Threshold based control of wheat diseases using the BAYER cereal diagnosis system after Verreet/Hoffmann

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Abstract

The practice of chemical plant protection as part of an integrated pest management scheme depends upon the availibility of active ingredients suitable for specific application and the use of scientifically based decision aids. This allows treatments to be based on biological criteria. At present growth stage-based routine treatments too rarely intervene specifically in the infection process. A crucial factor in ensuring the targeted use of fungicides is reliable diagnosis, especially of leaf pathogens which induce non-specific necrosis and of which fungal structures are not recognizable by the naked eye. Therefore, a diagnostic instrument has been developed which is easy to use and practical. The use of this instrument in allowing rational, biologically-based decisions to be taken on control is illustrated by work on *Septoria nodorum* Berk., *Septoria tritici* Rob. ex Desm., *Drechslera tritici-repentis* (Died.) Shoem., *Puccinia recondita* Rob. ex Desm., *Puccinia striiformis* Westend., *Pseudocercosporella herpotrichoides* (Fron.) Deighton and *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal.

Introduction

Current agricultural practice is to time treatments to control leaf and ear diseases of wheat in relation to plant development. This ignores changes in disease progress bet ween years. Because epidemics differ from year to year, depending on the biology of the pathogens and the weather, treatment that is related to the growth stage of the plant will affect the pathogen only if the disease is present at the time of application or if the sprays, accidentally, interfere with the epidemic. From an epidemiological point of view these growth stage-oriented treatments are randomly timed and only randomly successful. To follow the principles of integrated plant protection management certain preconditions must be met. Pathogen specific fungicides have to be available and the criteria must be known on which to base control measures. For several years we have been developing threshold values for disease control based on the population dynamics of the pathogens, the agronomy of the crop and environmental conditions. To realise this objective there must be accurate, qualitative and quantitative diagnosis of the pathogens present. While the diagnosis of the mildews and rusts is not a problem because of their prominent fruiting bodies, the diagnosis of leaf and glume blotch, tan spot and eye spot is subject to mistakes and misinterpretations because of non-specific necrotic symptoms

and only microscopically visible fruiting bodies. However, the recently developed and easy to use Bayer cereal diagnosis system after Verreet/Hoffmann (CDS)(Hoffmann et. al., 1988), which can be used in the field and the laboratory, has made the diagnosis of these diseases simple and reliable.

Thresholds

The threshold values that have been determined and tested so far are reported below.

Septoria nodorum - glume blotch (Verreet & Hoffmann 1989)
Septoria tritici - leaf blotch

Control threshold: >1 pycnidium/indicator leaf level (infection severity)

During the growing period, the number of the typical pycnidia of *S.nodorum* (honey-brown) and *S.tritici* (black) must be counted (CDS) on designated observation or indicator leaves (Table 1). For each sample 30 leaves collected at random from a diagonal across the crop are recorded. If an average of 1 or more pycnidia/indicator leaf are found then, from GS 37 onwards, an immediate treatment with a *Septoria*-specific fungicide e.g. tebuconazole, propiconazole, prochloraz or flusilazole, is necessary. These fungicides are effective for about 3-4 weeks and during this period no disease recording is required. With the possibility of a persistent infection and decreasing effectiveness of the fungicide due to its metabolization a second treatment may be necessary. The threshold remains the same and the indicator leaves are given in Table 2. Thus if the fungicide was applied at GS 37 a second treatment may become necessary from GS 51. If the first application is later then the need for a second treatment is also delayed but if the first treatment is at GS 47/51 or later then a second fungicide treatment is not justified.

Table 1: Threshold oriented decision model against *S. nodorum* and *S. tritici*

Growth stage (GS)	Indicator leaves	Decision
31-34	F-6/F-5	observation period: no treatment
37-39 43-47 51-69 71/73	F-5/F-4 F-4/F-3 F-3/F-2 F-2/F-1	decision period for 1st and 2nd treatment: threshold = > 1 pycnidia/indicator leaf

If the threshold value is not reached within the decision period (GS 37-71) on the designated indicator leaves, a *Septoria*-specific use of fungicides is not required.

Table 2: Chronology of primary and secondary threshold-based treatment

1st threshold oriented treatment (GS)	2nd threshold oriented treatment (GS)	Indicator leaves
37	3 weeks = 51/59 4 weeks = 59/65	F-3/F-2
39	3 weeks = 59/65 4 weeks = 59/73	F-3/F-2
43	3 weeks = 65 4 weeks = 73	F-3/F-2 F-2/F-1
From 47/51 to 71/73	no 2nd fungicide necessary	

Erysiphe graminis - powdery mildew

Control thresholds:
Primary : 60 - 80 % plants infected (infection frequency) (Käsbohrer et. al., 1988a)
Secondary: > 1% leaf area affected (infection severity) (Büschbell, 1990)

Because the fungus is easily visible an analysis of its development and population dynamics can be done on the whole plant, main shoot, stem, ear, single leaf. The plants to be assessed are marked, before tillering, at 3 points in a drill and 10 plants at each point assessed on each occasion (30 plants in crops, 10/replicate from plots). Assessments are made every 7 days and the percentage of infested plant parts or whole plants (infection frequency) and the percentage area covered with powdery mildew (infection severity), estimated. If, from GS 30/31 onwards, 60 - 80% of plants are infected then an immediate mildew specific treatment (tradimefon, triadimenol, fenpropimorph, propiconazole, triadimenol/triademorph) is necessary. If, after an early fungicide treatment, infection conditions remain favourable (favourable weather conditions, using much nitrogen, susceptible cultivar, decreasing effectiveness of the fungicide), then a second fungicide treatment is justified if more than 1% (infection severity) of the area of the upper organs (flag, second, third upper leaves and ear) is infected (smallest infestation unit - one pustule - is set equal 1%). This is evaluated by assessing the infection severity of these single organs and dividing it by the number of actually infected organs.

Pseudocercosporella herpotrichoides - eye spot

Control threshold: > 20 % plants infected (leaf sheaths) (infection frequency) (Käsbohrer et. al. 1988b)

Symptoms of infection on leaf sheaths and stems at GS 30-39 are not reliable enough to be included in the decision making process because of potential confusion with *Fusarium* spp., *Rhizoctonia cerealis* and *Septoria nodorum*. However, eyespot can be reliably diagnosed (CDS) on the basis of its typical mycelial pads (Mauler-Machnik & Nass, 1990). The mycelial pads are demonstrated by soaking the leaf sheaths from 60 plants collected at random for 15-30 min in a 5-10% ink-acetic acid solution before washing with tap water and examining at 60x magnification.

Infection frequency is determined using the agar dish test of Käsbohrer et. al. (1988b). From GS 20 up to GS 32 the three outermost leaf sheaths from 60 randomly collected plants are shaken with sterile water, centrifuged, then placed on water agar (ph 4.0) in Petri dishes and incubated at 15°C under u.v. illumination. The agar plates are examined after 14 days for conidia of *P. herpotrichoides* varieties. If there is an infection frequency of > 20% the use of the fungicide prochloraz or prochloraz plus carbendazim from GS 32 up to GS 37 is advisable.

Drechslera tritici-repentis - tan spot (Wolf, 1990)

Control thresholds:
Primary : > 5% leaves with conidiophores or conidia (sporulation frequency)
Secondary: > 5% leaves with spots (infection frequency)-
During the growing season the frequency of typical conidiophores and conidia is deter-

Table 3: Threshold oriented decision model for the 1st treatment against *Drechslera tritici-repentis* (1st threshold > 5% frequency of sporulation)

Growth stage (GS)	Indicator leaves	Decision
21-31	F-8 to F-5	Observation period without treatment
32	F-7/F-6/F-5	
37	F-6/F-5/F-4	Decision period for
49	F-5/F-4/F-3	1st treatment
59	F-4/F-3/F-2	
69	F-3/F-2/F-1	

mined (CDS) on indicator leaves (Table 3). If there is a 5% frequency of sporulation on the designated indicator leaves of 30 plants collected along a diagonal through the crop, an immediate treatment with a tan spot specific fungicide (tebuconazole, propiconazole) is justified.

These fungicides are effective for 3-4 weeks and no further observations are necessary in this period after treatment. If there is a continuing high risk of infection as a result of an especially susceptible cultivar, direct drilling, favourable weather and if the crop is a second or subsequent wheat crop, a second treatment may be necessary. This second treatment is justified if the designated indicator leaves show > 5% infection frequency. The time of the second treatment depends upon the time of the first treatment and whether the threshold is exceeded (Table 4).

Table 4: Chronology between 1st and 2nd treatments against *Drechslera tritici-repentis* (2nd threshold > 5% infection frequency)

1st threshold oriented treatment (GS)	2nd threshold oriented treatment (GS)	Indicator leaves
32	39-45	F-2/F-1/F
33	43-49	F-2/F-1/F
37	47-55	F-2/F-1/F
39	55-65	F-2/F-1/F
45	65-73	F-1/F
49	69-73	F-1/F
51	no second application necessary	

Puccinia recondita f. sp. tritici - brown rust
Puccinia striiformis - yellow rust

Control threshold: > 20 % infection frequency

Although rust fungi are easily identified control measures are often taken too late for optimum benefit. To ensure appropriate, early treatment, 30 plants from crops and 10/replicate from plots, are collected at random and diagnosed (CDS) for the incidence (% infected leaves on the main shoot) of the different rust fungi. If, from GS 34/37 > 20% infection (infection frequency) is recorded a rust specific fungicide (e.g. tebuconazole, fenpropimorph, propiconazole, cyproconazole) is necessary.

Discussion

The integration of these threshold-based systems was tested for 3 years in wheat cultivars Apollo, Ares, Basalt, Granada, and Kanzler sown on two dates, 21 September and 15 October given either 40 kg N/ha or 140 kg N/ha, and either grown in rotation or as consecutive crops. The pathogen that first reaches its treatment threshold is treated with the appropriate fungicide. This will also cause a shift in the populations of other pathogens (biological side effects of the fungicide) which may be supported by host plant tolerance or resistance. These side effects can be of such importance that other fungi present do not exceed the critical infestation values for the rest of the growing season. For example, a threshold treatment against eyespot with prochloraz at GS 32 or 37 also has a large effect on the population dynamics of *Septoria* spp. so that a *Septoria*-specific treatment may not be necessary. The establishment of such effects, however, requires a precise qualitative and quantitative diagnosis of the pathogens and the use of the decision model. The system described, with its integrated threshold treatments is able to react flexibly to any plant cultivation or environmental change, such as fertilizer use, sowing date, crop rotation, continuous cereals, variety tolerance or resistance, tillage and weather conditions. Consequently the use of this system means an optimisation of plant protection measures in every case because it uses the specific and current population dynamics of the pathogens. At the moment this system, named the wheat crop system Bavaria, is introduced on the instructions of the Bavarian Ministry of Agriculture at the official advisory bureaux and practical farms. In addition it has also been integrated in an expert system.

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DEVELOPMENT OF MONOCLONAL ANTIBODY-BASED IMMUNOASSAYS FOR DETECTION AND DIFFERENTIATION OF *SEPTORIA NODORUM* AND *S. TRITICI* IN WHEAT.

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ABSTRACT

Monoclonal antibodies specific for *Septoria nodorum* and *S. tritici* were developed and incorporated into highly sensitive immunoassays for detection of these pathogens in wheat leaves. Monoclonal antibody Sen15C6 reacted positively with isolates of *S. nodorum* and *S. avenae* f. sp. *triticea*, but not with *S. tritici*, or other related or unrelated species. Monoclonal antibody Set37B5 reacted positively only with isolates of *S. tritici*. The lower limits of detection, for double antibody sandwich enzyme-linked immunosorbent assays incorporating these antibodies for extracts of their target pathogens, were 1.0 μg protein/ml (*S. nodorum*) and 0.25 μg protein/ml (*S. tritici)*. On-site immunoassays were also developed and optimised using these immunoreagents. *Septoria nodorum* and *S. tritici* were detected specifically in symptomatic wheat leaves using both immunoassay formats.

INTRODUCTION

Early detection of pathogens is a critical component of effective plant disease control programs, one that has been often overlooked in the past as a result of the lack of adequate tools for rapid, in-field pathogen identification. The recent adaptation of monoclonal antibody (MAB) and immunoassay technology to plant pathogens, including fungi, however, has made it possible accurately to diagnose disease and quantify pathogens (Mitchell & Sutherland, 1986; Dewey, 1988; Hardham, 1989; Schots, 1990). Commercial immunoassays have already been developed for fungal diseases of turfgrass, field crops, woody and herbaceous ornamentals and fruit crops (Miller *et al.*, 1988; MacDonald *et al.*, 1990). This paper describes the development of MABs and immunoassays for early detection of *Septoria nodorum (Leptosphaeria nodorum)* and *S. tritici (Mycosphaerella graminicola)* in wheat.

MATERIALS AND METHODS

Monoclonal antibodies were produced by standard methods previously reported for other fungal species (Petersen *et al.*, 1989). Spleen cells from female Balb/C mice sensitized with either a mycelial extract of *S. nodorum* or an extract of germinated conidia of *S. tritici* were harvested and mixed with murine myeloma cells [P3/Ns1/1-A4-1 (NS1)] in a 1:4 ratio in the presence of

the chemical fusitive agent PEG 1500. After dilution of the fusitive agent the cells were plated in 96-well microtiter plates in the presence of Hypoxanthine-Methotrexate-Thymidine (HMT) medium. Cells were fed with HMT once, then with HT (HMT-methotrexate). Clusters of hybridoma cells began to appear 5-7 days after plating. Culture supernatants were screened by indirect enzyme-linked immunosorbent assay (ELISA) for reactivity against *S. tritici* or *S. nodorum* extracts bound at 5.0 μg protein/ml to the wells of microtiter plates. Cell lines secreting antibodies with desired reactivity toward the target fungi were identified, and hybridomas were isolated by limiting dilution. Further selections were made based on reactivity with primary screening panels consisting of isolates of related and unrelated fungi. Selected MABs were characterized to immunoglobulin subclass and reactivity with an extended panel of fungal isolates. Polyclonal antisera were produced in sheep using extracts of *S. tritici* and *S. nodorum* described above for mouse immunizations.

One MAB specific for *S. tritici* and another specific for *S. nodorum* were selected from separate fusion experiments on the basis of specificity and sensitivity in ELISA. Monoclonal antibody Set37B5 was purified from hybridoma culture supernatant by protein A affinity chromatography. Monoclonal antibody Sen15C6 and polyclonal antisera raised against *S. tritici* and *S. nodorum* were purified by specific affinity chromatography using extracts of the target pathogens. Polyclonal antisera were purified and conjugated with peroxidase for use as "tagged" reagents in the two assays, while the purified monoclonal antibodies were used as "capture" reagents. The immunoreagents were incorporated into multiwell (MacDonald *et al.*, 1990) and on-site (Miller *et al.*, 1988) formats. Optimal concentrations of *S. tritici* and *S. nodorum* capture and tagged antibody were determined for each assay system.

Fungal cultures used for evaluation of MAB specificity were grown on various liquid media as shake or still cultures until late log phase, harvested, washed and ground in the presence of glass beads. Soluble fractions were standardized as to protein concentration and used to coat microtiter plates for indirect ELISA, or were used directly in double antibody sandwich ELISA. For evaluation of diseased and healthy wheat leaves, plants were inoculated with various fungi and incubated in a greenhouse or growth chamber under conditions favorable for disease development. Non-inoculated plants were included as healthy controls. Extracts were prepared by grinding leaf pieces in extraction buffer.

RESULTS

Monoclonal and polyclonal antibody production and screening

The results of primary evaluations of MABs Sen15C6 (*S. nodorum*) and Set37B5 (*S. tritici*) for specificity are presented in Table 1.

Both antibodies demonstrated high reactivity with their target species and no cross-reactivity with non-target isolates. In secondary screening studies, Sen15C6 reacted strongly with 17 of 20 isolates of *S. nodorum* (mean OD_{405nm} for all isolates = 0.578) but not with any of the 81 isolates of taxonomically related and unrelated fungal species tested. Optical density values of $\leq$0.05 were observed for all of the fungi in the panel, which included species of *Alternaria, Aspergillus, Bipolaris, Botrytis, Chloridium, Cladosporium, Drechslera, Fusarium, Lambertella, Monilia, Mortierella,*

Penicillium, *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Sclerotium*, as well as *Colletotrichum graminicola*, *Curvularia lunata*, *Diplodia gossypina*, *Epicoccum nigrum*, *Helminthosporium sativum*, *Lanzia luteo-virescens*, *Leptosphaeria korrae*, *Myriosclerotinia denisii*, *Phialophora graminicola*, *Pseudocercosporella herpotrichoides*, *Rhizopus stolonifer*, and *Stemphylium vesicarium*.

TABLE 1. Monoclonal antibodies (MABs) produced against *Septoria nodorum* (Sen15C6) and S.tritici (Set37B5) and their reactivity by indirect ELISA to extracts (5 μg protein/ml) of isolates of several fungi.

		Optical Density (405 nm) for MAB†	
Species	Isolate	Sen15C6	Set37B5
Septoria nodorum	1	2.0+	0.01
S. tritici	3g	0.03	2.0+
S. tritici	4g	0.04	2.0+
Pyrenophora tritici-repentis	1	0.02	0.01
Negative control		0.01	0.01

†2.0+ = off-scale reading

Monoclonal antibody Set37B5 reacted strongly with all 14 isolates of *S. tritici* tested but did not react with any of the 62 non-*Septoria* isolates tested.

Multiwell immunoassay development

A sensitive and specific multiwell assay was developed for *S. nodorum* based on MAB Sen15C6 capture and sheep polyclonal antibody-peroxidase conjugate. The *S. nodorum* assay has a detection limit of approximately 1.0 μg/ml *S. nodorum* protein, with a linear dose-response curve through approximately 10 μg/ml protein (Figure 1).

The sensitivity of the *S. tritici* double antibody multiwell immunoassay to an extract of *S. tritici* is illustrated in the dose-response curve shown in Figure 1. A sensitivity of approximately 0.25 μg/ml of fungal protein is attained with a dynamic range up to a concentration of about 2.5 μg/ml.

Both assays demonstrate excellent specificity towards target *Septoria* species. In the *S. nodorum* assay, pure culture extracts of *S. nodorum* and *S. avenae* f. sp. *triticea* reacted strongly, while very little or no cross-reactivity to *S. tritici*, *P. tritici-repentis* and other fungi was observed (Table 2). Large positive values were obtained for *S. nodorum*-infected wheat leaves in the assay, while healthy wheat leaves and leaves severely attacked following artificial inoculation with non-target pathogens were not reactive (Table 3). Similar results were observed for the *S. tritici* assay (Tables 2, 3); *S. avenae* f. sp. *triticea* was negative in the *S. tritici* assay (Table 2). The *S. tritici* and *S. nodorum* assays could be done within 40 minutes.

FIGURE 1. Dose-response curves for the *Septoria nodorum* and *S. tritici* multiwell immunoassays using extracts of *S. nodorum* or *S. tritici*. Each point is the mean of six replicates.

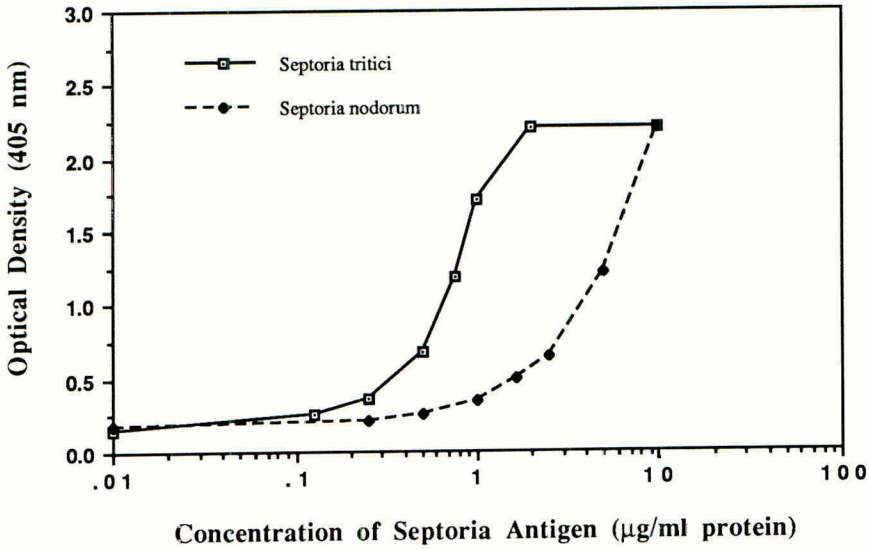


TABLE 2. Specificity of *Septoria nodorum* and *S. tritici* double antibody multiwell immunoassays against extracts of fungi associated with wheat. Each culture extract was tested at a concentration of 1.0 µg/ml fungal protein.

	Optical Density (405 nm)	
Isolate	*S. nodorum* Assay	*S. tritici* Assay
Septoria nodorum	<u>1.44</u>	0.14
Septoria tritici	0.14	<u>1.26</u>
Septoria avenae f.sp. *triticea*	1.37	0.00
Pyrenophora tritici-repentis	0.16	0.15
Alternaria sp.	0.22	0.08
Aspergillus fumigatus	0.07	0.09
Epicoccum sp.	0.12	0.14
Phoma sp.	0.29	0.14
Penicillium sp.	0.14	0.09
Pseudocercosporella herpotrichoides	0.00	0.08
Cladosporium sp.	0.14	0.12

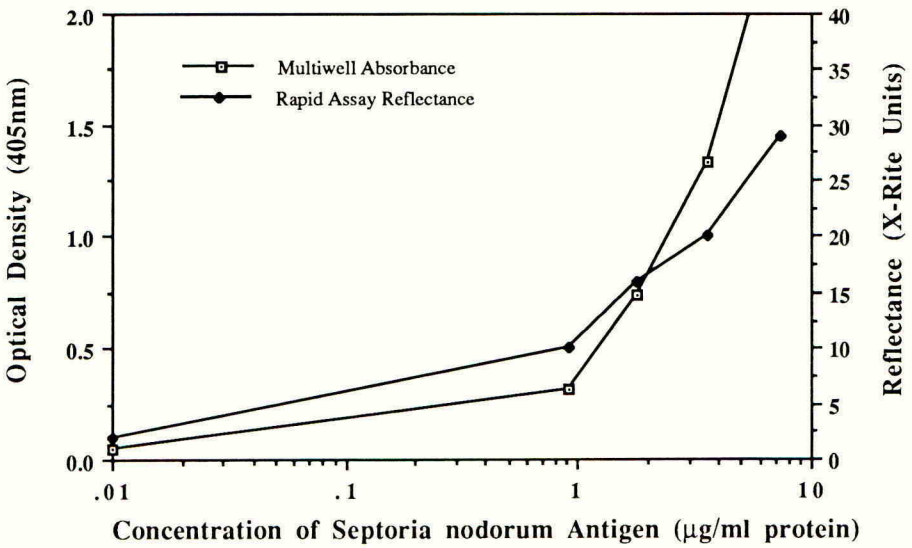
TABLE 3. Reactivity of *Septoria nodorum* and *S. tritici* multiwell immunoassays toward extracts of healthy wheat leaves and wheat leaves inoculated with target and non-target pathogens.

	Optical Density (405nm)†	
Sample description	*S.nodorum* Assay	*S. tritici* Assay
Septoria nodorum	> **1.50**	0.07
Septoria tritici	0.06	**0.81**
Puccinia striiformis	0.00	0.01
Puccinia recondita	0.03	0.04
Erysiphe graminis	0.04	0.13
Gerlachia nivalis	0.10	0.04
Fusarium culmorum	0.01	0.01
Fusarium graminearum	0.02	0.03
Non-inoculated (healthy) wheat leaves	0.01	0.07††

† O.D. values are the mean of five replicates
†† O.D. value is the mean of 30 wheat leaf samples

On-site immunoassay development

FIGURE 2. Comparison of on-site and multiwell assay sensitivities using dilutions of *Septoria nodorum* extracts in healthy wheat leaf samples.



Field usable immunoassays (Miller *et al.*, 1988) were developed for both *S. tritici* and *S. nodorum* analysis in infected wheat leaves. The same antibodies as described for the multiwell systems were incorporated into the on-site assay formats. The sensitivities of the *S. tritici* and *S. nodorum* on-site assays are very similar to the sensitivities of the multiwell assays. (Figure 2). A limited number of on-site assays were done in the USA and in Western Europe with field material and *Septoria* was reliably detected in infected leaves.

DISCUSSION

This is the first report of the production of species-specific MABs to detect and differentiate *S. nodorum* and *S. tritici*. The antibodies were incorporated into sensitive double antibody multiwell and on-site immunoassays capable of detecting these pathogens in wheat leaves. The multiwell assays have excellent sensitivity and reproducibility with only low background readings and are effective research tools for use in detecting and quantifying these pathogens and monitoring pathogen biomass in wheat crops. The on-site assays will permit rapid, accurate detection and diagnosis of *Septoria* diseases in the field, without the need for laboratory equipment or highly trained personnel. This easy and accurate method of *Septoria* identification means that the disease can be managed more effectively.

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FIELD RESULTS WITH A DIAGNOSTIC SYSTEM FOR THE IDENTIFICATION OF *SEPTORIA NODORUM* AND *SEPTORIA TRITICI*

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ABSTRACT

Monoclonal antibody-based immunoassays specific for *Septoria tritici* and *Septoria nodorum*, respectively, have been developed and evaluated for the detection of leaf diseases caused by *Septoria* spp. in wheat fields in Switzerland and England. The target pathogens were readily detected at an early stage of disease development. The *Septoria tritici* format was highly specific to *S. tritici* and the *Septoria nodorum* format specific to *S. nodorum*. There was no cross-reaction between the *Septoria* spp. nor to other non-target pathogens like *Erysiphe graminis* or *Puccinia* spp.. Both assays were very sensitive to the target diseases and their response was not influenced by cultivar, growth stage, leaf number, age of the leaf, previous fungicide sprays or environmental conditions.

Formats for the tests to be used in the field and based on the monoclonal antibodies used in the multiwell assays have been developed for both *S. tritici* and *S. nodorum*, and preliminary results are presented. These formats allow a test to be completed within about 10 minutes. The further development and possible uses of these immunoassays are discussed.

INTRODUCTION

S. nodorum and *S. tritici* are among the most important leaf spot pathogens of wheat causing considerable losses of yield and quality. Both pathogens infect the leaves; *S. nodorum* also attacks the ears. Several attempts have been made to establish thresholds for the control of the two diseases and to provide tools for better timing of fungicide sprays (Tyldesley & Thompson 1980, Shaw & Royle 1986, Verreet & Hoffmann 1989). A key requirement for better fungicide timing is to identify the pathogens and to quantify the amount of inoculum present. For identification of *S. tritici* the presence of dark pycnidia visible with the naked eye can be used whereas for *S. nodorum* pycnidia can be seen only

under a hand lense or microscope. Counts of pycnidia have been used to measure epidemics in crops. (Verreet & Hoffmann 1989).

Recently enzyme-linked immunosorbent assays (ELISA) first described by Engvall & Perlman (1971) and widely used as very specific and rapid diagnostic tools in medicine and virology, have been used also in plant pathology. There are several examples of the successful development of immunoassays to identify fungal pathogens or to quantify disease severity (Miller et al. 1988, Unger & Wolf 1988, Petersen et al. 1990).

This paper presents results using monoclonal antibody-based immunoassays to detect and differentiate *S. tritici* and *S. nodorum* in wheat leaves.

MATERIALS AND METHODS

The immunoassays described for detection of *S. tritici* and *S. nodorum*, are based on the principle of a double-antibody sandwich ELISA. Immunoassays were made with field grown plant material, using either single leaf pieces or bulk samples consisting of 5 to 30 leaves collected at random. Single leaves were macerated using an abrasive pad. Bulk samples were put into plastic bags, extracted in a 1 : 1 (weight : volume) tissue : buffer ratio, ground with a hand-held Tecan grinder and filtered through a 0.02 µ filter. 100 µl of the extract were added to two wells of the multiwell plates, incubated for 10 min at room temperature with constant agitation and washed 5 times with the wash solution. The same procedure was carried out after adding 100 µl antibody-peroxidase conjugate. 100 µl of the working substrate solution ABTS were added and incubated for 10 min with constant agitation. The reaction was stopped by addition of 50 µl of the stop solution to each well and the absorbance read at 405 nm.

Most of the on-site assays were done with single leaves using abrasive pads for grinding. The pads were put into bottles containing 2 ml of extraction buffer which after shaking were filtered through a 0.02 µ filter. Six drops of the filtered extract were put on an absorptive device coated with the capture antibody. After sequentially adding 2 drops each, of the enzyme-tagged antibody, a rinse solution and the enzyme substrate, the test circle turned blue when the fungal antigen was present in the sample. The addition of 2 drops of a stop solution stopped the colour reaction.

RESULTS

Septoria tritici and Septoria nodorum multiwell assays (STMA and SNMA)

Immunoassays for both *S. tritici* and *S. nodorum* were evaluated using field samples from various cultivars at different growth stages and from several locations. Table 1 shows results of the STMA with bulk samples of 12 asymptomatic leaves which were collected from TILT ^{R} treated plots to reduce natural infection with *Septoria* spp.. Independent of the growth stage and leaf levels tested, there was a consistently small OD 405 reading.

To see if cultivar influenced the assays single asymptomatic flag leaves of several cultivars were tested (Table 2). There was no influence of cultivar on non-specific binding to either the SNMA or the STMA.

TABLE 1. STMA from leaves of the cultivar Bernina at different growth stages (Bulk samples of 12 leaves)

	Optical density (OD_{405})			
Growth Stage Leaf	GS 34	GS 37-39	GS 61	GS 71-73
Flag leaf	-	0.015	0.007	0.000
F - 1	0.000	0.004	0.008	0.014
F - 2	0.000	0.015	0.046	-
F - 3	0.056	-	-	-

TABLE 2. SNMA and STMA from asymptomatic leaves of different cultivars at GS 39 - 49 (Mean of 10 leaves)

Variety	OD_{405} SNMA	OD_{405} STMA
Avalon	0.012	0.078
Apollo	0.003	0.029
Fortress	0.018	0.066
Galahad	0.009	0.050
Hornet	0.023	0.059
Kanzler	0.011	0.025
Slejpner	0.010	0.050
Rendezvous	0.002	0.057

Trials using field grown plants were carried out to evaluate the sensitivity of the multiwell assays and to check the specificity of the two assays. Bulk samples of 30 leaves were collected at random from different leaf levels within the crop canopy (Table 3). In the sample of leaf F - 2 only 4 leaves out of 30 showed a low level of *S. tritici*. This low infection was clearly detected by the STMA, whereas for the F - 1 and the flag leaves, which showed no sign of disease, the readings were within the range for asymptomatic leaves. There was also no reaction in the SNMA to *S. tritici*. Trials done in the UK confirm the high sensitivity of the STMA (Table 4).

TABLE 3. *Septoria* multiwell assay on bulk samples (30 leaves)

Leaf	*S. tritici*		OD_{405}	
	No. of leaves infected	% leaf area infected	STMA	SNMA
Flag leaf	0	0	0.000	0.021
F - 1	0	0	0.019	0.000
F - 2	4	0.2	0.298	0.000

Sampling on May 16, 1990, GS 37 - 39, cultivar Bernina

TABLE 4. Sensitivity STMA (Bulk samples of 20 F - 2 leaves, cv. Galahad)

Sampling date (1990)	% leaves infected by *S. tritici*	% leaf area infected by *S. tritici*	OD_{405}
16 May (GS 37-39)	0	0	0.033
23 May (GS 45)	5	0.02	0.143
30 May (GS 55)	5	0.05	0.563
07 June (GS 59)	10	0.60	0.216
13 June (GS 65)	30	0.27	0.110

Single *S. nodorum* infected leaves were used to confirm attack in field grown plants. Table 5 shows results achieved in the cultivar Tambo.

Table 5: SNMA using single *S. nodorum* infected leaves

Sample	% leaf area infected by *S. nodorum*	OD_{405} SNMA	OD_{405} STMA
1	5	0.809	0.002
2	10	0.318	0.000
3	5	0.630	0.003
4	3	0.297	0.041
5	20	> 1.5	0.000

Sampling on June 12, 1990, GS 59-61, cultivar Tambo

Septoria tritici assays using the on-site format

In a limited number of tests the same leaf extract of either single symptomatic leaves or bulk samples of several infected leaves were tested in parallel using the STMA and a first version of an on-site format which allows a test to be done without any specific laboratory equipment. Table 6 shows results achieved with the on-site format for *S. tritici* using samples of 6 leaves of the cultivar Bernina taken at GS 31-32. Leaves without symptoms resulted in very low readings on the multiwell assay and no colour formation in the on-site format. When all leaves in the sample had distinct *S. tritici* symptoms the multiwell assay resulted in off-scale readings and a clear positive reading expressed by a blue colour reaction of the test circle for the on-site kit. In a mixture of one *S. tritici* infected leaf with 5 leaves showing no *S. tritici* symptoms both formats gave a clear positive response.

TABLE 6. Confirmation of *S. tritici* attack using an on-site format of the *S. tritici* assay

Description of sample	OD_{405} STMA	On-site format*
6 asymptomatic leaves	0.000	-
6 asymptomatic leaves	0.000	-
6 S. *tritici* infected leaves	> 1.5**	+
6 *S. tritici* infected leaves	> 1.5**	+
1 *S. tritici* infected + 5 asymptomatic leaves	> 1.5**	+
1 *S. tritici* infected + 5 asymptomatic leaves	0.433	+

*) Scale on-site format: - = No colour formation; + = Intense colour formation

**) > 1.5 = off-scale on ELISA reader

The tests done using the on-site formats for *S. tritici* are preliminary and additional data using a broader range of varieties and environmental conditions are needed. The results achieved indicate that on-site formats of these monoclonal antibody-based ELISA tests can be developed and the results are very encouraging for further exploitation of this technology.

DISCUSSION

To increase and broaden the use of integrated disease control measures, rapid, easy and reliable methods to detect and differentiate disease symptoms are very important. Symptoms caused by *Septoria* spp. are often difficult to distinguish from symptoms caused by other biotic or abiotic factors. The monoclonal antibody-based ELISA tests

specific for *S. tritici* and *S. nodorum*, respectively, produced consistent results using either single leaves or bulk samples of several leaves. Low levels of *Septoria* leaf attack at an early stage of symptom expression were detected. To differentiate clearly symptoms caused by either *S. tritici* or *S. nodorum* from other leaf spots the immunoassays developed, offer a very useful and reliable tool.

Further tests are necessary to demonstrate fully the potential of either the multiwell assays or on-site kits in integrated disease control in wheat. As both *Septoria* multiwell assays detect low levels of infection at an early stage of the epidemic, a further interesting area for development is the use of these diagnostic kits as decision supports for fungicide treatments. Quantifying the level of *Septoria* attack in a field using appropriate sampling methods can help to optimise application timing of fungicides for the control of the disease in a more efficient way, or avoid unnecessary treatments. The results achieved with the multiwell assays and in preliminary tests with on-site formats indicate the large potential contribution modern disease diagnostic techniques can make to integrated disease control.

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IMMUNODIAGNOSTIC ASSAY FOR CEREAL EYESPOT: NOVEL TECHNOLOGY FOR DISEASE DETECTION

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ABSTRACT

A novel immunodiagnostic assay identified *Pseudocercosporella herpotrichoides* (cereal eyespot) in cereal plants. Laboratory tests indicate that the polyclonal antibody used in the assay is specific for *P. herpotrichoides* and does not cross-react with other important cereal pathogens. The assay detects the pathogen in wheat plants as early as tillering and before disease symptoms appeared. Extensive European field studies have demonstrated that *P. herpotrichoides* antigen levels rise during the growing season, with a significant increase occurring prior to extensive symptom expression. At harvest, assay results correlated well with severity of eyespot symptoms. These results emphasize that this diagnostic assay can identify the pathogen accurately, can rapidly monitor disease progression, and has the potential to contribute towards assessing the need for disease control measures.

