Project Title:	Studies on fungicides in mushroom casing in relation to disease control
Project Number:	M 41a
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First Interim Report:	1st July 2003
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Date Project commenced:	1st October 2002
Date Project Completed:	30th September 2005
Key words:	Prochloraz, Sporgon 50WP, *Verticillium fungicola, Agaricus bisporus*, mushroom

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PRACTICAL SECTION FOR GROWERS

Headline

Sporgon 50WP still gives good control of dry bubble disease, provided it is applied well and disease levels are kept in check with good disease management.

Background and expected deliverables

The fungicide Sporgon 50WP is the only chemical available for the control of dry bubble disease of mushrooms, caused by the pathogen *Verticillium fungicola*. Recent HDC funded work (project report M 14b) showed that in Britain over 60% of isolates tested were moderately tolerant of Sporgon 50WP. Cropping experiments have shown that Sporgon 50WP still gives good control of these isolates (project report M 14c) but analysis of casing throughout the cropping period showed that prochloraz levels (active ingredient in Sporgon 50WP) decreased with time. Thus control of dry bubble with Sporgon 50WP in later flushes may be less effective. Good disease management is therefore still very important in order to keep this disease under control. Expected deliverables from this project include:

1. knowledge on the activity of Sporgon 50WP in mushroom casing over time
2. knowledge on the key factors affecting Sporgon 50WP activity
3. knowledge on the most effective timing of Sporgon 50WP applications for the control of dry bubble disease
4. knowledge on the key factors influencing the development, spread and control of dry bubble disease

Summary of the project and main conclusions

This project will study the factors that affect the activity and persistence of the fungicide prochloraz (Sporgon 50WP) in mushroom casing. It will investigate how the timing of applications, the dose applied, and persistence of the chemical interact to determine the level of control achieved of the important pathogen *Verticillium fungicola*. Factors such as casing ingredients, casing microbiology (including *Agaricus* activity) and

environmental conditions such as temperature and moisture content will be studied to determine their role in controlling fungicide activity and persistence. Studies will be carried out to investigate ways to optimise fungicidal activity, and modifications to current management techniques will be studied in casing microcosms to determine whether they may be effective in commercial practice. Any positive results arising from laboratory studies will be tested on mushroom crops.

This report covers the first nine months of a three-year PhD project. In that time we have completed several small-scale experiments looking at:

- the degradation of prochloraz (a.i. in Sporgon 50WP) in casing soil under laboratory conditions
- the effect of *Agaricus bisporus* on prochloraz degradation in casing in a small scale study
- the effect of casing moisture content on the recovery of prochloraz from casing

In addition four essays were written by the key worker on the project (PhD student Mr. Georgios Papadopoulos), as required by Reading University, to gather together the relevant literature relating to the subject areas of the project.

The main conclusions of the first nine months work are:

- the introduction of the SPE cartridges in the analytical method improved the separation of prochloraz residues
- in all cases, prochloraz degraded slowly
- the presence of *A. bisporus* in the casing did not affect the degradation of prochloraz significantly (but a larger scale trial is in progress)
- casing moisture content was found to have no significant effect on the recovery of prochloraz residues

Financial benefits

It is important to maximise the disease-controlling effects of costly fungicides by identifying the important factors that influence efficacy. This project aims to deliver knowledge that will help to identify the most effective way to use Sporgon 50WP to control dry bubble disease.

Action points for growers

At this point in time there are no recommendations for change of practice as a result of this project, though we aim to be in a position to do so when the project is completed. Until such time the best practice for the control of dry bubble can be summarised as follows:

- Ensure Sporgon 50WP is applied correctly and evenly at the rates specified on the label
- Do not dry-sweep any areas or raise dust, ESPECIALLY when crops are being cased
- Ensure filters for fresh air at airing are in good condition
- Ensure diseasing-teams identify and treat dry bubble pieces quickly BEFORE any watering is done
- Do not dry-sweep cropping rooms, especially when there is disease in a crop
- Terminate badly-infected third flushes early to minimise the build up of background disease levels on the site
- Keep fly-numbers down

SCIENCE SECTION

M 41a: Studies on fungicides in mushroom casing in relation to disease control

1 Nine month report for Reading University

1.1 Introduction

Control of dry bubble disease (*V. fungicola*) relies heavily on prochloraz (Sporgon 50 WP) which is an imidazole demethylation inhibitor (DMI) fungicide, with protective and eradicant action. Recent HDC funded work (M14C, M30) suggested limited persistence of prochloraz in mushroom casing resulting in low concentrations when threats of disease are high.

1.2 Objectives

- To determine the factors affecting the persistence and activity of prochloraz in mushroom casing
- To investigate the effects of casing ingredients (brown peat, black peat, sugar beet lime) and casing micro-organisms (including *A. bisporus*) on prochloraz activity and degradation
- To examine the potential to manipulate certain factors in order to maximise prochloraz activity throughout the duration of the crop

1.3 Materials and methods

- Prochloraz was applied to mushroom casing in a series of small scale experiments using glass jars, 4cm high columns and spawned compost
- Fungicide residues were extracted with acetonitrile and measured by HPLC. A standard analytical method for the detection of prochloraz residues was established by introducing Solid Phase Extraction (SPE) cartridges in the extraction process improving the overall separation of prochloraz residues by the HPLC
- Prochloraz was applied as Sporgon® 50 WP on Day 3 and Day 21 to give concentration of 15ppm to each treatment (equivalent to 120g/100m^2)

- Casing material with moisture levels from 50-80% was incubated with 15ppm prochloraz for 24h in order to determine the effect of moisture content to the recoveries obtained.

1.4 Results

- Prochloraz was relatively stable with a slow degradation process and a half-life (DT_{50}) always above 40 days
- The recoveries obtained from casing colonised with *A. bisporus* were not significantly different from control pots with no *A. bisporus*
- The highest recovery was obtained from the driest (50% moisture) casing and the lowest from casing containing 60% water

1.5 Conclusions

- The introduction of the SPE cartridges in the analytical method improved the separation of prochloraz residues
- In all cases, prochloraz degraded slowly
- The presence of *A. bisporus* in the casing did not affect the degradation of prochloraz significantly in a small-scale experiment
- Water content was found to have no significant effect to the recovery of prochloraz residues

1.6 Current-Future work

- Further investigation of prochloraz degradation processes in large-scale experiments on the HRI-mushroom unit
- Investigations of the response of *V. fungicola* spores to increasing concentrations of prochloraz
- Disease management studies combining the dry bubble pathogen (*V. fungicola*) and the chemical (prochloraz), focusing on factors such as:- time of fungicide application, fungicide dose rate, quantity of disease inoculum applied and symptom expression.

2 Essay 1. The cultivation of *Agaricus bisporus*

2.1 Introduction

The cultivated mushroom *Agaricus bisporus* (Lange) Sing., is a Heterobasiodiomycetes fungus, which belongs to *Agaricale*s order. The botanical classification of the "button mushroom" or the " white cultivated mushroom" as this edible fungus is commonly known, was confirmed for first time by the International Botanical Congress in 1954 (Atkins 1974). There are 14 more genera of Basiodiomycetes with gastronomic appeal but for which complete cultivation methods are still unavailable (Chang and Miles 1989).
The first evidence of commercial production of mushrooms can be traced in Europe as early as 300 years ago but it was not until the end of second world war when the mushroom industry was boosted in countries like France, Holland and the UK as well the United States and Taiwan (Flegg, Spencer and Wood 1985). The world production of mushroom continues to increase and in 1991 reached 4.27 million of metric tones (Chang and Miles 1997). However, the years 1995-1999 there was a 39% reduction in the mushroom production area in UK due to a variety of reasons including increasing costs, competition from imported mushrooms and presence of virus X (Garthwaite, Thomas 1999).

2.2 Morphological Description

A simple morphological definition of a "mushroom" is given by Chang and Miles (1989) as a fleshy, spore-bearing fungal structure that is responsible for the production and dissemination of spores in numbers sufficient to assure the propagation of the species under a number of diverse environmental conditions. Most commonly, the dissemination of spores is airborne and the fruiting body is above the ground but in a few species the mushroom can be also formed underground. The spores develop root-like threads, called hyphae, in order to search for food sources. Unlike the higher plants, fungi contain no chlorophyll derive their energy by breaking down carbonhydrates realising CO_2 and many of those, which form mushrooms, are saprophytes, living on dead matter. Structural parts of the cultivated mushroom include the stalk (or "stipe"), the cap (or "pileus") and delicate membrane (or "veil") which covers the cap from below. At late growth stages, veil breaks up and numerous gills ("lamellae") develop under the cap. Spores are produced on the gills and are disseminated by wind and the life cycle goes on. The common mushroom produces around a million spores a minute for several days and it takes forty-five seconds in still air for each spore to reach the ground (Atkins 1972).

2.3 Growing Mushrooms

The nature and the requirements of the *Agaricus bisporus* cultivation are such that a variety of cropping methods and growing systems have been developed throughout the world. Cultivation techniques may vary in geographic areas but also vary in time as the methods are constantly developed from the primitive ones a century ago, to the computer software controlled growing conditions of the modern mushroom farms. Flegg *et al.* (1985) used the term, "evolution" of mushroom cultivation systems, to describe the continuous flow of developments in to mushroom technology until today. Despite the variation, a brief description of the procedures currently involved in growing a crop of A. bistros in UK will be attempted in this study.

