SESSION 4A

LINKING ENVIRONMENTAL FATE AND NON-TARGET EFFECTS OF PESTICIDES: TERRESTRIAL ECOSYSTEMS

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Papers	4A-1 to 4A-4

THE US EPA "NEW PARADIGM" GROWS UP: COMBINING TRADITION AND TECHNOLOGY FOR ENVIRONMENTAL ASSESMENT

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ABSTRACT

In the US, the EPA has recently made several product registration decisions involving products for which a “presumed risk” existed. Through innovative technologies, regulatory negotiation and environmental modeling or monitoring, the Agency and agribusiness have been able to reach mutual goals of risk assessment and new product registration. In this process, environmental risk, fate and impact assessment have grown from a fledgling science which estimated a predicted effect to a more mature process which quantifies risk, identifies environmental variability and utilizes modeling or monitoring to prove the underlying thesis about “risk”. The weakest link in the prediction of risk, however, is in the interpretation of a given event or condition with respect to its meaning for a broad geographic area. Along with other tools and strategies, Geographic Information Systems, which analyze data while maintaining their spatial relationships, and remotely sensed data, which define land cover variables, provide “real world” characterization of the environment for the region of interest and contribute data which can be used not only for probabilistic modeling but also for market development, product distribution analysis or test site selection. Through this approach, regulatory needs are met by data which then have further utility in product development and management.

INTRODUCTION

At the 1994 British Crop Protection Conference, Crabtree *et al.* (1994) introduced the use of spatial analytical technologies to the resolution of product environmental risk evaluations. Since that time, Geographic Information Systems (GIS) have been used to perform analysis of the spatial relationships between crop and non-target areas. Since the implementation of EPA’s “New Paradigm” in 1992, higher tiered ecological issues are more commonly being resolved by drawing upon modeling, mitigation and monitoring. Field toxicity testing, as was practiced prior to the New Paradigm, has become a less desirable alternative to the successful resolution of negotiations between the registrant and the registration authorities.

Input for stochastic modeling can be provided only by thoroughly considering the actual distribution and coincidence of environmental parameters such as soil type, hydrology, land cover, and site specific rainfall. Without statistical understanding of the coincidence of parameters important to the model in question, the output from the model may be highly variable and not truly reflect the behavior of the compound in the environment, nor represent the results produced by the real-world "worst case".

In a recent report on its findings, the United States Commission clearly stressed the identification of spatial relationships as critical to risk characterization (Commission on Risk Assessment and Risk Management, 1996). Their findings with respect to ecological risk characterization included the following points:

- Guidance in the use of qualitative and quantitative descriptions of uncertainty are needed
- Problem identification needs to be in a holistic context
- The spatial and temporal distribution of a stressor and an ecological component need to be predicted

In this paper, selected aspects of risk characterization are utilized to demonstrate the procedure which can be used to (1) reduce the uncertainty in modeling; (2) identify natural mitigation factors; and/or (3) site, record and interpret the results of monitoring studies. Such an approach reduces uncertainty, puts exposure in context and properly predicts the spatial and temporal distribution of events. This approach was adopted for a newly developed insecticide. As a result of the data generated and the current US regulatory climate, the need for providing a thorough understanding of the behavior of the compound which is proposed for use on a major field crop was recognized. In gathering and generating data, the registrant included the use of GIS and remote sensing to: 1) select study sites, 2) provide an understanding of mitigation (environmental characterization), and 3) generate statistics for model input. The analysis of avian and aquatic risk methodology used will be discussed here in general yet informative terms.

SITE SELECTION

The process of registering an agricultural chemical is quite involved and often requires companies to perform a multitude of studies ranging from ground water monitoring to assessment of environmental exposure. In many of these studies, the first step is to geographically position a study site in such a manner as to ensure adequate representation of the region under consideration. This is easier said than done. In the past, the persons performing the site selection process had the impossible task of attempting to collate statistics from different years, maps of different scales, and printed information from different sources. The result was often a selection process that was more qualitative than quantitative and the analysts seldom knew to what geographic extent the analysis could be extrapolated.

Recent advances in software technology and digital database availability are beginning to change the way site selections are performed. The advances in software technology include the use of GIS and high speed computers, which together enable the rapid manipulation and analysis of nationwide digital databases (Burroughs, 1986). The advances in data sets available for analysis include comprehensive databases such as the US Department of Agriculture's (USDA's) State Soil Geographic database (STATSGO), US Geological Survey's Digital Line Graph Hydrology (USGS DLG's), and USDA's 1992 Agricultural Census data.

The study presented in this paper took advantage of these new tools by employing the use of GIS technology and multiple nationwide databases to perform a site selection analysis for a project designed to quantify crop proximity to sensitive avian and aquatic habitat. The databases used included the following:

- USDA STATSGO
- USDA Major Land Resource Areas (MLRA)
- USEPA Ecoregions of the US
- 1992 Agricultural Census Data
- Satellite Based Land Cover Classification of the US
- Specific Cropping Practices and Pest Regions (From the registrant)

These data were combined using a GIS to answer the following questions: 1) How many study sites are needed to adequately represent the diversity of growing regions? and 2) To what extent can the study areas, once selected, be spatially extrapolated? For this project, twenty study areas were identified, each having unique environmental and agronomic characteristics based on soils, land cover, geomorphology, climate, agricultural practices, pest pressures, *etc*.

Once contiguous and representative areas were identified, the final step in site selection process was the placement of the study site within a study area. The procedure for study site placement can take on a variety of forms, however, it primarily involves a great deal of personal communication with agricultural groups, local and state agencies, and local grower groups. Unlike the study site in a field-based analysis, the GIS-based study site is used as the base unit for detailed mapping of environmental characteristics and typically ranges in size from a few hundred thousand acres to several million acres. Once the environmental characteristics of the study site have been compiled, they provide ample statistical information for reliable extrapolation over the entire study area.

ENVIRONMENTAL CHARACTERIZATION

The term "environmental characterization", as used in this project, referred to the mapping of a multitude of crop parameters that could be used, both qualitatively and quantitatively, to assess exposure. The crop parameters measured in this study included:

- proximity of crop to other land covers, including sensitive habitat
- field size (area / perimeter – min / max / avg.)
- field slope (min / max / avg)
- soil type (range of soil types on which crop is grown)
- buffer composition (land cover types typically between crop and sensitive habitat)
- buffer widths (typical width of different buffer compositions)

Data types

The measurement of crop and environmental parameters relies on a variety of data types. These data types include:

• satellite imagery	(general land cover mapping)
• USGS DLG data	(hydrology and road data)
• USDA STATSGO	(soils database)
• Digital Elevation Models (DEM)	(elevation and slope data)
• airborne imagery	(detailed land cover)

The satellite data used in this study were acquired by the SPOT Image Corporation satellite which has a frame size (field of view) of approximately 60 kilometers by 60 kilometers. The SPOT satellite acquires reflectance data in three different spectral bands; green (0.5 - 0.59 μ), red (0.61 - 0.68 μ), and near-infrared (0.79 - 0.80 μ) (SPOT, 1987). The spatial resolution (or minimum mapping unit) of the SPOT satellite data is 20 meters by 20 meters. Since the data are supplied in digital form, they can be processed through specially designed computer programs for generation of a detailed land cover map (*i.e.*, crop type, avian habitat, *etc.*) (Campbell, 1987; Colwell et al., 1983; Jensen, 1996).

The DLG data are line map data in digital form (USGS, 1989). The data are useful for the production of cartographic products such as base maps and are structured to support the analytical functions of a GIS. The typical use of base category digital cartographic data is to combine it with other geographically referenced data, enabling various automated spatial analyses to be conducted. The categories of DLG data used in this study included:

- intermittent streams
- perennial streams
- intermittent ditches and canals
- permanent ditches and canals
- lakes and ponds
- roads

Digital elevation models (DEM's) are elevation data generated by the USGS. These data can be geographically referenced to the other data layers and can be used to generate slope and aspect data in support of modeling.

The STATSGO database developed by the USDA consists of soil polygons at the soil association level and a database at the soil series level. These data are invaluable in ascertaining the range of soil types on which a crop is grown.

The airborne imagery is used for high resolution mapping of sensitive areas. The spatial resolution of the airborne imagery is approximately one meter and lends itself to detailed mapping of crop/habitat transition areas. The airborne imaging system is commonly flown over a stratified random sample of close crop/habitat proximity (Pearson *et al.*, 1992).

Compiling land cover data for avian exposure

Since most of the analyses for this project were based on the land cover data set generated from the satellite imagery, the land cover classes (classification scheme) had to consist of land covers that were meaningful with respect to an analysis of exposure. Therefore, each classification scheme needed to identify both the source of the pesticide and the potential sites of exposure. The source was the crop to which the insecticide would be applied, and, since the insecticide toxicity data indicated possible concern for both aquatic and avian habitats, these were determined to be potential sites for exposure. In addition to the crop of interest, avian habitat, and aquatic habitat, additional categories were included in the classification to account for land cover types that were not included above, *i.e.* other agricultural areas and roads, and to account for the areas within the satellite scene for which land covers were unidentifiable due to clouds or cloud shadows. Therefore, the classification scheme for each study site consisted of: 1) the crop of interest, 2) avian habitats, 3) aquatic habitats, and 4) supplemental land covers.

The avian habitats for each of the study sites were developed based on three criteria: 1) What general avian habitat types were identified in avian field surveys previously conducted by the registrant? 2) What types of habitats are listed in avian checklists, field guides and birding software that associate specific species with general habitats, and 3) What general habitats can be identified spectrally in the satellite imagery? It was important from the registrant's perspective to attempt to identify avian classes that were similar to those identified in previous avian field work. Because of the nature of fieldwork, only small areas can be included in any particular study. However, by identifying similar classes in the final image classifications, the registrant is able to understand the extent to which their field studies are representative of an area. It was also important to correlate the avian habitat classes with general habitats described in birding literature and software in order to be able to link the habitats in the classification to individual species.

Aquatic habitats were determined using the DLG data to distinguish between rivers, intermittent streams, lakes, canals, catfish ponds, and wetlands. Because the DLG data exist and already define the nature of water bodies in the area of study, separate habitat definition and classification, such as that conducted for avian habitats, was not necessary.

The classification scheme looked as follows:

- crop of interest
- other agriculture
- upland forest
- bottomland forest
- brush
- grass/pasture
- bare ground/urban
- rivers/streams
- lakes/ponds
- intermittent streams
- wetlands
- catfish ponds
- cloud/cloud shadows
- roads

GIS as an analytical tool (data analysis)

Once the land cover data set and other ancillary data sets have been developed and incorporated into a GIS, this can then be used to analyze these data sets in various ways, yielding results which quantify environmental characteristics (Aronoff, 1989; ESRI, 1992). First, environmental characteristics that directly influence modeling inputs can be quantified, *i.e.* field size and slopes and soils types in the crop if interest, and water body characteristics. The second type of analysis that can be conducted includes quantifying the proximity of sensitive habitats to the crop of interest. Not only does this include documentation of the proximity of sensitive habitats to the crop of interest, but also includes an analysis of buffer widths and composition that can significantly mitigate exposure of sensitive habitats (Webster and Shaw, 1996). Although the statistics generated in this type of analysis cannot, at this point, be used in models, they do put the modeling results in context when interpreted within the framework of an ecological risk assessment.

More specifically, with respect to avian exposure, the avian species/habitat database is designed to be analyzed in conjunction with the land cover data set in order to understand which avian species are most likely to be exposed to a product based on its use patterns and the temporal and spatial distribution of each species. For example, a given majority of the applications of the insecticide under evaluation in this study would be applied during the summer months, therefore avian species present in this area during the autumn, winter, and spring could be eliminated as potentially exposed species. This reduced the number of potentially exposed species by over half. Using the results of the sensitive habitat proximity analysis described above, we could see that bottomland forest, grass/pasture and urban areas accounted for most of the avian habitats adjacent to the crop of interest. So, from the list of avian species found in this study site during the summer only, those species that inhabit bottomland forests, grass/pastures or urban areas were selected. This reduced the number of potentially exposed species by nearly another half. At this point we incorporated the results of the avian census field studies which indicated that the only species that were observed in or around the crop of interest were species in the following feeding guilds: granivores, insectivores, and omnivores. All species within these feeding guilds were further selected from the previous subset to reduce the number of potential exposed species by yet another significant amount. It is important to note that although aquatic habitats are also avian habitats, the example provided here is an assessment of terrestrial avian habitats. A similar analysis was also conducted for aquatic habitats.

MODELING

Prediction of avian exposure

Given the example on the potential for avian species exposure and the analysis of habitat proximity to the crop of interest that was detailed above, one could then combine drift model results with terrestrial environmental effects. The drift models are based on the movement of pesticides off site and assume no mitigating landscape that may reduce drift. The proximity analysis identifies the avian habitats that are adjacent to the crop of interest and the buffers that exist between this and the avian habitats. With a knowledge of habitat adjacency, buffer width, and buffer composition, the argument can be made that these mitigating factors will reduce the exposure of avian habitats and that the models currently in use may overstate exposure. Although the extent to which the estimated environmental concentrations (EEC's) are reduced cannot yet be determined, due to the current capabilities of models, these data provide an environmental element that can be discussed qualitatively within the context of an environmental risk assessment. In addition, linking avian species to habitat provides more specificity on the species potentially affected.

Aquatic systems

The results from the GIS analyses will assist in refining or focusing many of the parameters used in models, especially those associated with aquatic systems. The problem with many of the model inputs is that the assumed worst case for a given parameter often is not associated with the crop of interest and in rare cases where the assumed worst case does occur, its actual frequency in nature is not well understood. For example, in Tallahatchie County, Mississippi there are some steep slopes of greater than seven percent associated with the natural levee systems. From a modeling standpoint, since the area contains slopes of this magnitude they would be potential model inputs. However, if one analyzes the cropping patterns throughout the county, it becomes obvious that these natural levees are predominately covered by natural vegetation, not agricultural crops. By using the GIS to overlay the land cover and slope data layers the range of slopes found only within the crop of interest can be identified, thereby narrowing the possible model inputs. The same can be done for the range of soil parameters that are often important model inputs. Once the range for each of these variables has been narrowed by use of the land cover layer, all possible "logical" combinations can be processed through the model to produce a range of more realistic output results.

APPLICATION OF RESULTS

Because GIS methods were used in the site selection process, it was possible to extrapolate the results from the study site to the entire study area. This extrapolation process enables one to understand a much broader picture of crop/habitat proximity and exposure. It is possible that the study will find that the growers in an area already have in place mitigation measures that reduce exposure. This has been found to be the case on several occasions, frequently by nature's rather than man's design. In some cases, the environmental conditions of an area are such that natural barriers exist and act as a primary mitigation measure.

Whatever the case, the result of a study such as the one described in this paper enable agricultural companies to establish reliable boundaries around similar ecological and agronomic regions. This can be valuable with respect to preparation of labels intended for use in varying regions. These data also can be of value to marketing departments as they attempt to understand how labels with restricted use buffer zones may adversely impact application acreage.

Finally, the data used to generate information on environmental characterization and model inputs may be valuable if monitoring studies should be required. The areas of highest potential concern or possibly the *typical case* for the study area can be easily identified and if necessary random sites located for ground surveys.

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POSSIBLE DEVELOPMENTS IN AGROCHEMICAL RISK ASSESSMENT USING MATHEMATICAL MODELS

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ABSTRACT

Considerable effort now goes into the assessment of the environmental fate and behaviour of pesticides and their ecotoxicological effects. Less consideration has been given to how these two areas can be brought together in the environmental risk assessment. Ways in which this can be done through the use of the Toxicity Exposure Ratio are considered.