INTRODUCTION

Eyespot, caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton, is an important stembase disease of cereals and can cause significant yield losses. The effectiveness of disease control measures has been limited by the difficulty of accurate diagnosis and in quantification of disease levels. Eyespot symptoms are easily mis-identified visually, and can be confused with other stem-based diseases including Fusarium footrots (*Fusarium* spp.) and sharp eyespot (*Rhizoctonia cerealis*). With a latent period of > 40 days between inoculation and symptom expression, eyespot can be present with no visible symptoms, making quantification of early disease levels difficult. Consequently fungicide applications are frequently made on a routine basis, and timed on host phenology rather than disease progress.

Accurate disease diagnosis would allow use of the most appropriate fungicide, whilst quantification of infection levels would help to establish disease thresholds for spray timing. Immunoassays can provide highly specific and sensitive tools to achieve these objectives (Miller *et*

al., 1988; Unger & Wolfe, 1988), but first an appropriately specific assay must be developed and its use demonstrated in the field. This paper reports the development of a highly sensitive, specific immunoassay for *P. herpotrichoides*, which can be used to accurately identify disease and monitor infection levels prior to symptom expression.

MATERIALS AND METHODS

Immunoassay reagents and format

The immunogen consisted of concentrated dialyzed malt extract broth culture supernatant derived from a wheat strain of *P. herpotrichoides*. Rabbit immunization protocols, immunoglubulin G (IgG) isolation protocols, immunoreagent development procedures used to prepare rabbit anti-*P. herpotrichoides* IgG (RAP IgG), and double antibody sandwich ELISA protocols have been described elsewhere (Saunders *et al.*, 1990). Briefly, plant samples were macerated in extraction buffer (5 ml/plant) and added to 96-well microtiter plate wells coated with immunoabsorbed RAP IgG specific for *P. herpotrichoides* antigen. Heavily infected samples were further diluted in extraction buffer prior to testing. Bound antigen was detected by the addition of biotin conjugated RAP IgG, avidin alkaline phosphatase conjugate, and p-nitrophenyl phosphate substrate. Colour development in the wells was measured spectrophotometrically at 405 nm.

Assay results were compared with one another using either optical density values or 'antigen units' (AgU). One AgU is defined as 2.26 ng (dry weight) of *P. herpotrichoides* culture extract (immunogen) prepared using the methods described above.

Target and non-target organisms

Diseased plant parts from both glasshouse and field grown wheat plants were tested. Plant materials included fresh, frozen or dried samples. Test samples from plants infected with stem pathogens consisted of 2.5-5 cm stem segments cut from between the stem base to just below the first leaf. Infected heads and leaves were also tested.

Growth cabinet experiments

Twenty-day-old wheat seedlings (cv. Avalon) were inoculated at the stem base with agar plugs of a rye pathotype (*P. herpotrichoides* var. *acuformis*, Nirenberg, 1981), and maintained at 10°C and a 16 h daylength. Seedlings were removed at intervals, assessed for eyespot, and the bottom 3 cm of stems, including leaf sheaths, were tested using the immunoassay. Flusilazole was applied as a root drench (50 mg/litre soil-less compost) 21 days after inoculation.

Field studies

Intensive sampling of field sites

Twenty-five winter wheat plants were collected per replicate on a uniform, but random, basis from each of four untreated plots (c. 10 m x 2.5 m) five times between tillering to harvest (March to July) at two sites in France, in FRG, and in the UK. Dead, outer sheaths were removed from samples. The lower 6 cm of each culm was scored for eyespot symptoms

(Scott & Hollins, 1974) and other stem-based diseases (*Fusarium* spp. and *Rhizoctonia cerealis*) and individually tested using the eyespot immunoassay.

Dead, outer sheaths (2-3 sheaths/plant) were removed from 10 plants collected at tillering (11 April 1989) in Westphalia, Germany. Dead sheaths and pseudostems were scored for eyespot severity and assayed using the immunoassay.

RESULTS

Antigen units

The use of 'antigen units' (AgU), rather than absorbance unit (AU values, provides a more reliable method of comparing immunoassay results generated on different days. Antigen standards were included on each assay plate and used to produce a standard curve for converting optical AU values to antigen units. A typical standard curve is shown in Figure 1. The average background AU value obtained from 50 uninfected plant samples was 0.081 AU (standard deviation (SD) = 0.062). Test samples resulting in AU values equal to background + 4 SDs were considered to be positive in the assay; therefore the lower limit of detection is approximately 0.320 AU, or 2 AgU/ml of extraction buffer (19 AgU/stem). Based on extensive disease surveys, antigen unit values may be combined into groups which roughly correspond to disease severity: > 5000 AgU - severe, 500-5000 AgU - moderate, 10-500 AgU - low severity eyespot. Samples with < 10 AgU/stem have either very low level infections for are not infected with *P. herpotrichoides*.

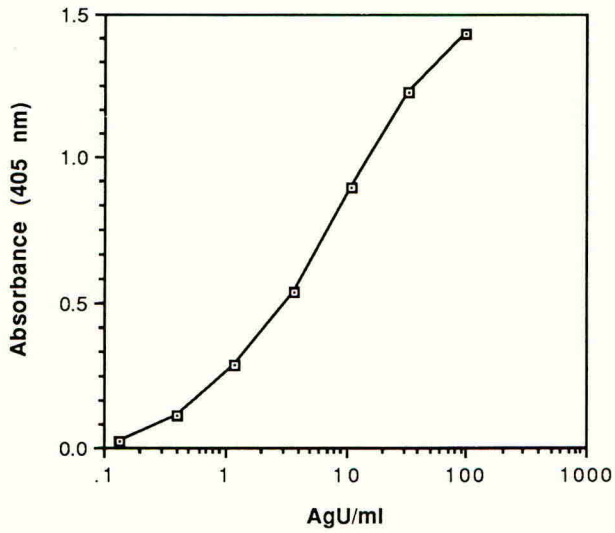

Figure 1. Example of a standard curve of *Pseudocercosporella* antigen

Target and non-target organisms

All *P. herpotrichoides* strains tested (*c.* 100), including wheat and rye pathotypes from France, FRG, UK and US were positive in the assay. Twenty ascospore progeny of *P. herpotrichoides*, grown *in vitro* but isolated from perithecia collected during 1989 showed < 5% variation in antigen levels. A *P. anquioides* isolate was also recognized. Plants infected with non-target fungi and healthy plants and soil were not recognized (Table 1).

TABLE 1. Plant samples tested with eyespot immunoassay

Disease	Absorbance units	AgU/plant
Uninfected	0.016	< 2
Eyespot (severe)	1.150*	35,100
Eyespot (moderate)	1.450	670
Eyespot (pre-symptomatic)	0.804	51
Sharp eyespot (*R. cerealis*)	0.030	< 2
Take all (*G. graminis*)	0.025	< 2
Root rot (*Fusarium* spp.)	0.040	< 2
Yellow rust (*Puccinia striiformis*)	0.025	< 2
Brown rust (*P. recondita*)	0.001	< 2
Powdery mildew (*Erysiphe graminis*)	0.050	< 2
Leaf blotch (*Septoria tritici*)	0.073	< 2
Glume blothc (*S. nodorum*)	0.033	< 2

* Sample diluted 1:729

Effect of flusilazole on eyespot development

In a growth cabinet experiment, antigen production was stopped by a single application of flusilazole before symptoms were observed. Antigen levels were constant after treatment for 5 weeks, whereas levels increased dramatically in inoculated but untreated seedlings (Figure 2).

Field studies

Eyespot diagnosis

The assay detected pre-symptomatic eyespot as demonstrated by the presence of > 10 AgU in culms with no observable eyespot symptoms (Table 2). Eyespot detection recorded by the immunoassay peaked at stem erect to the one node stage (GS 30). In addition, the assay provided accurate identification of eyespot, despite visual misdiagnosis, as indicated by false positives and false negatives. Assay results correlated well with visual eyespot symptoms as lesions became more pronounced.

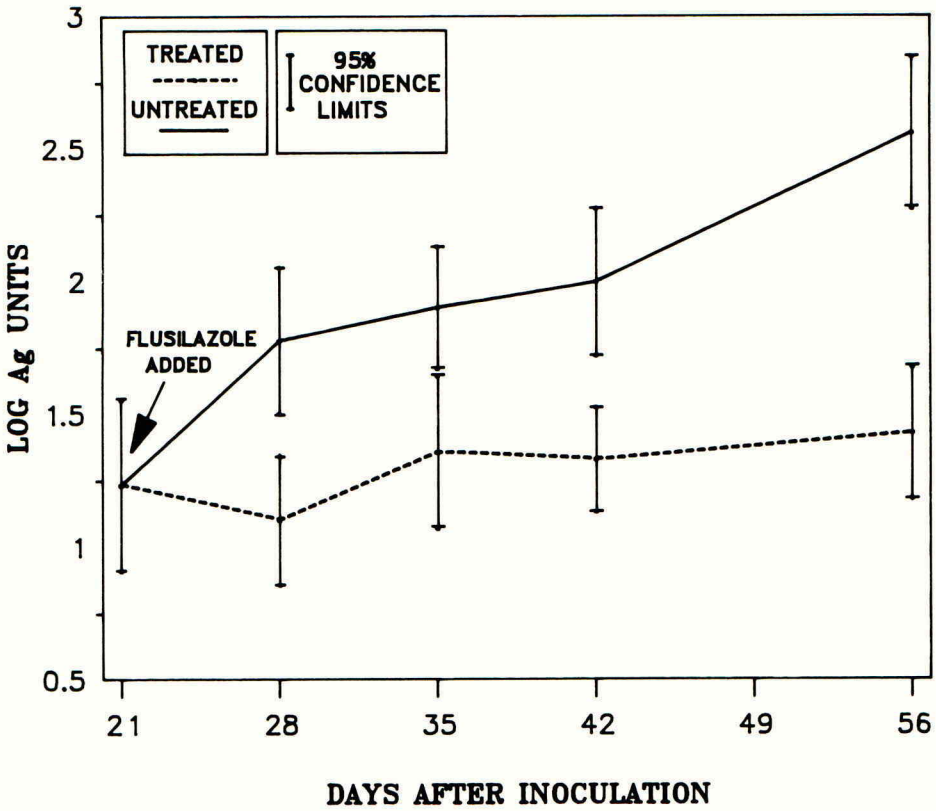

Figure 2. Antigen levels and eyespot control after treatment with flusilazole

Trends during the season

The diagnostic assay provided a quantification of eyespot levels. In France, the incidence of eyespot as recorded by the immunoassay increased from tillering to become widespread at harvest (Fig. 3) and the increase in eyespot antigen detected between GS 25 and GS 27 foreshadowed the subsequent increase in symptoms. In Germany, the assay detected eyespot only after GS 32, but again, infection was widespread at harvest. In both the French and German studies, eyespot antigen was detected before symptoms were seen.

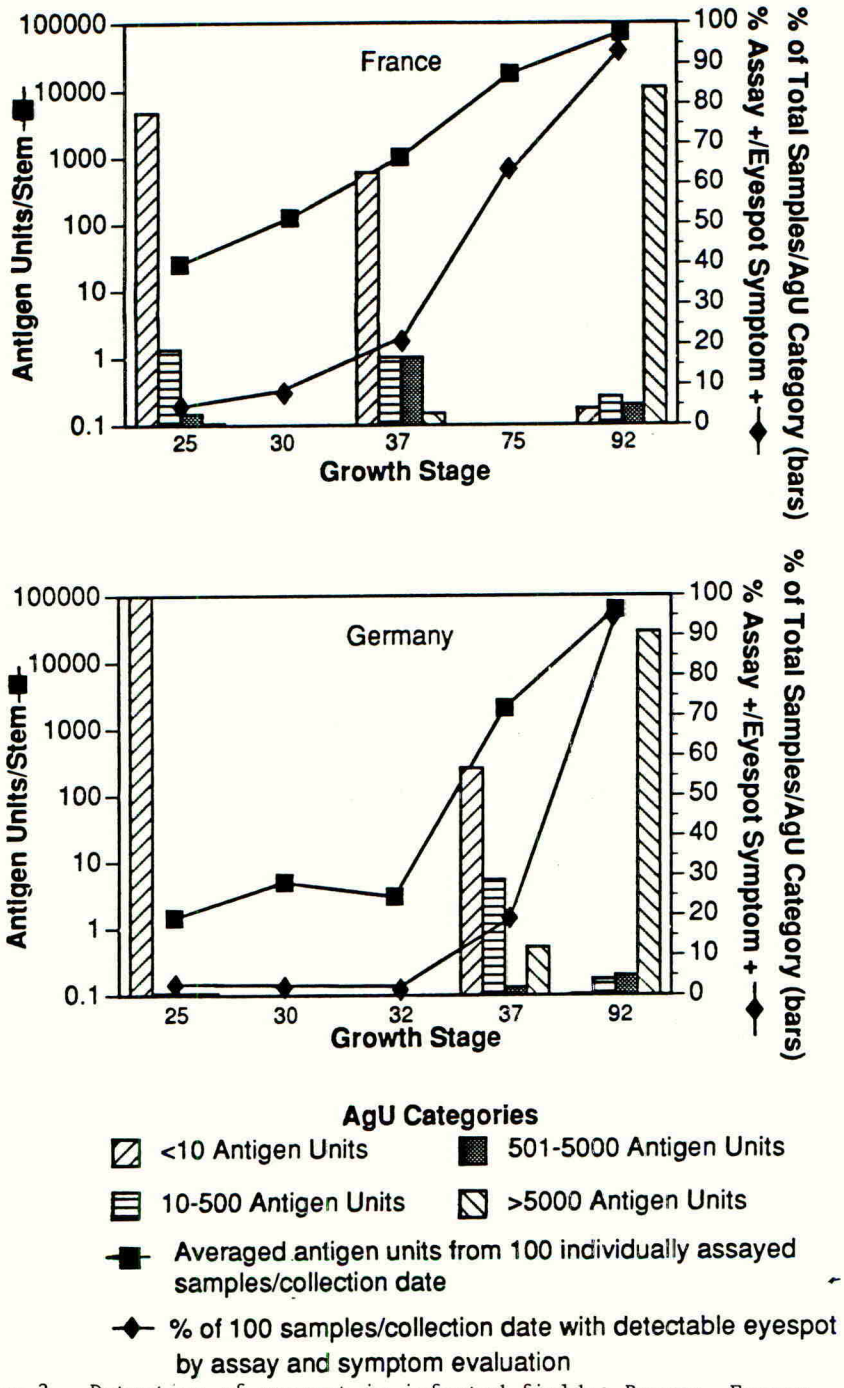

Figure 3. Detection of eyespot in infected fields; Beauce, France and Bavaria, Germany

Effect of removal of dead, outer sheaths on eyespot amount

Significantly greater amounts of eyespot antigen were present in dead, outer sheaths than in the pseudostems from which they had been removed. Both outer leaf sheaths and pseudostems gave positive eyespot assay results when there were no symptoms evident (Table 3).

TABLE 2. Comparison between immunoassay results and visual symptom assessment of cereal eyespot

	Percentage of samples[1]				
	Growth stage (GS)				
	25	30	37	75	92
Correct diagnosis[2]	79	27	68	85	91
Pre-eymptom eyespot	18	64	14	10	5
False positive[4]	3	0	16	4	0
False negative[5]	0	9	2	1	4

1 100 wheat stems sampled at each collection date (GS 25, March 23; GS 30, April 6; GS 37, May 11; GS 75, May 31; GS 92, July 7 1989) from Beauce, France.
2 Correct diagnosis: visual rating agreed with immunoassay results.
3 Pre-symptom eyespot: no visual eyespot symptoms but assay results ≥ 10 AgU/stem.
4 False positive: lesion visually diagnosed as eyespot but not confirmed by assay.
5 False negative: lesion visually diagnosed as *Fusarium* footrot or sharp eyespot but identified as eyespot by assay. May include mixed infections with presymptomatic eyespot.

TABLE 3. Eyespot infection of leaf sheaths

	Number of AgU	% Stems with visual eyespot
Outer sheath	593	0
Inner sheaths	49	0

DISCUSSION

The immunoassay for cereal eyespot described in this paper provides a novel method to accurately identify *P. herpotrichoides*, and to quantitatively monitor progress of eyespot, particularly before symptoms appear. Extensive laboratory studies have demonstrated the specificity of the assay which recognizes *P. herpotrichoides* and *P. anguoides*, but no other cereal pathogens or healthy plants. The ease and rapidity of the assay provides important benefits over traditional pathogen isolation techniques. In addition to allowing pre-symptom detection, the ELISA procedure has been useful in confirming visual diagnosis of eyespot particularly when symptoms of other diseases predominate on stems.

Our field data from 1989 show that in six different areas in Europe, infection levels increased significantly between tillering and flag leaf emergence, but the timing of this rise in infection depended on location. These results forehadowed the development of symptoms expressed in the crop at later stages. However, final levels of infection in the crop expressed in antigen units were remarkably similar for trial sites studied in France, FRG and UK (data not shown). The immunoassay may also be used to quantify the effects on eyespot development of fungicide treatment. Growth cabinet experiments also showed that the antigen was stable, even though pathogen development was stopped by flusilazole.

Early, more precise, quantification of eyespot infection levels provided by the immunoassay may contribute towards optimising control measures through better timing. However, immunoassay results must be considered in conjunction with all other variables that influence disease development before they can be used in disease forecasting. More development work is clearly required before economic thresholds can be established to use the immunoassay for spray decisions, and before sampling procedures can be optimised. However, this immunoassay has the potential to provide crop advisers and farmers with greater precision in eyespot detection and allow more effective use of disease control agents.

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EXPERIMENTS IN ENGLAND AND FRANCE ON FERTILISERS, FUNGICIDES AND AGRONOMIC PRACTICES TO DECREASE TAKE-ALL

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ABSTRACT

Seven factors reported to affect take-all were tested on winter wheat at risk from take-all in factorial experiments at Rothamsted, England, and split-plot experiments at Le Rheu, France. They were sowing date (Sept./early Oct. v. mid/late Oct.), soil fungicide (none v. nuarimol at 1.1 kg/ha), seed treatment (organomercury or mancozeb v. triadimenol), autumn nitrogen (none v. 50-60 kg/ha), nitrogen timing in spring (single v. divided application), form of nitrogen (calcium or ammonium nitrate v. ammonium sulphate) and seed rate (200 v. 100 kg/ha). Delayed sowing was usually the most effective single treatment in decreasing take-all. Reduced seed rate and soil fungicide also decreased the disease, but the latter was usually effective only where moderate or severe take-all developed and at Le Rheu it was then as effective as delayed sowing. Seed treatment (triadimenol) was sometimes effective. Fertiliser treatments had relatively little effect, except that ammonium sulphate was effective in early-sown plots at Le Rheu, where it was applied in autumn and spring (it was applied only in spring at Rothamsted). Significant interactions were few and no combination of treatments gave consistently better control than individual treatments.

INTRODUCTION

Despite frequent optimism in the scientific literature and the farming press, no one effective treatment has emerged for the control of take-all (causal fungus: *Gaeumannomyces graminis* var. *tritici*) in wheat. Methods currently available to the farmer include delayed sowing (Taylor *et al.*, 1983; Prew *et al.*, 1986), supplying adequate fertiliser nitrogen and adjusting its timing (Prew *et al.*, 1986), but they are at best only partially effective. Some fungicides can also decrease take-all but their effects are inconsistent or their commercial use unpractical (Bateman, 1989).

A number of treatments that have been reported to decrease take-all were tested at Rothamsted and Le Rheu to establish which was best and whether any combinations were better.

METHODS

In four annual experiments at Rothamsted in 1986-9, winter wheat cv.

Avalon was grown as a third consecutive cereal. In the experiments at Le Rheu, in 1988 and 1989, cv. Arminda was grown as a second wheat. Seven factors (Table 1) were each tested at one level which, apart from sowing date, approximated to normal farm practice (standard treatment), and at another level expected to decrease take-all (test treatment). A maximum of six factors were tested in any one experiment. At Rothamsted the treatments were tested in factorial combination using a single replicate, except in 1986 when there was a half replicate of 2^6. At Le Rheu, nitrogen treatments were applied to whole plots, and sub-plots tested all combinations of seed treatment and soil fungicide. The treatments representing the level of a factor were rarely identical in both countries but they are assumed to provide approximately similar comparisons for the purpose of this report.

TABLE 1. Factors and levels tested.

Factor	Standard treatment	Test treatment
Sowing date	Early: 21-25 Sept.(R); 5 Oct.(L)	Late: 18-31 Oct.(R); 30 Oct.(L)
Seed rate	Normal: 200 kg/ha	Low: 100 kg/ha
Soil fungicide	None	Nuarimol at 1.0-1.3 kg AI/ha into the seed bed
Seed treatment	Organomercury (R); mancozeb (L)	Triadimenol: at 0.5 g AI/kg seed + fuberidazole as 'Baytan' (R); 0.3 g AI/kg seed (L)
Autumn N	None	60 kg/ha as ammonium nitrate (R); 50 kg/ha as calcium nitrate or ammonium sulphate (L)
N timing in spring	Single: 200 kg/ha in April	Divided: 40 kg/ha in Feb./ March + 160 kg/ha in April
N form	Ammonium nitrate (R); calcium nitrate (L)	Ammonium sulphate in spring (R) or autumn and spring (L)

R, Rothamsted; L, Le Rheu

At Rothamsted the plots were 10x3 m, and at Le Rheu whole plots were 10.4x4.5 m and sub-plots 4.5x2.6 m. Plants were sampled from short lengths of row in 'W' patterns within plots at Rothamsted and in 'V' patterns within sub-plots at Le Rheu. The samples were taken at intervals through each season to assess plant growth, stem base diseases and take-all. Only take-all assessments in June (GS 69; end of anthesis) are described here, because they usually correlate best with grain yields. A take-all rating (TAR) per plot was calculated as % plants with slight take-all on the roots + 2(% plants with moderate take-all) + 3(% plants with severe take-all) (Dyke & Slope, 1978). Plots were combine harvested and grain yields measured.

RESULTS AND DISCUSSION

Late sowing significantly decreased take-all at GS 69 in four out of five experiments (Table 2), supporting advice in the UK to delay the sowing of wheat at risk. At Rothamsted in 1989, small patches of severe take-all developed regardless of sowing date and the low seed rate was more effective than delayed sowing. In factorial experiments elsewhere in 1984-6 the effects of sowing date persisted through the growing season in only one of the three years (Werker & Gilligan, 1990).

TABLE 2. Effects of test treatments on take-all ratings expressed as percentages of those for standard treatments.

	Rothamsted				Le Rheu	
Factor	1986 (95)[1]	1987 (129)	1988 (109)	1989 (101)	1988 (133)	1989 (148)
Sowing date	-13.7*	-35.9*	-45.8*	-13.1	-19.0*	NT
Seed rate	NT	NT	NT	-24.6*	NT	NT
Soil fungicide	-13.6	-23.4*	-13.7	-24.9*	-17.8*	-21.1*
Seed treatment	+13.5	-3.8	-30.2*	NT	-11.9*	-5.3
Autumn N	0	-5.3	-15.3	-2.7	0	+5.9
N timing	-4.1	+0.8	+19.0	NT	NT	NT
N form	-4.1	-3.8	-9.6	NT	-22.0*	-24.0*

[1]Mean take-all rating in each experiment.
* Significant (P=0.05).
NT, not tested.

Overall, soil fungicide was the second most effective treatment against take-all (Table 2). Treating seed with triadimenol had inconsistent effects (cf. Werker & Gilligan, 1990), but its effects on root symptoms in 1988 were greater than those seen previously on wheat at Rothamsted (Bateman, 1986). More reliable control might be achieved using higher concentrations of triadimenol applied as seed coatings (Cavelier & Lucas, 1985).

Although take-all has been decreased in some experiments by applying nitrogen in autumn (Werker & Gilligan, 1990), by dividing the spring dressing (Prew *et al.*, 1986), or by using ammonium sulphate instead of nitrate salts (Gutteridge *et al.*, 1987; Lucas *et al.*, 1988), the effects in these experiments were mostly small. The only exception was the sulphate treatment at Le Rheu; its application in autumn may have caused a gradual build up of antagonistic micro-organisms, perhaps related to a change in rhizosphere pH.

In the absence of other diseases and pests, later-sown crops often yield less than early-sown crops (Prew *et al.*, 1986). The yield benefits from delayed sowing (Table 3) probably, therefore, resulted mainly from decreased take-all. At Rothamsted, wet weather after sowing in October 1986 (i.e. the 1987 crop) resulted in poor emergence and a sparse crop, and yield was not increased. The low seed rate did not improve yield in 1989 even though take-all was less.

TABLE 3. Effects of test treatments on grain yields expressed as percentages of those for the standard treatments.

	Rothamsted				Le Rheu	
Factor	1986 (8.47)[1]	1987 (5.99)	1988 (7.65)	1989 (6.82)	1988 (3.73)	1989 (4.16)
Sowing date	+7.7*	-3.6	+17.9*	+3.9*	+2.8	NT
Seed rate	NT	NT	NT	-1.5	NT	NT
Soil fungicide	+3.2	+5.8	-0.1	+2.4*	+15.1*	+1.8
Seed treatment	-1.1	-0.7	+4.7	NT	+2.9	+3.8
Autumn N	+1.8	+7.3*	+6.6*	+3.6*	-5.8	-2.8
Divided N	+5.5	+1.2	+4.3	NT	NT	NT
N form	-0.1	-3.0	+2.5	NT	+3.0	+18.1*

[1]Mean grain yield (t/ha) in each experiment.
* Significant (P=0.05).
NT, not tested.

Soil fungicide usually had little effect on yield (Table 3). Slight yield depression by nuarimol has sometimes been noted and it is not, therefore, the ideal fungicide for soil treatment, although it was the best of those tested (Bateman, 1984). Seed treatment with triadimenol did not increase yield significantly, but it usually does so only where take-all is severe enough to cause extensive prematurely-ripened patches (Bateman, 1986). At Rothamsted in 1988 triadimenol seed treatment decreased such patches from 25% to 16% of the crop, but this was insufficient to affect yield.

At Rothamsted, autumn nitrogen often gave a yield benefit despite having no significant effect on take-all, but divided nitrogen in spring had no effect (Table 3). Ammonium sulphate, applied in both autumn and spring, increased yield at Le Rheu in 1989.

Yields may sometimes have been influenced by eyespot. It was prevalent at Rothamsted in 1988 only, and was unaffected at GS 69 by most treatments, except divided nitrogen, which increased the proportion of shoots infected from 54% to 69%. At Le Rheu, late sowing in 1988 decreased eyespot from 54% to 12% of shoots infected and in 1989 its incidence was increased from 45% to 64% by ammonium sulphate and decreased from 83% to 75% by soil fungicide.

The strength of interactions among factors varied from year to year, although sowing date was most often involved in the significant interactions. Combining two test treatments usually had no significantly greater effect than applying the treatments individually, but the occasions when it did were all at Rothamsted (Table 4). There were few significant second order (three-factor) interactions, but Table 5 shows the number of occasions on which each test treatment contributed to least disease or greatest yield in three-factor combinations. This generally reflects the performance of the treatments individually (Tables 1 and 2) although a more consistent contribution of triadimenol seed treatment against disease is apparent at Rothamsted.

TABLE 4. Significant first order interactions in which combinations of the two test treatments resulted in smallest take-all ratings (TAR) at Rothamsted.

1986 Treatments		TAR[1]	1987 Treatments		TAR[1]
Sowing date x N timing			*Sowing date x soil fungicide*		
Early-sown	Single N	93	Early-sown	None	181
	Divided N	111		Nuarimol	131
Late-sown	Single N	100	Late-sown	None	110
	Divided N	75		Nuarimol	91
Soil fungicide x autumn N					
None	None	90			
	60 kg N/ha	113			
Nuarimol	None	100			
	60 kg N/ha	76			
SED (10 d.f.)		9.1	SED (19 d.f.)		5.7

[1]Mean TARs are given in Table 2.

TABLE 5. Number of occasions each test treatment occurred in three-factor combinations that resulted in the smallest take-all rating (TAR) or the greatest yield.

Test treatment	Rothamsted 1987-89			Le Rheu 1988-89			Total		
	NI	ST	GY	NI	ST	GY	NI	ST	GY
Late sowing	23	21	13	6	6	3	29	27	16
Low seed rate	3	3	0	NT	NT	NT	3	3	0
Soil fungicide	23	23	13	9	9	9	32	32	22
Seed treatment	20	19	9	9	2	8	29	21	17
Autumn N	23	18	23	9	5	1	32	23	24
Divided N	20	5	14	NT	NT	NT	20	5	14
Amm. sulphate	20	17	9	9	9	8	29	26	17

NI, number of interactions.
ST, smallest TAR.
GY, greatest yield.
NT, not tested.

No simple 'package' of best treatments for minimising take-all has emerged from these experiments, but the importance of not sowing early those crops at risk from take-all is confirmed. Later sowing and autumn-applied nitrogen (which often increased yield), are against current advice for minimising environmental effects because they can increase amounts of nitrate available for leaching. The best option for controlling take-all is still to avoid risk situations by not growing second-fourth wheats, or by growing cereals in long sequences in which take-all decline is operative.

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EFFECTS OF DIFFERENT CROPPING SEQUENCES ON SOIL-BORNE INOCULUM OF *RHIZOCTONIA SOLANI* MEASURED USING MINI TUBERS OF *SOLANUM TUBEROSUM*.

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ABSTRACT

The trend for growers to practise more intensive potato cropping has reportedly led to an increase in inoculum of *Rhizoctonia solani* surviving in the soil. This possibility was examined on a site where a crop rotation experiment had previously tested twelve different cropping sequences over a period of seven years. The experiment involved the use of mini tubers (thereby excluding tuber-borne inoculum from the experiment) and provided clear evidence for the importance of soil-borne inoculum in the infection of *Solanum tuberosum*. Moreover, variation in the incidence and severity of disease could be attributed directly to the effect of different cropping sequences. The implications of these results, in relation to controlling soil-borne inoculum of *R. solani*, are discussed.

INTRODUCTION

Consumer demand for high quality ware tubers and an extended seasonal supply of new potatoes has resulted in growers practising closer crop rotations, and sometimes multiple cropping, to maximise use of the most suitable soils for growing the crop. This trend, which started in the early 1980s, reportedly increases the risk of damage by soil-borne pests and diseases, previously controlled by longer rotations (Forrest, 1989). As a consequence, soil-borne diseases such as *Rhizoctonia solani* are receiving an increasing amount of attention. *R. solani* is a serious pathogen of potato plants causing stem canker, stolon canker and black scurf on tubers. Infection can be initiated by tuber- and soil-borne inoculum (Scholte, 1987), although, until recently, the risk of infection from soil-borne inoculum of *R. solani* was considered to be comparatively minor (Bolkan, 1976; Weinhold *et al.*, 1978). Tuber-borne inoculum of *R. solani* can be controlled relatively easily through the use of chemical treatments such as 'Rizolex' or 'Monceren' but there is evidence to suggest that these treatments may not be effective against soil-borne inoculum. Hide & Read (1990) found that although satisfactory control from seed tuber treatments was usually obtained in a first crop of potatoes, chemical controls became increasingly less effective in second and subsequent crops grown on the same site. They attributed this effect to an increase in inoculum surviving in the soil as a result of frequent or continuous cropping with plants which are susceptible to *R. solani*. However, this contrasts with work described by Bolkan (1976) who did not find any build-up of soil-borne inoculum because of intensive potato cropping.

The experiment described here sought to provide conclusive evidence for the importance of soil-borne inoculum of *R. solani* by using disease-free mini seed tubers

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(Twygen Ltd, Dundee) as the planting material, to eliminate the possibility of infection from tuber-borne inoculum, and to compare the effects of different cropping sequences on the build-up of soil-borne inoculum, estimated by measuring the incidence and severity of stem canker in the crop.

MATERIALS AND METHODS

The original experiment was established at the Woburn Farm of Rothamsted Experimental Station in 1982 by Dr G.A. Hide to identify problems associated with growing potatoes in shorter and longer rotations than those used traditionally. The rotations investigated were: (i) potatoes susceptible to *Globodera rostochiensis* (cv. Desiree) grown in a two-year rotation, (ii) potatoes resistant to *G. rostochiensis* (cv. Maris Piper) grown alternately with susceptible potatoes (cv. Desiree) in a two-year rotation, (iii) potatoes (cv. Desiree) grown in a four-year rotation and (iv) potatoes (cv. Desiree) grown in the fifth, sixth and seventh year after the previous potato crop. Spring barley (cv. Triumph) was grown in all non-potato years. The two- and four-year rotations were each started in three consecutive years which gave a total of twelve different sequences (Table 1). There were two randomised blocks each containing twelve plots representing the twelve sequences. Each plot was 24.3m long with twelve rows 75cm apart. An additional (25th) plot, which had not been planted with potatoes for at least eight years, was included as an unreplicated control treatment. The initial experiment ended after seven years and the results are reported elsewhere (Hide & Read, 1990), however, the plots were maintained and used in the following season (1989) for the experiment described below.

TABLE 1. Summary of the cropping sequences

Sequence number	Year 1	2	3	4	5	6	7
1a	**P**	B	**M**	B	**P**	B	**M**
2a	B	**P**	B	**M**	B	**P**	B
3a	B	B	**P**	B	**M**	B	**P**
1b	**P**	B	**P**	B	**P**	B	**P**
2b	B	**P**	B	**P**	B	**P**	B
3b	B	B	**P**	B	**P**	B	**P**
4	**P**	B	B	B	**P**	B	B
5	B	**P**	B	B	B	**P**	B
6	B	B	**P**	B	B	B	**P**
7	B	B	B	B	**P**	B	B
8	B	B	B	B	B	**P**	B
9	B	B	B	B	B	B	**P**

P - potatoes (cv. Desiree) susceptible to *Globodera rostochiensis*.
M - potatoes (cv. Maris Piper) resistant to *Globodera rostochiensis*.
B - spring barley (cv. Triumph).

In 1989, the plots were again planted with twelve rows of potatoes. Commercial seed tubers (cv. Desiree) were planted by machine in all but the eighth row. In this row (the test row), 79 healthy mini seed tubers (cv. Pentland Squire), each weighing 4-8g were planted by hand into the ridges at a spacing of 30cm. The plants were harvested two weeks after emergence and scored for the incidence (presence or absence) of stem canker and its severity using a scoring system similar to the one devised by Adams *et al.* (1980). Each stem was assigned to one of five categories according to the proportion of the stem circumference affected by the lesion. The categories were: (1) no stem canker, (2) up to one-third of the stem circumference affected by the lesion, (3) one- to two-thirds of the stem circumference affected, (4) greater than two-thirds of the stem circumference affected (including lesions extending round the whole circumference), and (5) stems completely girdled and pruned by the lesion. A disease score for stem canker was then calculated by weighting the number of stems in categories 1-3 by 0, 1 and 2 respectively, and those in categories 4 and 5 by 3. The sums of the severity scores were expressed as percentages of the maximum possible (i.e. number of stems x 3) and these values were used in the analysis of variance. If susceptibility to *G. rostochiensis* is ignored, pairs of cropping sequences testing two-year rotations were identical (i.e. 1a+1b, 2a+2b and 3a+3b) and it is the mean values for these pairs that are presented below.

