Contract Report M23

Determination of the extent and degree of organophosphorous resistance in mushroom farm populations of sciarids

Final Report December 1995

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Project Leader	Philip F White
Project Co-ordinator	Harold Linfield
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Determination of the extent and degree of organophosphorous resistance in mushroom farm populations of sciarids

by

Philip F White and Jane E Smith

Horticulture Research International - Wellesbourne

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The objectives of project M23 were to establish the current pattern of (i) sciarid resistance to an organophosphorous (OP) insecticide; and (ii) species distribution within the mushroom industry. Knowledge of the pattern of OP resistance will aid insect pest control strategies as, from each grower's point of view, it would be extremely advantageous to know whether his sciarid population was OP-resistant or not. If susceptible, the sciarids could be treated successfully with relatively low-cost OP's. Whereas, if resistant, the grower would not waste time, money and effort on an ineffective treatment. A further objective was to fine-tune the resistance testing techniques from an existing well established base, so that the HRI Mushroom Clinic will be able to offer OP resistance testing to individual growers who have recalcitrant pest problems.

Summary

Pests are a constant threat to the successful production of mushrooms, as uncontrolled populations of any of the main mushroom pests can result in substantial losses in yield due to both direct larval action and associated disease spread by adult flies. Mushroom sciarids are the major pest and are capable of causing complete crop failures. Diazinon is the most frequently used of the five organophosphorous (OP) insecticides approved for its control. Work in 1986 showed that mushroom farm populations were variously resistant to diazinon. Changes in population type, agronomic practices and pesticide usage have all contributed to a change in the pattern of resistance since then. Without a knowledge of the degree of sciarid-resistance to diazinon, a grower will not be able to effectively protect his crop.

One hundred and seventeen farms were contacted to establish a pest occurrence/pesticide usage database. Sciarid populations were obtained from twenty of these farms, based on geographical position and pesticide usage, and tested for resistance to diazinon.

The main findings of this project were:

- All sciarid populations were resistant to diazinon.
- There was a wide variation in the degree of resistance
- With any farm population, the maximum kill achieved at the approved rate of diazinon (50 ppm) was only 69%.
- Increasing the concentration four-fold to 200 ppm only achieved 100% control in one farm population.
- There was no apparent correlation between age of farm and resistance.
- There was no apparent correlation between degree of OP usage and resistance.
- Overall resistance to diazinon has increased significantly since a similar study in 1986.
- On the farms tested (with a few exceptions), diazinon would be of very limited use for the control of mushroom sciarids.
- Growers should review their use of diazinon for sciarid control

1 INTRODUCTION

1.1 Project background

At the time of the project submission, five organophosphorous (OP) insecticides were approved for the control of larval and adult stages of the mushroom sciarid. The larvicides are: chlorfenvinphos, diazinon, malathion and pirimiphos-ethyl; with dichlorvos being, at present, the only approved adulticide. A further OP adulticide, pirimiphos-methyl, is in the process of being added to the approved list. Of these insecticides, diazinon is the most frequently used.

Resistance by sciarid larvae to chlorfenvinphos was first noticed in the 1970s, with cross-resistance by adults to dichlorvos being demonstrated in the early 1980s. From work done in 1986, when populations of sciarids from a small number of geographically-distributed mushroom farms were tested, it was found that there was also larval resistance to diazinon - with a wide variation in resistance level. Diazinon resistance has also been reported in a number of other countries including the USA. Changes in population type, agronomic practices and pesticide usage and even species, will all have contributed to a change in the pattern of resistance since the 1986 study. Without a knowledge of the degree of sciarid-resistance to diazinon, a grower will be disadvantaged with regard to efficient crop protection.

From each grower's point of view, it would be extremely advantageous to know whether his sciarid population was OP-resistant or not. If susceptible, the sciarids could be treated successfully with relatively low-cost OP's: for example, the current cost of incorporating diazinon in the compost at spawning is 19p/square metre (approx.) compared to 28p/square metre (approx.) for incorporating diflubenzuron in the casing (excluding labour costs). If a sciarid population is resistant, however, with the grower wasting time, money and effort on an ineffective treatment, the superfluous use of such compounds could be eliminated.

