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Project title:	Evaluation of existing and novel fungicides for the control of powdery mildew and other foliage/stem pathogens of cucumber.
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The results and conclusions in this report are based on a series of laboratory experiments. The conditions under which the experiments were carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

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

Signature ....

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Background and Objectives

The powdery mildew fungus (*Sphaerotheca fuliginea*) on cucumber is widespread and occurs every year where cucurbits are grown. Whilst it can be problematic on early crops, more usually it develops during April-May. As inoculum builds-up, it becomes particularly troublesome on the summer replants and, for this reason, many growers now rely on mildew tolerant cultivars eg. Enigma, for the later planted crops.

Unlike many pathogenic fungi powdery mildews prefer a drier environment and thrive under summer conditions in the UK. As a result the tendency has been for growers to apply mildew fungicides prophylactically with numerous repeated applications/crop. This intensive spray regime over a number of seasons is likely to have increased selection pressure towards insensitive or tolerant strains of the fungus. Fortunately, the availability of products from different groups with contrasting modes of action (eg. triazoles and pyrimidines) has, to some extent, alleviated the problem. It is now generally assumed that strains of *S. fuliginea* insensitive or tolerant to these widely used fungicides; imazalil (Fungaflor), fenarimol (Rubigan) and bupirimate (Nimrod) are commonplace in UK cucumber crops. Yet, it is some 10-15 years since any detailed resistance testing was carried out on mildew populations in UK cucumber crops. Unfortunately, *S. fuliginea* is an obligate parasite, which means it cannot be raised in artificial culture media. Instead, tests must be conducted on host tissues and securing consistent, reliable results can be difficult.

In other broad acre crops, eg. winter wheat, other powdery mildews eg. *Erysiphe graminis* are also problematic and numerous novel fungicides, some with contrasting modes of action, have been approved to maintain effective control. Currently, these novel products have not been evaluated in the UK for their efficacy and safety to the cucumber crop and currently none are approved for use on cucumber.

The purpose of this first year's investigation therefore was to:

a) investigate the sensitivity of a range of isolates of *S. fuliginea* to the widely used 'commercially available' fungicides,

b) determine the relative efficacy of a range of novel mildew fungicides in *in vitro* laboratory screening against *S. fuliginea, Botrytis cinerea* and *Mycosphaerella melonis* (syn. *Didymella bryoniae*),

c) determine the potential for phytotoxicity of the various novel fungicides to young cucumber plants in the greenhouse.

Summary of Results

A range of some 20 isolates of the cucumber powdery mildew fungus (*Sphaerotheca fuliginea*) were collected, courtesy of Mr D Hargreaves, from diverse UK locations and tested for their sensitivity to the commercially available fungicides (ie. Fungaflor, Rubigan and Nimrod). Several isolates exhibited a reduced sensitivity to fenarimol (Rubigan) though only one isolate was tolerant to imazalil (Fungaflor). A few isolates also showed reduced sensitivity to bupirimate (Nimrod) one of which was highly tolerant. There did not appear to be a consistent relationship with regard to resistance in the recognised fungicide groups ie. triazoles and pyrimidines.

In a preliminary efficacy screen using cucumber leaf discs in the laboratory, several novel fungicides were compared with the commercial standard products. Many were found to be highly effective against *S. fuliginea* in this test, though some evidence of phytotoxicity was observed on the leaf discs used in the study.

In separate detailed phytotoxicity studies on young rockwool grown cucumber plants (cvs. Pyralis, Enigma, Jessica and Bronco) several of the fungicides, particularly the triazoles and morpholines (eg. fenpropimorph) were phytotoxic to young plants causing a range of symptoms from leaf distortion, leaf scorch to plant stunting and even plant death. Further studies will be required at reduced rates of these products to determine an effective application dose which minimises crop damage whilst maintaining efficacy.

Finally, *in vitro* bioassay tests were devised and conducted to determine the performance of the novel fungicides against the cucumber stem pathogens *B. cinerea* and *M. melonis*. Several of the products evaluated were effective against one or both of these stem pathogens in these bioassay tests.

Action Points for Growers

Growers should maintain an alternating strategy to further minimise fungicide resistance until the new products identified, with efficacy against mildew, can be approved on the cucumber crop.