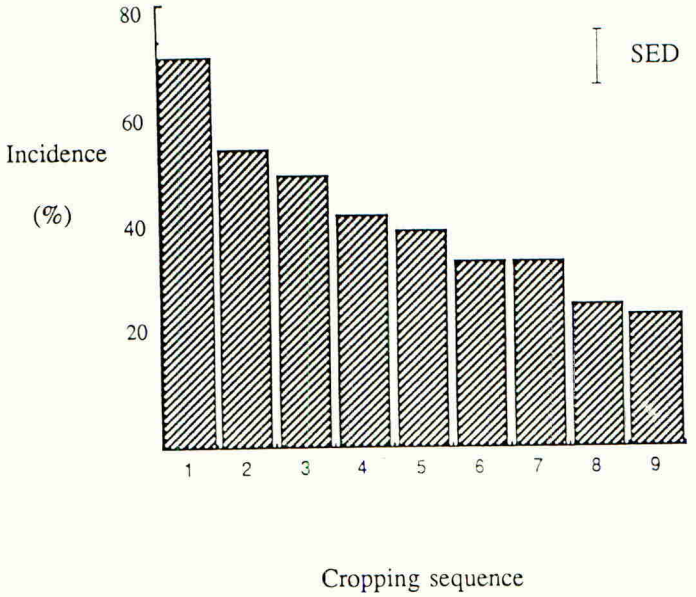
RESULTS

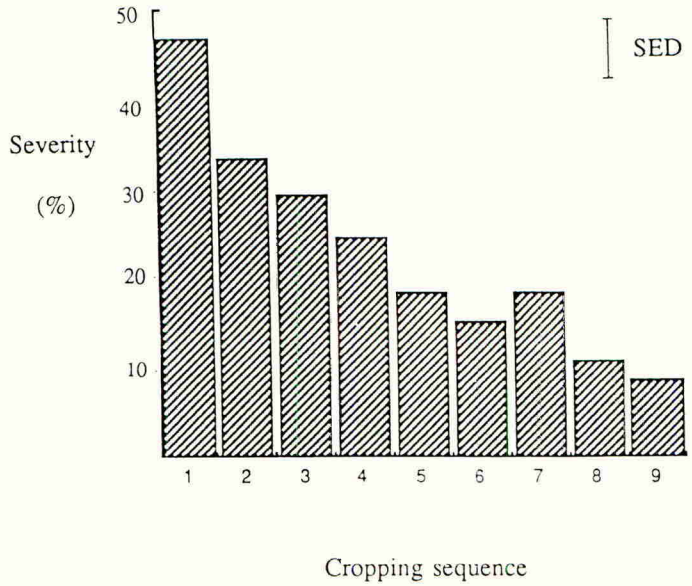
Stem canker developed in all of the plots except for the control plot where no potatoes had been grown for at least eight years. Both the incidence and severity of stem canker were also influenced significantly by cropping history (Fig. 1). Most stem canker (71% of plants infected) was seen in sequences 1ab, 3ab, 6 and 9, in which the test crop was preceded by a potato crop in the previous season, and least in the other sequences, which followed barley. Within each group, stem canker was also affected by the frequency of potatoes in the preceding seven years. In sequences with potatoes in the previous year, more stem canker occurred where potatoes were grown in alternate years (two-year rotations) than in the four- and six-year rotations. Where barley was grown in the previous year, there was most stem canker in the plots with potatoes in the year before that and the greatest frequency of potatoes in the sequence (two-year rotation) but less in plots with potatoes in the sixth year and smaller frequencies of potatoes in the sequence (four- and six-year rotations). Least stem canker was seen in plots which did not have potatoes after the fifth year (sequences 4 and 7). Thus, although the incidence and severity of stem canker were determined primarily by the crop grown in the previous year, the disease was also increased by increasing the frequency of potatoes in the rotation.

DISCUSSION

This experiment is the first reported use of mini seed tubers in the investigation of naturally occurring soil-borne inoculum of *R. solani*, and it provided convincing evidence for the importance of soil-borne inoculum of *R. solani* in the infection of *S. tuberosum*. Mini tubers have an advantage over chemically disinfected commercial seed tubers because they originate from micropropagated material and eliminate the possibility of tuber-borne inoculum affecting the experiment. By comparison, commercial seed tubers which appear to be 'clean',

FIGURE 1. The effect of different cropping sequences on the incidence and severity of stem canker (see Table 1 for details of cropping sequences).





may carry hyphae of *R. solani* which are only visible microscopically, and disinfected seed tubers may still carry some hyphae of *R. solani* just below their surface at sufficient depths to render chemical treatments ineffective.

Incidence and severity of stem canker was shown to vary markedly according to the cropping history of the plot. The factor that was most influential in determining the amount of stem canker was the crop (potatoes or barley) grown in the year preceding the experiment, irrespective of cropping frequency. This is in direct contrast to Scholte (1987) who found that the frequency of potatoes had much more effect on the occurrence of *R. solani* than did the immediately preceding crop. In agreement with the findings of Scholte (1987) and Frank & Murphy (1977), however, there was an increase in stem canker with increasing frequency of potatoes in the rotation, but the effect was significantly smaller than in the earlier reports. It is important to remember that these conclusions are based only on the incidence and severity of stem canker, and although it has been reported that stem canker is highly correlated with stolon canker and black scurf (Simons, 1989), other workers do not agree. Scholte (1987) and Lamers (1981) found that whilst the level of infection of stems by *R. solani* increased with increasing frequency of potatoes in a rotation, there was not necessarily a concomitant increase in the incidence of black scurf on progeny tubers. This inconsistency requires further investigation since an increase in stem canker may not result in an economic loss to the grower.

It is apparent from these results that the importance of soil-borne relative to tuber-borne inoculum of *R. solani* has, in some circumstances, been underestimated. The effect of different cropping sequences is almost certainly correlated with the density of inoculum surviving in the soil, and this has important implications for growers who practice closer crop rotations or multiple cropping. There is evidence to suggest that chemical treatment of seed tubers will be of little value in controlling soil-borne inoculum, in which case longer crop rotations, perhaps in conjunction with the use of mini or micropropagated seed tubers, appears to offer the only method for controlling soil-borne inoculum of *R. solani*. Unfortunately, there is no suitable method available for assessing the density of soil-borne inoculum in field soil, but a simple and efficient method for doing so might allow growers to adjust the frequency of cropping potatoes to take account of the risk. However, in considering crops which are suitable for rotation with potatoes, it is important to determine whether the alternative crop is susceptible to *R. solani*.

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EFFECTS OF PROPAMOCARB ON THE DEVELOPMENT OF PHYTOPHTHORA INFESTANS ON POTATOES

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ABSTRACT

In vivo studies with two strains of Phytophthora infestans known to be highly metalaxyl-sensitive (ED_{50} = 0.05 μg metalaxyl/ml) or resistant (ED_{50} = 1450 μg metalaxyl/ml) showed no difference in their sensitivity to propamocarb, with ED_{50} values of 1191 μg propamocarb/ml for the sensitive and 1104 μg propamocarb/ml for the resistant strain. Propamocarb applied 24 hours after infection gave control of P. infestans equal to that of treatments 24, 48 and 72 hours before inoculation. Application 48 and 72 hours after inoculation gave significantly less control. Tests using simulated rain suggested an increase in rainfastness of propamocarb with time. Control of P. infestans increased linearly as the interval between propamocarb application and rain treatment increased from 0.5 to 24.0 hours.

INTRODUCTION

The carbamate fungicide propamocarb was first used commercially in 1978 (Pieroh et al., 1978). The compound is marketed as propamocarb HCl [propyl-N-(ℓ-dimethylaminopropyl) carbamate-monohydro-chloride].

Propamocarb exhibits specific activity against many of the Oomycetes including Phytophthora spp (Pieroh et al., 1978) and is primarily used as a soil treatment in ornamentals and vegetables. It is mainly fungistatic possibly because it affects cell membrane function (Papavizas et al., 1978) and, although mainly used prophylactically, has curative action against some Pythium spp (Pieroh et al., 1978; Rapp & Richter, 1982). Propamocarb is taken up by plant roots, possibly facilitated by its high water solubility (Edgington 1981), and moves acropetally into the foliage. No evidence for symplastic transport has been found.

Propamocarb also shows promise for the control of Phytophthora infestans (Cooke et al., 1981), and its widespread use in Israel (Cohen, 1986) caused interest in developing propamocarb for this use and prompted the work described in this paper.

MATERIALS AND METHODS

The potato cultivar 'Arran Pilot', which is susceptible to late blight, was used in all experiments. Two strains of P. infestans were obtained from the culture collection at Chesterford Park Research Station. A metalaxyl-sensitive strain (with an ED_{50} of 0.05 μg metalaxyl/ml) was maintained on untreated potato plants. A metalaxyl-resistant strain (with an ED_{50} of 1450 μg metalaxyl/ml) had been maintained for at least 52 passages on potato plants treated with 100 μg/ml metalaxyl. After inoculation, stock plants were covered with polyethylene and incubated in a growth chamber with a 16/8 hours light/dark cycle at 12°C. Sporangia for experiments were removed from leaflets of stock plants by gently stirring in cold (4°C) distilled water and their concentration adjusted to 30 x 10^3 sporangia/ml. Adaxial leaf surfaces were inoculated with the sporangial suspension by spraying to run-off. Inoculated plants were placed on trays of water and covered with polyethylene. They were incubated for the first two days in a growth chamber with a 16/8 hours dark/light cycle (20°C/16°C) and subsequently in a 16/8 hours light/dark cycle (16°C/20°C).

The commercial 72.2% water-soluble concentrate formulation of propamocarb HCl was used as a solution in distilled water and applied to adaxial leaf surfaces using an automatic precision track sprayer fitted with two Teejet 8004E flat fan nozzles. The spray volume corresponded to 400 l/ha.

Six days after inoculation the percentage leaf area damaged was assessed visually. Analyses of variance (ANOVA) were performed on log transforms of percentage disease control, viz. $\log_e$ (100.5 - p), where $\log_e$ is the natural log and p is percentage disease. Percentages were subtracted from 100.5 before transformation to deal with the situation where p = 100%.

Sensitivity to propamocarb HCl was determined by linear regression, the resulting lines being used to calculate ED_{50} values (the effective concentration of fungicide in μg a.i./ml providing 50% disease control).

RESULTS

Response of metalaxyl-sensitive and resistant strains of P. infestans to propamocarb HCl

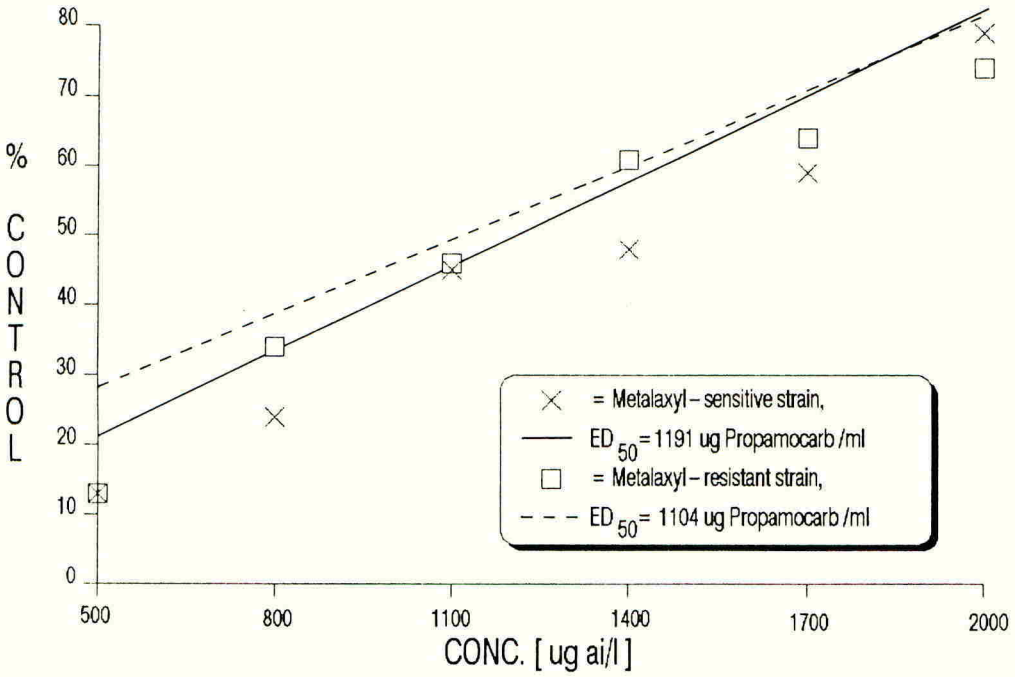
The sensitivity of the two Phytophthora strains was investigated by spraying potato plants with a dilution series of propamocarb HCl 24h before inoculation. The concentrations used increased from 500 to 2000 μg a.i./ml in 300 μg a.i./ml steps. For each strain there were six plants per treatment (fungicide concentrations plus inoculated control) arranged in a randomised block design.

Control plants inoculated with the sensitive and resistant strains had 87 & 70% infection respectively. For both strains the fungicide treatments gave a clear dose response (Fig. 1). The calculated ED_{50} values were 1191 μg a.i./ml propamocarb HCl for the metalaxyl-sensitive strain and 1104 μg a.i./ml propamocarb HCl for the metalaxyl-resistant strain and there were no significant differences between them ($P < 0.05$).

However, the results also show that neither strain was completely controlled at the rates tested. Activity ranged from 79% and 74% disease control (metalaxyl-sensitive and resistant strains respectively) at the highest concentration of 2000 μg a.i./ml down to 13% (both strains) at 500 μg ai/ml.

FIGURE 1.

Response of metalaxyl-sensitive and resistant strains of P. infestans to propamocarb HCl.



Effects of varying the interval between inoculation and fungicide treatment on efficacy of propamocarb HCl against P. infestans

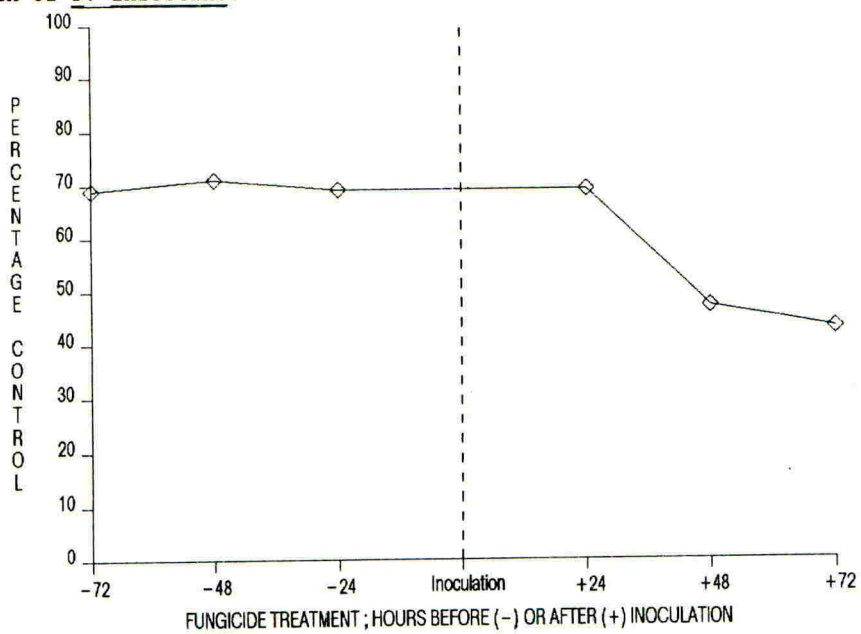
This experiment compared the protective and curative activity of propamocarb in controlling P. infestans. Potato plants were treated with propamocarb HCl at 1700 μg a.i./ml 24, 48 and 72 hours before and after inoculation with the metalaxyl-resistant strain. There were six plants per treatment (application intervals plus inoculated control) arranged in a randomised block design.

Twenty four hours after inoculation, most control plants were still symptom-free. After 48 hours, percentage leaf area affected was 15-30% and after 72 hours it had increased to 30-50%. Fungicide treated plants were scored after six days when 95% of the leaf area of control plants was blighted.

Disease control was identical whether plants were treated 24, 48 or 72 hours before, or 24 hours after inoculation (Fig 2), propamocarb HCl providing about 70% disease control. However, when the fungicide was applied 48 or 72 hours after inoculation, efficacy decreased significantly ($P < 0.01$) to 48% and 43% control, respectively.

FIGURE 2.

Effects of varying the interval between inoculation and fungicide treatment on efficacy of propamocarb HCl (1700 μg a.i./ml)against a metalaxyl-resistant strain of P. infestans.



Effects of simulated rainfall on the activity of propamocarb HCl against P. infestans

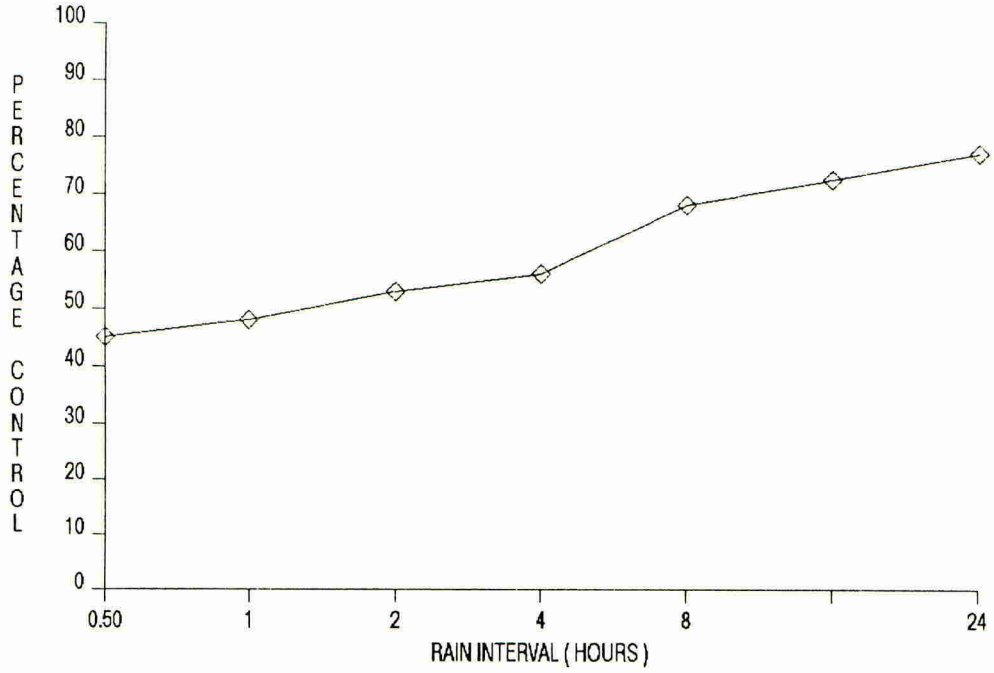
This experiment tested the hypothesis that the time of onset of rain after the application of propamocarb HCl affects the amount of chemical removed and, therefore, efficacy against P. infestans.

A 'raintower' consisting of an adjustable overhead sprinkler system was used as a rainfall simulator. The quantity of water delivered was measured by a rain gauge placed next to the potato plants. Plants were treated with 1700 μg a.i./ml propamocarb HCl. After intervals of 0.5, 1.0, 2.0, 4.0, 8.0 or 24.0 hours the plants were exposed for a period of one hour to 12 mm rain/h. To prevent chemical from the leaf surface being washed in to the soil, pots were covered with a polyethylene sheet which was wrapped around the stem-base of each plant. After the last rain treatment had been applied all plants were inoculated with the metalaxyl-resistant strain of P. infestans. Plants were then arranged in a randomised block design with five replicate plants for each rain interval and inoculated controls.

After incubation for six days the percentage leaf area blighted in inoculated control plants averaged 85%. Control averaged 45% when rain was applied 0.5 hours after the fungicide treatment but increased to 77% when rain was not applied until 24 hours after the spray (Fig 3). The fungicide spray had dried on the plants about 1.5 hours after application. Although there was no significant difference in control when rain was applied 8 or 24 hours after spraying it seemed that deposits that had aged generally provided better protection against P. infestans.

FIGURE 3.

Effect of simulated rain applied at different intervals after application of propamocarb HCl (1700 μg a.i./ml) on control of a metalaxyl-resistant strain of P. infestans.



DISCUSSION

The experiments described show that propamocarb was equally active against the metalaxyl-sensitive and resistant strains of Phytophthora infestans. A lack of cross-resistance between the two fungicides in the late blight fungus was also reported by Kendall & Carter (1984) and might be expected since the two compounds are chemically unrelated and presumably have different modes of action. There are no published reports of resistance among fungal pathogens to propamocarb, despite its prolonged and widespread use. This possibly implies that the compound has several sites of action in a fungal organism or acts in an indirect way on host physiology, inducing host defence mechanisms in a similar way to that suggested for fosetyl-Al (Bompeix et al., 1981).

Investigation of the protective activity of propamocarb against P. infestans showed that, irrespective of whether the chemical was applied 24, 48 or 72 hours before inoculation, equally good control was achieved. The application time after inoculation appears to be more critical. A curative propamocarb treatment 24 hours after inoculation was as effective as the protective treatments. When propamocarb was applied 48 and 72 hours after inoculation, control decreased significantly. However, infection pressure in the growth chambers was very high as the conditions were extremely favourable to late blight development and up to 50% leaf area was blighted 72 hours after infection. Against this background, the activity of the last two propamocarb applications must be interpreted with caution and they probably underestimate the curative effects of the compound.

The experiment on rainfastness of propamocarb revealed that there was a linear increase in percentage control with increasing intervals (log time) between propamocarb application and rain treatment. Since the spray had dried after only about 1.5 hours, further uptake probably occurred after this time, thus decreasing the amount of chemical on the leaf surface which was exposed to wash-off.

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THE CONTROL OF LATE DISEASES OF WINTER OILSEED RAPE IN SCOTLAND.

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ABSTRACT

Seven field trials carried out in east and north-east Scotland from 1983/84 to 1988/89 showed that in seasons when disease pressure was low, applications of flowering and post-flowering fungicide sprays for the control of late diseases (alternaria, botrytis and sclerotinia) of winter oilseed rape did not give significant yield benefits. In only eleven out of thirty three comparisons were there economic benefits from late sprays. Disease control was variable.

INTRODUCTION

The most important disease of winter oilseed rape in Scotland is light leaf spot (*Pyrenopeziza brassicae*) and most crops are routinely sprayed in autumn and at stem extension for its control (Wale *et al.*, 1990). Of less importance, but with the potential to cause severe yield reductions, are the late diseases of alternaria (*Alternaria brassicae and Alternaria brassicicola*), botrytis (*Botrytis cinerea*) and sclerotinia (*Sclerotinia sclerotiorum*). These late diseases are relatively common in England and Wales (Hardwick *et al.*, 1989) but in Scotland they are less prevalent. However, since these diseases cause premature pod ripening leading to seed shedding (Ward *et al.*, 1985), many crops in Scotland are routinely sprayed with fungicides at full flower or during pod developement to prevent infection. The data presented here are from seven field trials carried out by the Scottish Agricultural College, comparing the effects of fungicide treatments on yield responses, economic benefits and disease control.

MATERIALS AND METHODS

Seven field trials carried out in east and north-east Scotland (Edinburgh - sites in Borders and Fife and Aberdeen - sites in Grampian and Kincardineshire) from 1983/84 to 1988/89 compared several fungicide treatments for the control of late diseases of winter oilseed rape. Products used were MBC-compounds, prochloraz, iprodione and vinclozolin, applied at full flower (GS 4.5) (Sylvester-Bradley, 1985), 90-95% petal fall (GS 5.9) and two to three weeks after 95% petal fall (GS 6.0+)(Tables 1 & 2). Trials in 1983/84 and 1984/85 also received fungicide sprays at stem extension for light leaf spot control (Table 1) and in 1987/88 and 1988/89 all plots except treatment A were sprayed in autumn (MBC) and spring (MBC or prochloraz) to control this disease.

Fungicides were applied by hand held or tractor mounted sprayers in 194 - 240 litres of water/ha. Individual plot areas ranged from 40-77m^2 and plots were arranged in three to four fully randomised blocks. Five

cultivars were used, in different trials; Ariana, Bienvenu, Cobra, Mikado and Rafal. At Aberdeen in 1987/88 first and second year rapes were present within the same trial.

TABLE 1. Fungicide treatments tested at Aberdeen in 1983/84 and 1984/85.

Treatment	Stem Extension	Full Flower	95% Petal Fall
A	-	-	-
B	prochloraz	-	-
C	-	vinclozolin	-
D	-	-	iprodione
E	prochloraz	vinclozolin	iprodione
F	MBC	-	-
G	MBC	-	iprodione
H	MBC	prochloraz	iprodione

TABLE 2. Fungicide treatments tested in five trials in east and north-east Scotland during 1987/88 and 1988/89.

Treatment	Full Flower	95% Petal Fall	95% Petal Fall + 2/3 weeks
A*	-	-	-
I	-	-	-
J	vinclozolin	-	-
K	vinclozolin + MBC	-	-
L	vinclozolin + MBC+	-	-
M	iprodione + MBC	-	-
N	prochloraz	-	-
O	vinclozolin	iprodione	-
P	vinclozolin	-	iprodione
Q	-	iprodione	-
R	-	-	iprodione
S	iprodione + MBC	-	iprodione
T	prochloraz	-	iprodione
U	iprodione + MBC	iprodione + MBC	-

All chemicals normally applied at 500g AI/ha
* Untreated throughout, all others, including I, received light leaf spot sprays in autumn and spring.
+ MBC in this treatment applied at 1Kg AI/ha

Diseases were assessed on leaves, stems or pods two to four weeks after spraying to estimate incidence and percentage leaf or pod area affected. Plots were harvested and yields corrected to a moisture content of 91%. Economic benefits (£/ha) were calculated as (yield treated - yield untreated) x 265 - fungicide cost, based on a market price of £265/tonne rape seed and using fungicide prices (RRP) current in July 1990. Application costs and returns in subsidies were not included. Calculations for all trials except those in 1983/84 and 1984/85 excluded costs for light leaf spot sprays.

RESULTS

In 1983/84 and 1984/85, plots given late fungicide sprays yielded no better than untreated (Table 3) and none of the fungicides paid for themselves. This possibly reflected the absence of late diseases in 1983/84 and low levels of diseases in 1984/85, when 15% of untreated pods were infected with alternaria but only at trace amounts. An MBC spray at stem extension tended to reduce the incidence of alternaria (treatment F) and the addition of prochloraz at full flower (treatment H) further reduced levels, but these reductions were not significant.

TABLE 3. Yield, economic benefits and incidence of alternaria on pods at Aberdeen (Kincardineshire) 1983/84 and 1984/85.

	Yield (t/ha)		Economic benefits (£/ha)		Pods affected by alternaria
Treatment	83/84 cv. Bienvenu	84/85 cv. Rafal	83/84	84/85	in 84/85 (%)
A	5.05	2.94	-	-	14.7
B	5.12	-	-10.25	-	-
C	4.90	-	-64.36	-	-
D	4.94	-	-55.37	-	-
E	5.19	-	-24.23	-	-
F	-	2.75	-	-60.85	8.5
G	-	2.81	-	-71.17	8.6
H	-	3.03	-	-41.67	1.6
SED+/- (df)	0.12 (44)	0.16 (42)			9.24 (9)

Negative values represent losses

At Aberdeen in 1987/88 the application of only light leaf spot sprays (treatment I) gave yield increases in first and second year rape crops of 0.73 t/ha and 1.08 t/ha, respectively, compared to the untreated (treatment A)(Table 4). In none of the 1987/88 or 1988/89 trials did the addition of a full-flower spray of vinclozolin (treatment J) significantly increase yield over that achieved by light leaf spot sprays alone and at two of the five sites (Aberdeen 1987/88 and the first Edinburgh site in 1988/89) vinclozolin slightly depressed yields. Similarly, no significant yield increases were achieved by using vinclozolin plus MBC (at normal or twice normal rate), iprodione plus MBC or prochloraz at full-flower (treatments K,L,M & N). Treatments including iprodione after full-flower (with or without a treatment at full-flower)(treatments O-U) failed to give yield increases.

Calculations of economic benefits showed large margins for light leaf spot control (treatment I) of £185.25 for first year rape and £270.45 for second year rape (Table 5). Since little or no yield benefits were obtained with late applications of vinclozolin or vinclozolin plus MBC (treatments J,K & L), losses were in the range £3.31-£114.71. Full-flower sprays of iprodione plus MBC (treatment M) and prochloraz (treatment N), however, did give economic benefits ranging from £13.60-£74.55. Benefits of treatments including iprodione sprays after full-flower (treatments O-U) were mostly

TABLE 4. Yields obtained in field trials in 1987/88 and 1988/89 (t/ha).

Treatment	Ab 87/88 cv. Mikado 1st rape	Ab 87/88 cv. Mikado 2nd rape	Ed 87/88 cv. Rafal	Ab 8/89 cv. Rafal	Ed 1 88/89 cv. Cobra	Ed 2 88/89 cv. Ariana
A	3.70	3.90	-	-	-	-
I	4.46	4.98	3.64	3.92	4.12	2.68
J	4.23	4.64	3.66	3.96	3.90	2.95
K	4.38	5.07	-	4.04	-	-
L	-	-	3.71	-	-	-
M	-	-	-	4.16	-	-
N	-	-	4.03	4.08	4.50	-
O	4.14	4.62	4.01	4.02	4.01	-
P	4.23	4.75	3.88	-	-	3.14
Q	4.28	4.80	3.89	3.94	-	-
R	-	-	3.98	3.93	-	-
S	-	-	-	-	-	2.74
T	-	-	-	-	-	2.96
U	-	-	-	-	4.09	3.08
SED+/- (df)	0.208 (28)		0.200 (21)	0.119 (21)	0.280 (15)	0.230 (14)

Ab - Aberdeen (Grampian) Ed - Edinburgh (Borders and Fife)
1st - first year rape 2nd - second year rape

TABLE 5. Economic benefits of fungicide sprays in 1987/88 and 1988/89 (£/ha).

Treatment	Ab 87/88 1st rape	Ab 87/88 2nd rape	Ed 87/88	Ab 88/89	Ed 1 88/89	Ed 2 88/89
I	(185.25)	(270.45)				
J	-85.56	-114.71	-19.31	-14.10	-82.91	-46.94
K	-56.31	-11.26	-	-3.31	-	-
L	-	-	-27.06	-	-	-
M	-	-	-	34.40	-	-
N	-	-	74.55	13.60	71.90	-
O	-135.63	-146.23	47.22	-24.33	-79.98	-
P	-111.78	-111.78	12.77	-	-	71.07
Q	-73.92	-73.92	40.03	-20.92	-	-
R	-	-	63.88	-23.57	-	-
S	-	-	-	-	-	-39.52
T	-	-	-	-	-	19.18
U	-	-	-	-	-66.35	47.60

Values for treatment I (brackets) represent benefits of the light leaf spot spray alone compared to untreated (treatment A). All other benefits are calculated by comparison with treatment I. Negative values represent losses.

TABLE 6. Premature pod ripening and disease severity in 1987/88 and 1988/89.

Treatment	Ab 87/88 % Pod ripening 1st rape	Ab 87/88 % Pod ripening 2nd rape	Ed 87/88 % Incidence Leaf bot.	Ed 87/88 % Incidence Stem bot.	Ed 87/88 % Incidence Stem alt.	Ed 87/88 % Incidence Pod alt.	Ab 88/89 Number of plants/40m^2 with scler.	Ed 2 88/89 % severity leaf bot.
A	9.4	62.8	-	-	-	-	-	-
I	1.5	15.8	23.8	1.6	1.5	3.0	4.25	69
J	1.3	19.2	3.0	0.1	0.4	0.5	1.25	38
K	1.3	6.9	-	-	-	-	3.00	-
L	-	-	4.0	0.1	0.7	0.3	-	-
M	-	-	-	-	-	-	0.75	-
N	-	-	4.5	0.0	0.9	1.9	0.75	-
O	0.5	12.5	2.8	0.0	0.0	0.0	3.25	-
P	1.6	17.0	2.8	0.0	0.0	0.0	-	43
Q	0.5	4.7	12.8	0.6	0.2	0.0	3.0	-
R	-	-	25.0	0.5	0.1	0.1	3.0	-
S	-	-	-	-	-	-	-	38
T	-	-	-	-	-	-	-	80
U	-	-	-	-	-	-	-	50
SED+/- (df)	6.79(28)		2.3(21)	0.3(21)	0.2(21)	0.5(21)	1.8(21)	24(14)

Disease data indicate percentage incidence and severity of leaves, pods and stems affected by alternaria (alt.), botrytis (bot.) or sclerotinia (scler.)

mostly negative, with eight of the nineteen comparisons giving losses of greater than £50/ha.

The effects of full flower sprays on pod ripening and disease levels were variable. At the Aberdeen site in 1987/88 there was extensive premature pod ripening, particularly in the second rape (Table 6). The light leaf spot treatment (I) reduced premature pod ripening compared to untreated (treatment A) but the addition of vinclozolin at full-flower failed to give any further reduction, except when MBC was included (treatment K). The addition of a full-flower spray of vinclozolin, vinclozolin plus MBC at the double rate or prochloraz (treatments J,L & M) significantly reduced the incidence of botrytis on leaves and stems and alternaria on stems and pods at the Edinburgh site in 1987/88, but full-flower sprays failed to control diseases in 1988/89. The addition of very late sprays, at 95% petal drop or later (treatments O-U) gave no further disease control over that already achieved by full-flower sprays.

DISCUSSION

The seven trials described were intended to examine the efficacy of various products which are used prophylactically to control late diseases of winter oilseed rape. There is great pressure on farmers in Scotland to use these prophylactic sprays 'just in case' disease develops. However,

with other pressures to reduce pesticide usage, the use of fungicides unnecessarily is unacceptable. Results presented here show that in seasons when alternaria, botrytis and sclerotinia remain low, these late sprays mostly failed to give significant yield or economic responses. In only eleven out of thirty three comparisons between treatments containing late sprays was there an economic benefit: in only four of these was the benefit greater than £50/ha and in twelve of the comparisons losses were £50/ha or more. This lack of yield response, even where diseases were reduced by the use of full-flower sprays, agrees with findings in England and Wales (Gladders, 1987). How these late fungicides will perform under wet summers, when conditions are more conducive to late disease development (Ward *et al.*, 1985), has yet to be tested.

In some situations prophylactic sprays may be justified. For example, a large acreage of the variety Cobra is currently grown in Scotland. This variety is susceptible to botrytis and is weak strawed (Anon, 1990) and thus prone to early lodging. In lodged crops, humidities and temperatures can favour disease development, thus a prophylactic spray applied before lodging could prevent this. Prophylactic sprays may also be necessary for control of sclerotinia in fields that are at risk, either from previous oilseed rape crops or other susceptible crops (eg. potatoes, carrots and peas). Alternaria can occur on the leaves but is mainly a problem if the pods are infected. When disease is slight the use of fungicide sprays is not cost effective, but when the disease is present on the upper leaves or lower pods a spray is advisable.

In summary, although the use of routine fungicide sprays for the control of alternaria, botrytis and sclerotinia cannot be recommended on the basis of the results described, it is best to assess each crop individually and spray only when disease risk criteria are achieved.

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EFFECTS OF SEED TREATMENTS ON SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM*

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ABSTRACT

Twenty agrochemicals were applied to culture produced sclerotia of *Sclerotinia sclerotiorum* isolated from oilseed rape, peas, sunflower or potato. Three treatments completely inhibited myceliogenic germination *in vitro* of all isolates for more than 30 days, 11 treatments delayed germination, and 8 treatments had no effect. Treatments showed little effect on growth rate of mycelium from germinated sclerotia but the numbers of new sclerotia produced were sometimes reduced. Only five treatments reduced carpogenic germination, but another three reduced the numbers of apothecia produced per sclerotium of the oilseed rape isolate.

INTRODUCTION

Seed harvested from infected crops, e.g. linseed (Mitchell *et al.*, 1986), peas (Mitchell observations in 1988) and sunflower (Hoes & Huang, 1986) may be contaminated with sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. These sources pose a risk to crops grown from contaminated seed and could serve to widen the distribution of this pathogen. The risks might be reduced by treating the seed. This study examined the effects of various chemicals on the germination of sclerotia under laboratory and field conditions.

MATERIALS AND METHODS

Four isolates of *S. sclerotiorum*, from winter oilseed rape (JN1 - Mylchreest & Wheeler, 1987) (OSR); sunflower cv. Asmer 3, 1988 (Sun); peas cv. Bunting, 1988 (Pea) and potato cv. Ausonia, 1988 (Pot) were used and sclerotia of each produced on wheat grain (Mylchreest & Wheeler, 1987).

Cleaned sclerotia were airdried overnight and those of a similar size to wheat grain selected. Lots of 50 sclerotia of each isolate were placed individually in plastic bags and untreated wheat cv. Armada added to give a total weight of 200 g per bag. Seed and sclerotia were treated with liquid formulations of fungicide for 7s using a machine designed by I.C.I. Plant Protection and manufactured by Marline (General Engineers Ltd.). Dust treatments were added to wheat and sclerotia in a glass jar, shaken by hand and rotated on electric rollers for 20 min. After treatment the sclerotia were separated from the seed.

