Project title:	**The fate of Sporgon in mushroom casing**
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Project leaders:	**Helen Grogan and Richard Gaze** **HRI Wellesbourne** **Warwick** **CV35 9EF**
Location:	**HRI Wellesbourne**
Project Co-ordinator:	**Peter Woad**
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CONTENTS

PRACTICAL SECTION FOR GROWERS

Disease control in mushroom cultivation is heavily reliant on Sporgon at present. This is likely to remain so for some time as there are no new chemicals available to growers although research is being carried out to try and find new chemicals. In the meantime, there are worrying reports of disease outbreaks not being controlled by Sporgon but it is often difficult to establish what the underlying reasons for the lack of control are. One aspect to effective disease control is that the chemical is present **where** and **when** it is needed. The aim of this project is to ascertain where the active ingredient in Sporgon, prochloraz manganese, distributes itself following a number of different methods of application.

The results from this work indicated that the concentration of prochloraz manganese in the casing layer varied significantly depending on whether a deep dug black peat/sugar beet lime or a milled sphagnum/chalk casing was used. The active ingredient was almost totally concentrated in the upper layer of the milled sphagnum casing while it was more distributed in the black peat/sugar beet lime casing profile. This difference may have been due to panning of the milled sphagnum casing and further work is necessary to verify this result. In the black peat/sugar beet lime casing, a greater proportion of the active ingredient appeared to remain in the upper layer of casing when the fungicide was applied in 90 litres/100m^2 rather than 180 litres/100m^2. There was some suggestion, however, that the 90 litre treatments may have had a more phytotoxic affect on mushroom yield than the 180 litre treatment. Selected results are presented overleaf.

In general, there was little change in the concentrations of active ingredient in either casing up to Day 14 following the first dose. Following the second dose, however, (applied after the first flush), there was a steady decline in the amount of active ingredient so that by Day 35 less than half the total amount of fungicide applied to the casing remained.

Concentration of Prochloraz-Mn (a.i. in Sporgon) in two casings following two 120 gram doses applied in either 180 litres/100 square meters or 90 litres / 100 square metres. ● = upper half of casing layer; ○ = lower half of casing layer.

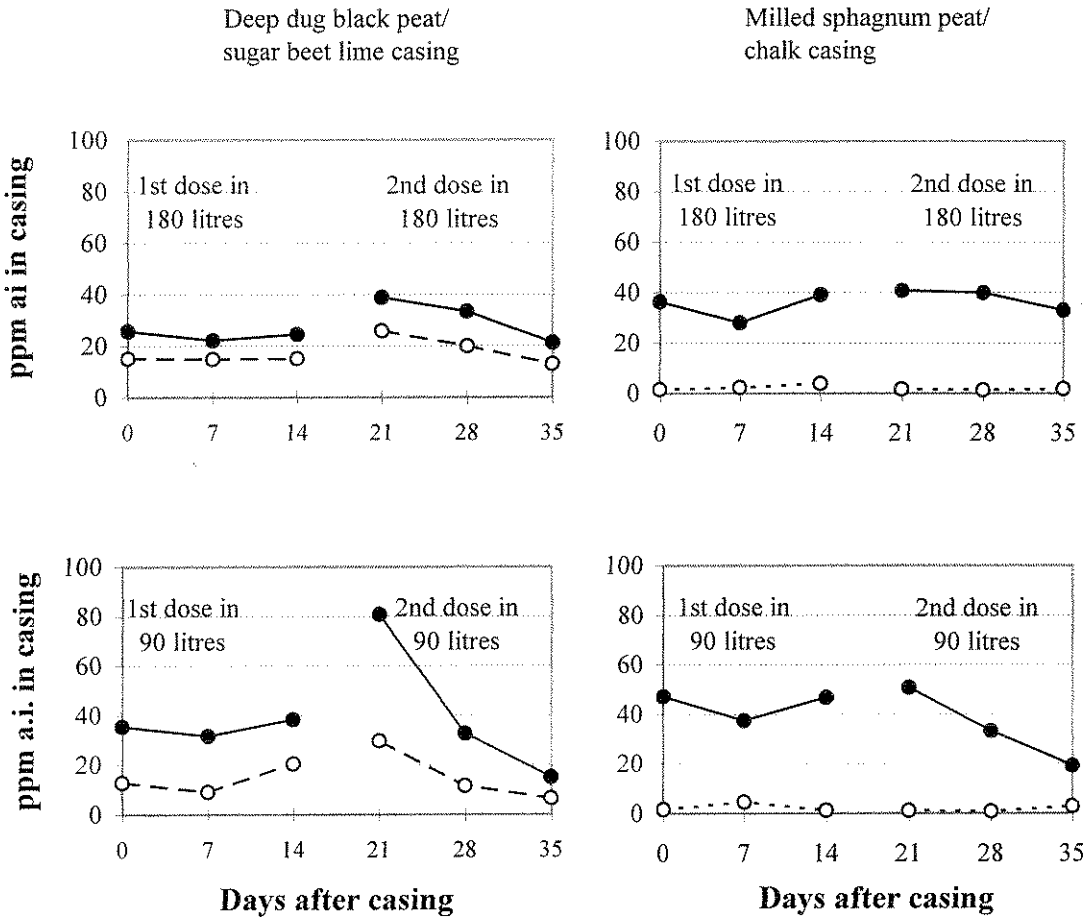
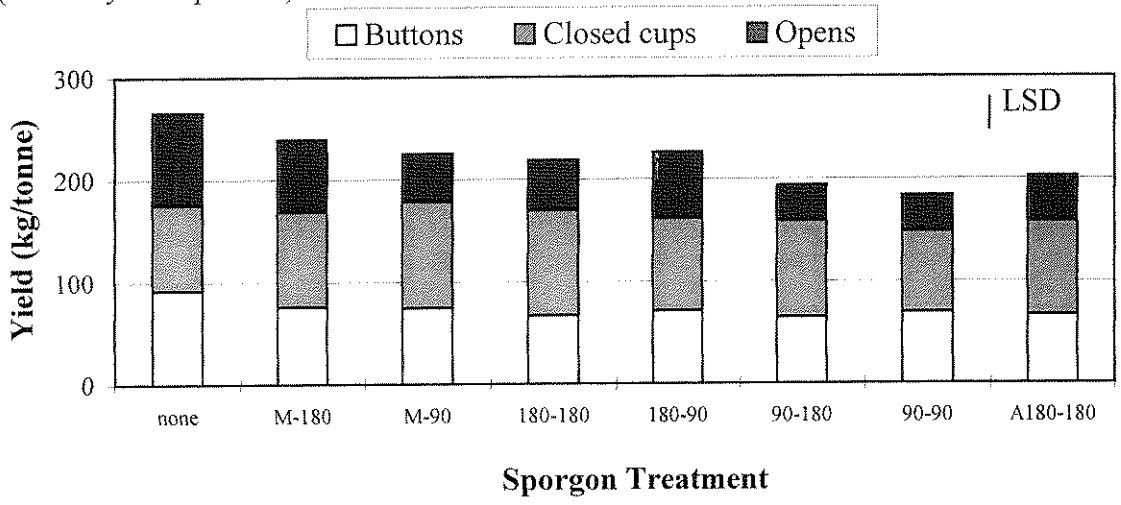


