SESSION 6
PATHWAYS FOR THE INTRODUCTION OF SEED TREATMENTS

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The regulation of pesticide seed treatments in the European Community and Great Britain

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ABSTRACT

Pesticides make a major contribution to pest and disease control in crops. However, before they can be approved it is necessary to show that they are safe to humans and the environment and effective for the desired purpose. The main data requirements for pesticide seed treatments within the European Community and Great Britain are outlined.

INTRODUCTION

Seed treatment pesticides make a significant contribution to disease and pest control in a range of crops. It is important that such pesticides are effective for the desired purpose and that they do not pose an unacceptable risk to human health or to the environment. In Great Britain (GB) a range of legislative and administrative controls cover the approval, storage, marketing and use of pesticides. The aim of these regulations is to protect the health of human beings, creatures and plants whilst safeguarding the environment and allowing the safe, effective and humane control of pests.

Pesticides are regulated under the Control of Pesticide Regulations, COPR (Anon. 1986) and its subsequent amendment. In addition, the EC Authorisation Directive (EEC Commission, 1991) is implemented in GB by the Plant Protection Product Regulations, PPPR (Anon. 1995) and its subsequent amendments. There is also legislation to control the level of pesticide residue in foods (Anon. 1999) and subsequent amendments.

At present there are two parallel systems for the approval of pesticides in GB. The first, under the COPR, is carried out entirely at national level. The second, under the PPPR, involves a system where the major part of the scientific evaluation is considered via harmonised procedures laid down by the European Commission. Under this latter system, active substances are assessed by a committee of Member States and, if acceptable, are entered onto a list of such substances (known as Annex I listing). Once an active substance is included on this list, in considering other applications, the individual regulatory authorities in Member States are expected to draw upon the scientific evaluation that has already been agreed. In GB there are transitional arrangements to allow COPR and PPPR to run in parallel, until all existing EC active substances are reviewed and placed on Annex I.

Sufficient data are required to allow a decision to be made on whether the active substance can be included in Annex I, and on whether any specific restrictions are required for the listing. The data and information required to support the authorisation of an active substance are laid down in the main framework EC Authorisation Directive (EEC Commission, 1991). A number of other Commission Directives provide details of the

and the requirements for testing (EC Commission 1995a). Guidance on data evaluation and decision making is given in the Uniform Principles (EC Council, 1997), which have been agreed by Member States to ensure a consistent approach. This is used, together with guidance documents produced at Commission level, in the decision making process on Annex I listing and on whether the product can be authorised in an individual Member State. Details of the procedures involved in the decision making process were given by Chaffey *et al.* 1999.

This paper focuses on the main issues that need to be addressed under the EC Directive (EEC Commission, 1991) before approval of an active substance can be given. In the UK, these are the requirements for a pesticide being considered under the PPPRs. In addition, the paper focuses on the requirements where the proposed first use is as a seed treatment. If the active substance is already approved for another use e.g. as a foliar spray, then it is possible that some of the requirements may be reduced. Since the requirements to demonstrate effectiveness and safety are extensive, this paper simply illustrates some of the main aspects that are examined in the process.

THE DATA REQUIREMENTS

Identity and Physical Chemical Properties of the active substance

Sufficient information is needed to allow the active substance to be identified with precision and to define it in terms of its specification and nature. A range of data are also required on the physical/chemical properties of the active substance. This includes information on melting point, solubility, octanol/water partition coefficient etc. These properties may be important in other parts of the assessment, e.g. the octanol/water partition coefficient is considered in relation to bioaccumulation in the environment. Refer to EC Commission, 1994a for further information.

Function

The intended use of the active substance, together with the dose and method of use needs to be described. This includes the function of the active substance e.g. fungicide, herbicide; together with its mode of action and the harmful organisms it controls. Details are also provided to allow the specification of any precautions necessary during the handling, storage and transportation of the active substance, as well as in the event of fire. Also, based on these data, measures should be proposed for use in emergency situations. Further guidance is given in EC Commission, 1994a.

Mammalian Toxicology

Sufficient data are required on the potential toxicity of the active substance, the product and any important metabolites to which humans may be exposed. This is required to allow an assessment of the risk to man associated with the handling and use of the pesticide and from residual traces remaining in food and water. It is also used to enable the hazard classification of the active substance and the identification of appropriate risk phases for packaging and to specify measures to be taken in the event of poisoning.

A range of data are required to assess potential human toxicity and these are discussed briefly. The metabolism and excretion of the active substance in mammals is examined. The acute toxicity of a single high dose of the active substance, together with the oral, dermal and inhalation exposure to the pesticide is also considered. Information is needed on the toxicity of the active substance when administered to animals over longer periods e.g. weeks. The potential of the active substance to cause cancer when administered over a lifetime is also examined, together with its potential to cause genetic damage. In addition, developmental toxicity i.e. potential to cause foetal death or malformation when administered to female animals during pregnancy, is examined. The reproductive toxicity of the active substance is also investigated by examining any effects when it is administered over two successive generations of animals over the course of their lives. The potential of the active substance and product to cause irritation and skin sensitisation is also examined. Where necessary, further tests may also be required to examine effects on particular organ systems e.g. on the nervous or immune system.

These data are used to establish the relevant acceptable daily intake (ADI) the acute reference dose (ARfD) and the acceptable operator exposure level (AOEL). The ADI is the amount of the chemical that can be consumed everyday for a lifetime in the practical certainty, on the basis of all known facts, that no harm will result. The ADI is generally derived from the lowest no observable adverse effect level (NOAEL) obtained in the toxicity studies. This is then divided by an uncertainty factor to allow for the possibility that animals may be less sensitive than humans and that individual sensitivity may vary. The ARfD relates to the amount of chemical that can be taken in one meal or on one day. It is normally derived by applying an uncertainty factor to the lowest no observable adverse effect level obtained from studies to assess the acute and developmental toxicity. The ARfD is used in conjunction with information on the likely exposure through the diet, to determine whether the risk from residues in food is acceptable (Section 5).

The AOEL is the daily exposure level that will not cause adverse effects in operators who work regularly with a pesticide over a period. Depending on the pattern of usage of the pesticide it may be necessary to derive a short term AOEL i.e. for exposure over several weeks and a long term AOEL i.e. for repeated exposure over the course of a year. The AOEL is normally derived from a short-term toxicity study or multi-generation study, together with the use of an appropriate uncertainty factor. The AOEL is used, together with information on exposure to the active substance and product, to determine whether the risk to operators is acceptable (Section 4). Further information on these requirements is given EC Commission, 1994b.

Operator Exposure

Human exposure to pesticides can occur during the course of their application or via contact with the treated crop. For example, in the case of seed treatments, operators may be exposed during application to the seed, as well as via seed handling and drilling. Information is therefore required to allow an assessment of the exposure likely to occur from the proposed use. This is used to undertake an assessment of the risk to operators and as a basis for the selection of appropriate protective measures including the use of personal protective equipment. It is also used to assess if there is a risk to bystanders and other workers.

Estimating exposure to pesticides is a complex process and affected by a range of factors e.g. the physical form of the pesticide and the extent of skin penetration. It may be necessary to undertake experiments to estimate the extent of exposure or alternatively, it may be possible to estimate it via mathematical modelling. Models have been produced for certain exposure scenarios, such as tractor mounted spraying of cereal crops. The use of pesticides as seed treatments generates a number of exposure issues specific to this method of use. In GB, a task force of companies generated data to estimate the exposure arising from the use of certain seed treatments. Non-members of this group are required to generate appropriate data to allow exposure to be estimated.

The exposure estimate generated is compared with the AOEL. If the AOEL is not exceeded, the extent of the exposure is considered acceptable and approval can be given. In some instances, acceptable operator exposure can only be achieved through the use of personal protective equipment e.g. gloves, face masks, and the necessity to use such equipment is specified on the product label. Guidance on the requirements to address the risk to operators is given in EC Commission, 1994b.

Pesticide Residues

Pesticides may pose a risk to humans through residues of the active substance or relevant metabolites occurring in food. The assessment examines whether the risk of residues is acceptable both in the short and long term. If the pesticide is acutely toxic i.e. causes toxic effects from a single dose, an estimate is made of the short term dietary exposure occurring in a single day or from a large portion of food. An estimate is also made of the long term dietary exposure of consumers by considering consumption over a prolonged period. This is based on the distribution of residues in food derived from treated crops. This is used in conjunction with data on national patterns of consumption of different foods, from surveys commissioned by the Food Standards Agency. UK guidance on undertaking estimates of dietary intake of pesticides is given in a PSD guidance document (1999b). The estimated short term dietary exposure is compared with the ARfD to determine whether or not the risk is acceptable. Similarly, the long-term dietary exposure to the pesticide is compared with the ADI. Approval is only given if the short term and long-term exposure is below the ARfD and ADI respectively. Since children and infants may be more susceptible than adults, separate calculations are also undertaken to ensure that the risk to them will be acceptable.

The following data are required to allow an assessment of the risk of any residues from seed treatments to humans. Metabolism studies are required in plants, unless it can be justified that no residues will remain in plant parts used as food or feeding stuff. Field trials may also be required to examine the level of residues resulting from the proposed use of the product. Where metabolism data indicate that translocation of residues to the consumable part of the crop does not occur, it may be possible to make a case for not requiring a supporting residues trials package. If residue trials are required but levels in the plants or plant parts are lower than the limit of determination, it may be possible to justify a reduction in the total number of trials needed. The risk from any significant residues in animal feed and in succeeding crops also needs to be considered. However, if the levels of residues are not significant, as may be the case for a seed treatment, then these data are not necessary. Proposals should also be made for the maximum residue levels (MRLs) and the residue definition. Calculations of a realistic prediction of daily intake may also be provided. Guidance on meeting the data requirements for residues is given in EC Commission 1997.

As indicated, several of these requirements are dependent on whether significant residues remain in plant parts used for human or animal consumption. This determines the extent of the data required to address the issues of residues. Applicants wishing to explore the various approaches possible may wish to consult PSD. Refer to EC Commission 1996b and c for further information.

Fate and Behaviour in the Environment

In considering the potential impact of a pesticide it is important to understand what happens in soil and water after its application. For instance, consideration needs to be given to how it degrades, and by what mechanisms. It is also important to determine where the pesticide ends up e.g. in surface or ground water and soil, and whether there are significant levels of degradation products (i.e. metabolites) which also need to be examined. These factors will affect the extent of exposure of non-target organisms, the possible contamination of ground and drinking water, as well as the potential for residues or effects in succeeding crops.

