Project Title	Protected lettuce: Generating decline curves for a range of pesticides under protection.
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Project leader:	Ms C Lambourne Plant Pathologist Stockbridge Technology Centre Cawood, Selby North Yorkshire YO8 3TZ
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Key staff:	Ms Carole Rockliff
Location of project:	Stockbridge Technology Centre Ltd
Project coordinator:	Mr Geoffrey Smith Mores Lane, Pilgrims Hatch, Brentwoood, Essex, CM14 5RZ
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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Ms C Lambourne
Project Manager
Stockbridge Technology Centre

Signature .... Date ....

Dr G M McPherson MBPR (Hort.)
Director – Plant Pathology
Stockbridge Technology Centre

Signature .... Date ....

Stockbridge Technology Centre Ltd
Cawood, Selby
North Yorkshire
YO8 3TZ

Tel: 01757 268275
Fax: 01757 268996

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Headline

- All three pesticides tested in this project showed an overall declining trend with time during low light conditions in a protected winter lettuce crop. Residue levels for iprodione (Rovral) and cypermethrin (Toppel 10) remained below their respective MRLs throughout the period of the trial. The residue levels for pirimicarb (Aphox) were high following application but declined rapidly however there was a slight exceedance of the MRL (0.1 mg/kg) at the 14 day harvest interval.
- The residue analysis for a fourth pesticide, propamocarb (Filex) did not show a clear decline curve as time elapsed however, results were variable and should be interpreted with caution.

Background and expected deliverables

The Pesticide Residue Committee (PRC) carry out regular independent surveillance monitoring of food commodities from both the UK and abroad to monitor produce and ensure that the food reaching our supermarkets is free from unacceptable residues and is safe to the consumer. In recent years there has been an unacceptable number of residues of approved and also one or two unapproved pesticides found on lettuce, particularly on winter grown lettuce and this has led to repeated enforcement monitoring in winter lettuce to try and bring about an improvement in the situation.

The results from pesticide residue monitoring programmes are published each year and include the names of retailers where produce has exceeded the MRL. Such 'naming and shaming' of retailers who are selling products with residues exceeding permitted MRLs incurs unacceptable publicity, and therefore the retailers are placing tighter restrictions on their suppliers to both minimise pesticide usage and demonstrate compliance with MRLs to try and maintain public confidence in the products they sell. Many of the large multiples are being more proactive and are setting their own standards with respect to pesticide use on food crops. Many supermarkets carry out their own pesticide residue analyses, particularly on fresh produce. They also form close relationships with their growers and help them to implement 'good agricultural practice' and follow the Assured Produce protocols to minimise pesticide usage.

The Assured Produce group commissioned two reports (Phase I and II) focusing on 'minimising pesticide residues in fresh produce supplied by assured produce growers' during 2005. Recommendations within these reports suggested that greater knowledge with regard to the decline curves for a large range of pesticides should be investigated further. This preliminary investigation was carried out to determine the fate of the active ingredients in four pesticides commonly used on winter lettuce crops in the hope that the data could be used by pesticide manufacturers, industry regulators, growers and their advisors/consultants to reduce the incidences of breaches of accepted residue levels on lettuce.

Summary of the project and main conclusions

Two plots of a short-day variety of lettuce - cultivar 'Brian' were sown in late September and planted at Stockbridge Technology Centre in late October 2005. When the crop was approximately one month away from maturity one fungicide and one insecticide were applied to each plot. Plot 1 was sprayed with Filex (propamocarb HCl) and Toppel 10 (cypermethrin) and plot 2 was treated with Rovral (iprodione) and Aphox (pirimicarb). All products used were applied at the manufacturers or SOLA label rate using a battery powered knapsack sprayer and boom at a constant pressure of 2 Bars. Samples of lettuce were collected 0, 1, 3, 5, 7, 14 and 28 days after application and sent to an accredited laboratory for residue analysis using a multi-residue screen where possible. A separate test was conducted for propamocarb which could not be recovered through the multi-residue analysis.

The results of the analysis for iprodione, pirimicarb and cypermethrin indicate a clear declining trend of the active ingredient as it is degraded presumably by a combination of microbial and photolytic (explain?) activity. The data for iprodione and cypermethrin show that at no time during the 28 day sampling period did the residue of their respective active ingredients exceed the MRL. The analytical data for pirimicarb shows that the residue was high following application, but dropped rapidly over the sampling period. However, there was a very slight exceedance (0.1mg/kg) of the MRL for pirimicarb (set at 1mg/kg in lettuce) by the time the 14 day harvest interval was reached. This information is important as it indicates that there is no leeway in the harvest interval time and that under low light winter conditions a breach of 1 or more days in the harvest interval could lead to an unacceptable exceedance of the MRL.

The results of the analyses for propamocarb were very variable with no clear downward trend of the active ingredient over the sampling period. A large peak in the residue was seen 3 days after application of the product, although the recorded level dropped and was measured at 10mg/kg (how does this relate to the MRL?) in the sample collected 28 days after application. It was not clear whether the variability in the residue levels was a result of the movement of the chemical within the plant, or was caused by problems with the analytical assay and it is recommended that this work is repeated to see if a more stable decline curve can be generated.

This relatively small-scale experiment has therefore been valuable in defining the rate of degradation of three of the selected pesticides during low light winter conditions on protected lettuce and should help ensure the industry avoids further MRL exceedances. However, it is recommended that further data is generated in different seasons to further validate the results.