Figure 5. Yield of mushrooms following two 120 g Sporgon doses applied in various ways (M = mixed in; 180 = 180 litres/100m^2; 90 = 90 litres/100m^2; A180 = applied at airing in 180 litres/100m^2); LSD = least significant difference at P = 0.05 (no *Dactylium* present).



EXPERIMENTAL SECTION

THE DISTRIBUTION OF SPORGON IN CASING FOLLOWING DIFFERENT METHODS OF APPLICATION

A. INTRODUCTION

One of the most important and effective crop protection chemicals available to mushroom growers is the product Sporgon which was developed for use on mushrooms in the late 1970's. With many disease organisms/isolates now showing resistance to the benzimidazole group of fungicides, Sporgon is being relied upon heavily, and sometimes it is the only effective chemical available. This complete reliance on a single product is potentially very dangerous as continuous, unbroken use of a crop protection chemical is recognised as a major factor in the emergence of resistant isolates, or isolates with increased/increasing tolerance to the chemical in question.

Serious outbreaks of disease during the mushroom production cycle invariably pose questions on the efficacy of the fungicides being used, particularly when there is an apparent lack of control. In such cases, lack of control is not always due to fungicide resistance *(per se)*. Disease outbreaks can persist, despite fungicide use, if hygiene standards slip. Loss of control can sometimes be related to fungicide resistance such as Benomyl-resistance in *Verticillium* and thiabendazole resistance in some *Dactylium* isolates. However, loss of control has also been related to the disappearance of the fungicide from the casing during cropping. This was shown to be the case when Benomyl no longer controlled *Mycogone* despite the fact that *Mycogone* isolates were still sensitive to the fungicide. Bacterial degradation of the fungicide was shown to occur during cropping. Degradation of carbendazim in casing may also be responsible for some cases where sensitive isolates are not being controlled.

If any fungicide is to be effective it is important that the concentration of active ingredient in the casing is at a level which will inhibit susceptible pathogens. While there is strong evidence to suggest that carbendazim disappears from casing as the crop progresses, little is known about what happens to prochloraz manganese, active ingredient in Sporgon, after it has been applied. This information is crucial before any inferences can be made about the

role of fungicide resistance where there is an apparent loss of control.

Many pathogen isolates demonstrate an ability to tolerate prochloraz-manganese at low concentrations under laboratory conditions. It is important to establish whether these concentrations are achieved in the casing layer. The label recommendations for the use of Sporgon are quite broad and are summarised as follows:

- **Disease anticipated prior to, or during first flush: (1 or 2 x 120 g doses)**

  (1) Incorporate 120 grams Sporgon/100 m^2 into the casing mix.

  or

  (2) Apply 120 g Sporgon/100 m^2 in up to 180 litres in the first watering after casing.

  (3) A second application of (2) can be applied if necessary between the first and second, or second and third flushes.