2.3.1 Composting

The first stage (phase **I)** of mushroom cultivation usually starts with compost preparation. Compost is the major substrate on which the *Agaricus* cultures will be grown. As it has been pointed out, saprophytic fungi such as *Agaricus* are capable of breaking down dead organic material of plant origin. The raw materials used are wheat straw mixed with chicken (or horse) manure. All sorts of vegetative waste can be used in composting but cereals and especially wheat is available in sufficient quantities, thus it is used as the basic material for compost in many parts of the world (Vedder 1978). The purpose of the manure is to start the composting process quickly in order that the microflora in the compost pile make use of the carbohydrates and nitrogen which are easily degradable. Very important is also the N: P: K ratio in the compost as *A. bisporus* requirements are 6.4:2.4:4.4 Chang and Miles (1989) reported that compost should contain 1.5-2% nitrogen and as wheat straw contains no more than 1%, the animal manure supplements the rest of the essential nitrogen.

Raw materials are wetted and constantly mixed together and the pile is re-stacked at intervals of two to three days. This wetting makes it possible for the micro-organisms that are naturally present in these materials to begin their degradative metabolic activities. Cellulose and hemicellulose of the straw are converted to sugars which along with the available nitrogenous substances, support further of bacteria and other fungi (Chang and Miles 1989). As the composting process in phase **I** progresses, many opportunist saprophytic fungi appear and start their activity: *Cladosporium herbaceum, Penicilium*

spp., *Aspergillus* spp., *Mucor hiemalis*, and *Rizomucor pusilus*. These fungi play only a minor role in composting and their number decreases after approximately 3 days of phase I.

As the temperature in the core of the stack rises different microbes start to dominate the composting process called "thermophylic" actinomycetes and bacteria.

Temperature is a key feature during the fermentation of composting. During composting heat is realised by microbial oxidation of organic material. This material acts as an insulator and the heat is retained and temperature zones are formed within the stack (figure 1.)

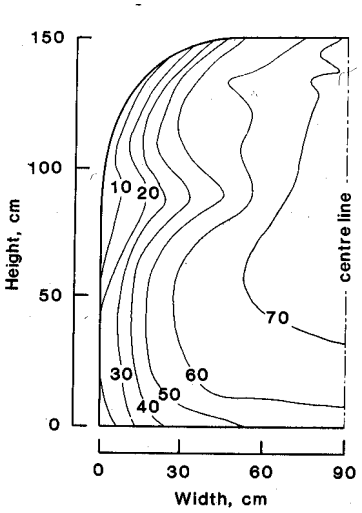


Figure 1. Temperature zones in a compost stack (taken from Flegg *et al.* 1985)

As the temperature rises to 50-60 ºC the mesophilic fungal population is gradually replaced by a thermophyllic population. The heat in the centre of a compost heap can sometimes reach 80ºC owing to the activities of the microbes (Atkins 1972). Phase I lasts no more than three weeks and is followed by Phase II which takes place under controlled (aerobic) conditions and which consists of two separated processes: pasteurisation and conditioning. The objective of the pasteurisation is to kill all the harmful organisms in the composts. This can be achieved by introducing heat and steam to compost increasing its temperature to 55-57 ºC in both air and compost for 5 to 6 hours. Conditioning, the other process of Phase II, aims to continue the heating of the compost. During the heating process fungi, actinomycetes and bacteria are constantly breaking down substances of the compost, producing nutrients favourable for *Agaricus.* The ideal temperature for conditioning is between 45 and 50 ºC.

2.3.2 Spawn and spawn running

The spawn is a cereal grain that has been sterilised and impregnated with selected inoculants made from *Agaricus* mycelium under aseptic conditions (Atkins 1972). Before

spawn is applied temperature must cool down to 25-30 ºC The amounts of spawn used as inoculum per unit surface area is variable, but, larger amounts of spawn result to a rapid and thorough mycelium build up (Chang and Miles 1989). Spawn running is the phase during which mycelium grows out from the spawn and permeates the substrate. Good mycelial growth and compost colonisation is essential for mushroom production. The mycelium is growing best in the compost when temperature is between 24-27 ºC and improper temperature and aeration can also cause poor spawn run. Chang and Miles (1997) stated that aeration is important as respiratory CO_2 can build up during spawn running and this can inhibit the formation primordia or later stages of mushroom development. Finally, RH on the air during spawn running should preferably be between 90-95%.

2.3.3 Mushroom casing

Normally, spawn-run compost is cased after 2 to weeks. Chang and Miles (1997) suggested that the main purpose of the casing layer is to stimulate and promote formation of fruiting bodies. They same authors reported that production does not occur unless the mycelium on the compost is covered by a casing medium. In a slightly different approach, Atkins (1972) mentioned that mushrooms tend to form on the surface of the compost but they are relatively heavy and with nothing to support them they may fall over and break. Another reason for casing the compost is to prevent the compost from drying out as casing materials have good water capacity and do not dry easily. In addition, vegetative mycelium is encouraged to fruit when it enters a medium deficient in food-the casing layer attempting to ensure its survival by producing fruits containing spores.

In good casing medium in the *Agaricus* cultivation should have open texture, good moisture capacity, freedom of pests and diseases and a pH between 6.5 and 8. In addition, it must not contain any undecomposed plant material, which will attract unwanted moulds. A peat moss mixture with pH adjust by lime, chalk or ground lime stone, fulfils the requirements of a good casing material and is now used by many mushroom industries.

Unlike France and Netherlands where casing materials are more standardised, in UK there is a wide range of materials (peat and lime or chalk sources) used in casing medium. In a survey conducted by Noble and Gaze (1995) in 48 different casing soils from 40 farms

throughout England, it was reported that casings were based on sphagnum peat and sedge peat, supplemented with chalk, limestone, lime or sugar beat lime. Quantitatively, chalk/lime were added in a range from 57 to 300 kg/m^3. pH and moisture content also varied from 7.3 to 8.1 the pH and 70-72% w/w the moisture. The peat used for casing in this study was classified according to its "darkness" which reflects its age and decomposition level. Finally, the same authors reported that many of the factors above affect the properties of final casing medium and have direct consequences on the quality and yield of mushrooms produced.

It has been pointed out that the fundamental property of the mushroom casing is the inducement of fructification to the mycelium. Basidiome induction in *Agaricus bisporus* is a complex and poorly understood phenomenon but it is accepted that the initiation and subsequent development of sporophores which represents the change from the vegetative to the reproductive phase of growth, is controlled by internal cellular, external factors or both (Miller et al. 1995). Miller *et al*. (1995) also reported in their study Eger's work from the early 1960's when bacterial population was found to be the key to basidiome initiation. These bacteria have been identified as a range of strains related to *Pseudomonas putida* and Rainey (1991) has suggested that the mycelium produce self – inhibitory compounds, which are removed by the bacteria below a threshold level permitting the transition to reproductive growth. The converse phenomenon i.e. *P. putida* may produce a substance which could stimulate the fruiting growth, is not likely because fructification can be achieved in sterile casing if activated charcoal is added (Miller *et al*. 1995). In that case volatile metabolites of the mushroom mycelium, which control vegetative growth, are absorbed by the activated charcoal removing the barrier to fructification. Finally, it is generally accepted that the interactions between the physical properties of the material used in casing, the environment in the growing room and management of the crop, will determine the crop progress.

2.3.4 Mushroom harvest

Depending on the method of growing, the strain used and the thickness of the casing layer, the first mushrooms will ready to be picked about 18-22 after casing (Vedder 1978). According the grades used in marketing, mushroom can harvested at different developmental stages. Food and Agriculture Organisation (FAO) issued in 1970, a mushroom classification according to their size and degree of maturity in which

mushrooms are classified as “buttons”, “caps” and “flats” or open mushrooms (Chang and Miles 1989). The overall duration of the cropping varies as well, as in the tray system, the cropping lasts 6 to 7 weeks enabling the farmers to obtained five crops a year. However, in the self-system it lasts 6 to 12 weeks (Atkins 1972).

2.4 Discussion and Conclusions

Mushroom growing involves processes with living organisms and, as such, is subject to numerous interactions which living organisms develop with their environment and with each other. Identical environmental conditions rarely exist in nature and thus, mushroom cultivation methods must be modified and developed for use in different locations. To do this effectively, knowledge of the basic principles is essential well as the practical cultivation techniques. In other words, everyone involved with mushroom cultivation must know the “how” and understand the “why” of every individual step of the complex events that constitute the mushroom growing.

2.5 References

Atkins F. C. (1972). *Mushroom growing today*. Faber and faber. London 1972.

Atkins F. C. (1974). *A guide to mushroom growing*. Faber and faber. London 1974.

Chang, S. T., Miles, P. G. (1989). *Edible mushrooms and their cultivation*. Boca Raton, Florida: CRC Press, 1989.

Flegg, P. B., Spencer, D. M., Wood D. A., (1985). *The biology and technology of the cultivated mushroom*. Chichester: John Wiley, 1985.

Garthwaite, D. G., Thomas, M. R., (2001). *Mushroom crops in Great Britain 1999*. DEFRA Pesticide usage survey report, 165; PB6167. London: DEFRA Publications, 2001.

Miller N., Gillespie J., Doyle O., (1995). *The involvement of microbiological components of peat casing materials in fruictification of Agaricus bisporus*. Science and cultivation of edible fungi, 1: 313-321.

Noble and Gaze (1995). *Properties of casing peat types and additives and their influence on mushroom yield and quality*. Science and cultivation of edible fungi, 1: 305-312.

Vedder P J C (1978). *Modern mushroom growing*. Culemborg, Netherlands: Educaboek / Cheltenham: Stanley Thornes, 1978.