Commercially available products used as seed treatments on rape, peas, beans and linseed were selected, plus some individual active ingredients and the two dicarboxymides used as sprays for *Sclerotinia* control in the UK. The treatments, and rate of application to 200 g seed were:- (1) None (2) bromophos + captan + thiabendazole (Tbz) ('Bromotex-T' at 0.80 g) (3) 'H-177' (supplied by Agrichem; at 0.73 ml) (4) Tbz + thiram ('Hy-Vic' at

0.50 ml) (5) thiram ('Agrichem Flowable Thiram' at 0.20 ml) (6) Tbz + thiram ('HY-TL' at 0.33 ml) (7) prochloraz ('Prelude 20 LF' at 0.40 ml) (8) vinclozolin ('Ronilan' at 0.80 ml) (9) fenpropimorph technical grade (97.2% AI at 2.80 ml) (10) fenpropimorph technical grase (97.2% AI at 5.60 ml) (11) metalaxyl + Tbz + thiram ('Apron Combi 453 FS' at 0.40 ml) (12) gamma-HCH + thiram + fenpropimorph ('Lindex Plus FS' at 4.40 ml) (13) triadimenol + fuberidazole ('Baytan' at 0.30 g) (14) tecnazene ('Fusarex' at 0.45 g) (15) drazoxolon ('Mil-col 30' at 0.33 ml) (16) ethirimol (4000 ppm at 1.34 ml) (17) hexaconazole (5% w:v at 1.0 ml) (18) flutriafol + Tbz + ethirimol ('Ferrax' at 1.0 ml) (19) ('Ferrax' at 2.0 ml) (20) flutriafol (30 g/l at 0.30 ml) (21) flutriafol (400 g/l at 4.0 ml) (22) fosetyl-al + captan + Tbz ('Aliette Extra' at 0.58 g) (23) iprodione ('Rovral Flo' at 1.60 ml) (24) 'San 793 F' (supplied by Sandoz; at 10.0 ml).

To measure effects on myceliogenic germination, treated sclerotia of all four isolates were placed on PDA and incubated in the dark at 20°C. There were four replicates of each treatment. The number of days before germination occurred, the colony diameter every 12 h thereafter, percentage germination after 14 days and number of sclerotia produced by the colony after 30 days were recorded.

To measure effects on production of apothecia, only treated sclerotia of OSR and Sun were used. Three samples of 10 sclerotia treated with each chemical, were tested in the laboratory for apothecia production (Mylchreest & Wheeler, 1987). For field tests, free draining pots (6.5 cm square x 9 cm deep) were filled with John Innes No. 1 compost and 10 treated sclerotia placed c. 1 cm below the surface. Pots were placed in the field in three completely randomised blocks in May 1989 and again in March 1990. As conditions were hot and dry, pots were sprayed every two days and in May 1990, all pots were shaded with netting (mesh size 2 x 2 mm). The number of weeks until apothecia were produced, percentage germination and total number of apothecia were recorded.

RESULTS

Effects of the treatments on myceliogenic germination are summarized in Table 1. The treatments could be divided into three groups according to their effects on number of days to germination. Three treatments (13), (14) and (16) had no significant effect whereas six treatments (7), (11), (12), (9), (10) and (24), with only one exception (treatment 9, OSR), significantly delayed germination of all isolates. Sometimes no germination was recorded within the 30 days of the experiment. Within an intermediate group of 14 treatments, sclerotial germination of the four isolates was affected differently. Thus, there were seven treatments which delayed germination of the potato isolate by 0.8-4 days, one treatment (21) that appeared to hasten germination of pot; two treatments which affected germination of both the potato and pea isolates by 4-19 days and four treatments which delayed germination of three isolates by 9-24 days, though not always the same isolates with each treatment.

Over all treatments germination was affected to some extent by the total quantity of fungicide applied. There was a significant negative correlation between ranks of % germination at 14 days and the total amount of chemical applied to 200 g sclerotia + seed ($r_s = -0.44$, $P < 0.05$; Siegal & Castellan, 1988).

Table 1. Effects of agrochemicals on germination of sclerotia to mycelium.

	Treatment	Mean number of days to germination of sclerotia				Mean number of sclerotia produced by mycelium			
		OSR	Sun	Pea	Pot	OSR	Sun	Pea	Pot
1	None	2.3	2.1	2.3	2.1	6.3	6.0	7.0	7.0
13	'Baytan'	2.8	3.9	NT	NT	2.3	8.3	NT	NT
14	'Fusarex'	2.5	2.5	8.9	2.1	4.3	8.0	6.8	6.0
16	Ethirimol	9.5	10.1	2.1	2.1	3.8	6.5	5.5	6.3
5	'Agrichem Thiram'	5.0	4.8	4.3	**4.6**	5.0	9.3	7.8	**3.5**
6	'HY-TL'	4.5	12.0	4.3	**6.8**	5.5	10.0	8.8	**6.5**
15	'Mil-col 30'	4.6	6.0	4.8	**4.9**	3.0	7.3	6.3	5.5
18	'Ferrax' (1 ml)	3.6	4.3	3.1	**4.0**	5.3	6.0	5.8	4.8
19	'Ferrax' (2 ml)	4.3	6.3	5.3	**3.9**	5.3	6.8	8.0	8.8
20	Flutriafol (0.3 ml)	2.6	3.5	2.0	**2.9**	4.8	4.3	6.8	4.8
21	Flutriafol (4.0 ml)	5.2	NT	12.1	**1.0**	5.3	NT	5.8	5.5
22	'Aliette Extra'	7.6	13.3	5.6	**6.0**	2.8	7.5	8.5	4.5
2	'Bromotex T'	6.7	**14.4**	6.0	**6.1**	6.0	9.0	6.8	**3.5**
3	'H-177'	8.0	**21.1**	12.3	**12.5**	4.3	11.0	7.0	**5.3**
4	'Hy-Vic'	9.5	**14.7**	**14.5**	**17.3**	5.3	5.8	5.8	4.8
8	'Ronilan'	**11.5**	**26.3**	11.0	**19.3**	3.0	1.5	*	**3.5**
23	'Rovral Flo'	**20.4**	**24.6**	13.5	**14.0**	4.0	**1.0**	**2.0**	**1.8**
17	Hexaconazole	9.5	**23.0**	**18.0**	**18.5**	3.5	1.8	4.0	**3.5**
7	'Prelude 20 LF'	**12.0**	**26.0**	**17.5**	**23.3**	5.3	**0.3**	5.0	**2.0**
11	'Apron Combi 453 FS'	**13.4**	**18.3**	**17.3**	**11.0**	3.3	8.8	7.8	**6.8**
12	'Lindex Plus FS'	**21.5**	**23.7**	*	*	5.8	**1.3**	*	*
9	Fenpropimorph (2.8 ml)	2.5	*	*	*	2.3	*	*	*
10	Fenpropimorph (5.6 ml)	*	NT	NT	NT	*	NT	NT	NT
24	'San 793F'	*	*	*	*	*	*	*	*

Values in **bold** are significantly different from the untreated at $P < 0.05$ using Duncans Multiple Range test, and $\sqrt{x + 0.5}$ transformation.
Data are based on observations over 30 days.
*, no germination of sclerotia or formation of sclerotia.
NT, not tested.

Despite the substantial effect of some chemicals in delaying germination of sclerotia, there were few effects on subsequent mycelial growth from treated sclerotia (not shown) or on production of sclerotia by these mycelia (Table 1). There were some reductions in mycelial growth and numbers of sclerotia with 'Ronilan' (8), 'Rovral Flo' (23), hexaconazole (17) and 'Prelude 20LF' (7) but not consistently with all isolates.

In the laboratory experiment measuring effects on apothecial production (Table 2) all except treatments (2) and (20) applied to Sun either inhibited carpogenic germination completely or significantly reduced the numbers of apothecia produced from sclerotia of the two isolates tested.

Sclerotia placed outside in 1989 produced few apothecia probably be-

Table 2. Effects of agrochemicals on germination of sclerotia to apothecia.

	Treatment	Total number of sclerotia producing apothecia						Mean number of apothecia produced per germinating sclerotium						Weeks after burying sclerotia that apothecia formed					
		Laboratory		Field				Laboratory		Field				Laboratory		Field			
		1990		1989		1990		1990		1989		1990		1990		1989		1990	
		OSR	Sun	OSR	Sun	OSR	Sun	OSR	Sun	OSR	Sun	OSR	Sun	OSR	Sun	OSR	Sun	OSR	Sun
1	None	26	30	28	25	30	26	4.0	4.1	6	2.4	4.2	2.7	14	7	49	21	11	13
13	'Baytan'	**0**	**0**	21	16	**0**	**0**	0	0	4.2	1.7	0	0	*	*	49	51	*	*
14	'Fusarex'	**0**	**8**	26	19	**0**	**7**	0	1.9	7.3	3.5	0	3.3	*	8	49	49	*	11
16	Ethirimol	**0**	**10**	25	15	0	7	0	2.5	8.8	2.6	0	2.9	*	8	50	51	*	11
5	'Agrichem Thiram'	9	**2**	27	23	**2**	16	3.0	2.0	4.7	5.4	4.0	2.9	10	10	49	49	11	12
6	'HY-TL'	4	**7**	22	24	**8**	16	3.0	5.7	4.1	5.8	2.3	3.1	12	8	50	21	13	12
15	'Mil-col 30'	0	**7**	25	22	**5**	**12**	0	8.0	6.0	4.1	3.0	3.8	11	8	49	51	11	12
18	'Ferrax' 1.0 ml	8	**11**	29	17	**4**	**23**	3.3	3.9	7.3	3.8	2.5	2.8	11	8	49	51	12	12
19	'Ferrax' 2.0 ml	NT	NT	21	19	NT	NT	NT	NT	4.5	5.3	NT	NT	NT	NT	49	51	NT	NT
20	Flutriafol 0.3 ml	10	30	25	15	**9**	24	2.6	6.0	6.6	3.5	2.0	3.0	10	7	49	51	10	10
21	Flutriafol 4.0 ml	0	**0**	**3**	NT	**0**	**0**	0	0	3.3	NT	0	0	*	*	50	NT	*	*
22	'Aliette Extra'	2	**10**	27	18	**14**	**18**	2.0	2.2	7.9	3.7	2.9	1.8	15	10	49	51	12	15
2	'Bromotex T'	6	**19**	24	25	**4**	**22**	2.4	2.7	6.4	2.6	2.0	2.1	12	8	50	49	12	12
3	'H-177'	1	**9**	21	22	**10**	**12**	1.0	2.4	6.3	4.7	1.4	3.0	14	10	49	49	12	15
4	'Hy-Vic'	2	**1**	20	16	**5**	**9**	5.0	1.0	5.5	3.3	1.2	1.2	16	13	49	49	16	16
8	'Ronilan'	0	**0**	26	19	**0**	**2**	0	0	5.6	2.5	0	1.5	*	*	49	49	*	15
23	'Rovral Flo'	**0**	**0**	21	13	**0**	**2**	0	0	8.6	2.1	0	1.0	*	*	49	51	*	17
17	Hexaconazole	**0**	**0**	**12**	**2**	**0**	**0**	0	0	1.9	5.0	0	0	*	*	50	53	*	*
7	'Prelude 20 LF'	**0**	**4**	24	23	**0**	**12**	0	3.8	4.2	4.8	0	1.8	*	14	49	51	*	15
11	'Apron Combi'	**3**	**19**	27	27	**1**	**17**	4.7	3.4	6.0	4.2	2.0	2.8	12	8	49	49	13	12
12	'Lindex Plus FS'	**0**	**0**	**7**	21	**0**	**0**	0	0	4.6	3.1	0	0	*	*	49	51	*	*
9	Fenpropimorph 2.8 ml	**0**	**0**	28	**0**	**0**	**0**	0	0	4.9	0	0	0	*	*	49	*	*	*
10	Fenpropimorph 5.6 ml	**0**	**0**	**1**	NT	**0**	**0**	0	0	7.0	NT	0	0	*	*	56	NT	*	*
24	'San 793F'	**0**	**0**	**2**	**7**	**0**	**0**	0	0	2.0	2.7	0	0	*	*	49	50	*	*

Values in **bold** are significantly different from the at $P < 0.05$ using Duncans Multiple Range test and $\sqrt{x + 0.5}$ tranformation.

*, no germination of sclerotia to apothecia.

NT, not tested.

cause of the hot dry summer. In the autumn a few apothecia were produced by sclerotia of the sunflower isolate (Sun) that were untreated or treated with 'HY-TL' (6) (Table 2). Following shading of the pots in May 1990 apothecia developed in most pots within 2 weeks and production then continued over the following 7 weeks. All mature apothecial disks were of similar size (< 1 cm diameter), and no malformations were observed.

No treatment prevented production of apothecia by the oilseed rape isolate (OSR) but the number of sclerotia that produced apothecia were significantly reduced (relative to the untreated) by fenpropimorph at 5.6 ml (10), 'Lindex Plus FS' (12), flutriafol at 4 ml (21) and 'San 793F' (24). With Sun, only sclerotia treated with fenpropimorph at 2.8 ml (9) did not produce apothecia (fenpropimorph at 5.6 ml was not tested), but numbers of sclerotia producing apothecia were reduced significantly by treatments with hexaconazole (17) and 'San 793F' (24). An analysis of the data for numbers of apothecia produced per germinating sclerotium was not possible because there were many zero values. However, there were particularly low numbers of apothecia from germinating sclerotia of OSR treated with hexaconazole (17), flutriafol at 4 ml (21) and 'San 793F' (24).

Sclerotia placed outside in 1990 produced apothecia in the same 7 week period as those of the 1989 set. Apothecia disks ranged from 0.5 to 1.8 cm in diameter and, again no malformed apothecia were seen. No apothecia were produced from sclerotia of isolates treated with fenpropimorph (9, 10), 'Lindex Plus FS' (12), 'Baytan' (13), hexaconazole (17), flutriafol at 4 ml (21) and 'San 793F' (24). Additionally no apothecia were produced from sclerotia of the oilseed rape isolate (OSR) treated with 'Prelude 20 LF' (7), 'Ronilan' (8), 'Fusarex' (14), ethirimol (16) and 'Rovral' (23). Sclerotia of this isolate seemed particularly susceptible since apart from these instances of complete inhibition, the production of apothecia was significantly reduced by all other treatments except 'Aliette Extra' (22). Seven treatments completely inhibited carpogenic germination of the sunflower isolate and a further seven significantly reduced production. These were 'H-177' (3), 'Hy-Vic' (4), 'Prelude 20 LF' (7), 'Apron Combi' (11), 'Fusarex' (14), ethirimol (16) and 'Rovral Flo' (23).

DISCUSSION

Research into the effects of herbicides and fungicides on mycelial growth of *Sclerotinia sclerotiorum in vitro* and to soil on carpogenic germination have been reported (Cerkauskas *et al.*, 1986; Yarden, 1986), but effects of fungicides applied as seed treatments have not been investigated.

Most fungicides used in this study decreased myceliogenic germination of sclerotia of all isolates, but 'Lindex Plus FS' (12), fenpropimorph (9, 10) and 'San 793' (24) caused complete inhibition. The potato isolate showed much less variability than the other isolates, so that relatively small effects of treatments on germination were significant. The reasons for this are uncertain but show that choice of isolate for testing chemicals could affect efficiency. Overall fenpropimorph (also present in 'Lindex Plus FS') was most effective. 'San 793', while effective was applied at a very high rate. These tests were done on a nutrient rich medium and those treatments which delayed germination beyond 14d might be very effective in soil, where nutrients are not so abundant. A number of seed treatments shown to be effective are already available to the farmer on other crops and

could be useful for control of S. sclerotiorum in sunflower crops (Holley & Nelson, 1985).

In the UK, ascospores are the primary source of infection by S. sclerotiorum but conditions in 1989, with 30 days in June and July with maximum temperatures above 27°C, were not condusive to apothecia production (Mylchreest & Wheeler, 1987). After shading in 1990, the treatments that affected myceliogenic germination, also decreased carpogenic germination in OSR ('Lindex Plus FS', fenpropimorph and 'San 793') but flutriafol (21) also had an effect. In Sun, only San 793 and fenpropimorph affected germination perhaps because they were more persistant. As sclerotia do not necessarily germinate in the first year after burial (e.g. if not conditioned or if it is too hot and dry), persistancy could be an important feature. The use of 'Lindex Plus FS' on oilseed rape seed may directly affect the development of Sclerotinia disease epidemics in the UK.

Laboratory tests were convenient to do and generally gave consistent results but choice of isolate may be important. They are therefore potentially useful for the initial evaluation of new treatments but it is important that further tests be done in the field under more realistic conditions and to measure longer term effects.

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EFFECTIVENESS OF CYPROCONAZOLE, ALONE AND IN COMBINATIONS, AGAINST A RANGE OF STEM BASE AND FOLIAR DISEASES IN WINTER WHEAT.

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ABSTRACT

Cyproconazole is a broad-spectrum triazole fungicide which has been evaluated throughout Europe since 1983. The molecule has been tested in the U.K., alone and in combination with complementary fungicides. It has shown good activity against rusts, powdery mildew and septoria and has significantly decreased eyespot. Cyproconazole has a more persistent effect than many other fungicides which may allow the grower more flexibility in planning a fungicide programme. Mixing with fungicides with different modes of action can increase efficacy and is a potentially effective resistance strategy.

INTRODUCTION

Cyproconazole is a broad-spectrum triazole fungicide discovered by Sandoz Limited, Switzerland and is one of the ergosterol biosynthesis inhibitors (EBIs). Cyproconazole exists as four active isomers all showing very high and more or less equal fungicide activity. It controls a wide range of diseases in several crops at low concentrations, gives a long duration of activity and shows exceptionally rapid penetration into plant leaves (Gisi et al., 1986).

Trials throughout Europe since 1983 have evaluated cyproconazole, alone and in combination with other fungicides, against winter wheat diseases. A number of trials in the U.K. are described in this report.

METHODS AND MATERIALS

Trials have tested cyproconazole against yellow rust, brown rust, powdery mildew, septoria and eyespot in winter wheat. Foliar disease sites were selected which had disease in the crop at a severity at which commercial treatment would be justified. Eyespot sites were in fields which had grown cereals for at least one previous season and where conditions were favourable for eyespot development.

Trials were of randomised block designs with three or four replicates per treatment and plot sizes of either 3m x 10m, 2m x 12m or 3m x 12m. Treatments were applied using a small-plot precision sprayer and water volumes were generally equivalent to 300-400 litres per hectare. Foliar diseases were assessed before and after fungicide application by visually estimating the percentage area infected on specified leaves using the ADAS disease assessment keys. Eyespot was assessed between GS 68 and GS 75 on 15 - 25 randomly sampled main stems per plot, also using the ADAS assessment key, and a disease index calculated.

RESULTS

Eyespot (Pseudocercosporella herpotrichoides)

In 1987 and 1990 cyproconazole was evaluated, alone and in combination with prochloraz, against eyespot in nine trials where the disease was moderate to severe (Table 1).

TABLE 1 Control of Eyespot

Treatment	Rate g AI/ha	Trial No.	1	2	3	4	5	6	7	8	9	Mean
		Crop GS	30-31	30	30-32	30-32	30-32	30-32	32	32-33	32	
			% control									
Untreated (eyespot index)			(81)	(75)	(29)	(40)	(62)	(36)	(32)	(66)	(29)	(50)
Cyproconazole	80		46	9	17	19	20	21	50	11	48	27
Cyproconazole + prochloraz	80 + 300		57	44	37	60	35	35	65	41	75	50
Prochloraz	400		58	53	27	40	49	34	30	-	73	46
Flusilazole + carbendazim	200 + 100		41	20	28	34	12	16	17	15	-	23

Cyproconazole at 80 g AI/ha was not as effective against eyespot as prochloraz applied at 400 g AI/ha but gave similar results to flusilazole + carbendazim. Cyproconazole + prochloraz at 80 + 300 g AI/ha performed at least as well as prochloraz at 400 g AI/ha.

Powdery mildew (Erysiphe graminis)

In eight trials between 1985 and 1990 (Table 2) powdery mildew was variable but was often severe (up to 46% on leaf 1). Cyproconazole at 80 g AI/ha was compared with propiconazole + tridemorph, flutriafol + chlorothalonil and flusilazole + carbendazim. It gave similar control of powdery mildew to propiconazole + tridemorph and was clearly superior to flusilazole + carbendazim and flutriafol + chlorothalonil.

In a further eight trials the duration of activity of cyproconazole at 80 g AI/ha, alone and in mixture with tridemorph at 350 g AI/ha, was monitored 3 to 4 weeks and again 5 to 6 weeks after treatment (WAT; Table 3). Cyproconazole provided more persistent control than either flusilazole + carbendazim or flutriafol + chlorothalonil. The addition of tridemorph to cyproconazole improved the control of mildew 5 to 6 weeks after application and the mixture was superior to propiconazole + tridemorph.

TABLE 2 Control of powdery mildew

Treatment	Rate g AI/ha	Trial No.	1	2	3	4	5	6	7	8
		Leaf assessed	L1	L1	L2	L2	L2	L2	L2	L3
			% control							
Untreated (% leaf infection)			(42)	(46)	(12)	(35)	(9)	(20)	(14)	(19)
Cyproconazole	80		93	94	92	64	85	95	98	60
Propiconazole + tridemorph	125 + 250		89	90	84	-	76	89	95	-
Flutriafol + chlorothalonil	117 + 750		-	-	-	52	63	73	79	-
Flusilazole + carbendazim	200 + 100		-	-	-	-	76	87	80	37

TABLE 3 Control of powdery mildew - duration of activity

Treatment	Rate g AI/ha	Mean % control [range] 3-4 WAT (4 trials)	5-6 WAT (6 trials)
Untreated (% leaf infection)		(14-46)	(4-42)
Cyproconazole	80	94 [89-98]	78 [56-92]
Cyproconazole + tridemorph	80 + 350	95 [91-99]	86 [72-93]
Propiconazole + tridemorph	125 + 250	90 [84-96]	78 [62-89]
Flusilazole + carbendazim	200 + 100	84 [80-89]	66 [44-77]
Flutriafol + chlorothalonil	94 + 600	74 [73-74]	57 [32-82]

Septoria (Septoria tritici)

In 20 trials between 1985 and 1989, cyproconazole was evaluated alone at 80 g AI/ha and in combination with chlorothalonil at 750 g AI/ha. These two formulations were compared with the standard triazoles, propiconazole, flutriafol + chlorothalonil and flusilazole + carbendazim (Table 4).

TABLE 4. Control of septoria

Treatment	Rate g AI/ha	Mean % control [range] 4-5 WAT (13 trials)	6-8 WAT (9 trials)
Untreated (% leaf infection)		(20-75)	(32-82)
Cyproconazole	80	65 [38-95]	60 [31-77]
Cyproconazole + chlorothalonil	80 + 750	80 [69-87]	78 [53-95]
Flutriafol + chlorothalonil	117 + 750	79 [54-97]	66 [44-97]
Flusilazole + carbendazim	200 + 100	74 [51-96]	54 [39-58]
Propiconazole	125	82 [67-93]	56 [43-82]

Cyproconazole gave poorer control than the standards when assessed 4 to 5 weeks after application but the mixture with chlorothalonil was generally equal or superior to the standards. In contrast, control 6 to 8 weeks after application was often better with cyproconazole alone than with propiconazole or flusilazole + carbendazim. However, the mixture with chlorothalonil was even better. Thus the main benefit of cyproconazole in controlling septoria appears to be its duration of activity.

Yellow rust (Puccinia striiformis)

Four trials in 1988 and 1989 with severe yellow rust in untreated plots (28 - 61%), evaluated cyproconazole at 80 g AI/ha. It was compared to the standard treatments flusilazole + carbendazim and flutriafol + chlorothalonil and was superior to both in controlling yellow rust (Table 6).

A further trial in 1990 evaluated the duration of activity of cyproconazole against yellow rust (Table 7). A single spray was applied at GS 37 when 40% of the tillers were infected and yellow rust on leaf 3 averaged 2.5%. The disease increased rapidly and 57 days later leaves 1 and 2 were completely destroyed in the untreated plots. Yellow rust and green leaf areas (GLA) were assessed on leaves 1 and 2 at 10 day intervals after treatment (DAT).

At the first assessment, yellow rust was present on leaf 1 where any of the standard treatments had been applied and had also started to appear on leaf 2 in the fenpropimorph and flusilazole + carbendazim treated plots. At the second assessment traces of yellow rust (0.2%) were found on leaves 1 and 2 in the cyproconazole-treated plots but there was up to 14% on leaf 2 and 42% on leaf 1 in the standard treatments. At the third assessment it was not possible to assess yellow rust but green leaf areas were much greater where cyproconazole had been applied than in plots given the standard treatments.

TABLE 6 Control of yellow rust

Treatment	Trial No.	1	2	3	4
	Leaf assessed	L1	L2	L1	L2
	Rate g AI/ha	% control			
Untreated (% leaf infection)		(41)	(61)	(43)	(28)
Cyproconazole	80	83	100	96	96
Flusilazole + carbendazim	200 + 100	59	97	86	94
Flutriafol + chlorothalonil	117 + 750	44	95	78	95

TABLE 7 Control of yellow rust - duration of activity, Suffolk 1990

Treatment	Rate g AI/ha	Leaf 1 % infection 35 DAT	Leaf 1 % infection 47 DAT	Leaf 1 % GLA 57 DAT	Leaf 2 % infection 35 DAT	Leaf 2 % infection 47 DAT	Leaf 2 % GLA 57 DAT
Untreated		(26.7)	(83.7)	(1.0)	(36)	(88.3)	(2.0)
Cyproconazole	80	0	0.2	50.0	0	0.2	53.3
Propiconazole	125	0.5	4.9	26.7	0	2.1	21.7
Flusilazole + carbendazim	156 + 78	2.5	19.0	9.3	0.3	3.6	16.0
Triadimenol + tridemorph	125 + 375	1.7	8.4	16.7	0	1.0	21.7
Fenpropimorph	750	7.2	41.7	3.7	0.9	14.2	4.3

Brown rust (Puccinia recondita)

There were four trials in 1987 and 1989 on brown rust, two of which had severe disease. Cyproconazole at 80 g AI/ha was compared with the standards propiconazole and flutriafol + chlorothalonil (Table 8). Cyproconazole gave effective control of brown rust for at least 6 to 7 weeks and at that time was comparable to the standards at 4 to 5 weeks.

TABLE 8 Control of brown rust

Treatment	Leaf assessed	L1	L1	L2	L1
	Rate g AI/ha		% control 4-5 WAT		6-7 WAT
Untreated (% leaf infection)		(5)	(50)	(3)	(31)
Cyproconazole	80	97	99	97	80
Flutriafol + chlorothalonil	94 + 600	79	53	86	34
Propiconazole	125	88	64	-	16

DISCUSSION

Where eyespot is present in winter wheat there is usually a mixture of the W (wheat) and R (rye) types. The W type was formerly dominant but the R type is now widespread in the U.K. (King & Griffin, 1985). It has been demonstrated that triazole fungicides are less effective on the R type than the W type unlike prochloraz which is known to control both equally well. (Leroux & Gredt, 1988). This might help to explain the lower level of activity achieved by cyproconazole and flusilazole + carbendazim and the benefit of using a combination with prochloraz.

The greater persistence of activity of cyproconazole against the foliar diseases (rusts, powdery mildew and septoria) should allow the grower much more flexibility in spray timing and may allow a reduction in the number of fungicide sprays applied.

The complementary use of cyproconazole with other fungicides such as prochloraz, tridemorph and chlorothalonil, with different sites and modes of action, provides not only greater efficacy against eyespot, powdery mildew and septoria, but also offers a potentially effective strategy against the problems of fungicide resistance.

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CONTROL OF YELLOW RUST WITH A TRIADIMENOL SEED TREATMENT ON A RANGE OF WINTER WHEAT CULTIVARS

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ABSTRACT

Replicated and unreplicated small-plot trials in harvest years 1989 and 1990 showed that a triadimenol/fuberidazole seed treatment is effective in controlling early infection by yellow rust, giving a mean of 96% control of the disease at GS30-32. This allowed easier management of the disease with subsequent spray treatments and resulted in yield responses of 0.55 - 0.83 t/ha. Two trials in 1990 demonstrated that a triadimenol/fuberidazole seed treatment gave superior control of yellow rust than a range of spray treatments applied in late autumn.

INTRODUCTION

Yellow rust *(Puccinia striiformis)* in winter wheat has been unusually serious in the UK since 1988. This has been a result of mild winters combined with the breakdown of specific resistances to yellow rust (WYR factors) in certain cultivars of winter wheat. The cultivars Slejpner (WYR9) and Hornet (WYR6,9) were particularly affected by the epidemics of 1988 and 1989 (Bayles *et al.* 1989).

Wainwright *et al.* (1979) previously reported that a triadimenol/ fuberidazole seed treatment was effective against the major seed-borne diseases of autumn-drilled wheat. In addition Wainwright and Morris (1989) reported control of soil-borne bunt of wheat. The control of septoria, mildew and brown rust during winter by the same treatment was reported by Clark *et al.* (1984). This paper describes a series of trials in harvest years 1989 and 1990 to investigate the effectiveness of a triadimenol/ fuberidazole seed treatment in controlling yellow rust.

MATERIALS AND METHODS

Results are given from a wide range of replicated and unreplicated trials on winter wheat using 'Baytan' seed treatment applied either as the dry powder or the aqueous suspension formulation at the rate of 37.5g AI triadimenol and 4.5g AI fuberidazole/100 kg seed. Both formulations are referred to in this paper as 'triadimenol ST' and were compared with the recommended rate of a phenylmercury acetate formulation (organomercury).

Details of the trials are summarised in Table 1. Series B, C and D were drilled with an Oyjord small-plot drill as were a number of the replicated trials in Series A. The unreplicated trials were drilled by farm drills. Target sowing depth was 2-4 cm and the drills were calibrated to achieve an identical sowing rate with each seed treatment.

TABLE 1 Details of the trials testing triadimenol ST in harvest years 1989 and 1990

Series	Harvest Year	Type	Number	Plot Size	Sowing Date
A	1989	Replicated (x4)	5	$21m^2$-$52m^2$	30/9 - 1/11
	1989,1990	Unreplicated	23	$50m^2$-$1000m^2$	22/9 - 7/11
B	1989	Replicated (x4)	1	$20m^2$	30/9
C	1989	Split split plot	8	$21m^2$	22/9 - 3/11
D	1990	Replicated (x4)	3	$21m^2$	21/9 - 5/10

Eight cultivars were compared at each site in Series C and more than one cultivar was present in the majority of trials in Series A. Series B and D were drilled only with the cultivar Slejpner.

Within a trial, identical spray programmes were applied during spring and summer to plots testing each seed treatment; in the majority of trials these were based on a co-formulation of triadimenol and tridemorph. Assessments were made of foliar diseases and particularly yellow rust. Yields were determined using various small-plot combine harvesters and were adjusted to moisture contents of 14 or 15%.

RESULTS

A total of 40 trials compared triadimenol ST with a standard organomercury treatment in 1989 and 1990. Table 2 shows the cultivars tested and the level of yellow rust control in Series A from soon after the disease was first seen in the plots treated with organomercury. Triadimenol ST gave excellent control of yellow rust ranging from 96% at GS30-32 to 72% at GS50 and later.

TABLE 2 Control of yellow rust by triadimenol ST in 1989 and 1990 (Series A)

GS at assessment	30-32	37-50	50+
No. of comparisons	17	14	9
Treatment			
Organomercury (% infection)	(14%)	(29%)	(26%)
Triadimenol ST (% control)	96	80	72
(Range)	(87-100)	(55-96)	(30-91)
Cultivars tested	Fortress(2)	Avalon(1)	Avalon(1)
(No.of sites)	Hornet(3)	Haven(2)	Druid(1)
	Riband(2)	Hornet(1)	Hornet(2)
	Slejpner(10)	Slejpner(10)	Riband(1)
			Slejpner(4)

The persistent effect of triadimenol ST on yellow rust is further illustrated by results from ADAS winter wheat surveys in 1988 and 1989. These show that over all varieties there was a more than ten-fold reduction in yellow rust on leaf 2 at GS75 (Thomas, 1988 and 1989) (Table 3). Furthermore, the treatment was no less effective when used in the very susceptible cultivar Slejpner.

TABLE 3 Yellow rust on second youngest leaves at GS75 in crops with and without triadimenol ST, surveyed in 1988 or 1989.

		1988		1989	
Triadimenol ST	Variety	No.of Crops	Yellow rust(%)	No.of Crops	Yellow rust(%)
Used	All varieties	47	0.02	29	0.02
Not Used	All varieties	306	0.21	261	0.92
Used	Slejpner	13	0.07	4	0.05
Not Used	Slejpner	76	0.71	49	3.94

Data from ADAS surveys; (Thomas, 1988 and 1989)

In a replicated trial at Morley Research Station in 1989 (Series B), yellow rust was first observed by Stevens and Nuttall (1989) in the plots treated with organomercury on the 21 February. Triadimenol ST gave over 90% control until GS31/32 thus reducing the infection pressure from yellow rust when the first foliar spray was applied on 26 April (Table 4). A second foliar spray was applied on the 23 May (GS39). Triadimenol ST plus two sprays increased yield by 0.49 t/ha compared with the organomercury treatment plus two sprays.

TABLE 4 Yellow rust and grain yield at Morley Research Station in 1989 (Series B; cv Slejpner)

Date assessed	14/4	5/5	12/5	19/5	26/5	2/6	Yield
Leaf	All	All	4	3	2	2	(t/ha)
GS	30	31/32	33	37/39	39/41	53	
Treatment	Mean % yellow rust infection						
1. Organomercury	17.6	36.3	21.3	21.8	31.8	40.0	3.04
2. Triadimenol ST	1.5	3.3	5.4	10.0	21.5	35.0	3.35
3. Organomercury + sprays	-	2.7	0.0	3.0	8.0	3.4	8.65
4. Triadimenol ST + sprays	-	0.1	0.0	1.1	4.1	2.6	9.14
LSD 5%	-	3.36	4.94	2.93	6.50	2.47	0.720

Leaf 1 was the top fully expanded leaf present at each assessment.
Sprays: triadimenol/tridemorph on 26/4, triadimenol/tridemorph + chlorothalonil on 23/5.

Control of yellow rust on the very susceptible cultivars Slejpner and Hornet in 1989 (Series C) and effects on grain yield, are summarised in Table 5. Assessments at GS59 to 70 showed that triadimenol ST followed by a spray at GS39 gave similar control of yellow rust and increases in yield to the organomercury treatment plus sprays at GS32 and 39. On both cultivars the triadimenol ST plus two sprays gave the best control of yellow rust and yield. The mean yield responses to triadimenol ST on Hornet and Slejpner were 0.70 and 0.87 t/ha, respectively, but 0.33 t/ha on the other six cultivars which were not substantially affected by yellow rust.

TABLE 5 Effects of triadimenol ST on yellow rust and grain yield in cvs Slejpner and Hornet in 1989 (Series C)

	Slejpner			Hornet		
GS at asessment	Y. rust (% control) 39	59-70	Yield (t/ha)	Y. rust (% control) 39	59-70	Yield (t/ha)
No. of trials	3	6	7	1	4	7
Treatment						
1. Organomercury (% infection)	(70%)	(63%)	6.0	(15%)	(23%)	7.5
2. Triadimenol ST	52	35	6.8	80	72	8.2
3. Organomercury + sprays at GS32+39	80	87	8.4	99	97	8.6
4. Triadimenol ST + sprays at GS32+39	86	95	9.4	100	99	9.2
5. Organomercury + spray at GS39	-	68	7.6	-	97	8.0
6. Triadimenol ST + spray at GS39	-	84	8.4	-	99	8.8

Sprays: triadimenol/tridemorph or triadimenol/tridemorph + prochloraz at GS32
triadimenol/tridemorph + chlorothalonil at GS39

Table 6 summarises yields obtained from all trials in Series A, B and C where yellow rust was present. The use of triadimenol ST in plots that were subsequently sprayed with fungicides gave yield increases of between 0.55 and 0.74 t/ha compared with plots treated with organomercury and given the same spray programme.