Practical and Financial Benefits from the Study

The resistance testing conducted, assuming it is representative, suggests that mildew may be better controlled using imazalil (Fungaflor) rather than either of fenarimol (Rubigan) or bupirimate (Nimrod). Clarification needs to be sought regarding the re-classification of these fungicides to ensure an effective anti-resistance strategy is maintained, as it is not possible to provide clear advice at this stage. (See 'Stop Press' at the back of the report).

Several promising candidate fungicides were identified in this initial screen, though many were damaging to young plants. None of these products are currently approved and therefore should not be used on the crop.

EXPERIMENTAL SECTION

Introduction

Powdery mildew (*Sphaerotheca fuliginea*) is a particularly common and damaging disease of cucumbers in the UK.

Fungicides are used routinely for control of mildew but, because the choice of effective products is limited, growers have to rely on the same products each year. Sprays are applied with increasing frequency and, even then, it is difficult to maintain effective control. As a result of this intensive disease pressure over a number of years it is widely assumed that a high level of tolerance to the fungicides (imazalil 'Fungaflor', fenarimol 'Rubigan', and bupirimate 'Nimrod') has developed. Yet, it is some 10-15 years since any structured resistance testing of mildew populations was undertaken in UK cucumber crops, and the significance of fungicide resistance in the control of powdery mildew has not been clearly determined.

It should be noted that for many years the approved fungicides have been grouped as follows:

imazalil (Fungaflor)]   DMI's
fenarimol (Rubigan)]

bupirimate (Nimrod)]   Pyrimidine

More recent information (Pesticide Register 11th Edition (1997)) suggests that imazalil is a triazole (or 'DMI' fungicide): bupirimate, a pyrimidine fungicide, whereas fenarimol is a pyrimidinyl carbinol. Further clarification is currently being sought in this respect as it may have important implications with regard to future strategies for disease control in the crop.

Powdery mildew has, to some extent, been alleviated, particularly in summer replants following the introduction of mildew tolerant cultivars, though at present there tends to be a small yield penalty incurred in their use. Previously they have not been used for early plantings because of problems with leaf necrosis and this maintains pressure on the existing range of fungicides.

In broad acre crops, particularly cereals, there have been many new developments in the control of powdery mildews. Yet none of these new products have been further developed on protected cucumbers in the UK. Most recently, the development of novel broad spectrum strobilurin fungicides has offered not only mildew control but effective suppression of many other target pathogens.

The objectives in the first year of this project were to:

(i) determine the sensitivity of a range of isolates of *S. fuliginea* from cucumber to the commonly used fungicides.

(ii) conduct preliminary efficacy studies in the laboratory using a range of novel fungicides in detached leaf disc studies.

(iii) investigate the relative phytotoxicity of a range of novel fungicides to a range of cucumber cultivars.

(iv) conduct *in vitro* efficacy studies in the laboratory using a range of novel fungicides against both *Botrytis* and *Mycosphaerella.*

Subsequent studies in 1998 will evaluate a short-list of the most promising fungicides in a commercial-scale trial whilst at the same time gathering more information on fungicide resistance and crop safety.

Materials and Methods

(i) Fungicide Resistance Tests

A range of isolates of the powdery mildew pathogen (*Sphaerotheca fuliginea*) were collected by Mr D Hargreaves from commercial crops in Yorkshire and the Lee Valley as follows:

Table I: Isolates of *Sphaerotheca fuliginea* submitted for resistance testing in 1997.

Reference No.	Date of Receipt	Location
cpm/01/97	02.09.97	Lee Valley
cpm/02/97	02.09.97	"
cpm/03/97	02.09.97	"
cpm/04/97	10.08.97	Yorkshire
cpm/05/97	04.09.97	"
cpm/06/97	04.09.97	"
cpm/07/97	10.09.97	"
cpm/08/97	10.09.97	"
cpm/09/97	11.09.97	"
cpm/10/97	11.09.97	"
cpm/11/97	11.09.97	"
cpm/12/97	10.09.97	"
cpm/13/97	10.09.97	"
cpm/14/97	10.09.97	Lee Valley
cpm/15/97	10.09.97	Lee Valley
cpm/16/97	11.09.97	Yorkshire
cpm/17/97	23.09.97	Lee Valley
cpm/18/97	23.09.97	Lee Valley
cpm/19/97	24.09.97	Yorkshire
cpm/20/97	24.09.97	Yorkshire
cpm/21/97	24.09.97	Yorkshire

A series of 1.5 cm leaf discs (16/isolate) were taken from healthy cucumber plants cv. Corona raised especially for the study, and placed in Petri dishes (4/dish) on moist filter papers. Leaf discs were either sprayed with fenarimol (Rubigan), imazalil (Fungaflor) and bupirimate (Nimrod) at rates 1 ml product/l, 0.5 ml product/l and 0.18 ml product/l respectively. Uninoculated and inoculated control discs were run alongside each isolate.