The breakdown and distribution of pesticides in the environment depends on a range of factors including the physical and chemical properties of the pesticide, as well as the climatic conditions following use. Specific data are required to allow an assessment of the environmental behaviour of the pesticide. A major objective of the assessment is to predict the environmental concentration of both the active substance and any major metabolites in soil and water, so that the extent of exposure can be quantified. The resulting exposure values are then used, for instance, in certain parts of the ecotoxicological, residues and efficacy assessments. In addition, any levels of pesticide in drinking water are determined with reference to the Council Directive on water quality (EEC Council, 1980) and any implications for human consumption are also considered.

The fate and behaviour of the pesticide in soil is examined, including the route and rate of degradation. Normally, both aerobic and anaerobic degradation together with soil photolysis are investigated. The rate of degradation is considered via laboratory studies and also, where necessary, by field studies. The relative importance of the different types of processes involved in degradation e.g. chemical and microbial degradation is also considered. Information is required on the adsorption, desorption and mobility of the pesticide in soil. Consideration of the fate and behaviour of the pesticide in water and air is also required. Again, the route and rate of degradation in both the aquatic system and air is examined together with the relative importance of the different degradation processes. In addition, based on the chemical composition of residues occurring in soil, water and air, a proposal is submitted for the definition of the residue. Finally, where monitoring data are available on the fate and behaviour of the active substance, these are examined. For further information refer to EC Commission, 1995b and 1996b.

Ecotoxicology

An assessment is needed of the potential risk of the pesticide to non-target organisms i.e. those which may be affected by its use, but which are not being deliberately targeted. Information is required to allow an evaluation and assessment of the acute and long-term risk to birds. A similar assessment is also undertaken to consider the risk to non-target mammals. This analysis makes use of the data submitted for the human mammalian toxicology assessment. It is also necessary to address the risk to aquatic organisms including fish,

aquatic invertebrates and algae. Again, both the acute and long-term (chronic) risk is assessed. The effect of the pesticide on bees as well as other non-target arthropods is also considered. Information is also required to address effects on earthworms as well as other soil macro and micro-organisms. Effects on other non-target organisms (flora and fauna) are also examined. The extent of the data required will depend on the potential risk identified.

The ecotoxicological risk assessment is based on a comparison between the toxicological effects data and the extent of exposure. Council Directive 97/57/EC (Anon 1997) sets out 'trigger' values for the acceptability of risk, based on the initial laboratory tests outlined above. Where a potential risk is identified using these 'trigger' values, it does not necessarily mean that the risk is unacceptable. Instead, further data may be required, using more refined testing methods, to allow an examination of whether there is an unacceptable impact under field conditions. For instance, if a potential risk to birds is identified, an avoidance study may provide additional useful information for use in the risk assessment. Risk management measures may also be used to reduce the risk e.g. labelling may be added that treated seed should not be broadcast. The ecotoxicological information provided is also used to evaluate the hazard classification of the active substance and to specify appropriate labelling on packaging. Refer to EC Commission, 1996a for further information on the requirements for ecotoxicology.

For seed treatments, the risk to birds and mammals may be a key issue. This is because certain species may use treated seed as a food source. It is therefore important that this risk is appropriately addressed and that it is shown to be acceptable before approval is given. In GB, labelling procedures for seed bags have been developed to help manage the risk to birds (PSD, 1998).

Efficacy and physical/mechanical requirements

In order to justify any potential risk from the use of a pesticide, it is necessary to demonstrate that it is effective for the desired purpose, and safe to both the treated and succeeding crops. So evidence is required to support the proposed claims made for each pest/disease on the product label. These data are usually obtained from field trials, but may be supplemented by data from artificially inoculated plots. The results of any preliminary tests e.g. to assess biological activity, should be submitted. Information is required on the possible occurrence and development of resistance to the active substance. Where the development of resistance is considered likely, baseline data must be generated on the sensitivity of the population to the harmful organism. In addition, an appropriate resistance management strategy is needed.

Evidence is required to show that the seed treatment does not adversely affect yield or quality. It is also important that treated seed can still germinate and emerge satisfactorily. Assessments of crop vigour and any phytotoxic effects in the treated crop are required. Evidence is also required to demonstrate safety to succeeding crops. To address this issue it may be possible to use information from the environmental fate and behaviour studies, together with crop screening studies. Finally, where treated seed is to be stored, evidence is required to show that effectiveness is retained and that the seed can still germinate and emerge satisfactorily.

The physical and mechanical data requirements for seed treatments cover two distinct areas. The first is the need to demonstrate the satisfactory retention of the chemical and the physical

properties of the products in its container after storage. Guidance on these requirements is given in a PSD guidance document (PSD, 1999a). Secondly, there is the need to show the ability to achieve the correct loading of the seed treatment onto the seed and to maintain this to the point of sowing. To demonstrate the achievement of these parameters, information from loading, distribution and adhesion studies are required. The satisfactory flow of treated seed through drill machinery also needs to be addressed. Guidance on meeting the requirements for seed treatment products in GB is given in Slawson & Gillespie, 1994 and an updated version is available on PSD's website (www.pesticides.gov.uk). Separate guidance is also available on the treatment of plant propagules i.e. tubers, bulbs etc. Refer to EEC Commission 1993 and EC Commission 1994a respectively for information on the efficacy and physical/mechanical requirements for pesticides.

CONCLUSION

The EC process is based on achieving a harmonised approach to the evaluation and authorisation of plant protection products. Considerable work has already been under taken to achieve this. This includes the development of data evaluation criteria (the Uniform Principles) and guidance to standardise the preparation of dossiers and monographs. In addition, a range of specialist guidance documents have been produced to assist in the interpretation and assessment of data. The Computer Aided Dossier and Data Supply (CADDY) project enables dossiers to be provided in a suitable electronic format, easing handling and increasing the accessibility of the large amount of information required, as well as assisting in archiving. The ECCO (European Commission Co-ordination) peer review process has also provided a basis for increased co-operation and confidence building between Member States. These steps have allowed a high degree of technical harmonisation to be achieved. Work is now focusing on speeding up the process of decision making on Annex I listing and guidelines on the criteria for listing are being developed to facilitate this.

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Filmcoating the seed of leek with fipronil to control onion thrips, onion fly and leek moth

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ABSTRACT

Research was conducted on the effect of seed filmcoating of winter leek (*Allium porrum* L.) with fipronil and some other insecticides on onion thrips, onion fly and leek moth. Trials were carried out in 1994, 1995 and 1996. Seeds film-coated with fipronil and imidacloprid showed effective control of thrips on the seedbed for twelve weeks and three weeks after transplanting. Diflubenzuron and methiocarb were not effective. Film-coating the seeds with fipronil, diflubenzuron, imidacloprid and teflubenzuron gave acceptable control of the larvae of the onion fly, whereas coating with benfuracarb and methiocarb was only moderately effective. The use of fipronil and imidacloprid film-coated seeds, resulted in sufficient protection against the leek moth, at low populations densities. The most effective insecticide, fipronil, was not phytotoxic.

INTRODUCTION

Increasing costs and increasing awareness regarding the environmental impact of insecticides have necessitated more economical and efficient application of insecticides. By applying insecticides as a filmcoating to the seed: cabbage root fly (*Delia radicum*), carrot root fly (*Psila rosae* F.) and the lettuce aphids can be controlled with reduced insecticide input (Ester & Brantjes, 1999). The seedcoat application corresponds well with the goals of the Multi-Year Crop Protection Plan of the Dutch Government concerning the periods 1990-1995 and 1995-2000 (Anonymous, 1990). The most important pests in leek in Western and Southern Europe are the onion thrips (*Thrips tabaci*), the onion fly (*Delia antiqua*) and the leek moth (*Acrolepiopsis assectella*) (Crüger & Hommes, 1990). Leek is grown year-round, including in winter, so host plants of the thrips are continually present.

'Mundial' is a fipronil-formulation specific developed for seed treatment with film-coating techniques. Film-coating gives seed treatment new and challenging possibilities: extending the activity, prevent burst-release, enhance selectivity, all of them offering growers new innovative ways of controlling pests: it will offer excellent control of root maggots: onion fly (*Delia antiqua*) in leek and onion, cabbage fly (*Delia radicum*) in cabbage and bean fly (*Delia platura*) in beans. In leek and onion, *Thrips tabaci* will be controlled up to 3 months after sowing, providing a healthy start for these crops with less aerial sprays.

This formulation of fipronil will also be developed in leeks, onions, cabbage and beans. As an example the development of fipronil as a Seed Treatment in leek will be presented.

Onion thrips

The onion thrips is a polyphagous, highly mobile insect and has high reproductive rates. Therefore, high population densities of onion thrips exist during extended periods of the growth cycle of leeks, which are grown in The Netherlands in all seasons, including winter. (Ester et al. 1997). The changes in leaf appearance decreases quality and thus causes economic damage (Crüger & Hommes, 1990). Especially in warm summers, heavy infestations can be difficult to control and spraying of insecticides is only partially effective (v.d. Steene, 1999). Eggs within leaf tissue and pupae in the soil and leaf litter are protected from most sprays (Lewis, 1973). The immature thrips may be well hidden between the inner leaves of the leek plant, which are folded over each other, so protecting them from sprays (Städler, 1995).

Onion fly

The onion fly is mainly a problem in the leek seedbeds (nursery) where plants are raised in high densities before being transplanted to the field about 12 weeks after sowing. The damage the larvae inflict to the based part of the stem can kill the plant. Also neighbouring plants are readily attacked, and this results in patches of collapsed plants. (Ester, 1999). The larvae pupate in the soil only. The onion fly is not a significant problem in the field because leek plants are widely spaced, this by contrast to an onion crop (Loosjes, 1976). However, when infected leek plants are transported into the production field, such plants become very susceptible to several fungal and bacterial diseases, such as *Erwinia*.

Leek moth

Considerable damage in terms of loss of quality can be caused by the leek moth. The eggs of the leek moth are deposited on the inner surface of the leaf, preferably in the top of the shaft. The young larvae feed mainly as miners within the leaf. Older larvae feed on the complete leaf, causing oval holes in the leaves which are readily recognised in the field as older leek moth feeding symptoms. We aimed at achieving complete protection of the leek plants against the onion fly, onion thrips and leek moth for 12 weeks, plus several weeks of protection against thrips and leek moth after transplanting, by applying an insecticide as a filmcoating on seeds (Ester, 2000).

