Evaluation of two formulations of permethrin for use against adult *Lycoriella mali* (Fitch) (Diptera: Sciaridae) in commercial mushroom culture in New South Wales

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Summary

Two formulations of permethrin, a dust (10 g a.i. kg^{-1}) applied to the mushroom beds and trays and a smoke generator (4.25 g a.i. to treat 400 m^3), applied as a volume treatment, were evaluated independently against the mushroom sciarid, *Lycoriella mali* (Fitch). Registered treatments, pyrethrins dust and dichlorvos aerosol, applied in the same use patterns, were included for comparison. Both permethrin and pyrethrin dusts remained active over at least 2 days, whereas the generated smoke showed residual activity similar to that found when dichlorvos aerosol was used. The use of generated smoke resulted in lower residues, 0.01–0.07 mg kg^{-1}, than the dust, 0.03–0.3 mg kg^{-1}. There was no increase in residues in mushrooms following repeated applications of either formulation of permethrin.

Introduction

Larvae of *Lycoriella* species are the major insect pests of cultivated mushrooms for which insecticide applications are made (Clift and Larsson 1984; Snetsinger and Rinker 1980; White 1981).

Both insecticidal dusts (pyrethrins) and aerosols (often dichlorvos or pyrethrins) can be used against the adult stage (Atkins 1974). Uniform application of dusts is difficult and much of the insecticidal activity results from flies contacting the dust after it has settled (Hussey 1979). With aerosols the droplets remain suspended for longer than the dusts, killing most flies active at the time of application. Activity after the droplets settle provides residual effectiveness against *Lycoriella* species (Hussey 1979).

The pyrethroid permethrin was evaluated for use against adult *Lycoriella* species in England (Hussey 1979) and in the U.S.A. (Snetsinger and Rinker 1980). Both reports indicated knockdown of adult sciarids effective over at least 24 h when permethrin was applied as an aerosol. Daily applications of permethrin aerosol against early generation *L. mali* (Fitch) reduced subsequent generations by 90% and the yield of mushrooms in an untreated control was 18% lower than that of the treated room (Snetsinger and Rinker 1980). High initial knockdown and residual activity over 3 days was reported when an aerosol formulation of permethrin was evaluated against *L. auripila* (Winnertz) (White 1981).

Permethrin is now used against adult *Lycoriella* species throughout the mushroom-growing cycle. Formulations available are aerosol or dust in the U.S.A. (Keil 1987) and generated smoke or an aerosol in the U.K. (Fletcher *et al.* 1986).

Although the use of permethrin as an aerosol against *Lycoriella* species is well documented (Hussey 1979; Snetsinger and Rinker 1980; White 1981; Keil 1987), less has been reported on use of the dust (Keil 1987). The permethrin smoke generator has been cleared for use in mushroom culture in England (Fletcher *et al.* 1986), but the results have not been published. Therefore there is limited information to demonstrate the relative effectiveness of the available formulations of permethrin against *Lycoriella* species in cultivated mushrooms.

This paper reports the separate evaluations of two formulations of permethrin, the smoke generator and dust, against *L. mali* in New South Wales. The dust was applied directly to the beds and sides of the trays, whereas the smoke generator was used as a whole-room treatment. Registered standard treatments, pyrethrins dust and dichlorvos aerosol, applied in the same use patterns, were included for comparison.

Materials and methods

Mushroom culture

The mushroom culture system used in this project was located in an experimental facility at the Biological and Chemical Research Institute, Rydalmere, New South Wales. The permethrin dust and smoke were evaluated in two growing rooms each 3 m × 5 m long × 3 m high. The dusts were evaluated at the same time in one room. The generated smoke and standard aerosol were evaluated as whole room treatments. It was not possible to compare the two evaluations directly, but indirect comparisons were made to the respective treated and untreated controls.

Mushrooms were grown using commercially prepared compost held in plastic trays (0.2 m^2) containing 16 kg compost. Both rooms contained 64 trays distributed over four trolleys each with four tiers. Two weeks after inoculating the compost with a pure white strain of *Agaricus bisporus* (Lange) Imbach, the trays were covered with a 4-cm layer of moist neutralized peatmoss (the casing). Adults of *L. mali* were released into the rooms 1 day after inoculation. The sciarids had established in the trays and adults were emerging daily during the insecticide evaluations.