- **Disease anticipated in the second flush: (3 x 60 g doses)**

  (4) Apply 60 g Sporgon/100 m^2/180 litres 7 days after casing - last watering before airing - and after the first and third flushes.

- **Disease anticipated in third or later flushes: (2 x 120 g doses)**

  (5) Apply 120 g Sporgon/100 m^2/180 litres between 7 and 9 days after casing (pre-airing) and again between second and third flushes.

(In addition to the above, a single 240 g/100 m^2 dose is used in the Netherlands but this is not approved for use in the UK).

The label specifies that the Sporgon can be applied in **'up to 180 litres'** and in general this volume, or near to it, would be used by most growers.

If a 120 gram dose of Sporgon is evenly distributed throughout a 50 mm layer of casing then in theory the concentration of active ingredient should be in the region of 15 to 20 parts per million (ppm). The aim of this project is to ascertain: (1) where the active ingredient in

Sporgon, prochloraz manganese, distributes itself in the casing and (2) what the concentration of active ingredient is in the casing following a number of different methods of applications. A related project (M26a) will examine whether or not a *Dactylium* isolate, partially-resistant to prochloraz in the laboratory, is controlled by Sporgon in a small-scale controlled inoculation experiment using Sporgon application treatments similar to those in this study.

B. MATERIALS AND METHODS

Fungicide

Commercial grade Sporgon 50WP was used throughout these experiments.

Compost and crop set up

Phase II compost produced by HRI Mushroom Unit was used in the experiment and compost analyses are presented in Appendix I. The compost is a straw and chicken manure based compost with Sporavite added. Spawn A12 (Sylvan) was used at the recommended rate and Diazinon insecticide was incorporated at spawning at a rate of 1.1 kg/tonne of compost. Fifty kg of compost was filled into 0.6 m^2 wooden trays. Compost was spawn run at 25°C for 17 days after which time it was cased, case-run and cropped for 3 flushes according to standard practises at HRI Mushroom Unit facility.

Casing

Two types of casing were used in the experiment as follows:

(a) A deep dug black peat/sugar beet lime mix. Commercially available from Harte Peat in 50 litre bags (No. 8).

(b) A milled sphagnum peat/chalk mix. One bale of Irish Moss Peat (horticultural grade) was mixed with 2½ bags of chalk and 50 litres of water. This was sufficient for about 5 m^2 of bed area.

Dimlin insecticide was mixed into both casing types at a rate of 4 g/m^2 of bed area.

Sporgon application

Seven different Sporgon treatments plus an untreated control were applied to both casing types. All treatments consisted of 2 x 120 g doses of which the second dose was applied between first and second flush. The first dose was applied either by incorporation into the casing mix, applied in the first watering after casing or applied in last watering before airing. The volume of water used for both the first and second dose was either 90 litres or 180 litres. The treatments are summarised in Table 1.

Table 1. Summary of 2 x 120 gram Sporgon treatments applied to two casings.

Treatment	**1st Dose (Variable)**	**2nd Dose (After 1st Flush)**
Mixed - 180	Incorporated into casing mix	Drenched on in 180 litres
Mixed - 90	Incorporated into casing mix	Drenched on in 90 litres
180 - 180	1st watering in 180 litres	Drenched on in 180 litres
180 - 190	1st watering in 180 litres	Drenched on in 90 litres
90 - 180	1st watering in 90 litres	Drenched on in 180 litres
90 - 90	1st watering in 90 litres	Drenched on in 90 litres
A - 180	Last watering before airing in 180 litres	Drenched on in 180 litres
None - none	No Sporgon	No Sporgon

Incorporation into casing

The correct amount of Sporgon for each casing mix was added to 5 litres of water and incorporated into the casing as it was being mixed.

First Watering

The 120 g dose of Sporgon applied in the first watering was mixed in either 90 or 180 litres of water. The fungicide solution was then drenched onto the cased mushroom trays using a hand held lance with a No. 2 Rose in conjunction with a 0.5 HP self-priming electric pump. The mushroom trays used had a surface area of 0.6 m^2 which required a fungicide dose of either 1.1 litres/tray (equal to 120 g/180 litres/100 m^2) or 0.55 litres/tray (equal to 120 g/90 litres/100 m^2).

Airing

The 120 g dose of Sporgon applied in the last watering before airing was mixed in either 90 litres or 180 litres of water. This was then drenched on at a rate of either 1.1 litre/tray or 0.55 litres/tray as described in the previous section.

Second Sporgon Dose

The second 120 g dose of Sporgon was drenched onto all trays between the first and second flush and was applied in either 90 or 180 litres as described earlier.

Determination of prochloraz-manganese concentration

Samples of casing were removed at weekly intervals in order to determine the concentration of prochloraz-manganese. Five x 26 mm (1 inch) cores of casing, 5 cm long, were extracted from the left and right side of each tray on days, 0 and 7. The number of cores was reduced from 5 to 3 on Days, 14, 21, 28 and 35. The cores were split in half transversely to give 'top' and 'bottom' sub-samples which were frozen (-15°C) until analysed.