Once the fungicide treatments had dried on the leaves a dry spore suspension of each isolate was cascaded down onto the untreated and treated leaf discs in Petri dishes enclosed in a perspex tower. After inoculation the leaves were misted with water and subsequently incubated. After 5-7 days incubation at 20°C the number of developing mildew pustules per disc were counted.

(ii) Preliminary Efficacy Screen

Eighteen fungicides (Table 2) were screened for their efficacy against the powdery mildew fungus, *S. fuliginea*, in an *in vitro* test using detached leaf discs.

A series of 1.5 cm leaf discs taken from healthy plants cv. Corona were placed on moist filter paper in Petri dishes and either sprayed with water (control) or sprayed with the fungicides listed in Table 2 at the rates specified. The sprays were allowed to dry on the leaf discs and then inoculated with a dry spore suspension of *S. fuliginea* and misted with water in a perspex tower. The isolate used for this study was selected at random from an infected crop at HRI Stockbridge House. After inoculation the leaves were incubated at 20°C for mildew development and scored for mildew after 5-7 days. Both the number of developing mildew pustules and the mean percentage area of leaf disc affected was determined.

Table 2: List of fungicides evaluated for efficacy and phytotoxicity in 1997.

Product	Active Ingredient	Rate of Use	Manufacturer
Fungaflor	imazalil (200 g/l)	0.5 ml product/l	Hortichem
Rubigan	fenarimol (120 g/l)	0.18 ml product/l	Dow Elanco
Nimrod	bupirimate (250 g/l)	1.0 ml product/l	Zeneca
Folicur	tebuconazole (250 g/l)	5 ml product/l	Bayer
Rocket	triflumizole	1 ml product/l	Hortichem
Genie	flusilazole (400 g/l)	2 ml product/l	Du Pont
Plover	difenconazole (250 g/l)	5.0 ml product/l	Novartis
Aura	fenpropimorph (750 g/l)	2.5 ml product/l	BASF
Topas	penconazole (100 g/l)	0.15 ml product/l	Novartis
Dorado	pyrifenox (200 g/l)	0.25 ml product/l	Zeneca
Calixin	tridemorph (750 g/l)	2.8 ml product/l	BASF
Alto 100	cyproconazole (100 g/l)	8 ml product/l	Sandoz
Unix	cyprodinil	0.67 g product/l	Novartis
Systhane 6 Flo	myclobutanil	3 ml product/l	Promark
Opus	epoxiconazole (125 g/l)	10 ml product/l	BASF
Bayfidan	triademenol (250 g/l)	1.25 ml product/l	Bayer
Amistar	azoxystrobin (250 g/l)	0.8 ml product/l	Zeneca
Stroby	kresoxim-methyl	0.5 g product/l	BASF
Milsana	extract of *Reynoutria sachaliensis*	20 ml product/l	-

(iii) Phytotoxicity Evaluation with Novel Fungicides

Young cucumber plants cvs. Pyralis, Enigma, Jessica, and Bronco grown in rockwool propagation blocks were raised in a disease-free environment to the first true leaf stage. Blocks of 4 plants/cultivar, selected randomly, were sprayed HV to run-off with the fungicides listed in Table 2 in the glasshouse. Plants were maintained for a further 7 days and monitored for symptoms of phytotoxicity. After this period plant height was measured, plants scored for the percentage leaf area with chlorosis, the severity of leaf curling using a 0-5 scale of severity and the leaves scored (+/-) for darkening ('hardening').

The exercise was repeated later under different weather conditions using older plants at the 2-3 leaf stage. In the repeat study only plant height was measured.

(iv) Evaluation of Novel Fungicides against *Botrytis cinerea* and *Mycosphaerella melonis* in *in vitro* studies

Young cucumber plants cv. Corona were propagated and grown to the 2-3 true leaf stage. Leaves of a similar age were detached and used in a series of *in vitro* inoculation tests with virulent isolates of *B. cinerea* and *M. melonis (D. bryoniae)* which had been selected at random.