Harvesting of mushrooms started 20 days after casing and continued for 5 weeks. Following commercial practice, mushrooms were harvested down to the size of a ten-cent piece, leaving mushrooms present at the time of treatment. The mushroom beds were watered prior to the application of the dust, use of the smoke generator or the dichlorvos aerosol. Mushrooms were harvested either 24 or 48 h after application of permethrin. When a weekend intervened, mushrooms were harvested after 72 h. The samples for residue determination were placed in sealed plastic bags and stored at −16°C. Mushrooms treated with the dust were randomly selected. Those treated by the generated smoke were selected from each of the four tiers of the trolleys nearest the smoke generator as four separate samples.

Residue analysis

Mushroom samples from each treatment were chopped and mixed, then duplicate subsamples were taken for analysis. Samples (50 g) were blended with anhydrous sodium sulfate (60 g) in 20% v/v acetone/hexane (160 ml) and filtered into a 1-l separating funnel, using hexane to wash the solids. The extract was washed with distilled water (3 × 150 ml), leaving an upper hexane layer which was then filtered through sodium sulfate and evaporated to dryness in a rotary evaporator at 40°C. The residue was cleaned up by chromatography with 5 g of activated florisil topped with 3 g anhydrous sodium sulfate, using 10% v/v diethyl ether in hexane as the eluting solvent. The elution pattern for permethrin was established with standard material for each batch of florisil used and checked at regular intervals. Permethrin (both isomers) was eluted with 120 ml of the solvent. The extract was evaporated to dryness and made to 5.0 ml with hexane.

After clean-up, the extracted permethrin was analysed by electron capture gas chromatography using 5% SE 30 on 80/100 mesh Gas Chrom Q. Column, injector and detector temperatures were 210, 240 and 290°C respectively, with nitrogen (40 ml min^{-1}) as carrier. Under these conditions, permethrin gave a single peak at a retention time of approximately 6 min.

When applied to samples spiked with permethrin at levels ranging from 0.01 to 1.5 mg kg^{-1}, this method resulted in recoveries from 65% (<0.06 mg kg^{-1}) to 85% (>0.3 mg kg^{-1}). The limit of detection was 0.01 mg kg^{-1}.

Aerosol application

The Insectigas (50 g kg^{-1} dichlorvos) or Pestigas (4 g kg^{-1} pyrethrins plus 20 g kg^{-1} piperonyl butoxide) was applied using the high pressure hand gun and hose supplied with the cylinder. Two 5-s applications of Insectigas were sufficient to fill the room with a fine mist. The ventilation in the room was turned off prior to application and was turned on after 2 h to clear the room.

Smoke generator

To include a considerable safety factor for residue purposes, the smoke generator was used at five times the recommended rate (4.75 g per canister to treat 400 m^{-3}) and for efficacy tests at both twice (3.5 g per canister to treat 284 m^{-3}) and at five times the recommended rate. The two different sizes of smoke generator were used to achieve the two rates. Ventilation in the room was turned off prior to lighting the smoke generator. After 2 h the ventilation was turned on to clear the room. Four applications of the generated smoke, at the higher rate, over 2 weeks were made. Two control runs, using no treatments were then done.

In addition, evaluation of the smoke generator at the recommended rate was done on two commercial farms. The first, using one room, was to obtain samples of treated mushrooms for residue determination, the second, using three rooms, was to evaluate the treatment for initial knockdown of adult sciarids.

Efficacy of volume insecticide applications

The effectiveness of the volume treatments was assessed by counting sciarids knocked down onto 14 white plastic trays, each 13.5 cm × 26.5 cm placed around the walls of the room (8), on the casing surface (2) or around the growing trays (4). The same arrangement of white plastic trays was used to assess the control mortality. Assessments were made 3 h after each application of each insecticide and at 24 and 48 h. The trays were emptied out after the 48-h count, prior to the next application. After each application of insecticide, the room was examined carefully for the presence of sciarids that had survived treatment. Subsequent examinations assessed the effects of the treatment on sciarids emerging after application. The insecticides were applied three times per week on Mondays, Wednesdays and Fridays.