Financial benefits

The objective of this investigation was to provide new information on the fate of specific pesticides in the glasshouse environment following application to winter lettuce. There are no immediate financial benefits for HDC members, however greater knowledge and understanding may help growers to implement pesticide application practices which lead to reductions in residue findings for tested produce.

More extensive and replicated studies would provide more information and may then lead to industry recommendations which may have a financial benefit for members in terms of reductions in pesticide usage, and increased confidence in the supply base.

Action points for growers

- The data generated from this study does indicate that it is vitally important for growers to adhere very closely to pesticide label recommendations and instructions. Failure to do so may lead to the detection of pesticide residues at or above MRL levels following routine surveillance and/or enforcement monitoring.
- In particular, care should be taken to ensure the harvest internal following application of pirimicarb is not breached.

Science Section

Introduction

Increased consumer awareness of the use of pesticides in food production has put growers, producers and supermarkets under increased pressure to provide fresh produce that is either free from pesticide residues or that does not exceed the established maximum residue levels (MRLs). At present, growers do not have ready access to information on the rate of decline of pesticides post-application and rely entirely on the manufacturers label recommendations in terms of the rate of application and pre-harvest intervals. Yet, if they are to minimise the risk of residues, especially those below the MRL, more information about the products that they are using and the fate of the active ingredients, especially the rate of breakdown, in the various products used would be extremely valuable.

The aim of this initial, short-term project was to investigate the rate of decline of four pesticides (2 insecticides and 2 fungicides) commonly used by the glasshouse lettuce industry. The products were applied to a flat or butterhead lettuce crop grown to a commercial standard. Samples of lettuce were collected over a 28 day period and analysed, primarily using a multi-residue screen[1], to monitor the fate of each of the active ingredients and assess the rate of degradation of the active ingredients relative to the prevailing climatic conditions.

Where naturally occurring pest and disease problems occurred they were observed and assessed to try and relate product efficacy with persistence of the active ingredients. However, as these data were likely to be somewhat limited, information on efficacy provided by the manufacturers of the products under investigation during the decline period has been included, where available.

Materials and Methods

Location

This single unreplicated study was carried out between October and March 2005/6 at Stockbridge Technology Centre Ltd in North Yorkshire.

Crop

Lettuce (*Lactuca sativa L var capitata*) – butterhead cultivar 'Brian'. Two plots of lettuce each approximating to 8m^2 were planted.

Treatments

Plot No.	Product	Active Ingredient	Rate of Product applied/ha	Water rate (l/ha)
2	Rovral WP	iprodione	0.5kg	1000
1a	Filex	propamocarb HCl	20.5l	1000
1	Toppel 10	cypermethrin	0.25l	1000
2	Aphox	pirimicarb	0.5kg	1000

[1] Propamocarb cannot be included in a standard multi-residue screen for technical reasons and therefore a separate analysis was conducted for this active ingredient.

A tank mix of either Rovral & Aphox or Filex & Toppel 10 were applied to each plot on one occasion when the crop was approximately 28 days pre-harvest. This application timing was used because the harvest interval for Rovral on winter lettuce is 28 days. The products were applied using a boom attached to a battery operated knapsack sprayer with an application pressure of 2 Bar. The boom width was 1.4m and was fitted with 3 nozzles, BCPC code F110/0.8/3.

Sampling
Samples of lettuce were collected on days 0, 1, 3, 5, 7, 14 and 28 following application. The lettuce heads were excised at soil level, and lowest leaves were removed. Fresh gloves and knife were used for each sample to reduce the risk of cross contamination. The day 0 sample was collected in the afternoon following application in the morning to ensure that the crop was dry. A total of 5, randomly selected, lettuce were collected from each plot and double bagged in labelled polythene bags. On the majority of sampling dates, the lettuce were frozen (-20˚C) overnight, prior to being packed in insulated boxes with ice-packs and couriered to the analysing laboratory (NRM, Bracknell). The only exception to this was the 3 DAT sample which was collected by courier and transported overnight to directly to an NRM employee's home (delivered on a Saturday) where it was frozen prior to being delivered to NRM on Monday.

A separate representative sample of lettuce was collected at each sampling date for measurement of mean head weight and mean leaf area.

Environmental data (temperature, humidity and sunshine hours) were recorded throughout the trial and are included in Appendix 1.

Crop Diary

28.09.05	Lettuce sown – cv 'Brian'
25.10.05	Lettuce planted
09.12.05	Pest and disease assessment carried out
04.01.06	Pest and disease assessment carried out
17.01.06	Pesticide applications carried out
17.01.06	0 DAT sample collected and frozen
18.01.06	0 DAT sample sent for analysis, 1 DAT sample collected and frozen
19.01.06	1 DAT sample sent for analysis.
20.01.06	3 DAT sample collected and sent by overnight courier
22.01.06	5 DAT sample collected and frozen
23.01.06	5 DAT sample sent for analysis
24.01.06	7 DAT sample collected and frozen
25.01.06	7 DAT sample sent for analysis
31.01.06	14 DAT sample collected and frozen
01.02.06	14 DAT sample sent for analysis
09.02.06	Pest and disease assessment carried out
14.02.06	28 DAT sample collected and frozen
15.02.06	28 DAT sample sent for analysis
27.02.06	Analysis results for Rovral, Aphox and Toppel 10 received
10.03.06	Analysis results for Filex received.

DAT – Days after treatment

Pest & Disease Surveillance

Pest and disease assessments were carried out in early December, January and February following the observation of any naturally occurring infections and infestations. During the final assessment on the 9th February the following disease severity scale was used to assess *Sclerotinia* and *Botrytis* infections.