From the detached leaves 10 cm^2 leaf sections were taken and placed in sterile dishes containing tap water agar (TWA) overlain with a filter paper moistened with sterile distilled water.

Fungicides were applied by spray application at the rates outlined in Table 2 and the product allowed to dry. 3 x 3 mm discs containing each pathogen were placed equidistant on duplicate leaf segments and incubated at 20°C. Inoculation sites were wounded to aid infection by these pathogens by inserting a sterile scalpel through the lamina to create a wound ca. 1 mm long. After 3-5 days the diameter of developing lesions was measured.

Statistical Analysis

No statistical analysis was conducted on the data in the first year of this study.

Storage of Data

All the raw data from this study will be retained for a period of not less than 5 years in the HRI Archive at Stockbridge House. Access to the data can only be made via the designated archivist.

Official Recognition

The experiments reported were conducted in accordance with the draft guidelines for official recognition of efficacy testing organisations, as outlined by the UK regulatory authority, PSD. See ref PRD 2400/2995 for more information.

A specific quality assurance audit was not undertaken.

Results

(i) Fungicide Resistance Testing

Of the 21 isolates of mildew collected infection was successfully achieved with 12. Mildew was not transferred on the remaining samples and this was assumed to be either because transit conditions had been poor or, more likely, that they had recently been treated with a fungicide which had reduced sporulation. The mean incidence of infection of the cv. Corona varied from 0.5-11.9 pustules per cm leaf area in the inoculated controls. All the uninoculated control treatments remained disease-free throughout the duration of the experiments.

In most cases, treatment of the leaf discs with fungicide reduced the incidence of mildew to a greater or lesser extent. In a few cases infection levels following fungicide treatment were as high, or higher than, the inoculated controls (Table 3). One isolate (cpm/08/97) from a nursery in Yorkshire was highly resistant to fenarimol (Rubigan) and a further 8 isolates from Yorkshire and the Lee Valley showed a slight tolerance to this fungicide. Only one isolate (cpm/19/97) from Yorkshire was found to be highly resistant to imazalil and this same isolate showed a reduced sensitivity to both fenarimol and bupirimate also. Only 3 isolates of powdery mildew developed on the bupirimate treated leaf discs (cpm/04/97, cpm/17/97 and cpm/19/97) suggesting a reduced sensitivity towards this fungicide in these tests.

(ii) Preliminary Efficacy Screen

Inoculation of the control leaf discs with a dry spore suspension of *S. fuliginea* sourced from HRI Stockbridge House was successful in infecting detached cucumber leaf discs. A mean number of mildew pustules/leaf disc of 5.0 was generated and this equated to a mean percentage area of leaf disc affected of 22.5 (Table 4). Interestingly, in this experiment imazalil (Fungaflor) was totally effective in eliminating infection. In contrast, fenarimol (Rubigan) provided a suppression of mildew though was not totally effective in preventing infection by *S. fuliginea*. Bupirimate (Nimrod) was more effective than fenarimol, but again did not completely control mildew infection. Interestingly these results correlate well with the overall results from the first year's resistance testing using isolates collected from commercial nurseries.

Of the experimental fungicides evaluated most were highly effective in preventing mildew infection in this detached leaf bioassay. Only one fungicide ('penconazole' Topas) allowed slight mildew development on the treated, inoculated leaf discs. Unfortunately, several of the novel fungicides evaluated appeared to cause a premature chlorosis or senescence of the leaf discs and this suggested that phytotoxicity may be problematic with their use (see below).

(iii) Phytotoxicity Evaluation with Novel Fungicides

A range of 4 different cultivars (Pyralis, Enigma, Jessica, and Bronco) were raised in rockwool propagation blocks to the first true leaf stage. The fungicides listed in Table 2 were applied HV to run-off, at the rates specified, on blocks of 4 plants/fungicide and compared with plants sprayed with water only. After 7 days the plants were examined for symptoms of phytotoxicity and the height of each plant measured.

Several of the applied chemicals, particularly the triazoles and morpholines, caused phytotoxicity in this evaluation. Symptoms of leaf scorching, curling, darkening and stunting was evident on the plants (Tables 5-8). The most damaging fungicides to the young cucumber plants were tebuconazole (Folicur), fenpropimorph (Aura), cyproconazole (Alto 100), tridemorph (Calixin), flusilazole (Genie), epoxiconazole (Opus), triademenol (Bayfidan), and difenconazole (Plover). Whilst there appeared to be some differences in sensitivity to the fungicides between cultivars, in general, the effects were broadly similar with the height of the plants most markedly affected.