MATERIALS AND METHODS

Fipronil: an active ingredient for seed treatments

Fipronil belongs to a new class of insecticides known as phenylpyrazoles. It has an effect on the Central Nervous System, where it interferes with the passage of chloride ions through the gamma-aminobutryc acid (GABA) regulated chloride channel, thereby disrupting CNS activity and, at sufficient doses, causing death. Target site specificity between insects and mammals provides useful selective toxicity.
Fipronil is active against a large range of agricultural pests: flies, thrips, soil insects, locusts, weevils etc. (Hymenoptera and Diptera spp.) Fipronil is known for its unique selectivity for plants and seeds: Unlike some other compounds fipronil-treated seeds can be stored and sown after a certain time (crop specific) without unacceptable germination-problems.

Seed treatments

In 1994 seeds of the cultivar Vrizo were used with a thousand kernel weight (tkw) of 2.36 g. In 1995 and 1996 seeds of the cultivar Farinto were used with a tkw of 2.80 g and 2.6 g, respectively. Seeds were film-coated by SUET (Saat-und Erntetechnik, Eschwege, Germany) using a dust-free polymer product. To obtain the same amount of insecticide per seed, rates are expressed per unit of seed, with one unit equalling 250,000 seeds (Table 1). All insecticide treatments also included a seed treatment with the fungicide thiram at a rate of 0.75 g (1994, 1995) or 0.65 g (1996) a.i. per unit of seed. The 'untreated' seeds were film-coated with fungicide only. The insecticides used are mentioned in table 1.

Table 1. Insecticides and rates (g a.i. per unit of seed) used in leek filmcoatings.

Insecticide	Formulation	1994	1995	1996
Benfuracarb	40 WP	-	20	-
Diflubenzuron	25 WP	25	-	-
		37.5	-	-
Fipronil (EXP 80415A)	FS 500	-	37.5	-
		50	50	50
		75	-	-
Imidacloprid	70% WS	42	-	-
		56	56	56
Methiocarb	500 FS	50	-	-
		75	-	-
Teflubenzuron	150 g/l	-	18	-
		-	27	-

Efficacy trials, at the seedbed

The trials were carried out in 1994, 1995 and 1996 at two locations (Breda and Meterik) but in 1995 also at eight additional locations in the main leek-growing areas in the southern part of The Netherlands. All sites had a sandy soil type. Because of the intensive leek cultivation all year round in these areas, onion thrips and onion fly could be expected. Thrips control is insufficient in practice, as they continually return to these areas. The experimental layout was in randomised blocks with four replicates. Data were analysed using analysis of variance (ANOVA) in Genstat 5. Means, least significant differences (LSD) and F – probabilities are presented. LSDs are calculated with Student's t distribution. The seeds were sown with a Miniair sowing machine as used by Dutch growers of winter leek. Plots consisted of eight rows (20 cm between rows) of 4 – 5 m length (1.7 cm between seeds). Sowing periods were mid – April in 1994 and 1996 and end of April in 1995.

Efficacy trial, at the production field

The treatments were randomised within five replicates, with plots of 3.40 x 6 m – an area of 20.4 m^2 each. Plots were six rows wide (50 cm between rows) and 6 m long (12.5 cm between plants). The soil was a sandy. The plant depth is 18 cm corresponding to growers' practice. The peg plants were sown at a row spacing of 20 cm and 1.7 cm between the seeds

in the seedbed nursery). The seeds were sown mid-April and transplanted mid-July (thirteen weeks after sowing).

EFFICACY ASSESSMENT

Onion thrips

Crop damage caused by thrips was assessed in mid-July, mid-August and end August, and early September by removing 12 plants at random from each plot. The plants were scrutinised visually leaf by leaf, and both larvae and adults were counted per plant. The damage caused by thrips was estimated for each plant using the following classification: Class 1, no symptoms; Class 3, slightly damaged plant, marketable product; Class 5, moderately damaged plants. Parts of the leaves with many symptoms, unmarketable product; Class 7, severely damaged plants, all the leaves with many symptoms; Class 9, very severe damaged plants, total plant grey.

Onion fly, leek moth

Damage to crops from onion fly attack was assessed by recording the percentage of plants per field found collapsed between seven and thirteen weeks after sowing. Damage to the crop caused by the larvae of the leek moth was assessed at 4 and 12 July 1995 by recording crop stand in a scale from 1 to 10, with scale 1 indicating a completely destroyed crop and score 10 representing an excellent and uniform crop without damage of the leek moth.

RESULTS

Table 2. Average number of immature *Thrips tabaci* and adults per leek plant 10, 16 and 19 weeks after drilling the film-coated seeds at Breda in 1995.

	g a.i. per	10		16		19	
Insecticide	unit of seed	immature	adults	immature	adults	immature	adults
Untreated	0	1.2	0.8	53	8.3	34	6.1
Fipronil	37.5	0.2	0.0	12	5.3	21	3.1
Fipronil	50	0.0	0.0	4	2.0	6	2.1
Imidacloprid	56	0.2	0.3	19	5.6	12	3.5
LSD ($\alpha = 0.05$)		0.54	0.2	26.3	ns	30.1	ns

Fipronil at 50 g a.i. per unit of seed gave excellent protection of leeks against the immature thrips for at least 16 weeks after sowing covering the period July-September (Table 2). Fipronil at a rate of 37.5 g and imidacloprid at 56 g a.i. per unit of seed were less effective than the higher fipronil rate but differences were not significant. None of the insecticides sufficiently controlled adult thrips.

In the eight field trials, almost no thrips could be found on plants from fipronil-coated seeds 11 weeks after sowing. (Table 3). Even at 21 weeks after sowing, these plants were less damaged and contained fewer immature and adult thrips compared with untreated plants.

However, at that time, the damage index was higher than the economic threshold level for marketable plants (Class 3).

Table 3. Damage index caused by thrips and average number of *Thrips tabaci* per leek plant; 11, 18 and 21 weeks after drilling the seeds (average of eight field trials, 1995).

Insecticide	g a.i. per unit of seed	11		18		21	
		Index	No.	Index	No.	Index	No.
Untreated (- coat)	0.0	1.7	1.4	5.6	20	5.6	24
Untreated (+ coat)	0.0	1.8	2.3	6.0	30	5.5	22
Fipronil	37.5	1.0	0.1	2.9	10	4.0	19
Fipronil	50	1.0	0.1	2.6	8	3.6	14
LSD ($\alpha = 0.05$)		0.4	1.6	0.8	9.3	0.5	6.3

Table 4. Average number of immature *Thrips tabaci* per plant and the damage index caused by thrips 1, 2 and 3 weeks after transplanting in 1996.

Insecticides	g a.i. per unit of seed	1		2		3	
		No.	Index	No.	Index	No.	Index
Untreated	0	9.9	5.0	17.	5.9	18.	4.8
Fipronil	50	0.4	1.4	5.	2.6	7.	2.5
Imidacloprid	56	0.4	1.7	6.	3.2	12.	3.5
LSD ($\alpha = 0.05$)		4.1	0.9	8.	1.34	6.	3.6

In 1996, until three weeks after transplanting a significant ($P<1\%$) control of thrips is shown after seed treatments with fipronil and imidacloprid (Table 4). Both seed treatments resulted in a lower damage index than the plants of the untreated seeds. Four weeks after transplanting, seed treatments still showed a significant reduction in damage index. However, this index was increasing and the plant quality moved to quality class II.

Table 5. Efficacy of the insecticides applied as a filmcoating to control leek moth. Crop damage caused by the leek moth. Crop stand (score 1-10) 10 and 11 weeks after sowing, 1995.

Insecticides	g a.i. per unit of seed	4 July	12 July
Untreated	0	5.2	3.3
Benfuracarb	20	6.6	3.3
Fipronil	37.5	7.1	5.3
	50	7.9	5.5
Imidacloprid	56	8.5	8.8
Teflubenzuron	18	6.9	4.0
	27	5.9	3.0
LSD ($\alpha = 0.05$)		1.17	0.87

We concluded that if there is a high population of the leek moth, plants from seed treated with fipronil and imidacloprid are protected. But a spray of a pyrethroid (deltamethrin) is

ultimately necessary in the longer terms (Table 5). On 12 July, imidacloprid showed a significant higher score of plant stand in comparison to the fipronil seed treatment

Table 6. Efficacy of the insecticides applied as a film-coat for controlling onion fly in leek crops. Percentages of damaged plants on the seed bed 13 weeks after drilling in 1994 and 10 weeks after drilling in 1995 at Breda.

Insecticide	g a.i. per unit of seed	1994	1995
Untreated	0	9.8	23.6
Benfuracarb	20	-	5.2
Diflubenzuron	25	1.0	-
	37.5	0.7	-
Fipronil	37.5	-	0.0
	50	0.2	0.0
	75	0.2	-
Imidacloprid	42	0.8	-
	56	0.4	0.0
Methiocarb	50	2.7	-
	75	1.8	-
Teflubenzuron	18	-	0.0
	27	-	0.0
LSD ($\alpha = 0.05$)		2.9	5.3

remarks: - = not tested

Coating the seed with insecticides reduced ($p = 0.05$) onion fly damage when compared to the untreated control (Table 5). However, methiocarb and benfuracarb did not provide sufficient crop protection.

Table 7. Laboratory germination and field emergence of seeds coated with fipronil or imidacloprid in 1995.

Insecticide	g a.i. per unit of seed	% normal germination in sand days after sowing			% field emergence weeks after sowing	
		12	16	21	3	7
Untreated	0	54.8	84.8	89.5	77.8	82.0
Fipronil	37.5	47.0	83.3	91.5	74.6	78.6
Fipronil	50.0	38.0	82.3	89.0	77.5	83.1
Imidacloprid	56.0	35.3	78.0	86.0	58.5	71.6
LSD ($\alpha = 0.05$)		11.8	6.2	5.6	6.4	4.0

In the laboratory test, germination was slower when the seeds were film-coated with imidacloprid or the highest rate of fipronil (Table 7). After 16 days, imidacloprid-coated seeds showed a significant lower percentage of normal germination, but final germination counts after 21 days were not significantly different. Fipronil at either rate did not affect emergence in the field, but imidacloprid at 56 g per unit of seed delayed and reduced emergence compared with the untreated control.