INTRODUCTION

Most agrochemical risk assessment schemes have a tiered structure which follows, in principle, the same route. If exposure of a non-target organism is possible then there is a progression from relatively simple, laboratory based tests to increasingly complex studies which move into the field. The initial laboratory tests assess the absolute potential for harm or toxicity of the chemical, producing a base-set of information for a range of indicator species. The more complex studies incorporate increasingly realistic elements of exposure, in order to measure the hazard of the chemical. At each step the available information can be assessed to see if the risk from the use of a pesticide can be determined or if further testing is necessary.

THE TOXICITY EXPOSURE RATIO

One tool that has been developed to aid the risk assessment process at the laboratory stage is the Toxicity Exposure Ratio (TER). This gives a measure of predicted risk, based on the relatively simple ecotoxicity data and which offers the opportunity for relevant fate and behaviour information to be incorporated into the assessment. The TER is given by the ratio of the toxicity measure and the Predicted Environmental Concentration (PEC). This allows a simple quantification of the toxicity levels obtained in relation to the predicted levels of exposure in the appropriate environmental compartment. A high degree of conservatism is built in to the interpretation of the TER, in order to accommodate the uncertainty associated with both the ecotoxicity and exposure information, i.e. using laboratory data to assess the risk under field conditions. Thus, in the EC Authorisations Directive, an assessment of low risk is assigned where the TER is greater than 100 for acute effects and greater than 10 for chronic effects.

CALCULATING PREDICTED ENVIRONMENTAL CONCENTRATIONS

There has been relatively little guidance on how to bring together ecotoxicity data and exposure assessments in a meaningful way with respect to the fate and behaviour of the chemical and the biology of the organism concerned. This applies particularly to the estimation of predicted environmental concentration. A number of approaches have been suggested:

1. Calculating a single value, the initial Predicted Environmental Concentration, (PECi). This is a relatively simplistic approach without reference to the properties of the chemical concerned. Thus, for terrestrial exposure the pesticide is assumed to be within-crop and the PECi is determined solely by the application rate. For spray treatments some amelioration is allowed for, if appropriate, by crop interception. Off-crop contamination is assumed to be principally via drift with crop type and distance being used to determine the proportion of the amount applied reaching the point of concern as 'fall out'.

This approach assumes that the maximum contamination occurs at application i.e. the maximum PEC is on day 0 which is probably appropriate for the terrestrial environment. It also assumes that the predominant risk arises from exposure on day 0. This may be appropriate for terrestrial non-target organisms where direct overspray is a reasonable worst-case scenario, e.g. honeybees, but where residual contact is the main route of exposure, e.g. from plant or soil residues, then some estimate of exposure via this route is required.

2. Dissipation of the pesticide in a medium such as soil can be accounted for in a basic form through the use of the time-weighted average concentration which assumes first-order kinetics for the decline in concentration, the rate of decline being determined solely by the use of an appropriate DT_{50} e.g. as determined in laboratory soil degradation studies.

This measure allows consideration of the average exposure over time periods relevant to those used in the toxicity tests, e.g. 14 days for an earthworm long-term TER based on the NOEC of the acute toxicity test. This results in a single long-term TER value which is assumed to be based on a 'worst-case' average exposure. However, it again assumes that the maximum PEC occurs on day 0 and also that all exposure starts at this point.

3. The more complex mathematical models now being developed to estimate the levels of environmental exposure, produce much more detailed information based on a range of parameters specific to a given chemical together with a number of standardised environmental characteristics. These models are thus capable of producing a more realistic picture of the dynamics of exposure in the different environmental compartments. They can incorporate all routes of contamination *e.g.* drift, run-off and crop interception, and take into account the use pattern, physical properties and subsequent fate and behaviour of the chemical of concern. This allows a similar, continuous picture of the TER to be produced, by incorporating the ecotoxicity data into the model's output. A number of opportunities for risk assessment at this early stage in the tiered scheme are provided by this approach:

- it can provide realistic worst-case TER values, based on the properties of the chemical and its interaction with the environment. These single, static values can then be compared with the existing threshold levels in the same way as the TER values produced using the initial environmental concentrations.
- more importantly, the dynamic picture provided can be used in a more complex risk assessment, incorporating not just the magnitude of the TER but also its pattern and duration over the period of concern.
- for chronic effects, the dynamic picture can be obtained using a moving average, which considers the average exposure during a 'time-window' of a length appropriate to the ecotoxicity data *e.g.* 14 days for the earthworm long-term TER. It considers this 'time-window' throughout the exposure event, i.e. those organisms initially present before and after t = 0 as well as during the application itself.

USING MODELS TO DETERMINE TER VALUES

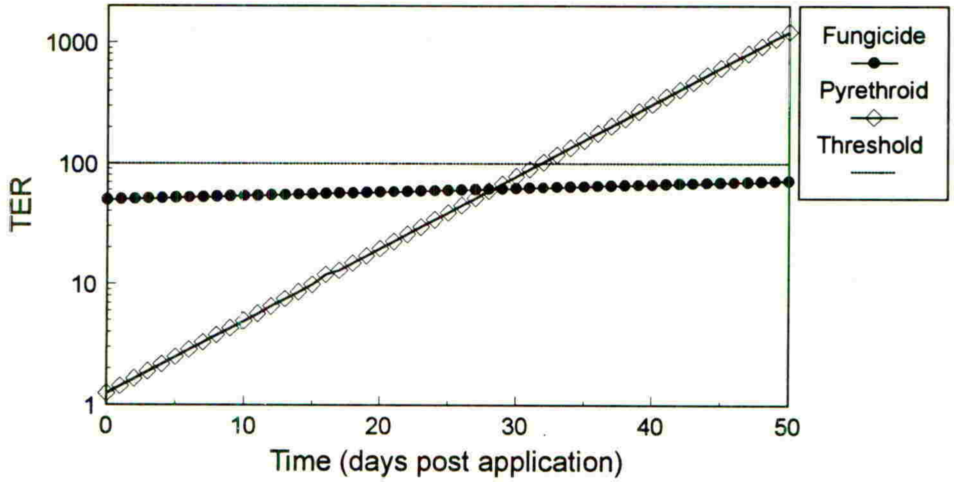
Examples are provided for two contrasting pesticides, a fungicide and a pyrethroid insecticide, in order to demonstrate the potential implications of this approach. A multi-compartment "level IV" fugacity model is used to provide PEC values in soil. However, there are currently a number of different models under development and their relative merits are the subject of much debate. While it is important that a consensus view is reached on the modelling approaches that should be used for regulatory purposes, the aim of this paper is not to discuss this but to consider how the information they generate might be used in the risk assessment process.

The chemical properties, fate and behaviour parameters and ecotoxicity data for the two hypothetical compounds have not been set on the basis of any known molecules but were designed to demonstrate the risk assessment process for two contrasting groups. The model pyrethroid used, degrades rapidly as a result of hydrolysis and photolysis and readily adsorbs to soil where it has a fairly short half-life. On the effects side, it is relatively toxic to many non-target organisms (notable exceptions being plants, birds and mammals). The fungicide, in contrast, is more persistent in terrestrial environments, with dissipation due to volatilisation and degradation being relatively slow. However, it is less toxic than the pyrethroid to most non-target organisms.

Example 1: Single application of product

The fungicide is applied at a rate of 750 g a.i./ha, resulting in an initial soil concentration of 0.5 mg a.i./kg (assuming 50% foliar interception and with mixing to a depth of 5 cm with a soil bulk density of 1.5 g/cm^3). Using an earthworm LC_{50} value of 25 mg a.i./kg the initial acute $TER_{(a)}$ is calculated to be 50 (Fig. 1) which in this case is the minimum TER_a (i.e. the maximum PEC occurs on day 0 as a result of the application). The TER_a subsequently drifts slowly up, not reaching the "acceptable" regulatory threshold of 100 until after 50 days.

Figure 1. Single application of product: earthworm acute TER over time

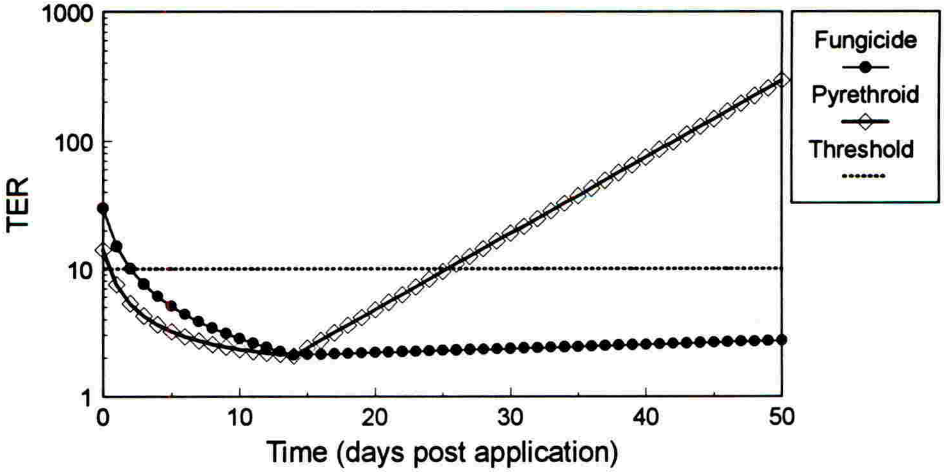


In the case of the pyrethroid there is a lower initial loading compared to the fungicide (an application rate of 120 g a.i./ha) and so a lower initial PEC (again, assuming 50% foliar interception). However, the pyrethroid is markedly more toxic, with an earthworm LC_{50} of 0.1 mg a.i./kg, so that the initial TER_a is much smaller (1.25). The TER_a subsequently rises more rapidly than for the fungicide, as the pyrethroid dissipates more quickly in the soil, rising above the 100 threshold after 31 days.

In both cases then, the regulatory threshold of 100 is crossed but the TER_a pattern over time varies due to differences in the fate and behaviour patterns in soil and thus also the PEC values. The two pesticides therefore present different potential risks. In the case of the pyrethroid, it is possible that there will be some effect expressed over a relatively short time period but there is clearly scope for recovery at the population level either by immigration into the system after a few days or through individuals that survive the initial impact (bearing in mind that there may also be chronic effects). In the case of the fungicide, the level of effect is likely to be less but will be more sustained and thus could become significant, especially in smaller populations. To be able to compare the relative impact of the two pesticides more precisely, further work is needed to be able to relate TER values to the level of effect that may be expected in the field.

The long-term $TER_{(lt)}$ (Fig. 2) is given by the acute 'no-observed-effect' concentration (NOEC) of 1 mg a.i./kg for the fungicide and the 14-day moving average soil PEC (the average concentration over the previous 14 days). There is a steady fall in the TER_{lt} as the 14-day time window moves into and through the exposure period, reflecting the initial increase in concentration on day 0 followed by the largely sustained high level over the time period considered in the example (50 days). The regulatory threshold for chronic effects is 10 and this is crossed after 3 days in the example given. The TER_{lt} trough is reached once the 14-day time window entirely encompasses the exposure period. The TER_{lt} subsequently increases only slowly and still remains below the threshold after 50 days.

Figure 2. Single application of product: earthworm chronic TER over time



The TER_{lt} for the pyrethroid shows a very different picture compared to the fungicide. The high soil concentration occurs over a much shorter period compared to the fungicide so that with an earthworm acute NOEC of 0.075 mg a.i./kg the TER_{lt} quickly drops to a level below the 10 threshold and then decreases further more slowly, as the 14-day time window moves into the exposure period, reaching a minimum TER_{lt} after 14 days. Over this period the low value is maintained by the high pyrethroid concentration over the first few days but as soon as the 14-day time window starts to move beyond this, the TER_{lt} rises sharply. It is thus taken well above the 10 threshold where it subsequently remains.

An assumption is made in the moving average approach that chronic exposure can be averaged out over the time period relevant to the effects data, in this case 14 days, in order to obtain an equivalent level of continuous exposure. This is particularly true in the case of the pyrethroid where the high concentration occurs for a relatively short time relative to the time period for the effect assessment. The 14-day average also backdates the exposure, immediately prior to application, and so is clearly providing a worst-case TER_{lt} up to 14 days. However, it does emphasise the potential for the delayed expression of chronic effects beyond the period of actual exposure. The dynamic TER picture also indicates the potential for a rapid recovery from the impact of a short-lived exposure event, as the input from individuals first exposed after the concentration has dropped (i.e. within a few weeks in the case of the pyrethroid) starts to come through. The pattern as presented is somewhat artificial and will depend on the age-structure of the population at the time of the initial exposure and the duration of the chronic effects on those organisms initially exposed.

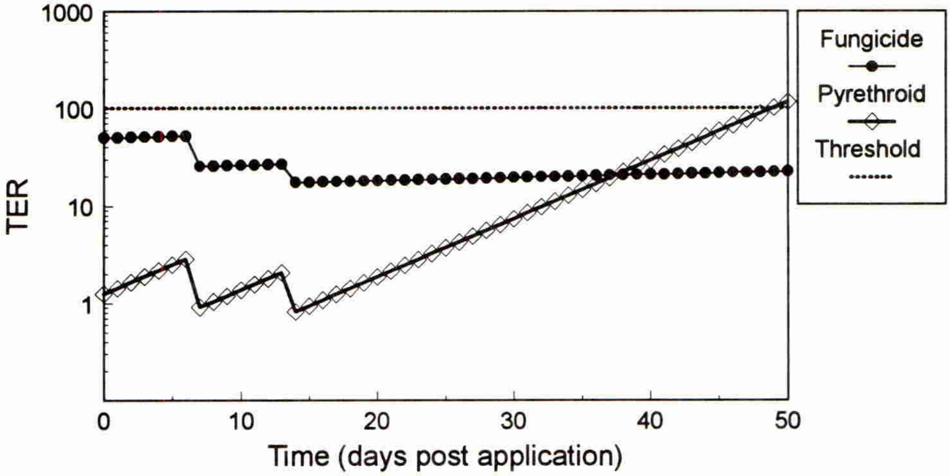
In the case of the fungicide, these considerations are less important as the exposure is more sustained. The picture presented for acute exposure is further emphasised with the minimum TER_{lt} not being reached for some time after application as a result of the delay in chronic effects, as measured over a 14-day period. Recovery is slow due to the longer maintenance of an effective soil concentration and even more delayed due to the potential continued expression of effects once the effective concentration has passed. Again, the precise pattern will be affected by the timing of the initial exposure in relation to the age-structure of the population and the duration of any effects.

In terms of the overall assessment of the two pesticides, the pyrethroid initially presents a greater potential risk, this time through chronic effects, but for a clearly limited period of time. However, while there is a lower immediate potential risk resulting from the fungicide application, it gradually reaches a similar minimum TER value, despite a lower toxicity value, due to the more sustained exposure resulting in a higher average concentration. The significance of this type of exposure pattern would need further investigation.

Example 2: Repeat applications of product

Three successive applications, 7 days apart, has a clear effect on the acute TER pattern shown by the fungicide (Fig. 3). There is very little dissipation over the intervening 7-day periods, and there is marked accumulation over the application regime so that the TER values show a marked proportionate decrease at each application. So while the TER_a starts at the same point below the threshold of 100 as with the single application it subsequently falls further below before drifting slowly up after 14 days (last application) and is still well below the 100 threshold after 50 days.

