Pesticide leaching models - past, present and future

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ABSTRACT

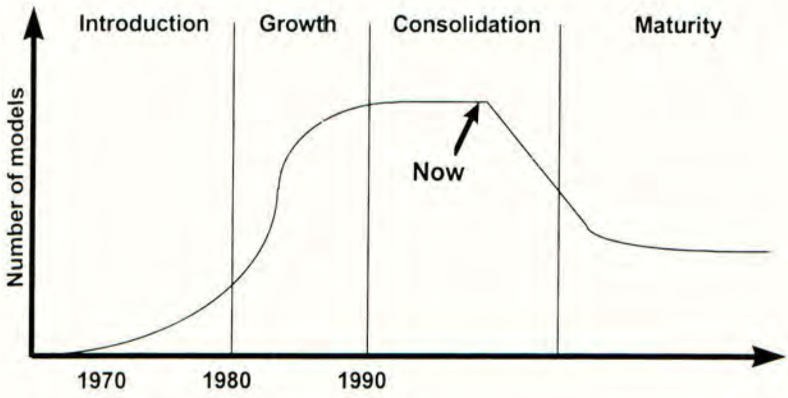
Pesticide leaching models have evolved as research tools and more recently as important components of regulatory decision-making procedures. A procession of recent model review exercises has resulted in a high level of consensus on what makes a good model, and differences between the models have greatly decreased. The most commonly used regulatory models in the EU are PRZM, PELMO, PEARL and MACRO, and the FOCUS group has recently developed a standard set of regulatory groundwater scenarios and corresponding input files for each of these models. A comparison of the state of the art with regulatory user requirements reveals that there has been a lot of emphasis on model accuracy in the past at the expense of practical needs like version control, input guidance, and user support - the FOCUS scenarios and associated support processes will greatly improve this situation. Global harmonisation of regulatory leaching models should be the next goal.

INTRODUCTION

Pesticide leaching models have come a long way in the last 30-40 years, and the year 2000 seems like a good opportunity to take stock. Where have we got to, and where are we going? How accurate are the current generation of models, and where is the next generation of models going to come from? I shall explore answers to these questions, and shall do this from the perspective not of a model developer, but from that of a model user with a keen interest in the use of models in the regulatory process. This paper is somewhat EU biased, which is partly a reflection of the target audience and partly because there is currently so much more activity in this field in the EU than there is in the USA or elsewhere.

PAST

Any new technoregulatory area tends to evolve according to set pattern (Figure 1). Someone thinks of a completely new idea, and the area gets started. Then everyone else thinks that is a good idea too, but they would like to modify it in their own image, and lots of competing ideas emerge from the primordial soup. Then people run out of new ideas, and the existing ones are compared, and evaluated. Then, if all goes well, a common view develops and everyone adopts it as the standard - harmonisation is achieved. This is a general pattern not just for leaching models, but also for regulatory pesticide study types - most study types are somewhere on this evolutionary path, with some (e.g. avian acute toxicity studies) more advanced than others (e.g. non-target plant testing).



COMPLEXITY	Low	Proliferation	Complex	Harmonised
REGULATORY ACCEPTANCE	V Low	Low/Patchy	Moderate	High

Figure 1. The evolution and current state of regulatory pesticide leaching models

The Netherlands and UK were early users of pesticide leaching models, with occasional uses going back to the 1960s and 70's. When the US-EPA was formed in 1970 they quickly recognised the importance of modelling and risk assessment, and funded an explosion of research models during the 1970s. Early models were typically skewed towards the personal research experience of the developer, and tended to be unbalanced in terms of the level of detail used to simulate different environmental processes, e.g. massive details on hydrology but no crop simulated. In the 1980s there emerged a number of models which had a better balance, learning from the good and bad features of preceding models. It is from this period that most current regulatory models originate. During the 1990s far fewer new models appeared, and most effort was spent on model review, comparison and validation exercises (Beusen *et al.*, 1997; van den Bosch & Boesten, 1995; Cohen *et al.*, 1995; FOCUS, 1995; Klein *et al.*, 1997, Vanclooster *et al.*, 2000, Walker *et al.*, 1995). It is hard to imagine that there is much more scope or energy for new review exercises, and my assessment is that we are now are about to enter a new phase. But before we gaze into the future, let's assess the present state of the art.

PRESENT

Which models are used?

The leaching models most commonly used in the pesticide regulatory process are PRZM (Carsel *et al.*, 1984), PELMO (Klein *et al.*, 1997), PEARL (Leistra et al., 2000) and MACRO (Jarvis, 1994). PRZM is a US model which is also used to some extent in the EU. PELMO was originally a German adaptation of an early PRZM version, and is in common use in the EU. PEARL is a merger of Dutch models PESTLA and PESTRAS, and is also commonly used in the EU. MACRO is a macropore flow model favoured in Denmark, and is also used elsewhere in the EU.

These models have certain things in common. They are all 1-dimensional, which means they are simulating leaching vertically below a single point in a field. All the models have their origins in the 1980s or earlier, though some are showing their age more than others. More interestingly the processes which they simulate, and even the equations which they use to simulate these processes, have a great deal in common. This reflects the high degree of consensus which has emerged over the last few years about what a good leaching model should include, and how it should do its calculations. During the many model evaluation and comparison exercises of the 1990s, the modellers have gradually moved towards a common standard, and differences between models have lessened. It has been said that if you switch off the preferential flow in MACRO, you have PEARL, which is an exaggeration rather than a lie. The German model PELMO is an adaptation of an early version of PRZM, but for a long time the code of these two models diverged. Now PRZM and PELMO have converged again, to the extent that it is possible with care to obtain the same results from the two models, often to two or more significant figures (FOCUS, 2000).

Still some differences remain between the models. The most important ones are to do with water and solute flow. PEARL, PELMO and PRZM only simulate chromatographic flow of water and solutes, i.e. homogeneous flow akin to that in a chromatography column, whilst MACRO can also simulate preferential flow down macropores. The second major difference is that PEARL and MACRO do a proper numerical solution of the Richards equation, which is the differential equation describing water and solute transport, whilst PRZM and PELMO use what is called a "tipping-bucket" approach. The tipping-bucket approach is a crude approximation to Richards equation, but does have the advantage of speed, allowing these models to run much faster than PEARL and MACRO. This speed makes PELMO and PRZM particularly suited to situations where hundred or thousand of simulations are needed, e.g. GIS-linked modelling or Monte-Carlo sensitivity analyses.

How do they match up to user requirements?

Pesticide leaching models have their origins as research tools, and they are still very valuable in this capacity. However they are also used in the pesticide regulatory process, especially in the EU, and recently the FOCUS group has developed a prescribed set of leaching scenarios and model input files for the evaluation of pesticides under the EU pesticide registration directive (FOCUS, 2000). So in my capacity as a customer for pesticide leaching models who uses them in the regulatory process, are the suppliers giving me what I want? Customers have shopping lists, so lets check my shopping list, and score the current models against each item (scores are generally only appropriate to the EU):

- **I want a model which gives accurate results.** Probably the most common and unreasonable customer demand of all, and the hardest to satisfy. This thorny topic will be explored in the next section. Score: 5/10.

- **I want a model that is easy to use and which runs quickly.** All four models now have graphical user interfaces, though in the case of PRZM this is only true for the EU FOCUS version. Overall these are quite good, but still have scope for improvement. PELMO and PRZM's crude transport routines mean they run at an acceptable speed. However, PEARL is slower, whilst MACRO runs in slow motion. Score 5/10.

- **I want a model which is free of bugs.** Any large piece of computer code will always contain bugs, some of which can be found and some of which successfully avoid detection. It is very hard to evaluate the relative "buginess" of the models. Perhaps the best re-assurance for the user is to put in place a reporting system for bugs, so that bug reports are compiled and the bugs fixed, whilst the users are notified. A system like this has just been set up for the EU FOCUS versions of these models, so this point is looking more promising now from this customer's perspective. Score 5/10.

- **I want to be provided with standard scenarios for soil, weather and cropping data, and also guidance on how to choose pesticide-specific input values in order to get sensible results.** The new FOCUS EU groundwater scenarios fit the bill perfectly. Nine basic scenarios with 20+ crop options and one happy customer. Better still, there is even detailed guidance on the selection of input values! Score 10/10.

- **I want a model which has a good manual.** Manuals are deadly boring to write, but very important to users. The original PRZM manual set the standard, but nowadays PEARL is the gold standard, whilst manuals for the other three models are OK but less than ideal. Score 6/10.

- **I want training in the use of the model.** There have been training courses for PRZM (in the US only) and PELMO in the past, but I know of no plans to repeat them regularly and know of no courses for PEARL or MACRO. There are training courses being run specifically for the EU FOCUS scenarios, but these will not cover the technical content of the models themselves. Score 2/10.

- **I want access to a helpdesk if I get in trouble**. No such system exists for the four models themselves, but there is a formal email helpdesk system run by ISPRA in support of the EU FOCUS leaching scenarios. This has just been set up and should be a great help, though it is a service only available to customers involved in the regulatory process. Score 9/10.

- **I want to use the same version of the model that the regulators use and I want to get hold of it easily.** Version control of leaching models has been very patchy in the past, but a formal version control process has been set up for the EU FOCUS groundwater scenarios and their component models and model shells. A version currently approved for regulatory use will always exist and be clearly flagged and readily available on an Internet site, along with all previous versions and descriptions of the differences between versions. Score 10/10.

- **I want to know that my modelling results will be accepted by regulators.** If you use the current version of the standard EU FOCUS scenarios along with the guidance on selection of pesticide-specific inputs then there should be a high chance of acceptance. Any argument should be about subtle differences in the interpretation of the studies on the pesticide in question. This is however unproven, and only time will tell for sure. Score 8/10

- **I only want one model.** The models are in many ways similar at heart, but a lot of effort is wasted in learning the peculiarities of each. Different regulators have different preferences, so I'm stuck with learning and using all four at present. Score 0/10.

A long shopping list, but remember - the customer is always right! The natural inclination of the model author is to get the model technically correct and as accurate as possible, but note that this is just one out of the 10 user requirements on my shopping list. The boring practical user requirements, like version control and a helpdesk have been largely unsatisfied in the past. But now the EU FOCUS scenarios are implemented, along with a managed process for their maintenance, version control and user support, around 30-35 points have been added to the score making a total of 60 out of 100. The customers are still not completely satisfied, but they are a lot happier than they were!

How accurate are they?

In order to judge the accuracy of the models, you must first say what it is you are comparing them with. It is important to realise that it is impossible to compare a model prediction with "reality". You can only compare model predictions with measurements which sample reality patchily in time and space, and which themselves are subject to uncertainty, error, bias and all the same things that bedevil modelling results. This clearly makes judging model accuracy quite difficult.

Spatial averaging is an important consideration. We know that leaching will vary widely from point to point across a field, so which point is the model supposed to be compared with (e.g. Figure 2)?

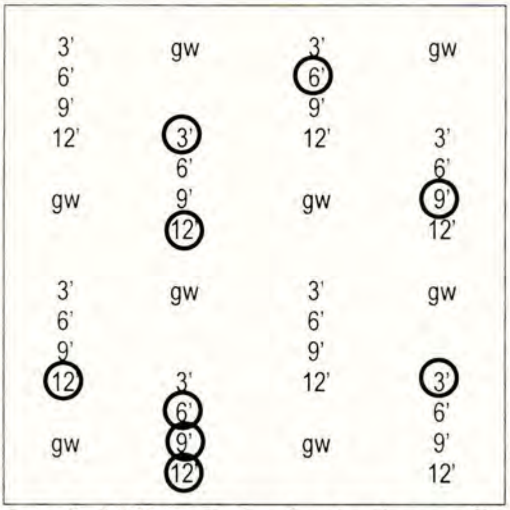


Figure 2. Overhead view of a 1.3 ha study site, showing the sporadic nature of leaching. The **3'**, **6'**, **9'** and **12'** symbols indicate the locations of suction cup porometers at these depths, and the **gw** symbols indicate a shallow monitoring well (typically 17'). Circled symbols indicate the only locations/depths where the study pesticide was detected during the course of the study (LOD=0.01µg/l).

The models are all 1-dimensional and so could be compared with any or all of the points in the field, but a deterministic model cannot give more than one result for a given set of inputs. In general, getting detailed soil and other data for all points across a field is bordering on the impossible. For these reasons PRZM was originally conceptualised to be representing average leaching across a field. But most measurements are averaged over a much smaller scale than the field - perhaps 0.01-0.1 m^2 for a suction cup porometer, and around 1m^2 for a very shallow monitoring well bridging the water table or for a typical lysimeter. Comparison with lysimeter results is perhaps the safest option, since all leachate is captured, and there is an averaging of the concentrations leaching over a 1 m^2 area. However, lysimeters can have their own problems, e.g. side-wall voids.

It has long been recognised that the accuracy of a model prediction is critically dependent on the quality of the model inputs. The old adage "rubbish in - rubbish out" is very apt here. The more sensitive a model is to an input variable, the more important it is that the value chosen is reliable. Typically, adsorption and degradation parameters are the most sensitive ones (e.g. Boesten, 1991), which is why there has been an increased emphasis in recent years on the quality and reliability of soil adsorption experiments and lab and field degradation studies, and new research like that on subsoil degradation and the kinetics of adsorption. A modeller once told me he had predicted with a model that a certain pesticide with Koc=10 and t1/2=100 days would leach through a lysimeter, but it did not do so and so the model was no good. A more appropriate conclusion would be that the model inputs bore no relationship to the behaviour of the pesticide in the lysimeter.

There have been a number of modelling "ring-tests", where each group of modellers have been given a set of data and been asked to make a prediction with a given model, and the results of the various groups predictions have then been compared to each other, and sometimes to a measured dataset. These ring-tests have proved to be very illuminating, but not for the reason originally intended. Instead of being a comparison of the accuracy of the models they have overwhelmingly been a comparison of the modellers (Boesten, 2000; Brown *et al.*, 1996). The conclusion is that the influence of the decisions made by the modeller has a greater effect than the choice of model. The conclusion for our purpose is that you have to eliminate the inaccuracies introduced by the modeller before you can say anything about the accuracy of the model itself. This emphasises again the need for really good quality guidance on the selection of model inputs.

But I won't allow myself to completely avoid the question of accuracy. My own belief is that with a good quality standard data package to work from, an experienced modeller, and with good advice on selection of inputs, a prediction of the concentration of pesticide reaching a certain depth can usually be made to within an order of magnitude, and often much better than this. With additional site-specific data, especially adsorption to and degradation rate in the soil in question, model predictions can often be within a factor of two. These statements refer to chromatographic flow situations - preferential flow is much harder to predict.

FUTURE

If pesticide leaching modelling as a discipline is going to flourish in the next 10-20 years then two things are needed. We need world-wide harmonisation on a single model for regulatory purposes, and we need to rediscover how to do basic scientific research on leaching models completely independently of regulatory considerations.