DISCUSSION

In 1995, thrips protection provided by the 37.5 gram was insufficient, but 50 gram gave good protection for at least 18 weeks. Field experiments with fipronil 50 g a.i. filmcoated leek seeds were sown at 1 cm (seed bed) and at 4 cm (direct drilling) seed distance. On plants sown at 4 cm seed distance significant ($p<.001$) more thrips were present in comparison to the plants at 1 cm (Ester & Evenhuis, 1998). Theunissen and Legutowska (1994) did not find any influence of plant size at different levels of thrips infestation as found at 1 cm and 4 cm.
Thrips tabaci was the dominant thrips species (99%) at the locations used in the field trials. The remaining species of thrips were *Anaphothrips obscurus*, *Antinothrips rufus*, *Frankliniella tenuicornis* and *Limothrips cerealium* (Vierbergen & Ester, 2000). During the study of thrips a minimum of predators were observed in leek crops (Vierbergen & Ester, 2000), so the observed differences regarding thrips populations are a consequence of the fipronil seedcoating.

Our results on onion fly demonstrate that coating seed with the insecticides diflubenzuron at a rate of 25 gram, teflubenzuron at a rate of 18 gram, imidacloprid at a rate of 42 gram and fipronil at a rate of 42 gram per unit of seed resulted in sufficient control of the onion fly in leek crops (Table 6). However, of the compounds tested, benfuracarb and methiocarb failed to give sufficient protection in several trials. The results with benfuracarb contradict to those recorded in 1991 and 1992 (Ester & de Vogel, 1994) and suggest that carbamate insecticides cannot always be relied upon to give sufficient protection against the onion fly in leek crops. Benfuracarb has been used to treat onion seeds for many years, so it is possible that the onion fly has built up resistance to this compound.

Infestation by the leek moth took place mainly in large plants (summer and autumn-leek) during June and July. Later in the season leek moth infestations were at a very low level corresponding to the observations of Theunissen & Legutowska, 1994. Using fipronil-coated seeds resulted in sufficient protection against leek moth, at low population densities. However, when the attack is severe an additional treatment is needed.

In winter leek, the seeds coated with fipronil were sown in April and protected the plants in the seedbed for twelve weeks and three weeks after transplanting. When the filmcoated seeds were drilled directly in the production field the protection appeared to be several weeks more (Table 2 and 4) longer. Based on numbers of thrips per plant at different times after sowing, it is suggested that the build-up of the thrips population was delayed when fipronil filmcoatings were used (Ester & Huiting, 2000). To keep the thrips population at an acceptable low level it is estimated that additional sprayings may be needed approximately 15 weeks after sowing. Thus, the number of sprayings is less when insecticide-coated seeds are used. For the additional spray applications against thrips a supervised control system is being developed (Villeneuve et al., 1996).

We recommend applying fipronil at a rate of 50 gram a.i. per unit of seed as filmcoating to control onion fly, onion thrips and leek moth in leek. Fipronil will be available in spring 2001 for use as a leek seedcoating in Belgium.

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Requirements for effective seed sampling in the application of treatment according to need strategies

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ABSTRACT

New diagnostic techniques in seed health testing can improve sensitivity, specificity and sample processing times which in turn allows a greater opportunity to target seed treatments effectively. However, these advantages are of little benefit unless the sample submitted adequately represents the bulk from which it was taken. Little is known about the distribution of seed-borne diseases of wheat within bulks, but this information is critical to the development of sampling strategies. Primary samples were taken from seed bulks and tested for the presence of *Microdochium nivale*. In the majority of bulks, infection appeared to be evenly distributed, but in others more heterogeneity occurred, and a higher sample number was required to ensure a correct treatment decision. Tests for *Tilletia caries* from a bulk where a high degree of heterogeneity had been deliberately created indicated that 40 samples on a 28 t bulk were necessary to detect a small volume of severely contaminated seed. The implications of this work on the management of these seed-borne pathogens by treating according to the health status of the seed are discussed.

INTRODUCTION

The development of rapid, sensitive and highly specific seed health testing techniques using immunological or DNA-based methodologies has increased greatly over the past five to ten years, and new tests continue to become available. The techniques offer advantages in many situations. Firstly, in cases where low initial levels of seed-borne disease can result in serious field epidemics, it is often not possible to detect relevant amounts of disease with conventional methods. Large numbers of seeds may have to be plated on agar, which renders the test impracticable or very costly. Secondly, disease complexes on seed can be composed of closely related organisms, only some of which may be serious pathogens, and a high level of skill is required to produce the correct identification. Finally, in situations where large numbers of seed lots have to be handled in a short period between harvest and the next planting, a rapid throughput test with high batch capacity is neccessary to avoid unacceptable delays to the supply of seed.

The disadvantages of conventional health tests in such situations are often cited as one of the main reasons for routine prophylactic use of seed treatments. However, both the cost of seed treatment, and continuing policy requirements for pesticide reduction, could increase the usage of new techniques and encourage management of seed-borne diseases through health

testing. If this proves to be the case, it is essential that samples submitted for tests are representative of the seed bulk from which it is derived.

In winter wheat, the two major seed-borne disease problems are seedling blight (*Microdochium nivale*) and bunt (*Tilletia caries*). Though *M. nivale* can reach high levels in wet seasons, it is frequently at much lower levels or absent in drier years. The incidence of bunt can be high, though the severity of infection is usually very low (Cockerell & Rennie, 1995). Low infection levels offer the opportunity to reduce seed treatment inputs, and provided new diagnostic techniques can deliver health test results within an acceptable time scale, seed treatments on this crop could become more effectively targeted. However, seed sampling methods are reliable enough to ensure that a test result based on a small submitted sample accurately reflects the disease risk in the seed bulk, and allow a decision not to treat to be taken with confidence.

This paper presents some results on sampling strategies for detecting *M. nivale* and bunt in wheat seed bulks, and discusses these in relation to the practical considerations of sampling wheat seed for health testing.

MATERIALS AND METHODS

Seed health tests

Tests for *M. nivale* were carried out by plating 200 seeds on potato dextrose agar, and incubating at 22 °C for 5 days under 12h uv, 12h dark. Results were expressed as % of seeds infected. Bunt tests were performed by shaking seed samples in water plus a wetting agent for 3 minutes, filtering the washing liquid under pressure, and counting the bunt spores collected on cellulose nitrate filter paper circles at a magnification of x 200. Results were expressed as spores per seed. Seed samples were prepared according to standard protocols specified by the Official Seed Testing Station, Cambridge, and included steps to minimise the risk of cross contamination between samples.

Seed sampling

Seed samples for *M. nivale* tests were taken from various wheat seed bulks harvested from the NIAB trial ground and a local seed producer in 1999 with a single chamber Neate stick sampler, capable of reaching a depth of about 3 m. All areas of the bulks were sampled at random, taking seed from different depths, until 40 primary samples were obtained.

Bunt heterogeneity trial

Untreated seed of cv Consort was mixed with spores of bunt at a rate of 2 g/kg. The contaminated seed was sown in a 1 m x 80 m strip at a rate of 210 kg/ha on 19 October 1999. The remainder of the field was sown with cv Consort treated with Sibutol. At harvest, a 0.5 m width of the strip was combined, followed by the rest of the field. Full trailers were emptied into a walled bay, and further grain from non-bunted crops added to give a bulk of about 28 t covering an area 6 m long, 4 m wide and about 1.5 m deep. The seed was then sampled at different depths (top, middle and base) covering all parts of the bulk using a Neate single

chamber stick sampler, with each sample consisting of three extracts from the same point in the bulk. A total of 40 separate samples were prepared.

RESULTS

M. nivale sampling

Details of cultivar, bulk size, and mean % infection with *M. nivale* derived from the 40 primary samples are shown in Table 1. Results of % infection with *M. nivale* are summarised in Table 2 as the number of samples in specific infection ranges.

Table 1. Cultivars and bulk sizes (t) sampled for *M. nivale* tests in 1999.

Bulk number	Cultivar	Estimated bulk size (t)	Mean % infection	± SEM
1	Shamrock	200	2.3	0.29
2	Abbott	800	1.9	0.19
3	Rialto	250	1.8	0.24
4	Consort	400	0.7	0.16
5	Chaucer	250	10.3	0.49
6	Equinox	30	2.7	0.23
7	Equinox	30	6.4	0.67

Table 2. Distribution of primary sample results with *M. nivale* in seven different wheat seed bulks.

Bulk number	Infection level		
	0-5%	6-10%	11-25%
1	40	0	0
2	39	1	0
3	38	2	0
4	40	0	0
5	2	21	17
6	39	1	0
7	15	19	6

In five of the seven bulks tested infection appeared very uniform, but in two bulks, primary sample results were distributed over all three infection level categories.

Bunt heterogeneity trial

Approximately 60% of ears contained bunt balls in the inoculated strip area, and an estimated 30 kg of grain and bunt balls was harvested from it. Numbers of samples from the 28 t bulk with different infection levels are shown in Table 3. Of the primary samples, 55% would have indicated that there was no serious bunt problem, whereas the mean of all samples was 96 spores per seed.

Table 3. Distribution of spores/seed results in 40 primary samples of wheat from a bulk with heterogeneous bunt infection.

Spores per seed	Number of samples
<1	14
1-3	8
4-10	7
11-100	8
>100	3

DISCUSSION

M. nivale sampling

The current advisory threshold for treating seed with *M. nivale* infection is 5% (Cockerell, 1995). Above this, losses in plant population may occur. Higher levels may be acceptable in cases where early sowing into a good seed bed is expected, though no safe higher limit for sowing untreated is yet defined. At levels above 35%, some products may be less effective in late sowing situations, and at very high levels it is probably advisable not to use seed. The accurate identification of these threshold levels is thus essential for effective management of this disease.

In the majority of wheat seed bulks tested, the distribution of *M. nivale* appeared to be uniform and a treatment decision would have been the same based on less than ten samples or 40 samples. Uniformity did not depend on bulk size, though some were very large, and it is possible that areas of variation within them were undetected, though it seems unlikely that serious losses would have resulted from a decision not to treat. However, in two bulks, a greater degree of variation was detected. In one of these, about one third of the primary samples were below the 5% treatment threshold level, but the mean of all samples indicated that the bulk should receive treatment. Combinations of 35 primary results would reliably predict that treatment was needed. In the other heterogeneous bulk, 95% of primary samples were above the treatment threshold, but below the level at which treatment choice or sowing date becomes relevant, and again fewer than ten primary samples would have predicted the correct treatment decision.