0-3 Disease Severity Scale

0 = No disease present
1 = Slight infection, bottom leaves affected
2 = Moderate infection, upper and bottom leaves affected
3 = Severe infection, whole plant collapse.

Aphid infestation was recorded as the number of aphids/plant. A total of 25 plants within a 'picture frame' of the plot were assessed.

Results

The residue data for the 2 fungicides and 2 insecticides is shown in Figures 1 and 2 overleaf (the results of the residue analysis of propamocarb appear somewhat anomalous and have therefore been shown on a separate chart). Full data sets are shown in Appendix 2.

The results of the analyses shown in Figure 1 all show a steady declining trend (with slight inconsistencies particularly around the 1 day after treatment [DAT] sample). Details of the agreed harvest intervals and Maximum Residue Levels (MRLs) are shown in Table 1.

It was not possible to gather useful data with regard to expected or observed efficacy of the products under investigation either from pest and disease assessments during the trial, or from data gathered from the product manufacturers. This was primarily due to only low levels of naturally occurring infestation. Where *Botrytis* did occur it was seen in both plots during early assessments (prior to the pesticide application), the incidence of the infection remained the same in the plot treated with Rovral and increased in the other plot (with no Botryticide application). This data does suggest that iprodione had maintained its efficacy throughout the 28 day post application, pre-harvest period.

Table 1. MRLs and harvest interval data (Source: PSD website & Liaison)

Product	Harvest interval	MRL (mg/kg)
Rovral WP	28 days (winter lettuce)	10.0 (UK/EC)
Filex	14days	10.0 (Codex)
Toppel 10	1 day	2.0 (UK/EC)
Aphox	14 days	1.0 (Codex)

The mean recovery level for each active ingredient is shown in Table 2 below.

Table 2. Mean recovery rate of the active ingredient during analysis.

Product	Mean recovery rate
Rovral WP	85%
Filex	Not provided by analytical laboratory
Toppel 10	94%
Aphox	85.4%

Table 3. Rates of application and concentrations of active ingredients

Product	Rate of application	% active ingredient in product	Concentration of ai at application (ppm)*
Rovral WP	0.5kg/ha	50% w/w	250
Filex	20.5l/ha	72.2%	14800
Toppel 10	0.25l/ha	10.0%	25.3
Aphox	0.5kg/ha	50.0%	250

* calculated from the dilution of the product in the spray mix.

Figure 1. Residue decline analysis for iprodione, cypermethrin and pirimicarb in winter lettuce