1.2 The project

The aims of the project were to establish the current pattern of (i) sciarid diazinon resistance and (ii) species distribution within the mushroom industry by obtaining sciarid populations from 20 geographically-distributed mushroom farms. Knowledge of the pattern of diazinon resistance will aid insect pest control strategies. The project will also fine-tune the resistance testing techniques from an existing well established base, so that the HRI Mushroom Clinic will be able to offer OP resistance testing to individual growers who have recalcitrant pest problems.

2 MATERIALS AND METHODS

2.1 Farm populations

One hundred and seventeen commercial mushroom farms were contacted to ascertain whether sciarids were present and these are indicated in Figure 1. From those farms that reported having a problem, a number were selected based, where possible, on geographical position and the farm's pesticide-use history. Such farms were visited to collect female flies for subsequent egg-laying. If possible, sufficient females were collected so that resistance testing could be carried out on their immediate larval offspring. This obviated subsequent rearing of populations - a time-consuming process which might have affected any resistance that the population may have acquired. Occasionally, insufficient numbers of flies were present on a farm at the time of the visit. In such cases, populations were bulked-up for one generation only.

The period of the initial telephone survey was from April to May 1995.

2.2 Resistance testing

For each farm population, the following protocol was used. Twenty-five grammes of moist peat:soya-flour medium (20:1), within a 170 ml plastic pot, was treated with a liquid

diazinon formulation (Diazitol Liquid, containing 17% diazinon) to produce one of five treatment concentrations: 12.5, 25, 50, 100 or 200 µg a.i./g (≡ ppm). A concentration of 50 µg a.i./g is equivalent to the approved label rate of 50 ppm diazinon. A further treatment, using the label rate of Dimilin (30 µg diflubenzuron/g), was also used for comparative purposes. There were three replicates of each treatment, with six untreated controls. Twenty sciarid eggs were pipetted into each pot which was then covered with a ventilated lid and placed in an incubator at 24°C. Subsequent emergence of adults was recorded and used to determine the degree of diazinon resistance.

2.3 Sciarid species

To ensure proper comparisons between farms, adult flies from each farm were preserved for subsequent species identification.

3 RESULTS

3.1 Telephone survey

The pest and pesticide history of the 117 farms contacted were determined and are shown in Table 1. Pest status was designated as being 'none' (flies absent); 'some' (a minor or intermittent problem); or 'many' (a major or continuous problem).

Although this table represents only a 'snapshot' of insecticide usage, a number of statistics can be gleaned from it. Sciarids were a pest on 74% of the farms contacted, compared to 22% for phorids. This confirms the view that sciarids are still the dominant insect pest.

Forty-nine percent of the farms used diazinon (53% including other OPs), which probably represents a higher figure than expected. In those farms which reported diazinon usage alone, sciarid populations were, with one exception, either totally absent or present in significant numbers - the ratio being about 50:50.

Where one of the insect-growth-regulator-type insecticides was used (Dimilin or Apex), either alone or in combination with an OP, the majority of farms (61%) fell into the 'some' category, with 18 and 21% in the 'none' and 'many' categories respectively. The reduction in the number of farms with a significant sciarid population would be expected as these compounds should exert more control over diazinon-resistant sciarids. The reduction in the number of sciarid-absent farms is, however, the opposite to what might be expected.

3.2 Farms visited

Sciarid populations from 20 farms were obtained over a 3 month period. These farms are shown in Figure 2. The farm numbers shown in the figure are not chronological but relate to the degree of resistance (see 3.3 below) and this is indicated further by the increase in size of the shaded circle - the bigger the circle, the more resistant are the sciarids to diazinon.

It was not possible to get populations from all geographic areas e.g. East Midlands, North-East England and North-West Scotland: some had no mushroom farms at all, whereas others had farms but these were not infested with sciarids at the time of the telephone survey. Twenty-eight farms were actually visited to provide the required number of populations. Some farms had 'phantom' populations which seemed to be present at the time of phoning but had disappeared by the time of the visit; and on other farms, it was apparent that there was an apparent confusion regarding identification!