Bunt heterogeneity trial

Current advice on bunt control is always to use treatment when levels exceed one spore per seed. Slightly higher thresholds have sometimes been used, particularly if the seed is being sown early under conditions when rapid emergence is expected, and seedlings escape infection. Though there are some differences in product efficacy against bunt, there is usually no need for an upper threshold at which product choice might change. Lots with more than one hundred spores per seed should be avoided (Paveley *et al.*, 1996).

In the bunted wheat bulk, only a relatively small amount of seed contained bunt balls, but seed significantly infected by the drifting spore cloud at harvest probably amounted to about 1 t which could have drilled an area of at least 4 ha, leading to a serious disease problem. However, many of the primary samples indicated that no treatment would be necessary and nearly all of the 40 samples taken were necessary to ensure that the correct treatment decision was made. Though the variability was created by adding non-bunted wheat to an infected bulk, this probably represents the worst case field situation where small areas of seed within a crop become infected by a spore cloud from a neighbouring crop while the rest of the field remains healthy. Severe infection pockets are therefore more likely to occur than a uniform infection throughout a seed bulk.

Implications of sampling wheat seed for health tests

If improved targeting of seed treatments on wheat is to be achieved without increasing disease risks from seed-borne diseases, robust sampling guidelines will be needed. At present, there is no information on the minimum number of primary samples for a specific bulk size which would give a reliable result on which to base a treatment decision. The Cereal Seed Certification Regulations (Anon., 1993) specify a maximum lot size of 25 t, and one primary sample for every 700 kg of seed. However, this sampling frequency has not been extensively tested when disease is the measured characteristic.

Scheel (1997) described the situation in the Nordic countries where treatment according to need strategies on cereals are being pursued, and outlined the difficulties of attempting to take samples after seed has been cleaned and divided into lots. Since most processors tend not to have much storage capacity, or keep seed in bins which are difficult to sample effectively, on-farm sampling was thought to be the most practical option. A similar situation exists in the UK. However, on-farm bulks can be very large, and impossible to sample adequately. Rennie *et al.*, (1993) showed a barley seed lot gave higher levels of leaf stripe (*Pyrenophora graminea*) in the field than the seed test predicted, and this was attributed to inadequate sampling of the bulk from which the seed lot was originally derived. Minimum bulk sizes which can be properly sampled still need to be defined. In Sweden, a 70 t limit is used (Sperlingsson, personal communication), and in further work in the UK during harvest 2000, bulk sizes of about 100 t were used when sampling seed intended for certification. Bulks intended for farm-saving are usually quite small, and here adequate sampling should be relatively easy to achieve.

The results presented here suggest that infection with *M. nivale* in seed bulks was usually quite uniform, and where more variation was detected in a 30 t bulk, a primary sampling frequency similar to that specified in seeds regulations was sufficient to ensure that a correct treatment decision was made. The epidemiology of *M. nivale* is perhaps more likely to lead to

relatively uniform field infection rather than foci of severe infection, in contrast to bunt, where pockets of infection could occur. However, the results presented here showed that a severe bunt infection could be detected in a 28 t bulk using a frequency of one primary sample for every 700 kg.

Further work during harvest 2000 has involved taking multiple primary samples from farm-saved seed where bunt has occurred as well as samples from certified seed where there has been no evidence of the disease. In addition, bulks where high levels of infection with *M. nivale* might be expected have been targeted in order to confirm whether or not the relative uniformity of this disease occurs over a wide infection range. The results will be used in the preparation of sampling guidelines for processors and growers.

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New generation seed treatment products for canola (*Brassica napus*, *B. campestris*) and mustard (*Sinapis alba*, *Brassica juncea*)

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ABSTRACT

Novartis Crop Protection Canada Inc. has developed and registered two water-based seed treatment products for canola and mustard. Both products are pre-formulated combinations of three fungicides and a novel neonicotinoid insecticide. The fungicides (difenoconazole, fludioxonil and metalaxyl-M) provide broad-spectrum control of *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp. and *Leptosphaeria* spp. The insecticide (thiamethoxam) provides systemic protection of flea beetles (*Phyllotreta* spp. *Psylliodes* spp.) under a wide range of growing conditions.

Trials also indicate that treated seedlings have a unique vigor effect which leads to rapid, healthy stand establishment, robust early season plant growth, earlier flowering and maximized yield potential. Both products are reduced-rate, environmentally friendly products. The formulations were developed in cooperation with the seed industry to be an easy-to-handle, odourless, quick drying, and low dust-off products. Testing has indicated that both products work well under a wide range of treatment conditions and do not require the addition of polymers to achieve uniform coverage or to prevent dust-off. Treated seed may be stored for up to 18 months with no negative impact on seed germination or efficacy. This feature enables seed companies to manage seed inventory and disposal issues. Both products were submitted in Canada and the U.S., as a joint scientific review between the Pest Management Regulatory Agency (PMRA) and Environmental Protection Agency (EPA). Simultaneous registration in both countries will help ensure free movement of treated seed.

INTRODUCTION

The majority of the canola seed treatment products in Canada are currently based on combinations of lindane (insecticide) and thiram/carboxin (fungicides). Growers typically choose a seed treatment based on the duration of flea-beetle control after seeding. Convention in areas with low-to-moderate flea beetle pressure has been limited to lindane-based seed treatments. Areas with more intense and prolonged flea beetle pressure commonly use a granular insecticide (e.g., terbufos), blended with lindane-treated seed, to extend the length of flea beetle protection up to 28 days. An alternative has been to apply a foliar insecticide (e.g., deltamethrin, lambda-cyhalothrin, chlorpyrifos etc.) to prevent crop loss from flea beetle attack on young plant stands.

The U.S. canola seed treatment market has two general segments. Approx. 50% receives an insecticide/fungicide (eg., imidacloprid + benomyl) seed treatment to control flea beetles and seed/soil-borne pathogens, while the remaining portion has been limited to fungicide only seed treatment. This portion of the U.S. market is expected to decline as flea beetles become established as a chronic pest.

Based on the fact that different seed treatments are registered in Canada and the U.S., and that the seed companies move seed between the two countries, cross border trade has become an issue. For example, canola seed treated with lindane cannot be shipped to the U.S., as no import tolerances have been established and lindane is not registered for use on canola. To help resolve this issue, the North American canola industry has embarked on a strategy to pursue new technologies to replace lindane-based products and register identical products in both countries. To meet this need, Novartis has developed two new seed treatments that are safe to seed, easy to apply and provide value-added features to support production goals.

Both products were submitted to the Canadian PMRA and the U.S. EPA as part of a work-share arrangement and were registered in December 2000, in time for introduction to the seed trade in 2001. This paper summarises data that was generated from four years of testing by universities, government agencies, seed-companies, third party consultants, and internal Novartis research personnel in Canada and the U.S.

METHODS AND MATERIALS

Formulations

The subject products, HELIX XTra (289FS) and HELIX (156FS), are ready-to-apply water-based formulations. The novel formulation technology in these products has resulted in treated seed with excellent seed-to-seed coverage, cold application characteristics and very low dust-off. The components and active ingredients for both products are identical, with the exception of the concentration of insecticide in the formulated product (Table 1).

Table 1. Formulation components / concentrations of 289FS and 156FS.

Active Ingredient	**289FS**		**156FS**	
	(gai/L)	% (w/w)*	(gai/L)	% (w/w)*
Thiamethoxam (CGA 293343)	266.6	20.70%	133.3	10.30%
Difenoconazole (CGA 169374)	16.0	1.25%	16.0	1.24%
Metalaxyl-M (CGA 329351)	5.0	0.39%	5.0	0.39%
Fludioxonil (CGA 173506)	1.7	0.13%	1.7	0.13%
Total pre-formulated a.i.	**289**	**22.47%**	**156**	**12.06%**

*at typical density of product.

Application Characteristics

The use rate for both products is 1,500 ml / 100kg seed which is 30 – 50% less than current commercial standards. At this use rate, treated seed has uniform coverage and favourable drying time before bagging. Due to the novel formulation characteristics of the products, no

additional colorant, dyes, binders, polymers or drying agents are required for optimal seed treatment application.
Other important features of a seed-treatment product are drying time and dust-off. Testing over a wide range of temperatures and seed sizes has indicated that both formulations dry quickly on treated seed and do not experience any significant levels of dust-off relative to current commercial standards. Both features are important to seed companies as treating often occurs during winter months under very cold conditions.

RESULTS

One of the greatest challenges in canola production is stand establishment. Maximised yields depend on uniform and vigorous early season crop development to help enable flowering prior to the onset of increased mid-season temperatures. The trend toward earlier seeding into conservation / reduced tillage systems has increased early season pathogen attack on crops that are seeded into cool/wet soils. It also increased the required duration of seed treatment insecticide protection as flea beetles attack young seedlings longer into the season.

Pest / Pathogen Spectrum

Both products provide excellent control of flea beetles (*Psylliodes* spp., *Phyllotreta* spp.), seed-borne blackleg (*Leptosphaeria maculans*), seed-borne *Alternaria*, (*Alternaria* spp.), and the seedling disease complex (damping-off, seedling blight, seed rot and root rot) caused by *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp. in canola and mustard (Table 2).

The dose rate of thiamethoxam is the distinguishing feature between the two products. The increased rate of insecticide in 289FS provides extended (4 – 5 week) protection, for areas that experience crop losses from intense and prolonged flea beetle attack. The reduced rate of thiamethoxam in the 156FS formulation provides 2 – 3 week protection for areas that experience light-to-moderate flea beetle pressure. As the fungicide components and use rates for both products are identical, both products deliver the same level of broad-spectrum disease control.

Table 2. Use rates and target pest(s) / pathogen(s) controlled by both products*.

Active Ingredient	**289FS**	**156FS**	**Target pest / pathogen**
	(gai / 100kg seed)		
Thiamethoxam	400	200	*Phyllotreta* spp., *Psylliodes* spp.
Difenoconazole	24	24	*Leptosphaeria maculans*
Metalaxyl-M	7.5	7.5	*Pythium* spp.
Fludioxonil	2.5	2.5	*Fusarium* spp., *Rhizoctonia* spp., and *Alternaria* spp.