Firstly harmonisation. In reviewing the past, my conclusion was that models of pesticide leaching are at a critical point in their evolution (Figure 1), the implication being that the next obvious step is harmonisation and stability with the obvious benefits that this would bring. But this will not happen by default - we will have to make it happen. Model evaluation and validation exercises have held the pesticide modelling community together through the 1990s, but things cannot continue indefinitely as they are. Existing models are becoming orphaned (e.g. GLEAMS, arguably PRZM, and perhaps MACRO), whilst other new ones are being developed from outside the close-knit community of modellers involved in the evaluation exercises of the 1990s. The current state is an unstable one, and if we do not actively seek harmonisation then my belief is that things will regress into the kind of uncoordinated model proliferation which we saw in the late 1970s and 1980s. The current models are so similar, and the current consensus so high, that the main barriers to harmonisation are undoubtedly political rather than technical.

If a single regulatory model can be created, then far from being the end of basic scientific research on the modelling of pesticide leaching, I see would see this as a new beginning. To date, we have been doing exploratory research work on the same models which we are using for regulation, and this has had unpleasant consequences. Regulatory considerations have had too great an effect on the models and how they are used. For example, the shift in the point of EU water regulation from the tap to the water-table has skewed thought towards macropore flow and has discouraged basic research on pesticide fate within aquifers. Laws tend to ignore real-world features like spatial variation, so is the correct logical endpoint for leaching modelling really the concentration dripping from the bottom of a single macropore, routing pesticide directly from soil surface to the water-table? The potential impact of macropore flow on water-table concentrations at isolated points in a field is clear, but whether it has any measurable impact on concentrations in tap-water is far more doubtful. A similar dilemma occurs for leaching modelling in rice, where modellers worry that the pore-water in the saturated paddy soil will be regulated against the drinking water directive!

We need to create some breathing space between models for research purposes and models for regulation. The version control which would accompany the harmonisation of regulatory models means that research versions of the model will emerge separately from the official, controlled version. Other models, including some of those whose regulatory purpose was superseded by the harmonised model, would re-emerge as research tools unfettered by regulatory considerations. There would be two separate strands to pesticide leaching work: a research strand where there is total freedom, and a tightly controlled regulatory strand. The regulatory strand would periodically review the research strand for useful new features and methods which could be incorporated into the harmonised regulatory model after appropriate testing. Wouldn't this be better than the current situation where modellers are overconstrained by regulation, and the regulatory process is at the mercy of a research idea which is conceived in the morning and is the basis for regulating pesticides by the afternoon?

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Advances in surface water modelling in the USA and Europe

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ABSTRACT

Current regulatory requirements for environmental fate modelling of surface water in the USA and Europe are summarized including prescribed models and modelling scenarios. Current issues in modelling are also addressed including obtaining needed chemical input data, creating scenarios, ensuring accuracy of results, model validation and defining of higher tier studies.

INTRODUCTION

Current regulatory requirements for modelling the environmental fate of pesticides in surface water include calculation of predicted environmental concentrations (USA, EU) as well as assessment of potential concentrations in drinking water drawn from surface water sources (USA). This paper reviews the various advances that have been made in regulatory modelling approaches in both the USA and Europe.

SURFACE WATER MODELLING IN THE USA

Ecotoxicological exposure assessment

For evaluation of exposure estimates for ecotoxicological risk assessment, the USEPA currently has four tiers of regulatory modelling of surface water. The first tier consists of using GENEEC (Parker *et al.*, 1995) to obtain screening (i.e. conservative, high) estimates of potential concentrations of pesticides in surface water. If this estimate results in an unacceptable level of risk, the next modelling tier consists of using PRZM-3 (Carsel *et al.*, 1997) and EXAMS (Burns, 1994) to provide probabilistic estimates of pesticide concentrations in a single representative scenario. Currently, regulatory Tier 2 modelling scenarios have been created for 20-25 key crops in the USA including apples, citrus, corn, grapes, potatoes, soybeans and wheat. Each scenario involves edge-of-field drift, runoff and erosion from a treated 10 ha field entering into a static 1 ha x 2 m deep pond with calculations extending over a period of 36 years of consecutive product use. The current regulatory endpoint is the 90^{th} percentile exposure value of the appropriate time-weighted-average which is determined by the duration of the various ecotoxicological studies. Most regulatory evaluations are not carried out further than Tier 2.

The next tier of exposure assessment consists of performing probabilistic PRZM-3 / EXAMS modelling for multiple scenarios which provides regional estimates of the various time-weighted-averages. A draft version of this approach has been published (Mangels, 1997) and the USEPA is currently developing their own version.

Relatively few pesticides have been evaluated using Tier 4 modelling. This tier consists of using a wide range of techniques to refine modelling input values (e.g. collection of additional laboratory and field data, use of ranges of input parameters, use of Geographic Information Systems (GIS) and remote sensing to guide parameter selection) as well as detailed probabilistic modelling to produce refined exposure estimates in a range of scenarios.

The relationships between the four tiers of regulatory exposure modelling used for ecotoxicological risk assessment are shown in Figure 1.

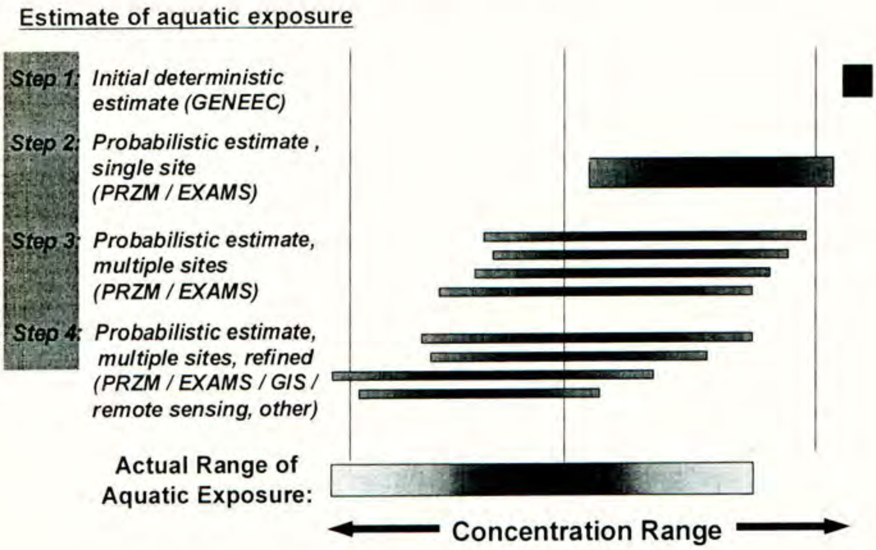

Figure 1. Relationship between modelling steps in USA approach

Drinking water exposure assessment

The USEPA is currently using two tiers of modelling to evaluate potential concentrations of pesticides in surface water and is working on developing a third tier. The first exposure estimate is performed using FIRST (Parker, 2000), which is a screening meta-model similar to GENEEC but adapted to the index reservoir scenario which will be described later. If the concentrations predicted by FIRST exceed the Drinking Water Level of Concern (DWLOC), then a second tier of modelling is performed using PRZM-3 and EXAMS to simulate a scenario in a representative drinking water reservoir (i.e. an "index" reservoir). In this scenario, edge-of-field drift, runoff and erosion from a small watershed (178 ha) is simulated to drain into a 5.3 ha reservoir which has a constant streamflow. To help refine the simulated results, the highest density of each major crop (called crop area factor) has been determined in representative watersheds across the USA and official values have been established for use in drinking water assessments.

Key assumptions of current regulatory modelling in the USA

Current regulatory modelling scenarios do not include potential effects of non-treated buffer zones and all runoff and erosion is assumed to directly enter receiving water bodies. Recently published data suggest that vegetated buffer strips can remove 30 to 50% of the pesticide that leaves the edge of a treated field via runoff and erosion (Misra *et al.*, 1996).

In EXAMS, the surface water hydrology is generally assumed to be steady-state to simplify the needed simulations. In actual field situations, the volume and flow rate of surface water bodies is highly dynamic and varies in response to changes in precipitation and upgradient drainage from the watershed.

The refinement of using crop area factors is used only for assessment of potential pesticide concentrations in small watersheds. This factor is not used for ecotoxicological assessments that are performed on the scale of a single field.

Worst-case values of chemical input data (i.e. 90th percentile degradation rate and lowest sorption) are generally selected to ensure conservative assessment of potential aquatic concentrations. The probability of experimentally observing the simulated worst-case concentrations is not known.

SURFACE WATER MODELLING IN THE EU

There are currently many approaches being used in the EU to estimate potential concentrations of pesticides in surface water for regulatory evaluation. The most dominant method estimates surface water concentrations resulting only from spray drift and ignoring potential contributions of runoff, erosion and tile drainage.

To help standardize the prediction of potential concentrations of pesticides in surface water, a sequence of three progressively refined modeling evaluations are currently being developed by the FOCUS Surface Water Working Group (Figure 2). The simulated results provide the exposure estimates needed for ecotoxicological risk assessment.

Step 1 and 2 exposure assessment

The first step consists of a screening calculation that represents the combined loading of drift, runoff, erosion and tile drainage into a static surface water body. This calculation provides actual and time-weighted average concentrations of pesticide in both the water column and in the benthic layer. This initial calculation has been implemented in both a spreadsheet format and a stand-alone executable model called the Step 1 Calculator. If the resulting exposure values result in unacceptable Toxicity-to-Exposure Ratios (TER), then the Step 2 Calculator is used to further refine the predicted concentrations by more accurately representing the time sequence of individual applications as well as modifying the sum of runoff, erosion and drainage as a function of region of the EU and season of application.

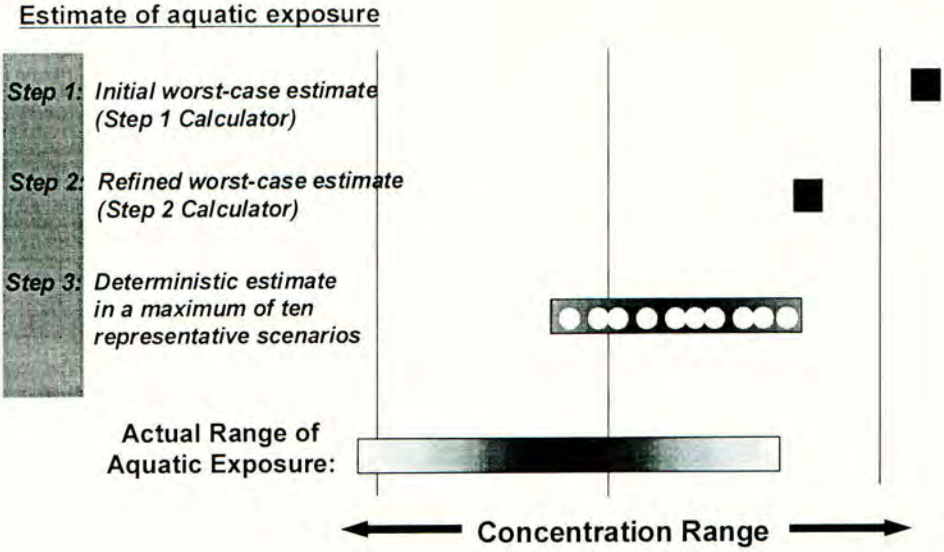

Figure 2. Relationship between modelling steps in EU approach

Step 3 exposure assessment: development of surface water scenarios for the EU

Further refinement of the predicted environmental concentrations (PEC) in surface water and sediment are made in Step 3 calculations using more detailed, mechanistic models. A total of ten surface water scenarios (combinations of soil, topography and climate) have been created to represent the range of agronomic settings for various crops across the EU (Figure 3). Specific crops included in the EU surface water scenarios include cereals, citrus, maize, potatoes, stone/pome fruit, sugar beets and vines.

Six of the scenarios are assumed to impact surface water primarily via drift and tile drainage. The remaining four scenarios involve drift, runoff and erosive loading of pesticide into surface water. Appropriate receiving water bodies (i.e. ditches, ponds and/or streams) have been identified for each scenario. The models that have been identified for use in simulating tile drainage include MACRO (Jarvis, 2000) and PEARL (Leistra *et al.*, 2000; Tiktak *et al.*, 2000). PRZM-3 or PELMO (Jene, 1998) should be used to simulate the four scenarios with runoff and erosion. The various surface water bodies should be simulated using either TOXSWA (Adriaanse, 2000) or EXAMS. To facilitate calculation of the various scenarios, FOCUS has developed automated shells to appropriately parameterize and run MACRO, PRZM-3 and TOXSWA.

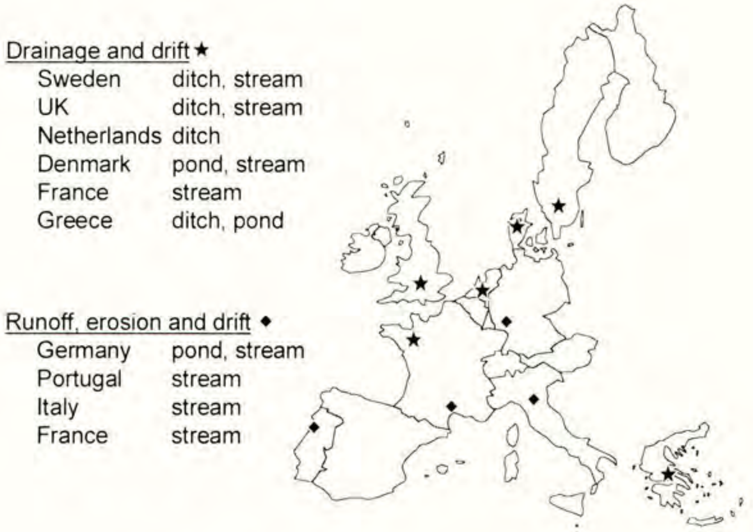

Figure 3. FOCUS surface water scenarios in the EU

Key assumptions of current regulatory modelling in the EU

Similar to the approach taken in the USA, the effects of buffer strips have not been included in the EU FOCUS simulations of surface water with tile drainage, runoff and erosion directly entering adjacent surface water from the edge of a treated field. In contrast with the USA approach, the hydrology of the various surface water bodies is assumed to be dynamic in TOXSWA, representing the runoff and drainage behaviour of a small watershed with an upgradient crop density of 20%.

The FOCUS group recommends that average chemical input values be used in simulating the potential concentrations of pesticide in surface water. As currently prescribed by FOCUS, the simulations of surface water are deterministic and cover a maximum period of 16 months. Step 3 calculations are intended to represent an overall probability of 70th percentile. If needed, more detailed probabilistic assessments can be performed as a higher tier evaluation of aquatic exposure in a Step 4 calculation.

CURRENT ISSUES IN SURFACE WATER MODELING

There are a number of significant issues which have not yet been resolved in regulatory modelling of surface water. A key issue is obtaining all of the chemical input data needed for a Tier 2 (USA) or Step 3 (EU) regulatory assessment. The standard suite of laboratory and field studies provides sufficient data to characterize the rate of degradation in soil and water, the extent of sorption to soil and sediments and the tendency to volatilize. For pesticides which are foliarly applied, it may be necessary to supplement this core dataset with additional information on the rate of degradation and washoff from crop canopies. Other special data needs could include nonlinear degradation kinetics as well as kinetics of sorption.