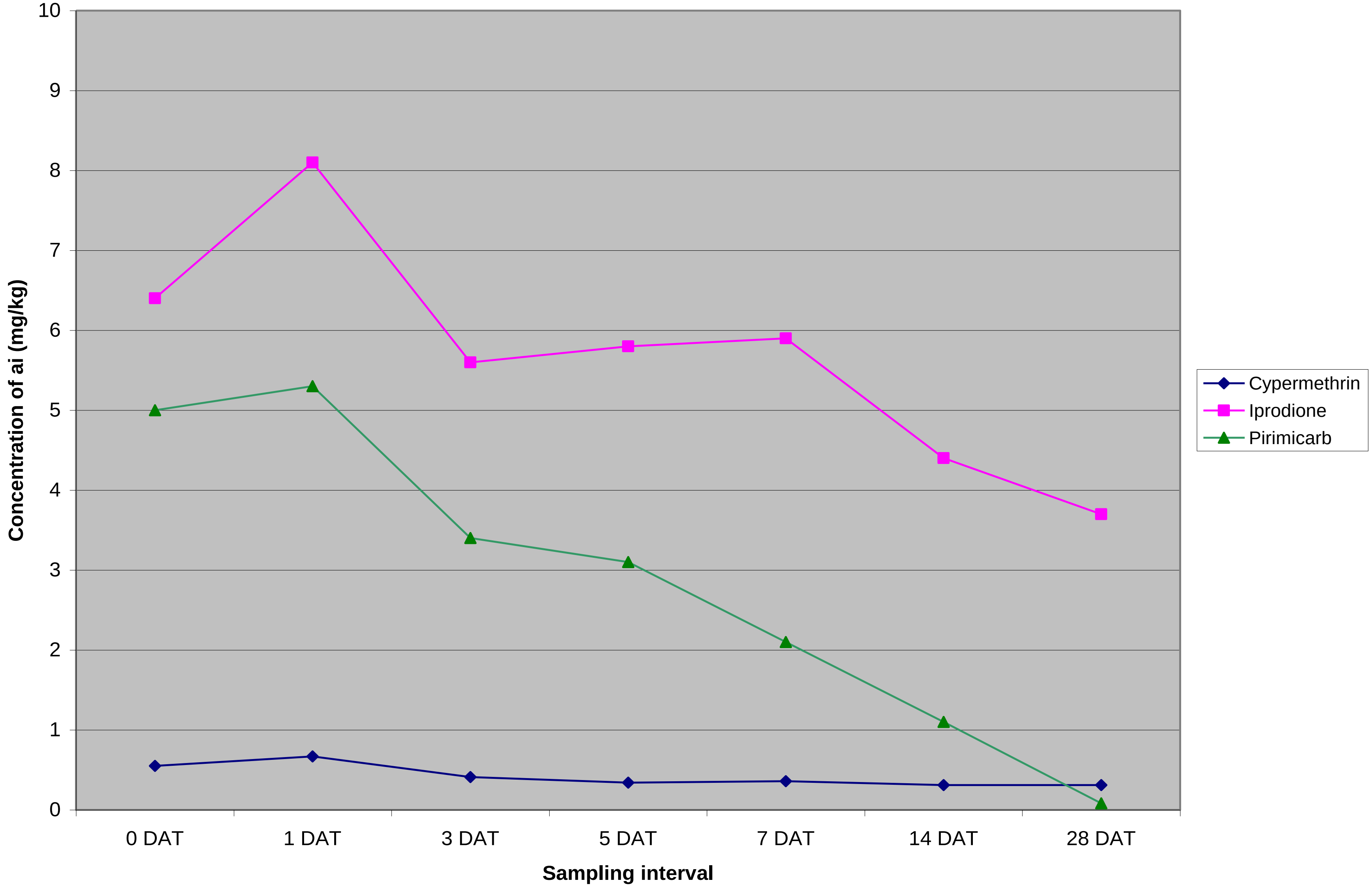
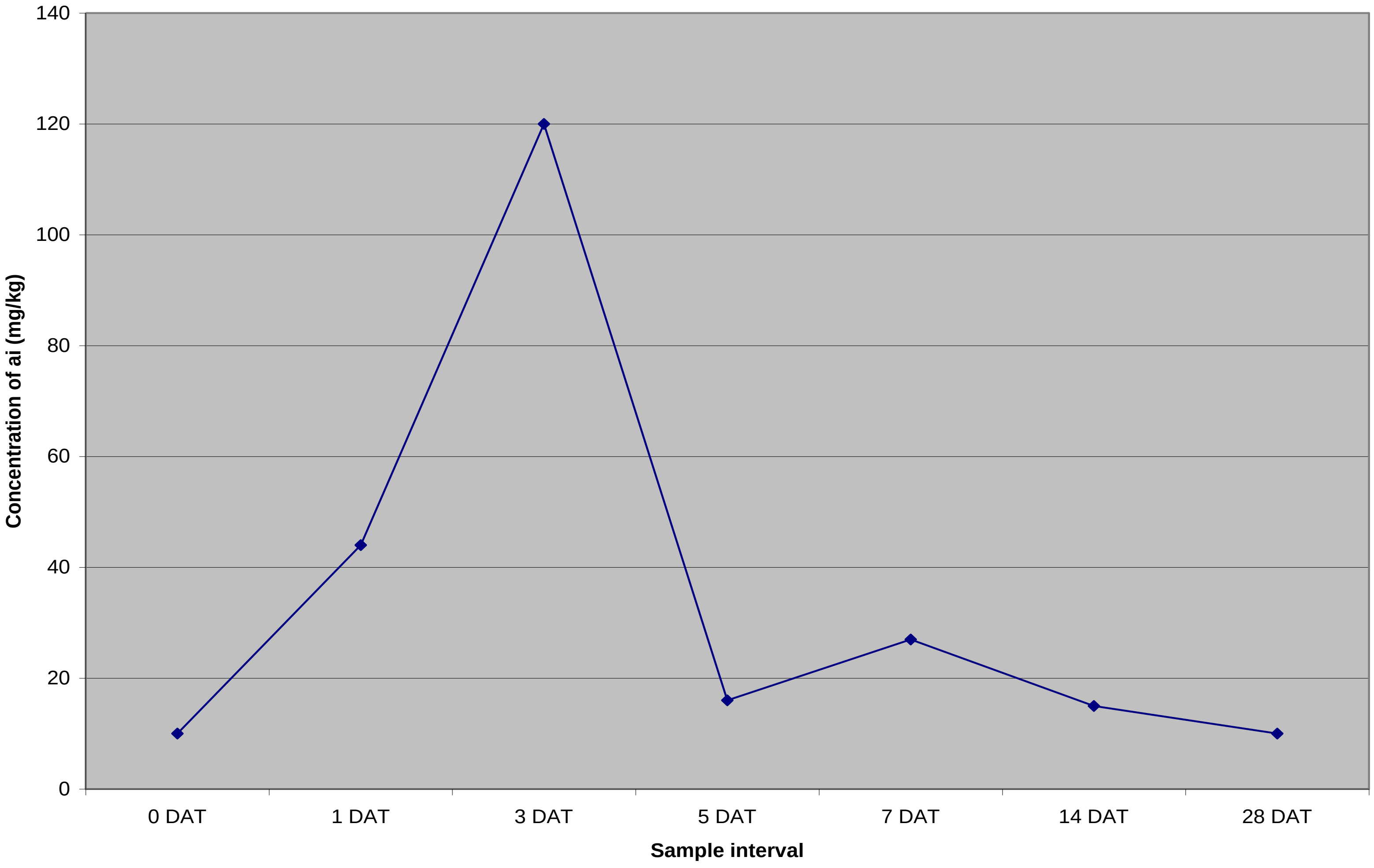


Figure 2. Residue decline analysis for propamocarb in winter lettuce



Pest and Disease assessments

Naturally occurring pest or disease infestations were observed at low levels during the trial period though were assessed and reported in the hope of providing useful data regarding product efficacy. It was further agreed that should data not be available, advice would be sought from manufacturers to ascertain the relative efficacy of the products at each stage in their decline.

The first pest and disease assessment was carried out on the 9th December, and repeated on the 4th January prior to the application of the chosen pesticides. A third assessment was carried out on the 9th February 2006. Low levels of both *Botrytis cinerea* and *Sclerotinia sclerotiorum* were observed in the crop during the first two assessments. However, due to the relatively small size of the plants and the lack of contact between plants, the infections did not spread from plant to plant, but instead caused the death of a small number of individual plants. As the crop matured and plant to plant contact increased, spread of *Botrytis* in plot 1, where no iprodione had been applied increased. No evidence of d. mildew infection was observed in the plots during the trial period. Low numbers of aphids were only observed in the crop in early February 2006, the majority of these were seen in plot 1.