*at application rate of 1.5L/100kg seed.

Flea Beetles

Both products provide improved flea beetle control relative to current commercial standards which is an important feature, as competitive products are often inconsistent under early season hot/dry conditions when seedlings are suffering the added stress of intense flea beetle feeding and delayed development.

To measure crop losses from flea beetle damage, and to evaluate efficacy so as to quantify duration and level of control, fresh weights were collected at the seedling stage (30 – 35 DAP, days after planting) and later in stand establishment (44 – 51 DAP) for all treatments tested in field development programs. Data collected over a three-year period indicated that 156FS (at 234 gai/100kg) and 289FS (at 434 gai/100kg seed) provided flea beetle protection that was equal to or better than commercial standard 1 (lindane 1826 gai/100kg); standard 2 (lindane + terbufos 1826 gai/100kg + 5000 gai/ha); and standard 3 (imidacloprid + thiram/carboxin at 800 + 303 gai/100kg) (Figure 1).

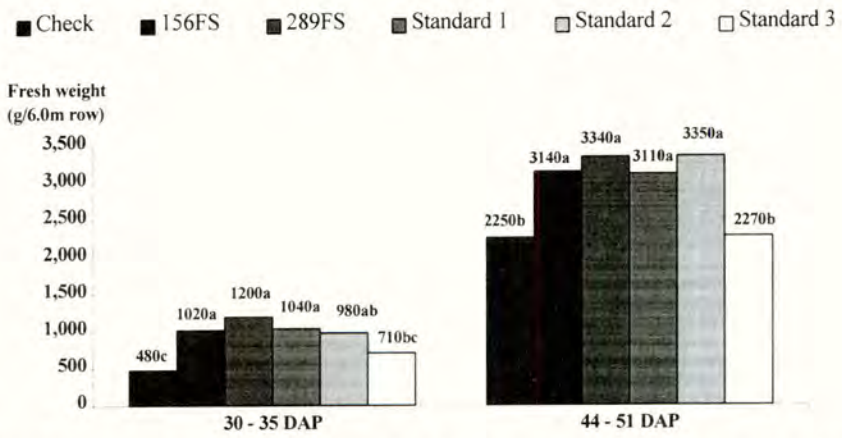

Figure 1. Summary of fresh weights taken from seven field trials conducted in 1998 - 2000. (Novartis Submission Data, 2000). Values followed by same letter are not statistically different (Duncan's test $p \leq 0.05$).

Seed and Soil-borne Pathogens

Laboratory, growth room and field studies on isolated pathogens conducted from 1996 to 1999 have indicated that, despite variable germination/growing conditions (i.e., reduced tillage, direct seeding equipment, cool, dry or wet soils, etc.), both formulations provided consistent broad-spectrum protection against seed- and soil-borne pathogens in canola and mustard. Research trials have also indicated that individual fungicide components in both products have overlapping disease spectrums.

In Vitro test results and calculated EC_{50} values (concentration required to reduce pathogen development by 50%) indicated that fungicide components within 289FS were more active on isolates of *Pythium paroecandrum, Rhizoctonia solani, Fusarium avenaceum* and

Lephtosphaeria maculans than the higher rate fungicide components in commercial standard 4 (carboxin + thiram + lindane) and standard 5 (thiabendazole + thiram + lindane) seed treatment products (Table 3) (Hall and Mooji, 1998-1). Test results indicated that each product was effective at inhibiting fungal growth, but 289FS was more potent and able to reduce colony diameters of *Pythium*, *Rhizoctonia*, *Fusarium*, and *Lephtosphaeria* isolates at lower concentrations.

Table 3. Summary of EC_{50} values from *In Vitro* toxicity studies (Hall & Mooji, 1998-1).

Fungal Isolate	**289FS**	**Standard 4**	**Standard 5**
P. paroecandrum	0.2	1.8	2.5
R. solani	0.68	1.62	2.73
F. avenaceum	0.12	1.38	2.22
L. maculans	0.19	0.31	1.30

Research conducted by Hall and Mooji (1998-2) with seeds that were either grown in or infested with target pathogens demonstrated that the fungicides within 289FS are very efficacious against seed and soil-borne infections caused by *Pythium, Fusarium Rhizoctonia* and *Leptosphaeria spp.* (Table 4). These results are supported by similar experiments conducted by Kharbanda and Ostashewski (1997-1; 1997-2; 1997-3; 1997-4) and Kharbanda (1998-1, 1998-2, 1998-3, 1998-4) who used seeds treated with 289FS and seeds inoculated with or grown in fungal infested soil. These observations were confirmed in the field test component of the respective studies. Growth chamber results were confirmed by field-tests.

Table 4. Summary of emergence results with canola seedlings grown in pathogen infested soil*. (Hall and Mooji, 1998-2).

Treatment	**Canola seedling emergence**			
	Pythium paroecandrum	*Rhizoctonia solani*	*Fusarium avenaceum*	*Leptosphaeria maculans*
Untreated / un-inoculated	22.8c	7.3a	8.7a	10.0a
Untreated / inoculated	16.8d	3.0c	5.3c	1.3b
289FS	30.8a	7.3a	8.7a	9.7a
Standard 1	28.4ab	5.3ab	6.3bc	8.0a
Standard 2	28.8ab	6.3ab	7.7ab	8.7a

* Numbers followed by same letter are not statistically different ($p \leq 0.05$).

Laboratory and growth chamber tests have also indicated that seed treated with 289FS and 156FS controlled of seed-borne *Alternaria* spp. in canola (Hall, Phillips, and Mooij, 1999).

Carry-over Seed Safety

Three varieties of canola seeds were treated with 289FS, 289FS + Cergard polymer and standard seed treatments and were stored over a period of two years under ambient conditions. Test results (Table 5) have indicated that 289FS had better storage safety than lindane-based seed treatments which demonstrated stunted growth, poor root-hair

development, thickened hypocotyls and curled-up shoots. In similar tests, seeds treated with 156FS or 289FS developed healthy root systems with normal root hair, hypocotyl development and plant stand establishment which was similar to untreated check.

Table 5. Summary of two – year warm and cold germination test results on three varieties of canola seeds* (Breadner, 2000).

Treatment	% Germination					
	45A71		Hyola 401		Reward	
	Warm	Cold	Warm	Cold	Warm	Cold
Untreated	96a	95a	90a	90a	94a	90a
289FS	96a	93a	89a	88ab	92ab	89a
289FS + Polymer**	94ab	93a	90a	85b	91ab	89a
Standard 1 + Polymer*	90c	86b	81b	76c	88bc	76b
Standard 2	91bc	86b	79b	74c	86c	73b

* Average of four replicates of 100 seeds per rep. ** Cergard polymer (commonly used seed adherent). Numbers followed by same letter are not statistically different (Duncan's multiple range test, $p \leq 0.05$).

Field Trial Results

Both products were evaluated on numerous crop varieties tested over several years under a wide range of conditions. Results have indicated that, regardless of crop (canola or mustard), canola type (e.g.. *Brassica napus* or *campestris*), canola variety, or location, seed treated with 289FS or 156FS had excellent crop tolerance with no observed decreases in germination, emergence counts, or crop development.

The high level disease and insect control combined with crop safety of both products became apparent in field observations of quick emergence, improved crop stand establishment, earlier flowering and crop yield/quality response. Figure 2 summarizes results from field trials that were conducted during 1997 to 1999 which shows that both 156fS (at 234 gai/100kg) and 289FS (at 434 gai/100kg) provided yields that were higher than untreated check and yields that were equal to or better than commercial standard 1 (lindane 1826 gai/100kg); standard 2 (lindane + terbufos 1826 gai/100kg + 5000 gai/ha); and standard 3 (imidacloprid + thiram/carboxin 800 + 303 gai/100kg).

Of the trials that were submitted for regulatory review, crop yield was statistically higher from plots receiving the 156FS treatment, compared with untreated check, in 6 of 20 trials (0 – 100.4 % yield increase in 20 trials relative to untreated check). Yields from plots receiving the 289FS treatment were statistically higher than untreated check in 8 of 20 trials (0 – 119.6% yield increase in 20 trials relative to untreated check). The greatest increases in yield were reported in trials where flea beetle pressures were highest. (PMRA, 2000).

Tests have also indicated that canola treated with both products often shows a unique 'vigor' effect demonstrated by quick emergence and vigorous seedling growth that leads to rapid,

healthy stand establishment and robust early season crop growth which, in turn, often leads to earlier flowering and maximized yield potential of treated crops. (Novartis, 1998; 2000).
Field observations have indicated that, on average, 156FS or 289FS treated canola begins the flowering period 2 – 3 days earlier than competitive seed treatments and five days prior to untreated check. Earlier flowering often results in a longer flowering period and the establishment of seed pods prior to increased mid-season temperatures which leads to earlier harvest, uniform ripening and improved seed quality with less immature (green) seed (Novartis, 1998; 2000).

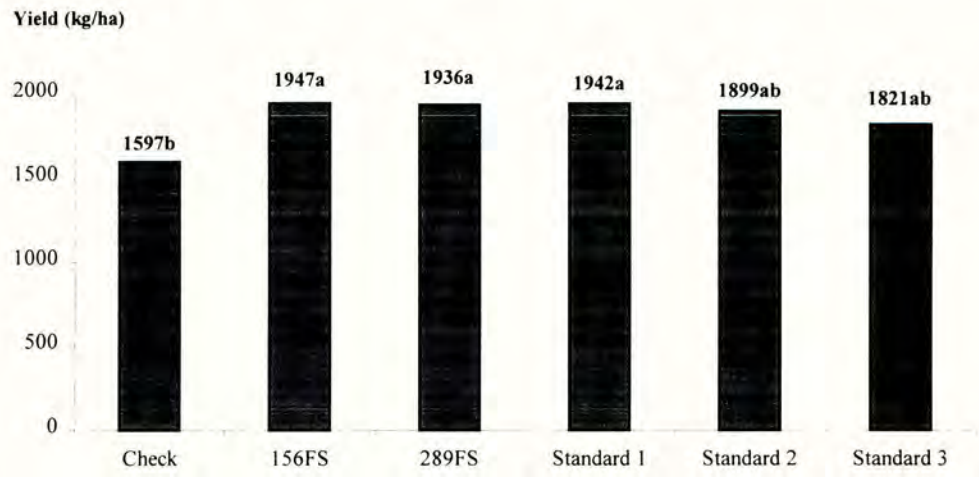

Figure 2. Average yield results of 18 Novartis efficacy field trials conducted from 1997 to 1999 submitted for regulatory review (Novartis, 2000). Values followed by same letter are not statistically different (one-tail t-test, $p \leq 0.05$).