In most regulatory modelling the goal is to provide either a "reasonable worst-case" evaluation or a probabilistic assessment with a recommended conservative endpoint (such as the 90th percentile of annual peak concentrations). It is critical to note that the overall probability of observing a specific chemical concentration in surface water is a function of the pesticide's chemical/physical properties, the timing of applications and the intrinsic vulnerability of the site based on crop, soil, topography and climate. As a result, it is not possible to select a specific site that has a fixed probability of surface water impact for all pesticides. The most reasonable approach is to select a range of vulnerable regulatory scenarios in which to evaluate all chemicals, with the goal of being adequately protective of the environment. The EU scenarios attempt to identify aquatic concentrations which would occur once every 3-4 years (i.e. 70th percentile) while the USA approach focuses on events that occur once every 10 years (i.e. 90th percentile).

Most of the models recommended for regulatory use have undergone some type of validation exercise to evaluate the accuracy of the model compared to experimental data. These efforts include validation studies of many of the current leaching, runoff and water models (e.g. Vanclooster *et al.*, 2000; Beulke, 1998; Van de Veen & Boesten, 1996; Jones & Russell, 2000; Westein *et al.*, 1998). One of the general conclusions is that the professional judgment of the modeler can have a significant effect on the accuracy of the modelling result. As a result, a sound technical approach is to create well-characterized regulatory scenarios together with guidance on input selection for the remaining parameters to minimize the variation caused by individual judgment. Using this approach, it is estimated that uncalibrated scenarios can provide exposure estimates within 4 to 10X of experimental values and within 2 to 6X if hydrologic calibration is performed.

Finally, it is critical to recognize that all current standardized regulatory scenarios include simplifying assumptions that tend to provide conservative results. For higher-tier evaluations, it is appropriate to consider refining many of the inputs used in modelling through the collection or analysis of additional data using additional experimentation, remote sensing and/or GIS. The mitigating role of buffer strips in capturing runoff and eroded sediment can be considered in higher-tier evaluations. It may also be useful to consider the actual rate of mixing of drift, runoff, erosion and drainage into surface water bodies. Particularly for larger water bodies, the mixing process is sufficiently non-uniform to create significant refugia for extended periods of time to allow partial protection of impacted populations. The accuracy of surface water modeling is generally improved if hourly rather than daily time steps are used to represent climatic events and to report resulting concentrations. Finally, probabilistic higher-tier evaluations can be performed using a range of chemical and soil properties rather than selected values in an attempt to obtain a more realistic range of observed concentrations.

CONCLUSIONS

Regulatory assessments of potential concentrations of pesticides in surface water include screening calculations as well as more detailed modeling evaluations using standard scenarios. This same approach is being taken in the USA and the EU.

Standard scenarios are generally intended to provide "reasonable worst-case" evaluations of surface water concentrations and can be used with either deterministic or probabilistic modelling.

Many advances have been made in the simulating the drift, runoff, erosion and drainage of pesticides from treated fields into adjacent surface water. The current models provide acceptably accurate estimates of surface water concentrations if reasonable input values are used together with standard scenarios.

Higher-tier evaluations can provide more realistic estimates of actual aquatic exposures through collection of additional experimental data (site-specific or regional, using remote sensing and/or GIS) and use of more detailed probabilistic modelling.

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Progress in simulating macropore flow through soil

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ABSTRACT

The development of macropore flow models over the last 5-10 years is an important advance which improves our ability to simulate the fate of pesticides in soil. A number of regulatory authorities are requesting the use of macropore flow models on a routine basis to assess the risk of contamination of ground and surface waters by pesticides. MACRO is the preferred macropore flow model for regulatory use and its predictive ability is reasonable for a range of intermediate soils but very variable for heavy clays. There are still significant problems with selection of input parameters which raise questions over the predictive use of such models for regulatory purposes. These problems must be considered in the light of similar issues for non-macropore flow models.

INTRODUCTION

There is clear evidence that macropore flow may be an important process for pesticide transport through a wide range of soils including both clays (Johnson *et al.*, 1994, Brown *et al.*, 1995) and intermediate soils (Aderhold & Nordmeyer, 1995; Flury *et al.*, 1995). A number of mathematical models have been developed to simulate macropore flow and its influence on pesticide fate. The incorporation of such models into the regulatory process appears desirable, particularly for higher tier risk assessment. However, incorporation has been limited by the need for evidence of their predictive ability and concerns over robust selection of key input parameters. This paper reviews progress in simulating pesticide transport via macropore flow.

APPROACHES TO SIMULATION OF MACROPORE FLOW

There has been a rapid development in the simulation of macropore flow over the last 10 years. Deterministic dual-porosity models are the most highly developed at present. The soil porosity is divided into a slow or immobile flow domain (the matrix) and a region of faster flow (the macropores). Examples include CRACK-NP (Armstrong *et al.*, 1995) and MACRO (Jarvis, 1994). Where water in the matrix is immobile, such models are only applicable to heavy clay soils. A much broader range of conditions can be simulated by including a mechanistic description of slow flow through this domain. Deterministic dual-porosity models are now widely accepted for modelling water flow and solute transport in heterogeneous soils and MACRO is generally used as the regulatory model of choice for macropore flow in Europe. The assumption of a biphasic pore system is a simplification for most soils, but strikes a balance between ease of use and rigour of approach.

A number of alternative approaches exist. Functional models adopt simple approaches to the simulation of macropore flow by incorporating semi-empirical relationships (e.g. Brown & Hollis, 1995). PLM is a capacity model which divides soil water into a mobile and immobile phase with the boundary at -5 kPa (Hall, 1993). The mobile water is then further divided by an empirical parameter into a 'slow' and 'fast' flow domain to account both for convective flow of soil solution through water filled pores and rapid transport through macropores or fissures. Macropore flow is a complex phenomenon and the ease-of-use which is a feature of functional models can often only be gained through a loss of robustness which means calibration is required in most situations. Whereas lack of knowledge currently precludes descriptions of finger flow and funnel flow in mechanistic models, functional models may simulate these processes on an empirical basis.

Two-domain models are gross simplifications of the porosity of most soils. A number of multi-porosity models have been developed whereby three or more distinct regions of the soil can be defined (e.g. TRANSMIT – Wagenet & Hutson, 1995). Such models allow greater flexibility in matching the observed behaviour of solute transport, but parameterisation becomes prohibitively difficult and extensive calibration is generally required. The logical extension of multi-porosity models is a genuine stochastic description of soil based on a continuous pore-size distribution function. Stochastic models describe water flow and solute transport in heterogeneous media statistically using a 'transfer function' (e.g. Grochulska & Kladivko, 1994) and may express aspects of variability not possible with deterministic models. Availability of data is a significant constraint for stochastic approaches.

LEVELS OF PREDICTIVE ACCURACY

It is rather dangerous to assign generalised levels of accuracy to a given model as these are likely to vary widely for different simulations. Nevertheless, this is a key piece of information for modellers carrying out risk assessments and for regulators who have to evaluate modelling submissions and is an important component in any attempt to build confidence in the credibility of modelling. SSLRC have undertaken a broad evaluation of the main macropore flow models (CRACK, MACRO, MACRO_DB, PLM and SWAT against four contrasting UK datasets (Beulke *et al.*, 1998). The evaluation concluded that MACRO should be the macropore flow model of choice for regulatory applications because it is broadly applicable, user-friendly, well documented and there are many reports of model tests in the literature. When applied to a lysimeter experiment with isoproturon in five intermediate soils, MACRO 4.0 predicted (without any calibration) maximum pesticide concentrations and total losses of pesticide in leachate which were within a factor of four of those observed. However, model predictions were much more variable in two heavy clay soils where there are inherent difficulties in predicting observed behaviour because of the extreme spatial and temporal heterogeneity in their structure. Figure 1 compares maximum concentrations of isoproturon observed in drainflow from a heavy clay (60% clay in the topsoil) at Brimstone Farm, UK with values simulated by MACRO. The model gave a good simulation of observed concentrations in some years, but over-predicted actual concentrations by one to two orders of magnitude in others.

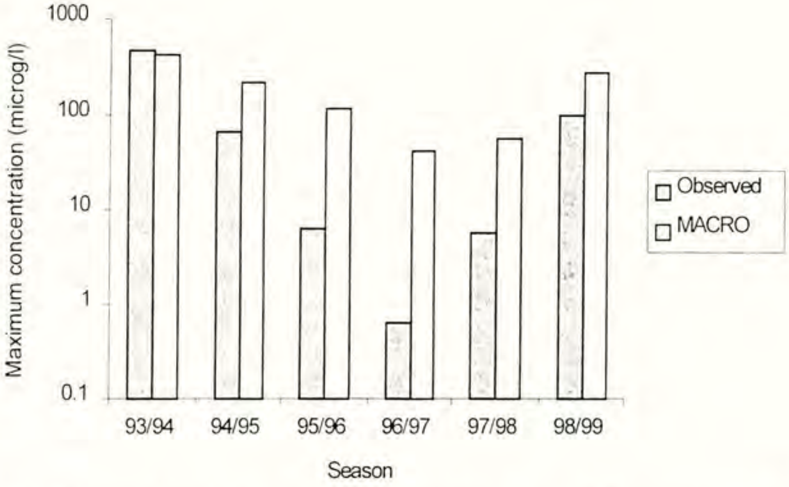

Figure 1. Observed maximum concentrations of isoproturon in successive seasons at Brimstone Farm and those simulated by MACRO 4.1.

SENSITIVITY ANALYSIS

One of the disadvantages to using macropore flow models which is often cited is the sensitivity of the model to parameters defining the domain of macropore flow which are very difficult to measure or estimate. The FOCUS surface water report concludes that "this may lead to high levels of predictive uncertainty compared to the use of models in non-structured sandy soils". Sensitivity analyses only partially support such statements. Figure 2 compares the sensitivity of MACRO 4.1 (macropore flow model) and PELMO 3.00 (non-macropore flow model) for total leaching of a mobile, slightly persistent pesticide through sandy loam and clay loam (MACRO only) soils. The fifteen most sensitive parameters from each model are ranked using a relative sensitivity index (MAROV) and also grouped according to type of parameter. MAROV is defined by:

MAROV = (output variation / input variation)

A MAROV value of 1 indicates exact correspondence between the change in an input parameter and the resulting change in model output. The larger the MAROV value for a given parameter, the greater its sensitivity.

Figure 2 clearly demonstrates that for these scenarios, model output is more sensitive to pesticide parameters than soil properties independent of whether or not the model includes a description of macropore flow. The sensitivity of the top ranked parameters in PELMO is somewhat greater than that in MACRO, but the latter has a far larger number of parameters with lesser, but still significant sensitivity (MAROV values of 0.1-1). Although most of the pesticide properties can be readily measured, experimental values carry a significant level of variability and thus uncertainty. Clearly, difficulties in estimating sensitive soil parameters for use in macropore flow models should not be under-played, but these must be considered in the light of other constraints for the use of non-macropore flow models which are routinely accepted for regulatory submissions.

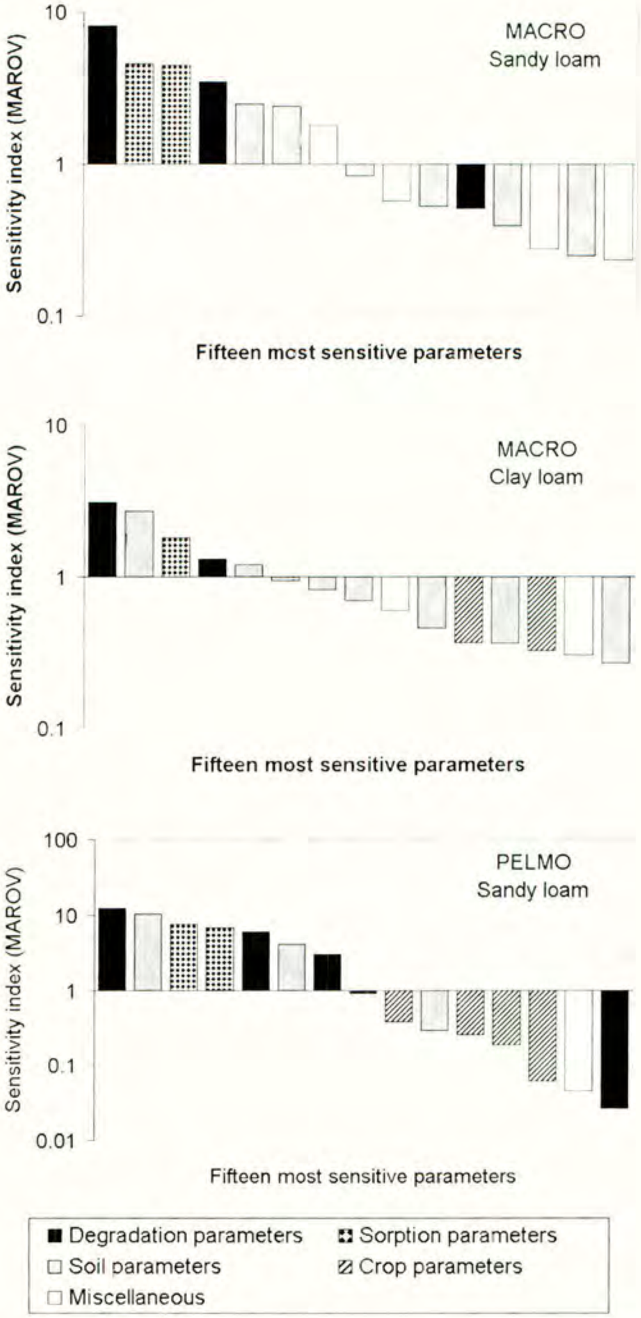

Figure 2 Comparison of the fifteen most sensitive parameters in MACRO and PELMO for loss via leaching of a pesticide with K_{oc} = 20 ml/g and DT_{50} = 20 days (at 20°C) from a sandy loam and a clay loam soil.

REGULATORY STATUS

There are three main routes of entry for pesticides into surface waters – drift, overland flow and drainflow. Each of these entry routes is usually characterised by transient pulses of pesticide. In the case of drainflow, this has been shown to result from macropore flow through cracks and fissures which is intercepted by the drainage system and rapidly transported to surface water (Johnson *et al.*, 1994; Brown *et al.*, 1995). Connectivity between macropore flow pathways and surface water has thus been clearly established and the impact of macropore flow on water quality can be readily monitored and studied. As a result, there is a clear need to simulate macropore flow when considering pesticide losses from drained clay soils, particularly at higher tiers in the risk assessment process. The FOCUS surface water scenario group has selected MACRO to simulate drainflow at Step 3 as the model can simulate the full range of drained soils from sands to heavy clays.

Evidence for connectivity between groundwater and routes of macropore flow through soil is much less clear. Rapid movement of pesticide to the base of the soil profile has been attributed to macropore flow in studies with a range of soils in lysimeters (Brown *et al.*, 2000) and in the field (Flury *et al.*, 1995). Equally, presence of residues in groundwater has occasionally been hypothesised to result from macropore flow (*e.g.* Ritter *et al.*, 1996) However, there is to date no direct evidence for connectivity and without this the impact of macropore flow on groundwater quality remains unquantified. In the absence of detailed scientific understanding of the processes involved, it is very difficult to simulate transport of pesticide to groundwater via macropore flow with any accuracy. Nevertheless, a number of regulatory authorities in Europe adopt a precautionary approach and request that macropore flow be considered in the calculation of predicted environmental concentrations for groundwater. The FOCUS groundwater scenarios group have included MACRO for one of the nine scenarios at Step 1 to provide a comparison with the non-macropore flow models recommended for the main assessment (PELMO, PEARL and PRZM).