Figure 3. Multiple applications of product: earthworm acute TER over time

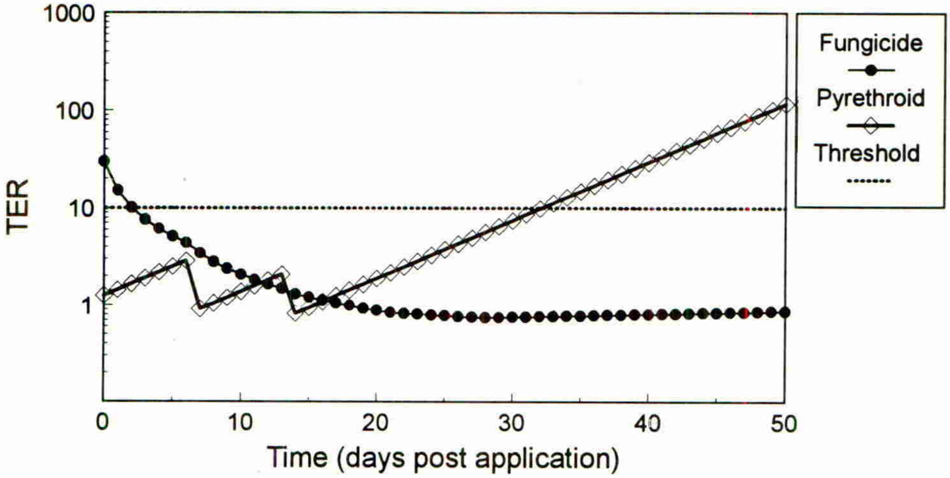


In the case of the pyrethroid, marked dissipation occurs over the 7-day interval between applications so that by the time of the second, soil residue levels are down to less than 50% of their initial values. This is reflected in the TER values which show little increase beyond the initial minimum value on day 0 at each subsequent application. The main effect of the additional loading is to increase the exposure concentration for a longer period such that the TER of 100 is not reached until after 48 days.

Three successive applications have a similar effect on the chronic TER pattern shown by the fungicide (Fig. 4). The three decreasing TER steps seen in the acute assessment are largely smoothed out by the averaging process of the 14-day time window. Compared to the results for the single application chronic TER, the minimum value is nearly 3-fold lower and occurs later, after 28 days (14 days after the last application). As with the acute assessment for three applications, the duration of the TER curve below the threshold of 10 is greatly increased.

In the case of the pyrethroid, the averaging process of the 14-day time window again smoothes out the impact of the three successive applications on the TER value. The dissipation that occurs over the 7-day interval between applications is reflected in the chronic TER - the minimum value shows only a 2-fold increase over the comparable value for the single application and it is only delayed by 7 days (to day 21). This is again followed by a marked decrease in predicted environmental concentration as the pyrethroid degrades, although the threshold of 10 is not reached until day 43.

Figure 4. Multiple applications of product: earthworm chronic TER over time



Multiple applications highlight the advantages of using the dynamic TER approach to risk assessment. With the pyrethroid there is clearly an increase in the net risk over time as a result of three successive applications (one way of quantifying this may be to take the area between the TER curve and the threshold). However, there is a proportionately greater increase in the risk presented by the three applications of the fungicide over time compared to the single loading. The slower dissipation results in the TER value decreasing by nearly 3-fold over the minimum value following the single application. Also, the duration below the 100 or 10 threshold is greatly increased so prolonging the period of potential concern.

DISCUSSION

In recent years, considerable effort has been directed towards the provision of reliable data required to assess the environmental fate and behaviour of pesticides and their ecotoxicological effects. This has been accompanied by the development of the methods necessary to produce this information. However, less consideration has gone into how these two areas of information should be brought together in the subsequent risk assessment, particularly at the early TER stage. The existing approaches do not make best use of the information available and tend to be over-simplistic using single time-point values for the PEC values. Assumptions are made about the timing of the maximum environmental concentration and the exposure of the organisms. This does not take into account the properties of the chemicals concerned and their interaction with the environment or with the biology of the organism(s) considered to be at risk.

As a consequence, conservative default values for the TER thresholds are used to trigger further work. This is done in order to take into account the uncertainty associated with both the estimation of the environmental concentration as well as in the laboratory toxicity data. As a result, there are probably a relatively high proportion of false positives which may incur unnecessary additional costs or, more importantly, may direct the available resources away from where it would be of most value. There is a need for more realistic trigger values to be determined which can reduce the number of false positives whilst ensuring that there are no false negatives. In order to do this, we need to have a better understanding of the systems involved. On the one hand we need better estimation of the levels of risk and one way of achieving this might be through the use of the dynamic assessment presented here. Also, we need to ensure that the ecological context is relevant, assessing the likely level of effects at the population level by taking into account such factors as recovery rates and the inherent variability shown by the species grouping of concern.

The mathematical models now being developed to produce estimates of pesticide concentrations in the different environmental compartments are a powerful tool which make better use of the information available at an early stage in the risk assessment process. The exposure estimates they produce are based on the use patterns and properties of the chemicals so allowing better differentiation between pesticides. They also take into account all routes of contamination (e.g. drift, run-off etc) and interaction between the various environmental compartments. Consequently a more accurate picture of exposure over time is produced which offers the potential for more realistic thresholds of concern. Adding in the ecotoxicity data, the resultant dynamic TER allows not only the magnitude of any potential impact to be considered but also its duration and pattern and if there is increased understanding of its ecological significance it can further improve the estimation of environmental risk.

Of course it must be accepted that all approaches have their limitations and that models predict risk only to certain levels of confidence. It may be necessary at some point to accept that further testing is required in order to help reduce the uncertainty or to refine the risk assessment. However, a dynamic TER risk assessment could still be useful in better targeting this work by identifying the uses and regions of concern. It could also aid in the design and interpretation of higher tier studies using site-specific PEC data , e.g. in determining the appropriate duration of such studies and helping to distinguish between direct and indirect effects. Finally, this approach could help at the risk management stage, identifying where mitigation measures might be necessary and in assessing their effectiveness.

CONCLUSION

Further work is needed. Any models used need to be validated in order to increase the confidence in the information they produce so that the safety thresholds for the resultant TER values can be set more appropriately. The standard environmental parameters used in the models need to be agreed and in this it is important that ecological considerations are taken into account in order to ensure that the output is appropriate from a biological point of view. The way in which a dynamic TER assessment could be used, as demonstrated in the examples given here, needs to be fully discussed and a consensus view reached if it were be implemented in a regulatory framework. The models could be built into a tiered risk assessment scheme at the TER level, starting with an empirical approach and then working up through higher tiers of increasingly sophisticated modelling where refined risk assessments lead to appropriate reductions in thresholds.

A possible forum for discussions on how to develop pesticide risk assessment, including the use of some of the ideas presented would be a work group set up under the auspices of SETAC Europe. This could follow similar lines to the risk assessment and mitigation dialogue groups set up in the US under SETAC.

EVALUATING THE EFFECTS OF MULTIPLE-APPLICATION PLANT PROTECTION PRODUCTS ON BENEFICIAL ARTHROPODS BY MEANS OF EXTENDED LABORATORY TESTS: CASE STUDIES WITH PREDATORY MITES AND HOVERFLIES, AND THE INSECTICIDES DPX-JW062 AND DPX-MP062

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ABSTRACT

A testing strategy was developed to determine the effects of multiple applications of the insecticides DPX-JW062 and DPX-MP062 (Du Pont Agricultural Products), both containing the active ingredient DPX-KN128 (CAS RN 173584-44-6), on two species of beneficial arthropod. Plants grown outdoors were treated on up to 6 occasions at 10-day intervals, and foliage from these was returned to the laboratory for bioassays after 1, 3, 4 or 6 applications. It is believed that this approach for testing multiple-application products is an effective means of determining potential hazards related to the accumulation of residues on foliage and that it could be successfully used to link product fate with the effects seen.

In tests with the hoverfly *Episyrphus balteatus* and treated cabbage plants, residues of DPX-JW062 did not have any significant harmful effects on either adults, larvae or eggs, even following six applications of the product. In tests with the predatory mite *Typhlodromus pyri* and excised apple tree leaves, residues of DPX-JW062 had slightly harmful effects on juveniles, with mortality increasing in relation to the numbers of applications made to a maximum of 37% after 6 sprays. Small reductions in mite fecundity were also seen, but this was not linked to application frequency, and no effects on egg hatch were detected. Residues of DPX-MP062 were not found to be harmful to the mites or to their eggs.

INTRODUCTION

To satisfy the requirements of EC Directives 91/414/EEC and 96/12/EC (Anon., 1991 and 1996), the effects of plant protection products on beneficial species of arthropod have to be assessed for all active ingredients using a representative product formulation. It is currently recommended that bioassays are carried out with species representing the four principal functional groups; predatory mites, parasitic wasps, ground-dwelling predators and foliage-dwelling predators (Barrett *et al.*, 1994). Testing follows a tiered approach (OEPP/EPPO, 1994), with laboratory bioassays initially carried out to determine harmful effects under "worst case" conditions of exposure, typically with a sensitive life-stage confined to fresh product residues on an inert surface of either glass or sand. Where effects are seen, higher levels of testing are triggered and these can involve semi-field or field experiments.

However, products that are applied to a crop on several occasions within the same season cannot be satisfactorily evaluated using the standard laboratory bioassays. This is because multiple applications to glass plates cannot accurately simulate the environmental fate of products with regard to processes such as weathering and chemical degradation which act on residues between applications. Consequently, the ESCORT guidelines (Barrett *et al.*, 1994) state that where a product is intended for use more than three times within a crop season, with applications being made at intervals of 14 days or less, then further testing under extended laboratory, semi-field or field conditions is required. For many species field-based tests are impractical, due to the difficulties in confining and/or relocating individuals after their release into a crop. An alternative approach, adopted for the present studies, was to treat crop plants outdoors and then transfer these to the laboratory for bioassays under controlled environmental conditions. In this manner, the environmental fate of a product over time may be directly linked to any effects observed.

Two insecticides containing the active ingredient DPX-KN128, (S)-7-chloro-3-[methoxycarbonyl-(4-trifluoromethoxy-phenyl)-carbamoyl]-2,5-dihydro-indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylic acid methyl ester, were tested. These were DPX-JW062 (a 50:50 racemic mix of DPX-KN128 and its inactive isomer, DPX-KN127) and a subsequent enriched form, DPX-MP062 (containing 75% DPX-KN128 and 25% DPX-KN127). Both test products were water dispersible granule formulations containing 30% *wt wt* DPX-KN128.

DPX-MP062 is currently being developed by Du Pont Agricultural Products for use in vegetable, pome fruit and vineyard crops. It is to be recommended for use up to a maximum of six times within a season, with minimum spray intervals of 10 days.

The aim of the present studies was to assess whether repeated application of the test products to crop plants at 10-day intervals resulted in residues that were harmful to beneficial arthropods. Bioassays were carried out using both the hoverfly *Episyrphus balteatus* on cabbage plants and the predatory mite *Typhlodromus pyri* on apple tree foliage. Other studies with the parasitoid *Aphidius rhopalosiphi* and the ground-active beetle *Aleochara bilineata* were also carried out but are not reported here.

MATERIAL AND METHODS

Bioassays with hoverflies

Cabbage plants (var. Pixie) being grown outdoors were treated with either a) DPX-JW062 at its maximum recommended rate of 167 g product/ha, b) Dimethoate 40EC (400 g/litre dimethoate) at a rate of 0.85 litres product/ha, as a toxic reference product, or c) water as a control. Up to six applications were made at 10-day intervals using a small-plot, compressed air sprayer (Azo Sprayers, Ede, The Netherlands). The first three sprays were made at a volume rate of 500 litres/ha, the second three at 1000 litres/ha to accommodate for increased foliar growth. The plants were dug up and returned in pots to the laboratory after they had received either 1, 3 or 6 applications. Bioassays were then carried out to determine the effect of treatment residues on adults, larvae and eggs of laboratory-bred *E. balteatus*.

To assess effects on adult syrphids, 2- to 3-week-old gravid females were individually confined over cabbage plants using transparent cylinders of clear acetate sheeting (30 cm wide x 60 cm tall). The tops of these cylinders were covered with nylon netting to allow ventilation, and the arenas were laid out on benching in a glasshouse (18-29°C). To assess pre-treatment levels of fecundity within the fly population, an untreated, aphid-infested cabbage plant was placed in each arena (9 per treatment) and the numbers of eggs laid over a two day period was recorded. The plants were then replaced with the freshly-treated plants being returned from the field (i.e. within 2 h of the last product application). Aphids (*Myzus persicae*) were placed on these plants to provide an ovipositional stimulus. The survival of the flies and their egg production was assessed for a further 2 days. In both the pre- and post-treatment assessments, counts were made of the number of eggs laid on the plants and the number laid on the walls of the arena (as possible evidence of repellent effects).

To assess whether residues on the plants were harmful to the eggs laid upon them, samples were taken on excised leaves, from both the treated and untreated plants after 2 days. These were stored in Petri dishes and the numbers of eggs hatching were recorded over a five-day period.

To assess whether fresh residues were harmful to developing larvae, leaves were cut from treated plants within 2 h of the final application. These were used to line the base of 9-cm-diameter glass Petri dishes, adaxial surface uppermost. Five 2- to 3-day-old larvae were placed in each dish and provided daily with an excess of food (live untreated pea aphids). The percentage of larvae that developed in to adult flies was assessed for five replicate dishes, *i.e.* 25 larvae per treatment. The larval and egg bioassays were carried out in a controlled environment room maintained at 19-23°C with a 16 h light photoperiod.

Bioassays with predatory mites

Two trials were carried out using young apple trees (*Malus* sp. var. Elstar), *c.* 1 m tall, which were grown outdoors in pots. In 1995, trees (6 per treatment) were sprayed with either a) DPX-JW062 at a concentration of 61.7 mg a.i. per litre water, b) Luxan Permethrin 250EC at a concentration of 47.4 mg a.i./litre water, as a toxic reference, or c) water as a control. In 1996, trees were sprayed with either DPX-MP062 at a concentration of 50 mg a.i. per litre water, or with Permethrin 250EC or water, as before. The treatments were applied to the individual trees using a hand-held spray gun (Guarany, Industria Brasileira) to the point of incipient run-off. Actual volume rates applied were measured at 900-1200 litres/ha (mean of *c.* 1000 litres/ha). In both trials, the toxic reference was applied to trees on one occasion, but the test product and control treatments were applied on six occasions at 10-day intervals. In 1995, bioassays were carried out using leaves taken from the trees after the 1st, 4th and 6th applications. In 1996, leaves were collected after the 1st, 3rd and 6th applications, immediately after the spray had dried.

After removing any natural populations of mites or insects from the foliage with a fine brush, under a binocular microscope, 5 leaves from each of 6 replicate trees in each treatment were placed abaxial surface uppermost in shallow trays. These were lined with water-saturated cotton wool which was kept moist throughout. Fifteen leaves from the same treatment were placed in each tray, with any that were over-sized being trimmed to an area of *c*. 5 cm x 5 cm. The leaf petioles were left intact since mites frequently rest and oviposit close to these. The replicates (*i.e*. 5 leaves) in each tray were separated by applying strips of a non-drying, sticky gel (Tanglefoot™) to the cotton wool. Pollen of the broad bean, *Vicia faba*, was placed on each leaf as a food source for the mites. The bioassays were carried out in a controlled environment room (25-26°C, 54-59% RH).