TABLE 6 Mean yield responses from all trials in Series A,B and C where yellow rust was present

	Mean yield (t/ha)			
Spray Programme	Organomercury	Triadimenol ST	Increase	(No.trials)
None	5.82	6.65	0.83	(18)
GS39	7.91	8.65	0.74	(12)
GS31 + GS39	8.94	9.49	0.55	(16)
GS39 + GS59	9.75	10.34	0.59	(3)
GS31+39+59	9.31	9.95	0.64	(6)

Spray programmes based on triadimenol/tridemorph.

In autumn 1989 a series of trials was sown to compare triadimenol ST with autumn-applied foliar sprays (Series D). At the two sites where yellow rust appeared, triadimenol ST was superior to the autumn sprays (Table 7) and especially at the Cambridgeshire site where yellow rust was first recorded in March.

TABLE 7 Effects of triadimenol ST compared with Autumn sprays on yellow rust (Series D)

	Mean % Yellow rust on leaves 2-4				
Site	Cambridgeshire				Lincolnshire
Assessment Date	15/3/90	28/3/90	17/4/90	2/5/90	25/5/90
Growth stage	30	31	32	37	45
Treatment (g AI/ha)					
1. Organomercury	18.0a	53.0a	50.0a	64.0a	12.3a
2. Triadimenol ST	1.0c	5.7c	6.2d	10.2e	1.5d
3. Triadimenol (125)	2.2c	23.9b	15.0c	26.7d	2.3d
4. Fenpropimorph(750)	5.4b	29.2b	35.8b	53.3b	5.8c
5. Flusilazole + carbendazim (156+78)	3.7c	31.8b	31.7b	45.9c	8.5b

Figures followed by the same letter do not differ significantly at P=0.05.
Leaf 1 was the top fully expanded leaf present at each assessment.
Sprays were applied on 29/11/89 (Cambridgeshire) or 11/12/89 (Lincolnshire).

DISCUSSION

Yellow rust is an obligate parasite which can infect winter wheat, usually from volunteers, in the autumn and then survive over winter in the plant. Triadimenol ST protects the young plant from infection by yellow rust in autumn and thus controls initial infection. This helps to explain the persistent control with triadimenol ST seen in some crops, even up to GS50 and beyond, which parallels the control of brown rust in wheat observed by Clark *et al.* (1984).

Triadimenol ST gave good control of yellow rust even in the susceptible cultivars Slejpner and Hornet in the epidemic years of 1989 and 1990, with a mean reduction of 96% up to GS30-32. This early spring control is a particular benefit because it allows easier management of yellow rust at a time of the year when the number of days suitable for spraying is often limited. Furthermore triadimenol ST plus a spray programme gave superior control of yellow rust throughout the season compared with an organomercury seed treatment plus the identical spray programme and resulted in additional yield responses of between 0.55 and 0.74 t/ha.

If the current trend of mild winters continues and acceptable wheat varieties resistant to yellow rust do not become available, then triadimenol ST will continue to be a useful tool in the control of yellow rust during the 1990s.

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ADVANCES IN THE DEVELOPMENT OF A PROCHLORAZ/CYPROCONAZOLE MIXTURE FOR BROAD SPECTRUM DISEASE CONTROL IN CEREALS

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ABSTRACT

Data from trials in the U.K. in 1989 and 1990 confirmed that CR 18015, a coformulation of prochloraz and cyproconazole (Sportak Delta®), is effective as a broad spectrum fungicide for stem-base and foliar disease control in cereals. Most major pathogens are controlled including *Pseudocercosporella herpotrichoides*, *Erysiphe graminis*, *Puccinia spp.*, *Rhynchosporium secalis* and *Septoria spp.*.

Data are also presented which demonstrate the excellent persistency of disease control of CR 18015 compared with similar products. The implications of enhanced persistency of effect are considered in relation to flexibility of usage and options for reduced input programmes.

INTRODUCTION

Prochloraz, an imidazole fungicide, and cyproconazole, a triazole fungicide, have complementary activity spectra (Harris *et al.*, 1979; Gisi *et al.*, 1986). A particular combination of both molecules was developed by Schering Agriculture and initial trials results reported at this Conference in 1988 (Bush and Bardsley, 1988). The broad spectrum of activity covers all important fungal pathogens of cereal stems and foliage including *Pseudocercosporella herpotrichoides* (eyespot), *Puccinia striiformis*, *P. hordei* and *P. recondita* (rusts), *Erysiphe graminis* and *E. hordei* (powdery mildews), *Rhynchosporium secalis* (leaf scald), *Pyrenophora teres* (net blotch) and *Septoria tritici* and *S. nodorum* (septoria). The breadth of disease control and consequential benefits in yield were confirmed in trials in 1989 and 1990 and the results are summarised here.

Another key feature of the mixture is the excellent persistency of effect contributed by cyproconazole. Interim results of specific trials designed to investigate this property are presented in this paper.

MATERIALS AND METHODS

Commercial crops of wheat and barley were used for all trials which were of randomised block designs replicated sixfold. Plots were 2-3 m wide and 10 m long.

Details of the fungicides, applied only once for each trial, are given in the result tables. They were sprayed using backpack sprayers at volumes of 200-220 l/ha. Spray nozzles and pressures were selected to provide a medium spray quality.

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TABLE 1. Eyespot control and grain yields (as % of untreated) from a single application made at GS 31-33 to winter barley and winter wheat trials in 1989 and 1990.

Treatment	g AI/ha	1989 Winter Barley % eyespot control	1989 Winter Barley yield	1989 Winter Wheat % eyespot control	1989 Winter Wheat yield	1990 Winter Barley % eyespot control	1990 Winter Barley yield	1990 Winter Wheat % eyespot control	1990 Winter Wheat yield
Prochloraz/cyproconazole	400/60	82	117.8	41	108.5	57	114.8	54	107.9
Prochloraz	400	72	104.3	33	104.5	50[1]	112.6[1]	56[1]	107.1[1]
Flutriafol/carbendazim	118/188	11	107.2	-1	103.6	18	106.8	9	103.7
Propiconazole/carbendazim	125/100	26	110.9	5	103.7	12	114.2	19	101.5
Flusilazole/carbendazim	200/100	53	118.1	18	105.5	40	116.3	31	105.8
untreated (disease index		(36)		(49)		(30)		(52)	
yield t/ha)			(5.0)		(8.0)		(5.3)		(6.9)
number of trials		4	4	3	3	2	2	6	6

[1] Prochloraz + fenpropimorph 400 + 563 g AI/ha

TABLE 2. Foliar disease control 4-8 weeks after a single application at GS 37-55 and grain yield (as % of untreated) in winter wheat trials in 1989 and 1990.

Treatment	g AI/ha	1989				1990					
		PUCCST	ERYSGT	SEPTTR	YIELD	PUCCST	PUCCRE	ERYSGT	SEPTTR	YIELD	
Prochloraz/cyproconazole	400/60					97	98	70	84	112	112.1
Prochloraz/cyproconazole + tridemorph	400/60 + 263	91	56	53	108.0	98	96	78		109	
Prochloraz/cyproconazole + fenpropimorph	400/60 + 375	92	82	61	107.5	98	93	83		112	
Flutriafol/chlorothalonil	118/750	76	33	58	108.5	97	79	28	98[2]	110	108.7[2]
Propiconazole + fenpropidin	125 + 563	97	77	65	105.2	97	80	90		110	
Flusilazole/carbendazim +											
tridemorph	156/78 + 263	100[1]	48[1]	61[1]	103.3[1]						
or fenpropimorph	156/78 + 375					96	91	78	90	114	112.3
untreated (% leaf area infected		(11.5)	(3.3)	(12.3)		(5.8)	(8.5)	(11.9)	(9.3)	(5.9)	(6.8)
yield t/ha)					(8.4)						
number of trials		2	4	3	6	4	1	4	3	6	8

[1] One trial only.
[2] Plus fenpropidin 375 g AI/ha in 2 trials.

PUCCST *Puccinia striiformis*, ERYSGT *Erysiphe graminis f.sp. tritici*,
PUCCRE *Puccinia recondita*, SEPTTR *Septoria tritici*

TABLE 3. Foliar disease control 2-7 weeks after a single application at GS 39-55 and grain yield (as % of untreated) in winter barley trials in 1990.

Treatment	g AI/ha	PUCCHD	ERYSGH	YIELD
Prochloraz/cyproconazole	400/60	91	93	111
Propiconazole + fenpropidin	125 + 375	91	99	114
Propiconazole/tridemorph	125/250	89	98	110
Fenpropimorph	750	85	99	108
untreated (% leaf area infected		(10.0)	(23)	
yield t/ha)				(5.8)
number of trials		2	3	3

PUCCHD Puccinia hordei
ERYSGH Erysiphe graminis f.sp. hordei

Assessments of foliar and stem-base diseases were made with reference to appropriate ADAS disease assessment keys using a minimum of 10 leaves or stems per plot. A minimum of 4 replicates were assessed for disease control and all 6 replicates for yield, which was measured after cutting with small-plot combine harvesters and subsequently corrected to 15% moisture. In persistency trials disease assessments were made at weekly intervals until crop senescence precluded further assessment. An assumption has been made that disease control declined logarithmically. Data were, therefore, transformed to $\log_{10}$ before calculating linear regressions on time (weeks after treatment).

RESULTS

The superiority of CR 18015 over other standards for eyespot control and/or yield increases is confirmed. The differences are particularly evident when comparisons are made with products reliant upon mbc for eyespot control (Table 1).

Data from winter wheat and winter barley trials also confirm the broad spectrum activity of CR 18015 against foliar diseases (Tables 2 and 3). All important cereal diseases were well controlled. Where powdery mildew is well established addition of a morpholine to CR 18015 can give enhanced eradicant control. In the persistency trials discussed below preventative applications were made and CR 18015 was at least equivalent to standard products.

TABLE 4. Summary of linear regression analyses on persistence of control of *Puccinia striiformis* and *Erysiphe graminis* after spraying with different fungicides.

		SLOPE[1] (10^{-3})	CONSTANT	*P*	duration of decline (WAA)
	g AI/ha				
Puccinia striiformis 1988					
Prochloraz/cyproconazole	400/60	-77.1	2.21	0.2-0.1	)
Flusilazole/carbendazim	160/80	-103.6	2.25	0.05-0.02	) 2-11
Propiconazole/carbendazim	125/100	-115.4	2.30	0.02-0.01	)
Puccinia striiformis 1990					
Cyproconazole	80	no decline			
Cyproconazole	40	-1.89	2.01	0.1-0.05	)
Flutriafol	125	-5.17	2.02	0.1-0.05	) 4-9
Flutriafol	63	-13.43	2.06	0.01-0.001	)
Flusilazole/carbendazim	200/100	-7.83	2.04	>0.001	)
Flusilazole/carbendazim	125/63	-11.97	2.05	0.01-0.001	)
Erysiphe graminis 1990					
Cyproconazole	80	-11.0	1.97	0.2-0.1	)
Cyproconazole	40	-21.8	1.92	0.4-0.2	)
Flutriafol	125	-44.0	2.05	0.2-0.1	)
Flutriafol	63	-66.2	1.99	0.4-0.2	) 4-7
Flusilazole/carbendazim	200/100	-15.0	1.73	<0.2	)
Flusilazole/carbendazim	125/63	-50.8	1.84	0.2-0.1	)

WAA - weeks after application
1 - a negative value indicates decline in disease control over time and increasing numerical value implies increasingly rapid rate of decline.

Persistence of control of *Puccinia striiformis* (1988 and 1990) and *Erysiphe graminis* (1990) was investigated. Without exception the slopes for decline in control of *P. striiformis* by cyproconazole are substantially less than for other treatments (Table 4). Indeed at 80 g AI/ha disease control remained at 100% 9 weeks after application in this particular trial. Although statistically significant at a low level, probably because of the erratic course of disease development, similar trends in persistence of control of *E. graminis* were observed.

DISCUSSION

Data presented in this paper show that CR 18015 is a good cereal fungicide. It has a broad spectrum of activity, controlling all major cereal diseases but of equal significance is its persistency of effect which can outlast alternative products by several weeks. The precise advantage depends on a number of factors such as disease pressure and the reference level of

disease control selected to make the comparisons. Similar results have been obtained by Beck & Wiedmer (pers. comm.). Superior spectrum of activity and persistence of CR 18015 should make it attractive to farmers and advisers. It is a single product able to control all major diseases thereby simplifying the process of product selection. The persistency of effect allows spray programmes to be devised that should enable a reduction in inputs to be made with no loss in efficacy. These two important properties - spectrum and persistency - make CR 18015 an extremely flexible product to use in spray programmes for early, mid and late season disease control.

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FLUTRIAFOL/CHLOROTHALONIL: CONTROL OF SEPTORIA TRITICI IN WINTER WHEAT

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ABSTRACT

Flutriafol, a flutriafol/chlorothalonil formulated mixture, chlorothalonil and prochloraz were compared in field trials for the control of Septoria tritici in winter wheat, before infection and during the incubation period of the disease. Under field conditions, observations were made on S.tritici development following rainsplash.

Leaf spot symptoms developed 30-35 days after infection and flutriafol, flutriafol/chlorothalonil and prochloraz gave good control when applied up to 29 days after infection, although all three tended to be slightly less effective when applied towards the end of the latent period. Flutriafol-based products were generally more effective than prochloraz. Chlorothalonil showed good protectant activity but gave no significant control of the disease when applied after infection. Flutriafol and flutriafol/chlorothalonil offered the highest and most consistent long term reductions in leaf spot symptoms.

INTRODUCTION

Leaf spot caused by S.tritici is potentially the most damaging disease of winter wheat in the UK, and is spread within crops by rainsplash. A period of 25-42 days then elapses between infection and the development of symptoms. This is known as the latent period. It has been suggested that information on the frequency and intensity of rain related to growth stage, could allow growers to forecast the build up of S.tritici and to plan a strategy of cost effect control (Shaw & Royle, 1986).

The timing and choice of fungicide is critical to achieve optimum control. Chlorothalonil has proved over many years to be an excellent protectant material, and has been shown (Jordan, et al., 1986) to reduce the viability of S.tritici pycnidiospores for up to 42 days after application, presumably as a result of redistribution of the chemical by rainfall.

A number of broad spectrum fungicides from the DMI group have both a protective and curative effect. The relative curative abilities of certain of these fungicides have been assessed previously in laboratory studies. One of these, flutriafol is highly systemic (Shephard, 1988) and has been shown to give excellent curative control of S.tritici up to 28 days into the latent period under laboratory conditions (Jordan et al., 1986).

Three trials were undertaken by ICI Agrochemicals in the UK in 1988 to evaluate the abilities of flutriafol, a flutriafol/chlorothalonil formulated mixture, prochloraz and chlorothalonil to control S.tritici at recommended volumes and rates of application under field conditions.

These were applied before infection and at intervals during the latent period, to assess their curative ability and persistence of fungicidal effect.

MATERIALS AND METHODS

'Impact Excel' a flutriafol/chlorothalonil formulated mixture and a flutriafol formulation were evaluated in winter wheat trials in the UK in 1988. These were compared with commercial formulations of prochloraz and chlorothalonil. Trials were fully replicated using a randomised block design. Plot sizes were 72 m^2. Winter wheat cultivars susceptible to S.tritici were selected. Treatments were applied with CO_2 pressurised sprayers fitted with F110-02 fan-jets. Operating pressure was 200 KPa and the application volume was 200 l/ha.

The following fungicide formulations were evaluated:

Active Ingredients	Formulation g ai/l	Rate Applied g ai/ha
flutriafol/chlorothalonil	47/300	94/600
flutriafol	125	125
prochloraz	400	400
chlorothalonil	500	1000

Flutriafol/chlorothalonil, flutriafol and prochloraz formulations were applied at Z31 and at intervals following a suitable infection period - the trigger rain 'event'. Timings of these applications were 21, 28 and 35 days after infection (DAI). Growth stage Z31 treatments were made irrespective of infection period. Chlorothalonil was applied at Z31 only.

Rainsplash infection periods were measured using a simple 'splash meter' placed within the crop, a device similar to that suggested by Shaw et al., (1986). Rainfall of a certain intensity splashed dye onto an absorbant surface, indicating the potential height of S.tritici infection. The quantity of rain was measured using a rain gauge.

Disease severity on leaves was recorded as percentage area affected using an average of 20 leaves per plot. Yields were taken from 52 m^2 of crop and expressed as percent response relative to the untreated.

All data were analysed statistically using ANOVA. Disease control data were arcsin transformed prior to analysis. Values in any assessment with no letter in common are significantly different at $P < 0.05$. Efficacy of the treatments is expressed as the % reduction in disease compared to the untreated. Yields and disease levels quoted for untreated plots are actual values. Growth stages quoted follow the Zadoks Decimal Code (Zadoks et al., 1974).

TABLE 1. Trial locations, rainfall and application details.

Trial Ref	Location	Cultivar	Application GS	Application Date	Rainfall Events Date	Amount (mm)	Splash height (mm)
GB14	Hambridge, SOMERSET	Slejpner	Z31	22/04	16/04*	12	270
			Z32	10/05	02/05	8	340
			Z39	20/05	27/05	10	550
			Z45	01/06			
GB15	Wootten Wawen, WARWICKSHIRE	Slejpner	Z31	13/04	16/04*	12	350
			Z33-37	10/05	26/04	11	450
			Z37-39	17/05	03/05	3	600
			Z45	27/05	11/05	5	400
GB26	Hillington, NORFOLK	Fortress	Z31	11/04	19/04	12	480
			Z37	20/05	26/04	3	330
			Z39	27/05	30/04*	6	450
			Z45	03/06	05/05	11	440
					10/05	10	480
					20/05	2	380

* Rain events which 'triggered' the 21, 28 and 35 DAI treatments.

RESULTS AND DISCUSSION

Disease development related to rain event

Infection periods were most clearly identifiable at the Somerset site (GB14), where a latent period of 30-35 days elapsed before the first lesions of S.tritici could be seen on the upper leaves. These were initially circular, chlorotic spots between 0.5 and 3 mm in diameter on the upper leaf surface.

Lesion enlargement and pycnidial formation were enhanced during periods of leaf wetness resulting from light rain or dew. New lesions did not always appear on leaves at the upper limit of the rainsplash range, indicating that the severity of new infections was influenced by the distance from the inoculum source.

Protectant and curative ability of the fungicides

The first fungicide application at each site was made at Z31 (first node detectable), the traditional timing of an early cereal foliar fungicide. Rainfall patterns at this time differed markedly between the trials and produced differences in product efficacy that were related to the protectant and/or curative properties of the treatment.

TABLE 2. Control of leaf spot (%) related to fungicide application before or after rainsplash infection.

Treatment	Rate (g ai/ha)	GB15 42 DAA 39 DAI	GB14 26 DAA 32 DAI	GB26 46 DAA > 46 DAI
Chlorothalonil	1000	96 d	0 a	11 a
Flutriafol/ chlorothalonil	94/ 600	86 d	78 b	58 b
Flutriafol	125	93 d	62 b	53 b
Prochloraz	400	57 c	73 b	16 a
Untreated (actual % disease on leaf 4)		(10.1)ab	(17.4)a	(4.5)a

NB: DAA = Days After Application; DAI = Days After Infection.

Chlorothalonil gave no significant reduction in disease expression when applied after infection, but when applied before, gave high levels of S.tritici control equivalent to the systemic products.

The relative curative ability of the systemic fungicides

Periods of rainfall between the 'trigger' event and the 21 DAI applications obscured the relationship between symptom expression and infection period in much of the data. In all three trials however, leaf 2 was subjected to only one infection period prior to the 21 DAI application. Applications to leaf 2 were made from 7 to 29 days after that infection period.

TABLE 3. Leaf spot control (%) from fungicides applied at three timings within the latent period.

Treatment: Rate (g ai/ha)			Flutriafol/ chlorothalonil (94/600)	Flutriafol (125)	Prochloraz (400)	Untreated (-)
Trial		DAI				(actual % disease) on Leaf 2
GB14	T2	8	99 f	95 def	82 cde	
	T3	18	98 ef	91 def	80 cd	(14.6)a
	T4	29	65 bc	75 bcd	41 ab	
GB15	T2	7	84 f	61 cd	46 bc	
	T3	14	75 ef	64 de	45 b	(36.9)a
	T4	24	76 ef	52 bcd	58 bcd	
GB26	T2	10	86 e	61 cd	36 b	
	T3	17	80 de	64 cde	58 c	(82.9)a
	T4	23	66 cde	64 cd	47 bc	

Flutriafol/chlorothalonil gave consistently good control of leaf spot, at least matching that achieved by flutriafol alone, and often significantly better than prochloraz. All treatments tended to be slightly less effective when applied towards the end of the latent period.

Persistence of Control

At sites GB14 and GB26 assessments of S.tritici were carried out at intervals up to 100 days after the Z31 (T1) treatments. Good levels of control were still evident up to 100 days after treatment, especially with the flutriafol/chlorothalonil mixture and flutriafol alone. Control with prochloraz was generally poorer throughout.

TABLE 4. Leaf spot control (%) from treatments applied at Z30-31.

Trial:	GB14			GB26		
DAA :	47	69	100	53	79	96
Leaf Assessed:	L2	L2	L1 (flag)	L4	L2	L2
Flutriafol/ chlorothalonil	63 bc	71 b	63 c	40 b	65 b	54 c
Flutriafol	83 c	82 b	57 c	36 b	67 b	40 bc
Prochloraz	47 b	56 b	37 b	0 a	51 b	24 b
Untreated (Actual % disease)	(8.5)a	(14.6)a	(66.3)a	(32.4)a	(20.6)a	(82.9)a

Yield responses

Yield responses to application timing and individual fungicides were analysed factorially. The timing of application had no significant effect on yield in trials GB14 and GB15, but in trial GB26 treatments applied after flag leaf emergence gave the greatest response.

TABLE 5. Yield response to application timing (%).*

Timing		GB14	GB15	GB26
T1 Z30-31		12	18	25 b
T2 (21 DAI)		15	16	29 ab
T3 (28 DAI)		13	18	32 a
T4 (35 DAI)		9	16	30 a
Untreated		0	0	0 c
(Actual t/ha @ 85% d.m.)		(6.07)	(5.75)	(4.91)
Growth stage application	T2	Z32	Z33-37	Z37
	T3	Z39	Z37-39	Z39
	T4	Z45	Z45	Z45

* Data are means of flutriafol/chlorothalonil, flutriafol and prochloraz treatments.

Flutriafol/chlorothalonil and flutriafol alone, averaged over timing, generally gave higher yields than prochloraz. At trial GB26 the flutriafol/chlorothalonil mixture yielded significantly more than flutriafol alone.

TABLE 6. Yield response to fungicide (%).*

Fungicide	Rate (g ai/ha)	GB14	GB15**	GB26
Flutriafol/ chlorothalonil	94/600	15	17	34 a
Flutriafol	125	14	16	29 b
Prochloraz	400	09	18	22 c
F-test probability (%)		(8.84)		(0.01)

* Data are means of T1 (excluding chlorothalonil), T2, T3 and T4 timings.
** >93% lodging occurred in all treatments at site GB15.

CONCLUSIONS

These trials demonstrate the benefits of controlling S.tritici at an early stage of its development. The knowledge of infection periods is clearly critical in deciding on the choice of chemical and the timing of application. Protectant fungicides have little effect once infection has taken place and, in practice, the vagaries of the UK climate and the need for flexibility in crop management mean that the grower must select products which will achieve effective control of S.tritici under most conditions and crop growth stages.

The formulated mixture of flutriafol and chlorothalonil proved to be at least as effective a protectant as full rates of flutriafol alone or chlorothalonil, and equally as effective as full rate flutriafol when applied during the latent period.

The use of this mixture allows a flexible approach to S.tritici control. Substantial reductions in symptom expression were achieved from applications as early as GS Z31 until late into the latent period. These high initial levels of control were reflected in long term reductions of S.tritici and significant increases in yield.

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CGA 173506: A NOVEL FUNGICIDE FOR SEED TREATMENT

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ABSTRACT

CGA 173506 is a new phenylpyrrole fungicide being developed by CIBA-GEIGY as a seed treatment and for foliar uses. It is highly active as a seed treatment against target pathogens at low rates of use. In cereals, 5 g A.I./ 100 kg seed gave control of *Gerlachia nivalis (Fusarium nivale)*, *Fusarium culmorum*, *Tilletia caries* and seed-borne *Septoria nodorum*, equivalent to that given by the best commercial products.

CGA 173506 is also active in a wide range of non-cereal crops, controlling a broad spectrum of fungi among Ascomycetes, Basidiomycetes and Deuteromycetes; 5 g A.I./ 100 kg seed was effective against *Fusarium graminearum* on maize, and 25 g A.I./ 100 kg seed was effective against *Gibberella fujikuroi* on rice.

Strains of fungi resistant to products from other chemical classes (e.g. benzimidazoles) show no cross-resistance to CGA 173506. This is particularly important for a seed treatment to control MBC-resistant *Fusarium* spp. (e.g. *Gerlachia nivalis*, *Gibberella fujikuroi*).

INTRODUCTION

Seed treatment provides an effective and efficient way of applying small quantities of fungicides where they are most needed, and is the only effective method for controlling many seed-borne diseases. Widespread use of seed treatments in modern agriculture has led to only a very few cases of resistance problems. However, some fungicides have become subject to loss of efficacy due to development of fungicide resistance, notably resistance of *Fusarium* spp. to benzimadazoles (Locke *et al.*, 1987).

CGA 173506 is a new non-systemic phenylpyrrole fungicide which, although chemically related to fenpiclonil (®BERET), is unrelated to other fungicides currently in common usage (Gehmann *et al.* 1990; Nevill *et al.*, 1988). This fungicide is being developed by CIBA-GEIGY for foliar and seed treatment uses in a wide range of crops and is characterised by having a broad spectrum of activity at low rates of application. For seed treatment, it is highly active against *Fusarium* spp. with no cross-resistance to benzimidazole fungicides. In addition, no adverse effects on emergence or development have been detected on any crop.

This paper presents results from international field trials with CGA 173506 as a seed treatment demonstrating the high efficacy of this fungicide in the major pathosystems and in particular on cereals, rice and maize.

BIOLOGICAL ACTIVITY

Field trials in 1987 - 1990 determined the spectrum of activity of CGA 173506. At the rates shown in Table 1, disease control is equal to the best commercial standards. In addition, CGA 173506 is also active on peas (*Ascochyta* spp, *Fusarium* spp.), potatoes (*Rhizoctonia solani, Fusarium* spp., *Helminthosporium solani*) and oilseed rape (*Alternaria brassicae, Phoma lingam*).

TABLE 1. Main spectrum of activity of CGA 173506 as a seed treatment, determined in field trials in 1987-1990.

Crop	Pathogen	Rate (g A.I./ 100 kg seed)
Wheat	*Tilletia caries*	2.5 - 5
	Gerlachia nivalis	5
	Fusarium culmorum	5
	Septoria nodorum	5
Barley	*Gerlachia nivalis*	5
	Ustilago hordei	5
	Helminthosporium gramineum	5 (approx. 85% control)
	Cochliobolus sativus	5 (approx. 70% control)
Rye	*Gerlachia nivalis*	5-10
	Urocystis occulta	5
Maize	*Fusarium graminearum*	5
Rice	*Gibberella fujikuroi*	25
	Helminthosporium oryzae	25

Control of *Gerlachia nivalis (Fusarium nivale)* on cereals

CGA 173506 was evaluated against snow mould (*Gerlachia nivalis)* on winter wheat in 24 field trials in Switzerland, Germany and Denmark during 1987-1989. Heavily infected seed was obtained either from natural infections or by artificial inoculation of ears during flowering, and was sown at sites known to be favourable for the disease development.

In these trials, CGA 173506 at 5 g A.I./ 100 kg seed gave control of disease equal to or better than the current best commercial standards as evaluated by stand increases in the autumn (GS 11) and in spring (GS 13-21). Results of the spring evaluations are shown in Fig. 1. Standard products used were guazatine + fenfuram at 90 + 30g A.I./ 100 kg seed in Switzerland or at 60 + 20g in Germany, and guazatine at 60g in Denmark. Stand increases of up to 725 % were recorded in comparison to the untreated check.

Field trials in Switzerland with *G. nivalis* on winter rye showed that CGA 173506 at 5-10g A.I./ 100kg seed gave control of severe attacks of this disease equal to that given by guazatine + fenfuram at either 90 + 30g or 60 + 30g (Table 2). These trials with highly

infected seed were carried out at high altitude to ensure prolonged snow cover to favour development of the disease.

FIGURE 1. Stand increases in spring with CGA 173506 treatments (5 g A.I./ 100 kg seed) plotted against stand increases with standard treatments in 24 field tests of *G. nivalis* on winter wheat. The line shows performance equivalent to standards and points lying above the line indicate improvement over the standards.

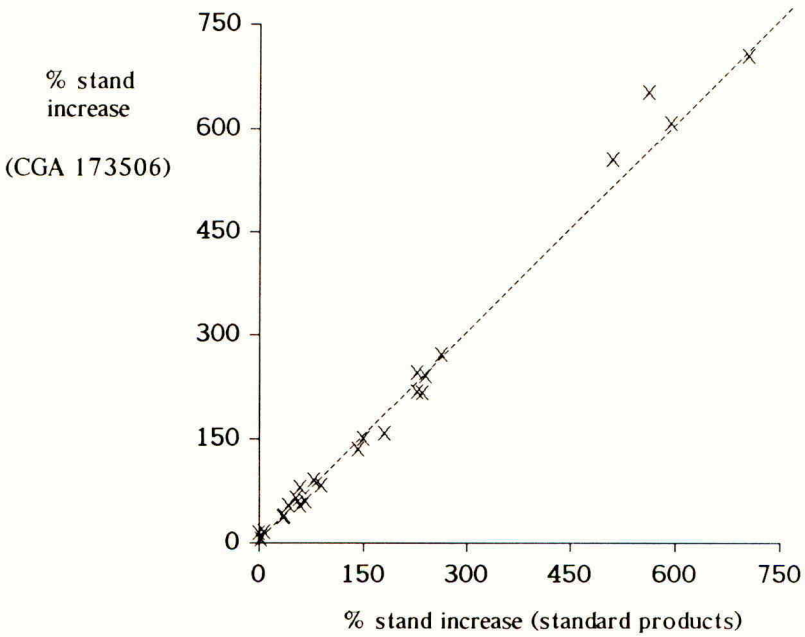

TABLE 2. Control of *G. nivalis* on winter rye, Switzerland 1988 & 1989.

Treatment	Rate (g A.I./ 100 kg seed)	% diseased leaf area in March 1988	1989a	1989b
Untreated	--	43.3	58.9	29.1
Guazatine+fenfuram	60+30*	3.0	9.9	3.8
Benomyl	112.5	14.0	20.6	11.1
Fenpiclonil	20	3.0	11.6	3.2
CGA 173506	5	4.0	8.2	5.1
CGA 173506	10	2.0	7.2	1.6

* = in 1989 trials rate of guazatine + fenfuram was 90 + 30 g A.I./ 100 kg seed.
1988 = trial FR013, 1989a = trial FR019, 1989b = trial FR020

Laboratory analysis confirmed that *G. nivalis* in these trials (Table 2) was largely resistant to benomyl. The activity of CGA 173506 against MBC-resistant *G. nivalis* was confirmed by *in vitro* tests.

Control of common bunt (*Tilletia caries*) on winter wheat

CGA 173506 was tested in 35 field trials for activity against common bunt of wheat (*Tilletia caries*) in Switzerland, Germany, Denmark, Great Britain, Holland and USA in1987 -1989 (Table 3.). The seed used was infected either naturally or by direct inoculation with spores at 2 - 5 g per kg seed before treatment. In these trials, CGA 173506 at 2.5 g A.I./ 100 kg seed gave 98-100% control and at the 5g rate favoured for commercial development control was 99-100% in all trials.

TABLE 3. Control of *T. caries* on winter wheat with CGA 173506.

Rate (g A.I./ 100 kg seed)	Number of trials	% disease in untreated	% efficacy
2.5	15	3-49 (mean=18)	98-100
5.0	30	3-49 (mean=18)	99-100

Control of leaf stripe (*Helminthosporium gramineum*) on barley

CGA 173506, when applied at 5 g A.I./ 100kg seed provides good control of *H. gramineum* on winter barley. In a series of 31 field trials throughout Europe during 1987-1990, control averaged 86% (range 80-100%) and was achieved across a wide range of disease incidence (Table 4). Although this was usually weaker than the performance of the best commercial treatments (e.g. those containing imazalil), it was clearly superior to such products as copper oxyquinolate (20g) as used in France. Where performance requirements are higher, for example in Germany or Great Britain, a mixture of CGA 173506 with imazalil (5 + 4 g A.I./ 100 kg seed) will be recommended.

TABLE 4. Control of *H. gramineum* on winter barley, 1987-1990.

% tillers attacked on untreated	Number of trials	Mean % efficacy of: CGA 173506 5g	Baytan ** 37.5+4.5+50g	copper* 20g
0.9-3%	8	82.6 (80-100)	96.4 (82-100)	---
4-20%	17	87.7 (82-100)	99.5 (98-100)	---
21-69%	6	86.3 (83-93)	---	37.9 (7-90)

**®Baytan = triadimenol + imazalil + fuberidazole (registered trademark of Bayer AG).
* = copper oxyquinolate

Control of *Fusarium graminearum* on maize (corn)

CGA 173506 was tested in field trials in USA in 1989 - 1990 against *F. graminearum* on hybrid corn and sweet-corn varieties. In trials with either natural or artificial infection, CGA 173506 at 5 g A.I./ 100 kg seed gave stand increases at least equal to captan at 150g (Table 5). Average stand increases over untreated in 1990 field trials were 38% from CGA 173506 compared with only 15% for captan. Artificial inoculation was made by sowing infected oat grains with the maize seed and all maize seed was also treated with metalaxyl (®APRON) at 30 g A.I./ 100 kg seed to eliminate the influence of *Pythium* spp.

TABLE 5. Control of *Fusarium graminearum* on maize, USA 1990.

Treatment	Rate (g A.I./ 100 kg)	Mean final plant stand per plot			
		25/90*	106/90*	26/90	24/90
Untreated	---	41	48	176	76
Captan	150	78	87	186	103
CGA 173506	5	143	87	184	137

* = trials 25/90 and 106/90 artificially inoculated.

Control of bakanae disease (*Gibberella fujikuroi*) on rice

CGA 173506 has been extensively evaluated as a seed slurry treatment and as a seed soak treatment for the control of seedling diseases of rice, including bakanae disease. Trials were carried out during 1989 at 10 different official testing stations in Japan (Iwate, Miyagi, Niigata, Ibaraki, Aichi, Shiga, Hyogo, Shimane, Ehime and Sagu). CGA 173506 as either a seed slurry treatment at 25 g A.I./ 100 kg seed, as a dip to pre-germinated seed for 10 minutes at 1.25-2.5 g/l, or for 24 hours at 0.125-0.25 g/l was more effective than the standard benomyl + thiram as either seed slurry or dip treatments at rates significantly lower than this standard (Tables 6 and 7).

These trials were carried out with MBC-sensitive and resistant strains of *G. fujikuroi* and CGA 173506 was always highly active. The efficacy of benomyl+thiram suggests that resistant strains were present only in low proportions.

TABLE 6. Control of *G. fujikuroi* in seed slurry treatment, Japanese official trials 1989.

Treatment	Rate (g A.I./ 100kg seed)	mean % seedlings infected	
		MBC-R* (6 trials)	MBC-S* (1 trial)
Untreated	----	35.7	67.3
Benomyl+thiram	100+100	2.3	4.5
CGA 173506	25	0.3	0.3

* R = trials where resistance to MBC was detected. S = no resistance detected.

TABLE 7. Control of *G. fujikuroi* in seed dip treatment, Japanese official trials 1989.

Treatment	Rate (g/l)	Time	mean % seedlings infected MBC-R* (6 trials)	MBC-S* (2 trials)
Untreated	---	---	44.6	46.7
Benomyl+thiram	10.0+10.0	10min	4.4	4.5
Benomyl+thiram	1.0+1.0	24h	9.1	14.2
CGA 173506	1.25 to 2.5	10min	0.4	0.1
CGA 173506	0.125 to 0.25	24h	0.5	0.1

* R = resistance to MBC was detected; S = no resistance detected.