3.3 Resistance

For each farm population, the numbers of flies emerging from each treatment was used to ascertain the level of resistance to diazinon. This was done by determining the LC_{50} for each population *i.e.* the concentration (C) of diazinon which was lethal (L) to 50% of the population - the higher the LC_{50} value, the more resistant the population. These values are shown in Table 2. It is also normal to determine the LC_{95} but this was not possible to do with any confidence as, in almost half of the cases, the LC_{50} values actually exceeded the highest concentration. Instead, two other values are shown in Table 2. These are the

expected percentage mortalities at 50 ppm (the approved label rate) and 200 ppm (the top concentration).

From Table 2 it can be seen that the LC_{50}s range from 10.1 to almost 12,000 ppm, possibly indicating that Farms 1-6 may be susceptible to diazinon. However, although these farms have LC_{50}s less than 50 ppm, the maximum mortality at this concentration for any population was only 68.7%. This rises to 100% in only one farm (6) at 200 ppm - 4 times the approved rate. In this laboratory test, a susceptible population would be expected to have 100% mortality at 25 ppm or less. These results, therefore, demonstrate that ALL of the farms tested were resistant to diazinon, although there was a wide variation in the degree of resistance. Growers at Farms 1-6 might still envisage using diazinon as a supplementary control aid but probably should not rely on it to any great extent.

There appears to be little correlation between current diazinon usage and the degree of resistance. For example the farm with the lowest LC_{50} used diazinon, whilst that with the highest, although having a long history of diazinon usage, has not used it for 5 years. Similarly, there is little correlation between age of farm and degree of resistance, with the two oldest farms tested being at opposite ends of the resistance spectrum and one of the youngest farms (9) having an LC_{50} in excess of 100 ppm.

3.4 Comparison to 1986 study

It is interesting to compare the degree of resistance found in this study to that of a previous study carried out in 1986. In the 1986 study, fewer farms were visited and tested although they were still geographically-distributed. The results from this survey are reproduced in Table 3 and Figure 3.

From Table 3 it can be seen that, although there was still a variation in resistance, the extent and degree of resistance was very much less than has occurred in this current study. For example, populations on Farms 1 - 3 could be said to be genuinely susceptible, whilst the highest LC_{50} was only 109.9 ppm. In addition, four further farm populations gave 100% mortalities at all concentrations tested (50 ppm and above). Figure 3 shows the

position of the 1986-study farms: as with Figure 2, the farms are listed in increasing order of resistance and with increasing size of shaded circle - the 'scale' of the circles in each figure being equivalent. The only farm to be tested on both occasions was farm 11/13 (1986/1995). This farm had an LC_{50}<50 ppm (100% kill at this lowest-used concentration) in 1986, compared to 240 ppm in 1995 - at least a 5-fold increase.

3.5 Species distribution

Identification of sciarids to species level is notoriously difficult and is mostly based on the reproductive structures on the male abdomen. Samples of male flies were sent away to a recognized authority on Sciaridae for identification. His report is currently awaited, so species identification will be described in a subsequent addendum.

4 CONCLUSIONS

- All sciarid populations were resistant to diazinon.
- There was a wide variation in the degree of resistance
- With any farm population, the maximum mortality achieved at the approved rate of diazinon (50 ppm) was only 68.7%.
- Increasing the concentration four-fold to 200 ppm only achieved 100% control in one farm population.
- There was no apparent correlation between age of farm and resistance.
- There was no apparent correlation between degree of OP usage and resistance.
- Overall resistance to diazinon has increased significantly since a similar study in 1986.

- With the possible exception of Farms 1-6, diazinon would be of very limited use for the control of mushroom sciarids.