Table 4. Pest and Disease assessment 9th December

Plot No.	**Total no. of plants infected**	
	Botrytis cinerea	***Sclerotinia* sclerotiorum**
1 (Filex & Toppel 10)	4	0
2 (Rovral & Aphox)	0	0

Table 5. Pest and Disease assessment 4th January

Plot No.		
	Botrytis cinerea	***Sclerotinia* sclerotiorum**
1 (Filex & Toppel 10)	5	0
2 (Rovral & Aphox)	5	1

No evidence of aphid infestation was observed during either assessment.

Table 6. Pest and Disease assessment 9th February

Plot No.	***Botrytis cinerea***		***Sclerotinia sclerotiorum***		**Mean no. Aphids/plant**
	No. plants affected	**Mean severity score/plot (0-3 scale)**	**No. plants affected**	**Mean severity score/plot (0-3 scale)**	
1 (Filex & Toppel 10)	15	1.1	0	0	6.3
2 (Rovral & Aphox)	5	0.2	3	0.3	0.04

Details of 0-3 scale: 0 = No disease, 1 = Bottom leaves affected, 2 = Upper and bottom leaves affected, 3 = whole plant collapse.

Head Weights and leaf area

At each sampling date 4 extra lettuce were cut to allow records of mean head weight and leaf area to be made (Table 7).

Table 7. Mean head weight and leaf area at each sampling date

Sampling date	Mean head weight (g)	Mean leaf area (cm^2)/head
0 DAT – 17.1.06	122.3	NR
1 DAT – 18.1.06	NR	NR
3 DAT – 20.1.06	NR	NR
5 DAT – 22.1.06	123.3	1850.7
7 DAT – 24.1.06	125.0	1960.3
14 DAT – 31.1.06	145.0	2206.0
21 DAT – 14.2.06	175.0	2600.6

NR – Not recorded – missing data

Discussion

MRLs are set by the Pesticide Safety Directorate (PSD), or Codex or the EU for each pesticide on a wide range of fruit and vegetables, cereals and animal products. They are the legal limit on the residues of that pesticide that are permitted for a particular food stuff. MRLs are not safety limits but are instead effectively a trading standard for treated food and ensure that Good Agricultural Practice has been followed. There are set guidelines for acceptable residue levels. Where there are different MRLs (EU, UK and Codex) the EU MRLs take precedence with the UK MRL second and Codex serves as a default value if others are not available.

A comparison of the residue analysis to their respective MRLs and harvest intervals indicates that the residue for iprodione (Rovral) was well below the regulatory level set even on the day of application and throughout the sampling period. Cypermethrin (Toppel 10) also remained well below its MRL throughout the sampling period. The analysis for pirimicarb (Aphox) was of slightly more concern as it was slightly above its MRL (0.1mg/kg over) at the expiry of the 14 day harvest interval, and residue levels were very high prior to the harvest interval. This could represent a serious risk of breaching of MRLs if harvest interval dates were not adhered to. The results of the analysis for residues of propamocarb were also of concern (Figure 2). The recorded values were very variable with no obvious trend over time. The MRL of 10mg/kg was exceeded at the designated recommended harvest interval, as it was for the entirety of the trial period. It was unclear why the analytical values were so high throughout and also so variable and no explanation can be given for this[2]. The unreplicated nature of this study means that it was not possible to analyse additional lettuce samples to investigate whether the analysis for propamocarb always results in such a variable data set, or whether this was a true anomaly.

The mean head weight and leaf area data shows that the crop was developing as would be expected with a winter crop, with a reasonable and acceptable head weight being achieved by the final sample date (expected harvest date). A comparison of the mean number of sunshine hours/day in each month, along with the mean daily maximum and

[2] Clarification of these variable results was sought from the analytical laboratory. They confirmed the results as correct i.e. no experimental error had occurred during the analytic methodology and they were unable to speculate about the variability of the results.

minimum temperatures/day is presented in Appendix 1. Lower than average numbers of sunshine hours per day were observed in December and January during this winter, along with a decrease in the average daily maximum in temperature in January and February. These factors will have influenced the growth of the lettuce, perhaps reducing the head weights. They may well have had an effect on the rate of degradation of the active ingredients applied. It should also be remembered that growth of the lettuce following application of the various pesticides would also result in a dilution, or decline in the residue of the active ingredient that could be determined following analysis. This aspect of the natural decline of the active ingredient is particularly important in products with longer harvest intervals which allow for greater increases in growth of the plant.

The pest and disease data collected is of limited use. It does indicate the development of *Botrytis* in the crop as it matured from similar early levels of infection in each plot and demonstrates effective control following the application of Rovral in plot 2 in comparison to plot 1 where no Botryticide products were applied. It also demonstrates that the *Botrytis* population remained highly sensitive to this fungicide and that resistance was not a problem here. A clear difference in the mean number of aphids seen per plant was also observed between the plots, with much lower levels of infestation seen in plot 2 which had been treated with Aphox, than in plot 1 treated with Toppel 10. However, this may be due, in part, to uneven early infestation numbers or the foci of the infestation being higher in one plot than the other, and not merely a measure of product efficacy, in this unreplicated experiment.