To confirm these effects and to re-evaluate the fungicides on slightly older, hopefully less susceptible, plants the experiment was repeated with the same fungicides but using larger plants at the 2-3 leaf stage instead. In this case only the plant height was measured 7-10 days after treatment.

The results of this repeat phytotoxicity study on the older plants using the same 4 cultivars, were broadly similar. They confirm that, at the rates tested, tebuconazole (Folicur), fenpropimorph (Aura), cyproconazole (Alto 100), tridemorph (Calixin), epoxiconazole (Opus), and difenconazole (Plover) were all phytotoxic to young cucumber plants (Table 9). In addition, there was evidence that some of the cultivars were more sensitive to particular fungicides than others. For example cv. Enigma appeared particularly susceptible to damage from imazalil (Fungaflor) whereas the other cvs. tested were unaffected. Similarly, cyprodinil (Unix) caused a marked sensitivity on the mildew-tolerant cv. Enigma whereas no adverse effects were observed with cvs. Pyralis, Jessica or Bronco.

(iv) *In Vitro* Efficacy Studies against *B. cinerea* and *M. melonis*

In a series of *in vitro* inoculation studies using detached leaves the various fungicides listed in Table 2 were also evaluated for their efficacy against both *Botrytis* and *Mycosphaerella*.

Botrytis cinerea

Isolates of *B. cinerea*, selected at random from commercial crops, produced moderately aggressive leaf lesions on the inoculated control leaves (Table 10). The commercially available mildew fungicides provided a moderate suppression and, fenarimol (Rubigan) was most effective against of *B. cinerea* in this evaluation. Imazalil (Fungaflor) was the least effective of the three standard products used. Several of the novel fungicides tested were effective in preventing the development of *Botrytis* lesions. The most effective products in this preliminary screen were tebuconazole (Folicur), flusilazole (Genie), fenpropimorph (Aura), tridemorph (Calixin), cyproconazole (Alto 100), cyprodinil (Unix), myclobutanil (Systhane), epoxiconazole (Opus) and azoxystrobin (Amistar).

Mycosphaerella melonis

The isolates of *Mycosphaerella melonis*, selected at random from affected commercial crops, produced very aggressive leaf lesions on the inoculated control leaves (Table 10). The commercial standard products, particularly fenarimol (Rubigan) and imazalil (Fungaflor) were moderately effective in reducing lesion size on the detached leaves. Bupirimate (Nimrod) was the poorest of the three standard products tests in this respect.

Again, several of the experimental fungicides were very effective in minimising the development of *Mycosphaerella* lesions. The most effective products were tebuconazole (Folicur), flusilazole (Genie), cyproconazole (Alto 100), myclobutanil (Systhane), epoxiconazole (Opus) and triademenol (Bayfidan).

Table 3: Evaluation of fungicide sensitivity using a range of isolates of *S. fuliginea* from commercial cucumber crops collected during 1997.

Isolate No.	Mean No. of Mildew Colonies per cm Leaf Area				
	Uninoculated	Inoculated	Imazalil	Fenarimol	Bupirimate
cpm/01/97	0.0	3.8	0.0	0.9	0.0
cpm/02/97	-	-	-	-	-
cpm/03/97	0.0	2.5	0.0	1.3	0.0
cpm/04/97	0.0	11.9	0.0	0.0	0.6
cpm/05/97	0.0	6.1	0.0	1.2	0.0
cpm/06/97	0.0	0.5	0.0	0.0	0.0
cpm/07/97	0.0	5.4	0.0	0.4	0.0
cpm/08/97	0.0	2.6	0.0	7.9	0.0
cpm/09/97	0.0	4.4	0.0	0.5	0.0
cpm/10/97	0.0	1.5	0.0	0.1	0.0
cpm/11/97	-	-	-	-	-
cpm/12/97	-	-	-	-	-
cpm/13/97	-	-	-	-	-
cpm/14/97	-	-	-	-	-
cpm/15/97	-	-	-	-	-
cpm/16/97	-	-	-	-	-
cpm/17/97	0.0	9.9	0.0	3.5	0.2
cpm/18/97	0.0	2.5	0.0	0.0	0.0
cpm/19/97	0.0	7.4	10.4	2.2	3.1
cpm/20/97	-	-	-	-	-
cpm/21/97	-	-	-	-	-

Table 4: Incidence and severity of powdery mildew on detached fungicide treated cucumber leaf discs artificially inoculated with *S. fuliginea*.