After defrosting the samples were weighed and mixed. Dry weights were determined by drying samples of casing to constant mass in a microwave oven. Residues of prochloraz were extracted from casing (20 g) with methanol (50 ml, hplc grade) by tumbling end-over-end for 1 hour. After filtering through filter paper (Whatman No. 5) the extracts were analysed by high performance liquid chromatography using a Spectra Physics system. A 25 cm Spherisorb C_8 column was used with a mobile phase of acetonitrile:water:methanol (15:20:65, 1.5 ml/min) which gave a retention time for prochloraz of 4.1 min. Detection

was by a Cecil variable wavelength uv detector set at 220 nm which gave a linear response in the range of 0.5-20 ug/ml. Analytical efficiencies were assessed by fortifying untreated casing with prochloraz or sporgon at levels of 4-176 mg a.i./kg dry casing. Recoveries were always within the range of 90-110% and results were not corrected for analytical loss. Prochloraz concentrations were calculated and expressed as mg of prochloraz manganese per kg fresh weight of casing (ppm). Mean concentrations of prochloraz manganese were calculated for the top and bottom halves of the casing layer for each tray, and these means were then used to calculate treatment means.

Phytotoxicity

Mushroom yield was recorded over 3 flushes for all treatments and compared to a non-Sporgon treated control in order to ascertain any phytotoxic effects.

C. RESULTS

Concentration of prochloraz manganese in the casing profile

The concentration of prochloraz manganese in the casing layer varied significantly depending on which type of casing was used and how the Sporgon had been applied. These results are summarised in Figures 1 and 2. The actual concentrations of active ingredient were higher than expected on occasions but this was largely due to over-delivery of fungicide to some treatments. In order to compare treatments more accurately therefore the proportion of active ingredient in the top half of the casing layer was expressed as a percentage of the total amount of active ingredient recovered from both the top and bottom halves of the casing layer and this information is presented in Figures 3 and 4.

In general, there was little change in the concentrations of active ingredient in either casing up to Day 14 following the first dose. Following the second dose, however, (applied after the first flush), there was a steady decline in the amount of active ingredient so that by Day

Figure 1. Concentration of Prochloraz-Mn (a.i. in Sporgon) in the top and bottom layers of a deep-dug Black Peat/Sugar beet lime casing following two 120 g doses of Sporgon applied in various ways.