Due to the limitations of the pest and disease data in terms of efficacy of the product over time, efforts were made to gather further information on product efficacy from the manufacturers of the products used in the trial. Responses that have been received suggest that if efficacy data relating to the decline of the active ingredient does exist, it may be considered confidential and not open to the public domain. This provides further support for this study as manufacturers keep relevant data as ‘commercial – in confidence’. In some cases e.g. Aphox, the product is now quite old and data of this sort was generated only in terms of how often the product had to be reapplied to maintain control. The organisational changes such as mergers and buy-outs that have occurred in many of the agrochemical companies also mean that data of this sort is likely to have been lost or archived and is now not readily obtainable. It is difficult to envisage other routes by which this information may be available, although there is merit in acquiring the data to support recommendations to alterations in harvest intervals and so help reduce the risks of pesticide residues occurring, particularly on high risk crops such as winter grown lettuce.

Conclusions

This unreplicated experiment was carried out successfully at Stockbridge Technology Centre during the period October 2005 to March 2006. The lettuce grew well and normally for a winter crop, though the growth rate was perhaps slightly slower than in other seasons due to slightly lower than average radiation levels, particularly in December and January, along with lower than average daily maximum in temperature during January and February. The spray applications were carried out accurately and with care and in line with the principles of GLP. Samples of lettuce were collected on the prescribed dates and forwarded to NRM laboratories for analysis of the active ingredient residues. Residue data was provided for all the samples, although some anomalies within the reported results for propamocarb do present some difficulties with regard to describing the expected decline curve for this fungicide.

A clear downward trend in the residue data for iprodione, pirimicarb and cypermethrin was observed over the time period of the trial. Analysis for iprodione and cypermethrin showed that residues of both were well below their MRL even on the day of application, and both declined further over the 28 day sampling period. Inconsistent residues of pirimicarb were well above the MRL (1mg/kg) following application, and were still slightly above on the 14 days after treatment sample (1.1mg/kg). This suggests that either the harvest interval for pirimicarb should perhaps be extended to ensure that breaches of the MRL do not result in future or the rate of application should be reduced. Further R&D would be required to validate these initial findings and ensure that efficacy of the amended treatment regimes is not compromised.

The analysis carried out for propamocarb is much more difficult to interpret and does not show a clear trend. It is apparent that further investigation of the fate of the active ingredient is required to clarify the situation of whether there is a flaw in the analysis, or some other explanation for the data.

The data generated from this study provides valuable information on a range of active ingredients, applied to one lettuce crop, under one set of environmental conditions in one season. Clearly there are numerous environmental and edaphic factors that could influence pesticide breakdown and this may result in an entirely different set of residue analyses. If the horticultural industry wishes to reduce the risk of pesticide residues it must start to consider generating robust data sets to establish decline curves, especially during high risk cropping periods e.g. winter months with a view to minimising or reducing pesticide applications. In addition consideration of alternative growing practices or the use of alternative crop protection products e.g. biopesticides should be considered as a component of an integrated crop protection strategy to further reduce the potential residue burden in these crops.

Technology transfer

The information obtained following this study is available to growers and industry representatives. Further studies and investigations are required before robust industry recommendations can be made.