CONCLUSIONS

289FS and 156FS are ready-to-use, water-based flowable seed treatment products based on three fungicides (difenoconazole, fludioxonil, and metalaxyl-M) and a neonicotinoid insecticide (thiamethoxam) which provides broad-spectrum control of pests and pathogens to increase yield and quality potential in treated crops. Research trials have demonstrated that both products control diseases caused by *Fusarium* spp., *Rhizoctonia* spp, *Alternaria* spp. *Leptosphaeria* spp. and *Pythium* spp. in canola and mustard under a wide range of growing conditions. Field trials have shown that the insecticide component provides consistent flea beetle control for periods of initial crop growth (2 – 3 weeks [156FS]) or extended duration (4 – 5 weeks [289FS]) for areas that experience intense and prolonged flea beetle attack. Both formulations have unique features that provide benefits for commercial seed treatment application. Another feature with both products is that, treated seed can be stored for at least 18 months with no negative impact on seed germination, vigour, crop development, or product efficacy. This feature enables seed companies to manage inventory and helps to eliminate the need for disposal of treated seed.

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Quantifying the benefits of seed treatment for foliar disease control

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ABSTRACT

Treatment of winter wheat seed with fluquinconazole was shown to delay primary infection by airborne ascospores of *Mycosphaerella graminicola*. As a consequence inoculum pressure was reduced and this effect was still evident five months from sowing. The biological activity of the fungicide did not persist beyond the emergence of the fourth true leaf. The mechanism for this long-lasting control was due to a delay in epidemic onset. Significant site and variety interactions were demonstrated. In particular, the effect of temperature on crop phenology was demonstrated to have a substantial influence on epidemic progress, and thus the efficacy of the seed treatment. These preliminary results have provided new insights about the importance of the winter epidemic and provide a strong basis from which strategies can be devised for product support and development.

INTRODUCTION

Fungicides with good activity against *Septoria tritici* (anamorph of *Mycosphaerella graminicola*), the cause of septoria leaf blotch, are used routinely in UK wheat production. However, optimal control depends on well-timed applications at appropriate doses (Paveley, 1999). Vagaries of weather, work schedules and the desire to reduce input costs, can conspire to cause sub-optimal control.

Advances in fungicide design and formulation may offer opportunities to improve the foliar disease control provided by seed treatments. However, exploiting the full benefit of such improvement will depend on understanding the extent and limits of long-term control. In particular, growers must be given information that allows them to achieve the potential benefits reliably. Thus, they need to know where use of the seed treatment is appropriate and also how use affects the need for subsequent foliar applications. Product stewardship depends on reliable quantitative data upon which recommendations for use can be developed. In recent years, quantitative understanding of the epidemiology of *S. tritici* has improved greatly (Lovell *et al.*, 1997; Parker et al., 1999). In particular, the importance of varietal characteristics other than genetic resistance are now recognised to influence epidemic progression. Features of canopy growth and architecture, such as the rate of stem extension and leaf insertion angle affect the potential for disease escape (Lovell *et al.*, 1997). Disease escape prevents or reduces contact between pathogen spores and the yield forming upper crop canopy. Substantial differences in the expression of escape have been demonstrated in wheat varieties (Lovell *et al.*, 1997). Previous studies have demonstrated long-lasting control of septoria leaf blotch by fluquinconazole (FQ) seed treatment (Wenz *et al.*, 1998). One

explanation for this may be that this treatment contributes to disease escape by reducing inoculum build-up on the rosette leaves. If this putative explanation is correct, it is likely that the efficacy of the seed treatment will be affected by differences in crop architecture and rainfall.

In this paper we describe preliminary results from mechanistic experiments, designed with reference to the epidemiology of *S. tritici,* to provide quantitative information about disease control from fluquinconazole seed treatment. We discuss how such data might be used to develop strategies for product support and development.

METHODS AND MATERIALS

Design

Field experiments were located at two sites, which contrasted for weather and septoria leaf blotch risk.

(1) Long Ashton Research Station (LARS), North Somerset - high risk, early disease development.
(2) ADAS-High Mowthorpe (HM), North Yorkshire - moderate risk, late disease development.

Fields (following a non-cereal crop to minimise take-all) were ploughed disced and harrowed, before planting on 29 October. Varieties were grown in plots that were a minimum of 2 × 18m.
A randomised block design was used, consisting of four varieties providing orthogonal contrasts for escape and resistance and three fungicide treatments (Table 1).

Table 1. Experiment design

Treatment		
Variety	Resistance (NIAB rating)	Escape
Consort	Susceptible (4)	Poor
Cadenza	Susceptible (5)	Good
Claire	Resistant (7)	Poor
Spark	Resistant (7)	Good
Fungicide	Seed treatment	Foliar treatment at growth stage 31
(a) Control	Sibutol[a]	None
(b) Seed treated	Sibutol + fluquinconazole[b]	None
(c) Foliar	Sibutol	Flamenco[c] 1.25 l c.p. ha^{-1}

[a]bitertanol + fuberidazole (375:23g l^{-1}); [b]75g a.i. per 100kg seed; [c] fluquinconazole 100g l^{-1}

Measurements

Leaf emergence was recorded twice a week on ten tagged plants per plot in treatments (a) and (b). Leaves were numbered in order of emergence. Thus the first true leaf to emerge was leaf 1 and the last to emerge was the flag leaf. Crop development stage was measured using the key reported by Tottman & Makepeace (1979).

Disease pressure and expression was measured through:

1. Frequent assessment of disease symptoms both pre- and post- stem extension in fungicide treatments (a) and (b) and at key growth stages in treatment (c).
2. Inoculum quantity
   - in crop (pycnidiospores) by spore washings (Lovell et al., 1997) from sampled leaves at frequent intervals in treatments (a) and (b).
   - daily air spora counts (ascospores) trapped using suction traps and confirmed by plating techniques (Hunter *et al.*, 1999).
3. Lesion heights [the distance between inoculum (disease symptoms) and the yield forming leaves, flag to leaf 3] twice weekly during stem extension in all treatments.
4. Plants sampled from treatments (a) and (b) of Consort and Spark, at LARS, were inoculated with a conidial suspension (1.5×10^5 spores ml^{-1}) or distilled water. After inoculation the plants were maintained at 17°C and constant humidity of 90% for 72 hours, and then moved to an unheated glasshouse with capillary matting.

RESULTS

Weather

The sites differed greatly for temperature, HM was considerably cooler than LARS. This had a significant effect on the rate of crop growth and development. On average, plants grew 9 leaves at HM compared to 12-13 leaves at LARS.

Total rainfall was broadly similar at the two sites. However, large differences in rainfall between the sites were recorded for December and February (when rainfall was greater at LARS) and June (when rainfall was greatest at HM).

Daily ascospore counts

Large differences were measured in the ascospore risk at the two sites. Although the patterns of release were broadly similar, substantially more ascospores were trapped at LARS (Table 2).

Table 2. Number of ascospores trapped each month in the 1999/2000 growing season.

Month	Ascospore counts	
	LARS	HM
November	457	24
December	94	15
January	106	6
February	474	7
March	195	2
April	32	5
May	217	66

Days from leaf emergence to symptoms

The FQ seed treatment caused a delay in disease onset. This effect was greatest on the first three leaves that emerged. At LARS, the delay was consistent across varieties; approximately 30 days on leaf 1 and 10 days on leaves 2 and 3 (Fig. 1). Similar delays were observed at the HM site for leaves 1 and 2. Some delay was also observed, at this site, on leaf 3 for the two susceptible varieties, Consort and Cadenza, but onset was identical from leaf 3 onwards on the resistant varieties.

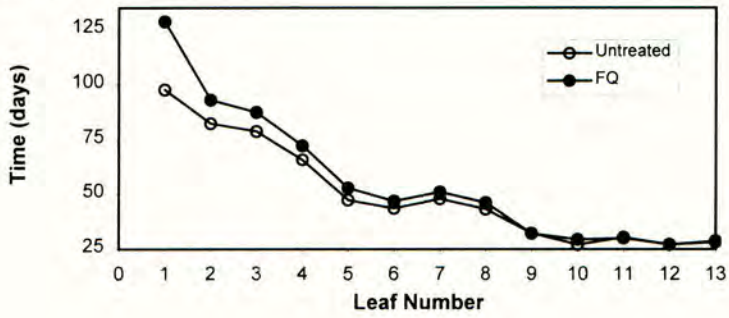

Figure 1. Mean number of days from leaf emergence, of 4 varieties, to first symptoms of *S. tritici*, at Long Ashton. Leaf 13 is the flag leaf.

The first records of disease on each emerging leaf layer were recorded substantially earlier at Long Ashton than High Mowthorpe (Fig. 2A). However, considered on a thermal time scale accumulated from sowing (above a base temperature of 0°C) the epidemic progress was broadly similar at the two sites (Fig. 2B).

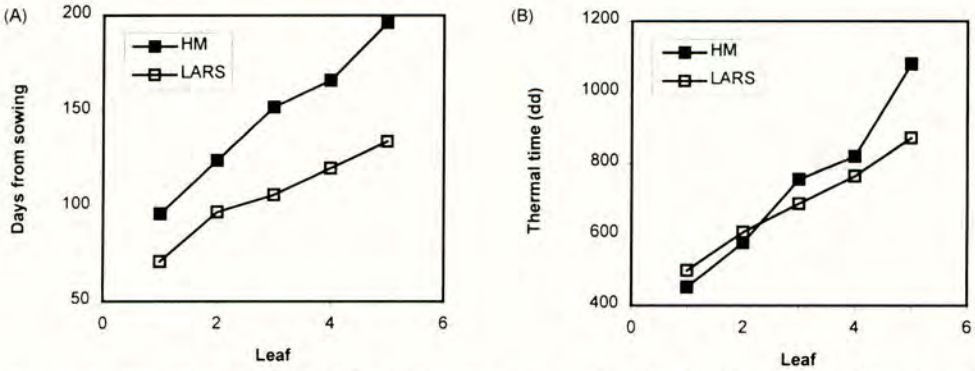

Figure 2. Time from sowing to first disease symptoms observed in the crop on (A) daily and (B) thermal scales

Disease incidence

At LARS, the incidence of infected Consort plants was 100% on all leaves from leaf 3 onwards and leaf 4 onwards for the other varieties. The level of control reduced substantially on each subsequent leaf layer. For example, 90% control was achieved on leaf 1 of Claire compared to 20% on leaf 3. The same general pattern of control was observed at HM, but the levels of control were substantially smaller *e.g.*, 15% control on leaf 1 of Consort.