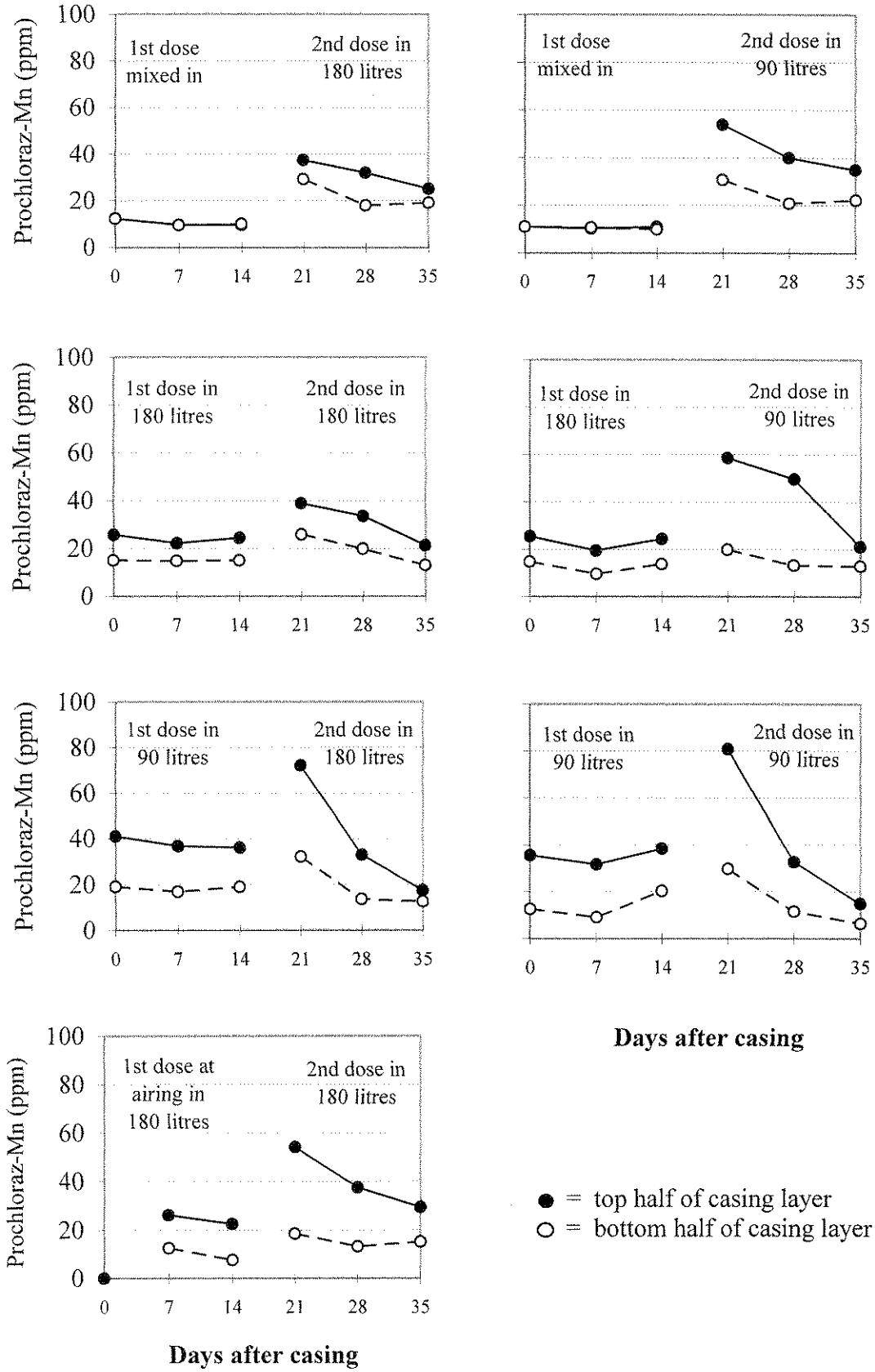


Figure 2. Concentration of Prochloraz-Mn (a.i. in Sporgon) in the top and bottom layers of a milled Sphagnum Peat/Chalk casing following two 120 g doses of Sporgon applied in various ways.

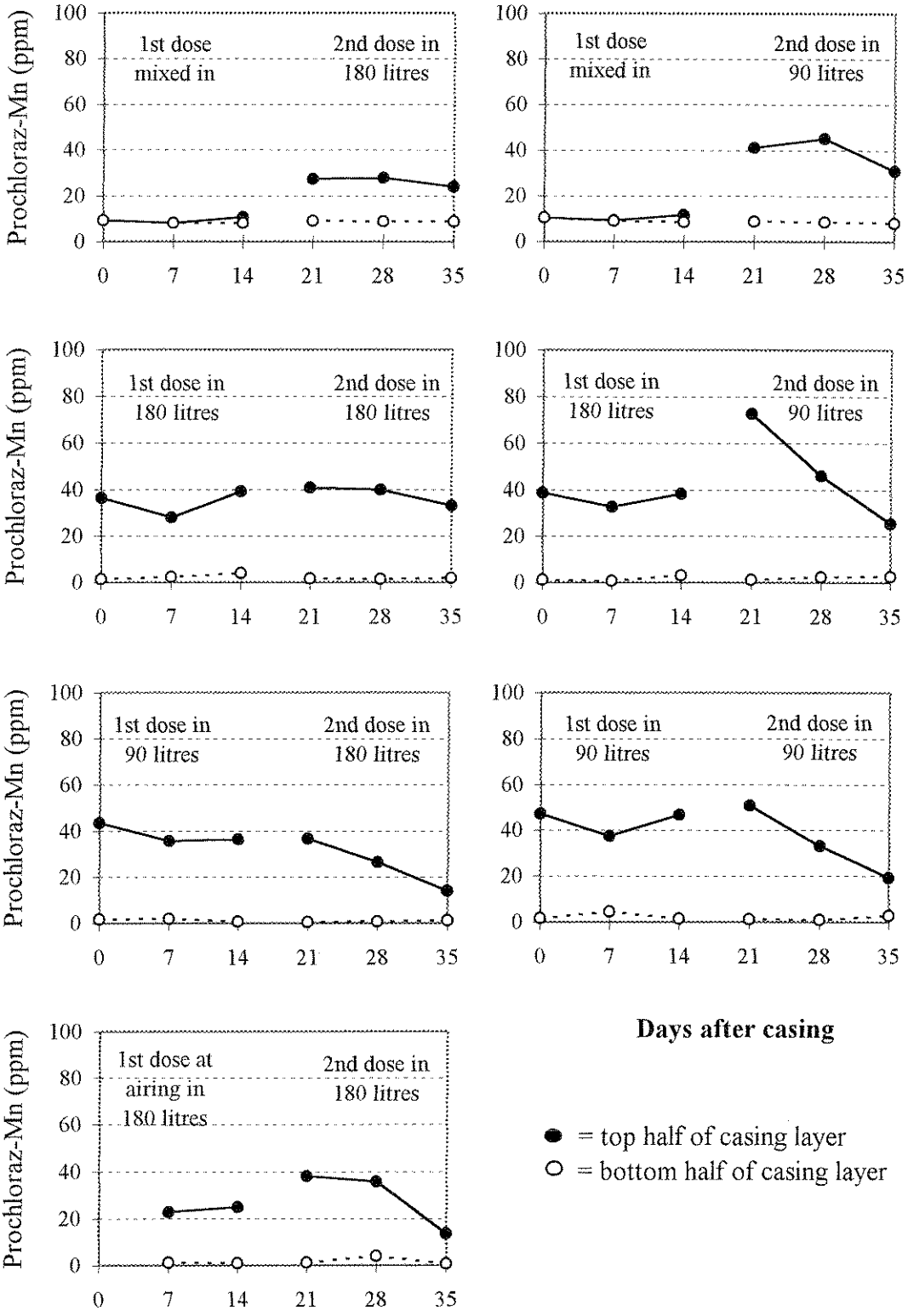


Figure 3. Percentage of active ingredient in top half of a deep dug black peat & sugar beet lime casing following two 120 g doses of Sporgon applied in various ways (M = mixed in; 180 = 180 litres/100m^2; 90 = 90 litres/100m^2; A180 = applied at airing in 180 litres); data are means ± standard error.