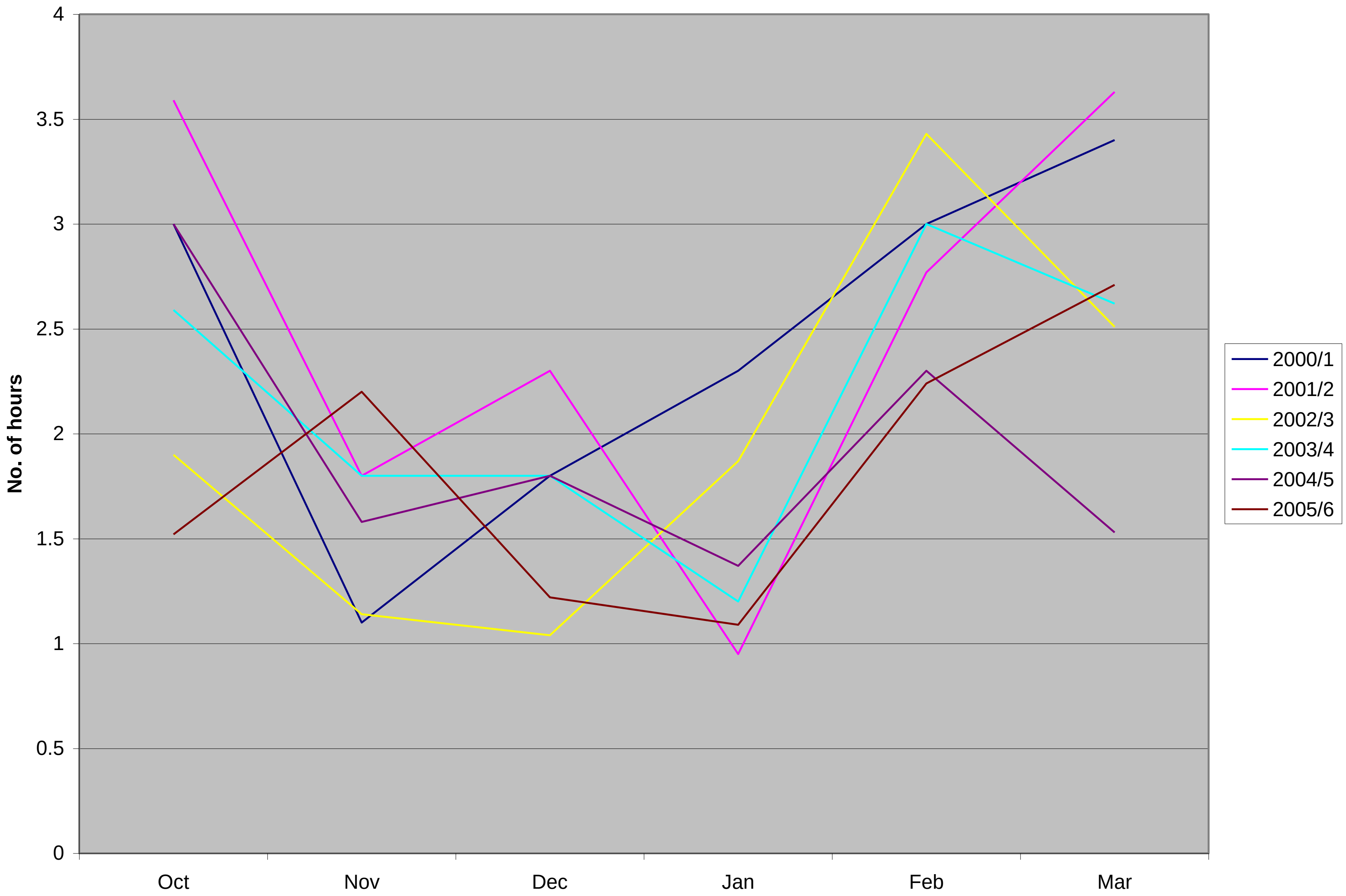
Appendices

Appendix 1 – Environmental data for trial period – shown as weekly means

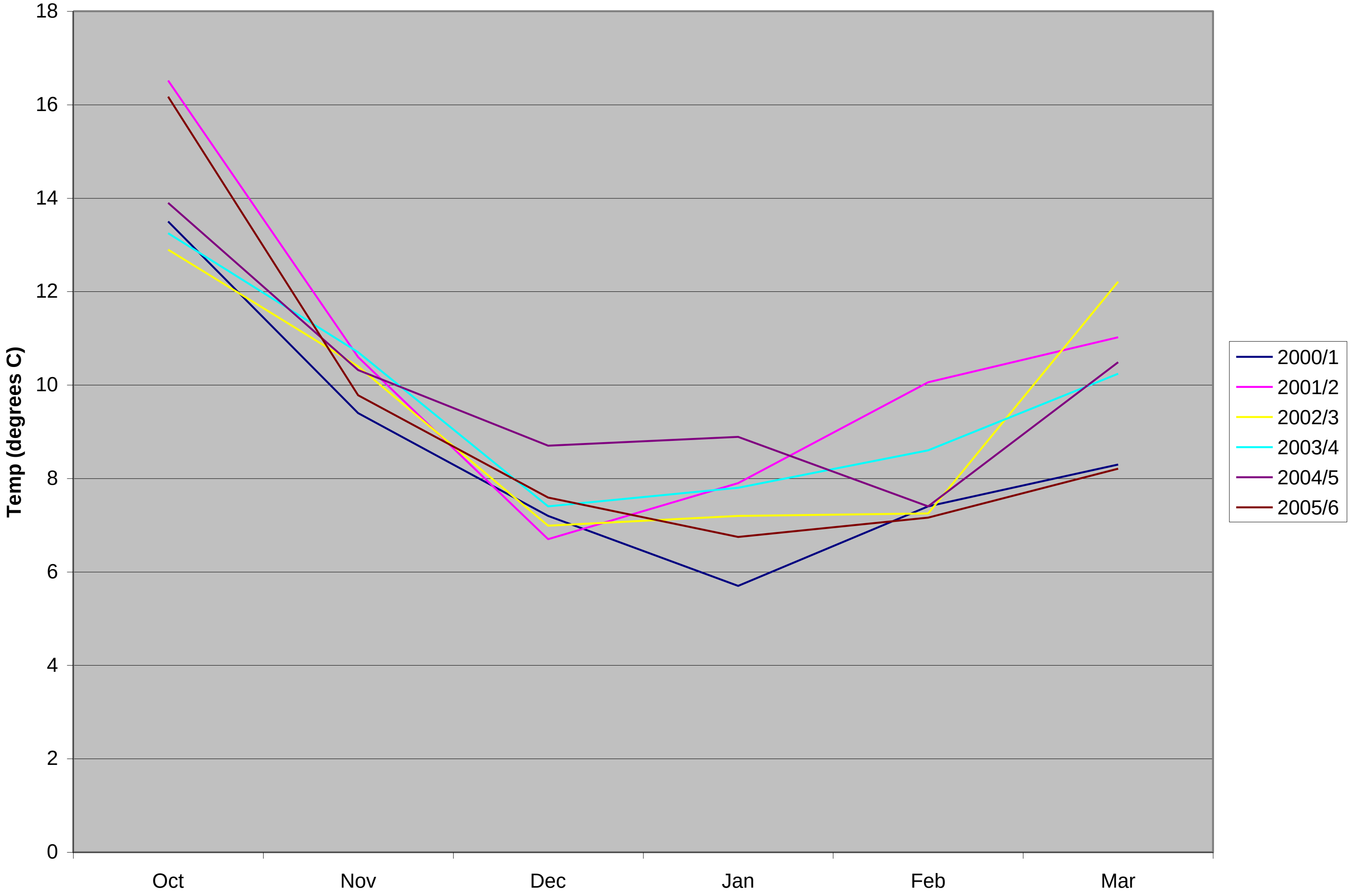
Week No.	Mean glasshouse temp (ºC)	Mean relative humidity*	Mean no. sunshine hours/day
44 (31.10.05 – 6.11.05)	12.5	-	1.66
45	10.8	-	2.52
46	7.5	67.7	4.79
47	6.0	72.4	0
48	6.5	77.7	0
49	7.4	79.7	0.88
50	6.8	78.0	2.7
51	7.3	82.5	1.97
52	5.6	83.9	0
1	5.8	-	0
2	7.1	-	0.83
3	7.7	-	1.7
4	6.0	-	2.07
5	5.9	-	0.09
6 (6.2.06 – 12.2.06)	7.1	-	2.56