Rainey P. B. (1991). *Effect of P. putida on hyphal growth of A. Bisporous*. Mycological Research. 95 (6) 699-704

3 Essay 2. Environmental behaviour of pesticides and the fate of prochloraz in the soil

3.1 Introduction

Pesticides-or agrochemicals in a wider context- are special case of widely used chemicals. In less than half century the tremendous increase in agricultural productivity has coincided with the advent of pesticide use. At present, over a thousand organic and inorganic chemical substances with pesticidal activity are registered and regularly applied on a global scale (Vink J. 1997).

The environment serves as a massive but finite reservoir for xenobiotics that reach it intentionally or unintentionally as contaminants and pesticides constitute a significant portion of this load. The detection of pesticide chemicals in all compartments of the environment and the determination of their magnitude and their ecotoxicological significance is essential in assessing their influence on crop plants, on target pests and on humans and other animals.

3.2 Pesticides in the soil

The soil is main recipient of all types of pesticides and plays a leading role in the environmental fate of these chemicals as well as in the protection of ground and surface waters. The environmental fate of pesticides in the soil is viewed with great concern today mostly due to the problems resulting from the use of persistent and mobile molecules affecting the surface and the ground water quality. Along with the increasing concern about chemical contamination of various ecosystems, much emphasis has been put on designing suitable methods to characterise the different processes affecting the fate of pesticides in soil (Cornejo et al. 2000)(Figure 2). Ideally the chemical compounds used in crop protection, should persist long enough to control target organisms and then degrade into inert products. Leaching and run-off losses, however, lead to inadequate pest control as well as pollution of surface and ground waters (Vink J 1997))

Cornejo et al. (2000) suggested that soil functions as an active filter, where the chemicals are degraded by biological and non biological processes and as a selective filter because it is able to retain some chemicals to prevent their leaching to ground water. Both the fates

of the agrochemicals in the soil and their dispersion in the environment mainly depend on the characteristics and the overall functioning of the ecosystem. In the soil, pesticides are affected by the simultaneous influence of transfer, adsorption-desorption, physicochemical degradation and biodegradation phenomena. All these processes are dynamic and non-linear.

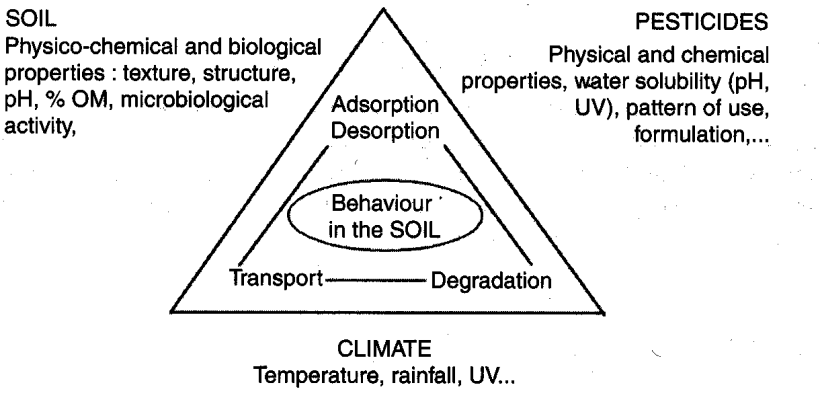


Figure 2. Factors and processes affecting the behaviour of pesticides in the soil (Cornejo et al. 2000).

3.2.1 Pesticide transfer

Cornejo et al. (2 000) reported that the processes involved with the pesticide transfer are volatilisation, leaching and run-off. **Volatilisation** is an important pathway for the dissipation of pesticides from treated areas. Through volatilisation, chemicals leave the application surfaces in the vapour phase and move into the atmosphere. Airborne transportation of pesticides is probably the principal method for their widespread dispersion in extraordinary long distances in the environment (Spencer W. 1987). Volatilisation usually occurs during application, especially when spraying and it concerns the quantities of the compound that remain at the surface of the soil. The vapour pressure, the temperature on the soil surface and the partition coefficients air/water and air/solid phase, are the main factors affecting this process.

During the **leaching** process, pesticides can move in the soil in two directions, vertically and laterally and the two principal transport mechanisms are mass flow and molecular diffusion. Green and Khan (1987) defined mass flow as the transport of a pesticide using water or air as vehicle and diffusion as the random movement of molecules which tends toward uniform distribution of solute molecules within a solution or a volume of air. It should be stressed that mass flow can move solutes to long distances in the soil profile in

a relatively short period of time while the process of diffusion is rather slow and it moves solutes only in small distances. Both processes can, and usually do, occur simultaneously. Soil a complex porous medium, consisting of minerals from weathered rocks, organic matter (dead or alive) and pore space containing water and air in proportions that change over time. The individual minerals and organic particles clump together into aggregates. The combined effects of micropores within aggregates and macropores between aggregates can result in the deep penetration of small quantities of slightly sorbed pesticides, while most of the residual pesticide is held within aggregates near the soil surface and thus protected from rapid leaching (Green and Khan 1987).

As leaching process is a significant transport mechanism of pesticides in the liquid phase, **run-off** losses are rather a small fraction of the amount applied and in most cases less than 5% of the amount applied (Green et al. 1977). In addition, surface run-off generally deposit pesticides in surface waters where degradation is relatively rapid and therefore, contamination problems arising from run-off and erosion are generally transitory. Run-off depends on the slope of the soil, the rainfall characteristics, the farming practices and the crop cover. It also depends on the water solubility of the compound as well as the adsorption of the soil components Cornejo et al. (2000)

3.2.2 Physicochemical properties and environmental fate

The environmental behaviour and fate of pesticides and other chemicals depend to a large extent on their molecular structure and consequently, on their physicochemical properties. Polarity or ionisation, water solubility and octanol-water partition coefficient affect directly the adsorption-desorption phenomena and eventually have significant effect on the overall fate of the compound in the soil. Monfort et al. (2000) defines sorption as a key process governing the distribution of pesticides between soil solution (available fraction) and soil particles (sorbed non-available fraction). The dame author stress that the factors influencing the sorption of chemicals in the soil are those that affect the activity of the chemicals towards target and non-target organisms and their degradation in the soil. An adsorbed molecule is not generally bio-available, carried away by the movement of water in the soil and is less rapidly degraded. On the other hands desorbed molecules is bio-available and it can be easily degraded and carried away by run-off water (Cornejo et al.2000).

3.3 The fate of prochloraz in the soil

3.3.1 Physicochemical properties

Prochloraz [N-propyl-N-{2-(2,4,6-trichlorophenoxy)-ethyl}imidazole-1-carboxamide] belongs to the group of imidazole fungicides that inhibit ergosterol biosynthesis. It is widely used to control eyespot disease and powdery mildew on cereals and it is also effective against a broad spectrum of fungal diseases on fruits as well as vegetables (Kapteyn et al. 1992; Tomlin 2000). Prochloraz is weakly basic, with pK_a 3.8. It exhibits a low water solubility of 34.4 mg/l and a high lipophilicity with a $logP_{ow}$ of 4.38. In aqueous solution its half-life is days and dissipation of half-lives in soil range 5 and 37 days under field conditions whereas laboratory studies showed half-lives between 92 and 171 days (Hollrigl-Rosta et al. 1999). The main metabolic pathway starts with prochloraz-formylurea which then hydrolysed to prochloraz-urea. Both substances have isolated from rats as well as from soil.

3.3.2 Soil moisture content and sorption of prochloraz

Sorption of organic chemicals on soils, substantially influences their fate because mechanisms such as transport, microbial uptake or metabolism, are operative only on the fraction of non-sorbed molecules (Pignatello 1989)

Gaillardon et al. (2000) studied the effect of soil moisture content of a loam soil (46.3% sand, 39% silt, 17.4% clay and 2.44% organic matter), on the sorption of five sterol biosynthesis inhibitors (SBIs) including prochloraz. In this study the Wershaw's humus model (humic substances have a membrane-like structure) was adapted and based on this, it was assumed that low soil moisture content could modify the structure of humid substances and generate hydrophobic surfaces which favour sorption of hydrophilic fungicides. The effect becomes adverse for more hydrophilic compounds, which are more, sorbed at high soil moisture due to their higher affinity for hydrophilic regions of humus and due to their diffusion.

Specifically prochloraz was found to partition rapidly into the liquid-like interior of humus at low soil moisture content but increased diffusion at high soil moisture caused additional sorption by ion exchange at colloid surfaces. The mechanism of sorption of prochloraz involved both physical and ionic processes depending on the pH surrounding

the chemical. The neutral form of prochloraz could follow the partition first into the hydrophobic interior of the membrane-like humus, especially at the lowest soil moisture. As molecules diffuse throughout the soil particles, they come into contact with water of lower pH at the colloid surface and small portion can protonate. The ionised species might then be strongly adsorbed to clay and humus cation exchange sites. The diffusion process could be responsible for the highest level of long-term sorption observed at the high soil moisture by increasing the probability of neutral molecules to ionised at the surface. The changes in prochloraz concentrations in different soil moisture contents are shown in Figure 3.