PRIORITIES FOR FUTURE RESEARCH

Dual-porosity, deterministic models (particularly MACRO) are the most widely used for simulating macropore flow for regulatory purposes. They represent a compromise between the complexity needed to represent the processes and the ease of parameterisation needed to allow their use. The most important requirement to support existing regulatory use of such models is the development of guidance for modellers on how to select difficult parameters from readily-available information. This is particularly important for parameterising soil properties from basic soil analyses and describing pesticide behaviour in dual-porosity systems on the basis of standard regulatory sorption and degradation studies.

Further improvement of macropore flow modelling is hampered by limits to our understanding of key processes and their influence on pesticide transport rather than by our ability to incorporate existing knowledge into model code. A number of priority areas can be identified for further research. These include: a) development of techniques to routinely characterise the extent of macropore flow and subsequent application to a range of soils to establish relative vulnerability for pesticide transport; b) work to characterise the micro-scale processes of pesticide sorption and degradation in relation to macropore flow; and c) development of models specifically applicable at larger scales for application in screening tools and/or product stewardship.

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The effect of windbreak height and air assistance on exposure of surface water via spray drift

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ABSTRACT

In a series of field experiments spray drift was assessed when spraying a sugar beet crop. Next to the crop, the field margin was planted with a 1.25m wide strip of *Miscanthus* (Elephant grass). To evaluate the effect of different heights of such a windbreak crop on spray drift it was cut at different heights just before spraying. Heights varied between: not planted (0m), at crop height level (0.5m), 0.5m above crop height (being sprayer boom height, 1.0m) and 1m above crop height (1.5m). Spraying was performed with a conventional and an air-assisted sprayer. Spray volume was 300 l/ha using Medium spray quality nozzles. Spray drift was measured as soil deposit up to 15m from the last nozzle using surface collectors. The height of the windbreak had a clear effect on spray drift deposit. Spray deposit at 3-4m distance from the last nozzle decreased significantly with increasing heights of the *Miscanthus*. When *Miscanthus* was cut to equal height as the sugar beet spray drift reduction was 50% compared to spray drift on the same distance when no windbreak was grown. With the 0.5m and 1.0 above crop height levels of *Miscanthus* spray drift was reduced by respectively 80% and 90%. On average the air assisted sprayer reduced spray drift by 70%. The effect of these results on predictions of surface water exposure is discussed.

INTRODUCTION

The Multi Year Crop Protection Plan (MYCPP, 1991) of the Dutch government formulates objectives for a reduction in plant protection products to be used and for an application practice for these products which is safe and more compatible with the environment. The emissions of plant protection products to soil, (surface)water and air should be reduced. Regulations are embedded in both the Pesticide Act and the Water Pollution Act. Based on the spray drift deposition level in surface water, the width of crop-free bufferzones can be set and impacts on the registrability of agrochemicals determined. A general reduction in spray drift to surface water next to the sprayed field can be achieved by improvements in spray application techniques. For the last 10 years an intensive measuring programme on spray drift has been performed (Van de Zande *et al.*, 2000). Porskamp *et al.* (1994) concluded that a windbreak hedgerow next to an orchard reduced spray drift by 70-90%. For the Dutch situation De Snoo & de Wit (1998) showed that leaving the outer 3m of the crop unsprayed gave a drift reduction of 95% to adjacent ditches. Miller *et al.* (2000) suggest that canopy structure and height has its effect on spray drift reduction. Because no data were available on the reduction in spray drift of the effect of the height of a windbreak crop next to an arable crop, field experiments were performed. A comparison was made between a conventional field sprayer and an air-assisted field sprayer and three heights of a windbreak crop (Elephant grass *Miscanthus ssp.*) when spraying sugar beet.

MATERIALS AND METHODS

Spray drift was measured spraying a downwind swath of 24m of a sugar beet field. The length of the sprayed track was 35m. A minimum of nine replications were made in time. Measurements of spray drift were performed using a reference situation i.e. a field sprayer, with Medium spray quality nozzles (Southcombe *et al.*, 1997), applying a volume rate of 300 l/ha and a sprayer boom height of 0.5m above crop canopy. Specifications of the sprayer used in the experiments are as summarised in Table 1.

Table 1. Settings of the field sprayer during spray drift field experiments

sprayer	Hardi Commander Twin Force
working width [m]	24
nozzle type	XR 110.04VP
spray pressure [bar]	3.0
spray quality	Medium
nozzle flow rate [l/min]	1.58
driving speed [km/h]	6.0
spray volume [l/ha]	316
air assistance oil pressure (bar)	240

Average height of the sugarbeet crop was 0.5m. The windbreak crop of *Miscanthus* (Elephant grass) was planted in two rows. Row spacing of the *Miscanthus* was 0.65m, average canopy width was 0.95m, and distance between last sugar beet row and the first *Miscanthus* row 1.0m. Total windbreak canopy was therefore on a zone of 2.25m, virtually between the last crop row and the ditchbank. The windbreak crop was cut at heights equal to the sugarbeet crop (0.5m), 0.5m above crop height (is sprayer boom height) and 1.0m above crop height, respectively 1.0 and 1.5m high. Spray drift measurements were performed for a standard sugarbeet field lay-out, a crop free zone of 2m, and three heights of a *Miscanthus* windbreak crop of 1.25m width on this crop free zone.

A fluorescent tracer (Brilliant Sulfo Flavine: BSF 1g/l) and a non-ionic surfactant (Agral; 1g/l), solubilised in the spray liquid, were used to analyse spray deposit. Spray deposit was measured next to the field up to 15 m from the last nozzle. Ground deposit was measured on horizontal collection surfaces placed at ground level in a double row downwind of the sprayed swath. Collectors used were synthetic cloths with dimensions of 0.50x0.08 and 1.00x0.08 m. The collectors were placed at distances 0-0.5, 1-1.5, 1.5-2, 2-3, 3-4, 4-5, 5-6, 7.5-8.5, 10-11, 15-16 m from the last downwind nozzle. For the situations with the windbreak crop collectors were at the same distances starting at 2m from the last nozzle. The last nozzle of the field sprayer moved in the middle between the last and last but one crop-row of sugarbeet, 0.25m inside the last crop row. After spraying, the dye was extracted from the collectors. The deposited dose was measured by fluorimetry and expressed per surface area of the collector. The spray drift was expressed as percentages of the application rate of the sprayer (spray dose).

Meteorological conditions during the measurements were recorded. Wind speed and temperature were recorded at 5 s interval at 0.5 and 2.0 m height, using cup anemometers and Pt100 sensors, respectively. Relative humidity (RH) was measured at 0.5 m height and wind direction at 2.0 m height. Average weather conditions during the field experiments were as summarised in Table 2.

Table 2. Average weather conditions during spray drift field experiments

	temperature [°C]		RH [%]	wind angle	windspeed [m/s]	
	0.5m	2.0m		° to square	0.5m	2.0m
conventional	9,3	9,2	72	15	3,6	4,7
air assistance	9,8	9,7	69	17	3,5	4,6

Statistical analysis of the data was done on spray deposition on different zones next to the field, using analysis of variance (ANOVA 5% probability; Payne, 1993).

RESULTS

Spray drift deposition next to the field

The differences in spray drift deposition for the conventional field sprayer and the air-assisted field sprayer when spraying the standard sugarbeet field layout, and the field layout with different heights of *Miscanthus* next to it are presented in figures 1 and 2. When spraying conventionally, the increasing height of the *Miscanthus* decreases spray drift deposition behind the windbreak. With air assistance these effects remain the same. Levels of spray drift are for the air assisted field sprayer clearly lower than for the conventional operated field sprayer. In general spray drift deposition decreases with increasing distance. However figures 1 and 2 show that for the 1.0m high *Miscanthus* spray drift is very low on the zone 4-8m from the last nozzle and increases slightly again on larger distances, remaining at lower levels than with the other objects. As spray drift decreases with increasing distance, a statistical comparison is performed on discrete zones next to the field. In table 3 the spray drift deposition on different zones next to the field is presented with results of the statistical analysis (ANOVA $\alpha < 0.05$). These zones were chosen as being most important for the effect of drift deposition on the surface water area.

Table 3. Spray drift deposition (% of dose) next to the field on surface water distance, when spraying a sugar beet crop conventionally or with air assistance in combination with a crop-free bufferzone of 2m, or a windbreak crop of *Miscanthus* of 1.25m width of equal height, or 0.5m and 1.0m higher than crop canopy (0.5m)

bufferzone	conventional	air assistance
no	2.4 a	0.46 a
2m crop-free	1.5 b	0.20 b
Miscanthus, equal height	1.1 c	0.20 b
Miscanthus, + 0.5m high	0.40 d	0.11 c
Miscanthus, + 1.0m high	0.28 e	0.05 d

means within one column followed by the same letter do not differ significantly (ANOVA $\alpha < 0.05$)

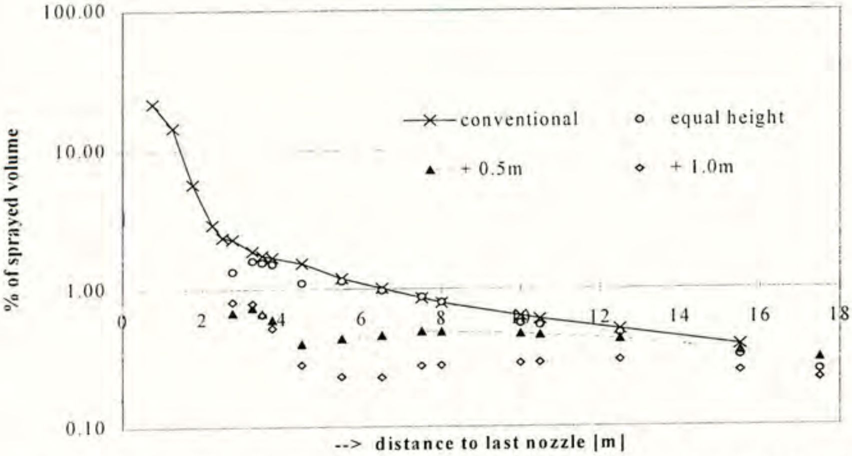

Figure 1. Spray drift deposition (% of sprayed volume) next to a sugar beet field sprayed conventionally with a standard field layout (conventional) and with a 2m strip of *Miscanthus* next to it of equal height, 0.5m (+0.5m) and 1.0m (+ 1.0m) higher than the sugar beet crop

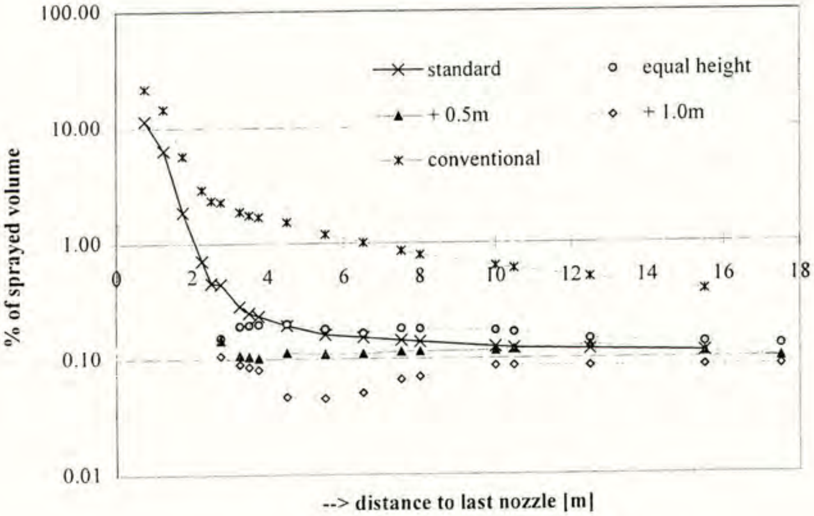

Figure 2. Spray drift deposition (% of sprayed volume) next to a sugar beet field sprayed with the aid of air assistance on the field sprayer with a standard field layout (standard) and with a 2m strip of *Miscanthus* next to it of equal height, 0.5m (+0.5m) and 1.0m (+ 1.0m) higher than the sugar beet crop, compared to standard field layout sprayed conventionally (conventional)

Spray drift reduction

The effect on spray drift for the different spray techniques can be expressed as the spray drift reduction relative to the spray drift deposition of the 'conventional" field sprayer for the standard field layout. Spray drift reduction on the areas next to the field is expressed as percentage of the deposition of the conventional field sprayer on the same area (Table 4).

Table 4. Average spray drift reduction (%) to the ground next to the field, on 4-5m distance from the last nozzle when spraying a sugarbeet crop using a conventional and an air-assisted field sprayer using Medium spray quality nozzles for a standard field layout (2 m crop-free) and when growing a *Miscanthus* windbreak of equal height, and 0.5m or 1.0m above crop canopy (0.5m) on this 2m zone.

bufferzone	soil deposition	
	conventional	air assistance
2m crop-free	36	92
Miscanthus, equal height	53	92
Miscanthus, + 0.5m high	83	95
Miscanthus, + 1.0m high	88	98

The growing of a strip of *Miscanthus* on a zone next to an arable crop like sugar beet decreases spray drift deposition on the surface water area next to the sprayed field. Spray drift is reduced with increasing height of the windbreak crop. On the distance 4-5m from the last nozzle average spray drift reduction is up to 88% for conventional spraying and up to 98% using an air-assisted sprayer. Air assistance reduced spray drift in general 70-80%.

DISCUSSION

In the Netherlands crops like sugarbeet are usually grown close to the the border of the field, in case of a ditch close to the ditch bank. When the distance between the last crop row and the ditchbank is 0.5m, for sugar beet the distance of the last nozzle of the field sprayer to the surface water is 2.25m (Figure 3).

Figure 3. Typical lay-out of a field sprayer above a sugarbeet crop in the Netherlands. When the ditch-bank is 1.5m wide, the distance between the last crop-row and the surface water is 2.0m.

On 2-3 m distance from the last row of a sugarbeet crop, the area where in the Netherlands the surface water is, the spray drift deposition is significantly lower when crop protection products are applied with an air assisted sprayer. Applying on the downwind 24m of a sugarbeet field agrochemicals with an air assisted field sprayer, reduces spray drift with 85 to 90%, compared to a boom sprayer equipped with nozzles producing a Medium spray quality (300 l/ha). In combination with a 1.25m wide windbreak crop like *Miscanthus* grown on a zone of 2m width between the ditch-bank and the first crop-row, spray drift reduction can be increased depending on the height of the windbreak crop. Drift reduction increases from 53% for an equally high windbreak crop sprayed conventionally to 98% when spraying air-assisted in combination with a 1.5m high windbreak crop (1.0m higher than crop canopy).