- Growers should review their use of diazinon for sciarid control

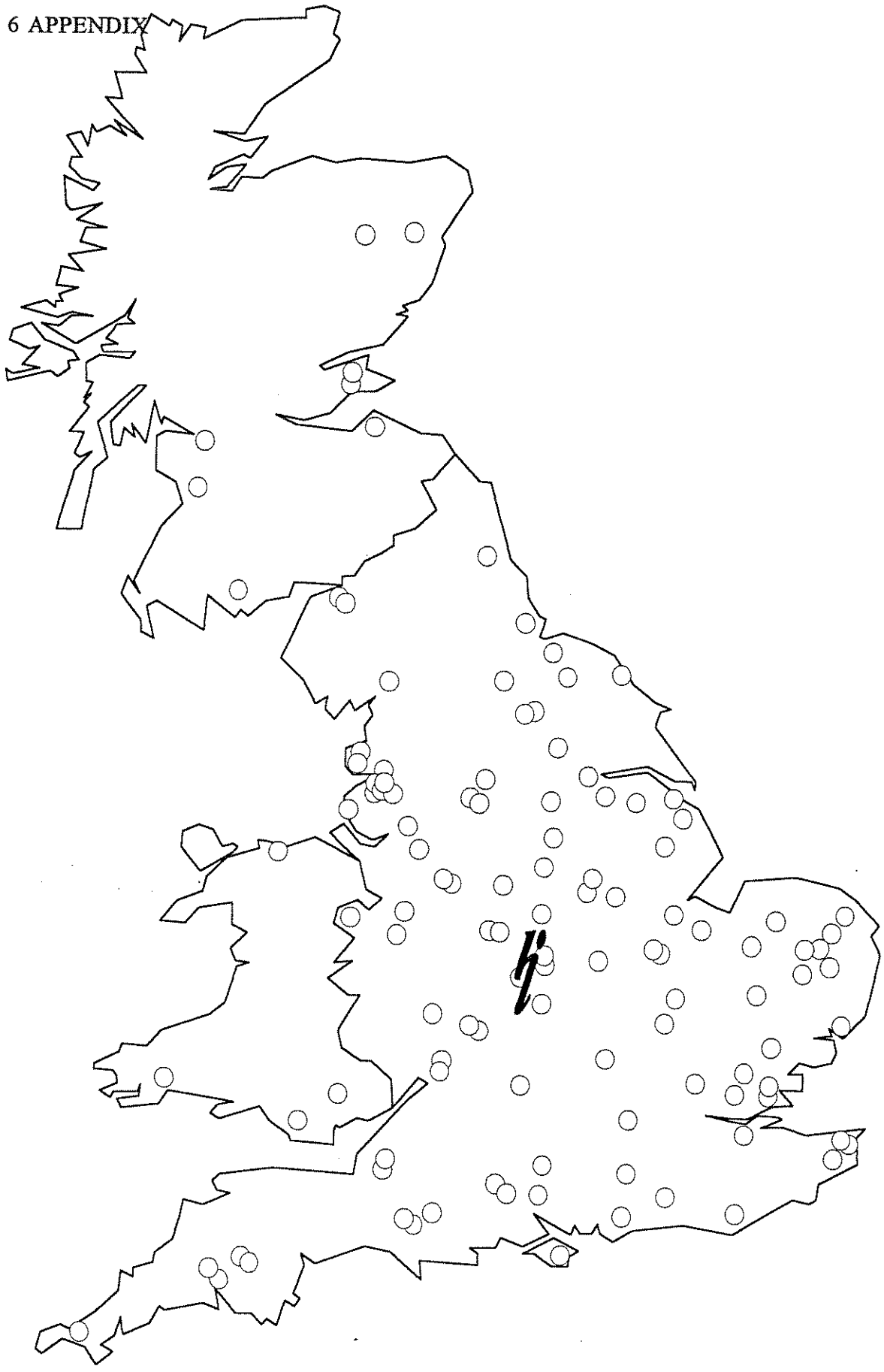

Figure 1. Location of farms - telephone survey

Chemical Usage	Sciarid problem category			Phorids
	None	Some	Many	
Diazinon	14	1	12	0
Diazinon/Dimilin	7	16	5	3
Diazinon/Apex	1	0	1	0
OP*/Dimilin	1	2	2	3
Dimilin	3	18	4	8
Apex	1	6	0	5
Dimilin/Apex	1	5	4	4
Nemasys	0	4	6	3
Unknown	3	0	0	0
TOTALS	31	52	34	26

Table 1. Chemical usage and pest status of 117 farms contacted during telephone survey.
* OPs other than diazinon.