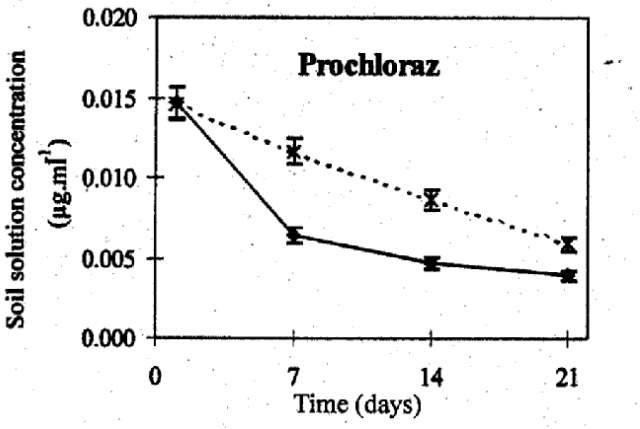


Figure 3. Changes over time in the concentrations of prochloraz in the soil solution at (x) 26.1% and at (♦) 46.6% soil moisture content (Gaillardon *et al.* 2000).

It was concluded that all five SBI fungicides are strongly and rapidly sorbed onto soil and the bioavailable fungicide concentrations for soil organisms (earthworms, micro-organisms and plant roots) are very low. Finally, no degradation was reported for any of the five fungicides (prochloraz included), during the 3-week period of equilibration and the lowest rate of recovery of all fungicide tested was 95% (Gaillardon et al. 2000).

3.3.3 Sorption of prochloraz in different soils

Sorption of prochloraz was studied also by Hollrigl-Rosta et al. (1999) in order to characterise the behaviour of this compound in six different soils (two silt loams, three loamy sands and one sand) and examine the influence of pH, organic carbon and clay content to the sorption isotherms constructed.

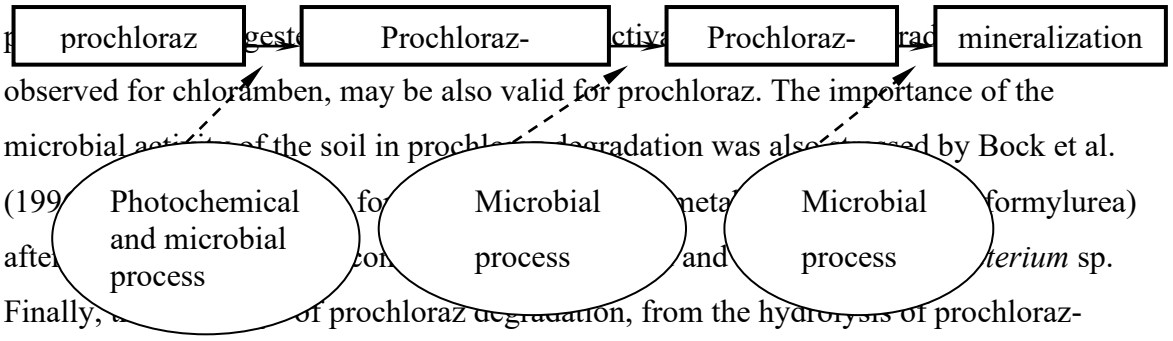
Experimental results in this study confirmed that prochloraz is sorbed on soil through a two-step process as rates of sorption are highest within the first hour and significantly lower over the subsequent course of time. This was interpreted as a weak binding of the

molecules to outer sorption sites in the first and subsequent diffusions to inner sorption sites in the second step (Hollrigl-Rosta ET al.1999).

Concerning clay and organic matter content, a higher clay content appeared to have no enhancing influence on the sorption of prochloraz while organic carbon content distribution coefficients K_d were positively correlated. In poorly water soluble, lipophilic compounds, sorption is governed by organic carbon content rather than clay content. This may be due to the fact that organic matter often covers the surfaces of clay minerals or shielded by a lipophobic hydration shell and hence are not directly accessible for lipohylic organic compounds.

Hollrigl-Rosta et al. (1999) also studied the influence of soil pH to the sorption of prochloraz and found that K_d and K_{oc} are increased when pH is decreased. In laboratory degradation experiments in the dark, it was found slightly slower dissipation of prochloraz in acidic than in neutral to basic soils. Stronger sorption of the molecules at lower pH suggests reduction of bioavailability of prochloraz to degrading micro-organisms. In conclusion it was reported that this compound is strongly adsorbed on all soils tested in this study and that is not likely to leach into deeper soil layers despite its relatively long half-life time.

3.3.4 Degradation of prochloraz

Degradation research studied by Hollrigl-Rosta et al. (1999) reported that both biotic and abiotic degradation occur concurrently for prochloraz. The overall fate of this chemical in soil is determined by a combination of photochemical and microbial degradation p


observed for chloramben, may be also valid for prochloraz. The importance of the microbial activity of the soil in prochloraz degradation was also stressed by Bock et al. (199 fo meta formylurea) after con and *terium* sp. Finally, of prochloraz degradation, from the hydrolysis of prochloraz-formylurea to prochloraz-urea until the its mineralization depend only on the microbial metabolism. The degradation process of prochloraz as it is presented in Hollrigl-Rosta's study, can summarised in the following figure:

3.4 Discussion

Despite the significance prochloraz has gained in agricultural practice since its introduction in 1977, few studies have focused on its fate in soil. As a widely used fungicide approved for use mainly in cereals and one of the few fungicides for use in mushrooms crops, further research is needed to investigate its fate in the environment and to determine the factors controlling its behaviour in the soil.

3.5 References

Bock C, Diekmann H., Kroppenstedt R. M., Schmidt U., (1996). Degradation of prochloraz and 2,3,6-trichlorophenol by environmental bacteria strains. Applied Microbiology and Biotechnology. 45: 257-262 1996.

Cornejo J., Jamet P., Lobnik F. (2000) Pesticide/soil interaction. Introduction. INRA, Paris 2000.

Green and Khan 1987. Volatilisation of pesticides. Fate of pesticides in the environment. Division of agriculture and natural resources, university of California. 1987.

Green R. E., Goswamin K. P., Mukhtar M. 1977. Herbicides from cop water sheds in stream and estuarine sediments. Journal of Environmental Quality. 6:145-150.

Hollrigl-Rosta A., Kreuzig R., Bahadir M., Rutters H., 1999). Sorption of prochloraz in different soils. Journal of Agricultural Food Chemistry. 1999. 47, 1242-1246.

Kapteyn J. C., Pillmoor J. B., De Waard M. A., (1992). Biochemical mechanisms involved in selective fungitoxicity of two sterol 14-α-demethylation inhibitors, prochloraz and quinconazole: Accumulation and metabolism studies. Pesticide Science. 1992, 36, 85-93.

Pignatello J., (1989). Sorption dynamics of organic compounds in soils and sediments. Reactions and Movements of Organic Chemicals in Soils, 2nd ed.; Sawhney B. L., Brown K., Eds.; SSSA Special Publication 22; SSSA: Madison, WI 1989; 45-50.

Spencer W 1987. Volatilisation of pesticides. Fate of pesticides in the environment. Division of agriculture and natural resources, university of California. 1987.

Tomlin C. (2000) The Pesticide Manual, A world compendium. 12th ed. Farnham: British Crop Protection Council (BCPC), 2000.

Vink J 1997. Pesticide biotransformation and fate in heterogenous environments.

4 Essay 3. The casing layer

4.1 Introduction

It has been known for at least two hundred years that mushroom beds or trays must be covered with a layer of "soil" to induce fruiting and it was also known that those soil based casing materials varied in their performance, some giving good yields while other did not (Hayes 1973). A compost, full grown with mycelium, produces hardly any mushrooms while a layer of casing soil on the top of the compost initiates the production of mushrooms in reasonable quantities. Kligman (1950) underlined the influence of casing soil on yield, as poor soil will definitely reduce the yielding capacity of good spawn run compost substrate. In addition to the stimulation of fruiting, the casing soil also provides the substrate for the developing sporophores and the water-holding reserves essential for high yields necessary for economic mushroom production (Miller *et al*. 1995).

The application of the casing soil, -which is commonly known just as *casing*- and the treatment of it during the life of the crop, have a great influence on the quantity and the quality of mushrooms obtained. The cultural measures following the *casing* are to ensure that the mushroom mycelium grows through casing soil, forms small aggregations of mycelial threads called initials, and eventually produces mushrooms.

4.2 Functions of the casing layer

In relation to the compost, the casing soil is seeing as a protective, covering layer which stimulates the production of mushrooms and which acts as a water reservoir with sponge-like properties retaining large amounts of water and releasing it again as required. Generally speaking, the casing will keep fully-grown or spawn-run compost, in good condition preventing it from drying.

More specifically, Visscher (1988) refers a number of different functions the casing has:

- To protect the compost layer from drying-out and from too rapid disappearance of metabolic products
- To supply water for the growth and development of mycelium and fruit bodies
- To regulate water evaporation in such way that the climate in the growing room meets the minimum air humidity standards

- To provide certain bacterial species which will stimulate fructification.
- To create a low osmotic value environment as the compost itself has too high osmotic value to produce enough mushrooms, even after ventilation.

Clearly the casing soil through its various functions, is associated with water regulation in mushroom crops, with certain bacterial species which stimulate fructification of mycelium and with a CO_2 gradient from the compost to the air.

4.2.1 Water regulation and the casing

Because large quantities of water are required over a period of 4-5 days for the undisrupted growth of pins, the casing material must be able to absorb a relatively large amount of water and return it gradually. On average, 2 litres of water are required for the formation of 1 kg of mushrooms (Vedder 1978). The same author reports that the ventilation required uses an additional litre of water per kg of already formed mushrooms. Since the casing layer supplies this moisture, regular watering of the casing must take place in order to maintain the required levels of moisture. The casing layer acts as a water reservoir. Without this layer above the compost it would be impossible to maintain the moisture content of the compost: Ventilation would dry out the surface of the beds completely and the watering of uncased beds would be risky, if not impossible.