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SESSION 2C

CEREAL STEM-BASE DISEASE: ECONOMIC TARGET OR ACADEMIC CHALLENGE?

Chairman:	Dr B D L Fitt *IACR-Rothamsted, Harpenden, UK*
Session Organiser:	Dr G L Bateman *IACR-Rothamsted, Harpenden, UK*
Papers:	2C-1 to 2C-4

Cereal stem-base disease – a complex issue

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ABSTRACT

PCR assays were used to evaluate the accuracy of visual assessment of stem-base diseases of winter wheat and identify the predominant stem-base pathogen species present in the UK. At early growth stages, pathogenic fungi were identified in the absence of symptoms. At later growth stages, there was generally good agreement between occurrence of symptoms and causal pathogens identified by PCR. Quantitative PCR was evaluated as a means of determining the efficacy of fungicides and cultivars against stem-base pathogens and for risk assessment. Cyprodinil controlled both eyespot pathogens, *Tapesia acuformis* and *T. yallundae*, but prochloraz gave consistent control only of *T. yallundae*. Despite significant control of eyespot, yield increases were moderate and were only achieved on the most susceptible cultivars. Azoxystrobin increased yields most consistently but there was no evidence that this occurred through control of stem-base diseases. It was active against sharp eyespot, which was present only at low levels. Brown foot rot was associated only with *Microdochium nivale*, which was affected little by fungicides.

INTRODUCTION

Stem-base disease of wheat and barley commonly has three components: eyespot, sharp eyespot and brown foot rot. Although sharp eyespot is associated with a single causal agent, *Rhizoctonia cerealis*, eyespot may be caused by either *Tapesia yallundae* or *T. acuformis* (formerly referred to as W-type and R-type *Pseudocercosporella herpotrichoides* respectively). Brown foot rot is associated with two varieties of *Microdochium nivale* (vars *majus* and *nivale*) and a number of *Fusarium* species (predominantly *F. culmorum* and *F. avenaceum*).

Current strategies for management of stem-base disease require estimation of disease incidence and severity in plants during stem extension (GS 30-37) (Goulds & Polley, 1990). Unfortunately, visual diagnosis is complicated by the fact that stem-base disease is frequently a complex of eyespot, sharp eyespot and/or brown foot rot. Their symptoms are often indistinct and may be confused, particularly during early growth stages. Even where the diseases are correctly identified, it is not possible to determine visually the species of *Tapesia* present in an eyespot lesion or to differentiate and identify the *Fusarium* species or *M. nivale* varieties based upon brown foot rot lesion appearance.

The accuracy of early diagnosis of disease could have potentially significant repercussions where the causal agents differ in their sensitivity to fungicides, susceptibility to host resistance or ability to reduce yield or quality. A number of molecular techniques have been developed to overcome many of the perceived problems associated with diagnosis and quantification of stem-base disease. Among the most sensitive and potentially specific techniques available is the polymerase chain reaction (PCR). Assays based on this technique have been developed for the major pathogens involved in stem-base disease. Although PCR is an extremely sensitive technique it has generally been used only to detect the presence of pathogens and not to quantify the amount of pathogen present (e.g. Nicholson & Parry, 1996). Quantitative PCR assays have, however, recently been developed in our laboratory and elsewhere for the major stem-base pathogens (e.g. Nicholson *et al.*, 1996).

The quantitative diagnostic PCR assays provide an opportunity to identify which species are prevalent in crops early in the season and, when compared with results from visual assessment, to highlight those visual diagnoses most prone to error. The ability to detect and quantify the amount of fungus present in a crop early in the season might also provide information to enable the risk posed to the crop by that fungus (or fungi) to be determined. Thus, these new tools might aid crop management and the development of strategies to combat stem-base disease. This work reports comparisons of visual assessment with PCR diagnosis and the use of PCR to identify the predominant stem-base pathogens present in winter wheat crops in the British Isles. In addition, quantitative PCR was evaluated in field experiments as a tool in disease forecasting and risk assessment and as a means of determining the efficacy of selected fungicides and cultivars against stem-base pathogens.

METHODS AND MATERIALS

Field monitoring and survey sites

Winter wheat (cv. Beaver) was sown in October 1993 in two replicate plots at sites in Hingham, Norfolk and Little Stonham, Suffolk. Forty-six seedlings or main shoots were sampled at each site throughout the season (GS 25, 30, 37, 65 and 85). Samples (30 shoots) were also taken as close to GS 31 as possible from fields across the UK and Eire in 1997 (409 samples) and 1998 (348 samples).

Field experiments

Nine field experiments were carried out in a collaborative project at three sites in England over three years (1996/7, 1997/8, 1998/9). The sites were Harper Adams University College (west midlands), Rothamsted Experimental Station (southeast midlands) and Morley Research Centre (East Anglia). Each experiment consisted of four randomised blocks in which the effects of fungicides were compared on cultivars of winter wheat, grown as second wheat crops. The cultivars, differing in their susceptibilities to eyespot according to NIAB ratings, were: Brigadier (rating 5) (omitted in 1998/9), Lynx, (rating 8, but not appearing in the Recommended Lists), Mercia (rating 5) and Soissons (rating 4). The fungicide treatments (full rates), applied at approximately GS 31, were: none (no-fungicide control), azoxystrobin (Amistar), cyprodinil (Unix), flusilazole (Sanction) in 1996/7 only, prochloraz (Sportak), HGCA1 (undisclosed formulation) in 1997/8 and 1998/9. Husbandry was standard for the farms and included additional fungicide applications to control foliar diseases where appropriate. Plant samples (30 per plot) were taken at GS 22, GS 31 (immediately before fungicide application) and 2-3 weeks after fungicide application. Two further samples (in May and at late ripening) were taken in 1997 and one (at early ripening) in 1998 and 1999. Disease assessments were carried out immediately after sampling and were followed by DNA extraction.

Visual and PCR diagnoses

Symptoms on the plants from monitoring and survey sites were recorded as present or absent for eyespot, sharp eyespot and brown foot rot using the key of Goulds & Polley (1990). DNA was extracted from each sample and analysed by PCR for stem-base pathogens as described in Turner *et al.* (1999). In the field experiments, symptoms on stems were usually assessed as slight, moderate or severe. The pathogens were then detected and quantified by competitive PCR using the primers and methods described in Nicholson & Parry (1996) and Nicholson *et al.* (1996, 1997 and 1998).

RESULTS

Disease monitoring sites

Eyespot was not widespread until GS 85 when its incidence, averaged over both sites, increased to 72% of stems. Sharp eyespot was scarce at both sites. The incidence of brown foot rot increased considerably after GS 37 to reach 87% by GS 85. PCR revealed that the predominant eyespot pathogen throughout the season at both sites was *T. acuformis* while *T. yallundae* was not detected at Stonham and was found only very rarely at Hingham. The prevalent brown foot rot pathogen was *Microdochium nivale* var. *majus* with *M. nivale* var. *nivale* also common at Hingham (particularly later in the season); *F. avenaceum* and *F. culmorum* were rarely detected. *Rhizoctonia cerealis* was uncommon at Hingham and was rarely detected at Stonham.

Pooling the various stem-base pathogens into the categories of eyespot (*Tapesia* spp.), sharp eyespot (*R. cerealis*) and brown foot rot (*M. nivale*, *F. culmorum*, and *F. avenaceum*) allowed comparisons between visual disease assessments and molecular diagnoses. Most plants diagnosed with eyespot contained one or other of the *Tapesia* species, usually *T. acuformis* (Table 1). However, the incidence of *Tapesia* spp. detected by PCR was much greater than that for eyespot diagnosis, particularly early in the season. *Tapesia acuformis* was detected at both sites at GS 25 although no eyespot was diagnosed at either site. The agreement between visual diagnosis of eyespot and PCR, however, improved throughout the season. PCR analysis also detected, at early sampling times (GS 25, 31 and 37), one or more of the brown foot rot causal organisms in many more plants than were diagnosed as having brown foot rot symptoms. As for eyespot, there was poor agreement between PCR and visual diagnosis before GS 37. At later sampling times (GS 65 and 85) the vast majority of visual diagnoses were accompanied by positive PCR diagnoses for one or more of the brown foot rot pathogens. In the case of sharp eyespot, correlation between PCR and visual diagnosis remained very poor throughout the season and there was no correlation between sharp eyespot diagnosis and the presence of any of the stem-base pathogens.

Eyespot diagnoses were correlated, not only with the presence of *T. acuformis* and *T. yallundae*, but also with *M. nivale* var. *majus* (statistical details not shown). Similarly, brown foot rot diagnoses were positively correlated with the two *Tapesia* species as well as *M. nivale* varieties and *F. culmorum*.

Table 1. Comparison of visual disease assessments and PCR diagnosis for eyespot, sharp eyespot and brown foot rot on 92 seedlings or individual shoots at different growth stages (GS)

GS	Eyespot			Sharp eyespot			Brown foot rot		
	Visual	PCR[a]	Both	Visual	PCR[b]	Both	Visual	PCR[c]	Both
25	0	34	0	2	3	0	4	24	1
30	6	35	6	10	4	0	13	21	4
37	13	55	9	4	0	0	30	50	26
65	21	79	19	6	15	3	82	75	67
85	66	70	59	4	7	1	80	78	59

[a] *Tapesia* spp. [b] *Rhizoctonia cerealis*. [c] *Microdochium nivale* or *Fusarium* spp.

Disease survey

The results from the surveys revealed that incidence and relative abundance of stem-base pathogens differed markedly between 1997 and 1998 (results not shown). Only three species, *T. acuformis*, *R. cerealis* and *M. nivale* var. *nivale*, were abundant in 1997. Notably, while *T. acuformis* was found in 75.6% of samples, *T. yallundae* was detected in only one. In 1998, *M. nivale* var. *majus* was the most common pathogen (66.1% of samples) and *T. acuformis* was also common (58.3% of samples). In addition, the incidences of *F. culmorum* and *F. avenaceum* were greater in 1998. As in 1997, the incidence of *T. yallundae* was very low. These results confirmed the predominance of *T. acuformis* over *T. yallundae* in winter wheat in the British Isles and also revealed significant differences between years in the overall, and relative abundance of stem-base pathogens in winter wheat at approximately GS 31.

Field experiments

A summary of the results from this large-scale project is presented. Details are in Bateman *et al.* (2000). The comparison of visual assessment with PCR, using bulk samples (30 seedlings or individual shoots) was largely similar to those with single plants reported above. Disease assessments before GS 31 often did not agree with pathogen diagnoses using PCR although agreement improved later in the season. The use of bulked samples reduced the overall sensitivity of the PCR assessment through the dilution effect resulting from relatively few diseased plants among a majority of uninfected ones.

T. acuformis was the only pathogen that occurred in quantifiable amounts in all nine experiments. This species was detected in some early samples but generally developed late and was only present in quantifiable amounts 200 days or more after sowing. In contrast, where *T. yallundae* was present it tended to accumulate earlier than *T. acuformis*.

There were often discrepancies between the amounts of pathogen DNA and incidence or severity of disease symptoms. Sharp eyespot generally had the strongest relationship to DNA of its pathogen, *R. cerealis*. Brown foot rot was associated primarily with *M. nivale* but the amount of this pathogen tended to decline as the crops ripened. This resulted in weaker relationships between brown foot rot and pathogen DNA in summer than in the spring. Where a significant regression was observed between the disease and DNA of the causal organism(s) it often accounted for only a small proportion of the variance, suggesting that other factors contributed to the effects. These factors might include the presence of other pathogens or a decrease in the amount of the pathogen after symptoms have developed.

Table 2. Number of significant ($P \leq 0.05$) decreases in eyespot by fungicides on four cultivars at GS 69-85, compared with no-fungicide controls (numbers of tests in parentheses)

Fungicide	Lynx	Brigadier	Mercia	Soissons
Prochloraz	1 (9)	3 (6)	2 (9)	2 (9)
Cyprodinil	4 (9)	5 (6)	7 (9)	8 (9)
Azoxystrobin	0 (9)	0 (6)	0 (9)	0 (9)
Flusilazole	0 (3)	0 (3)	1 (3)	1 (3)
HGCA1	0 (6)	0 (6)	0 (6)	1 (6)

The most effective fungicide against eyespot was cyprodinil, which resulted in significant decreases in disease (Table 2) whether caused by *T. acuformis* or *T. yallundae* (Table 3). Prochloraz gave less consistent control and was generally most effective at Rothamsted where *T. yallundae* was more prevalent. The benefit of fungicide application was dependent upon the cultivar. Whereas cyprodinil significantly reduced eyespot at GS 69-85 in Soissons on eight out of nine occasions, the level of disease in Lynx (which usually had least disease in untreated plots) was reduced significantly on only four occasions (Table 2). Similarly, cyprodinil reduced the amount of both *Tapesia* species on all occasions in Soissons whereas levels of *T. yallundae* were unaffected on Lynx and *T. acuformis* was reduced on only three out of eight occasions (Table 3).

Azoxystrobin was the only fungicide to reduce *R. cerealis* and sharp eyespot at GS 69-85. This occurred, however, on relatively few occasions over the four cultivars (16 and 21% of occasions respectively). None of the fungicides significantly reduced *M. nivale* var. *majus* at the last sample time although cyprodinil and HGCA1 each reduced *M. nivale* var. *nivale* in Mercia on a single occasion.

Table 3. Number of significant ($P \leq 0.05$) decreases in *Tapesia acuformis* (*T.a.*) and *T. yallundae* (*T. y.*) by fungicides on four cultivars at GS 69-85, compared with no-fungicide controls (numbers of tests in parentheses)

Fungicide	Pathogen	Lynx	Brigadier	Mercia	Soissons
Prochloraz	*T. a.*	0 (8)	0 (5)	1 (8)	2 (8)
	T.y.	1 (4)	0 (1)	2 (4)	4 (4)
Cyprodinil	*T.a.*	3 (8)	3 (5)	5 (8)	8 (8)
	T.y.	0 (4)	0 (1)	2 (4)	4 (4)
Azoxystrobin	*T.a.*	0 (8)	0(5)	0 (8)	2 (8)
	T.y.	0 (4)	0 (1)	0 (4)	0 (4)
Flusilazole	*T.a.*	0 (2)	0 (2)	0 (2)	1 (2)
	T.y	0 (0)	0 (0)	0 (0)	0 (0)
HGCA1	*T.a.*	0 (6)	0 (3)	0 (6)	0 (6)
	T.y.	0 (4)	0 (1)	0 (4)	0 (4)

Table 4. Number of significant ($P \leq 0.05$) increases in yield (t/ha) by fungicides on four cultivars, compared with no-fungicide controls (numbers of tests in parentheses)

Fungicide	Lynx	Brigadier	Mercia	Soissons
Prochloraz	0 (9)	0 (6)	1 (9)	0 (9)
Cyprodinil	0 (9)	0 (6)	2 (9)	3 (9)
Azoxystrobin	2 (9)	3 (6)	3 (9)	3 (9)
Flusilazole	0 (3)	0 (3)	0 (3)	0 (3)
HGCA1	0 (6)	0 (6)	0 (6)	0 (6)

Eyespot is generally regarded as the most damaging of the stem-base diseases. Despite significant reductions in eyespot and its causal agents by cyprodinil, this was rarely accompanied by significant increases in yield (Table 4). Significant increases in yield resulting from application of cyprodinil were observed only on the most eyespot-susceptible cultivars (Mercia and Soissons). Azoxystrobin was the most effective fungicide at increasing yield but this occurred in only a third of instances across the whole experiment. The levels of sharp eyespot and *R. cerealis* were generally low throughout the experiments and it is therefore unlikely that the yield increases with azoxystrobin were due to effects on this or other stem-base diseases.