These results were confirmed by trials conducted by CIBA-GEIGY in Japan where the activity of CGA 173506 at the above rates has been found to be at least equal to that of prochloraz or triflumizole at recommended rates and a wide range of pre-sowing regimes. In *in vitro* studies, CGA 173506 was effective against MBC-resistant isolates.

Further trials in Japan showed that CGA 173506 used as above was highly active against *Helminthosporium oryzae*, giving control superior to the benomyl+thiram standard and equal to triflumizole at recommended rates. In addition, good activity was found against *Pyricularia oryzae* although it was not equal to the best standards.

CONCLUSIONS

CGA 173506 represents a new chemical tool for the control of a wide range of economically important seed-borne diseases. It is highly active at low rates, especially against *Gerlachia nivalis*, *Gibberella fujikuroi* and other related *Fusarium* species, and is equally effective against MBC-sensitive and resistant forms of these fungi.

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EXPERIENCES WITH BAS 480 F, A NEW TRIAZOLE FUNGICIDE, FOR THE CONTROL OF CEREAL DISEASES IN WESTERN EUROPE

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ABSTRACT

BAS 480 F is a new systemic triazole fungicide with a broad spectrum of activity. Results from field trials in Denmark, France, Federal Republic of Germany and United Kingdom during the years 1987-89 showed that BAS 480 F at 125 g AI/ha gave very effective control of *Erysiphe graminis*, *Puccinia spp.*, *Septoria tritici*, *Leptosphaeria nodorum*, *Pyrenophora tritici-repentis*, *Pyrenophora teres* and *Rhynchosporium secalis*. With 187 g AI/ha, good control of *Pseudocercosporella herpotrichoides* was achieved. Yield increases reflected the good disease control obtained.

INTRODUCTION

BAS 480 F (SC, 125 g/l) is a new systemic triazole discovered and patented by BASF Aktiengesellschaft Ludwigshafen (Ammermann et al., 1990). The compound showed excellent control of the most important cereal diseases of wheat, barley and rye. The following results were obtained from trials in 1987-89.

MATERIALS AND METHODS

Field trials were performed in Western Europe in United Kingdom (UK), France (FR), Denmark (DK) and the Federal Republic of Germany (FRG).

The trials were laid out in randomized blocks with 4 replications and the size of plots varied from 12 to 20 m^2. All trials were sprayed at the beginning of disease attack using either a small plot tractor-spray equipment or a hand-held precision plot sprayer. Treatments were applied in 200 - 400 l water/ha.

Visual assessments of infected leaves or green tissue were made as percentage for the plot as a whole. Assessments of foot rot in FRG and UK were done according to BBA Richtlinie (Bauers et al., 1986). In FR, the stem was cut and the percentage of necrosis on the cross-section assessed.

The trials were harvested using a small plot combine harvester and yields corrected to 86 % dry matter content. Cereal growth stages (GS) are described after Zadoks et al. (1974).

Numbers of trials (n) are given and letters following yields indicate significant differences in a Duncans multiple range test ($p < 0.05$). Treatments with the same letter are not significantly different.

RESULTS

Pseudocercosporella herpotrichoides

The efficacy of BAS 480 F against eyespot is shown in Table 1. Increasing the amount applied from 125 to 188 g AI/ha improved the biological activity against *P. herpotrichoides* but did not completely reach the value of prochloraz. The addition of carbendazim to BAS 480 F resulted in similiar or superior foot rot control compared with the standards used.

Treatment with BAS 480 F improved yield which was significantly increased by up to 1 t/ha and was superior to the standard prochloraz.

TABLE 1. Control oF eyespot (Pseudocercosporella herpotrichoides) on winter wheat, 1987 - 89.

Treatment	g AI/ha	FR n = 5 % attack	FR n = 5 Yield t/ha	UK *) n = 7 Ia	UK *) n = 7 Yield t/ha	FRG n = 15 Ia	FRG n = 15 Yield t/ha
Untreated	-	71	6,1 a	24,55	7,18 a	40	6,60 a
BAS 480 F	125			19,86	8,07 de		
BAS 480 F	156			18,81	7,98 cd		
BAS 480 F	188	36	6,9 c	17,13	8,11 de	31	7,40 c
BAS 480 F + carbendazim	188 + 150	24	7,0 c				
Flusilazole	300			18,24	7,78 bc		
Flusilazole + carbendazim	300 + 150	30	6,9 c				
Prochloraz	400			14,90	7,72 b	25	7,32 bc
Prochloraz + carbendazim	450 + 120	35	6,4 b				

Treatment: GS 30-32 *) Index of attack

Erysiphe graminis

Two treatments with BAS 480 F at GS 32-37 and 55-61 greatly reduced powdery mildew attack on wheat. The activity of BAS 480 F at 125 g AI/ha was comparable with the ready-mix tebuconazole + triadimenol (250 + 125 g AI/ha) and flusilazole + tridemorph (160 + 350 g AI/ha). Also efficacy was improved when combined with fenpropimorph (125 + 375 g AI/ha). The good disease control was reflected in the values for green tissue (Table 2).

The combination BAS 480 F + fenpropimorph gave the highest yield increase of 2.03 t/ha.

TABLE 2. Control of powdery mildew (Erysiphe graminis) on wheat, 1988 - 89, FRG (n = 16).

Treatment	g AI/ha	% leaf area infected 30 DAT	Green tissue GS 85	Yield t/ha
Untreated	-	22	11	6,99 a
BAS 480 F	125	8	29	8,71 c
BAS 480 F + fenpropimorph	125 + 375	1	34	9,02 d
Flusilazole + tridemorph	160 + 350	10	27	8,51 b
Tebuconazole + triadimenol	250 + 125	6	31	8,85 cd

Treatment: GS 32-37, GS 55-61

Puccinia recondita and P. striiformis

BAS 480 F was highly effective against rust (Tables 3 and 4). With brown rust, 125 g AI/ha on wheat was comparable to tebuconazole + triadimenol (250 + 125 g AI/ha). Both products increased the yield significantly. Similiar results were obtained against brown rust on rye.

In DK, BAS 480 F (125 g AI/ha) and tebuconazole (250 g AI/ha) gave similar control of yellow rust and were better than flusilazole (200 g AI/ha), fenpropimorph (750 g AI/ha) and propiconazole (125 g AI/ha). All treatments increased yield.

TABLE 3. Control of brown rust (Puccinia recondita) on winter wheat and rye 1989, FRG.

Treatment	g AI/ha	% leaf area infected 30 DAT		Green tissue GS 86		Yield t/ha	
		wheat n=15	rye n=3	wheat	rye	wheat	rye
Untreated	-	13	66	10	2	6,89 a	5,74 a
BAS 480 F	125	0	0	18	45	7,81 c	7,06 de
Tebuconazole + triadimenol	250 + 375	0		17		7,70 c	
Propiconazole + fenpropimorph	125 + 375		2		39		6,89 cde

Treatment: GS 39 - 61

TABLE 4. Control of yellow rust (Puccinia striiformis) on wheat 1987 and 89.

Treatment	g AI/ha	% leaf area infected 31 DAT DK n=4	FRG n=5	Green tissue FRG	Yield t/ha DK	FRG
Untreated	-	73	13	17	7,46 a	5,18 a
BAS 480 F	125	2	0	50	8,94 ab	7,60 e
Flusilazole	200	9			8,63 a	
Tebuconazole	250	5			8,87 ab	
Fenpropimorph	750		2	38		7,08 d
Propiconazole	125		2	38		7,07 d

Treatment: GS 39 - 61

Septoria tritici and Leptosphaeria nodorum

Very strong attacks of leaf spot and glume blotch were highly suppressed by two applications of BAS 480 F (125 g AI/ha) and the control was equal to or better than the standards used. Yields were significantly increased (Tables 5 and 6).

TABLE 5. Control of leaf spot (Septoria tritici) on wheat 1989, UK n=3.

Treatment	g AI/ha	% infected leaf area 35 DAT
Untreated	-	29
BAS 480 F	125	9
Flusilazole	200	11
Propiconazole	125	12

Treatment: GS 39

TABLE 6. Control of glume blotch (Leptosphaeria nodorum) on wheat, 1987 and 1989, FRG.

Treatment	g AI/ha	% infected area I n=11 GS 87	II n=8 GS 75	Green tissue II GS 85	Yield t/ha I	II
Untreated	-	37	34	5	5,94 a	7,10 a
BAS 480 F	125	11	8	19	7,11 c	8,01 c
Propiconazole	125	16			6,98 b	
Flusilazole + tridemorph	160 + 350		21	16		7,98 bc
Tebuconazole + triadimenol	250 + 375		9	18		7,93 b

Treatment: GS 39 and GS 61 I = 1987 II = 1989

Pyrenophora tritici-repentis

Tan spot has spread in the southern area of Germany in recent years and caused severe losses. In 5 trials (Table 7) BAS 480 F and propiconazole applied at GS 39 gave similar disease control and yield increase.

TABLE 7. Control of tan spot (Pyrenophora tritici-repentis) on wheat, 1989, FRG (n=5).

Treatment	g AI/ha	% infected leaf area 30 DAT	GS 87	Green tissue GS 75-87	Yield t/ha
Untreated	-	30	30	12	6,81 a
BAS 480 F	125	4	14	33	7,84 c
Propiconazole	125	3	19	27	7,85 c
Prochloraz	480	12	19	22	7,46 b

Treatment: GS 31-39

Rhynchosporium secalis

BAS 480 F at 125 g AI/ha decreased scald on barley from 40 % to 8 % in France and 44 % to 12 % in Germany. Control was similar with tebuconazole + triadimenol (250 + 125 g AI/ha) and flusilazole + tridemorph (160 + 350 g AI/ha). The green tissue and yield responses reflect control of *R. secalis*. In FR, BAS 480 F gave yields significantly larger than flusilazole and in Germany it was equivalent to the standards.

TABLE 8. Control of scald (Rhynchosporium secalis) on barley, 1988 - 89.

Treatment	g AI/ha	% infected leaf area FR n=3 35 DAT	FRG n=10 GS 75	Green tissue FRG GS 85	Yield t/ha FR	FRG
Untreated	-	40	44	19	3,5 a	7,59 a
BAS 480 F	125	8	12	45	6,2 de	8,52 b
Flusilazole	200	8			5,6 c	
Flusilazole + tridemorph	160 + 375		8	47		8,57 b
Tebuconazole + triadimenol	250 + 125		12	45		8,55 b

Treatment: GS 32-37

Pyrenophora teres

Results from trials in FR and FRG showed that BAS 480 F (125 g AI/ha) gave results similar to propiconazole (125 g aI/ha) and the other products tested.

Yield increases were similar with BAS 480 F, flusilazole and the combination with tridemorph. Yields from BAS 480 F matched those from propiconazole.

TABLE 9. Control of net blotch (Pyrenophora teres) on barley 1988 - 89.

Treatment	g AI/ha	% infected leaf area 30 DAT				Green tissue FRG	Yield t/ha	
		FR	n=3	FRG	n=3	GS 85	FR	FRG
Untreated	-	33		18		3	6,1 a	7,85 a
BAS 480 F	125	11		7		30	7,3 d	9,05 bc
Flusilazole	200	5					7,3 d	
Flusilazole + tridemorph	160 + 350			5		34		9,18 cd
Tebuconazole + triadimenol	250 + 125			7		28		8,97 b
Propiconazole	125	13					6,6 b	

Treatment: GS 32 and GS 51

CONCLUSIONS

Results presented in this paper show BAS 480 F to be a highly effective fungicide for controlling economically important cereal diseases and increasing yields.

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UPTAKE, TRANSPORT AND MODE OF ACTION OF BAS 480 F, A NEW TRIAZOLE FUNGICIDE

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ABSTRACT

The properties of BAS 480 F, (2RS,3RS)-1-[3-(2-chlorophenyl)-2-(4-fluorophenyl)-oxiran-2-ylmethyl]-1H-1,2,4-triazole, were investigated with respect to its uptake and transport and effects on fungal sterol biosynthesis and morphology. Radiotracer studies showed the fungicide was systemic and well distributed within the plant tissue. It was an exceptionally potent inhibitor of fungal sterol 14α-demethylase in whole cells and a cell-free enzyme system and bound to the target enzyme with very high affinity. Cytological studies with *Uromyces appendiculatus* showed that treatment with low levels of BAS 480 F completely arrested fungal growth and development 2 days after inoculation.

INTRODUCTION

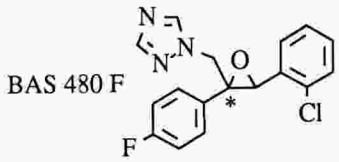
Public concern for health and environmental issues is likely to place ever increasing demands for information on the manufacturers of plant protection products. In addition to fulfilling the statutory requirements for the registration of new products, it is also desirable to obtain a fuller understanding of their physiological effects and modes of action.

Azole fungicides are generally able to penetrate the plant surface (for a review see Kuck & Scheinpflug, 1986) and their systemic properties confer both prophylactic and curative activities. They also all inhibit the same step in the sterol biosynthetic pathway, the removal of the 14α-methyl group (for a review see Mercer, 1984). In the light of these similarities, new compounds introduced into this well established group of fungicides must compare favourably with existing products. They should exhibit high biological activity for an effective period of time at low application rates. Protection against disease can thus be maintained when significantly lower levels of fungicide are released into the environment. High biological activity is the net result of a complex interaction between many different factors. However, it ultimately depends upon the concentration of active ingredient encountered by the sensitive fungal structures and on its intrinsic capacity to inhibit the target enzyme, thus initiating the disruption of fungal physiology and morphology. We therefore investigated the uptake and translocation of BAS 480 F by wheat plants and its capacity to inhibit fungal sterol 14α-demethylase activity in yeast cultures and a cell-free enzyme system. Binding to the target enzyme was determined spectrophotometrically. The *in vivo* effects of the fungicide on the infection process of *U. appendiculatus* were observed by fluorescence microscopy and scanning electron microscopy.

MATERIALS AND METHODS

Uptake and transport studies

[^{14}C]BAS 480 F, specific activity 0,67 MBq/mg, labelled in position 2 of the oxiran ring (*), was used as an aqueous solution in 0,5 % (*V/V*) methanol. Different formulations were tested by integrating [^{14}C]BAS 480 F into concentrated stock solutions before diluting with water to the final spraying concentrations.



Plant material: Wheat (cv. Kanzler) was cultivated in the glasshouse at ca. 20°C and 60% r.h. until growth stage 11. Additional HQI-light was given for a 16h day. During experiments, the plants were incubated in growth chambers with a 16-h-day at 20°C and 60% r.h. and 16°C night (similar humidity).

Adsorption test: The effects of different formulations and adjuvants on BAS 480 F adsorption by wheat leaves were determined by incubating leaves with test solutions in capped vials on a roller mixer. After 15 min, the distribution of active ingredient was determined by measuring the radioactivity remaining in the test solutions, in superficial methanol washings of leaf material and in CH_2Cl_2-extracts.

Penetration and translocation test: 5µl-droplets of test solutions (stained with aniline blue for a better visual control) were applied between the basal and the middle third of wheat leaves. The time course of penetration of epicuticular wax layers was monitored by removing surface residues of BAS 480 F at intervals using cellulose acetate stripping (Silcox & Holloway, 1986). Treated leaves were then cut into 5mm sections and the distribution of [^{14}C]BAS 480 measured by liquid scintillation counting after combustion of the dried leaf segments. Translocation was also determined by autoradiography and quantified by scanning the optical density of the autoradiograms.

Inhibition of sterol biosynthesis

The high sterol yeast strain SC 739 was grown for 16h at 28°C with shaking in a complete liquid medium. BAS 480 F was added in ethanolic solution before inoculation. Control cultures received ethanol only. Cells were harvested by centrifugation and refluxed for 90 min. in methanolic KOH. Sterols were extracted from the saponification mixture with hexane:*tert*butylmethylether (10:1, *V/V*) and analysed by gas chromatography/mass spectrometry with additional structural confirmation by nmr when necessary.

The effects of BAS 480 F on sterol biosynthesis in a cell-free enzyme system (Gadher *et al.*,1983) were also determined. Cells grown in liquid culture were harvested by centrifugation and homogenised in a glass bead mill. A fraction which incorporated [2-^{14}C]mevalonic acid into ergosterol with high efficiency was obtained by differential centrifugation. Inhibitor was added in ethanolic solution. After incubation for 2h sterols were extracted as above and separated by tlc. The distribution of radioactivity incorporated in the sterol fractions was determined using a Berthold Linear Analyser.

The binding of BAS 480 F to the sterol 14α-demethylase, a cytochrome P-450, was estimated spectrophotometrically (Aoyama *et al.*,1983). Inhibitor was added in increments to solubilised yeast microsomes obtained by differential centrifugation and the preparation was scanned immediately between 350 and 550 nm. Solvent only was added to the enzyme in the reference cuvette.

Cytological studies

Urediospores of the bean rust fungus, *U. appendiculatus*, were produced on garden bean (cv. Fori) at 20-24°C under greenhouse conditions. Protective fungicide applications were made by spraying the primary leaves with an aqueous suspension of BAS 480 F, 25% WP,

(1 & 5 mg/l AI) until a deposit was clearly visible. Approximately 24 h later the lower leaf surfaces were inoculated with freshly harvested urediospores, incubated overnight at high humidity then returned to the greenhouse. Leaves were sampled at 2, 5 and 8 days post inoculation (dpi) and prepared for fluorescence microscopy (FM) (Hänßler & Kuck, 1987). Specimens for scanning electron microscopy (SEM) were rapidly frozen in liquid propane at 9h post inoculation and evaluated using Cryo-SEM techniques (Müller *et al.*,1986).

RESULTS

Adsorption of BAS 480 F on the plant surface

The degree of adsorption of BAS 480 F by wheat leaves in contact with a large surplus of spray solution was strongly affected by the type of formulation and the addition of surfactant (Table 1).

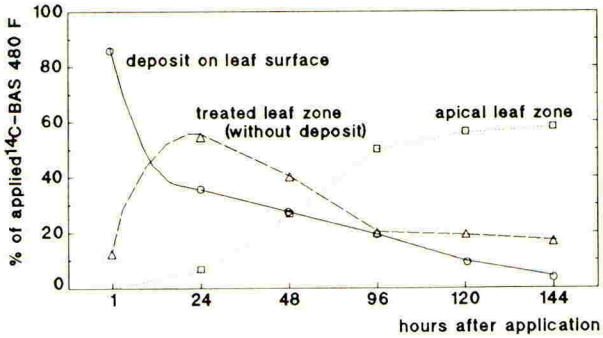
TABLE 1. Effect of Formulation and Surfactant on the Adsorption of BAS 480 F.

Type of formulation	SC	SC	EC	EC
addition of surfactant	-	+	-	+
active ingredient remaining in solution	98 %	25 %	91 %	60 %
recovery in leaf methanol-washings	2 %	75 %	7 %	29 %
recovery in leaf CH_2Cl_2-extracts	0 %	0 %	2 %	11 %

Penetration of BAS 480 F into the leaf and its translocation

The uptake and subsequent translocation of [^{14}C]-labelled BAS 480 F (drop application of diluted SC) in wheat leaves is demonstrated in Fig. 1. There was no apparent basipetal transport. The [^{14}C]-activity found in the basal leaf segments did not exceed 1 % of the initial activity in the application zone.

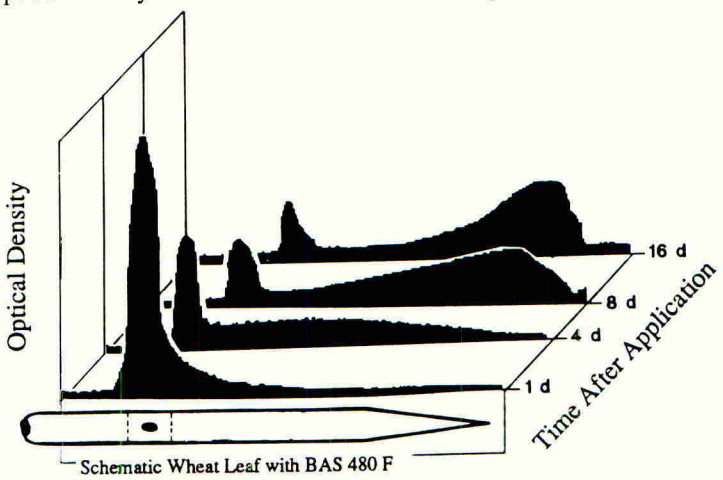
FIGURE 1. Distribution of [^{14}C]BAS 480 F in Wheat Leaves.



Translocation through the leaf was also monitored by autoradiography (Fig. 2) which demonstrated that 4 days after applying a single drop of diluted BAS 480 F, the whole

acropetal part of wheat leaf was uniformly labelled. Subsequent further translocation was slower, with about 20 % of the activity still remaining in the treated leaf segment.

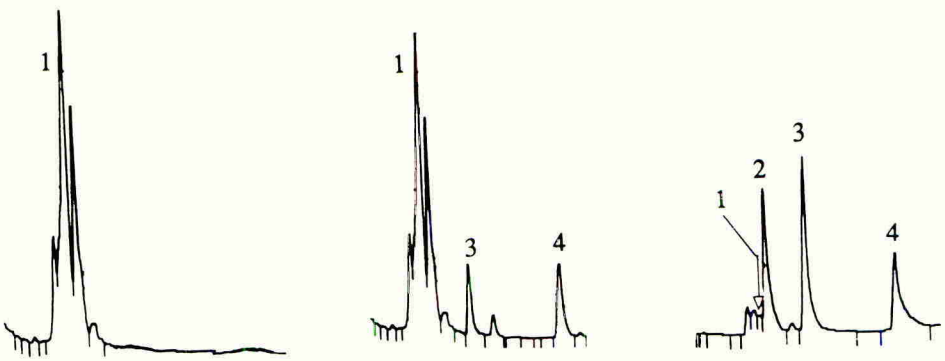
FIGURE 2. Optical Density Scans of Wheat Leaf Autoradiograms.



Inhibition of sterol biosynthesis

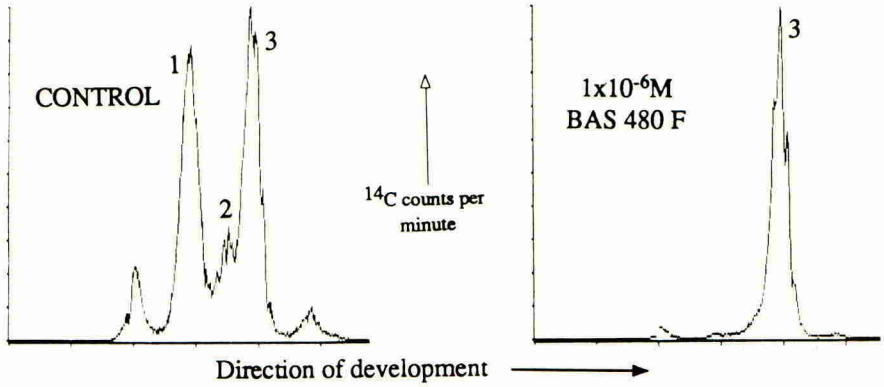
The growth of yeast cultures was strongly inhibited by extremely low concentrations of BAS 480 F (<0,5µM). This was reflected by the sterol profiles of treated cultures, as shown by gas chromatography (Fig.3). At $5x10^{-8}$M BAS 480 F there was very clear accumulation of sterols which retained the 14α-methyl group. Increasing the concentration to $1x10^{-7}$M caused these to become by far the dominant sterols and ergosterol was reduced to a trace level, indicating almost complete inhibition of the target enzyme at this very low fungicide concentration. The main sterols to accumulate were 14α-methyl fecosterol, lanosterol and 14α-methyl-ergosta-8,24(28)-diene-3β,6α-diol.

FIGURE 3. Gas Chromatograms of Yeast Sterol Extracts.
1=ergosterol, 2=14α-methyl fecosterol, 3=lanosterol, 4=14α-methyl-ergosta-8,24(28)-diene-3β,6α-diol.



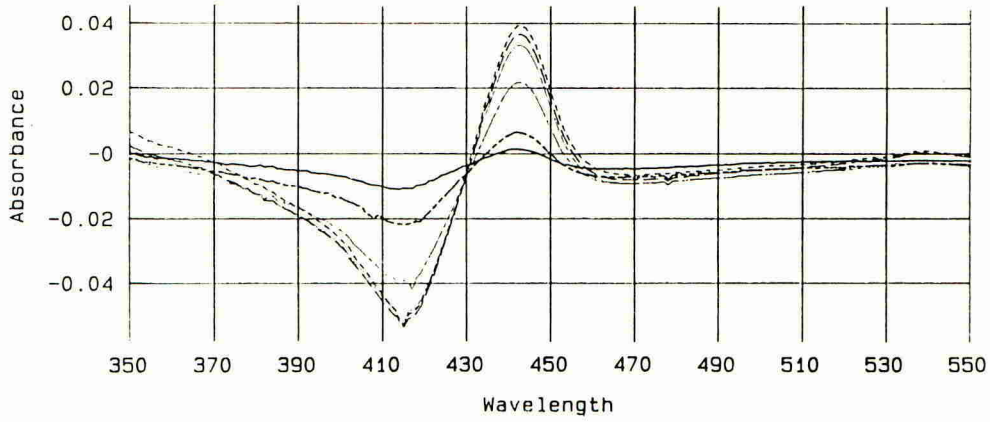
In the cell-free enzyme system $1x10^{-6}$M BAS 480 F totally prevented the incorporation of [^{14}C]mevalonic acid into 4,4-demethyl sterols, including ergosterol. Incorporation into 4,4-dimethyl sterols increased in proportion(Fig.4).

FIGURE 4. Scans of [^{14}C]sterols from the Cell-free Enzyme System, Separated by tlc.
1=4,4-demethyl sterols, 2=4α-monomethyl sterols, 3=4,4-dimethyl sterols.



The cytochrome P-450 content of solubilised yeast microsomes was determined from the CO difference spectrum (Omura & Sato, 1964). Incremental additions of BAS 480 F to freshly prepared enzyme caused an immediate, concentration-dependent Type II change in the difference spectrum until the concentration of BAS 480 F equalled that of the cytochrome P-450 (Fig.5). Further additions induced no further change, indicating that BAS 480 F binds stoichiometrically with its main target enzyme.

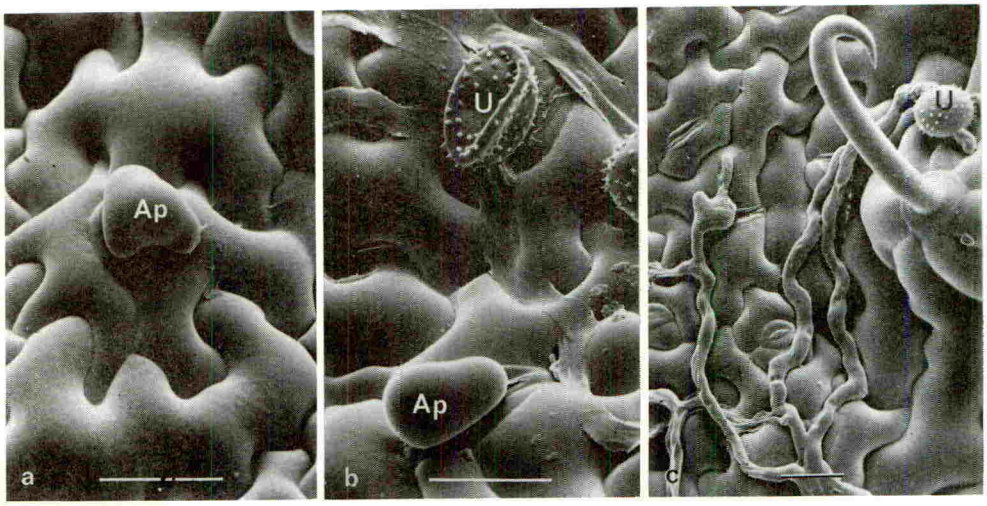
FIGURE 5. Difference Spectra of Yeast Cytochrome P-450 with Incremental Additions of BAS 480 F.



Cytological studies

BAS 480 F prevents sporulation at 1mg/l AI, but it does not affect urediospore germination at concentrations much higher than this (Fig.6).

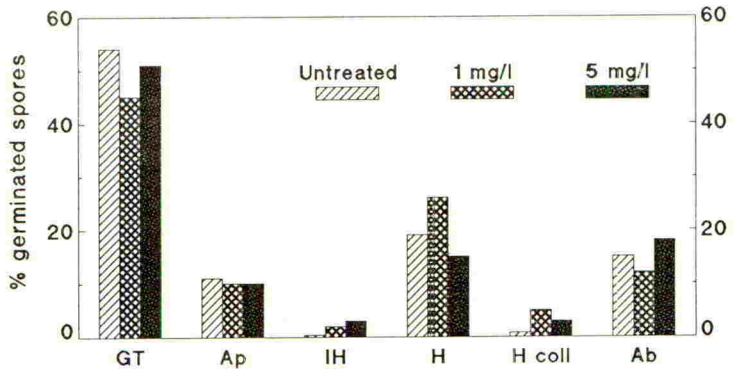
FIGURE 6. Cryo-SEM of *U. appendiculatus* Urediospore Germlings on Bean Leaves 9 hpi. (a) untreated control, (b) 5mg/l BAS 480 F, (c) 250mg/l BAS 480 F. (Bar=25µm).



On control and 5mg/l-treated leaves urediospores (U) form germ tubes and differentiate appressoria (Ap) on contact with the stomatal lip of the guard cell. On 250mg/l-treated leaves spores germinate, but typically fail to form appressoria.

Even at 2 dpi, no inhibitory effects on germ tube differentiation and formation of infection structures were observed after treatment with 1 and 5mg/l BAS 480 F (Fig.7).

FIGURE 7. Influence of BAS 480 F on Early Stages of Infection Structure Formation of *U. appendiculatus* 2 dpi. GT=germ tube, Ap=appressorium, IH=infection hypha, H=haustorium, H coll=haustorium collapsed, Ab=abnormal germling.



At 5 and 8 dpi the fungistatic effect of BAS 480 F on mycelial growth and morphology was clearly evident. Mycelial growth had apparently halted at 2-3 dpi(Fig.8). Many of the hyphae were branched and/or swollen and showed irregular patches of strongly fluorescent material (Fig.9). Haustoria were typically enlarged and irregularly branched.

FIGURE 8. Influence of BAS 480 F on Mycelial Growth of *U. appendiculatus*

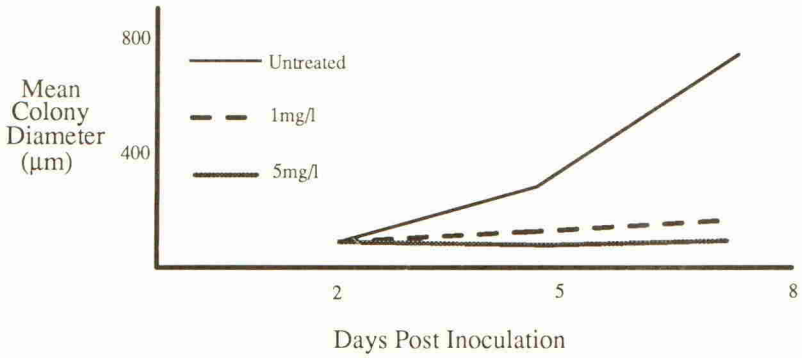
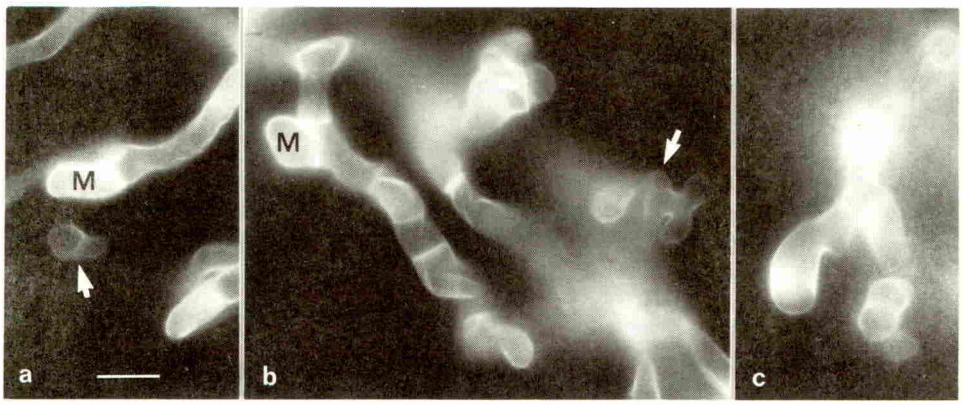


FIGURE 9. Effects of BAS 480 F on *U. appendiculatus* Mycelial Morphology in Bean Leaves 5 dpi. (a) untreated control with haustorial mother cell (M) and haustorium (arrow). Fluorescence is brighter in the mother cell due to its thicker cell wall. (b) 1mg/l BAS 480 F caused distortion of the mother cells and the formation of enlarged, branched haustoria (arrow). (c) distorted growth habit resulting from treatment with 1mg/l BAS 480 F; note the intensely fluorescing hyphal cells.



DISCUSSION

Appropriately formulated and applied as a foliar spray, BAS 480 F adsorbs very effectively to the plant surface. Low losses by run off during the spray treatment and good rain fastness of active ingredient deposits on the leaf surface can thus be expected. Most of the BAS 480 F permeates the plant cuticular structures after the spray deposits have dried. Following penetration, the export of BAS 480 F from treated into untreated wheat leaf segments correlated with its rate of decrease on the plant surface, as determined by cellulose acetate stripping. The range of performance of formulations with different surfactants added was found to be identical for the decrease of active ingredient on the plant surface and for its translocation (data not given). Further evidence that penetration of the plant surface is the rate-limiting factor for BAS 480 F transport within the plant is provided

by the fact that the influence of surfactants was far less when BAS 480 F was applied to wheat leaves without epicuticular waxes (removed by prior stripping; data not given),

Like most other triazoles, BAS 480 F is transported acropetally via the transpiration stream and there is no apparent basipetal transport which exceeds the diffusion rates, even in senescent leaves. However, unlike other systemic fungicides, BAS 480 F distribution over the leaf area is relatively uniform for a prolonged period before acropetal accumulation. This effect supports overall fungicidal protection. Deviation from regular distribution in the form of local enrichment can be observed at sites of fungal infections due to their sink character. This effect is desirable, since eradicative treatment is improved.

The sterol profiles of yeast cultures treated with BAS 480 F show that marked inhibition of sterol biosynthesis occurs at exceptionally low inhibitor concentrations, ergosterol being reduced to trace levels at concentrations below 0,5µM. They also show that there is a large accumulation of the sterol 3β,6α-diol first noted by Ebert *et al.*(1982) in cultures of *Ustilago maydis* after prolonged treatment with etaconazole. The presence of this sterol has recently been proposed as one of the major reasons for the fungitoxicity of azoles (Watson *et al.*,1989). In the cell-free enzyme system BAS 480 F completely suppressed the incorporation of [^{14}C]mevalonic acid into demethyl sterols at a concentration of only 1µM. The difference spectra of BAS 480 F and the target cytochrome P-450 show that it rapidly binds to the enzyme in a 1:1 ratio. These results indicate that BAS 480 F has an affinity for its target enzyme that is probably close to the maximum that can be achieved.

The cytological studies showed that low concentrations (≤5 mg/l) of BAS 480 F do not influence the growth and formation of infection structures of *U. appendiculatus* up to 2 dpi. This is in agreement with several reports on sterol biosynthesis inhibitors in a wide range of fungi (see Scheinpflug,1986; Hänßler & Kuck, 1987). At higher concentrations (>60 mg/l) however, BAS 480 F strongly reduced the formation of functional appressoria, suggesting a disturbance in the stomatal recognition and/or differentiation process. This is the first report of a triazole inhibiting an early differentiation event in the infection process of an obligate plant pathogen. The main effects on fungal development became clear after 2 dpi. BAS 480 F strongly inhibited mycelial growth and altered the morphology of intercellular hyphae and haustoria. The hyphal cell walls were often irregularly thickened, as evidenced by the brighter fluorescence of specific areas of the mycelium. Furthermore, the haustoria were also enlarged and abnormally branched. These effects suggest deregulation of chitin synthesis and deposition as discussed by others (Pring,1984; Roberson *et al.*,1989).