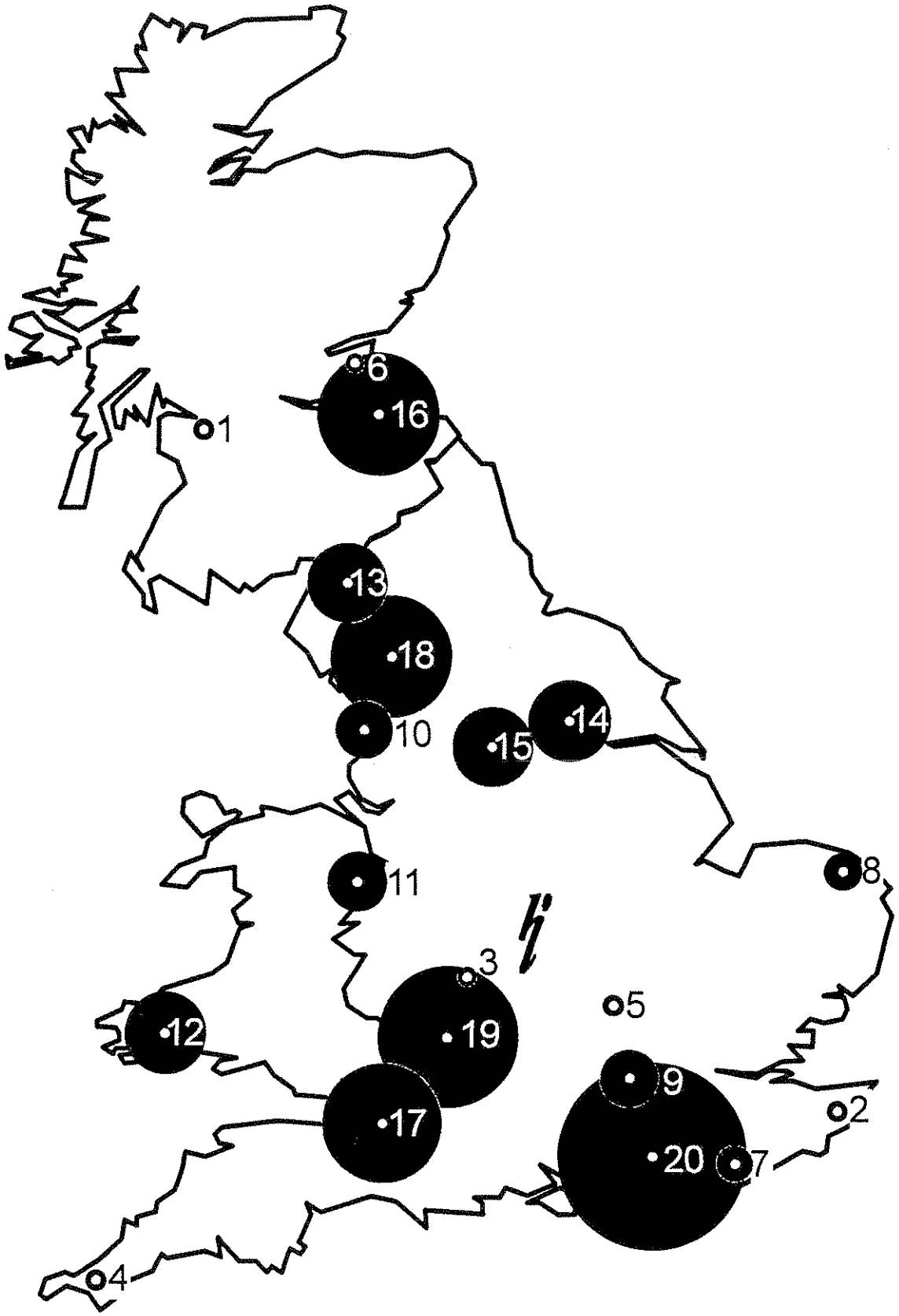

Figure 2. Location of farms from which sciarid populations were obtained for resistance testing - 1995 survey. The higher the number and the larger the shaded circle, the greater the resistance to diazinon.