The mean increase in yield resulting from azoxystrobin was 0.43 t/ha while that due to cyprodinil was 0.24 t/ha. The mean increase achieved by prochloraz, the only other fungicide to increase yield significantly in any experiment, was 0.05 t/ha.

DISCUSSION

Results from these studies confirm the difficulty in making accurate diagnoses of stem-base disease, particularly early in the season when crop protection decisions need to be made. The sensitivity of the PCR assay enables detection of disease prior to the appearance of symptoms, making it a useful aid for early diagnosis of the presence of particular fungal pathogens within the crop.

Results from the disease surveys highlight the predominance of *T. acuformis* over *T. yallundae* in winter wheat in the British Isles. The surveys also revealed the prevalence of *M. nivale* within crops early in the season and showed how the relative incidence of its two varieties may alter dramatically between seasons. The causes and implications for disease of such shifts are not known.

Comparison of early and late samples from the field experiments to test the effects of fungicides and cultivars (Bateman *et al.*, 2000) were in agreement with the findings of the above studies. This research also confirmed that, while *T. acuformis* may infect early in the season, it tends to increase late relative to *T. yallundae*. It has been suggested that the later development of *T. acuformis* may result in smaller yield losses than those caused by the once predominant *T. yallundae* (Royle, 1998). It was concluded that, where eyespot develops relatively late, the use of PCR to determine the amount of pathogen DNA at GS 31 does not provide a precise means of assessing risk. The detection of significant quantities of eyespot pathogen at this stage (when symptoms may not be clearly recognisable) may, however, indicate that infection is sufficiently extensive to warrant fungicide treatment.

Quantitative PCR for stem-base pathogens generally confirmed which diseases were affected by fungicides and cultivars as well as identifying which of the possible pathogens were contributing to those diseases. Cyprodinil was found to be the most effective fungicide against eyespot, being active against both *Tapesia* species. Control by prochloraz was erratic and tended to be more effective against *T. yallundae*. Control of eyespot by fungicides was, however, dependent upon the cultivar. While cyprodinil reduced both eyespot pathogens on all occasions in Soissons (susceptible), it failed to reduce *T. yallundae* in Lynx (resistant) and was effective against *T. acuformis* on less than 50% of occasions in this cultivar. Azoxystrobin was active against sharp eyespot and its pathogen and was the most effective fungicide in increasing yield. It is doubtful, however, that this was achieved through its effect on sharp eyespot, which occurred at generally low levels.

Even where significant yield increases were achieved they were modest across cultivars and it is most doubtful that this would offset the cost of treatment. Overall, the findings from the present studies indicate that, even on susceptible cultivars, the use of fungicides against stem-base disease was of limited value. A resurgence of eyespot-resistant cultivars, such as Lynx, could dramatically reduce or even eliminate the need for fungicide application to control stem-base disease.

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The use of PCR diagnostics in determining eyespot control strategies in wheat

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ABSTRACT

The progress of eyespot epidemics in winter wheat was assessed both visually and through the use of fully quantified PCR diagnostics over four seasons in south-east Scotland. The effect of two fungicides (cyprodinil and prochloraz) on the eyespot pathogen populations was determined. The PCR results showed that *Tapesia acuformis* was the dominant strain in each season. There was no correlation between visual eyespot symptoms on the stem base at stem extension and eyespot levels at the end of the season. Amounts of *T. acuformis* DNA early in the season also did not correlate with those late in the season. A threshold approach for determining the need for treatment based on visual symptoms or amounts of DNA at stem extension would not have been successful. Significant control of eyespot and a yield response were achieved with fungicides but the optimum timing for control was different for the two fungicides.

INTRODUCTION

A complex of diseases can occur on the stem base of wheat, of which eyespot is the most damaging. Two species of eyespot pathogen predominate in the UK, *Tapesia yallundae* and *Tapesia acuformis* (Robbertse *et al.*, 1995). *T. yallundae* was the most common species in the UK until the early 1980s but *T. acuformis* has increased in prevalence so that it became the dominant species (Hollins & Scott, 1987).

Eyespot severity in crops is governed by agronomic factors and is greatest where cereals are grown in close rotation and in cool, moist conditions. Losses in yield and quality as a result of eyespot infection can be serious (Burnett & Oxley, 1996). Fungicides applied at stem extension will reduce eyespot levels and give yield benefits in some, but not all situations. Fungicides with activity against eyespot represent an additional cost in the winter wheat spray programme and identifying crops that will give a cost-effective response is critical. A visual threshold of 20% infected shoots at stem extension is often used to predict where a cost-effective response to a specific eyespot fungicide would be expected (Anon., 1987). PCR-based diagnostic tests are now available (Nicholson *et al.*, 1997) which allow the levels of eyespot fungus DNA to be quantified throughout the season.

The aim of the work reported was to use both visual and PCR-based methods to assess the effects of the fungicides prochloraz and cyprodinil on disease development and to determine the usefulness of these diagnostic techniques in assisting with decision making and predicting eyespot risk in winter wheat crops.

MATERIALS AND METHODS

Field trials were carried out from 1995 to 1998 by superimposing plots in commercial crops of winter wheat (cultivar Riband) at a site in East Lothian. The crops were all second wheats. Plots were 40 m^2 and were laid out in randomised blocks with four replicates of each treatment. Fungicide treatments were applied using a hand-held Cooper Pegler CP3 sprayer calibrated to deliver a water volume of 220 l/ha at a pressure of 2.5 bars. The plots were sprayed with fungicides at GS 39 and GS 55 (Tottman, 1987) in 1995, 1996 and 1997 to minimise any influence of foliar diseases on plot treatment yields. In 1998 no foliar disease sprays were applied. Except for fungicides, the trial areas received the same inputs as the surrounding commercial crop, which were typical of agronomy practices in the area.

At each sampling date, 25 separate plants from each plot (prior to growth stage 31) or 25 shoots after this growth stage were chosen at random from each plot. Eyespot was assessed visually at each sampling and the results expressed as a percentage index (Burnett and Oxley, 1996). The same stems were used for quantification of eyespot pathogen DNA at each assessment. Roots were removed close to the crown and the stem base was cut to 2-3 cm in length. This was rinsed in tap water followed by distilled water, then freeze dried for 48 hours. The tissue was removed to plastic storage boxes containing silica gel and stored at -80°C until DNA could be extracted.

Prior to DNA extraction, the freeze-dried weight of each pooled sample of 25 stem bases was recorded. The sample was ground in liquid nitrogen to a fine flowable powder. This was transferred to a centrifuge tube and DNA extracted using a commercially available kit designed for plant DNA extraction (Nucleon Phytopure, Scotlab Ltd, Coatbridge, Strathclyde). Final re-suspension of the DNA was made in 500 µl TE (tris-EDTA buffer, pH 8.0) in plastic eppendorf tubes. A competitive PCR technique was used at the John Innes Centre, Norwich, which enabled quantification of the PCR products (Nicholson *et al.*, 1997). Results were expressed as ng fungal DNA per mg dry weight of stem base and used to quantify the amount of *T. acuformis* and *T. yallundae* present at each sampling date.

Yield (t/ha corrected to 85% moisture content) was determined at harvest. The crop growth stages and treatment dates are detailed in Table 1 and the spray programmes listed in Table 2. No eyespot-specific fungicides were applied in 1997 and in that season samples were collected only from untreated plots. In 1998 only treatments 3 and 9 were applied.

Table 1. Treatment spray dates and crop growth stages in each season

Target growth stage	1995		1996		1998	
	Spray date	Growth stage	Spray date	Growth stage	Spray date	Growth stage
22	14 Feb	22	21 Feb	24	-	-
25	14 Mar	27	18 Mar	25	25 Feb	25
30	14 Apr	30	26 Apr	31	-	-
32	1 May	32	15 May	32/33	11 May	32

Table 2. Eyespot spray programmes evaluated in winter wheat trials

Target growth stage	22	25	30	32
Treatment no.				
1	-	-	-	-
2	P	-	-	-
3	-	P	-	-
4	-	-	P	-
5	-	-	-	P
6	C	-	-	-
7	-	C	-	-
8	-	-	C	-
9	-	-	-	C

Full commercial doses of the following products were used

Active ingredient	*Product*	*Manufacturer*	*g a.i./ha*
P = prochloraz	Sportak 45	Aventis	405
C = cyprodinil	Unix	Novartis	750
- = untreated			

RESULTS

Eyespot fungus DNA was extracted from plants in untreated plots over the four seasons and the average results plotted in Figure 1. *T. acuformis* was the dominant strain. Eyespot pathogen DNA tended to increase gradually until stem extension when levels declined. After stem extension the amount of DNA increased rapidly until July, after which there was a sudden and rapid decline coinciding with crop senescence.

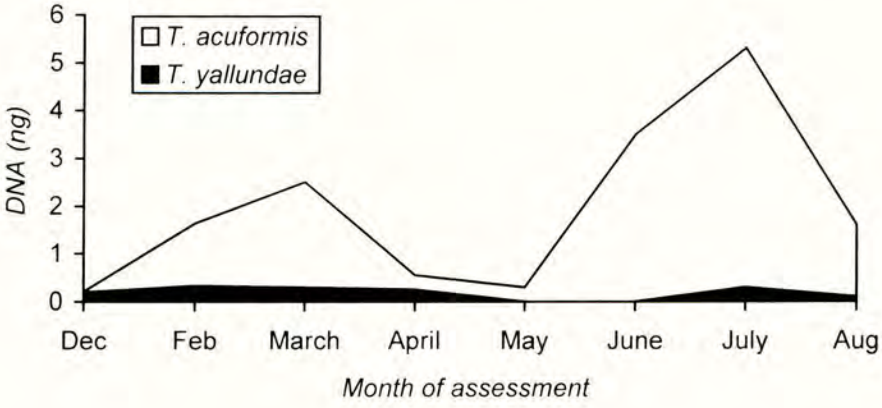

Figure 1. *Tapesia* in untreated plots 1995 -1998

Using data from the untreated plots, there was no significant correlation between visual eyespot levels at stem extension and the visual eyespot index at the end of the season (Table 3). There was also no significant correlation between the amounts of *T. acuformis* DNA measured at stem extension and the amounts recorded at the end of the season. There was a highly significant correlation between the amounts of *T. yallundae* DNA measured at stem extension and those recorded at the end of the season ($P<0.001$).

Table 3. Correlation coefficients for eyespot fungus DNA in untreated plots 1995-1998

	T. yallundae ng DNA at GS 32	*T. acuformis* ng DNA at GS 32	Visual eyespot % index at GS 85
T. yallundae ng DNA at GS 85	0.924	-	0.148
T. acuformis ng DNA at GS 85	-	-0.068	0.153
Visual eyespot % incidence at GS 32	-0.204	-0.321	0.428

The eyespot indices and the yields from each season are presented in Table 4. At some of the treatment timings prochloraz and cyprodinil significantly reduced the level of visual eyespot and increased yield compared to the untreated controls.

Table 4. Effect of fungicide treatment on visual eyespot and yield

	1995			1996			1998		
Treat-ment	Eyespot % index GS 32	Eyespot % index GS 87	Yield t/ha	Eyespot % index GS 31	Eyespot % index GS 85	Yield t/ha	Eyespot % index GS 31	Eyespot % index GS 90	Yield t/ha
1	2.0	49.7	9.9	1.2	26.1	11.8	9.5	51.3	3.67
2	0.8	17.7	11.0	1.0	10.7	11.1	-	-	-
3	1.5	21.0	11.0	0.8	21.0	11.0	8.0	41.3	3.75
4	1.0	25.7	11.1	1.2	19.3	10.6	-	-	-
5	1.8	23.7	10.7	2.2	21.0	11.3	-	-	-
6	1.8	41.0	10.7	2.2	24.3	11.3	-	-	-
7	0.8	44.0	10.5	1.0	26.0	10.6	-	-	-
8	0.8	41.3	10.7	2.2	18.8	11.5	-	-	-
9	0.8	31.0	10.9	1.8	16.3	11.7	-	44.7	4.59
SED	0.60	5.43	0.29	0.89	6.01	0.590	2.683	8.54	0.172
d.f.	24	24	24	24	24	24	16	16	16
P	0.199	0.008	0.038	0.253	0.004	0.213	0.645	0.977	<0.001

In 1995 and 1996 the most effective treatment timing for reduction of eyespot levels with prochloraz was at early tillering (GS 22-24). Cyprodinil gave most effective control when applied at GS 32 (second node detectable). In 1995 there was a yield response to these treatments of approximately 1 t/ha. There was a smaller yield response in 1998 and no yield response in 1996.

The quantities of eyespot fungus DNA extracted at stem extension and at the end of the season for the different treatments are shown in Table 5. Amounts of DNA of both eyespot fungi were small at stem extension. In 1998, no *Tapesia* DNA was identified at stem extension. The amounts of *Tapesia* DNA extracted tended to vary widely even between replicates of the same treatment and so differences were seldom significant. Among the treated plots, however, the smallest amounts of *T. acuformis* DNA in 1995 and 1996 were recorded after the prochloraz treatment applied at early tillering (treatment 2) and after the cyprodinil treatment applied at GS 32 (treatment 9). The smallest amounts of DNA overall were recorded in the untreated samples.

Table 5. Quantities of *Tapesia* DNA (ng/mg) extracted at stem extension and at the end of the season

	1995				1996				1998			
Treatment	W GS 32	R GS 32	W GS 87	R GS 87	W GS 31	R GS 31	W GS 85	R GS 85	W GS 31	R GS 31	W GS 90	R GS 90
1	0.10	0.06	0.02	0.09	0.23	0.32	0.24	1.28	0	0	0.001	0.19
2	0.00	0.05	0.00	0.19	0.24	0.33	0.41	4.47	-	-	-	-
3	0.00	0.05	0.00	0.21	0.21	0.20	0.28	6.99	0	0	0.000	0.09
4	0.00	0.06	0.00	0.27	0.24	0.88	0.27	5.63	-	-	-	-
5	0.00	0.06	0.02	0.28	-	-	0.32	5.29	-	-	-	-
6	0.00	0.04	0.01	0.37	0.23	0.86	0.22	2.33	-	-	-	-
7	0.00	0.03	0.01	0.16	0.10	0.39	0.27	1.48	-	-	-	-
8	0.00	0.05	0.01	0.25	0.20	0.88	0.68	0.53	-	-	-	-
9	0.00	0.05	0.01	0.16	-	-	0.17	0.02	0	0	0.001	0.13
SED	0.003	0.038	0.006	0.119	0.008	0.970	0.324	5.29	-	-	0.0051	0.222
d.f.	24	24	24	24	18	18	24	24	-	-	16	16
P	0.008	0.383	0.001	0.285	0.789	0.075	0.791	0.823	-	-	0.001	0.008

W = *T. yallundae*, R = *T. acuformis*

DISCUSSION

The results show that while significant reductions in eyespot could be achieved with fungicides, the optimum timings for prochloraz and for cyprodinil differed. Both prochloraz and cyprodinil gave significant reductions in visual eyespot levels at the end of the season compared to the untreated controls but prochloraz gave the largest reductions in visual eyespot when applied at early to mid tillering and cyprodinil gave the largest reduction when applied at the end of the stem extension window, at GS 32.