4.2.2 Stimulating bacteria

It has already been pointed out that a fundamental property of the mushroom casing is the inducement of fructification to the mycelium. Basidiome induction in *Agaricus bisporus* is a complex and poorly understood phenomenon but it is accepted that the initiation and subsequent development of sporophores which represents the change from the vegetative to the reproductive phase of growth, is controlled by internal cellular, external factors or both (Miller *et al*. 1995). Miller *et al*. (1995) also reported in their study Eger's work from the early 1960's when bacterial population was found to be the key to basidiome initiation. The fundamental study by Eger is also mentioned as the "the half dish test" in which compost and casing were placed next to each other in a Petri dish. When the compost was spawned, the mycelium began to grow over the casing material. The results showed no fructification on sterile casing in contrast to the appearance of fruiting bodies on non-sterile casing soil. Compared with the mycelial growth in the sterile casing, the growth in non-sterile casing was slowing down and then fructification was initiated. When a casing suspension was filtered through cotton wool or paper, the active principle

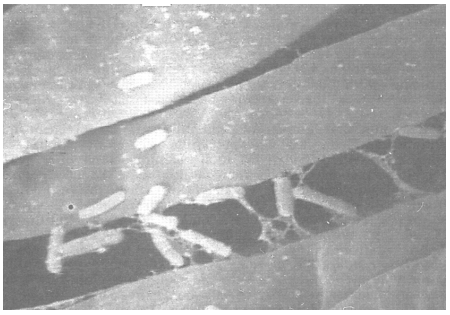
remained. But if the suspension was filtered through a bacteria filter, the fructification capacity of the casing suspension disappeared (Visscher 1988). Hayes *et al*. (1969) has identified these bacteria as a range of strains related to *Pseudomonas putida* and *Pseudomonas* GroupIV on the peat based casing mixtures. However, in a recent study bacterial populations were found to vary greatly according to different materials used in the casing mixture. In dry coir, for example, *Corynbacteriaceae* and *Bacillus spp* dominated. In wet peat was found mainly *Pseudomonas spp* from which 40% belongs to *Pseudomonas putida* (Fermor *et al*. 2000).

Concerning the mechanism, with which these bacteria cause fructification, Vedder (1978) reports that various volatile metabolic by-products of the growing mycelium enhance the development of these bacterial populations and that fructification occurs when the bacteria oxidise volatile metabolic products that inhibit the production of fruiting bodies. Such gases produced during *Agaricus* metabolism have been identified and include: acetone, acetaldehyde, ethyl alcohol, ethylene and ethyl acetate (Lockard & Kneebone 1963). Further experimental work, demonstrated that the bacteria could survive in a mixture of such products as the sole source of carbon Hayes *et al*. (1969).

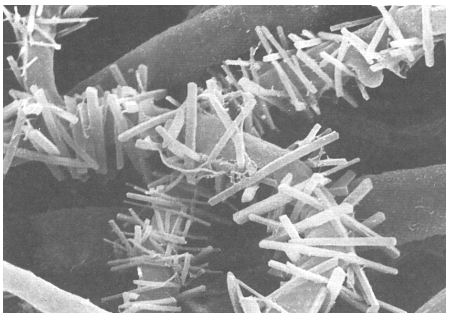
In a nutrient poor environment such as peat casing, the production of such metabolites by *A. bisporus* would create nutrient gradients to which *Pseudomonas* bacteria could respond in a chemotactic way. The ability to move quickly toward these nutrients would probably give *Pseudomonas* bacteria a survival advantage over non-chemotactic species. Perhaps this migration of pseudomonads towards mycelial exudates of *A. bisporus*, assists in the subsequent colonisation and proliferation which appears to be necessary before the bacteria provide *A. bisporus* with the stimulus to initiate basidiome formation (Grewal & Rainey 1991).

In an electron microscopy study, Miller *et al*. (1995) found that pseudomonads adhere rapidly, firmly and specifically to the hyphal walls of *A. bisporus*. The specificity of this attachment was demonstrated by the inability of *E. coli* to adhere to the fungal hyphae in the same manner. It is also proposed that calcium oxalate deposits occur along the hyphal walls as a result of calcium detoxification efforts of the mycelium. The same authors

finally concluded that the removal of the calcium oxalate crystals from the hyphal walls is probably a determining factor in the attachment of pseudomonads to *A. bisporus*.



P. putida attached to the hyphal walls of *A. bisporus*



Calcium oxalate formation on the hyphal walls of *A. bisporus*

4.2.3 CO_2 gradient and casing soil

The activity of the growing mycelium releases also a significant amount of CO_2 and therefore, the CO_2 content in the compost and between compost in casing appears to be increased. The vegetative growth of the mushroom mycelium remains good when the CO_2 in the air above is about 2% (Vedder 1978). However, a well-ventilated growing room will bring CO_2 levels close to the fresh air (as low as 0.06%). Given that fructification occurs at a CO_2 content 0.08-0.15%, the layer of casing can create an intermediate space with a CO_2 gradient, favouring fructification. According to Visscher (1988), the "gradient" theory alone cannot justify fructification and it is likely to be complementing the bacterial activity. On that point, Long & Jacobs (1968) investigated the effect of various CO_2 levels on fructification and showed that non-sterile casing combined with a 0.03-0.10% CO_2 content causes maximum fructification while sterilised casing did not produce any fruit bodies regardless of CO_2 concentration.

However, if the mode of action of carbon dioxide is be thoroughly understood, it should also be studied the influence of its chemical breakdown products such as the bicarbonate ion, on the process of initiation. The chemical reaction, which represents the relationship between the carbon dioxide and bicarbonate ion, is shown below:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 + H_2O \Leftrightarrow H_3O^+ + HCO_3^-$$

The concentration of the bicarbonate ion depends on the pH value as well the initial CO_2 concentration. Hayes (1973) investigated the effect of the bicarbonate ions on initiation

through their impact to *Pseudomonas* populations. He found a significant increase of the *Pseudomonas* populations in samples amended with sodium bicarbonate compared with sodium chloride or unamended (controls) samples.

4.3 Raw materials used in casing soil

Depending on the country and the availability, a number of different materials that can used in preparation of casing soil but mixtures of black/brown peat, clay, sugar beet lime, marl and gypsum are used predominantly by most mushroom industries. Those materials are very important for quality and the type of the casing blend produced. In the past many materials with good water-holding capacity have been tested as alternatives that could substitute peat due to environmental and economical reasons. Such materials include coconut fibres (coir), coal sludge, paper waste, perlite and vermiculite. Several materials provided yield that was substantial enough to compete with peat but they were too expensive for practical applications. Others were less expensive but they gave lower yields that casing made with peat. The main ingredients of a common casing soil are discussed below.

4.3.1 Peat

Of all raw materials used in the casing soil, peat is the most common. It originates from countries like Ireland, north Germany, Scotland and Finland. Through to its nature, of different degree of decomposed organic matter, peat can retain extremely large amounts of water up to 85%. The peat is responsible for the sponge-like texture of the casing adsorbing and retaining large amounts of moisture during the drench-watering cycles of cropping. Also, with a low-very low pH (3.5-4) value, peat is free of soil born pathogens. Brown peat-together with a pH regulator-can be used as casing soil in a ratio: 70-75% brown peat and 25-30% sugar beet lime. (Vedder 1978).

4.3.2 Clay

In the old mushroom farming systems, clay was very popular as the main casing raw material (Kligman 1950). It is capable of retaining relatively large amounts of water (30-40%) and it can produce a satisfactory yield. However, it tends to turn to sludge when watered and it cannot compete with peat in moisture capacity and in texture. For example, the moisture holding capacity of river clay can be as high as 38% but it can also contain

too much sand which is undesirable or too many soil pathogens which unacceptable (Vedder 1978).

4.3.3 Sugar beet lime

The spent lime is a by-product of the sugar beet industry containing mainly $CaCO_3$, beet impurities, magnesium and phosphates. It also contains 2% sugar and the organic matter is 8-10% in total. Its pH value is around 8 and therefore it is used in casing blends to increase the pH of the peat. It can also replace marl as pH regulator of the peat. In addition the amount of sugar beet lime added can influence texture of the casing soil as more lime gives a denser structure of casing, resulting in fewer mushrooms in every flush (Visscher 1988). Finally, there are a number of other materials used to raise the pH of the casing mixture, including gypsum ($CaSO_4$), lime stone [$Ca(OH)_2$] and ground chalk.

4.4 Physic-chemical properties of casing soil

As it has been pointed out in the functions of the casing material, the water holding and the water-releasing capacity is very important in a mushroom cropping system. The ability of the casing to retain moisture is predominantly defined -unless a peat alternative is used- by peat. Taking account that the moisture content of a peat soil is around 80% (or above) and depending on the total peat percentage of the casing mixture, the casing is expected to contain around 70% moisture on ruffling (Visscher 1988).

The optimum pH value for *Agaricus* growth is 7 and the acidic environment that peat induces to the casing, is corrected with the lime to give a casing blend with a pH of 7-7.5. A pH slightly above seven is preferable in order to reduce risk of *Trichoderma spp*. In addition, as soon as the mycelium starts to grow in the casing layer, pH decreases slightly because of the formation calcium oxalate crystals but this fall in pH, has little or no effect on the *Agaricus* growing cycle. The heavy watering cycles straight after casing release enough Ca^{++} from the lime source to bring the acidity to desirable levels.