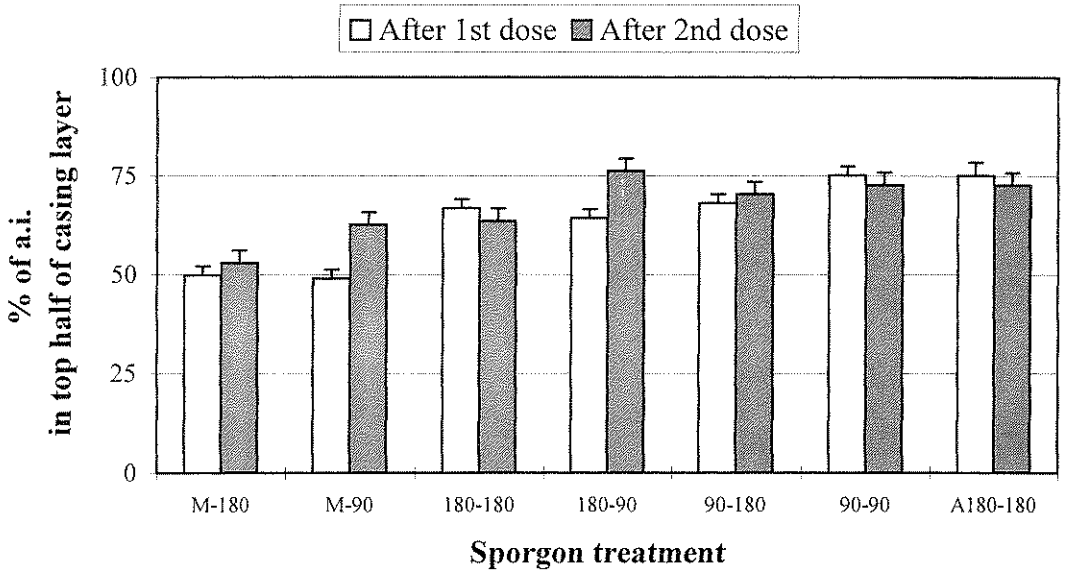
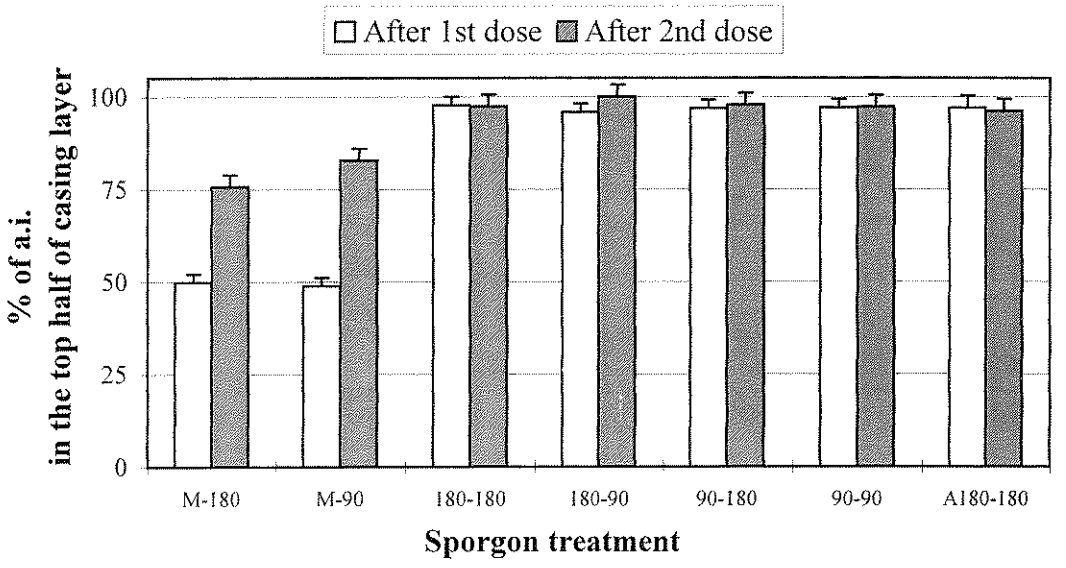


Figure 4. Percentage of active ingredient in top half of a milled sphagnum peat & chalk casing following two 120 g doses of Sporgon applied in various ways (M = mixed in; 180 = 180 litres/100m^2; 90 = 90 litres/100m^2; A180 = applied at airing in 180 litres); data are means ± standard error.



35 less than half the total amount of fungicide applied to the casing remained.