In summary, BAS 480 F is rapidly and strongly adsorbed on the plant surface and is subsequently well distributed within the tissues. Its exceptionally high activity against the target enzyme inhibits fungal growth and development at very low concentrations.

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INFLUENCE OF PROCHLORAZ ON THE EARLY PATHOGENESIS OF *Pseudocercosporella herpotrichoides*

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ABSTRACT

Microscopic techniques have been used to assess the effects of the sterol biosynthesis inhibitor (SBI) prochloraz on infection stages involved in the early pathogenesis of W- and R-pathotypes of the cereal eyespot fungus *Pseudocercosporella herpotrichoides* on wheat seedlings. Protectant sprays made prior to inoculation offered good control, whereas eradicant sprays were less effective due to re-growth of the pathogen from structures within host tissues not directly exposed to the fungicide. Some differential effects on W- and R-types were observed during colonisation of the coleoptile, but infection structures formed by both types on leaf sheaths were similarly affected by prochloraz.

INTRODUCTION

Two main pathotypes of the cereal eyespot fungus *P. herpotrichoides*, most commonly classified as W- and R-types, are routinely isolated from stem base lesions in the field. These may be differentiated on the basis of several criteria, outlined in Table 1. W- and R-types differ in their sensitivity to many SBI fungicides, with the exception of prochloraz (Cavelier *et al.*, 1987, 1990), and also in the development of specialised infection structures formed as part of a morphogenetic programme involved in the early pathogenesis of the disease on wheat plants (Daniels *et al.*, 1990), summarised in Fig. 1.

Light and electron microscopic techniques have been used to assess the effect of protectant and curative applications of the imidazole prochloraz on the pre-lesion infection processes of W- and R-pathotypes on intact wheat seedlings.

MATERIALS AND METHODS

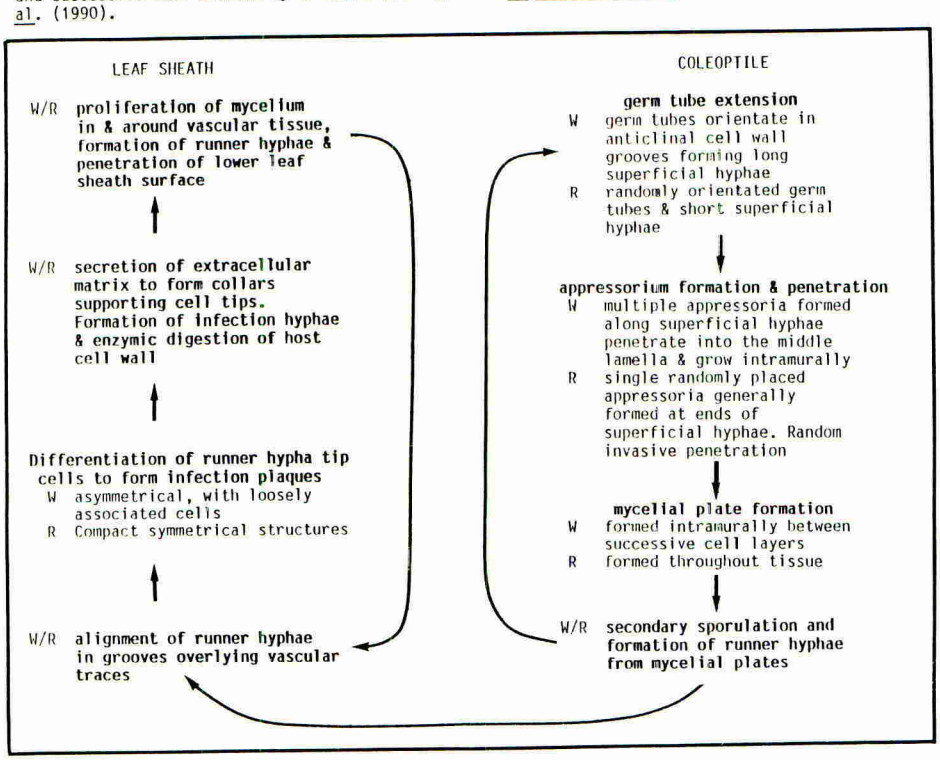
Details of inoculation methods and microscopic techniques are given in Daniels *et al.* (1990). Soil grown wheat seedlings (cv. Avalon) were inoculated with spore suspensions using polyvinyl collars, and grown under controlled conditions.

Prochloraz (as Sportak 40 EC, Schering Agrochemicals Ltd.) was applied as a high volume spray at a field application rate equivalent of 200g a.i/ha, either 24h prior to inoculation as a protectant spray, or at selected intervals thereafter as curative applications. A non-fungicide formulation was used as a control.

TABLE 1. Summary of characteristics and tests currently used to differentiate W- and R-pathotypes of *Pseudocercosporella herpotrichoides*.

W-type	R-type	Reference
P. herpotrichoides var. *herpotrichoides*	*P. herpotrichoides* var. *acuformis*	Nirenberg (1981)
fast growing, even edged colonies	slow growing, feathery edged colonies	Hollins *et al.* (1985)
more pathogenic to wheat than R-type (wheat>barley>rye)	more pathogenic to rye than W-type (wheat≥barley≥rye)	Scott *et al.* (1975)
curved or straight spores with 4 septa, 35-80µm long	Straight spores with 4-6 septa, 43-120µm long	Nirenberg (1981)
colony pigmentaion on maize-meal agar under near UV light at 13°C greenish-black	brownish-pink	Creighton (1989)
isozyme polymorphisms for several enzymes		Julian & Lucas (1990)
	Greater incidence of MBC resistance	Brown *et al.* (1984) King & Griffin (1985)
	Reduced sensitivity to some SBIs	Leroux *et al.* (1988) Cavelier *et al.* (1987)

FIGURE 1. Summary of infection structures formed during the initial colonisation of the coleoptile and successive leaf sheaths by W- and R-pathotypes of *P. herpotrichoides* (adapted from Daniels *et al.* (1990).



RESULTS AND DISCUSSION

Coleoptile infection

The importance of the coleoptile in establishing infection is well known (Bateman & Taylor, 1976; Higgins, 1984). This tissue sheaths the young seedling base, and although short-lived, may remain physically intact in the field up to ear emergence.

Spore adherence

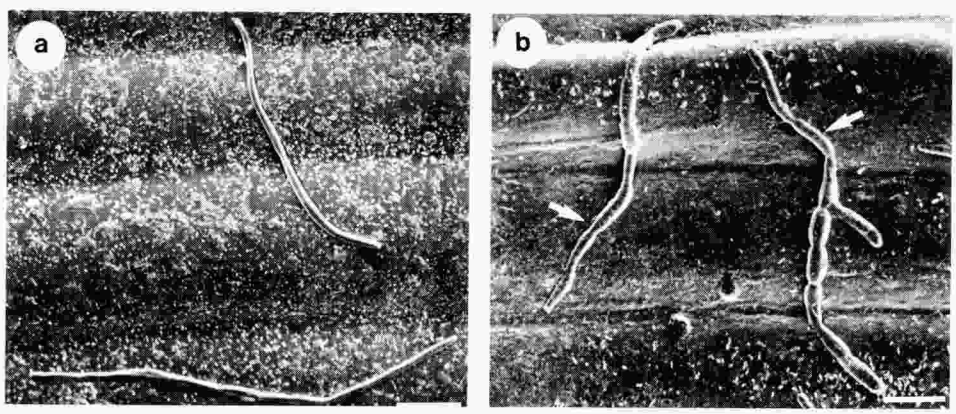
Spores adhere to the coleoptile by means of an extracellular mucilage which mediates nonspecific binding to a variety of inert hydrophobic surfaces (Daniels et al., 1990). Spore adherence was greatly reduced (ca. 80%) following a protectant application of Sportak 40 EC, due to wetters and stickers in the formulation which create a hydrophilic surface on the coleoptile. There was no apparent change in texture of coleoptile surface waxes following sprays with Sportak 40 EC.

Spore germination and germ tube extension

Although germination of W- and R-type spores on the coleoptile was unaffected by prochloraz at field application rates, inhibition of subsequent germ tube extension was noted in W-type isolates. This may reflect the enhanced growth capacity of W-types, which rapidly extended to form long superficial hyphae oriented in anticlinal cell wall grooves (Fig. 1). R-types showed only limited germ tube extension, and may thus have a reduced chance of encounter with surface fungicide deposits.

Inhibition of germ tube growth was accompanied by hypertrophy (cf. Fig. 2a & 2b) and subsequent lysis of individual cells, with adjacent cells often retaining normal morphology. This differential effect of prochloraz on discrete hyphal cells was characteristic of protectant sprays, and was manifested in a variety of infection structures formed during the developmental sequence proposed in Fig. 1.

FIGURE 2. Low-temperature SEM of germinating spores on the coleoptile. Bars = 10µm **a.** untreated control at 2d post inoculation. **b.** following a 24h pre-inoculation spray with Sportak 40. Arrows indicate spore mother cells.



Appressorium formation and host cell penetration

A proportion of spores were able to grow and establish superficial hyphae and appressoria in the presence of fungicide. This may reflect patchy distribution of fungicide on the seedling base. Appressorium formation was not significantly affected by prochloraz as shown by quantitative assessments of numbers of penetration sites formed 4-5d after a protectant spray. Sometimes, numbers of penetration sites were elevated on treated coleoptiles, which may have been due to an enhanced ability to penetrate in the absence of prochloraz-sensitive competing microorganisms.

Formation of mycelial plates

Following successful penetration of the coleoptile, reserves of mycelium were formed as discrete plates within and on the host surface (Fig. 1). Laterally aggregated hyphal strands (runner hyphae) were formed from mycelial plates which initiated leaf sheath infection. In addition, large numbers of spores were produced at the peripheries of mycelial plates, which may have further contributed to the infection cycle. Once established, mycelial plates were apparently relatively unaffected by prochloraz. However, a protectant spray curtailed their development, and also seemed to delay secondary sporulation cycles.

Leaf sheath infection

Runner hyphae

Runner hyphae initiated leaf sheath infection by very rapid oriented growth in grooves overlying vascular traces, followed by tip cell differentiation to form compact masses of inflated cells called infection plaques (Fig. 3a). Runner hyphae were also formed by lateral association of hyphae formed at the margins of growing plaques (Fig. 3b). Runner hyphae formed on coleoptile and leaf sheath surfaces were sensitive to the high concentrations of prochloraz resulting from curative sprays, and underwent rapid cell lysis (Fig. 3c). Runner hyphae formed in the presence of low fungicide concentrations following a protectant spray were often morphologically abnormal, with highly thickened cell walls (Daniels & Lucas, 1990). This is thought to have resulted from perturbed secretory processes involved in the synthesis and export of cell wall polymers.

Infection plaques

During their early development, these were morphologically distinct in W- and R-types (Fig. 1). During subsequent growth on leaf sheaths, R-type infection plaque populations persisted for a longer period before natural degeneration as shown in Fig. 4. Only a small proportion (ca. 25% in the most aggressive isolate) achieved successful penetration through to the underlying leaf sheath during the period in which the leaf sheath remained physically intact. However, the runner hyphae resulting from a single successfully penetrating infection plaque may initiate numerous further plaques. The spread of infection in this manner is much greater in the more rapidly growing W-types.

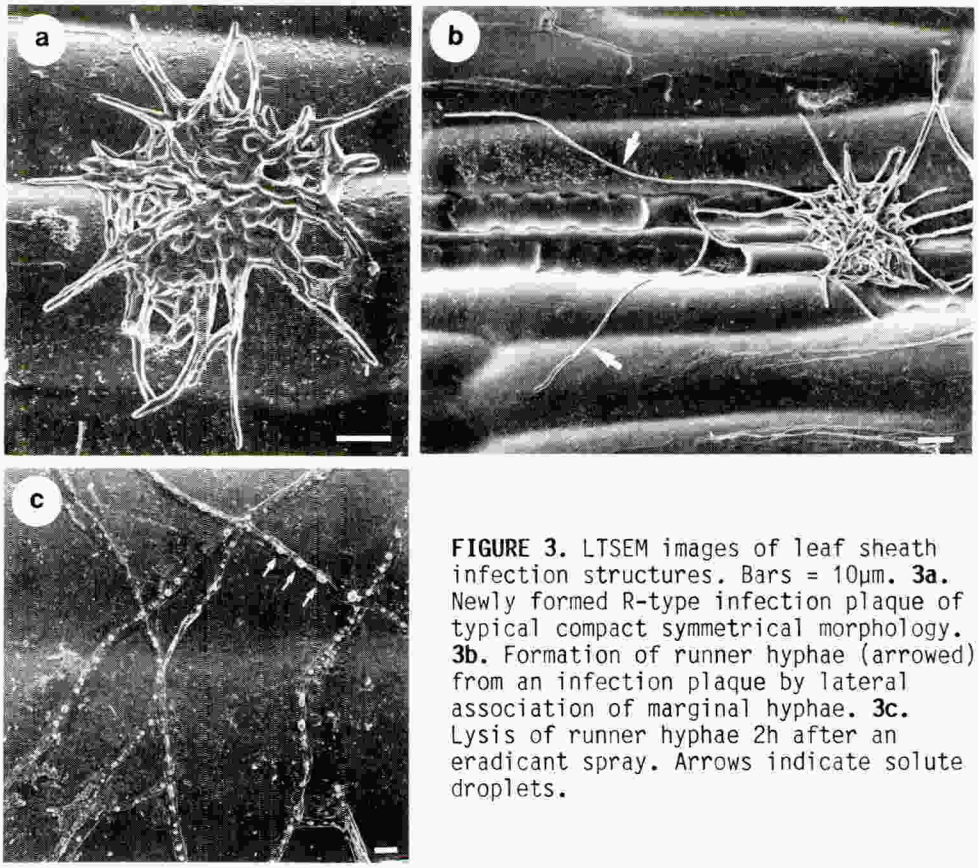


FIGURE 3. LTSEM images of leaf sheath infection structures. Bars = 10µm. **3a.** Newly formed R-type infection plaque of typical compact symmetrical morphology. **3b.** Formation of runner hyphae (arrowed) from an infection plaque by lateral association of marginal hyphae. **3c.** Lysis of runner hyphae 2h after an eradicant spray. Arrows indicate solute droplets.

The effect of 24h pre-inoculation protectant and 8d post-inoculation curative sprays on infection plaque populations is summarised in Fig. 5a & b. This shows that a protectant spray offered good long-term control of both W and R types up to 20d post-inoculation, with only minimal regrowth after 10d. Ultrastructural analyses of cells within plaques formed following a protectant spray revealed abnormalities in the endomembrane system, which indicate long-term fungistatic effects (Daniels & Lucas, 1990).

Although the extent of control offered by curative sprays timed to coincide with the onset of leaf sheath infection was much reduced compared to a protectant spray, both pathotypes showed a similar response (Fig. 5a and b). Substantial regrowth was evident 4-7d after spraying, and reflecting that only a proportion of the total plaque population was affected by prochloraz, which induced rapid cell lysis. This was not apparently due to patchy distribution of fungicide, as a similar differential susceptibility of infection plaques to prochloraz was also observed following immersion of detached leaf sheaths in fungicide solution. Such observations indicate that the developmental status of infection plaques may be an important determinant of sensitivity to fungicide.

FIGURE 4. Light microscopic analysis of infection plaque populations on the first leaf sheath showing different growth patterns of W- and R-types over a 20 day period.

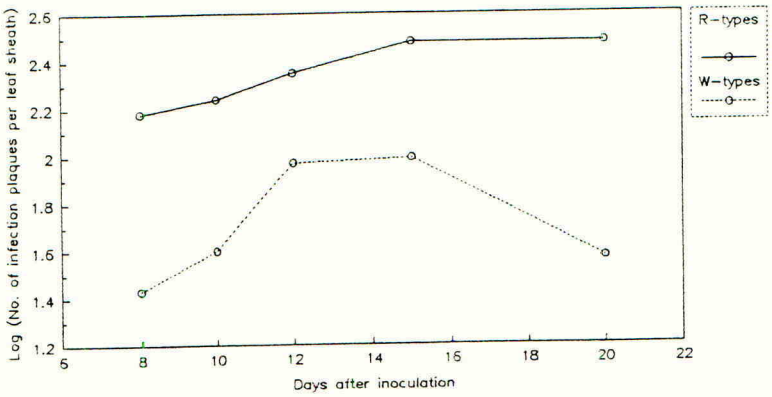
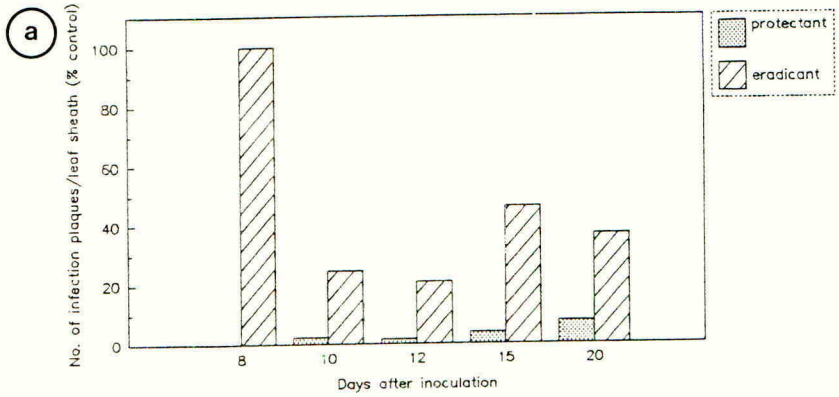
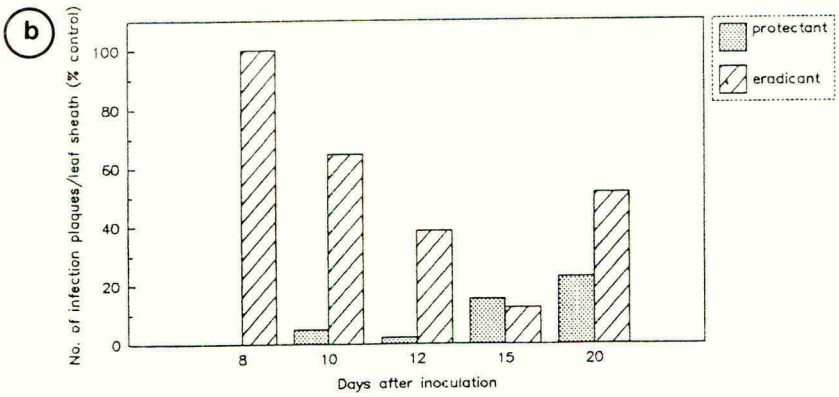


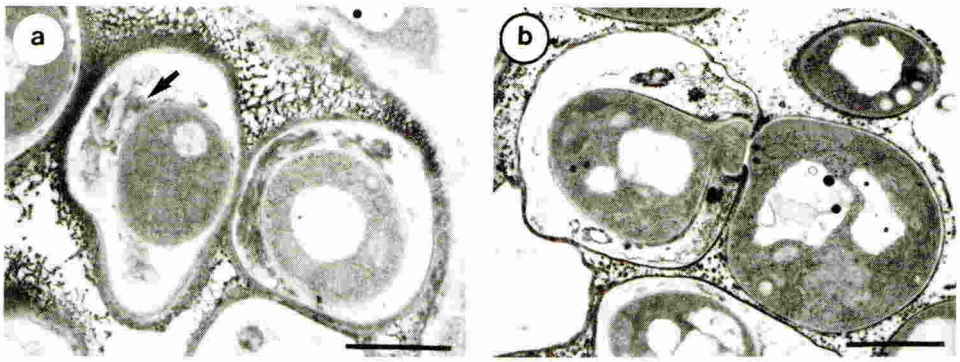
FIGURE 5. Effects of a 24h pre-inoculation protectant and an 8 day post-inoculation (arrowed) curative spray with Sportak 40 on infection plaque populations of the first leaf sheath. **5a** = R-types, **5b** = W-types. Data averaged for three representative isolates in each case, n = 10.





Following penetration into the leaf sheath tissue, infection plaque cells naturally senesced, leaving empty cell lumens. Cells at plaque peripheries involved in the formation of runner hyphae, often regrew into dead cell lumens to form double-walled hyphae. Extracellular matrix was subsequently secreted into the dead cell lumen, forming a 'protected' cell (Fig. 6a). Such structures are thought to account for the long-term survival of the pathogen on stubble, with hyphal regrowth occuring when suitable conditions prevail (Fig. 6b).

FIGURE 6. Transmission electron micrographs of cells at the periphery of successfully penetrating plaques. Bars = 2µm. **a.** TS hyphae growing within dead cell lumens into which extracellular matrix is secreted (arrowed). **b.** showing either regrowth into a dead cell (left), or growth (right) out of an existing 'protected' cell.



SUMMARY

Prochloraz has good long-term protectant activity against W- and R-pathotypes of P. herpotrichoides, apparently inducing fungistatic modifications during early development of the pathogen, which effectively arrest growth. Curative sprays offer significant control of both pathotypes, but are only effective against a proportion of infection structures present on the plant at any one time. Curative sprays thus delay the course of infection, and will offer subsequent protectant control if redistributed to underlying tissues; shown by Cooke et al. (1989) to occur after rainfall.

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THE ACTIVITY OF FLUSILAZOL AGAINST POWDERY MILDEW OF WHEAT

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ABSTRACT

The activity of flusilazol, a sterol biosynthesis inhibitor, was compared with related fungicides against powdery mildew to wheat. Its activity was very similar of flutriafol and propiconazole but relatively large differences in activity occurred when the chemicals were applied as single droplets to foliage or to soil. Differences were detected in the rate of translocation and uptake.

Vapour activity was determined on conidia *in vitro* and on leaf pieces. It was an important component of the activity of flusilazol and fenpropimorph but not of flutriafol and propiconazole.

INTRODUCTION

The activity of the new triazole fungicide flusilazol (Moberg et al., 1985) was compared with two other triazole fungicides, flutriafol (Skidmore et al., 1983) and propiconazole (Urech et al., 1979), and with the morpholine fungicide fenpropimorph (Bohnen & Pfiffner, 1979). The compounds were subjected to tests which would distinguish between intrinsic activity and the components of activity arising from translocation or vapour.

MATERIALS AND METHODS

Wheat (cv. Brimstone) was grown in 4 cm or 7 cm diameter pots of John Innes Compost No 2 to the 2 leaf stage in a glasshouse. Leaf pieces were cut from the second leaf and placed on moist filter paper in a petri-dish before inoculation and chemical treatment. Four replicates were used for all treatments. Soil treatments consisted of a 10 ml aliquot in a dish placed under each 4 cm pot containing one plant.

To assess movement along the leaf a droplet of chemical was placed at the proximal end of an attached leaf and the whole inoculated while pinned flat on a polystyrene board. Plants were inoculated with *Erysiphe graminis* by allowing a cloud of conidia to settle on foliage, either in the open glasshouse or in a settling chamber in the laboratory. The interval between treatment and inoculation was varied between

experiments. Disease was assessed as the percentage leaf area with disease seven days after inoculation but in tests for vapour activity the width of the inhibition zone was measured.

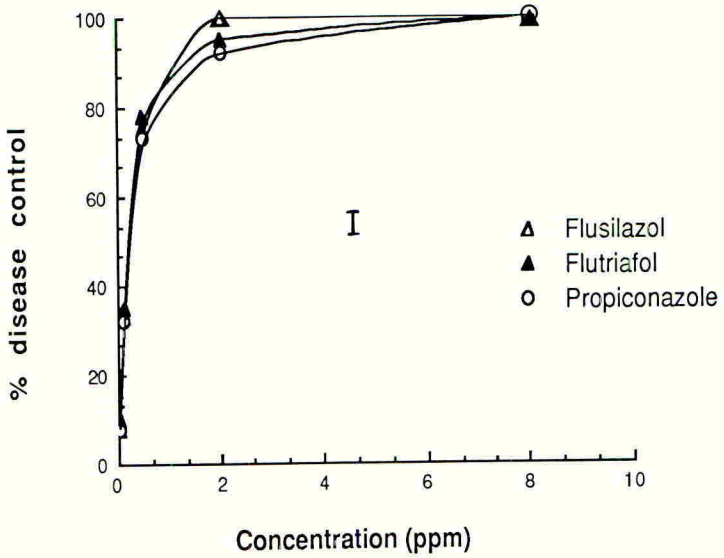
Chemicals were applied as aqueous suspensions at the specified rates. Tween 20 (300 ppm) was added to materials applied to leaves as droplets. Petri-dishes were sprayed with an atomiser in a spray settling tower at a rate of 200 l/ha. Flusilazol (DPXH6573 40 % experimental formulation) was supplied by Du Pont UK, flutriafol ('Impact ', 12.5 % SC) by ICI, UK, propiconazole ('Tilt', 25 % EC) by Ciba-Geigy and fenpropimorph ('Mistral'75 % EC) by May & Baker. To asses vapour activity the chemicals were placed in small separate containers within closed Petri-dishes or beakers covered with 'Cling film'.
Results were analysed using Statistical Analysis System (SAS). L.S.D. values (P = 0.05) are represented by bars on the figures.

EXPERIMENTAL

Activity of fungicides on leaf pieces

Leaf pieces were sprayed with chemicals one day after inoculation with *E. graminis* and incubated in Petri-dishes. No differences in activity were detected between the three fungicides (Fig 1.).

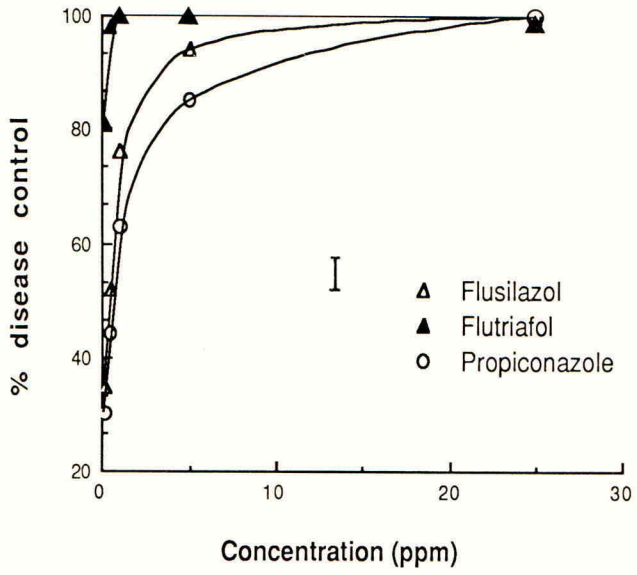
FIGURE 1. Activity of fungicides against *E.graminis* on detached leaf pieces.



Activity of fungicides as soil treatments

Plants were inoculated three days after treatment with the chemicals and the results (Fig. 2) show that flutriafol applied as a soil treatment was significantly more active than flusilazol and propiconazole.

FIGURE 2. Disease control following soil application.



Movement along leaves

Leaves of intact plants were fixed horizontally, inoculated with *E.graminis* and one droplet of chemical was placed on the proximal section of the leaf two days later (Fig.3). The plants were then incubated in the glasshouse until assessment. All fungicides controlled the disease on several sections of the leaves but the results (Fig.4) indicate that flusilazol and propiconazole were translocated less than flutriafol.

FIGURE 3. Bioassay method to observe translocation of fungicide along a leaf.

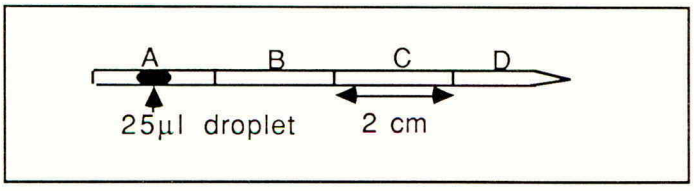
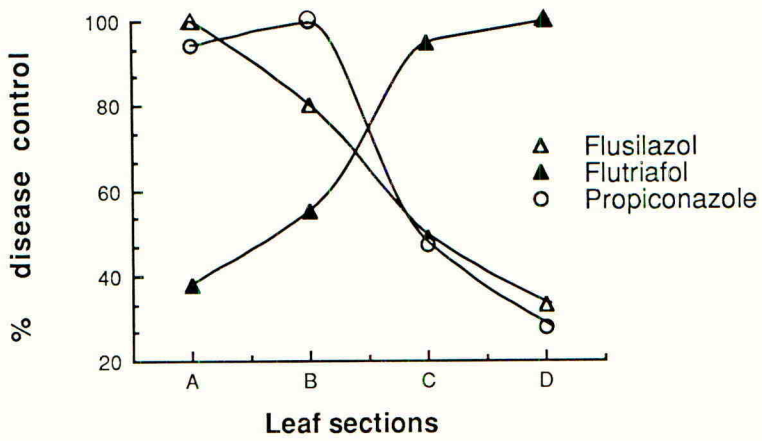


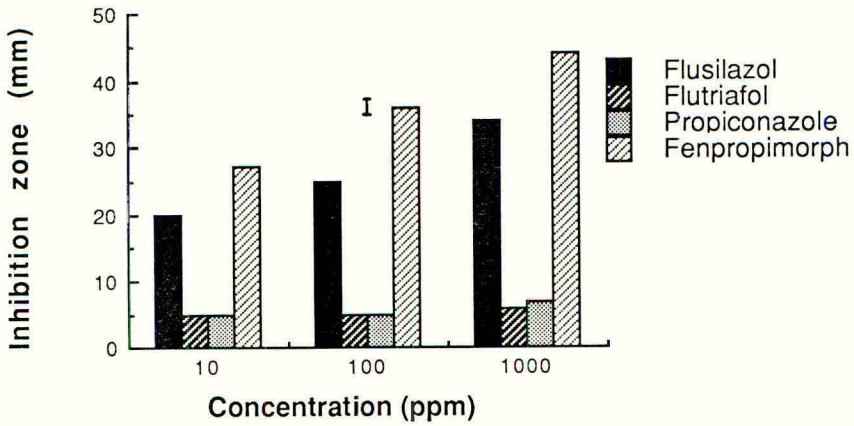
FIGURE 4.Disease control in different parts of the leaf.



Activity in the vapour phase

Inoculated leaf pieces were placed in petri-dishes as described by Shephard (1988). After 24 hours, chemicals were added and the dishes incubated in a growth cabinet at low light intensity (14 hours light; 10 hours dark) at 16°C for 6 days. Inhibition zones (Fig.5) indicate that flusilazol had vapour activity approaching that of fenpropimorph whereas flutriafol and propiconazole had negligible vapour activity.

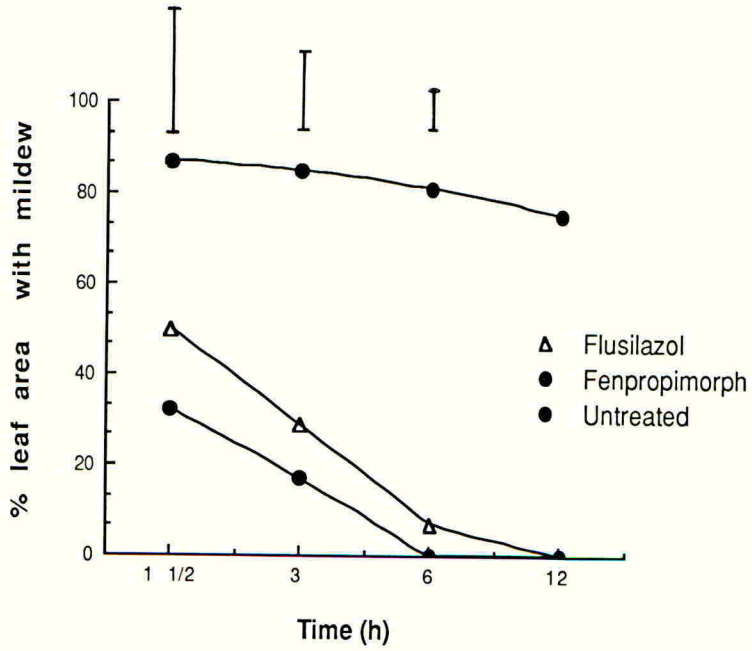
FIGURE 5. Relative vapour activity of four fungicides.



Effect on conidial viability

Conidia on glass slides were exposed to chemical vapour (for 1.5 - 12 h) in a sealed beaker. They were then transferred to untreated leaf pieces and viability assessed by the percentage disease which developed. The results (Fig.6) indicate a rapid decline in viability of conidia with exposure to vapour of either flusilazol or fenpropimorph.

FIGURE 6. The effect of the chemicals on fungal conidia.



DISCUSSION

Flusilazol, flutriafol and propiconazole had similar activity against *E.graminis* on detached leaves where the chemicals were applied directly to the whole of the infected leaf piece. However, the differences in disease control observed in the other experiments suggest that there is probably differential uptake and translocation. Thus the greater activity of flutriafol when applied as a soil treatment resulted from greater uptake and translocation leading to higher concentration in the foliage. This agrees with Shephard (1988) who demonstrated rapid uptake and accumulation of C^{14} flutriafol in barley foliage.

Flusilazol and propiconazole were less effective when applied to the soil and were presumed to be translocated less freely. Similarly, as found by Shephard (1985), a leaf application of flutriafol moved rapidly along the leaf, whereas flusilazol and

propiconazole moved more slowly. When used in the field it is likely that flusilazol and propiconazole would move only slowly to provide disease control on unsprayed parts of the leaves, whereas flutriafol would do this more quickly. However, the concentration of fungicide remaining in the treated part of the leaf would decrease more rapidly for flutriafol thus providing a shorter period of protection there.

The vapour activity was much higher for flusilazol than flutriafol or propiconazole. In the field the vapour activity coupled with the direct effect on conidial viability could result in disease control on unsprayed parts of leaves and in a dramatic reduction in the available inoculum for crops already infected with *E. graminis*.

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COMPARATIVE ACTIVITY OF FUNGICIDES AGAINST *SEPTORIA NODORUM* AND *S.TRITICI* ON WHEAT

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ABSTRACT

A comparison of the growth of *Septoria nodorum* and *S.tritici* on agar showed differences in the activity of five cereal fungicides. Chlorothalonil was much less active than the SBI fungicides prochloraz, hexaconazole, flusilazol and tebuconazole. *S.nodorum* appeared to be inhibited at lower concentrations than *S.tritici*. The SBI fungicides reduced leaf damage on wheat by both fungi when applied up to 10 days after inoculation. Pycnidial development was inhibited by application after the appearance of leaf symptoms and sporulation of *S.tritici* was inhibited by application after the appearance of pycnidia. Chlorothalonil had no effect on pycnidial development when applied after the appearance of lesions.

INTRODUCTION

It was reported by Jordan *et al.*, (1986) that some fungicides reduced sporulation of *Septoria tritici* when applied after inoculation. The present study was made to confirm this observation, and to investigate whether *S.nodorum* is affected in the same way. Experiments were made with a range of modern fungicides using *in vitro* and *in vivo* tests.

MATERIALS AND METHODS

The fungicides used were chlorothalonil (Bravo, 500 g/l EC, BASF), prochloraz (Sportak, 400 g/l SC, Schering), hexaconazole (JF9480, 50 g/l SC, ICI), tebuconazole (HWG 1608, 250 g/l EC, Bayer) and flusilazol (F6573 224, 400 g/l EC, Du Pont).

For *in vitro* experiments, chemicals were incorporated into Czapek Dox V8 juice agar (Cooke & Jones, 1970) to assess their effect on fungal growth. Amended agar (20 ml) was placed in a 90 mm petri-dish and inoculated with an 8 mm plug cut from an actively growing colony. Four replicates were used for all treatments. Plates were incubated in the dark at 20°C and growth was recorded as colony diameter, less inoculum plug, after 18 d with *S.nodorum* and 42 d with *S.tritici*.

In vivo experiments were made on winter wheat cv. Hornet graded as moderately susceptible to *S.nodorum* and *S.tritici* (NIAB

1989 ratings 6 and 4 respectively). Seeds, sufficient to give 3 plants per pot were sown in John Innes Compost No.2 in 4 cm pots and kept in a heated greenhouse until inoculation. Pots were arranged as randomized blocks with four replicates for each treatment. At GS 12-13 a spore suspension was atomised onto plants. The suspensions were obtained by washing spores from actively sporulating cultures and adjusting the concentration to 10^6 spores/ml. Tween 20 was added at 300 ppm. Trays of plants were then enclosed in large polythene bags for 72 hours to maintain a high humidity before transferring them to a cool-shady greenhouse (15-25°C). At various times after inoculation, plants were sprayed to maximum retention with fungicides using a DeVilbiss paint atomiser. All fungicides were used at concentrations equivalent to recommended rates in 250 l/ha (Table 1).

