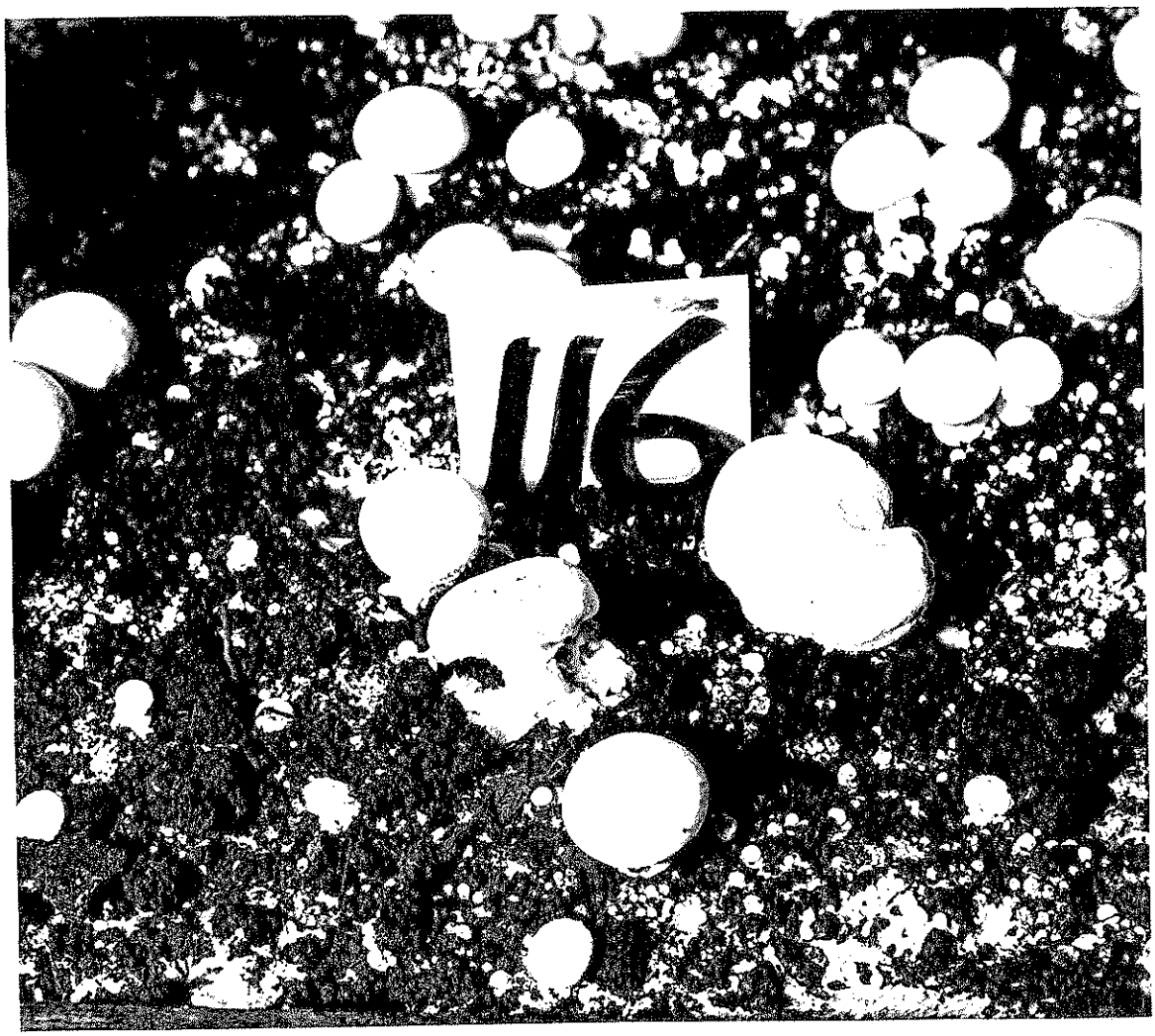

Plate 3: Third flush on casing treated with Chemical D at a rate of 0.7 g a.i./m^2 and inoculated with a concentrated spore suspension after the first flush. Note heavily colonised mushroom to right of centre and restricted growth of cobweb onto casing at base of mushrooms to left of centre.