On more feature of a good casing soil is its texture. It should have been light and open textured in order to prevent too much CO_2 during fructification and harvesting. Visscher (1988) suggested that a dense casing or one which has been compacted during the vegetative phase of the mycelium, gave best yield provided that the dense texture was loosened up again before the transition to the generative phase by ruffling the casing

layer. A dense, compacted casing soil, which is not loosened up, clearly reduces the final yield obtained.

4.5 Casing the compost

The decision when to case the spawn-run compost depends on the degree to which the mushroom mycelium has grown through the compost. Normally, two weeks after spawning will be the most suitable day to case the compost although Atkins (1974) suggested that most of the growers case 12-13 days after spawning. Taking account the fact that the casing layer mainly protects the compost below from drying out, its the thickness depends on the depth of the compost. Noble *et al*. (1997) determined the ideal depth of the casing layer as 50mm deep but it may vary between 30mm to 60mm on commercial farms. Although the 60mm depth of casing gave significantly higher yields compared to the 30mm, there is no evidence suggesting any difference between 50mm and 60mm (Noble & Gaze1995).

For a number of reasons, it is important that the depth of the casing soil is uniform. Unless the casing layer is even, the pinheads could form deep in the casing soil in some spots and on the surface in other places were the layer is thinner. Even the watering and the pesticide spraying will not have the desirable effects (Vedder 1978). To make the casing layer level, the compost underneath should also be spread evenly on the beds and tamped down firmly. The casing can be applied mechanical with special machinery and the casing material can spread, levelled and raked automatically without any manual labour. On the casing lines of tray farms, the trays are cased continuously as they move with a constant speed through the casing machine. Special care should be taken when handling casing in order to maintain the casing its moisture and its coarse structure.

4.6 Discussion

Notwithstanding, the importance of the other components of a mushroom cultural processes such the compost or the spawn, it is the casing layer, which generates fructification and ultimately determines the yield and productivity of the system (Hayes 1973). There is also evidence to suggest that the growth of the crop is not only dependent on the activity of some micro-organisms associated with growth but that the extent of their activity is linked to economically important features of production such as size and weight of individual mushrooms harvested and yield. It is therefore of great importance to

understand every aspect of the casing layer such as its microbial ecology or its physico-chemical properties. These aspects relate directly to the overall productivity and behaviour of the crop and also can provide possible guidelines by which culture systems can be improved to introduce a further degree of control over the growth of the crop. In addition, the limitations and the inherited dangers of some pest and disease control measures faced in growing conditions, can be evident in studies on the ecology of the casing layer.

4.7 References

Atkins, F. C., (1974). Guide to mushroom growing. London: Faber & Faber.

Fermor T, Lincoln S, Noble R, Dobrovin-Pennington A, Colauto N, (2000). Microbiological properties of casing. *Science and cultivation of edible fungi*. 447-454.

Grewal S. I. S., Rainey P.B., (1991). Phenotypic variation of *Pseudomonas putida and P. tolaasii* affects the chemotactic response to *Agaricus bisporus* mycelial exudate. *J. Gen. Microbiol*. 137: 2761-2768

Hayes W. A., Randle P. A., Last F. T., (1969). The nature of the microbial stimulus affecting sporophore formation. In Agaricus bisporus (Lge.) Sing. *Annals of Applied Biology* 64, 177-187.

Hayes, W. A., (1973). The casing layer. Proceedings of the 1st Seminar in Mushroom Science held in the Department of Biological Science, The University of Aston in Birmingham, November.

Kligman, A. M., (1950). Handbook of mushroom culture. 2nd ed. Kennett Square, Pa, USA: J.B. Swayne.

Lockard J., Kneebone J., (1963). Investigations on the metabolic gases, produced by *Agaricus bisorus* (Lge.) Sing. *Mushroom Science* 7, 373-384.

Long P. E., Jacobs L., (1968). Some observations on CO_2 and sporophore initiation in the cultivated mushroom. *Mushroom Science* 7, 373-384.

Miller N., Gillespie J., Doyle O., (1995). *The involvement of microbiological components of peat casing materials in fruictification of Agaricus bisporus*. *Science and cultivation of edible fungi*, 1: 313-321.

Noble R., Gaze R. H., (1995). Properties of casing peat types and additives and their influence on mushroom yield and quality. *Science and Cultivation of Edible Fungi,* 1: 305-312.

Noble R., Rama T., Miles S., (1997). Influences of compost and casing layer depths on the mechanical properties of mushrooms. *Ann. Appl. Biol*. 131 (1): 79-90.

Visscher, H. R., (1988). Cultivation of mushrooms. Rustington, W Sussex: Darlington Mushroom Laboratories.

Vedder, P. J. C., (1978). Modern mushroom growing. Culemborg, Netherlands: Educaboek / Cheltenham: Stanley Thornes.

5 Essay 4. The dry bubble disease of the cultivated mushroom, *Agaricus bisporus*, caused by *Verticillium fungicola*

5.1 Introduction

As with all cultivated plants, mushrooms are susceptible to diseases and pests. Similarly to crops, *A. bisporus* is also subject to a number of diseases of fungal, bacterial or viral origin which are parasites of the mycelium or its fruiting body: the mushroom. *A. bisporus* has to struggle against the competitive or pathogenic fungi, which will either reduce the food available or alternatively excrete toxic substances, retarding growth (Atkins 1974, Seth 1977). Many fungi that may cause significant economic damage can occur in commercial mushroom cultivation and over the years a number of different fungal species have been named as pathogens (parasitic) or weeds moulds (competitive) of the cultivated mushroom (Zaayen 1982, Geijn 1982). The weed moulds are also known as competitive moulds, affect the growth of *Agaricus* mycelium during the spawn run of the substrate (compost and/or casing) competing for nutrients, water, O_2 and space. Although the parasitic moulds can also exhibit competitive behaviour, they mainly cause damage to the mycelium or the fruiting bodies of the mushroom without necessarily being lethal (Van Griensven 1988).

The required hygienic measures and the proper pasteurisation and steaming of substrates at crop termination (cooking out) will normally keep fungal infections to a minimum but fungal diseases can develop at any phase or growth stage affecting adversely the mushroom yield. If harmful fungi appear in the compost during growing, Van Griensven (1988) suggests that it is no longer possible to control them. On the other hand, slight fungal infections on the casing during mushroom growth can be effectively controlled with certain pesticides.

The number of mushroom fungal diseases encountered on modern farms has been considerably reduced in the recent years due to better composting techniques, extended pest/disease research and better technical equipment for environmental control. However, there are still fungal diseases, which cause significant economic losses and one those is dry bubble disease caused by *Verticillium fungicola* var. *fungicola*.

5.2 The pathogen

The disease caused by *Verticillium fungicola* (Preus.) Hassebr. has been a continuing problem for *A. bisporus* mushroom growers for many years (North & Wuest 1993, Gandy 1973). As with other fungal disease, crop losses due to by *V. fungicola* have been minimised in the recent years due to new cultural practises, effective sanitation measures and the use of chemical. However, this pathogen is still a significant threat to mushroom farms with many recent outbreaks reported and new improved control measures are expected to benefit the industry (Collopy et al 2001, North & Wuest 1993).

The pathogen, *V. fungicola*, which was formerly known *V. malthousei* (Ware 1933), is the common casual agent of the dry bubble disease. The taxonomic revision by Gams & Zaayen (1982) has placed *V. fungicola* into section Prostrata of the *Verticillium* genus and distinguishing three different varieties: *fungicola, aleophilum* and *flavidum* based on morphological taxonomy and pathogenicity studies. The conidia appear unicellular with thin walls but there is a great variation in the size and the shape of those asexual structures of all three varieties (Figure 4):

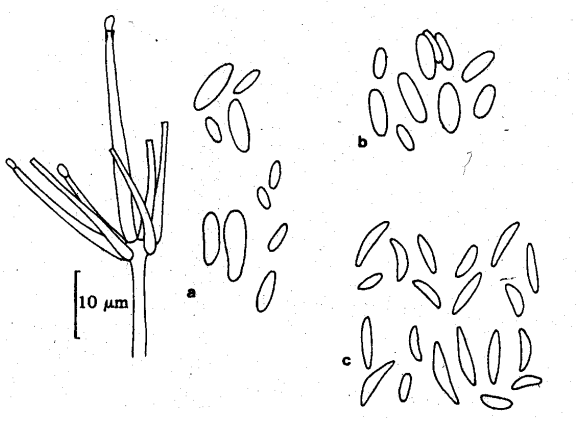


Figure 4. Conidia, variable size and shape, of *Verticillium fungicola* var. *fungicola* from different isolates a. CBS 648.80 b. type material in Herb.

The variety *flavidum* differs distinctively from the other two in the production of sclerotia, the lower optimum temperature, the pungent odour, the yellow colour of its colonies and its inability to cause disease in *A. bisporus.* On the other hand the only consistent difference between *Verticillium fungicola* var. *fungicola* and *Verticillium fungicola* var.

aleophilum, is the temperature of optimal growth. *Verticillium fungicola* var. *fungicola* has optimum growth temperature 20-24 ºC while *Verticillium fungicola* var. *aleophilum* grows better at 24-27 ºC. *V. f.* var. *fungicola* was found to have maximum temperature for growth 27ºC in contrast to *V. f.* var. *aleophilum*, which has a maximum growth temperature of 33ºC (Figure 5). From the disease perspective these differences in the growing temperatures can explain why var. *fungicola,* which is the cause of dry bubble in *A. bisporus* and which can grow at 25 ºC, does not cause disease in other cultivated *Agaricus* species such as *A. bitorquis,* which grows at higher temperatures. In that case *A. bitorquis* appears to be susceptible to *Verticillium fungicola* var. *aleophilum*.