TABLE 1. Fungicide concentrations.

Fungicide	Rate (g AI/ha)	Equivalent concentration in 250 l/ha (ppm)
Chlorothalonil	1,000	4,000
Prochloraz	400	1,600
Hexaconazole	250	1,000
Tebuconazole	250	1,000
Flusilazol	250	1,000

Eighteen days after inoculation with *S.nodorum*, plants were transferred to a bench out of doors for 5 days to promote pycnidial formation (King & Cook, 1983). After assessment of leaf damage, the three infected second leaves from each pot were cut, soaked in water for 1 min and placed on tap water agar in a petri-dish. Dishes were incubated in a growth cabinet at 20°C for 9 hours during the middle of the day and under natural light from 6 pm to 9 am for 3 d (*S.tritici*) or 7 d (*S.nodorum*).

The percentage leaf area with disease was recorded on the second leaf 22-23 d after inoculation using standard keys (Anon, 1976; James, 1971). The percentage leaf area with pycnidia was recorded for *S.tritici* and the presence or absence of pycnidia recorded for *S.nodorum*. Where pycnidia were present, dishes were flooded with water, the leaf pieces rubbed with a glass rod to release pycnidiospores and the resultant suspension counted using a haemacytometer.

RESULTS

Comparison of the activity of the fungicides *in vitro* (Table 2) shows that the sterol biosynthesis inhibitors (SBI) inhibited fungal growth at low concentrations. Relatively high concentrations of chlorothalonil were required to inhibit growth and this was never complete. Activity of all the SBI fungicides

was similar against *S.nodorum* whereas hexaconazole appeared to be slightly more active and prochloraz less active against *S.tritici*. Usually the rates required to inhibit *S.nodorum* were lower than those for *S.tritici*.

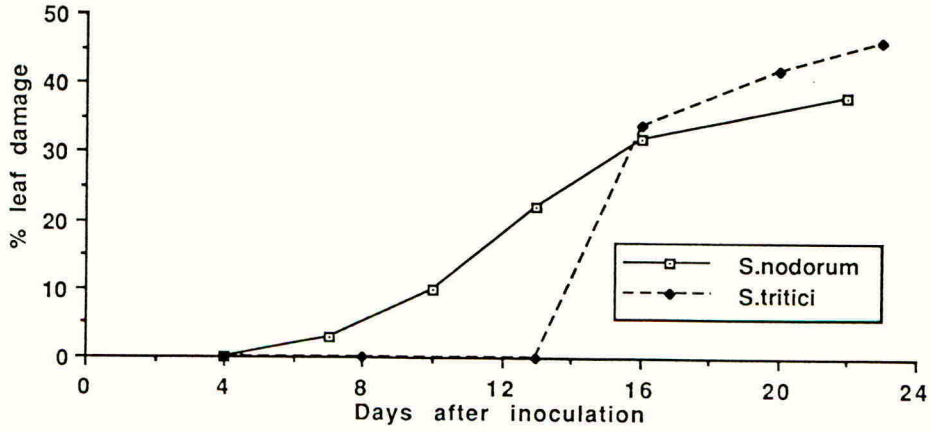
TABLE 2. Mycelial growth *in vitro*.

Compound	Fungus	% growth of colony ppm AI in agar 0.08	0.4	2	10	50	250
Chlorothalonil	S.nod.	-	-	85.6	68.7	46.0	26.1
	S.tri.	-	-	95.2	-	58.2	52.7
Prochloraz	S.nod.	28.3	15.4	3.8	0.3	-	-
	S.tri.	54.6	45.4	27.0	10.8	-	-
Hexaconazole	S.nod.	43.6	12.8	2.8	0.0	-	-
	S.tri.	34.2	7.1	5.8	2.6	-	-
Tebuconazole	S.nod.	34.1	9.7	1.7	0.0	-	-
	S.tri.	51.0	24.5	12.9	4.1	-	-
Flusilazol	S.nod.	54.3	32.9	3.0	0.0	-	-
	S.tri.	57.0	39.6	10.7	3.4	-	-
Untreated	S.nod.	100.0 (68.2)					
	S.tri.	100.0 (18.2)					

( ) growth in mm

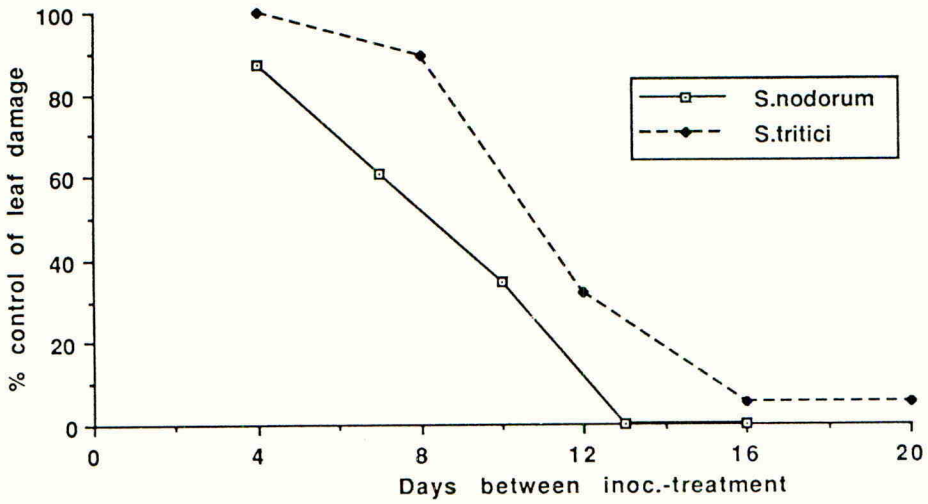
Lesions of *S.tritici* appeared after 13 d and those of *S.nodorum* after 6 days (Figure 1). This was reflected in the extent to which leaf damage could be prevented by fungicidal treatment at different intervals after inoculation. Good control

FIGURE 1. Development of leaf damage on untreated plants.



of leaf damage was achieved by application of prochloraz 7-8 days after inoculation but not after 13-16 days (Figure 2).

FIGURE 2. Curative activity of prochloraz.



Comparison of the effect of fungicides applied 7 and 13 d or 8 and 16 d after inoculation indicated the type of effect on an epidemic which might be expected from treatment at different times. The amount of leaf area damaged by *S.nodorum* was greatly

TABLE 3. Activity of fungicides against *S.nodorum*.

Fungicide	D.between inoc. and treatment	% leaf area diseased	Presence of pycnidia	Spore prod. per leaf ($*10^4$)
Chlorothalonil	7	30 a*	+++	102
	13	33 a	+++	66
Hexaconazole	7	19 b	0	0
	13	31 a	0	0
Flusilazol	7	20 b	0	0
	13	37 a	0	0
Prochloraz	7	15 b	0	0
	13	38 a	0	0
Tebuconazole	7	19 b	0	0
	13	34 a	0	0
Untreated		38 a	+++	81

* Newman & Keuls test (p=0.05).

reduced by application of all SBI fungicides 7 d after inoculation (Table 3) but chlorathalonil was ineffective. Where infection and leaf damage had occurred, the production of pycnidia was completely inhibited by the SBI fungicides tested but not by chlorothalonil. Leaf damage caused by *S.tritici* was markedly reduced by the SBI fungicides and to a lesser extent by chlorothalonil with applications at 8 d after inoculation (Table 4).

TABLE 4. Activity of fungicides against *S.tritici*.

Fungicide	D.between inoc. and treatment	% leaf area diseased	% area with pycnidia	Spore prod. per leaf ($*10^4$)
Chlorothalonil	8	31 b+	5.9 c	9.8 b
	16	44 a	25 b	154 a
Hexaconazole	8	6.5 c	0 d	0 c
	16	42 a	3.7 c	0.3 c
Flusilazol	8	*	0 d	0 c
	16	*	4.0 c	0.4 c
Prochloraz	8	4.8 c	0 d	0 c
	16	44 a	4.8 c	0 c
Tebuconazole	8	*	0 d	0 c
	16	*	4.4 c	0 c
Untreated		46 a	31 a	164 a

(+) Newman & Keuls test (p=0.05).
(*) assessment not possible because of phytotoxicity.

With later applications of the SBI fungicides, pycnidial development was still severely restricted and spore production was almost completely inhibited, although leaf damage was not controlled. Leaf damage from disease was difficult to assess with flusilazol and tebuconazole treatments because they caused phytotoxicity.

DISCUSSION

This study demonstrated a similarity in the performance of the SBI fungicides tested. It also showed excellent curative activity of these fungicides against both *S.nodorum* and *S.tritici*. Leaf damage was reduced by applications 10-12 d after inoculation and sporulation was reduced or prevented by even later applications.

These results confirm observations of Jordan *et al.*, (1986) and extend their findings to other fungicides. They also show that similar effects occur with *S.nodorum* and *S.tritici*. It appears that epidemics of either fungus should be greatly

curtailed by spraying SBI fungicides 16 or more days after the primary infection, and that the damage caused by primary infections should be reduced by sprays up to the time at which the first lesions appear.

Chlorothalonil had little curative activity and would therefore be expected to have little effect on the early stages of an epidemic if applied after infection. It could however still be effective in preventing secondary infections.

This study did not include any assessment of protectant activity but observations *in vitro* suggested a high level of activity of the chemicals against both fungi; chlorothalonil was less active. *S.nodorum* was more sensitive than *S.tritici* to the fungicides *in vitro* and this was reflected in the relative responses *in vivo*. For example, *S.nodorum* was unable to produce spores under conditions in which *S.tritici* did. The differences in activity between the SBI fungicides against *S.tritici in vitro* were not reflected *in vivo*, possibly because different field rates were used.

In these experiments the plants received a higher than normal dose of fungicide having been sprayed to maximum retention and would have been relatively soft having been grown in the greenhouse. This presumably accounts for the phytotoxicity as such symptoms have not been experienced over several seasons of field trials (Heatherington & Meredith, 1988; Austin, 1986). Also the degree of disease control may have been higher than would be achieved in the field. Differences in the re-distribution of fungicides in or on plants could also be an important factor affecting their field performance.

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USE OF PHOTOAFFINITY LABELLING TO IDENTIFY THE BINDING SITE OF THE NOVEL β-METHOXYACRYLATE AGROCHEMICAL FUNGICIDES

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ABSTRACT

An affinity labelled analogue from the β-methoxyacrylate series of exploratory fungicides has been designed and synthesised so that the binding site of these fungicides can be determined. The experiments described show that the inhibition of cytochrome bc_1 function by the β-methoxyacrylate fungicides is due to specific binding at the "o-centre" of cytochrome b.

INTRODUCTION

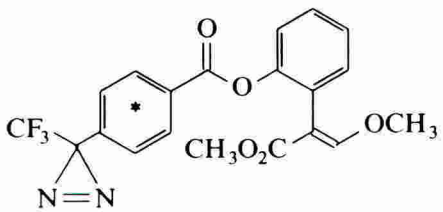
A synthetic chemistry programme based on the natural product mucidin (strobilurin A) has yielded a novel series of exploratory agricultural fungicides. Their mode of action is to block mitochondrial electron transport by inhibiting the function of the cytochrome bc_1 complex (Becker et al., 1981; Wiggins, unpublished).

To aid the design in this series of fungicides the long term aim of elucidating the structure of the β-methoxyacrylate binding site was undertaken. Although there is no X-ray crystal structure of the cytochrome bc_1 complex biochemical data are available in the literature on which to base a structural study. Primary sequences from a diverse range of species have been compared to identify conserved structural features, such as transmembrane α-helices and haem ligands (Saraste, 1984; Widger et al., 1984; Roa & Argos, 1986). Mutations of the cytochrome b gene which confer resistance to inhibitors of the cytochrome bc_1 complex have been identified (di Rago & Colson, 1988; di Rago et al, 1989; Daldal, 1990; Howell, 1988). From these studies models of the structure of cytochrome b have been proposed (Brasseur, 1988; Esposti, 1989; Crofts et al, 1987). To provide additional data on the amino acids involved in binding, and to confirm that the inhibitor resistant mutations occur at the binding site an affinity labelling study was undertaken. The first phase of this affinity labelling study is to identify to which subunit of the cytochrome bc_1 complex forms the β-methoxyacrylate binding site.

MATERIALS and METHODS

The procedures outlined here are discribed more fully in Mansfield & Wiggins (1990). A carbene-generating photoaffinity label was selected following the rational of Bayley & Knowles (1977). Data from structure activity and UV-light stability studies were used in to design affinity labelled analogues. The structure of the selected analogue is shown in Fig 1.

FIGURE 1 Structure of the affinity labelled β-methoxyacrylate.



* - ring uniformly ^{14}C-labelled

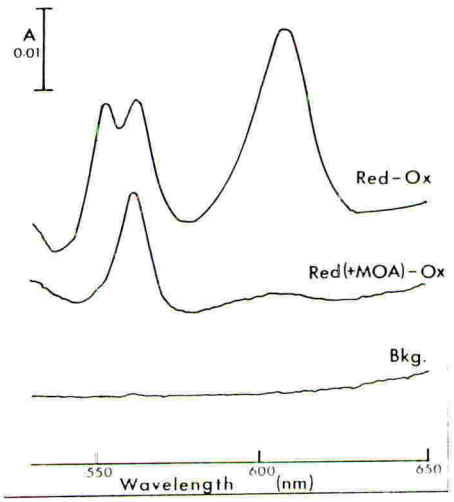
Inhibition of mitochondrial electron transport can be investigated spectroscopically, by determining the redox state of the cytochromes in the presence of an inhibitor. This is possible because reduced cytochromes have characteristic α-band absorbances, which are not present in the oxidized cytochrome spectrum. A suspension of beef heart mitochondria was placed in sample and reference cuvettes of the spectrophotometer and the base line zeroed electronically. Succinate was then added to the sample cuvette as substrate to bring about the reduction of the cytochromes. The spectrum was recorded upon oxygen depletion, as this gave full reduction of the cytochromes. The β-methoxyacrylate was added to the sample cuvette and the spectrum again recorded to determine the effect on the redox state of the cytochromes.

The ^{14}C-photoaffinity labelled β-methoxyacrylate was added to cytochrome bc_1 complex prepared from beef heart (Hatefi & Rieske, 1967; Rieske, 1967), which had been preincubated with "o and i-centre" inhibitors. The affinity label was activated by photolysis in an Hanau Suntest machine. The cytochrome bc_1 complex was then precipitated by addition of ammonium sulphate (to 50% saturation). Following centrifugation the precipitate was collcted and dissolved in solubilization buffer prior to separation of the individual subunits by SDS-PAGE. Protein bands were identified by coomassie blue staining, and covalently bound ^{14}C-affinity labelled β-methoxyacrylate was detected using an autoradiography.

RESULTS

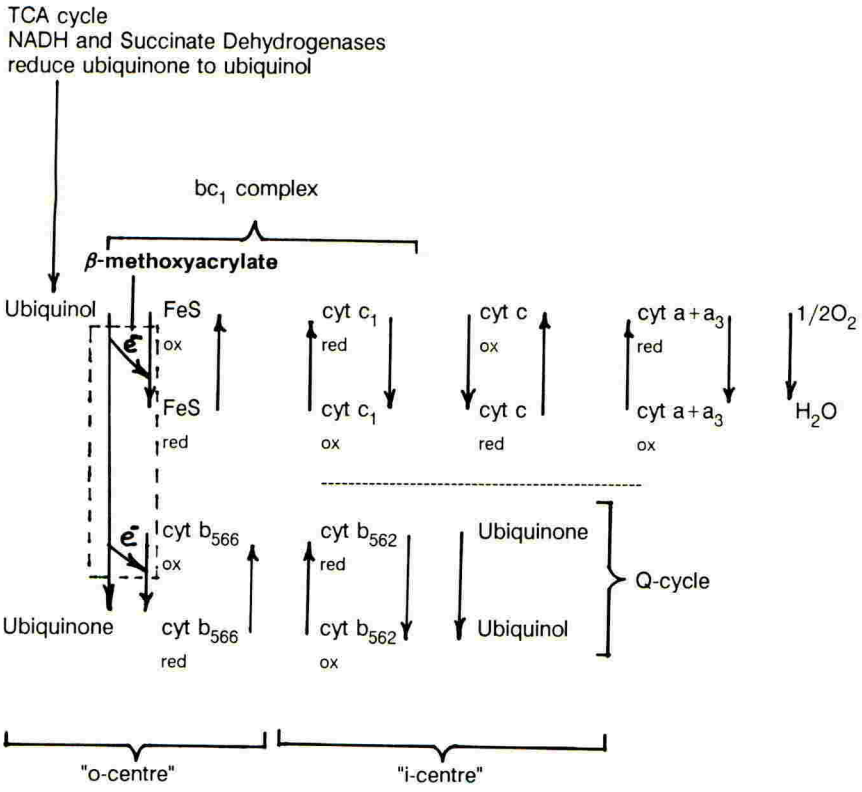
With succinate as substrate the spectrum of reduced cytochromes between 650 - 530nm revealed the presence of α-bands corresponding to cytochromes a, b, and c. After addition of the β-methoxyacrylate the absorbance due to the α-bands of the reduced cytochromes a and c disappeared (Fig 2). This is because cytochromes a and c have been re-oxidized whereas cytochrome b remains reduced. Thus the β-methoxyacrylate caused a block in electron transport at the cytochrome bc_1 complex (Fig 3).

FIGURE 2. Demonstration of the inhibition of mitochondrial electron transport by the affinity labelled β-methoxyacrylate.



Bkg: baseline sample - reference, both oxidized.
Red - Ox: addition of succinate to sample cuvette to reduce cytochromes - oxidized reference.
Red(+MOA) - Ox: β-methoxyacrylate added to reduced cytochromes in sample cuvette - oxidized reference

FIGURE 3. Schematic representation of the mitochondrial electron transport chain. Showing the position where the β-methoxyacrylate blocks the cytochrome bc_1 complex.



Electrons released by the oxidation of tricarboxylic acid cycle intermediates are used to reduce ubiquinone to ubiquinol. The pathway by which electrons are transfered and protons translocated from ubiquinol through the cytochrome bc_1 complex is known as the Q-cycle (Mitchell, 1975). Ubiquinol is then oxidized at the "o-centre" of the bc_1 complex. One electron is transfered to the FeS protein (Rieske or iron-sulphur) and on via cytochromes c and a to reduce molecular oxygen to water. The other electron first reduces the b_{566} and then the b_{562} haems of cytochrome b. The b_{562} haem is subsequently re-oxidized by the reduction of ubiquinone to ubiquinol. The stoichiometry is for 2 quinols to be oxidized at the "o-centre"; one quinone reduced to quinol at the "i-centre" ; $4H^+$ to be translocated across the inner mitochondrial membrane to form a proton gradient to drive ATP synthesis and 2 e- transfered through to the terminal oxidase.

The labelling of cytochrome bc_1 subunits by the β-methoxyacrylate is shown in Fig 4. The proteins of the cytochrome bc_1 complex were identified by coomassie blue staining (Fig 4a). Fig 4b shows the location of the bound ^{14}C-affinity labelled β-methoxyacrylate. The radioactivity was found predominately bound to cytochrome b. Smaller amounts were associated with the cytochrome b dimer and a low molecular weight subunit. The labelling of the low molecular weight subunit was approximately double the background, whereas the cytochrome b labelling was about 30 times greater than the background. Competition experiments with characterized inhibitors (von Jagow & Link) were carried out to investigate the specificity of the β-methoxyacrylate binding to the cytochrome bc_1 complex. Track 1 shows the labelling of the cytochrome bc_1 complex by the ^{14}C-affinity labelled β-methoxyacrylate following photolysis. In track 2 the sample was not photolysed and no labelling occurred. Tracks 3, 4,5 and 6 were preincubated with with antimycin A, HQNO ("i-centre" inhibitors) and two rates of DBMIB

(inhibits at Rieske protein) respectively. None of these inhibitors competed for the β-methoxyacrylate binding site as they did not prevent the labelling of cytochrome b. Tracks 7,8 and 9 were preincubations with "o-centre" inhibitors, trans-stilbene methoxyacrylate (Bushell et al., 1986), myxothiazol, and stigmatellin respectively. These inhibitors did compete for the same binding site as they prevented the labelling of cytochrome b.

FIGURE 4. Binding of ^{14}C-affinity labelled β-methoxyacrylate to subunits of the cytochrome bc_1 complex, separated by SDS-PAGE.

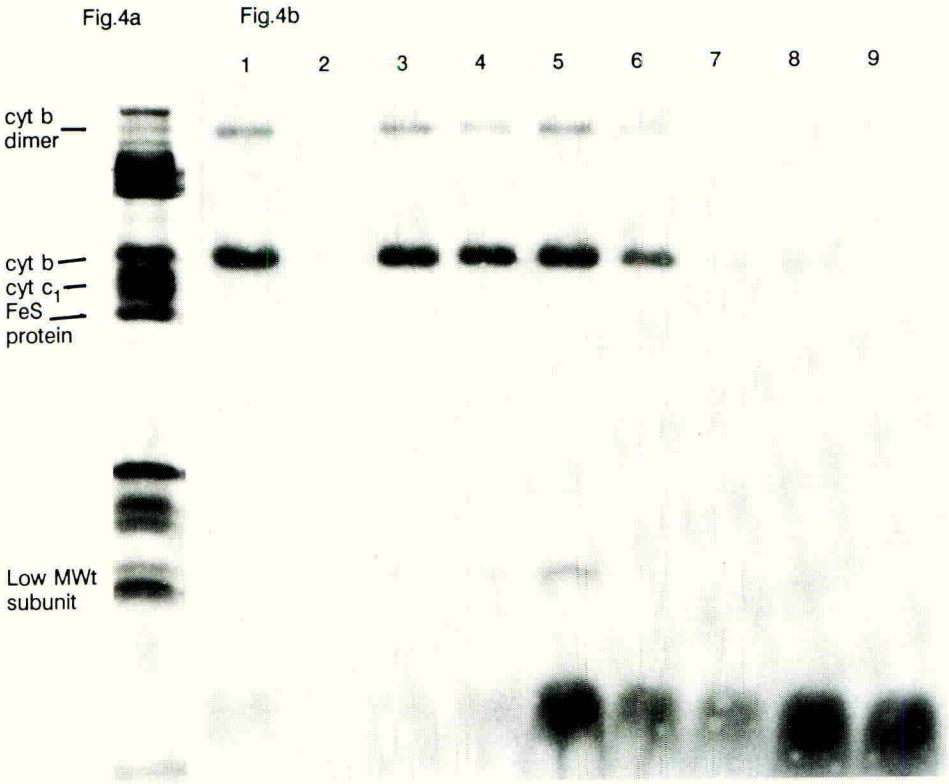


Figure 4a - gel stained to identify protein bands
Figure 4b - Radio-scan to detect bound ^{14}C-affinity label.
Track 1 - bc_1 complex plus affinity label, photolysed.
Track 2 - no photolysis (kept in dark).
Track 3&4 - "i-centre" inhibitors Antimycin A and HQNO respectively.
Track 5&6 - Rieske iron-sulphur protein inhibitor DBMIB.
Track 7,8&9 - "o-centre" inhibitors trans-stilbene methoxyacrylate, myxothiazol, and stigmatellin respectively.

CONCLUSION

The dark and inhibitor competition results show that the ^{14}C-affinity labelled β-methoxyacrylate bound specifically to cytochrome b at the "o-centre" site. These results confirm the biochemical (Becker et al,1981) and molecular biology (di Rago & Colson,1989) studies that cytochrome b is involved directly in the binding of β-methoxyacrylates. The second phase of this project to identify the amino acids of cytochrome b involved in binding β-methoxyacrylates can now proceed.

ACKNOWLEDGEMENTS

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MICROSCREEN TESTING FOR AGRICHEMICAL ACTIVITY

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ABSTRACT

Ricerca, Inc. has developed a microscreen for the agricultural chemicals market. Three key characteristics of the screen are that only a small amount of a test chemical is needed, results are obtained quickly, and it is relatively inexpensive. Only four milligrams of chemical are required for evaluation against one bacterial and four fungal plant pathogens, one nematode, and a series of tests to evaluate herbicidal potential. Results of the assays can be evaluated in seven days. Many of the assays are conducted in 24-well tissue culture plates, which conserves chemical, time and space. Consequently, the microscreen is less expensive than standard whole plant screens. The method is also flexible because target bacterial, fungal, and weed species can be selected from a variety of organisms. The Ricerca microscreen offers many advantages to companies wishing to evaluate large numbers of compounds for potential agrichemical uses.

INTRODUCTION

The most valuable resource of a chemical company is the file of novel compounds which have been prepared in the search for new market opportunities. Once the compounds have been prepared it is important that they are examined for any potential use beyond what they were designed to do. As the amount of novel compound is often limited, it is important to define the potential activity of a compound within an area using a minimum quantity. Should the compound show potential, more compound can be committed to advanced evaluation or resynthesis may be justified.

Ricerca has developed a microscreen for the agricultural chemicals market which requires only four milligrams of the test substance. This screen provides evaluation for activity against four fungal plant pathogens, one bacterial plant pathogen, a nematode and a series of tests which evaluate herbicide potential.

DESCRIPTION OF MICROSCREENS

Microscreen for evaluating fungicidal/bactericidal activity

This *in vitro* microscreen is a technique for testing chemicals for their ability to inhibit the growth of fungi and bacteria. Two important features of the microscreen are that only a small quantity of test chemical is needed and a screen can be completed within one week. The procedure can be used for any microorganism that grows in nutrient media or produces propagules that germinate in nutrient media.

The microscreen is conducted in tissue culture plates containing 24 wells, each well having a volume of about 3 mL. Test chemicals are dissolved in an appropriate solvent if necessary. Aliquots of each chemical, the nutrient media, and the test organism are placed in each well. Control wells contain an aliquot of media and the organism. If a solvent other than water is used, a solvent blank control well is included which contains an aliquot of media, the solvent and the organism. Well plates are incubated under conditions appropriate for growth of the organism. The test is evaluated by observing the percent growth inhibition in the chemical well compared to the control well.

Microscreen for potential nematicidal compounds

The free-living, soil-inhabiting nematode, *Caenorhabditis elegans*, is used in nematicide screens because of its relatively short life cycle (7 days vs. 25-30 for root knot nematode) and the ease with which it can be cultured. Most plant parasitic nematodes, including the root knot nematode (*Meloidogyne incognita*) can only be cultured inside plant roots, necessitating labor and space-intensive greenhouse or plant tissue culturing. *C. elegans* is similar to most plant-parasitic nematodes in size, basic morphology, and habitat.

The compound is added in the appropriate solvent to a 24-well tissue culture plate and the solvent is evaporated. A total of 900 µL of *C. elegans* culture and media (ca. 300 worms) are then added to each well. After eight days the mean percent kill is determined.

Herbicide Microscreen

The herbicide microscreen is composed of a matrix of four tests. These include a *Lemna minor* growth inhibition test, a germination test, a root elongation test and a whole plant test. In the latter three tests, the species can be selected to detect compounds specific for broadleaf or grass targets.

The *Lemna* test is based on the sensitivity of *L. minor* to many compounds at very low levels. Inhibition of growth and morphological damage are readily detected and growth can be quantified for statistical analysis.

The germination test is based on the ability of certain small-seeded species to germinate on water agar amended with a test substance. This system can accommodate water insoluble compounds which are tested at saturation level.

The root elongation test utilizes a special pouch developed for the seed industry which allows for rapid evaluation of linear root elongation. This test also permits visual observation of root abnormalities as a result of treatment. Discrete data points allow for statistical analysis of the results.

The whole plant test typically uses tomato seedlings which are sensitive to a wide variety of phytotoxins. A wide range of effects including necrosis, chlorosis, stunting, formative effects and alteration of growth are observable in the tomato within 7 to 10 days. Using small seedlings increases the sensitivity of the test.

A scoring system is applied to all tests which result in a total test score. Generally, active compounds will score points in at least three of the assays and often all four. The point scale can be easily adjusted to reflect a preference for any of the assays. A minimum threshold can be established for detecting active compounds based on experience with a particular set of compounds.

VALIDATION

As validation of this new screen, our sample handling group selected 37 compounds, 24 of them randomly selected from the Ricerca chemical file. These compounds, including standards, were evaluated as blind samples by the appropriate biological evaluation group. Results are summarized in Table 1.

TABLE 1. Summary microscreen of thirty-five compounds for biocidal potential.

No.	Compound	Fungicidal/[1] Bactericidal Activity RS	PY	BC	CG	PP	Nematicidal [2] Activity 10 ppm	100 ppm	Herbicidal [3] Rating
RIC1		0	0	0	0	0	4	5	8
RIC2		0	0	0	0	0	2	2	2
RIC3		0	0	0	0	0	2	6	5
RIC4	aldicarb	0	0	0	0	0	20	98	4
RIC5		0	0	0	0	0	8	5	6
RIC6	DCPA	0	0	0	0	0	2	2	3
RIC7		0	0	0	0	0	0	0	1
RIC8		0	0	0	0	0	0	0	2
RIC9	benomyl	5	0	5	4	0	68	80	4
RIC10		0	3	0	0	0	0	0	5
RIC11		0	5	0	0	0	0	0	5
RIC12	fenamiphos	0	0	0	0	0	25	90	5
RIC13		0	0	0	0	0	0	0	3
RIC14	atrazine	0	0	0	0	0	0	0	13
RIC15		0	0	0	0	0	0	0	4
RIC16	chlorothalonil	5	5	4	5	0	0	0	2
RIC17		0	0	0	0	0	0	0	15
RIC18		0	0	0	2	0	5	10	5
RIC19	2,4-D	0	2	0	1	0	0	0	17
RIC20		0	0	0	1	0	0	0	5
RIC21		0	0	0	0	0	0	0	8
RIC22		0	0	0	0	0	0	0	5
RIC23		4	4	5	3	0	0	0	2
RIC24	iprodione	5	5	5	4	0	0	0	2
RIC25		5	5	5	3	5	0	0	0
RIC26		0	0	0	0	0	0	0	1
RIC27		0	0	0	0	0	0	0	2
RIC28		0	5	0	0	0	0	0	2
RIC29	glyphosate	0	5	0	0	0	0	0	8
RIC30		0	0	0	0	0	0	33	2
RIC31		0	5	0	0	0	0	0	5
RIC32		5	0	0	3	0	0	10	7
RIC33		0	1	0	0	0	0	0	0
RIC34	alachlor	0	0	0	0	0	0	0	9
RIC35	mebendazole	4	0	0	0	0	55	75	5
RIC36	metalaxyl	0	5	0	0	0			
RIC37	streptomycin	0	4	0	0	5			

[1] Plant Pathogen Code

Fungi

RS = *Rhizoctonia solani*
PY = *Pythium aphanidermatum*
BC = *Botrytis cinerea*
CG = *Colletotrichum graminicola*

Bacterium

PP = *Pseudomonas syringae* pv *phaseolicola*

Fungicidal /Bactericidal Growth Inhibition Scale

0 = 0 %
1 = 1 - 14 %
2 = 14 - 44 %
3 = 44 - 74 %
4 = 75 - 99 %
5 = 100 %

[2] Percent kill of *Caenorhabditis elegans*

[3] Herbicidal Rating (0 - 17)

0 = no control
17 = maximum points possible most active

Fungicide evaluation

Standard commercial fungicides (benomyl, chlorothalonil, iprodione and metalaxyl) were identified as active in the microscreen. In addition, two compounds, RIC23 and RIC25, were identified as very effective. These two compounds were advanced to a primary *in vivo* screen and tested against seven diseases at a single rate of 200 ppm a.i. plus an additional *in vitro* screen against three fungi.

Results of the primary screen (Table 2) show that RIC23 had no activity against any of the diseases and minimal activity against the fungi in the *in vitro* test. For RIC25, activity against *Botrytis* (gray mold) was confirmed and it also showed activity against *Pythium* and *Rhizoctonia* in the *in vitro* test. This compound also gave some control of Apple scab, but little or no activity was seen against the other diseases.

TABLE 2. Percent control of seven diseases in an *in vivo* screen and three fungi in an *in vitro* screen of two active compounds identified in the microscreen.

	In vivo (200 ppm a.i.)							*In vitro* (25 ppm a.i.)		
Compound	Apple scab	Gray mold	Peanut Leaf-spot	Rice blast	Grape Downey mildew	Wheat rust	Powdery mildew	Ph[1]	Pu	Rs
RIC23	0	0	0	0	0	0	0	50	50	0
RIC25	70	80	0	40	0	0	0	0	80	60

[1] Ph = *Pseudocercosporella herpotrichioides* ; Pu = *Pythium ultimum* ; Rs = *Rhizoctonia solani*

Three compounds structurally related to RIC25 were screened in the *in vivo* and additional *in vitro* tests. This series was active against *Botrytis* (Table 3) and Analog #2 also gave good control of *Cercospora* , making it a good candidate to be tested against related diseases such as black sigatoka. The microscreen provided several leads after running only two small primary screen assays as compared to one lead after assaying all compounds through a primary screen.

TABLE 3. Percent control of seven diseases in an *in vivo* screen and three fungi in an *in vitro* screen of three analogs of RIC25.

	In vivo (200 ppm a.i.)							*In vitro* (25 ppm a.i.)		
Compound	Apple scab	Gray mold	Peanut Leaf-spot	Rice blast	Grape Downey mildew	Wheat rust	Powdery mildew	Ph[1]	Pu	Rs
Analog 1	50	75	0	40	0	0	0	100	60	0
Analog 2	0	90	80	20	0	0	0	0	0	0
Analog 3	0	100	10	10	0	0	0	85	80	70

[1] Ph = *Pseudocercosporella herpotrichioides* ; Pu = *Pythium ultimum* ; Rs = *Rhizoctonia solani*

Bactericide and nematicide evaluation

Streptomycin, aldicarb, fenamiphos and merbendazole were identified as active compounds by their respective targets.

Herbicide evaluation

Atrazine, 2,4-D, glyphosate and alachlor standards were identified as active compounds in the microscreen. Three additional compounds (RIC1, RIC17, and RIC21) scored 8 points or above, were advanced to *in vivo* screens and results (Table 4) indicate that two of the compounds showed efficacy against a number of weeds. Therefore, to identify two lead compounds, only three *in vivo* screening assays were necessary because of the microscreen data.

TABLE 4. Percent control of six weed species in an *in vivo* screen by three compounds identified in the microscreen.

Compound	Dose kg/ha	Pigweed	Velvet-leaf	Mustard	Red millet	Fox tail	Barnyard grass
RIC1 Primary *in vivo*	4	0	0	0	0	0	0
RIC17 Secondary *in vivo,* pre-emerge	8	100	97	100	100	100	100
	4	97	87	97	100	97	98
	2	95	79	94	96	96	97
	1	85	45	90	85	78	83
	0.50	80	29	89	67	86	84
	0.25	76	25	75	49	62	61
	0.12	47	7	77	27	70	30
RIC 17 Secondary *in vivo,* post-emerge	32	35	50	75	55	65	55
	8	35	55	70	40	45	45
	2	33	37	57	37	47	47
	1	20	30	50	40	50	40
	0.50	30	20	50	45	47	40
	0.25	30	20	50	50	50	40
	0.12	30	20	40	40	50	40
RIC21 Secondary *in vivo,* pre-emerge	8	100	10	40	80	90	95
	4	90	0	0	80	70	90
	2	70	0	10	30	50	90
	1	10	0	0	0	10	0
RIC21 Secondary *in vivo,* post-emerge	8	0	0	10	10	30	10

CONCLUSIONS

Of the 37 compounds tested in the microscreen, the commercial fungicide, bactericide, nematicide and herbicide standards were identified as active compounds. One fungicide lead was found by performing only two primary *in vivo* fungicide assays and two herbicide leads were found by performing only three *in vivo* herbicide assays.

From one preweighed four milligram sample, Ricerca, Inc. can provide an inexpensive evaluation of compounds in major agrichemical markets. This microscreen is a very efficient indicator of biological potential in the agricultural area.