Casing effects

When a deep dug Black Peat/Sugar beet lime casing was used, in the region of 60-75% of the active ingredient was present in the top half of the casing layer following drench applications with 25-40% percolating down to the bottom half; when the chemical was incorporated into the casing during casing preparation however there was an even distribution of the active ingredient within the casing profile as might be expected (Figures 1 and 3).

When a milled Sphagnum peat/chalk casing was used 90-100% of the active ingredient was present in top half of the casing layer following drench applications with less than 10% percolating down to the bottom half. As expected, however, there was an even distribution in the casing profile when the fungicide was incorporated during casing preparation (Figures 2 and 4).

Fungicide application effects

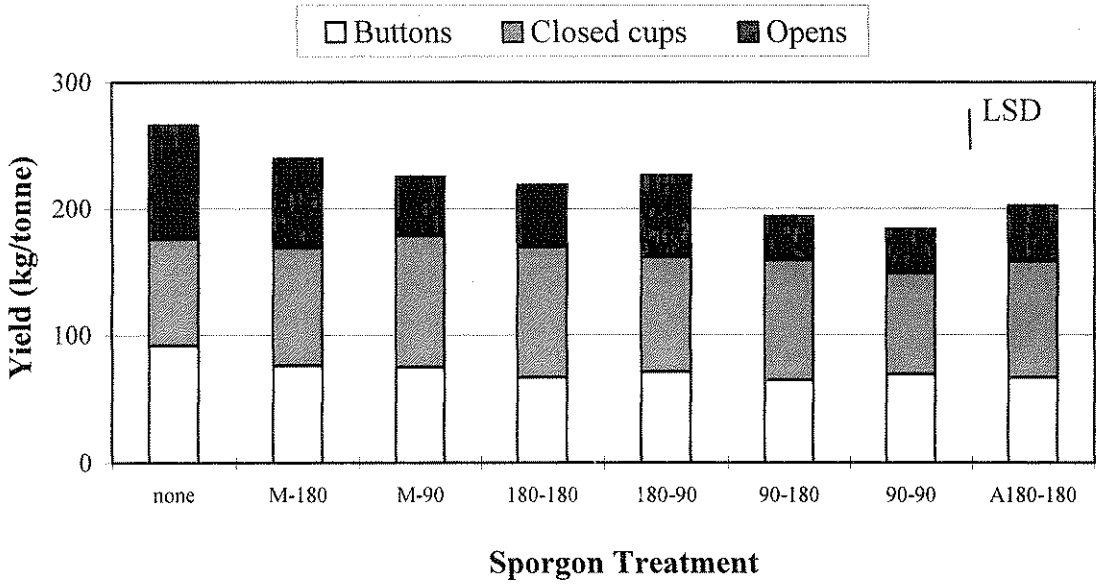
The distribution of prochloraz manganese in the casing profile varied significantly depending on how the fungicide was applied. As mentioned earlier when the first dose of the product was incorporated, there was an even distribution of the active ingredient, as might be expected, irrespective of casing type. When the first dose was drenched on in either 180 or 90 litres/100 m^2 there were no differences between application treatments for the Sphagnum peat/chalk casing as almost all the active ingredient remained in the top half of the casing layer. In the black peat/sugar beet lime casing however, 64-67% of the active ingredient remained in the top half when the drench volume was 180 litres/100 m^2 compared with 68-75% when the drench volume was 90 litres//100 m^2 (lease significant difference at $P = 0.05$ is 4.9%). This concentration of the active ingredient in the top layer following a 90 litre/100 m^2 drench is also observed following the second dose (compare treatments M-180, M-90 and 180-180 with 180-90 in Figure 3. When casing was drenched with Sporgon at airing the proportion of active ingredient in the top half of the casing layer seemed to be higher than for a similar drench applied just after casing.

Yield and phytotoxicity

The average yield of mushrooms was higher from the black peat/sugar beet lime casing compared with the Sphagnum peat/chalk casing at 244 kg/tonne compared with 192 kg/tonne respectively (least significant difference at P = 0.05 is 11.7 kg). There was a significant reduction in yield from all Sporgon treatments compared with the controls (Figure 5). Some of this was due to a higher yield of open mushrooms from the untreated crop which was ready for picking at an earlier stage. Nonetheless there appears to have been a significant reduction in yield when the first Sporgon dose was applied in 90 litres compared with other Sporgon application treatments apart from the airing treatment which had the third lowest yield.

There was no enhanced yield reduction in the Sphagnum/chalk cased treatments due to higher concentrations of prochloraz manganese in the upper layer of casing.

Figure 5. Yield of mushrooms following two 120 g Sporgon doses applied in various ways (M = mixed in; 180 = 180 litres/100m^2; 90 = 90 litres/100m^2; A180 = applied at airing in 180 litres/100m^2); LSD = least significant difference at P = 0.05 (no *Dactylium* present).