Quantifying the DNA from each treatment showed that, of the fungicide treatments, those where visual eyespot was most reduced at the end of the season were also those with least

T. acuformis DNA. The untreated plots, however, had even less *Tapesia* DNA, although the visual eyespot index was significantly higher. One possible explanation for this is that in very severe lesions, as the stem base senesced, the recoverable fungal DNA also declined. Mahuku *et al.* (1995) reported a similar decline when quantifying *Leptosphaeria maculans* DNA in severe lesions on oilseed rape.

The yield response to specific eyespot fungicides was variable, emphasising the need to be able to predict which crops will give a cost-effective response to eyespot treatment. There was no significant correlation between visual eyespot levels at stem extension and those at the end of the season, so that a threshold approach to determining the need for treatment would not have been successful. The use of PCR diagnostics at stem extension did not help to predict final eyespot levels in this study. There was no correlation between amounts of *T. acuformis* DNA at GS 32 and those measured at the end of the season and this was the dominant eyespot type in all seasons. There was, however, a significant correlation between amounts of *T. yallundae* DNA at GS 32 and those measured at the end of the season. This may indicate why the threshold of 20% stems infected at stem extension was a useful guide to treating crops in the 1970s, when this fungus predominated in the UK, and why it has less value in forecasting now that *T. acuformis* is predominant eyespot.

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Stem-base diseases of wheat in Ukraine

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ABSTRACT

Stem-base diseases of winter wheat have an uneven distribution in Ukraine and occur mainly in the central and northern parts of the country. Eyespot and sharp eyespot significantly influence the productivity of the crop. Fungi isolated from diseased plants were *Pseudocercosporella herpotrichoides*, *Rhizoctonia cerealis*, *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. oxysporum*, *F. solani* and *F. sporotrichioides*. There were differences in distributions of the R-type and W-type isolates of *P. herpotrichoides*. The R-type was isolated mainly from winter wheat grown in central and northern regions, the W-type from wheat grown in southern regions. *Fusarium* species differed in their pathogenicity on inoculated wheat seedlings: *F. culmorum* and *F. graminearum* were most pathogenic. Pre-symptom diagnosis of stem-base diseases, based on the appearance of hyphae in leaf sheaths, is proposed for correct forecasting.

INTRODUCTION

Winter wheat is the most important crop in Ukraine. It is grown on about 6 million ha, which comprises 50% of the area under cereal crops and 20% of total arable land. The crop is grown in steppe (45%), forest-steppe (42%) and forest zones (13%). The potential yield of commercial cultivars is 11-12 t/ha, but only 25-40% of their productivity is realised. The main factors reducing the yield of winter wheat are inadequate nutrition and ineffective pest and disease control. Loss from diseases were estimated at 8.5% in 1997 (Seal & Baronowski, 2000).

There is an increasing demand for estimations of the economic importance of every disease. Stem-base diseases are among the most important diseases of winter wheat in Ukraine. Surveys of winter wheat during 1975-1988 showed that eyespot, sharp eyespot and brown (fusarium) foot rot all occurred on stem bases, as well as take-all, fusarium root rot and common root rot (Novokhatka *et al.*, 1990). Lesovoy & Parfenyuk (1995) reported that eyespot leads to enormous economic losses in some regions of Ukraine. Losses from fusarium foot and root rot are more controversial. Losses due to sharp eyespot have not been estimated.

Because, for a long time, stem-base diseases of cereal crops were investigated under the common name "root rot", the same measures (cultural practice and seed treatment) were used for their control. Breeding eyespot resistant cultivars is now conducted but the results are not yet successful (Lesovoy *et al.*, 1999). Chemical control by spray application of fungicides is currently proposed as most effective. Methods of early disease diagnosis are necessary in order to improve forecasting and to make fungicide use more economical.

MATERIALS AND METHODS

Field surveys were conducted during 1996-1999 on commercial winter wheat at three sites located in central (site I: research enterprise "Velykosnitynskyi"), northern (site II: collective agricultural enterprise "Baryshivskyi") and southern (site III: Mycolayiv research station) parts of the country. The first two sites represent the forest-steppe zone, the other is steppe. Samples of plants for disease assessment were collected at maturity (growth stage, GS, 73; Zadoks *et al.*, 1974). The disease assessment method depended on the disease concerned. Stems with symptoms of sharp eyespot or fusarium foot rot (affected sample) were selected at random together with the nearest apparently unaffected stems (control sample). Stems with eyespot were separated into three groups according to the scale used by Goulds & Polley (1990): 1, slight infection; 2, moderate infection; 3, severe infection. The nearest unaffected stems served as controls. The losses in yield due to eyespot and fusarium foot rot were estimated in site I where these diseases predominated. The estimation of yield loss due to sharp eyespot was made in site II, where this disease prevailed. The yield of each ear, mean number of grains per ear and 1000-grain weight were recorded.

Isolations of fungi were made to confirm that the causes of symptoms had been correctly identified. Small pieces of stem were cut off and surface-sterilised in sodium hypochlorite (5-10%) for 5 min, rinsed twice in sterilised water and placed in a moisture chamber for 2 weeks. Single-conidium isolates were obtained from the fungal growth and were subcultured on potato dextrose agar (PDA) for species identification and tests for pathogenicity. Single-conidium isolates of *P. herpotrichoides* were identified as W-type or R-type on PDA by the rates of colony growth and morphology of the margins (Creighton & Bateman, 1991). These may represent the teleomorph species *Tapesia yallundae* and *T. acuformis*, respectively.

Artificial inoculation of 10-d-old wheat seedlings (experiment 1) was made with agar culture of each fungus in small plant pots containing sterile sand. A colonised agar ring was placed over each plant and touching the coleoptile. The rings were covered with sand. In experiment 2, a ring of agar culture of a *Fusarium* sp. was placed on sterile sand and a seed was placed on top of it and covered with sand. The pots were placed in a cool growth chamber for 4 weeks, after which the plants were removed, washed and assessed for stem-base disease symptoms. A disease severity score (0-4) was used. Leaf sheaths with symptoms that were not identifiable were cut up into small pieces (1-2 cm) and placed into a mixture of 96% ethanol (74%), chloroform (25%) and trichloroacetic acid (1%) for several hours. They were then put into a solution of 0.3% Serva Brilliant Blue R250 in 99%-methanol for 5 min, rinsed in water and observed using a microscope (x150).

RESULTS

Incidence of eyespot, sharp eyespot and fusarium foot rot

In site I, eyespot, sharp eyespot and fusarium foot rot were detected annually on more than 5% but less than 60% per cent of stems (Table 1). In site II, where sharp eyespot appeared on more then 60% of stems, the wheat was severely affected by take-all (as patches). Up to 30% plants were affected by eyespot in this site. In site III, stem-base diseases were uncommon, eyespot and fusarium foot rot affecting only individual wheat plants.

Table 1. Incidence of stem-base diseases on winter wheat at three sites in Ukraine

	Sites		
Disease	I	II	III
Eyespot	M	L	S
Sharp eyespot	L	H	-
Fusarium foot rot	L	S	S

H – high (>60%), M – moderate (30-60%), L – low (5-30%), S – single (<5%).

Loss of productivity

No lodging or whiteheads associated with any type of lesions were observed. It would appear, however, that some loss can be associated with infection even in the absence of such acute effects. Moderate eyespot decreased grain weight but slight eyespot caused an increase in grain weight and number (Table 2). Fusarium foot rot did not affect yield (Table 2). Sharp eyespot slightly decreased grain weight outside take-all patches but did not affect yield inside patches (Table 3).

Table 2. The effect of eyespot and fusarium foot rot on yield of wheat (site I)

Disease	Grain weight per ear (mg)	No. grains per ear	1000-grain weight (g)
Control	541±72.1	20.5±1.64	24.5±1.50
Slight eyespot	782±82.2	26.0±1.69	29.2±1.88
Moderate eyespot	367±67.4	19.3±2.02	17.9±1.81
Fusarium foot rot	554±63.9	22.7±1.58	23.6±1.63

Characterisation of fungi

P. herpotrichoides and *R. solani* were isolated from lesions. The former was identified as R-type or W-type (Table 4). Isolates of W-type on PDA produced colonies with smooth or rough margins and colony diameters of 24.3-26.8 mm after 2 weeks. Colonies formed by R-type isolates had feathery and indented margins and diameters 11.0-17.7 mm. The W-type was isolated from wheat stems from site III (steppe zone) and the R-type from sites I and II (forest-steppe zone). Two isolates (3,7) were intermediate in appearance.

Table 3. The effect of sharp eyespot on yield of wheat (site II)

	Outside take-all patch			Inside take-all patch		
	Grain wt/ear (mg)	No. grains per ear	1000-grain wt (g)	Grain wt/ear (mg)	No. grains per ear	1000-grain wt (g)
Control	885±105.5	20.5±2.19	41.7±1.23	403±108.0	10.0±2.13	36.8±2.38
Infected	750±87.2	17.8±1.84	41.1±0.79	447±48.2	11.1±1.06	39.4±1.11

Table 4. Colony growth and margin characteristics of isolates of *Pseudocercosporella herpotrichoides*

Isolate	Site	Colony margin Score (1-4)	Colony diam. after 2 weeks (mm)	Type
1	II	1.5	13.0	R
2	II	2.7	14.2	R
3	III	3.0	14.5	?
4	III	4.0	25.5	W
5	III	4.0	26.7	W
6	III	3.3	24.3	W
7	II	4.0	14.8	?
11	II	1.0	13.3	R
12	II	2.7	11.0	R
13/3	II	1.3	15.9	R
13/4	II	2.6	13.9	R
14	II	1.0	17.7	R
15	II	1.8	11.5	R
17	II	2.0	13.9	R
18	II	2.0	15.4	R
19	II	2.3	14.3	R
20	II	2.0	13.8	R
21	II	2.3	11.4	R
22	III	4.0	26.8	W
23	I	2.0	13.5	R
24	I	2.0	12.7	R

Table 5. Species of *Fusarium* isolated from stems of winter wheat

Species	No. isolates yielded	Frequency of isolation*
F. oxysporum	43	5
F. culmorum	16	3
F. avenaceum	5	1
F. graminearum	7	2
F. sporotrichioides	10	4
F. solani	6	2

*Frequency of isolation: 1 – isolated from<10% samples, 2 – from 10-30% samples, 3 – from 30-50% samples, 4 – from 50-75% samples, 5 – from > 75% samples.

Isolates of six species of *Fusarium* were obtained from brown foot rot lesions (Table 5). *F. oxysporum* was the predominant species in all samples. About 60% of isolates of *F. oxysporum* caused disease on winter wheat seedlings after artificial inoculation (Table 6). *F. culmorum* was less common, but all isolates, as well as those of *F. graminearum*, were more pathogenic on average than *F. oxysporum* or *F. solani*. The pathogenicity of *F. solani* was equal to that of *F. oxysporum*.

Table 6. Pathogenicity of *Fusarium* isolates under artificial inoculation

	Experiment 1				Experiment 2			
	No. isolates		Severity score (0-4)		No. isolates		Severity score (0-4)	
Species	Total	Patho-genic	Coleo-ptile	Root	Total	Patho genic	Coleo-ptile	Root
F .oxysporum	4	3	0.6±0.25	0	14	8	0.2±0.14	1.3±0.43
F. culmorum	6	6	1.2±0.30	0	8	8	1.3±0.42	1.5±0.34
F. graminearum	2	2	2.6±0.25	0.2±0.15	2	2	0.5±0.50	1.8±0.75
F. solani	6	6	1.1±0.35	0.2±0.17	6	2	0.1±0.05	0.1±0.05

Early diagnosis of stem-base diseases

Pre-symptom diagnosis of stem-base diseases was attempted by microscopic examination of leaf sheaths. Hyphae were present before symptoms were distinguishable visually. There were differences in the appearance and width of the hyphae. Hyphae of *R. cerealis* were broad (2.5-8.0 µm), those of *P. herpotrichoides* were narrow (2.5-3.5 µm) and those of *Fusarium* spp. were intermediate (3.0-6.0 µm). *P. herpotrichoides* also produced distinctive infection cushions.

DISCUSSION

The field results indicated that yield reductions resulted from eyespot and sharp eyespot. There were regional differences in the presence of W-type and R-type isolates of *P. herpotrichoides*. Other papers in this session suggest that the faster-growing W-type may cause greater yield loss but it is not known if greater losses from eyespot occur in the south, where the W-type is predominant. This population difference is not associated with MBC-sensitivity since fungicide application is not common here.

After artificial inoculation, all species of *Fusarium* caused seedling disease on wheat but the species differed in their pathogenicity. The most severe symptoms were caused by *F. culmorum* and *F. graminearum*. These species are likely to be the most important causes of fusarium foot rot in Ukraine although the disease may not be important.

As fungicides become more widely used it is necessary that they be used effectively and efficiently. Microscopic examination of the hyphae on pre-symptomatic leaf sheaths may be a way of ensuring that treatments are targeted correctly. This was earlier proposed as a means of early detection of eyespot (Verreet & Hoffmann, 1990).

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Towards better understanding and management of cereal stem-base diseases

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ABSTRACT

The recent histories of actual and perceived changes in the behaviour and importance of stem-base diseases are reviewed. Some recent research aimed at understanding and explaining these changes is presented. The prospects for more rational, integrated control of stem-base diseases, based on this research, are considered.

INTRODUCTION

This paper concerns those diseases that affect stem-bases of cereals in the UK and much of continental Europe. They are: eyespot, the most important of them, caused by *Tapesia yallundae* and *T. acuformis*; sharp eyespot, caused by *Rhizoctonia cerealis*; brown (fusarium) foot rot, caused by *Fusarium* spp. and *Microdochium nivale*. The brown foot rot fungi also cause ear blight and can be seed-borne. *M. nivale* also causes snow mould, a serious disease of overwintering cereals in northern latitudes. This group as a whole therefore has complex epidemiology that presents difficulties in the choice of control measures. The paper is not concerned with *Fusarium* as a component of the root disease complex that occurs in the USA, or with crown rot, caused by *F. graminearum*, that occurs in Australia, although research on effects of straw management practices on the latter (Burgess *et al.*, 1993) is relevant to some of what follows.

Fungicides have been used very widely for about thirty years to control eyespot, rather than the minor diseases with which it is often associated. Other contributors to this session (Burnett, 2000; Nicholson & Turner, 2000) have inferred from field experiments that, in recent years, applying fungicides may often not have been cost-effective, even when a highly active fungicide such as cyprodinil was used. This paper examines, by reference to recent research, the reasons for this and the prospects for the situation continuing. It also considers recent and new research that may contribute to more cost-effective, environmentally sound disease control using an integrated approach.