To determine knockdown times and the initial uniformity of the treatments, adults were confined in glass tubes (5 cm diam.) connected to a suction device to draw treated air over the test insects. The tubes were plugged with cotton wool at one end and mesh at the other. A tube was left on a mushroom bed on each of four tiers of the trolley. The test insects were examined every 5 min until complete mortality was obtained. The cotton wool plugs were analysed for permethrin after one of the applications. This test was done three times for the permethrin smoke generator, twice for the dichlorvos aerosol and once for the pyrethrins aerosol.

Dust application

The two dusts used were: Pybuthrin Grain Protectant Powder (3 g pyrethrins kg^{-1} and 16 g piperonyl butoxide kg^{-1}) as a standard treatment and Coopex Insecticidal Dusting Powder (10 g permethrin (75:25) kg^{-1}). The dusts were applied with a hand-held plunger duster at a rate equivalent to 10 g product m^{-2} to the casing layer, the mushrooms and to the sides of the plastic growing trays. To simulate wooden trays, as used in commercial farms wooden boards 10 × 30 × 1.5 cm thick were dusted and used to determine residual activity. Eight applications of the dust over 3 weeks were made during the trial. The left rear trolley was treated with Coopex Dust, the right rear trolley was treated with Pybuthrin and the other two trolleys were left untreated as controls. Plastic sheets were used to separate the trolleys during application of the dusts, which was done three times per week.

Table 1 Cumulative mortality of *L. mali* at various intervals after application of the indicated insecticides inside a mushroom-growing room

Treatment	Rate applied	Number adult flies killed per tray[A]		
		3 h	24 h	48 h
Pybuthrin dust (2)[B]	30 mg a.i. m^{-2}	1.38 ± 0.56[ns]	4.50 ± 1.79*	7.50 ± 0.87**
Permethrin dust (4)[B]	100 mg a.i. m^{-2}	1.20 ± 0.50[ns]	4.25 ± 1.18*	7.90 ± 1.45**
Control (2)[B]	–	0.38 ± 0.18	1.25 ± 0.38	2.97 ± 0.82
Dichlorvos aerosol (2)[B]	30 mg a.i. m^{-3}	2.34 ± 0.21**	2.80 ± 0.44**	3.73 ± 0.52*
Permethrin SG (3)[B]	36 mg a.i. m^{-3}	1.74 ± 0.47**	2.27 ± 0.48*	2.45 ± 0.67[ns]
Permethrin SG (3)[B]	60 mg a.i. m^{-3}	1.44 ± 0.25**	1.96 ± 0.33*	2.57 ± 0.34[ns]
Control (2)[B]	–	0.28 ± 0.16	0.78 ± 0.37	1.40 ± 0.68

[A] Based on mean count from each evaluation ± standard error.
[B] Number of applications evaluated.
[ns] Not significantly different from control.
* Different at the 0.05 level.
** Different at the 0.01 level.

Efficacy of dust applications

The effectiveness of the dust applications was assessed using the same white plastic trays from the assessment of the volume treatment. However, seven were used per treatment, three on the casing surface and four beneath the dusted wooden boards. These boards were located on the trolleys, next to the mushroom beds. The same arrangement was used in the control, except neither the beds nor the boards were dusted. Assessments were made 3 h after each dust application, and at 24 and 48 h. The white trays were emptied after the 48-h assessment, prior to the next application.

Statistical analysis of sciarid counts

The counts of sciarids knocked down into the plastic trays were variable between each insecticide application due to variation in daily emergence of sciarids from the mushroom beds. An analysis of variance procedure was used to remove variation between trials. The variation remaining within each treatment was used to determine the significance of the difference between each treatment and its respective control.

Results and discussion

The dusts had settled out within 15 min of application, whereas both aerosols and smokes required 2 h. The mean cumulative numbers of dead adult *L. mali* collected after each insecticide test together with those from their respective untreated controls at 3, 24 and 48 h after initial application are given in Table 1. Both Pybuthrin and permethrin dusts showed limited initial knockdown but considerable residual activity (Table 1). The difference between the cumulative knockdown associated with both dusts compared to that of the untreated control (Table 1) indicates that the dusts remained active over the 2 days tested.