D. DISCUSSION

When Sporgon is incorporated into casing during casing preparation, the active ingredient is uniformly distributed throughout the depth of the casing, irrespective of casing type, until the second dose is drenched on. However, if the first dose is drenched on, the distribution of active ingredient can vary significantly depending on the casing type used, and the volume of water (per $100m^2$ of bed area) in which the fungicide is mixed. In a deep dug Black peat/sugar beet lime casing mix, 64-67% of the active ingredient was found in the top of the casing layer relative to the bottom when a 120 g dose was applied in 180 litres of water/$100m^2$. This rose to 68-75% when the same dose was applied in 90 litres/$100m^2$. However, when a Milled Sphagnum peat/chalk casing mix was used 90 - 100% of the active ingredient remained in the top of the casing profile irrespective of the volume of water used. This is a very significant difference between the two casing types in terms of the distribution of the active ingredient within the casing profile and it may explain why there have been so many reports of prochloraz manganese not controlling disease outbreaks in recent years. These reports seem to have increased alongside the increasing widespread use of deep dug Black Peat casing mixes. These new casing ingredients have different properties to the milled sphagnum casings which were almost universally used in the UK up until recently (HDC reports M20a and M20b). It is not inconceivable therefore that fungicides may also behave differently in them. The Milled Sphagnum casing used in these experiments panned somewhat during the watering of the crop so that the accumulation of the active ingredient in the surface layer may simply be a reflection of this; the longer it takes for the fungicide drench to percolate into the casing, the greater the likelihood that the active ingredient binds or settles out at the surface. This is an area of work worthy of further study as such information would be invaluable to our understanding of how we might best apply fungicides for maximum disease control.

There was some indication that applying prochloraz manganese in a 90 litre/$100m^2$ volume of water may had a phytotoxic effect on yield. Some of the yield difference in treated plots was due to a greater proportion of open mushrooms being picked from the control crop which was a little in advance of the fungicide treated crop. It would be useful to establish a more accurate assessment of the potential yield reduction associated with this fungicide treatment

The average concentrations of active ingredient recorded during this experiment varied from treatment to treatment. In theory they should have been the same - in the region of 17-20 ppm per dose for the whole of the casing depth; i.e.(concentration in the top + concentration in the bottom)/2. Some difficulties were experienced in applying precise volumes to the experimental trays so that some treatments received a slightly higher volume than was desired. Although the mean concentration of fungicide was therefore higher in some treatments than it should have been, the subsequent distribution of that fungicide within the casing profile would, nonetheless, have reflected the application method. Similarly, the concentration of fungicide following incorporation of Sporgon at casing preparation was less than expected; 9-12 ppm instead of 17-20 ppm. A review of our incorporation technique is required to see where the fungicide is being lost. A possible explanation is the settling-out of the fungicide in the mixing container, prior to incorporation. This variability emphasises the need to be very aware of the importance of good fungicide-application technique so as to ensure accurate dosing. Regular checking of fungicide concentration in the casing should be done in order to pick up any tendency towards miss-dosing for one reason or another.

E. CONCLUSIONS

1. The concentration of active ingredient following incorporation of Sporgon into casing at casing preparation is uniform throughout the profile and should be in the region of 17-20ppm if properly mixed.

2. If Sporgon is drenched onto a milled sphagnum/chalk casing, 90-100% of the active ingredient appears to be concentrated at the surface. This result needs to be verified in view of its significance.

3. If Sporgon is drenched onto a deep dug black peat/sugar beet lime casing in 180 litres/100m^2, 64-67% of the active ingredient is to be found in the top half of the casing layer with the remainder being further down the casing profile. If the same dose is applied in 90 litres/100m^2 then 68-75% of the active ingredient will be found in the top. For a single 120 g dose this should translate into concentrations of 26-27 ppm, and 27-30 ppm, respectively.

4. Irrespective of casing type or application method, the concentration of prochloraz manganese in the casing remains stable in the first 14 days after casing.

5. Irrespective of casing type or application method, the concentration of prochloraz manganese in casing decreases steadily during the cropping period (after two 120 g doses) to less than half the total amount applied. In view of this, it is not recommendable to apply only one 120 g dose.

F. RECOMMENDATIONS

- It would seem **essential** to apply **both** Sporgon applications not just the initial one as is often the case.

- There would seem to be advantages in applying the fungicide in less than the maximum permitted quantity of water suggested on the label. EXERCISE CAUTION. There is clearly a danger of unacceptable phytotoxicity and growers should proceed carefully in this area. Project M30 will explore this further but in the meantime if water quantity is to be reduced it should be done progressively, in stages, and assessed for phytotoxic effect.

- It should be recognised that at the moment drenching at the last watering before ärg and **after the 1st flush** is not a label recommendation. The label states that the second dose should be applied **after the 2nd flush**.

- To minimise the possibility that lower than expected levels of prochloraz manganese occur in the casing care should be taken to ensure that the correct dose is used in the correct volume of water and applied to the appropriate bed area. Care should be taken to ensure adequate agitation in the spray tank to prevent fungicide being precipitated before application. Care should also be taken to avoid excess spillage of the drench onto the floor.

F. ACKNOWLEDGEMENTS

We would like to thank Andrew Jukes, HRI Wellesbourne, for analysing the casing samples for prochloraz manganese.