* missing humidity values caused by fault with data-logging equipment

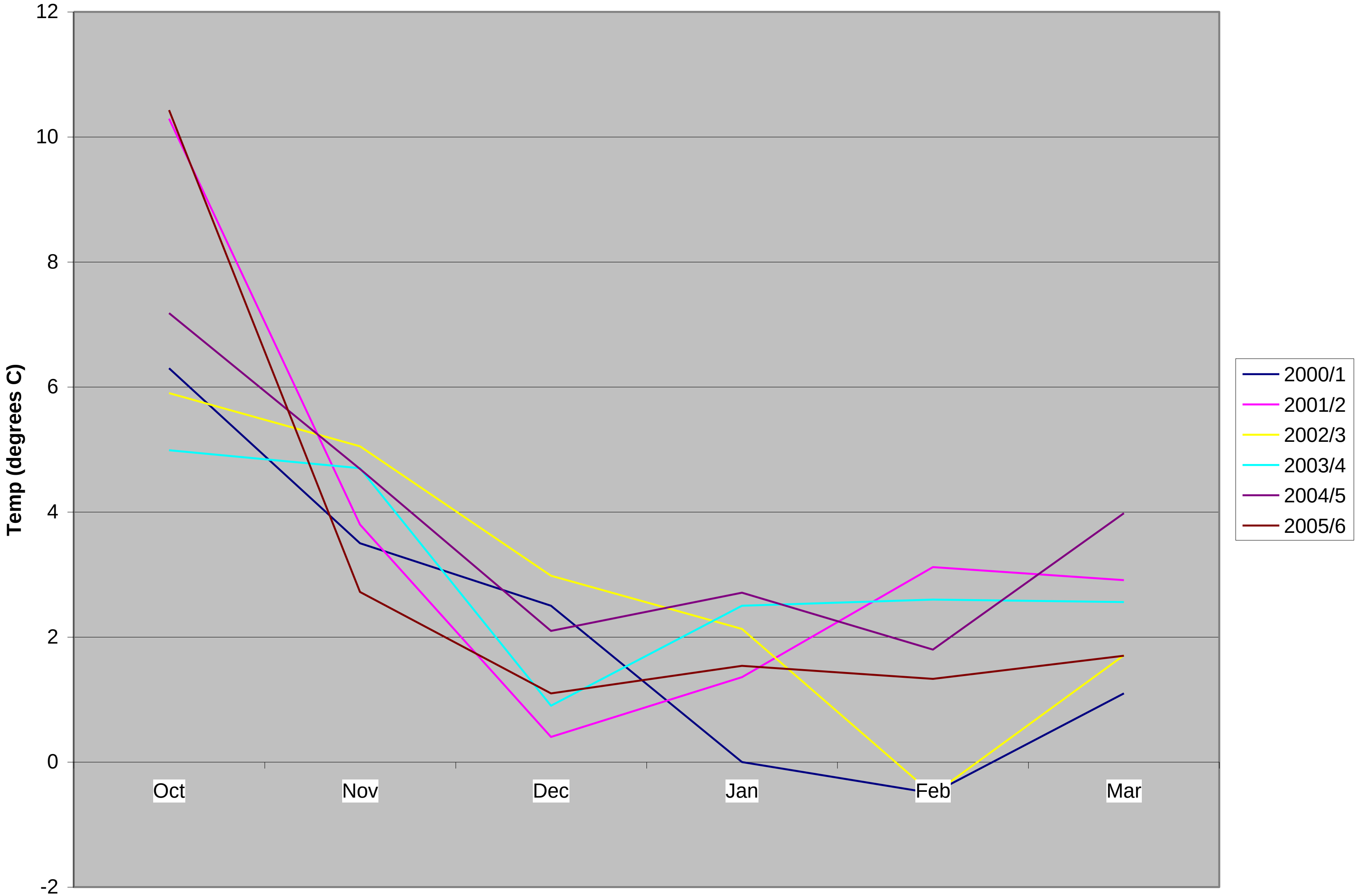
Appendix 1a – Comparison of mean sunshine hours per day during winter periods 2000-2006



Appendix 1b. Comparison of mean maximum daily temperature during the winter periods 2000-2006



Appendix 1c. Comparison of mean minimum daily temperature during the winter periods 2000-2006



Appendix 2 – Full Data set for residue analysis

Product	Active Ingredient	MRL (mg/kg)	Residue Concentration (mg/kg)						
			0 DAT	1 DAT	3 DAT	5 DAT	7 DAT	14 DAT	28 DAT
Rovral	iprodione	10.0	6.4	8.1	5.6	5.8	5.9	4.4	3.7
Filex	propamocarb HCl	10.0	10.0	44.0	120.0	16.0	27.0	15.0	10.0
Toppel 10	cypermethrin	2.0	0.55	0.67	0.41	0.34	0.36	0.31	0.31
Aphox	pirimicarb	1.0	5.0	5.3	3.4	3.1	2.1	1.1	0.082