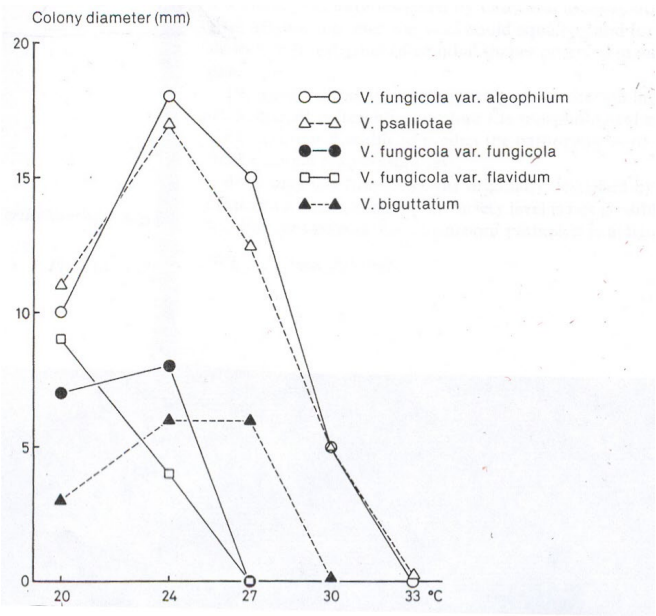


Figure 5. The relationship between the diameter of the colonies and the temperature, for three *Verticillium* species and three varieties grown for 4 days on malt extract agar. (Modified from Gams

5.3 The disease

Malthouse gave one of the first descriptions of dry bubble disease and its cause in Edinburgh in 1901, by 1938 the disease had already spread from UK to farms in the Netherlands. In England, Gaze & Fletcher (1975) showed that 56% of the farms sampled, had the disease and that the incidence was severe in 33% of them. Nowadays, it causes considerable losses (up to 30% without any control measures) worldwide and it is considered to be the most important fungal disease of the cultivated mushroom (Nair and Macauley 1987, Russell 1984, Zaayen 1982).

A mushroom infected with *V. fungicola* may have a very swollen stipe, but the stipe is often still recognisable. If the infection occurs at an early stage, the mushroom becomes onion-shaped but the most characteristic symptom of the disease is that the mushroom grows lop-sided because it is affected on the one side only. Vedder (1978) and Van

Griensven (1988) refer to this symptom as the “harelip” when a partially grown pinhead is infected on one side only and the mushroom is encouraged to grow locally, causing it to become crooked and the outer layer to be torn off. In the case of a late infection, the cap shows brown spots that resemble the bacterial blotch and ultimately, a grey mycelium grows out of the spots. Unlike the wet bubble caused by *M. perniciosa*, mushrooms infected with *V. fungicola* do not rot and do not smell. Mushrooms infected by *Verticillium* remain dry, with a leathery texture. The disease develops in clusters, rather than singly on the beds/trays (Zaayen 1982, Ware 1933). Delayed symptom development is very common in this disease and North & Wuest (1993) showed in their study that almost all mushrooms inoculated with 2×10^5 spores per ml, displayed symptoms after a post harvest incubation period. Mycelium and phialospores of *V. fungicola* was found in and around the host issue before symptoms appear. On the other hand at higher density (2×10^6 spores per ml) symptoms were developed by the time of harvest. This is a very important aspect of disease epidemiology as it suggests that the percentage of harvested mushrooms on which symptoms develop will be higher than the incidence of disease at the time of harvest.

North & Wuest (1993) concluded that pathogenesis is irrespective of the *Agaricus* cultivar used. They also reported that the pathogen has not any specialised penetration structures and thus, no direct penetration occurred. Also, hyphae of *V. fungicola* were observed growing in close association with *A. bisporus* hyphae and where contact was made between the two fungi, *Verticillium* caused adherence and eventually collapse of the host mycelium. Finally, North & Wuest (1993) suggested that an enzyme might be involved in the pathogenesis of *V. fungicola* on A. *bisporus* without defining its function or its nature.

The disease can spread very rapidly in a crop, particularly when temperature is left relatively high (above 20 ºC) during the growing stage. High relative humidity also promotes the occurrence of this pathogen, the spores of which are mainly spread by watering. *Verticillium* can be carried and established in the a farm by infected casing soil, although the possibility of spores being carried by dirty packing containers, tools, clothing or even the ventilation system, cannot be ruled out. Mushroom pests can also act as vectors of the disease as the sticky spores can be transmitted by mites, sciarid and phorid flies, especially during the summer period. The spread of dry bubble is quicker than that

of wet bubble, particularly through the fact that the small conidia are easily spread by sciarid flies and picking work. The reason for this is the sticky mucilage with which the clusters of *Verticillium* conidia are surrounded which enhance transmission the disease by its vectors (Seth 1977, Van Griensven 1988, Zaayen 1982, Ware 1933). Wong & Preece (1987) studying the sources of inoculum in a commercial mushroom farm in UK reported that walls, floors exposed structural woodwork in growing rooms and even symptomless mushrooms could act as sources of inoculum. However, the main bulk of inoculum existed in the casing (peat and limestone) material. Although viable airborne spores were detected in the air, inside and around the growing rooms, the disease could not spread through the air in long distances.

5.4 Control

5.4.1 Ecological control

Environmental factors such as temperature, relative humidity, and ventilation are closely controlled in a commercial mushroom farm. Taking account the primary sources of inoculum and the main ways of the transmission of the pathogen, it should be possible to alter the crop environment to favour the growth of the mushroom and not the pathogen. This way of disease control is mentioned by Sinden (1971) as "ecological" control. For example, a lowering of crop air temperature from 20 ºC to 14 ºC and RH from 90% to 80% can reduce significantly the number of infected mushrooms (Nair & Macauley 1987). It appears that *A. bisporus* can survive better at a lower temperature than *V. fungicola*. The lowered relative humidity (and therefore less condensation of water on the mushrooms) may also have reduced the spread of the pathogen, because the presence of water is known to aid in the dispersal of *Verticillium* spores. However this method of control is unlikely to be feasible in modern mushroom farms.

Hygiene in and around the farm, is also very important for the control the dry bubble disease. The remnants of the compost, the casing soil and the waste mushroom stalks should be removed from the farm as soon as possible in plastic bags or in closed containers (Zaayen 1982). In addition it is equally important to control the mushroom sciarid flies because they contribute in the disease transfer from one crop to another.

Spore filters in ventilation openings, cleans tools, disinfected picking boxes and clean clothing, can reduce further the possibility of an infection.

5.4.2 Biological control

Gandy (1979) stated that more than 300 isolates of fungi and over 100 isolates of actinomycetes obtained from soils and vegetation had little effect on *V. fungicola* when tested in *vitro*. However, in the same study is reported that *Penicillium nigrans* (Brainer) Thom syn. (*P.janczewskii*) caused marked curling of *V. fungicola* due to griseofulvin production. It also demonstrated that several *Streptomyces spp* isolates and one Bacillus sp isolate, has also displayed some antagonism to the pathogen. But the strongest antagonists appeared to be Trichoderma spp often causing plasmolysis of *V. fungicola* mycelium as in the Trogoff & Richard (1976) study, in which *Trichoderma viride* spray was tested on commercial mushroom production. The compound Binab T (*T. viride, T. polysporum*) was applied in 2 and 5g/m^2 doses, one week after casing, plus another 5g/m^2 after the first flush. The trial was done in two growing rooms containing a healthy and a diseased crop inoculated with *V. fungicola* four days after the application of Binab T. The results showed a small portion of *Verticillium*-free mushrooms developed a small brown lesions which turned dark green, indicating weak parasitism by the *Trichoderma spp*. inoculant. In the diseased crop heavy infections of *V. fungicola* developed and there were no significant differences between the number and the weights of healthy mushrooms produced from treated and untreated plots.

5.4.3 Control with chemicals

Unfortunately, even when hygiene is strictly observed, the fly control is properly carried out and the casing used is pathogen-free, the disease still occurs. In that case it seems inevitable that the measures above should be combined with the use of chemicals in order to minimise the disease incidence. The introduction of benzimidazole fungicides in 1969 gave the mushroom industry the first reliable means of control of dry bubble (*Verticillium fungicola*) as well wet bubble (*Mycogone perniciosa*) and the cobweb (*Cladobotryum spp*). Holmes *et al*. (1971) showed effective control of *Verticillium* in mushroom beds using a benzimidazole fungicide. But *Verticillium* developed resistance to this group of fungicides (benomyl, carbendazim, and thiabendazole) fairly rapidly and by 1973 the dry bubble disease became a major problem to the industry once again (Russell 1984). The use of chlorothalonil as an alternative to the benzimidazole fungicides for the control of

dry bubble showed advantages over zineb and thiabendazole where pathogen populations were already resistant (Fletcher *et al*. 1983) but this no longer has label approval for mushrooms in the UK.