Within 1-8 h of the leaves being collected, 5 larvae/protonymphs of *T. pyri* were placed on each of the leaves (*i.e*. 25 per replicate, 150 per treatment). After 7 days the number surviving was recorded and the mites were transferred to a new set of leaves. These were freshly collected from the treated plants in the field and had again been cleaned of any natural mite infestations. Males from the stock culture were added to the individual leaves if no males were already present. During the second week of the test, the leaves were inspected on a further three occasions (on days 10, 12 and 14) and the numbers of females present and eggs laid were recorded on each occasion. The eggs were removed and on days 10 and 14, up to 30 of these from each replicate were set aside to assess their viability. For this, the eggs were placed on glass plates under humidified conditions (after Bakker *et al*., 1993).

RESULTS

Effects on hoverflies

As a large number of eggs were laid on the treated plants in the three DPX-JW062 treatments, the hoverflies were clearly exposed to product residues. During the bioassay no adult flies died in any of the DPX-JW062 treatments, whereas all of those in the toxic reference treatments died within 24 h (Table 1). More eggs were produced in the post-treatment period than in the pre-treatment period, perhaps as a result of the flies becoming accustomed to their environment (Table 1). The increases seen for the individual treatments were variable but were similar for both the control (36-115% improvement) and DPX-JW062 treatments (9-118% improvement). The proportion of eggs laid on the treated plants, rather than the walls of the arenas, did not differ significantly between the individual DPX-JW062 treatments and their corresponding control treatments (one-way ANOVA of arcsine-square root transformed data for the % of eggs laid on plants, $P > 0.05$). This indicated that the treated plants were not repellent to the egg-laying flies.

The numbers of larvae obtained from the eggs collected from the test arenas were low in all treatments, including the control (Table 1). It was believed that this was due to cannibalism of unhatched eggs by the first larvae to emerge. Since the level of cannibalism was unknown, no analysis of these data was carried out but there was no evidence that the viability of eggs laid on plants treated with DPX-JW062 had been affected.

DPX-JW062 did not significantly affect the numbers of adult syrphids which developed from larvae confined on freshly-treated leaves (Chi^2, $P > 0.05$). The survival rates in the three DPX-JW062 treatments were 92% (1x), 86% (3x) and 76% (6x), and these were similar to values in the control treatments of 76% (1x) and 92% (3x). All of the larvae in the 6x control treatment died for no apparent reason and no larvae survived in the three dimethoate treatments.

Table 1. Results of the bioassays with adult *E. balteatus*. Hoverflies (n = 9 per treatment) were initially confined over untreated cabbage plants for 48 h (pre-treatment period) and then over treated plants for a further 48 h (post-treatment period). The treated plants had previously been sprayed either 1, 3 or 6 times at 10-day intervals.

Treatments		Pre-treatment period			Post-treatment period			% mortality of adult flies by the end of the bioassay
		No. eggs laid	% laid on plants	% eggs hatching	No. eggs laid	% laid on plants	% eggs hatching	
Control	1x	531	94	52	1139	75	32	11
	3x	672	88	41	991	85	23	22
	6x	773	87	53	1057	79	31	0
DPX-JW062	1x	678	86	28	1289	67	29	0
	3x	763	75	35	832	61	8	0
	6x	632	96	38	1377	83	27	0
Dimethoate	1x	551	82	44	91	56	0	100
	3x	440	99	53	90	94	2	100
	6x	491	97	56	78	45	0	100

Effects on predatory mites

In the 1995 study with DPX-JW062, juvenile mite mortality increased in relation to the number of applications made, to a maximum after 6 applications of 37% when data were corrected for control mortality using Abbott's formula (Abbott, 1925) (Table 2). The differences between the individual treatments and the control was only significant for the 6x application regime (one-way ANOVA and Tukey's HSD test, $P < 0.05$), but linear regression analysis indicated that there was a significant relationship between the number of applications of DPX-JW062 and the mortality seen (coefficient of determination = 0.391, n = 29).

In all DPX-JW062 treatments, a reduction in the number of eggs produced per female was observed. However, this reduction was only statistically significant when the DPX-JW062 data were pooled and compared with the controls (one-way ANOVA, $P = 0.015$). For multiple comparisons involving the individual treatments and controls, differences were not significant ($P > 0.05$) and there was no apparent relationship with the number of applications preceding the test.

Table 2. Results of the bioassays with adult *T. pyri* carried out with DPX-JW062 (1995 trial) and DPX-MP062 (1996 trial). The mites (150 per treatment) were confined on leaves taken from apple trees that had previously been sprayed either 1, 3, 4 or 6 times at 10-day intervals. The percentage mortalities of adult mites 7 days after treatment (DAT) were corrected for any control mortality using Abbott's formula and the total treatment effect (E) was derived from the Overmeer-Van Zon formula.

Year	Treatments		% mortality of adult mites by 7 DAT	corrected % mortality	Number eggs per female	Mean % eggs hatching	Total effect (E)
1995	Control	1/4x	12	-	7.1	88	-
		6x	15	-	8.8	93	-
	DPX-JW062	1x	21	10	4.5	91	37%
		4x	23	12	6.2	90	25%
		6x	46	37	8.4	88	47%
	Permethrin	1x	100	100	-	-	-
1996	Control	1/3/6x	11	-	11.4	78	-
	DPX-MP062	1x	11	0	11.9	82	-5%
		3x	8	-3	12.6	79	-14%
		6x	12	1	12.1	83	-5%
	Permethrin	1x	89	88	-	-	-

In line with other testing guidelines for predatory mites (Overmeer and Van Zon, 1982), the overall effect (E) of the individual treatments was calculated using the following formula:

$$E = 100 - \left\{ \left[100 - \left(\frac{m_t - m_c}{100 - m_c} \right) * 100 \right] * \left(\frac{R_t}{R_c} \right) * \left(\frac{H_t}{H_c} \right) \right\}$$

where t = treatment, c = control, m = % mortality at day 7, R = total reproduction (eggs/female) and H = % egg hatch success. The range of values obtained for E (Table 2) was 25-47% and indicated that the product should be classified as being 'slightly harmful' to *T. pyri*. However, it is not possible to say how such apparent effects would translate into effects at the population level in the field, since no validation data are at present available for this novel test approach. It should be noted that for *T. pyri*, products with total effects of up to 55% in the laboratory can be of 'low risk' (*i.e.* < 25% effect) to predatory mites in the field (Bakker and Jacas, 1994).

In the 1996 study, DPX-MP062 was not found to have any significant effects on juvenile survival, adult fecundity or egg viability (same analyses as above, $P > 0.05$). The values derived for total effects were all negative, indicating that the product was not harmful under these test conditions.

DISCUSSION

When attempting to evaluate the effects of multiple product applications on beneficial arthropods, the approach adopted here of returning treated crop plants to the laboratory for extended laboratory bioassays has many practical advantages over ordinary field-based studies. In carrying out experiments under controlled environmental conditions, direct comparisons of the results from individual tests are easier and the data are independent of the variable environmental factors that may be encountered in the field. This will increase the sensitivity of the experiments and small increments in treatment effects are easier to detect, as was shown in the 1995 mite study with DPX-JW062.

However, in order to make a direct link between fate and effect, it is likely that foliage residue analysis would have to be carried out alongside any bioassays. In previous residue analysis studies conducted at three separate trial sites, four applications of DPX-JW062, each equivalent to 75 g DPX-KN128/ha, were applied to apple trees at 7-day intervals. The dissipation of residues following the fourth application varied between trial sites, with 25% to 75% still remaining after 35 days. However, such variation may have been due, in part, to differences in environmental conditions between the three sites. In the present studies, residues of DPX-JW062 on apple leaves had a small effect on *T. pyri* during the 1995 trial. However, in the 1996 trial there was no evidence that residues of DPX-MP062 had any harmful effects on the mites.

The bioassay procedures described here satisfy current European guidelines (Anon., 1991) in that they provide data on the effects of accumulated residues on plants treated under worst-case conditions, *i.e.* where the test product is applied at its maximum recommended rate and with the minimum recommended interval between sprays. It was also possible to evaluate the impact of residues on different life stages of the test species, something that is not always practical in field-based studies. However, this type of experimental approach will not directly provide information on changes being seen at the population level, as might occur where a test product was only affecting a small proportion of the arthropod community with each application. Further validations of the test methods are still required before such extrapolations can be made.

Improvements to the bioassays

Although the evaluation of plant residues at different stages of the spray program increases the number of bioassays that need to be carried out, it can help with the interpretation of data where natural anomalies occur. For instance, the ovipositional behaviour of the individual hoverflies proved to be variable, with 17 of the 81 test insects (21%) failing to lay eggs at all. For future studies it would be advisable to make a greater pre-selection of flies, perhaps using only individuals laying a pre-requisite number of eggs during the pre-treatment period. To enhance the percentage of individuals producing eggs, older females (perhaps a minimum of 3-4 weeks in age) should perhaps be used, as recent studies have shown that the mean time before the onset of egg-laying may be as much as 24 days after emergence from pupae (Dunkley, unpublished data). In retrospect, the method used for assessing syrphid egg viability was flawed since the eggs sampled were laid over a two-day period. The lack of synchrony in larval emergence exaggerated problems with egg cannibalism and this impaired accurate assessments of viability. It would have been better to sample eggs laid after just the first day.

In the mite study, the immigration of natural mite populations on to the trees during the spray program caused some problems. It did not prove possible to eliminate all of these prior to the experiments, in spite of a careful inspection of the leaves carried out beforehand. There was no apparent means of excluding these mites without the use of cages, which in turn might have influenced rates of product weathering.

Bioassays with other species

Although experiments with only two species have been described here, a similar testing approach has already been successfully applied to other species, such as the parasitic wasp *A. rhopalosiphi* and the ground-active rove beetle *A. bilineata*. There is no reason, therefore, why this experimental approach could not be used for most of the species of beneficial arthropod that are currently being used as indicator species for ecotoxicological studies.

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AZOXYSTROBIN: FATE AND EFFECTS IN THE TERRESTRIAL ENVIRONMENT

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ABSTRACT

Detailed laboratory and field studies have been conducted investigating the fate and effects of the new broad spectrum fungicide azoxystrobin in the terrestrial environment, establishing suitability for use in integrated pest management (IPM) programmes.

Foliar uptake of azoxystrobin is low and slow with absorbed compound moving only in the xylem to achieve an even systemic redistribution profile with no accumulation at leaf tips or margins. When used as directed, residues of azoxystrobin and/or its breakdown products are often non-detectable or low in most food products. Azoxystrobin is degraded rapidly in soil under field conditions with an initial half-life generally in the range of one to four weeks. The soil dissipation occurs by both photolytic and microbial processes leading ultimately to complete mineralisation of the compound. Azoxystrobin and its degradates demonstrate low mobility in soil.

The effect of azoxystrobin on non-target organisms has been extensively studied, and no risk to non-target plants, birds and small mammals has been demonstrated. Laboratory toxicity studies, reflecting 'worst case' situations with maximum exposure to the highest recommended application rates, have shown that azoxystrobin has minimal effects on a range of important beneficial arthropods. Numerous field studies have been conducted across Europe demonstrating the safety of repeated azoxystrobin applications to predatory mites in vineyards. With a benign environmental profile and no significant adverse effects on terrestrial non-target organisms, azoxystrobin is highly suitable for inclusion in IPM programmes.

INTRODUCTION

Azoxystrobin is a new broad spectrum fungicide based on naturally occurring compounds (strobilurins) with a novel mode of action. The preventative, curative, eradicant, translaminar and systemic properties of azoxystrobin facilitate control of major Ascomycete, Basidiomycete, Deuteromycete and Oomycete plant pathogens (Godwin *et al.*, 1992).

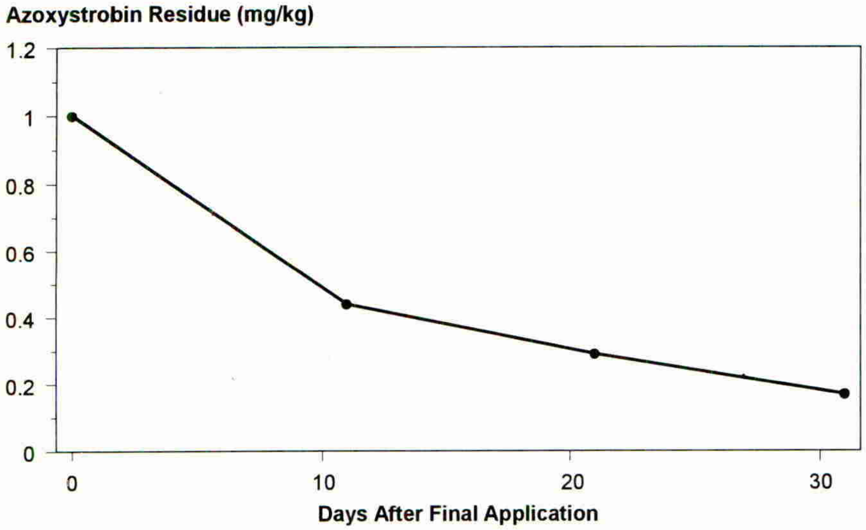
As part of a comprehensive ecological risk assessment, the terrestrial fate and effects of azoxystrobin have been investigated. To assess which non-target ecological groups will be exposed to chemicals in the environment, information regarding degradation in, and movement through, successive environmental compartments is required. The combination of fate and exposure data with relevant information on toxicity, allows evaluation of potential risks to non-target groups. In the terrestrial environment, establishing safety to non-target organisms such as birds, small mammals and plants is important. Also, beneficial arthropods that provide additional natural control of pest species, are a significant component of integrated pest management (IPM) systems. IPM is now a well established practice in many existing and future outlets for azoxystrobin, e.g. vines, glasshouse crops and rice. It is therefore necessary to assess potential risk to beneficial arthropods and thus suitability of azoxystrobin for use in IPM programmes.

ENVIRONMENTAL FATE

Fate in the crop

Following application to the crop, foliar uptake of azoxystrobin is low and slow with absorbed compound moving only in the xylem to achieve an even systemic redistribution profile with no accumulation at leaf tips or margins. When used as directed, residues of azoxystrobin or its breakdown products are often non-detectable or low in many food items. Residue decline data clearly demonstrate that azoxystrobin is not persistent on crop foliage (Fig. 1), the decrease being due to a number of processes including plant growth, photolysis, uptake and plant metabolism. Fungicidal activity is maintained despite this decline because of uptake of azoxystrobin and its high intrinsic activity. Indeed, a very good persistence of fungicidal effect is a key feature of the product on cereals and other crops. The metabolism of azoxystrobin in plants is extensive and complex and independent of crop type.

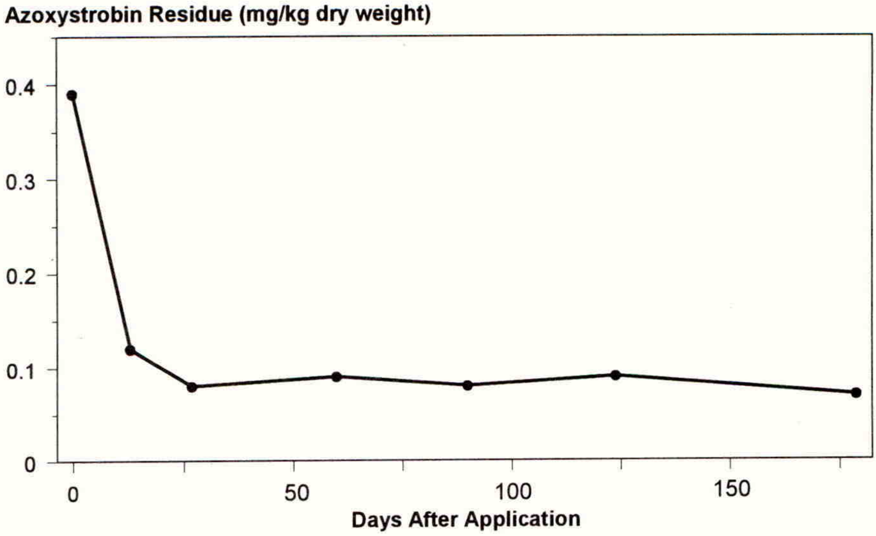
Figure 1. Typical residue decline in forage following three applications of azoxystrobin to cereals.