Treatment	Mean No. of Mildew Pustules per Leaf Disc	Mean % Leaf Disc Area Affected
Water control	5.0	22.5
Fungaflor (imazalil)	0.0	0.0
Rubigan (fenarimol)	1.5	5.7
Nimrod (bupirimate)	0.2	0.4
Folicur* (tebuconazole)	0.0	0.0
Rocket (triflumizole)	0.0	0.0
Genie (flusilazole)	0.0	0.0
Aura* (fenpropimorph)	0.0	0.0
Topas (penconazole)	0.2	1.7
Dorado (pyrifenox)	0.0	0.0
Calixin* (tridemorph)	0.0	0.0
Alto 100 (cyproconazole)	0.0	0.0
Unix* (cyprodinil)	0.0	0.0
Systhane (myclobutanil)	0.0	0.0
Opus* (epoxiconazole)	0.0	0.0
Bayfidan (triademenol)	0.0	0.0
Amistar (azoxystrobin)	0.0	0.0
Stroby (kresoxim-methyl)	0.0	0.0
Milsana* ('plant extract')	0.0	0.0

* Some evidence of phytotoxicity on the leaf discs following fungicide treatment.

Table 5: Evaluation of phytotoxicity following various fungicide treatments on the commercial cucumber cultivar cv. Pyralis.

Treatment	Mean Plant Height (cm)	% Leaf Chlorosis	Severity of Leaf Curling (0-5)	Leaf Discoloration (+/-)
Pyralis				
Water control	35.3	0	0	-
Fungaflor (imazalil)	29.3	0	0	-
Rubigan (fenarimol)	31.5	0	0	-
Nimrod (bupirimate)	32.1	0	0	-
Folicur (tebuconazole)	0.0	100	*	+
Rocket (triflumizole)	32.3	0	0	-
Genie (flusilazole)	10.3	5	3	+
Plover (difenconazole)	12.0	0	3	+
Aura (fenpropimorph)	12.3	15	3	+
Topas (penconazole)	32.1	0	0	-
Dorado (pyrifenox)	35.0	0	0	-
Calixin (tridemorph)	12.0	10	2	+
Alto 100 (cyproconazole)	10.6	10	3	+
Unix (cyprodinil)	28.2	0	0	-
Systhane (myclobutanil)	19.6	0	3	+
Opus (epoxiconazole)	15.0	5	4	+
Bayfidan (triademenol)	18.0	10	3	+
Amistar (azoxystrobin)	26.6	0	0	-
Stroby (kresoxim-methyl)	32.5	0	0	-
Milsana ('plant extract')	33.8	0	0	-

* No green leaf tissue remaining.

Table 6: Evaluation of phytotoxicity following various fungicide treatments on the commercial cucumber cultivar cv. Enigma.

Treatment	Mean Plant Height (cm)	% Leaf Chlorosis	Severity of Leaf Curling (0-5)	Leaf Discoloration (+/-)
Enigma				
Water control	30.5	0	0	-
Fungaflor (imazalil)	27.4	0	0	-
Rubigan (fenarimol)	29.0	0	0	-
Nimrod (bupirimate)				
Folicur (tebuconazole)	6.9	40	3	+
Rocket (triflumizole)	27.9	0	0	-
Genie (flusilazole)	12.0	5	3	+
Plover (difenconazole)	16.6	10	3	+
Aura (fenpropimorph)	9.3	30	4	+
Topas (penconazole)	18.6	0	2	-
Dorado (pyrifenox)	36.8	0	0	-
Calixin (tridemorph)	22.4	45	3	+
Alto 100 (cyproconazole)	20.5	20	4	+
Unix (cyprodinil)	16.3	2	2	+
Systhane (myclobutanil)	10.8	5	3	+
Opus (epoxiconazole)	11.1	5	4	+
Bayfidan (triademenol)	19.9	0	3	+
Amistar (azoxystrobin)	25.7	0	0	-
Stroby (kresoxim-methyl)	21.0	0	0	-
Milsana ('plant extract')	29.2	0	0	-

Table 7: Evaluation of phytotoxicity following various fungicide treatments on the commercial cucumber cultivar cv. Jessica.