In the search for benzimidazole alternatives, Zaayen & Adrichem (1982) reported that prochloraz was a very effective fungicide against *Verticillium* disease. Also in Russell's experimental work (1984) prochloraz-manganese was confirmed as the most effective fungicide achieving 98% control of dry bubble at the rate of 1.5g a.i./m^2 (in addition to 99% control of wet bubble and 100% control of cobweb) and increasing the obtained yield by 55%. Two years later, Nair & Macauley (1987) found that all *V. fungicola* isolates tested, had low ED_{50} values (<2ppm) when treated with this fungicide. They also found that in the rate of 1.5g a.i./m^2 sufficient control of dry bubble can be achieved and in the same time, the levels of prochloraz residues in the sporophores were well below the official maximum residue limit (MRL) established for mushrooms in Australia, where the study was carried out. But more recently *Verticillium* isolates in Britain, exhibit different sensitivity to prochloraz-manganese and Grogan *et al*. (2000) tested the in vivo response of two isolates with different EC_{50} values (isolate 182 with EC_{50} 5.9ppm, and isolate 620 with EC_{50} 2.7ppm) to Sporgon 50WP (prochloraz-manganese). It was found that this fungicide gave good control of both isolates compared to untreated controls and also that the control was better in the more sensitive isolate (620) although this isolate was more aggressive in the absence of the fungicide. However, the levels of prochloraz dropped to 25% of what had been applied by day 28 after casing. This loss of prochloraz active ingredient from the casing layer combined with the aggressive behaviour of fungicide-sensitive isolates could be a serious problem in disease control in the British mushroom industry.

5.5 Discussion

According to Fletcher *et al*. (1983) the dry bubble disease caused *by Verticillium fungicola* (Preuss.) Hassebr., is the most important disease of the cultivated mushroom, *A. bisporus* in the United Kingdom. Today despite the strictly controlled growing environment, the extended hygienic measures, the use sophisticated equipment, and the use of fungicides, *Verticillium* continues to be the major pathogen affecting the British mushroom industry with estimated annual losses of 2-3 million (Grogan 2001). At present

much of dry bubble control relies on prochloraz despite the risks of resistance build up. For such an important crop in UK, *Verticillium* disease management is still necessary and in the same time disease research must continue towards all directions including alternatives to prochloraz.

5.6 References

Atkins, F. C., (1974). Guide to mushroom growing. Faber and Faber London.

Collopy, P. D., Largeteau-Mamoun, M. L. Romaine, C. P. Royse, D. J (2001). Molecular phylogenetic analyses of *Verticillium fungicola* and related species causing dry bubble disease of the cultivated button mushroom, *Agaricus bisporus*. *Phytopathology*. **91**: 9, 905-912.

Fletcher, J. T. Hims, M. J. Hall, R. J (1983) The control of bubble diseases and cobweb disease of mushrooms with prochloraz. *Plant Pathology*. **32**: 2, 123-131.

Gandy, D. G., (1973). Observations on the development *of Verticillium malthousei* in mushroom crops and the role of the cultural practises in its control. *Mushroom Science* **8**: 171-182.

Gandy, D. G., (1979). Investigations into biological control of *V. fungicola*. Annual Report of the Glasshouse Crops Research Institute. Glasshouse Crops Res. Inst,Littlehampton, Sussex, UK: 145-148.

Gaze, R.H., Fletcher J.T., (1975). ADAS survey of mushroom diseases and fungicide usage 1974/75. *Mushroom journal* No**35**, 370-376.

Geijn, J., van de, (1982). Fungal diseases: in practise. *The Mushroom Journal*. No **113**: 157-161.

Grogan, H. (2001). Screening new chemicals for *Verticillium* control. HDC report M33a.

Grogan, H. M., Keeling, C., Jukes, A.,(2000). A In vivo response of the mushroom pathogen *Verticillium fungicola* (dry bubble) to prochloraz-manganese. *The BCPC Conference: Pests and diseases*, Volume **1**: 273-278.

Holmes, J., Cole, H. Jr., Wuest, P. J.,(1971). Control of the *Verticillium* disease of the cultivated mushroom *Agaricus bisporus*, with benomyl spray applications ta cased trays. *Plant disease Reporter* **55**: 684-687.

Nair, N. G., Macauley, B. J., (1987). Dry bubble disease of *Agaricus bisporus* and *A. bitorquis*, and its control by prochloraz - manganese complex. *New Zealand Journal of Agricultural Research*.. **30**: 1, 107-116.

North, L. H., Wuest, P. J., (1993). The infection process and symptom expression of *Verticillium* disease of *Agaricus bisporus*. *Canadian Journal of Plant Pathology*.. **15**: 2, 74-80.

Russell, P., (1984). Sporgon on mushrooms. *The Mushroom Journal*.. No.**141**, 299-300.

Seth, P. K., (1977). Pathogens and competitors of *Agaricus bisporus* and their control. *Indian Journal of Mushrooms*. **3**:1, 31-40.

Sinden, J. W., (1971). Ecological control of pathogens and weed-moulds in mushroom cultivation. *Annual review of phytopathology* **88**: 143-154.

Trogoff, H. de, Richard, J. L., (1976). Biological control of *Verticillium malthousei* by *Trichoderma viride* spray on casing soil in commercial mushroom production. *Plant disease reporter* **60**: 8, 677-680.

Van Griensven, L. J. L. D., (1988). The cultivation of mushrooms. Rustington, W Sussex: Darlington Mushroom Laboratories.

Vedder, P. J. C., (1978). Modern mushroom growing. Culemborg, Netherlands: Educaboek / Cheltenham: Stanley Thornes.

Ware, W. M., (1933). A disease of the cultivated mushroom caused by *Verticillium malthousei*. *Annals of Botany* **47**: 763-785.

Wong, W. C., Preece, T. F., (1987). Sources of *Verticillium fungicola* on a commercial mushroom farm in England. *Plant Pathology*.. **36**: 4, 577-582.

Zaayen, A. Van., (1982). Fungal diseases. Literature and research. *The Mushroom Journal*. No **113**: 149-156.

Zaayen, A. Van., Adrichem, J. C. J., (1982). Prochloraz for control of fungal pathogens of cultivated mushrooms. *Netherlands Journal of Plant Pathology*.. **88**: 5, 203-213.

Zaayen, A. Van., Gams, W., (1982). Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. II. Pathogenicity. *Netherlands Journal of Plant Pathology*.. **88**: 4, 143-154.

Studies on fungicides behaviour in mushroom casing in relation to disease control

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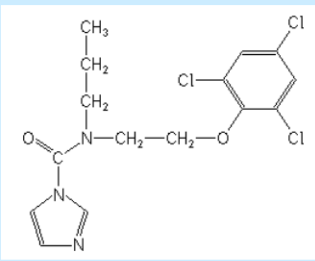
Introduction

Control of dry bubble disease (*V. fungicola*) relies heavily on prochloraz (Sporgon 50 WP) which is an imidazole demethylation inhibitor (DMI) fungicide, with protective and eradicant action. Recent HDC funded work (M14C, M30) showed that prochloraz levels in casing were significantly reduced by the end of the second flush and this may make disease control more difficult

Objective

- To determine the factors affecting the persistence of prochloraz in mushroom casing

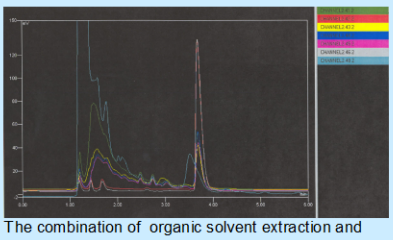


Prochloraz: [N-propyl-N-{2-(2,4,6-trichlorophenoxy)-ethyl}imidazole-1-carboxamide]

Dry bubble disease (*V. fungicola*) on mushroom crops

Materials and methods

- Prochloraz was applied to mushroom casing in a series of small scale experiments using glass jars, 4cm high columns and spawned compost
- Fungicide residues were extracted with acetonitrile and measured by HPLC. Solid Phase Extraction (SPE) cartridges were used to improve the separation of prochloraz residues
- Prochloraz was applied as Sporgon 50 WP on Day 3 and Day 21 to give concentration of 15ppm to each treatment (equivalent to 120g/100m^2)

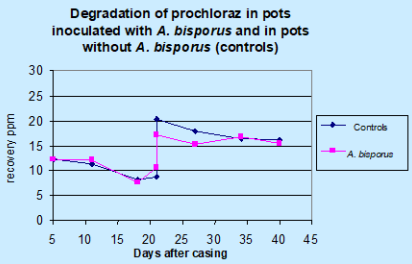


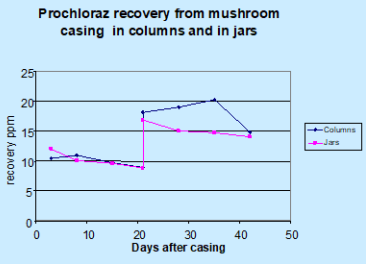
The combination of organic solvent extraction and use of SPE cartridges (samples 41-46) optimised the separation of prochloraz in the HPLC analysis, compared to acetonitrile extraction alone (sample 47)

Results

- Prochloraz was relatively stable with a slow degradation process and a half life (DT_{50}) always above 40 days
- The recoveries obtained from casing colonised with *A. bisporus* were not significantly different from control pots with no *A. bisporus*

Experiment to determine the effect of *A. bisporus* on prochloraz degradation in a controlled environment cabinet





Conclusions

- The introduction of the SPE cartridges in the analytical method improved the separation of prochloraz residues
- In all cases, prochloraz degraded slowly
- The presence of *A. bisporus* in the casing did not affect the degradation of prochloraz significantly

Future work

- Further investigation of prochloraz degradation processes in large scale experiments in HRI-mushroom unit
- Studies on disease management (*V. fungicola*) management