Fate in Soil

On soil surfaces azoxystrobin is rapidly degraded by light. Under laboratory conditions, the pattern of products formed is complex with the major product being carbon dioxide. Azoxystrobin is dissipated rapidly in soil under field conditions with an initial half-life generally of one to four weeks (Fig. 2), and a DT_{90} of less than one year. The dissipation occurs by both photolytic and microbial processes leading ultimately to almost complete mineralisation of the compound. Photolysis is frequently the dominant dissipating process resulting in relatively short persistence in the field.

Figure 2. Typical field soil dissipation of azoxystrobin.



Azoxystrobin is primarily adsorbed onto organic matter in soil. Adsorption studies also suggest that soils of higher clay content adsorb azoxystrobin more strongly, probably as a result of the increased organic matter surface area available for adsorption. K_{oc} values range from 300 in loamy sand to 1690 in silty clay loam, corresponding to a McCall classification (McCall *et al.*, 1980) of between ‘medium’ and ‘low’ potential mobility in soil. Under field conditions, data from many soil dissipation trials demonstrate azoxystrobin is not detectable below the top 15cm of soil surface and does not substantially move out of the 0-5cm soil. Azoxystrobin does not volatilize from the soil surface.

ENVIRONMENTAL EFFECTS

Beneficial arthropods

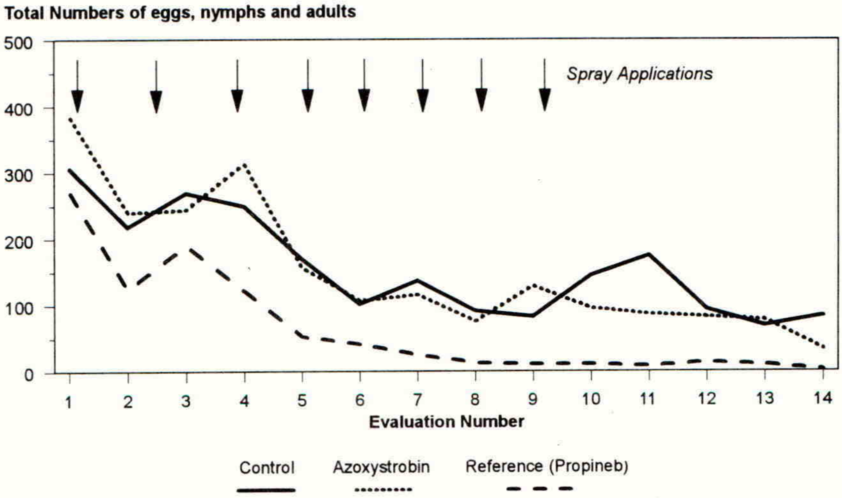
Laboratory invertebrate toxicology studies, reflecting 'worst case' situations with maximum exposure to the highest recommended application rates (Barrett *et al.*, 1994), have demonstrated the safety of azoxystrobin to a range of important beneficial arthropods and other terrestrial invertebrates (Table 1).

Table 1. Effects of azoxystrobin on terrestrial invertebrates.

Species	Testing Method	Effects
Predatory mite *Typhlodromus pyri*	PVC plate limit tests at 250 and 500 g ai/ha (SC and WG formulation).	No mortality or fecundity effects
Parasitic wasp *Aphidius rhopalosiphi*	Extended laboratory limit test at 250 g ai/ha (SC form.).	No mortality or fecundity effects
Parasitic wasp *Trichogramma cacoeciae*	Glass plate limit test at 150 g ai/ha (WG form.).	No mortality or fecundity effects
Carabid beetle *Poecilus cupreus*	Direct application onto adults, their food and test substrate, limit test at 250 g ai/ha (SC form.).	No mortality or feeding effects
Hoverfly *Episyrphus balteatus*	Extended laboratory limit test at 250 g ai/ha (SC form.), direct application onto larvae.	No mortality effects
Lacewing *Chrysopa carnea*	Glass plate limit test at 250 g ai/ha (SC form.).	No mortality effects
Spider *Pardosa* spp.	Direct application onto adults, their food and test substrate, limit test at 250 g ai/ha (SC form.).	No mortality or feeding effects
Honey bee *Apis mellifera*	Laboratory acute contact and oral dose-response studies (SC and WG form.).	Contact and oral LD_{50} > 200 µg ai/bee
Earthworm *Eisenia fetida*	Laboratory 14 day LC_{50} artificial soil test (SC and WG form.).	LC_{50} > 880 mg ai/kg

It is recommended that azoxystrobin is applied up to 6 times within a crop season with spray intervals of less than 14 days, therefore field trials were conducted to assess the risk of repeated applications on important predatory mite species in vineyards. Predatory mites in the family Phytoseiidae provide natural control of some pest mite species particularly important in vineyard and orchard crops (Moreton, 1969). The results from numerous studies conducted across Europe all demonstrate no effects of azoxystrobin on populations of *Typhlodromus pyri* (Fig. 3) and *Amblyseius aberrans*, compared to the toxic reference product propineb.

Figure 3. Effect of multiple applications of azoxystrobin (SC formulation) on predatory mites (*T. pyri*) in vineyards.



Birds and small mammals

Birds and small mammals may be exposed to residues of azoxystrobin through consumption of treated food items. Azoxystrobin is of low toxicity to birds and small mammals (Table 2).

Table 2. Toxicity of azoxystrobin to mammals and birds.

Species	Acute oral LD_{50} (mg kg^{-1} bodyweight)	Dietary LC_{50} (mg kg^{-1} diet)
Rat	>5,000	-
Mallard duck	>2,000	>5,000
Bobwhite quail	>2,000	>5,000

The maximum residue levels on vegetation immediately after application have been estimated for a range of food items based on Hoerger and Kenaga (1972). Comparison of these exposure estimates with toxicity values for both birds and small mammals produce risk quotients that are well below levels of concern, indicating no risk. As azoxystrobin is rapidly metabolised and excreted in animals and does not bioaccumulate (supported by a Log P of 2.5), there is little potential for exposure to predators and transfer of residue through the food chain.

Terrestrial plants

The potential risk of azoxystrobin to a range of non-target terrestrial plants was assessed in glasshouse studies considering both pre-emergent and post-emergent exposure. Species tested represented a wide range of plant diversity (dicotyledon and monocotyledon) likely to be found in both agricultural and non-agricultural situations (Table 3). Azoxystrobin had no effect at the rates tested (up to 1120g a.i./ha) and is therefore unlikely to effect seedling emergence or vegetative vigour of non-target plants.

Table 3. Non-target plant species tested for azoxystrobin safety.

Family	Species
Dicotyledons	
Amaranthaceae	*Amaranthus retroflexus* (pigweed)
Chenopodiaceae	*Beta vulgaris* (sugarbeet)
	Chenopodium album (fat hen)
Compositae	*Bidens pilosa* (hairy beggarticks)
	Xanthium strumarium (common cocklebur)
Convolvulaceae	*Ipomoea lacunosa* (white morning glory)
Cruciferae	*Brassica napus* (oilseed rape)
Euphorbiaceae	*Euphorbia heterophylla* (spurge)
Leguminosae	*Glycine max* (soybean)
Malvaceae	*Abutilon theophrasti* (velvetleaf)
	Gossypium hirsutum (cotton)
Polygonaceae	*Polygonum aviculare* (knotgrass)
Rubiaceae	*Galium aparine* (goosegrass/cleavers)
Monocotyledons	
Cyperaceae	*Cyperus esculentus* (yellow nutsedge)
	Cyperus rotundus (purple nutsedge)
Gramineae	*Alopecurus myosuroides* (blackgrass)
	Avena fatua (common wild oat)
	Digitaria sanguinalis (crabgrass)
	Echinochloa crus-gallii (barnyardgrass)
	Oryza sativa (rice)
	Setaria viridis (green foxtail)
	Sorghum halapense (johnsongrass)
	Triticum aestivum (winter wheat)
	Zea mays (field corn)

DISCUSSION

A detailed study of azoxystrobin fate has shown which environmental compartments the fungicide will move through after application, and at what predicted concentrations. The combination of this information with relevant toxicity data allows a detailed ecological risk assessment to be conducted. It is clear from the environmental fate data that azoxystrobin rapidly dissipates in the terrestrial environment. This results in the formation of a number of complex metabolites but the ultimate product of degradation is carbon dioxide.

Extensive studies have been conducted to assess the effects of azoxystrobin on non-target organisms in the terrestrial environment. The combination of low toxicity to birds and small mammals with low persistence on potential food items and lack of residue transfer through the food chain, indicates azoxystrobin will present no risk to terrestrial wildlife. Also, no effects on a wide diversity of non-target plant species were seen, indicating low risk of azoxystrobin on the emergence and subsequent development of plants either within or outside the target area.

As azoxystrobin is recommended for use on crops where IPM is commonly practised, safety to beneficial arthropods is important. Detailed laboratory and field studies have been conducted and clearly demonstrate that azoxystrobin is harmless to beneficials. This feature of azoxystrobin's environmental safety makes it ideal for use in IPM programmes.

ACKNOWLEDGEMENTS

The authors would like to thank all Zeneca staff and collaborators who worked on azoxystrobin environmental studies.

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SESSION 4B

PEST AND DISEASE CONTROL IN FRUIT AND VEGETABLES

Chairman	Dr I Crute *HRI, Wellesbourne*
Session Organiser	Dr T Locke *ADAS Rosemaund, Hereford*
Papers	4B-1 to 4B-4

ESTIMATING THE ECONOMIC COSTS AND BENEFITS OF PESTICIDE USE IN APPLES

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ABSTRACT

Commercial apple production in the UK is intensive in terms of artificial inputs, with high levels of fertiliser and pesticides applied to maintain yields and farm profitability. However, in recent years there has been increasing concern about the effects of such inputs on the environment and human health, and alternative means of production, ranging from low input systems to organic and biodynamic agriculture, have been proposed. If production systems became less intensive, it may be assumed that such negative effects would be reduced. However, if growers are currently maximising profits, any change to a new system is likely to result in lower incomes to growers and reduced economic welfare for society as a whole. Thus a key issue is the costs which would be incurred if growers were to move to less intensive systems. This paper investigates the costs to both growers and society of such changes using data relating to Cox's Orange Pippin which is the dominant apple variety in the UK. Scenarios are constructed for "current commercial practice", "integrated cropping systems" and "no pesticide". On the basis of these, aggregate estimates are made of the benefits to the use of varying levels of pesticide in apple production.

INTRODUCTION: COST BENEFIT ANALYSIS

Cost Benefit Analysis (CBA) is concerned with the appraisal of investment projects or policies. The technique is used to identify the optimal choice from two or more alternatives (Gittinger, 1984), which are often formalised as the "with" and "without" project (or policy) scenarios. In this study we compare the production of crops under conventional farming practices with their production under low input systems. The comparison is made by calculating the welfare gains of each of the scenarios, using consumer prices as a measure of social welfare. However, optimality depends on the perspective of the CBA: the farmer's optimal level of pesticide application may differ significantly from that of society as a whole. This may be due to externalities from the production process which are not taken into account when resource use decisions are made. A common example is pollutants where clean-up costs or other environmental disbenefits may not be directly involved in the application decision.

APPLE PRODUCTION IN THE U K

Apples are the most important fruit crop in the UK in terms of area of production and require relatively high levels of pesticide inputs. Profitability is marginal in many years, as evidenced by the decline in the area of apple orchards (dessert and cooking) from 25,000 ha. in the early 1980's to about 19,300 ha in 1993 (Anon., 1996). Cox's Orange Pippin is the dominant variety, accounting for about 40% of the total apple area, and almost 70% of the dessert apple area. This variety is used as a proxy for all apple varieties in this study. The response to different pesticide usage scenarios is cultivar-specific, and thus the selection of other cultivars would have yielded different results, but the pattern of responses reported is unlikely to differ significantly. There are no consistent differences in either crop production techniques or yields between regions in the UK, and the UK crop is thus considered in its entirety.

High levels of pesticides are applied to apples in the UK which is at the edge of the ecological range over which the crop can be grown. Fungicides are the major pesticide, accounting for almost half of the total weight of active ingredients. Table 1 shows that the amount of pesticides applied to apples has declined considerably in that last ten years largely due to (i) improved application technology; (ii) the use of new active ingredients which are active at lower dosages (eg triadimefon); and (iii) the use of various forms of integrated pest management (eg predator friendly pesticides). However, the rate of decline has slowed between 1987 and 1992, indicating that there may be limits to the reductions in pesticide applications which these improvements cause.

Table 1. Pesticide Applications on Apples: kg a.i. per ha.

	Fungicides	Herbicides	Insecticides	Growth Regulators	Total
1983	13.61	4.43	3.53	0.18	21.75
1987	5.84	4.31	2.03	0.85	13.03
1992	6.60	2.91	2.89	0.33	12.72

Source: Davis *et al.* (1991); Thomas & Gaithwaite (1994)

SELECTION OF SCENARIOS AND MODEL TREATMENTS

In order to study the costs and benefits of pesticide use on apples, it is necessary to set up production scenarios.

Current Commercial Practice (CCP)

CCP is defined for the purposes of this study as "crop husbandry which maximises profitability using external inputs applied within permitted limits to overcome constraints on production". This represents, in the view of the national extension service, ADAS, the pest control strategy adopted by the "average" commercial grower in the UK in 1994. CCP assumes a routine programme for the control of major diseases and weeds but a supervised approach for pest control (Carden, 1987). Having identified the target organisms and the pesticides effective against them, the precise choice of pesticide is governed mainly, but not exclusively, by price and not by selectivity against the target organism.

Integrated Farming System (IFS)

We define IFS as "a crop husbandry system which aims to maximise profits thorough the application of reduced levels of inputs". Environmental benefits are thus secondary to profit maximisation. A large minority of growers are already using an IFS for the control of at least some pests, due to the high costs of pesticides and insect pest resistance, although few growers apply these techniques to all pests. This approach relies on detailed and regular monitoring of pests and diseases; meteorological data to determine spray timings; relating herbicide dosages precisely to the weed population density; and biological control agents (eg predatory mites). Those pesticides which target specific pests have been selected in preference to broad-spectrum and persistent pesticides. In some cases lower application rates than those in CCP have been used. This system was based on guidelines agreed by IOBC (International Organisation for Biological Control (Dickler & Schafermeyer, 1993).

No Pesticide (NP)

This scenario allows comparison of the other two scenarios with a "control". It should be distinguished from organic production, which would not involve the use of artificial fertiliser, and thus would not isolate the specific effects of changes in pesticide usage. The scenario is thus hypothetical, in that it would not be used by commercial farmers, who would opt for organic crop production which would allow them to gain a premium for their produce. It is unlikely that any saleable crop of dessert apples would be obtained, and the fruit would only be of value for juicing. An adjustment period of several years would be required to allow the ecology of the orchard to reach equilibrium before even this could be expected.