All the visible *L. mali* adults were knocked down on both occasions when the dichlorvos fog was used and on five out of six occasions for the permethrin smoke generator. With both materials, considerable numbers of live adult *L. mali* could be seen within 1 day of use. The differences in numbers of sciarids collected 3 h after each insecticide was used reflects differences in numbers present rather than effectiveness. The trends, compared to the untreated controls are more meaningful. There was some residual activity from both the dichlorvos fog and permethrin smoke applications (Table 1). The level of residual activity is difficult to assess beyond noting that some newly emerged *L. mali* were killed by the deposit left after the smoke or aerosol had been used.

In U.K. tests, use of permethrin aerosol resulted in almost complete suppression of *L. auripila* adults for 2 days, with some live flies present on the third day (White 1981). This differs from our results using generated smoke in that, although effective knockdown of *L. mali* occurred, considerable numbers of recently emerged flies could be found within 24 h of treatment. The limited residual activity of dichlorvos applied as an aerosol can be explained by its rapid degradation in the moist alkaline conditions in mushroom sheds (Hussey and Hughes 1964).

Keil (1986) reported up to 92% control of *L. mali* using a permethrin dust in whole-room treatments. Our results were based on part-room treatments and so are not directly comparable. Hussey (1979) noted residual activity of pyrethrins dust similar to our results.

When used at the recommended rate in three commercial growing-rooms, volume

300–800 m³, the permethrin smoke generator provided >95% knockdown of the sciarid adults present. Most survivors were found resting on the undersides of the wooden trays. As previously noted, within 1 day after treatment, considerable numbers of *L. mali* could be found within the room. Both samples of mushrooms taken from one room had residues below 0.01 mg kg^{-1}.

Residue analyses on mushrooms treated with permethrin dust suggest that there is no increase in the levels of permethrin found after repeated applications directly onto the mushrooms (Table 2). This is best illustrated by the last three sets of data in Table 2 which show a progressive drop in levels from 0.3 to 0.09 mg kg^{-1} despite continued additions of dust. The marked decline in permethrin residues (from 0.2 after 1 day to 0.05 mg/kg after 2 days) following the fourth application was attributed mainly to dilution due to mushroom growth and, to a lesser extent, chemical degradation.

Table 2 Permethrin residues in randomly selected mushrooms at various time intervals between application and harvesting following each of eight applications of permethrin dust

Application number	Interval (days)	Permethrin[A] residue (mg kg^{-1})
1	2	0.03
2	3	0.08
3	2	0.08
4	1	0.2
4	2	0.05
5	3	0.1
6	2	0.3
7	2	0.2
8	3	0.09

[A] Means of duplicate analyses, corrected for recoveries.

Despite using five times the recommended rate, residues resulting from the smoke generator treatments (Table 3) again showed no obvious accumulation with multiple applications of permethrin. Samples were collected at 1- and 2-day intervals following both the first and fourth applications and in both instances there was a noticeable drop in residue levels over the 2-day period. Again, this was attributed to growth dilution and degradation of the chemical. There were no consistent differences in residues between samples from each of the four tiers of the growing trolleys. This suggests that the smoke generator produces a more uniform distribution.

A noteworthy difference in the two sets of residue data (Tables 2 and 3) was that the smoke generator generally produced much lower residues than the dust. The highest mean value obtained in the former case was 0.05 mg kg^{-1}, while dust samples exhibited mean residue levels as high as 0.3 mg kg^{-1}, with 50% in the range of 0.1 to 0.3 mg kg^{-1}. These differences, although not unexpected, highlight the practical difficulties in applying the chemical uniformly as a dust and demonstrate one advantage of the smoke generators.