Fungicide activity

Disease severity on FQ seed treated plants recovered from the field and inoculated with distilled water was substantially lower than on untreated plants. Only small amounts of disease developed on leaves 2 and 3, and leaf 4 exhibited no symptoms. In contrast, disease developed on all leaves of plants inoculated with a spore suspension of *S. tritici* (Fig. 3A). Disease severity on leaves 2 and 3 was lower for plants seed treated by FQ, but on leaf 4 disease levels did not differ significantly from untreated (Fig. 3B).

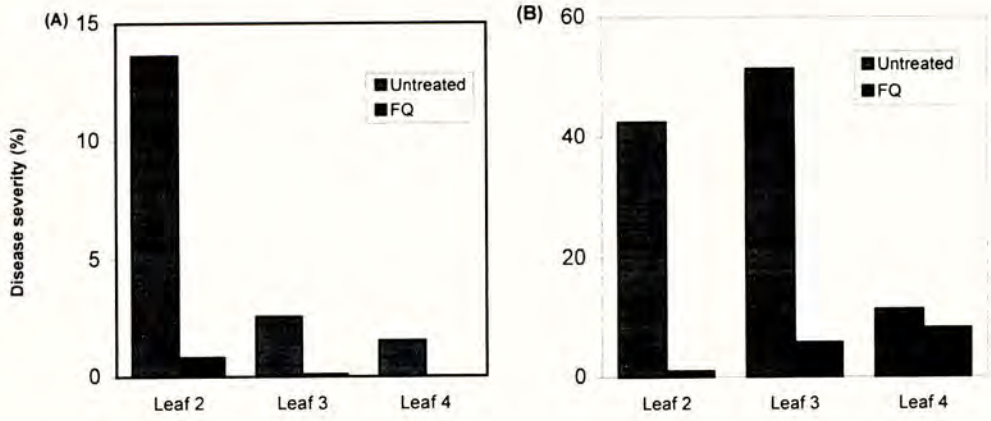

Figure 3. Disease severity on plants recovered from the field and inoculate with (A) distilled water and (B) a suspension of *S. tritici* (1.5×10^5 spores per ml)

Inoculum development

Measurements of spore production within crop canopies showed that FQ seed treatment suppressed inoculum production substantially at both sites. This suppression was still evident 5-6 months from sowing (Fig. 4A). At both sites, the least effect on inoculum production was measured on Spark, the most resistant variety. Inoculum measurements by calendar date indicated that the progress of the epidemic was greatest at LARS (Fig. 4A). However, conversion to a thermal timescale showed that the onset of the epidemic earlier and that rate development was greater at HM (Fig. 4B).

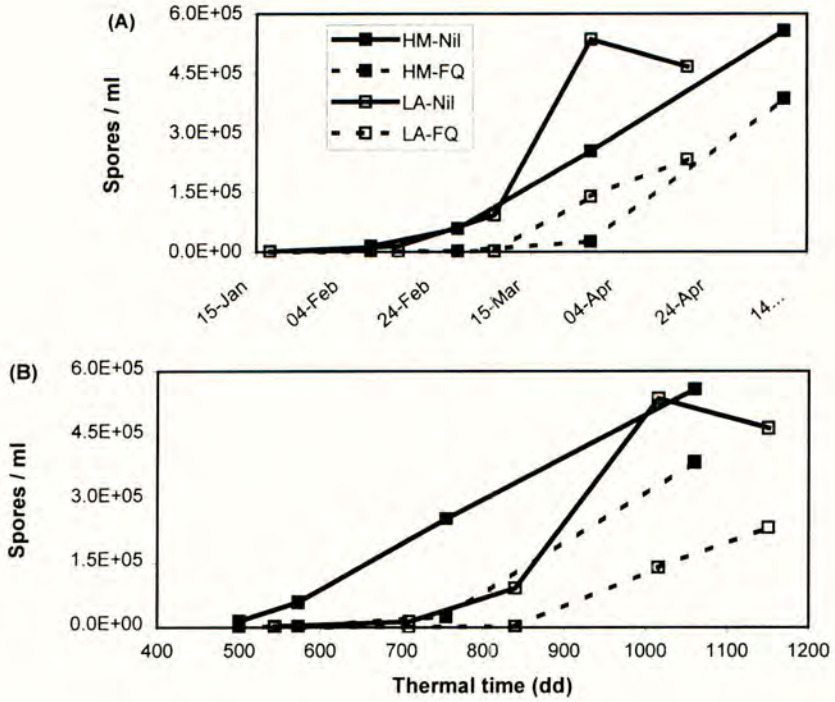

Figure 4. Inoculum potential by (A) date and (B) thermal time accumulated from sowing.

Yield Response

At HM there was no evidence of significant yield benefit from FQ, seed treatment (Table 3). For the two susceptible varieties the yield benefit was not significant even for a full rate application of FQ applied at GS31 (Table 3). A trend for improved yield above the untreated was measured for the seed treatment at LARS. This improvement was significant for the most susceptible variety, Consort. The response to seed treatment at LARS was not due to take-all control.

Table 3. Yield response to treatment. Letters indicate significant differences (p=0.05) for comparisons within a site and variety.

Site	Treatment	Yield t ha^{-1}			
		Consort	Cadenza	Claire	Spark
LARS	Control	5.70**a**	5.96**a**	7.52**a**	7.05**a**
	Seed treated	6.56**b**	6.57**a**	8.19**a**	6.98**a**
	Foliar	7.44**c**	7.53**b**	8.72**b**	7.87**b**
HM	Control	8.84**a**	8.15**a**	9.71**a**	8.35**a**
	Seed treated	8.79**a**	7.96**a**	10.07**a**	8.43**a**
	Foliar	9.16**a**	8.35**a**	10.22**b**	8.91**b**

DISCUSSION

Ascospores of *M. graminicola* cause primary infection of UK wheat crops during the autumn (Shaw & Royle, 1989) and are the primary source of inoculum in the USA (Schuh 1990). This study measured large differences in ascospore risk at the two sites. But these differences did not appear to have any significant affect on the onset or progress of the epidemics. Fluquinconazole seed treatment provided effective protection against primary infection. This has previously proved difficult to achieve reliably using foliar applications of fungicide in the winter (Lovell & Parker, data unpublished). Such foliar applications are in any case impracticable for commercial disease management. However, previous studies suggest that suppression of the winter inoculum pool by cold temperatures can reduce the severity of the summer epidemic (Parker *et al.*, 1997; Gladders *et al.*, 2001).

Evidence from observations of disease incidence and from inoculations of plants recovered from the field suggests that the seed treatment did not persist at a biologically active level beyond the emergence of leaf 4. However, large reductions in inoculum pressure were maintained through to spring by FQ seed treatment at both sites. The mechanism appears to have been through delay of initial crop infection and a subsequent knock on delay to inoculum production. In this respect, the seed treatment might mimic the suppression of the winter inoculum pool by cold temperatures.

Despite enormously different primary inoculum pressure from ascospores, epidemic progress was broadly similar at the two sites when measured by thermal time. The best and most long-lasting control was obtained at Long Ashton, the high disease pressure site. Two reasons may explain this. First the soil type at HM was a shallow silty clay loam overlying chalk compared to a deep sandy loam at LARS. Fluquinconazole is relatively immobile in the soil, so it is unlikely the effective dose at HM was reduced by leaching (P. Cavell, Ongar; pers. comm.). The more likely explanation therefore probably relates to the physiological differences in crops at the two sites caused by the large differences in temperature. Three to four fewer leaves developed at the colder site, HM. This observation is supported by estimates of phyllochrons (leaf emergence rates) for wheat (Kirby, 1994). Slow plant development and growth rates may have reduced plant uptake of the fungicide during the winter phase. Furthermore, due to the greater number of days from leaf emergence to senescence at HM, there was a greater opportunity at this site for infection to result in pycnidial production prior to natural leaf death. Thus greater levels of crop inoculum were available at HM for dispersal and re-infection, and this probably reduced the capacity for disease escape.

No yield benefits were evident from the seed treatment at HM and foliar application at GS31 also failed to provide a significant benefit on the susceptible varieties. For both treatments we suspect that this was due to high disease pressure and low leaf production, which prevented the plants from out-growing disease. At Long Ashton, the observation of reduced inoculum pressure was supported by a significant yield benefit on Consort, the most susceptible variety, and the other varieties showed a trend for improved yield above the untreated.

Disease escape can be promoted by applying fungicides to create a 'clean barrier' between rosette leaves and the yield forming upper canopy leaves. However, successful enhancement of disease escape depends on fungicide choice and application timed accurately to the emergence of final leaf 5 (*i.e.* flag leaf minus 4). Inappropriate fungicide choice and variation from the specified timing leads to a substantial decrease in the efficacy of this treatment. In

part, we believe this explains the variable response measured for GS31 applications, because they coincide with final leaf 5/4 emergence. An additional problem with this approach is that disease established on the earlier rosette leaves provides a substantial inoculum source. The long-lasting control provided by FQ seed treatment might therefore be valuable in providing growers greater opportunity and flexibility in optimising the benefits of disease escape. Based on these data from one season, it remains difficult to quantify exactly how the seed treatment might affect the fungicide requirement at the T1 timing. The 2000 harvest season proved particularly challenging for management of septoria leaf blotch in the UK. Losses due to the disease in this season were estimated to be around £29m, despite substantial fungicide use (pers. comm., Hardwick, CSL York). Despite these favourable conditions for septoria leaf blotch development, significant levels of control were achieved by the seed treatment at both sites. At LARS this control provided clear trends for yield improvement. Further supporting data are now necessary so that strategies for use can be developed.

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