FARM-LEVEL GROSS MARGINS

The effect of some changes in enterprise size or production method on farm profits can be estimated by analysing the change in gross margins, assuming that fixed costs are not affected by these changes. If farmers adopted new pesticide usage techniques, there would be changes in the cost of pesticides, pest and disease monitoring, pesticide application (fuel, machinery depreciation and labour), fertiliser, and planting material, eg more resistant cultivars. All of these are thus variable costs. It should be noted that the labour used to apply pesticides would normally be permanent (as opposed to casual), and thus not included in the calculation of gross margins. However, for the purposes of this study, this factor is included in the gross margin calculations since changes in pesticide usage scenarios would have significant consequences for farm labour costs.

Yield and Output

Apple yields vary considerably between farms and cropping years. A similar average yield of 19 t/ha has been assumed for both the CCP and IFS scenarios. Of crucial importance to the output is the grade, or quality of the produce, since apples are sold largely on appearance. Class 1 fruit is the only type acceptable to most of the major buyers (e.g. supermarkets), and the price of Class 2 fruit can be half of that obtained for Class 1 fruit. For this study, it is assumed that 80% of the harvest under Scenarios 1 and 2 will be of Class 1. The NP option would yield fruits of juicing quality only. Prices of £533/t for Class 1 fruit and £243/t for Class 2 fruit have been assumed (August 1994). The price used for NP is that received for juicing, £50/t.

Variable Costs

Fertilisers costs are assumed similar for each system:(N 60 kg. @ £0.27/kg = £16.20; P 20 kg. @ £0.25/kg = £5.00; K 40 kg. @ £0.17/kg = £6.80). Pesticide application costs are estimated to be £10.50/ha. (Anon, 1993). The number of spray rounds required is considerably less than the number of active ingredients applied since tank mixes are possible. The use of an integrated system would not lead to a reduction in the number of spray rounds (as opposed to a reduction in the amount of pesticide applied); and it is estimated that the use of non-systemic herbicides will increase from 2.5 to 3 the number of herbicide applications required in the IFS. Pest and disease monitoring is a vital part of both CCP and IFS production systems. However, the latter would require more intensive monitoring for a wider range of pests. It has been assumed that monitoring of a 4 hectare block takes 30 minutes at £4.70/hr, ie £0.59/ha/visit. The IFS scenario would require weekly monitoring, amounting to 25 visits during the growing season, ie £14.75/ha/season. This may be compared to 8 visits under the CCP scenario, with a total cost of £4.72.

Table 2. Apple Gross Margins (£/ha).

			CCP	IFS	NP
Output	Yield, t/ha	Class I	15.20	15.20	0
		Class II	3.80	3.80	0.00
		Juicing	0.00	0.00	10.00
	Price, £/t	Class I	533.00	533.00	533.00
		Class II	243.00	243.00	243.00
		Juicing	50.00	50.00	50.00
	Output, £/ha	Total	9,025	9,025	500
Variable Costs, £/ha	Pesticides		747.00	648.00	0.00
	Application costs		183.75	189.00	0.00
	P &D monitoring		4.72	14.75	0.00
	Fertiliser		28.00	28.00	28.00
	Sundries		61.25	61.25	0.00
	Picking		418.00	418.00	200.00
	Packing		950.00	950.00	0.00
	Marketing		3,145.00	3,145.00	650.00
	Total v.c.		5,537.72	5,454.00	878.00
Gross margin	£/ha		3,487.28	3,571.00	-378.00

Other production costs include an allowance for sundries such as the hire of bees, and the purchase of incidentals such as protective clothes, picking buckets, etc. Labour requirements for harvesting under CCP and IFS are about 5.8 hr/t. for picking and 2.6 hr/t. for related activities (cartage, marking, supervision, etc.). This is mainly piecework and is charged at £2.60/hr, giving a total of £22/t. Packing costs are approximately £50.00/t. under current conditions. Marketing costs are estimated to be £165/t. for eating apples and £13/t for juicing apples.

Gross Margins

Table 2 indicates that IFS appears just slightly more profitable than CCP, due to lower pesticide costs, but that NP results in a negative gross margin. This implies that, other things being equal, producers would grub their orchards immediately.

COSTS AND BENEFITS AT THE NATIONAL LEVEL

If pesticides were not used, apple production would thus not be commercially viable, and the market shortfall would be made up by imports at a similar price. With a negative gross margin apple producers would leave the industry and find other uses for their land. The net benefits to pesticides should therefore take account of the returns from the next best alternative land use. In a world where pesticides were banned for apple production it is also likely that similar bans would apply to other crops.

Table 3. Net Annual Economic Benefits of Pesticides on Apples.

	With pesticides (Apples, CCP)	With pesticides (Apples, IFS)	Without pesticides (Wheat, NP)	notes
Area, '000 ha	19	19	19	(1)
Financial gross margin, £/ha	3,487	3,571	601	(2)
Economic gross margin, £/ha	3,487	3,571	160	(2) (4)
Fixed costs, £/ha	2,395	2,395	565	(3)
Net economic margin, £/ha	1,092	1,176	-405	
Total economic net margin, £m	20.7	22.3	-7.7	
Benefits to Pesticides, £m	28.4	30.0	-	

Notes: (1) No pesticide option assumes apple growers convert to No pesticide wheat.
(2) Source: Table 2, includes Area Payments for NP Wheat.
(3) Source: Nix (1994)
(4) Economic price of wheat = £75.36/t

We therefore argue that "no pesticide" wheat would be a reasonable alternative. Under such conditions the CBA must include changes in fixed costs, since "with pesticide" apples would have a very different profile of fixed costs as compared with "no pesticide" wheat. After fixed costs, excluding rent, have been deducted, the net margin from wheat (using an economic price of £75.36/t) is negative (table 3). But producers would still grow wheat since the area payments and supported price would give positive financial gains.

Table 3 shows that under these conditions the net economic benefit of pesticides on apples is estimated at £28.4 million, which is the difference between the benefits of the "with pesticide" policy (£20.7m) and the "without" policy (£-7.7m). It is, of course, open to debate as to what impact on domestic apple prices there would be if domestic production were scaled down. But as the price of NP apples increases, so the benefits to pesticides decline. These benefits would be just extinguished if, as a result of the ban, the price of NP apples rose from its present £50/t (for juicing) to approximately £440/t, [(£3487+£878)/10t, from table 2]. Under such scenarios the benefits of moving from conventional to integrated systems appear to be relatively small at less than £2 million nationally.

Finally, these figures could be regarded as the breakeven value of the environmental benefits, such as reduced pollution and human health costs foregone, which would need to result if society were to gain if pesticide inputs were to be reduced in these ways.

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PRELIMINARY INVESTIGATIONS ON APPLE SAWFLY CONTROL WITH FUNGICIDES

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ABSTRACT

In a three year study the fungicides fenarimol, cyproconazole + captan and thiophanate-methyl were found to be highly effective in reducing damage to apple fruit due to attacks by the apple sawfly. The fungicides were found to be most effective when applied at the peak of the flight of the pest, which usually occur at the pink bud growth stage. Fungicides applied at petal fall were less effective and could not be economically justified.

INTRODUCTION

The apple sawfly (*Hoplocampa testudinea* Klug.) is a species commonly occurring in Poland, as well as in many other European countries. In recent years the pest population has considerably expanded, causing serious damage in some orchards in certain regions. Such an effect can be of economic importance in seasons or regions when fruit set is poor or moderate.

Effective control of this pest is determined by two essential factors: an efficient pesticide and an appropriate timing of the treatment. Based on research by Stępniewska (1961) and Niezborała (1978) a treatment against apple sawfly a few days after petal fall had been considered adequate for many years in Poland. However, according to Karabas (1965, 1967) pre-blossom spraying can provide better results. Also, the use of sticky traps helps to determine the appropriate timing of sawfly control (Coli *et al.*, 1985; Wildbolz & Staub, 1986; Galli *et al.*, 1993).

Most of the recommended insecticides, such as diazinon, fenitrothion and phosalone, are of translaminar action and although effective against the pest are also harmful to most beneficial fauna (predators and parasitoids). According to some authors (Jaworska, 1982, 1987; Babandreier, 1996) parasitoids in particular can potentially suppress populations of apple sawfly. Consequently, the use of these insecticides may not be acceptable in a system of integrated fruit production (IFP). However, since the 1970s it has been known that benzimidazole-generating fungicides, especially thiophanate-methyl, when applied before blossom, may also significantly reduce populations of apple sawfly (Prędki & Profic-Alwasiak, 1976). Unfortunately, the common occurrence of strains of scab (*Venturia inaequalis*) resistant to this group of fungicides has considerably limited or even eliminated their use in apple orchards.

The overall aims of the research project, of which some results are reported here, were to assess the effectiveness of white sticky traps in catching adult apple sawflies, to determine the flight dynamics of this pest in Poland and to find efficient pesticides for its control which would meet IFP requirements.

MATERIALS AND METHODS

The research was carried out during 1994-96 in commercial apple orchards located in various regions of Poland (Figure 1). Swiss white sticky traps of the "Rebel^R bianco" type were used for determining the number of caught sawflies and the dynamics of their flight. Shortly before the pink bud growth stage the traps were hung on randomly selected trees in the central part of the section to be monitored in each orchard. Depending on the orchard area or number of cultivars 2-6 traps were used. Traps were inspected every 1-3 days, specimens were identified, counted and removed on each occasion.

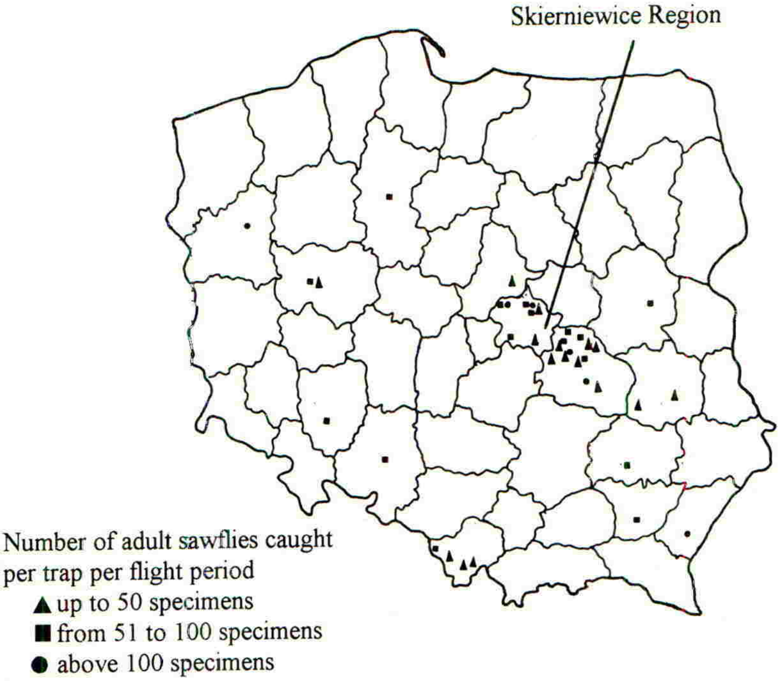

Figure 1.Population of the apple sawfly in some orchard growing regions of Poland in 1994-1996

Application of sawfly control with selected pesticides was undertaken in several of these commercial orchards, however, only in two of them was accurate analysis of damage made. The fungicides were applied at the moment when the economic treshold was exceeded, as determined by the sticky traps. Such situation usually occurred at pink bud stage. The effectiveness of the treatments was compared with the results obtained from the use of insecticides applied directly after petal fall. Each treatment was applied to 0.5-1.0 ha section of the orchard, but unsprayed sections comprised only about 0.25 ha. Treatment effectiveness was assessed prior to June fruitlet fall by the inspection of 800 fruitlets (200 each from 4 randomly selected trees) collected within the central part of each section, to record the level of apple sawfly damage. The effectiveness of the treatments was calculated by Abbott's formula only (Abbott, 1925).

RESULTS

In all of the orchard growing regions under study (Figure 1) the populations of apple sawfly exceeded the economic damage threshold, which is considered to be 20-30 adults per trap (Höhn *et al.*, 1993), at the pink bud and/or bloom stage.

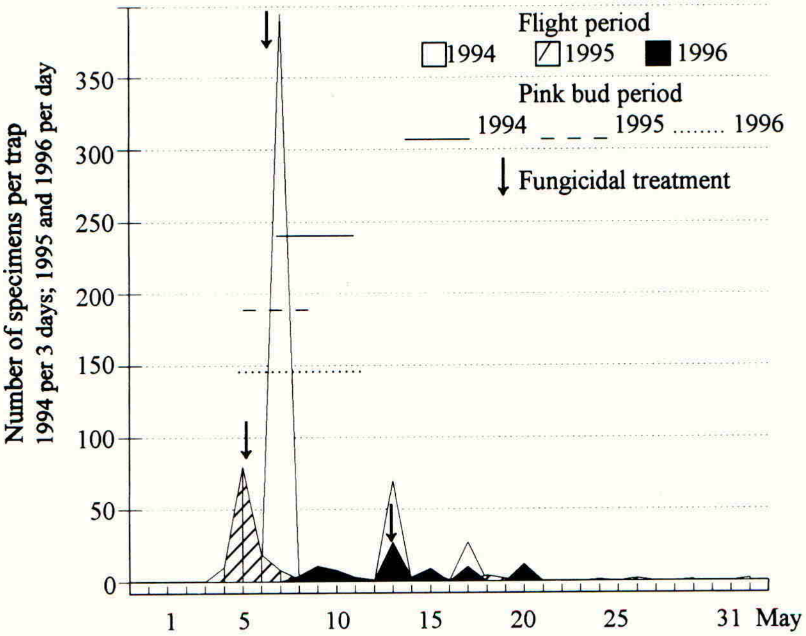

Figure 2. Apple sawfly caught in sticky traps at Skierniewice

Studies on the flight dynamics of the pest in each year showed the presence of adult sawflies in the period from pink bud until petal fall. The data for Skierniewice in 1994-96 are shown in Figure 2. The most intensive flight was observed mainly during pink bud or a few days before full blossom.

Fungicide tests 1994

Initial trials conducted in 1994 showed that among the 5 fungicides tested the best results were provided by fenarimol and cyproconazole + captan, when applied at the peak of pest flight (Table 1). Their effectiveness was similar to that of phosalone used at the same time and diazinon when applied at petal fall. Fungicides applied at petal fall did not provide satisfactory control of pest damage.

Table 1. Effectiveness of fungicides in reducing apple sawfly damage in 1994 at Skierniewice

Active ingredient	Product	Product dose rate l or kg/ha	% control Period of treatment pink bud	 petal fall
cyproconazole+captan	Atemi C	1.5	68.9	-
tolyfluanid	Euparen 50 WP	5.0	62.0	11.2
dithianon	Delan 750 SC	1.2	22.6	-
thiram	Thiram Granuflo 80 WG	3.0	60.4	-
fenarimol	Rubigan 12 EC	0.45	94.7	25.2
diazinon	Basudin 25 EC	2.25	-	66.0
phosalone	Zolone 35 EC	1.8	70.4	97.2

Damaged fruitlets in the control - 13.2%

Fungicide tests in 1995 and 1996

Subsequent trials in 1995 and 1996 were also conducted at the peak of the pest flight and included only those fungicides which had appeared most promising in 1994, plus an evaluation of bitertanol and thiophanate-methyl. All of the fungicides showed a high efficacy which was equal to that of the standard insecticides phosalone and etofenprox applied at petall fall (Table 2).