Table 3 Permethrin residues in mushrooms following use of a permethrin smoke generator

Application number	Interval between last application and harvesting (days)	Mean[A] residues (mg kg^{-1})	Range (mg kg^{-1})
1	1	0.03	0.02–0.04
1	2	0.01	0.01–0.02
2	3	0.02	<0.01–0.07
3	2	0.02	0.01–0.03
4	1	0.05	0.02–0.06
4	2	0.03	<0.01–0.04

[A] Means of duplicate analysis of samples taken from each of four tiers. All values have been corrected for recoveries, the limit of detection being 0.01 mg kg^{-1}.

Permethrin residues in this study did not exceed 0.3 mg kg^{-1} (Tables 2 and 3) compared to values three times higher found by Ingratta and Braun (1981) for several pyrethroids on mushrooms. They were using EC formulations of the pyrethroids applied as drenches and so it is not surprising that they found higher residue values.

The times for complete knockdown of test *L. mali* exposed to permethrin smoke at five times the recommended level, and to dichlorvos and synergized pyrethrin aerosol at recommended rates are given as Table 4. The permethrin smoke generator produced an initially uneven treatment, showing shorter knockdown times on the top two tiers than to the lower two (Table 4). This difference is reflected in the permethrin levels found in the cotton wool plugs used in the bioassay (Table 4). The dichlorvos and pyrethrin fogs were considerably more uniform, but similar trends were evident (Table 4). Observations made in commercial growing-rooms treated with permethrin smoke also suggested uneven treatment of areas between trays next to walls.

In the U.S.A. *L. mali* has started to develop resistance to permethrin of between 8 and 50 fold (Brewer 1986; Keil 1987), resulting in control effectiveness of 65–92% (Keil 1987). The U.S.A. has a history of using DDT to control *L. mali*, which can confer cross-resistance to pyrethrins and pyrethroids (Brewer 1986). The use of permethrin aerosols (Snetsinger and Rinker 1980; Keil 1987), which seems to result in residual activity lasting 2–3 days (White 1981), may accentuate the problem. Denholm *et al.* (1983) found that, whereas regular use of persistent surface sprays of permethrin rapidly selected resistant *Musca domestica* L., less frequent use of non-persistent space spray did not.

In our work use of permethrin smoke at five times the recommended rate resulted in limited residual activity compared to that reported by White (1981) and also residue values in the mushrooms below 0.1 mg kg^{-1}. The use of the dust provided residual activity and higher residues, but still below 0.5 mg kg^{-1}. The provisional maximum residue limit for permethrin in mushrooms is 2 mg kg^{-1} with a 3-day withholding period (Commonwealth Department of Health 1986). The absence of increasing residues despite repeated applications of either formulation indicates medium-term persistence is not a problem. The work of Denholm *et al.* (1983) suggests that occasional use of permethrin smoke, rather than regular frequent use of the smoke or dust, is required to reduce long-term selection for resistance.

Our work used three applications of permethrin smoke per week. Fletcher *et al.*

Table 4 Time (min) to complete knockdown of test *L. mali* bioassay of whole-room space treatments using three insecticides

Level[A]	Permethrin residue in cotton wool plug	Knockdown time (min)		
		Permethrin smoke generator[B]	Dichlorvos fog[C]	Pyrethrins fog[C]
I (Top)	15600 mg kg^{-1}	7	<5	<5
II	20000 mg kg^{-1}	10	<5	<5
III	2800 mg kg^{-1}	25	10	10
IV	2000 mg kg^{-1}	35	10	10

[A] Four shelves holding the growing trays in the room.

[B] Based on three bioassays.

[C] Based on a single bioassay each.

(1986) does not believe two applications of an aerosol per week would be effective in the absence of other treatments. Snetsinger and Rinker (1980) used daily applications of permethrin EC to achieve 90% control of *L. mali*. However, we believe that effective control of *L. mali* is possible with three applications of generated smoke per week.

L. mali populations show distinct peaks early in the infestation of a culture room (Clift and Larsson 1984). It would be possible to time applications of the generated smoke during the first-generation emergence (10 days to 3 weeks after casing) eliminating most of those flies, with a consequent reduction of the next generation. The dust, having greater residual activity, could be used to knock down *L. mali* that gain entry while the mushroom mycelium colonizes the compost or casing. This would require two applications of the dust.

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