Farm	Age of farm (years)	Diazinon use	LC_{50} (ppm)	Percent kill @ 50 ppm	Percent kill @ 200 ppm
1	12	Yes	10.1	68.7	85.0
2	50	No	13.3	64.7	79.0
3	2	Yes	13.5	65.4	81.7
4	18	Yes	15.0.	68.1	88.9
5	44	Recently	26.2	63.1	91.2
6	40	Yes	28.8	64.4	100
7	40	Yes	86.2	43.1	60.7
8	24	Yes	94.6	40.3	61.4
9	2	Yes	100.5	37.0	62.8
10	8	Yes	133.0	35.7	56.0
11	3	Yes	157.5	41.4	51.8
12	13	Recently	223.3	29.6	48.5
13	12	No*	240.3	30.0	47.7
14	20	No**	295.5	19.0	43.2
15	6	Yes	298.9	25.6	44.5
16	6	Yes	874	28.7	39.0
17	4	Recently	1077	37.6	43.2
18	8	Yes	1188	27.4	30.3
19	25	No*	1691	21.3	32.6
20	70	No***	11914	31.7	36.3

Table 2. LC_{50}s and percentage mortalities at 50 and 200 ppm for the 20 farms tested. The number of years mushroom production is also shown. See Fig.2 for farm locations. *, malathion users; **, had used malathion up to 1 year ago; ***, stopped diazinon 5 years ago after long history of use.

Farm	Diazinon use	LC_{50} (ppm)	LC_{95} (ppm)
1	No	0.8	14.1
2	No	4.4	85.1
3	Yes	10.4	36.3
4	Yes	57.5	124.5
5	Yes	67.3	195.0
6	Yes	91.0	171.4
7	Yes	109.9	193.6

Table 3. LC_{50}s and LC_{95}s of farms tested in 1986.

Figure 3. Location of farms from which sciarid populations were obtained for resistance testing - 1986 survey. The higher the number and the larger the shaded circle, the greater the resistance to diazinon. Circle sizes are on same scale as Fig.2.

Contract between HRI (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project

1. TITLE OF PROJECT

Contract No: M23
Contract date: 13.2.95

Determination of the extent and degree of OP resistance in mushroom farm populations of the sciarid *Lycoriella auripila*

2. BACKGROUND AND COMMERCIAL OBJECTIVE

Five organophosphorous (OP) insecticides are currently approved for the control of larval and adult stages of the mushroom sciarid, *Lycoriella auripila*. The larvicides are: chlorfenvinphos, diazinon, malathion and pirimiphos-ethyl; with dichlorvos being, at present, the only approved adulticide. A further OP adulticide, pirimiphos-methyl, is in the process of being added to the approved list. Of these insecticides, diazinon is the most frequently used. The Pesticide Safety Directorate has recently scrutinized the use of these products and there appears to be no impediment to the future use of these chemicals by the mushroom industry.

Resistance by larvae to chlorfenvinphos was first noticed in the 1970s, with cross-resistance by adults to dichlorvos being demonstrated in the early 1980s. From work done in 1986, when populations of sciarids from a small number of geographically-distributed mushroom farms were tested, it was found that there was also larval resistance to diazinon - with a wide variation in resistance level. Diazinon resistance has also been reported in a number of other countries including the USA. Changes in population type, agronomic practices and pesticide usage will all have contributed to a change in the pattern of resistance since the 1986 study. Without a knowledge of the degree of sciarid-resistance to diazinon, a grower will be disadvantaged with regard to efficient crop protection.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

From each grower's point of view, it would be extremely advantageous to know whether his sciarid population was OP-resistant or not. If susceptible, the sciarids could be treated successfully with relatively low-cost OP's: for example, the current cost of incorporating diazinon in the compost at spawning is 19p/square metre (aprox.) compared to 28p/square metre (aprox.) for incorporating diflubenzuron in the casing (excluding labour costs). If a sciarid population is resistant, however, with the grower wasting time, money and effort on an ineffective treatment, the superfluous use of such compounds could be eliminated.

4. SCIENTIFIC/TECHNICAL TARGET

This project will establish the current pattern of sciarid OP resistance and species distribution within the mushroom industry and will significantly extend the earlier survey made in 1986. Knowledge of the pattern of OP resistance will aid insect pest control strategies. The project will also fine-tune the resistance testing techniques from an existing well established base, so that the HRI Mushroom Clinic will be able