IMPORTANCE AND CHANGES IN PERCEIVED SIGNIFICANCE IN THE UK

Eyespot has not always been considered by farmers to be one of the major cereal diseases in recent years despite estimates, from MAFF surveys, that the cost of yield losses in England and Wales amounted to £16 million in 1999, averaging £17 million over the preceding 10-year period (N. Hardwick, pers. comm.). Estimated losses in 2000 were the largest for 25 years and may have cost up to £22 million at current grain prices (N. Hardwick, pers. comm.). These estimates, and the history of fluctuations in the importance of eyespot, confirm that farmers' recent perception of eyespot's importance should not deter the development of new fungicides, research on alternative control practices or integration of eyespot control into overall disease management strategies. Eyespot was also considered relatively unimportant in the 1960s, mainly because of effective resistance in Cappelle-Desprez and wheat cultivars derived from it. It was maintained at low levels subsequently by fungicides, particularly the MBC group (benzimidazoles and including thiophanates), and host resistance was less important. The benzimidazoles were largely replaced by other fungicides, such as prochloraz, when MBC-resistance developed and led to increased crop losses. This was a period, in the 1980s, when eyespot was a research priority and much of the important epidemiological work was done (e.g. Goulds & Fitt, 1991). In many recent field trials, fungicides applied to control eyespot have often failed to increase yields significantly (Bateman *et al.*, 2000; Burnett, 2000; Nicholson, 2000). The small, non-significant effects on yields seen in individual trials suggest that the large total losses estimated from survey data (see above) were mainly a consequence of modest losses in many fields rather than very variable, but sometimes large, losses.

Sharp eyespot can cause losses in localised patches within crops but has never been considered as a major problem. Losses in the UK have never been great (estimated losses in England and Wales were £4 million in 1999, with a 10-year average of £3 million, N. Hardwick, pers. comm.), despite the absence of effective fungicidal control. The contribution of *Fusarium* species to stem-base disease has long been recognised, but it came to prominence in the 1990s when research proliferated as interest in eyespot was declining. Data are not available for losses but these are unlikely to be large. The main threat from *Fusarium* is ear blight, for which stem-base disease may be an important inoculum source. So far, mycotoxins that can occur in *Fusarium*-infected grain are at a low level in the UK but that situation could change if, for example, the climate changes.

EYESPOT PATHOGEN POPULATIONS AND THEIR SIGNIFICANCE

The sexual stage

The fast- and slow-growing pathogenic types of the eyespot fungus are referred to in much of the recent literature as W-type and R-type of *Pseudocercosporella herpotrichoides*. These asexual fungi are now redesignated *Ramulispora herpotrichoides* and *R. acuformis* (Robbertse *et al.*, 1995). These, in turn, have the teleomorph (sexual) stages *Tapesia yallundae* and *T. acuformis* (names that are used subsequently in this paper). *T. yallundae* was found to occur naturally on infested, overwintering stubble in the UK (Hunter, 1989) soon after its discovery in Australia. *T. acuformis* has been found only rarely in the field (Dyer *et al.*, 1994). Most of the epidemiological research on eyespot has concerned epidemics arising from conidia, asexual spores that are mostly dispersed over short distances by rain-splash. The production of air-borne ascospores adds a new dimension to the epidemiology of eyespot, especially as they develop readily in uncultivated set-aside fields, but their significance has not yet been determined. Ascospore production provides the opportunity for sexual recombination and so their dispersal has implications for transmission of undesirable traits such as fungicide resistance, as yet little investigated.

Epidemiology

Differences in the epidemiology of the two *Tapesia* spp. arise mainly from their different rates of development following infection. *T. yallundae* develops more quickly and so is often found earlier in crops (Goulds & Fitt, 1991). Weather can influence these differences: for example, it was thought that a cold winter caused loss of basal leaf sheaths that delayed development of *T. acuformis* that had not yet penetrated them (Goulds & Fitt, 1991). Differences between the species were less marked on the less susceptible host, barley, than on wheat, and on the less susceptible wheat cultivars grown in controlled environments (Bateman *et al.*, 1990b) although the latter effect was not clearly evident in the field (Bateman *et al.*, 2000).

Effects on yield

T. yallundae and *T. acuformis* are both capable of causing severe disease in wheat crops by the end of the growing season (Goulds & Fitt, 1991). However, the slower development of *T. acuformis* means that it is less likely than *T. yallundae* to become severe. In an experiment using artificial inoculum to test the effects of the two species, mean percentages of straws with moderate or severe symptoms at grain filling were larger with *T. yallundae* (64.4%) than with *T. acuformis* (50.0%). There was a negative and significant (P=0.02) relationship between grain yield and severity of eyespot (% straws with moderate or severe symptoms), the fitted regression explaining 38.5% of the variation. A simple test for coincidence and parallelism showed that the data for the two species were explained by a single regression equation, indicating that symptoms of similar severity caused by the two pathogens are similarly damaging.

Tapesia populations

During the 1980s it became apparent that populations of the eyespot fungus in the UK generally changed from being predominantly the fast-growing type (now known as *T. yallundae*) to predominantly the slow-growing type (*T. acuformis*) (King & Griffin, 1985). A recent survey suggests that *T. acuformis* still predominates in UK populations (West *et al.*, 1998). It was relatively more frequent in wheat crops in Scotland and northern England than in the south. Putative causes and significance of population changes are discussed in the following sections and are listed in Table 1.

Table 1. Putative factors affecting population changes in *Tapesia* spp.

Factor	Suggested effect	Assessment
Fungicides	MBC or DMIs select for *T. acuformis*	Not proven for MBC; good evidence for DMIs
Crop	Barley, rather than wheat, selects for *T. acuformis*	Good experimental evidence
Sowing date	Late drilling favours *T. yallundae*	Good evidence from epidemio-logical research
Weather	Various, e.g. spring frost delays *T. acuformis*	Some evidence but likely to be important only in individual crops

Effects of fungicides

Fungicides have undoubtedly been important in selecting *Tapesia* spp. The use of MBC fungicides was implicated in the dramatic change in the early 1980s to a predominance of *T. acuformis*, which occurred along with resistance to these fungicides (King & Griffin, 1985), but this was not proven experimentally (Bateman *et al.*, 1990a). Long-term experimentation suggested that this selection can occur as a result of using DMI fungicides such as prochloraz (e.g. Bateman *et al.*, 1995). These fungicides were not used against eyespot until after the population change had occurred but it is possible that their use against foliar diseases, at growth stages when application would not be expected to affect eyespot, may have affected the eyespot pathogens. Resistance to prochloraz has been found, particularly in France (Migeon *et al*., 1993) but its significance there and in the UK is unclear. Prochloraz has been applied to the same plots each year in an experiment at Rothamsted in which wheat has been grown continuously since 1984 (except for set-aside with natural regeneration in 1993/4). The pathogen population in these plots is almost entirely *T. acuformis*. Prochloraz treatment has usually resulted in less eyespot than occurred in continuously untreated plots, which have retained a significant, but minority, *T. yallundae* population (Bateman *et al*., 1995). In the 1998 crop, stems with moderate or severe eyespot in July in plots that were repeatedly untreated or treated with carbendazim, prochloraz or carbendazim + prochloraz were, respectively, 81%, 86%, 9% and 55% (SED=7.5, df=3, *P*=0.006). However, prochloraz did not decrease eyespot significantly in 1999 or 2000 although there was no evidence of resistance to prochloraz, which was tested for in the 2000 crop. Prochloraz-treated plots have often yielded most but the effects were never significant. This lack of significance may be a consequence of the pathogen population having a large proportion of the slower-developing *T. acuformis*; damage in the untreated plots may have been limited by its reaching moderate severity at a late date. This in turn suggests that the widespread use of prochloraz, whilst not always controlling eyespot, may have decreased losses and potential losses caused by eyespot by maintaining *T. acuformis* as the predominant fungus.

Effects of crop species

Increased barley growing was suggested as a possible contributory cause of the change to predominantly *T. acuformis* after 1980. It was demonstrated subsequently that barley selects for populations with greater proportions of *T. acuformis* than does wheat (Bateman & Gutteridge, 1996). The proportion of *T. acuformis* in wheat is greater in Scotland, where barley constitutes a greater proportion of the cereal acreage, than in southern England (West *et al*., 1998). This appears to be consistent with the experimental evidence although evidence from crop sequences sampled in that survey was less convincing. Where a large proportion of the cereal acreage is barley, selection for *T. acuformis* may contribute to decreased potential losses in wheat crops.

Effects of husbandry

Husbandry practices may affect the two eyespot pathogens differently because of differences in their epidemiology. Earlier sowing of winter cereals is likely to be the main factor. Greater proportions of *T. acuformis* were found in earlier drilled wheat crops (West *et al*., 1998) which allowed more time for the slow-developing R-type epidemics to become severe. A less likely explanation for the early-drilling effect is that the two pathogens have different abilities to survive in the absence of a crop. This was investigated by frequent sampling, over a year from October, of infested debris on the surface of a fallow site (Bateman & Creighton, unpublished). At the October and April samples, respectively, frequencies were: W-type, 18.1% and 6.5% of straw pieces; R-type, 32.0% and 14.8%. The similar decreases over this period suggest that neither short breaks between cereals nor set-aside would favour either pathogen. Pathogenic *Fusarium* spp. were also identified on the same material. Percentages of *F. culmorum* (31.9% and 36.8%) and *F. avenaceum* (35.4% and 32.0%) on the same respective dates demonstrate their better survival abilities.

BIOLOGICAL INTERACTIONS

Fungicides

There is often an inverse relationship between eyespot and sharp eyespot and fungicides that decrease eyespot can cause increases in sharp eyespot (Jenkyn & Prew, 1973). A strobilurin fungicide such as azoxystrobin, which is active against sharp eyespot, can prevent damage by this disease, which is likely to be small, if it is included in a stem-base treatment. Other effects of fungicides on pathogen populations, less concerned with interactions between pathogens, are discussed above.

Cultivations and straw management

Eyespot and sharp eyespot were often more severe after ploughing than after non-inversion tillage but ploughing usually decreased fusarium foot rot (Prew *et al.*, 1995). Burial of inoculum of the crown rot pathogen *F. graminearum* (occurring mainly as hyphae on stubble) caused inoculum to decrease as straw decomposed more quickly (Summerell & Burgess, 1988).

Straw management also affected eyespot and sharp eyespot, both of which were typically more severe where straw was burnt, despite depletion of inoculum sources, than where it was incorporated (Prew *et al.*, 1995). Incorporating straw decreased these diseases only with non-inversion tillage. Mixing infected stem bases with chopped straw was found to reduce conidium production (Jalaluddin & Jenkyn, 1996) but other research suggests that less inoculum is produced in burnt than in non-burnt areas (Jenkyn & Jalaluddin, unpublished). Brown foot rot tends to be increased by incorporating straw (Prew *et al.*, 1995). However, in a year with favourable conditions for foot rot caused by *F. culmorum* (i.e. warm and dry in early summer), this disease was less severe after straw incorporation than after burning, despite more propagules of the fungus in the soil after straw incorporation (Bateman *et al.*, 1998). This suggests that straw may sometimes interfere either with production or dispersal of inoculum (as conidia) or with the infection process. Inhibition of infection may occur if fungitoxic substances are produced by decomposing straw or if antagonism or competition from straw-decomposing microorganisms occurs. Phytotoxic chemicals are produced as straw decays (Harper & Lynch, 1981); although these seem usually to cause no significant damage to the crops they may affect micro-organisms. Another report showed that removal or burial of straw did not affect fusarium foot rot (Colbach *et al.*, 1996), indicating that these effects are variable. The ability of wheat straw to suppress fusarium foot rot has been demonstrated consistently in glasshouse experiments (Bateman, unpublished), which also showed that the effect tended to be greater with straw that had been allowed to begin decomposing than with fresh straw. Suppression of fusarium foot rot in the glasshouse (Bateman, unpublished) and of eyespot in the field (Jenkyn & Gutteridge, unpublished) by straw of oilseed rape or field beans has also been demonstrated.

INOCULUM SOURCES AND TARGETS FOR CONTROL

A severe outbreak of eyespot in wheat grown after ploughing following a long period of non-inversion tillage, during which buried inoculum was not expected to have survived up to its return to the surface by ploughing, provided indirect evidence for the importance of ascospore inoculum. On the other hand, the continued prevalence of *T. acuformis*, not known to produce ascospores commonly in the field, suggests that the widespread production of *T. yallundae* ascospores, particularly in set-aside fields, has had little general impact.

Infected straw is an important source of primary inoculum for stem-base diseases and straw management would therefore seem to offer prospects for contributing to their control. In particular, it may be possible to exploit the disease-suppressive properties of straw whilst managing it in such a way as to minimise its role as an inoculum source. Eyespot fungi are poor colonisers of straw but previously infected stem bases can remain effective reservoirs for infection for up to 3-years when buried (Macer, 1961). *Fusarium culmorum* is a more effective straw coloniser but evidence suggests that populations of this fungus fluctuate, building up very rapidly when conditions allow; rapid increases in soil also occur when water stress allows rapid development of stem-base disease (Bateman *et al.*, 1998). Therefore the benefits of targeting infected straw as a means of control are less clear than for eyespot. Fungicides for stem-base disease control are unlikely to provide adequate control of rapidly developing brown foot rot unless they can be applied later in the season than is normal, when only a small proportion of chemical applied using conventional spray equipment usually reaches the stem base. Later applications would have additional, perhaps greater, value in protecting ears from inoculum originating from stem bases.

Table 2. Present and prospective management options for cereal stem-base diseases

Management option	Potential value	Problems and practicality
Host resistance	Very good	Stem-base diseases not a breeding priority
Crop sequence	Moderate	Effects of break crops variable. Economics may favour intensive cereal growing
Sowing date	Good	Late sowing can reduce potential yield and often presents practical difficulties
Cultivation	Variable	Ploughing and non-inversion tillage affect different diseases differently
Fungicide	Good	Risk assessment not always reliable; over-use can lead to resistance; expensive
Straw management	Variable	Excessive straw residues on the surface can affect drilling and crop establishment
Biological control	Not known	Effective treatments not available

DISCUSSION AND CONCLUSIONS

The evidence suggests that applying fungicides to control eyespot may often have small and not significant effects in individual fields. Widespread small effects appear sometimes to explain the relatively large estimated losses on a national basis. For the individual farmer, the economic case for controlling eyespot may often, therefore, be doubtful and a fungicide that controls leaf diseases as well as eyespot might be easier to justify. Relatively small effects on yield are likely to result from the prevalence of the pathogen *T. acuformis*, which may be being sustained by the use of DMI fungicides that are less effective against this species than against *T. yallundae*. Since the effectiveness of fungicides can not be assured, additional control measures based on husbandry, that may also be effective against other, minor, stem-base diseases, should be implemented wherever possible. Crop-debris management may be a useful component of this integrated approach but further research is needed so that the effects seen in some experiments can be exploited in a consistent and effective way. Table 2 lists some current and prospective options that might be considered as components in an integrated management system for stem-base diseases.

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