Table 2. Effectiveness of fungicides in reducing apple sawfly damage in 1995 and 1996 at Maurzyce

Active ingredient	Product	Product dose rate l or kg/ha	% control Period of treatment pink bud 1995	 1996	 petal fall 1995	 1996
cyproconazole+captan	Atemi C	1.5	100.0	100.0	-	-
bitertanol	Baycor 25 WP	2.25	0.0	-	-	-
fenarimol	Rubigan 12 EC	0.45	96.5	91.2	-	-
thiophanate-methyl	Topsin M-70 WP	1.5	93.6	91.2	-	-
phosalone	Zolone 35 EC	1.8	89.3	-	100.0	-
etofenprox	Trebon 10 SC	0.9	-	-	97.2	97.0

Damaged fruitlets in the control - 17.7% (1995); 8.5% (1996)

DISCUSSION

The study on apple sawfly dynamics showed that the period of peak flight was at a time when insecticidal spraying of orchards was not possible because of the risk of injury of bees. The results from these trials show that some fungicides, when applied at the correct timing, can give excellent control of damage due to this pest. It has been previously reported (Prędki & Profic-Alwasiak, 1976) that thiophanate-metyhl was an effective product for the control of this species but several applications were required to achieve a satisfactory reduction in damage. This was because no work was undertaken to determine exactly the peak of the pest flight. In the present study such monitoring was possible with the use of the white sticky traps and this helped to establish the appropriate time for a single treatment.

The fungicides applied fully meet IFP requirements and, if used at the right time give an opportunity to control the apple sawfly as well as fulfilling their standard role in controlling scab and powdery mildew. However, the use of thiophanate-methyl is considerably limited due to the wide-spread occurrence of benzimidazole-resistant strains of *Venturia inaequalis*. Instead, fenarimol and cyproconazole + captan are already widely recommended and used. So, in forthcoming years the application of these fungicides will be advised for apple sawfly control especially in the IFP farms.

Up to now the exact mechanism of the effect of these fungicides on *H.testudinea* is not understood. The preliminary observations have shown that on treated trees egg development was restrained and larval hatch strongly reduced. However, other modes of action (repellents or antifeeding) could also be possible.

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CONTROL OF TRICHODERMA HARZIANUM - A WEED MOULD OF MUSHROOM CULTIVATION

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ABSTRACT

An aggressive strain of *T. harzianum*, molecular type Th2, has been associated with serious yield reductions of the cultivated mushroom *A. bisporus*. Experiments show that a single *Trichoderma*-inoculated mushroom spawn grain in a 45 kg tray of compost can reduce yield by 12 to 46%. Existing fungicide treatments and label recommendations are ineffective against this new problem for mushroom growers so a number of fungicide treatments were tested. Carbendazim applied to spawn grains gave the best control. A specific off-label approval has now been obtained for this fungicide treatment.

INTRODUCTION

Aggressive strains of *Trichoderma harzianum* have been associated with a condition known as compost green mould which can result in serious reductions in the yield of cultivated mushrooms (*Agaricus bisporus*). In the UK the most aggressive strain has been designated as "Th2" using molecular techniques and is one of three *T. harzianum* strains found on mushroom farms (Muthumeenakshi *et al.*, 1994). Compost green mould is a relatively new problem of mushroom cultivation. The first devastating outbreaks occurred in 1985/86 in the UK and Ireland (Seaby, 1987) and since then it has been recorded in Canada (Rinker, 1994), Australia and the USA although other strains are associated with these outbreaks.

Trichoderma harzianum Th2 colonises mushroom compost, usually during spawn run, and prevents the mushroom mycelium from becoming established. Fletcher (unpublished) demonstrated that the cereal grains which provide nutrition (and support) for the mushroom inoculum are an essential food base for *Trichoderma* and without these grains green mould does not establish in compost. *T. harzianum* spores added to unspawned compost do not grow but they remain viable for a considerable time - at least 12 weeks (Fletcher, unpublished). Control of compost green mould has been hampered by the fact that once the condition has become apparent it is too late to prevent massive yield losses. In addition, it is a relatively new problem which is unlike other problems of mushroom cultivation and none of the fungicides approved for use can be applied at spawning. All fungicides are applied at a much later stage in the crop cycle when disease outbreaks are more likely.

Experiments were carried out jointly by HRI and ADAS to study the effect of inoculum type and concentration on the establishment of a known aggressive *T. harzianum* Th2 isolate in mushroom compost and its effect on mushroom yield. Attempts were made to control the problem by preventing the colonisation of the spawn grains by *T. harzianum*. Three fungicides with known differential toxicity to *T. harzianum* Th2 and *A. bisporus* were used at very low doses on the spawn.

MATERIALS AND METHODS

Compost

Mushroom compost from a commercial farm was used in all experiments. It was a dense, degraded compost made from wheat straw, pig, horse and chicken manure and water and was spawned using Somycel 609 spawn at a rate of 0.5% (w/w). The compost was spawn-run at 27⁰C rather than the standard 25⁰C in order to favour *T. harzianum*. The spawn-run compost was then cased and case-run at 25°C and aired and cropped at 18°C according to standard mushroom cultivation practices (Fletcher *et al.*, 1989).

Inoculum

Compost was inoculated using one of two types of inoculum. The first consisted of mushroom spawn grains which were coated in spores of *T. harzianum* Th2 (isolate T7). Spawn grains were placed in Petri dishes containing sporulating cultures of *T. harzianum* Th2 and gently shaken to coat the spawn grains with spores. Coated spawn grains were then placed into trays of spawned compost at rates ranging from 1 to 200 inoculated spawn grains per 45 kg tray of compost. The second inoculum type consisted of a *T. harzianum* Th2 spore suspension at concentrations ranging from 3.4×10^1 to 2.5×10^7 spores/ml. Using 2 x 50 ml syringes, 100 ml of spore suspension were sprayed into 45 kg of spawned compost while trays were being filled to give final concentrations in compost (to the nearest power of 10) of 10^2 to 10^8 spores/kg compost.

Fungicides

Three benzimidazole fungicides were tested, benomyl and carbendazim - active ingredients in 'Benlate' and 'Bavistin DF', respectively, - and thiabendazole in an experimental liquid formulation provided by Agrichem. The content of active ingredient in all three was 50% and the recommended rate of use for the first two products for the control of mushroom diseases is 240 and 250g/100 m^2 respectively. There is no label recommendation for incorporation of fungicides into compost or onto spawn. Benlate no longer has a label recommendation for use on mushrooms.

Experiment 1

Compost was inoculated with 1, 5, 50, 100 or 200 *T. harzianum* Th2 coated spawn grains over a period of 4 mushroom crops. The number of inoculation points used in the first crop was 50, 100 and 200 but these treatments reduced yields similarly so in subsequent crops progressively fewer inoculation points were used. Consequently, inoculum treatments were replicated from 1 to 3 times over 4 crop cycles. Assessment of *T. harzianum* Th2 colonisation was carried out at first flush by estimating the % cover. The yield of mushrooms was recorded and the compost was analysed at the end of the crop for the presence of *T. harzianum* Th2 propagules.

Experiment 2

This was the same as experiment 1 except that a spore suspension was used as inoculum rather than spore-coated spawn grains. The number of spores added to trays of compost was in the region of 10^2, 10^5, 10^6, 10^7 and 10^8 spores/kg of compost.

Experiment 3

Three fungicides were applied in two different ways to see whether or not they were effective in controlling compost green mould. The first method consisted of fungicides being incorporated into compost at a rate of 70g/tonne of compost to give a concentration of active ingredient of 35 ppm (this approximates the concentration of carbendazim in casing following label recommendations). The relatively small amounts of fungicide were bulked up so that there would be a more even distribution of product throughout the compost. Benomyl and carbendazim were bulked up in 2.2 kg chalk/tonne of compost and the liquid thiabendazole was dissolved in only 800 mls of water/tonne of compost as mushroom spawn does not tolerate free water in compost very well. The second method of fungicide application consisted of treating the spawn grains with a fungicide chalk mix at the rate of 115 mg ai/10g chalk/kg spawn. This rate is low when compared with seed treatments which average about 1g ai/kg seed but the short time interval to harvesting mushrooms (6-8 weeks) and the relatively high moisture content of spawn compared to seed prompted the use of a lower rather than a higher rate. This rate was also demonstrated to be effective in laboratory tests (Fletcher, unpublished). The fungicide was gradually bulked up in the chalk carrier prior to gently mixing it with the spawn as very rough handling of the spawn can damage the mycelium and impede the spawn run. Trays of compost (45 kg) were inoculated with *T. harzianum* Th2 using 100 ml of a spore suspension containing 3 x 10^6 spores/ml resulting in approximately 10^7 spores/kg compost. First flush mushrooms were sampled from fungicide treated and control plots and analysed for carbendazim residues by Oxford Analytical Ltd.

In all experiments 8 replicate plots were prepared and laid out in a randomised block design. Data were analysed by ANOVA with some data being transformed prior to analysis.

RESULTS

Spore coated spawn grain inoculum

All plots which had received inoculated spawn grains experienced a reduction in yield due to compost green mould (Figure 1). The presence of 1 inoculated spawn grain reduced yield by 12-46% while 5 inoculated spawn grains or more reduced yield by 51-98%. Control yield over 4 crops averaged 194 $\pm$ 25 kg/tonne.

Spore suspension inoculum

Similar trends were observed when a spore suspension was used as inoculum except that no yield reduction was recorded at the lowest inoculation rate of 10^2 spores/kg of compost (Figure 2). Yield reductions of over 90% were recorded at the highest level of inoculation (10^8 spores/kg compost) while they were more variable at intermediate inoculation rates (62 - 99% yield reduction at 10^5 - 10^7 spores/kg compost).

Figure 1. Yield of mushrooms from 45 kg of compost containing *T. harzianum*- inoculated spawn grains

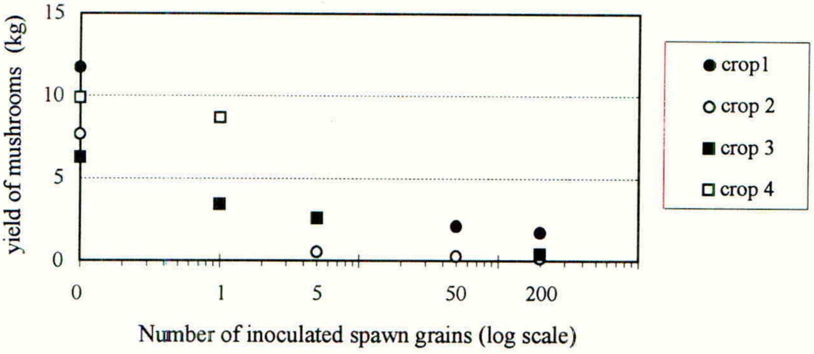
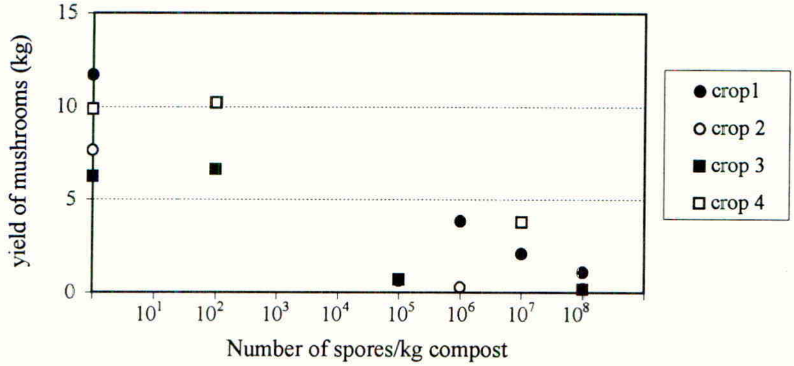


Figure 2. Yield of mushrooms from 45 kg of compost inoculated with *T. harzianum* Th2 spores



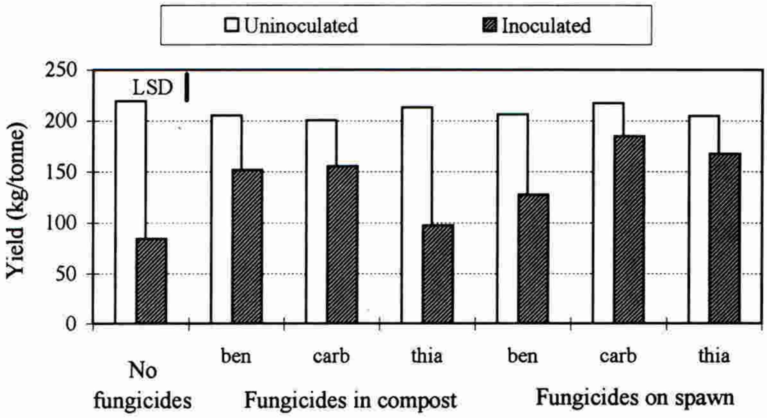
Manifestation of *Trichoderma*

Sporulating *T. harzianum* was observed on the cased surface of the compost. Mean % cover was in the region of 20% for crops 1 and 2 but individual plots varied from 0 to 90% cover. There was no consistent relationship between sporulation levels on the casing surface and either inoculum type or rate. High numbers of *T. harzianum* propagules were recovered from the compost at the end of the crop cycle. Compost from crop 2 contained a higher number of *T. harzianum* propagules at the end of the crop - 6.2 x 10^{10} propagules/kg fresh weight of compost - compared with 2.9 x 10^{10} propagules/kg fresh weight of compost from crop 1.

Fungicide control of compost green mould

Carbendazim on spawn gave the best control of compost green mould with mushroom yields of 84% being recorded compared to 100% (219 kg/tonne) for uninoculated compost and 38% for inoculated compost with no fungicide treatment (Figure 3). The thiabendazole spawn treatment also gave good control with a yield of 77% being obtained. The benomyl spawn treatment only gave a yield of 58%. Two of the compost fungicide treatments, benomyl and carbendazim, gave reasonable yields of 69 and 71% respectively. The thiabendazole in compost treatment yielded only 44% of control which was not significantly different from inoculated compost with no fungicides. None of the fungicide treatments had any significant phytotoxic effect on mushroom yield.

Figure 3. Effect of three fungicides on the mushroom yield from compost inoculated with *T. harzianum* Th2 spores (ben = benomyl; carb = carbendazim; thia = thiabendazole)



DISCUSSION

A single spawn grain coated in spores of a compost colonising *T. harzianum* isolate was sufficient to reduce mushroom yields by up to 46%. This finding has very important implications in crop hygiene practices both when handling spawn and during the spawning operation. Low levels of spore inoculum did not have a significant effect on yield whereas higher levels did, but were somewhat erratic. This may indicate that the added spores need to encounter spawn grains before they can seriously colonise the compost. The chances of this happening are greater when the number of spores added are relatively high. It is not yet known if there is a threshold spore load required per spawn grain in order for compost colonisation to occur. In theory, it only requires one spore per spawn grain but factors such as vigour of the spawn, compost quality and compost temperature may all influence the relative success of any single *T. harzianum* spore.

Compost factors appear to have some influence on the progression of compost colonisation by *T. harzianum*. Results from another experiment in this project, which are not presented here, indicated that a less well degraded compost showed no visible signs of green mould during cropping and had a significantly lower number of *T. harzianum* propagules present at the end of the crop compared with the more degraded compost used in these experiments. This paucity of *Trichoderma* expression in the less degraded compost was also associated with slightly better yields although dramatic yield reductions in response to inoculation still occurred. Work is in progress to try and identify what compost factor(s) are responsible for these observed differences in *T. harzianum* expression and effect.

The best fungicidal control of *T. harzianum* colonisation was obtained when spawn grains were treated with carbendazim. Some yield reduction was still observed and this may reflect the presence of spawn grains with less than optimum fungicide coverage. Refinement of the fungicide application method should lead to improved performance. Carbendazim incorporated into the compost also gave good control but not as good as when applied to the spawn (84 and 71% respectively). Considering that only 1.15g of fungicide is needed to treat the spawn for 1 tonne of compost, spawn treatment represents a more economical use of the fungicide when compared with a compost treatment which would utilise 70g of product/tonne of compost. These results also demonstrate the importance of the cereal grain base used in spawn on the development of *Trichoderma* in the crop. Thus, by protecting the grain for a short time enabling the mushroom mycelium to grow vigorously on the grain, the problem of compost green mould can be prevented

Fungicide residues in mushrooms harvested from the fungicide treated crops described above were found to be less than the M.R.L. (maximum residue level) allowable for carbendazim in fungi which is currently 1 mg of ai/kg of mushrooms (Anon, 1988). The results from these and subsequent experiments were used to obtain a specific off label approval (SOLA) for the use of carbendazim as a spawn treatment to control compost *Trichoderma* from the Pesticide Safety Directorate, MAFF (document number 1144/95).

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SPRAYING IN FIELD VEGETABLES: DEPOSIT AND BIOLOGICAL EFFICACY, THE EFFECTS OF VOLUME RATES, DOSE, SPRAY INTERVAL AND AIR ASSISTANCE ON DISEASE CONTROL

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ABSTRACT

In a series of experiments the deposition of spray liquid and the biological effects were determined in arable crops using a sprayer equipped with an air assisted system to convey the spray droplets to and in the crop. In four growing seasons the use of air assistance was compared with conventional spraying. The biological effects were determined in randomized field tests, with spray concentrations of the active ingredient varying from 0 to 100% of the dose recommended, and volume rates of 100 l ha^{-1} and 200 l ha^{-1} for onions.

The amount of deposition of the spray liquid was established by washing paper strips suspended at different heights in the crop, using the dye Brilliant Sulfo Flavine (BSF).

Biological effects of the sprayings were investigated by quantifying the percentages of the leaf area covered with 'leaf spot' (*Botrytis squamosa*) in onion. In each growing season the number of infected leaves or level of infected area of the plants was measured on each plot at weekly intervals from the time of first infection.

The use of air assistance resulted in an even distribution of the spray in the crop, and a more evenly spread distribution over the crop. The total deposition on the plants was significantly higher for air assisted spraying. Significant differences were found in the biological efficacy between volume rates, spray interval and dosage.

INTRODUCTION

Because of environmental contamination a general reduction in the use of pesticides is required. The aim in The Netherlands is to reduce the use of pesticides by 50% by the year 2000. Drift of spray to surface water next to cultivated land should be reduced by more than 90% (Tweede Kamer der Staten-Generaal, 1991). In accordance with the Multi Year Crop Protection Plan research has been set up to develop improved application techniques for pesticides. Improvements in spraying application techniques can contribute to these goals by better deposition on the leaves and reduction of drift to soil, surface water and air (DLO, 1995).

If the essential aspects of dose-effect relations of the chemicals are not well understood, this is often compensated by an overdosage of the active ingredient. Reduction in the use of chemicals now being a top priority, more attention needs to be paid to achieving a better leaf coverage with less chemical. Furthermore, emission of crop protection chemicals is a major problem in crop protection. New spray application techniques might improve the deposition and reduction of drift. In a series of experiments spray-deposition and biological effect were determined in a onion crop using a sprayer equipped with an air assisted system. In the design volume rates, the rate of active ingredient, spray interval and the use of air assistance were compared.

MATERIALS AND METHODS

During four growing seasons (1991-1994) field trials were established in a crop of onions (cv. Hysam) at PAGV Research Station. Plots, measuring 4.5 m wide and 19 m long, were marked out in a randomized block design incorporating three replicates. A "Hardi Twin" sleeve boom sprayer was used. For air assistance the sprayer was operated at one third of its maximum air flow with nozzles kept vertical,as in conventional practice. Because of its ability to be operated without air assistance, the "Hardi Twin" was also used to apply the standard conventional non air assisted spray treatments, the air curtains on the machine being folded.

Hardi 4110-12 flat fan nozzles were used for sprays applied at 2.5 bar pressure. A rate of 200 l ha^{-1} was produced using the tractor speed of 4 kph and the 100 l ha^{-1} was achieved at 8 kph. Sprayer boom height was 0.50 m above the crop canopy.

Spray deposition

During 1991 and 1992 growing seasons spray deposition measurements were done in equal sized plots within the field trial area. At the time of fungicide application deposition measurements were carried out by adding the fluorescent dye Brillant Sulfo Flavine (BSF) to the spray agent (0.5 g l^{-1} water). The detergent Agral N was added in a concentration of 1 g l^{-1} water to simulate a pesticide formulation. After the spraying the dye was extracted from the leaves or collectors. The collectors used were chromatography paper strips 10 cm long and 2 cm wide folded around leaves at three heights in the plant canopy. On the soil surface and over the crop canopy 100 x 8 cm filter tissues were used. Collectors were placed systematically on three places across the sprayer boom. A single spray pass was made across each target. The rate was measured by fluorimetry and expressed per surface area of the collector. The measured deposits were expressed as percentage of the application rate of the sprayer (spray dose). After log-transformation results of the deposition measurements were statistically evaluated using Genstat statistical software (Payne, 1993). In addition to absolute deposition (quantity of chemical), the results included the coverage and the droplet spectrum on the target area. For this part of the research, video recordings were made of the spray deposition on water sensitive paper that was suspended in the crop. The video recordings were analyzed by means of vision technology but these are not reported here.

Biological effect

Biological effects of the sprayings were investigated in randomized field trials during four consecutive growing seasons (1991-1994). In each growing season the level of *Botrytis squamosa* infection was measured. Levels of disease in the crop were recorded at weekly intervals from first infestation (time of first application) until fall-over of the leaf. This was done by taking five randomly selected plants per plot, assessing leaf disease as number of spots per leaf. If more than 150 spots occurred, numbers were assessed using key-figures and classified accordingly.

Fungicide treatment (chlorothalonil as Daconil M at 2 kg ha^{-1} product) was applied every week or fortnight, starting when first spots were found and ending at desiccation of the onion leaf. Dosages varied from 100% to 50% and an untreated control was also included. The total field received normal farm inputs for fertilizer, weed control and growth regulation. At harvest time in each plot 15 m^2 were harvested to determine crop yield.

RESULTS

Spray deposition

From the deposition measurements in 1992 it became clear that 100 l ha^{-1} gave proportionally lower rates of deposition on the three leaf levels of the onion plant than 200 l ha^{-1} (Table 1). With both 100 l ha^{-1} and 200 l ha^{-1} there were differences between with and without air assistance. In general least deposit was from 100 l ha^{-1}, more occurred with 200 l ha^{-1} and most at 100 and 200 l ha^{-1} with air assistance. At 200 l ha^{-1} there was a difference between with and without air assistance in deposition on in- and outward directed leaf side of the plant. With both 100 and 200 l ha^{-1} there was a difference in inward leaf side deposition on all leaf levels between with and without air assistance. Deposition on the top leaf level between treatments was not statistically significant ($P<0.05$). On the middle and bottom leaf level air assistance deposited proportionally more spray volume than without air. Deposition on the top, middle and lower leaf levels was different for all spraying systems.

Table 1. Mean deposition on inward and outward directed leaf sides of the top, middle and lower leaf levels on onions as % of sprayed volume, 1992.

Volume (l ha^{-1})	Air assistance	Leaf level					
		Top		Middle		Lower	
		In	Out	In	Out	In	Out
200	none	22.9	6.9	17.8	2.8	16.1	4.6
200	1/3 of full	23.0	12.4	16.6	9.5	18.3	9.2
100	none	21.2	5.9	17.0	2.8	11.2	1.9
100	1/3 of full	27.8	5.8	22.9	2.3	26.6	2.7

In 1992 the deposition on the soil surface with 200 l ha^{-1} was more with air assistance than without air assistance (not shown). At 100 l ha^{-1} the differences in soil deposition were not statistically significant ($P<0.05$). (Deposit measurements in 1991 had shown similar results when measurements were then only done with a 200 l ha^{-1} spray volume).

Biological effect

During the individual growing seasons leaf diseases occurred in untreated as well as in treated plots; in general disease levels were low in 1994, moderate in 1992, and severe in 1991 and 1993. In all years but 1994 all spraying systems and dosages reduced the mean level of *B. sqaumosa* significantly ($P<0.05$) (Table 2)..

Table 2. Mean number of leaf spots of *B. squamosa* on onions close before fall-over of the leaves for the treated and untreated plots, 1991-1994.

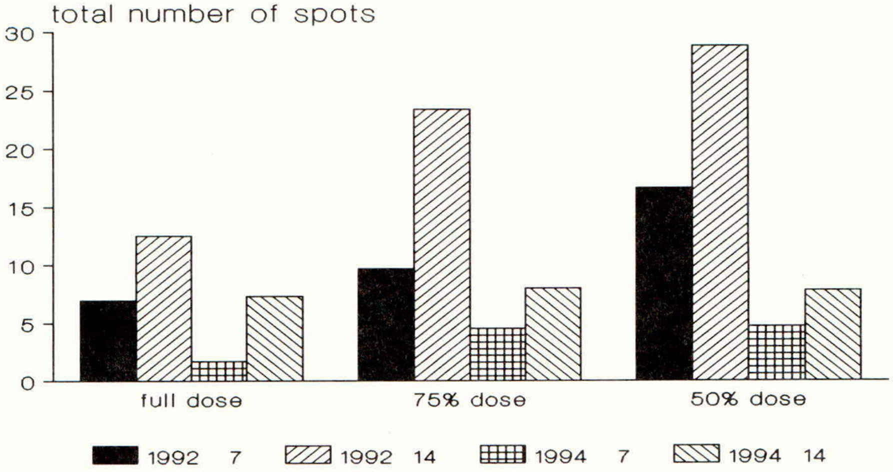
		1991	1992	1993	1994
treated		19	15	932	6
untreated	519	98	1287	11	

Averaged for the four growing seasons data for the number of leaf spots for the total plant are given in Table 3.

Table 3. Number of leaf spots of *B. squamosa* on onions, 1991-1994 mean.

		Spray interval (days)	7	7	7	14	14	14
		dose (%)	100	75	50	100	75	50
Year	Volume (l ha^{-1})	Air assistance						
1991	200	none	7.5	8.7	13.7	55.3	30.6	79.2
	200	1/3 of full	11.2	10.0	11.9	62.3	64.7	58.2
1992	200	none	6.2	5.5	11.7	6.3	13.2	29.9
	200	1/3 of full	2.2	10.1	14.4	8.5	12.2	2.5
	100	none	9.2	12.7	25.3	13.7	40.0	30.6
	100	1/3 of full	10.2	10.3	15.2	21.7	29.1	26.3
1993	200	none	+	+	+	198.8	244.1	207.6
	200	1/3 of full	+	+	+	297.4	305.6	270.8
	100	none	+	+	+	404.1	324.4	461.2
	100	1/3 of full	+	+	+	403.3	431.4	309.1
1994	200	none	0.9	2.7	6.0	4.3	11.8	6.0
	200	1/3 of full	1.8	2.2	5.6	9.2	8.7	9.6
	100	none	2.3	10.3	3.2	7.6	6.3	7.8
	100	1/3 of full	1.8	2.9	4.0	8.2	5.2	7.9

From Table 3 effects can be distinguished on spray intervals, dosages, spray volumes and air assistance. In Figure 1 the mean number of leaf spots averaged per plant for spray interval and dosage is presented for the years 1992 and 1994. It became clear that a 14 day spray interval had a higher level of infestation than a 7 day interval. Effect of dosage was only significant in 1992, a year with moderate infestation pressure. Mean number of leaf spots per plant for the 200 l ha^{-1} spray volume was lower than for the 100 l ha^{-1}. For both 100 and 200 l ha^{-1} there seemed to be no effect of the use of air assistance on the number of leaf spots per plant.



(Chlorothalonil as 2 kg/ha Daconil M)

Figure 1. Mean number of *B. squamosa* spots per leaf for 7- and 14-day spray interval, 1992-1994.

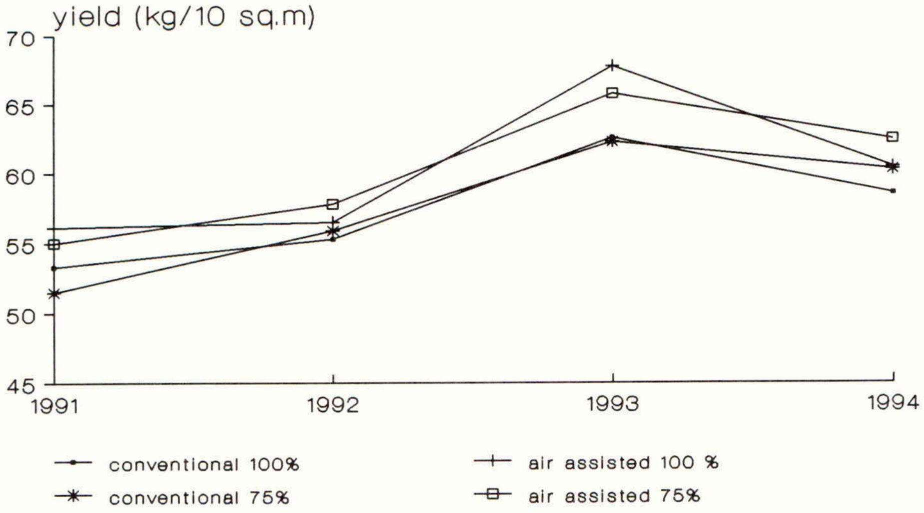


Figure 2. Mean crop yield (kg/10 m^2) of onions at 14-day spray interval for conventional and air-assisted spraying, full and 75% dose, 1991-1994.

In Figure 2 mean crop yields are presented for the four seasons for the effect of spraying technique and dosage at a 14 day spray interval (averaged for spray volumes). From this graph it becomes clear that air-assisted spraying results in higher crop yields both at full and reduced dosage of fungicides. In all years yields for plots sprayed with air assistance and 75% dose were higher than yield of plots sprayed conventionally at full dose. However, these differences are statistically not significant.

At high infestation levels the 7 day spray interval resulted in higher crop yields than the 14 days interval (data not presented here).

DISCUSSION

In general, a spray volume of 200 l ha^{-1} gave better control of *Botrytis squamosa* in onions than a 100 l ha^{-1} volume. A 7 day spray interval resulted in lower levels of infestation of *B. squamosa* than a 14 days interval. With high infestation levels a 14 day spray interval also resulted in lower crop yields.

The use of reduced dosage for disease control is possible, but a 50% dose reduction resulted in unaceptably high levels of disease.

The use of air assistance resulted in a higher spray deposit on the onion plant. Also, distribution over the crop and on plant leaf levels was more even. Penetration of the spray into the crop was also higher with air assistance. However air assistance can also result in a higher deposit of spray onto the soil beneath the crop.

Crop yields are effected by fungicide dose and spraying technique. However, a relationship between spray deposit and number of leaf spots is difficult to make. Although a higher spray deposit does not implicitly mean better disease control, it had a positive effect on crop yield. It appears that other parameters should be looked at to give a better explanation for this situation. Analysis of spray quality, e.g. droplet distribution and drop sizes on the leaf tissue, could be valuable in combination with quantitative measurements of spray deposit.

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