Treatment	Mean Plant Height (cm)	% Leaf Chlorosis	Severity of Leaf Curling (0-5)	Leaf Discoloration (+/-)
Jessica				
Water control	31.5	0	0	-
Fungaflor (imazalil)	27.3	0	0	-
Rubigan (fenarimol)	35.8	0	0	-
Nimrod (bupirimate)	40.1	0	0	-
Folicur (tebuconazole)	2.0	95	4	+
Rocket (triflumizole)	35.8	0	0	-
Genie (flusilazole)	19.1	2	3	+
Plover (difenconazole)	20.6	30	2	+
Aura (fenpropimorph)	13.8	40	2	+
Topas (penconazole)	31.6	0	0	-
Dorado (pyrifenox)	30.4	0	0	-
Calixin (tridemorph)	15.9	65	3	+
Alto 100 (cyproconazole)	14.0	20	3	+
Unix (cyprodinil)	32.3	5	1	-
Systhane (myclobutanil)	26.6	0	1	+
Opus (epoxiconazole)	13.9	10	4	+
Bayfidan (triademenol)	16.0	5	0	+
Amistar (azoxystrobin)	27.6	0	0	-
Stroby (kresoxim-methyl)	33.2	0	0	-
Milsana ('plant extract')	26.9	0	0	-

Table 8: Evaluation of phytotoxicity following various fungicide treatments on the commercial cucumber cultivar cv. Bronco.

Treatment	Mean Plant Height (cm)	% Leaf Chlorosis	Severity of Leaf Curling (0-5)	Leaf Discoloration (+/-)
Bronco				
Water control	30.1	0	0	-
Fungaflor (imazalil)	29.1	0	0	-
Rubigan (fenarimol)	33.7	0	0	-
Nimrod (bupirimate)	29.6	0	0	-
Folicur (tebuconazole)	8.5	70	4	+
Rocket (triflumizole)	26.9	0	0	-
Genie (flusilazole)	19.6	2	3	+
Plover (difenconazole)	14.4	20	4	1
Aura (fenpropimorph)	12.0	30	4	+
Topas (penconazole)	17.2	0	2	+
Dorado (pyrifenox)	30.3	0	0	-
Calixin (tridemorph)	13.2	15	3	+
Alto 100 (cyproconazole)	9.1	25	4	+
Unix (cyprodinil)	28.8	10	0	-
Systhane (myclobutanil)	26.9	0	1	+
Opus (epoxiconazole)	12.2	20	5	+
Bayfidan (triademenol)	17.1	0	0	-
Amistar (azoxystrobin)	31.6	0	4	+
Stroby (kresoxim-methyl)	33.6	0	0	-
Milsana ('plant extract')	28.6	0	2	+

Table 9: Repeat evaluation of phytotoxicity following various fungicide treatments on a range of commercial cucumber cultivars in 1997.

Treatment	Mean Plant Height (cm)				Comments
	Pyralis	Enigma	Jessica	Bronco	
Water control	88.8	63.6	58.8	68.3	Healthy.
Fungaflor (imazalil)	68.6	26.4	55.5	60.3	Generally healthy but leaf chlorosis on Enigma.
Rubigan (fenarimol)	70.2	57.6	58.9	70.3	Healthy.
Nimrod (bupirimate)	75.3	50.7	74.0	74.5	Generally healthy, slight distortion on Enigma.
Folicur (tebuconazole)	-*	5.6	1.8	7.0	Leaves scorched, dying or dead.
Rocket (triflumizole)	75.4	64.7	68.0	56.3	Healthy.
Genie (flusilazole)	14.0	31.9	32.5	33.6	Leaves shrivelled, distorted, plants stunted.
Plover (difenconazole)	28.3	23.6	32.3	19.1	Leaves shrivelled, plant stunted.
Aura (fenpropimorph)	13.3	11.8	12.3	11.8	Leaves curled, chlorotic, dying.
Topas (penconazole)	66.8	53.1	71.4	23.5	Generally healthy but dark stunted leaves on Bronco.
Dorado (pyrifenox)	62.3	42.7	50.8	47.9	Fairly healthy, but small.
Calixin (tridemorph)	13.3	12.0	14.4	13.1	Leaves curled and shrivelled, plants dying.
Alto 100 (cyproconazole)	11.8	12.0	12.8	8.6	Leaves small, chlorotic, dying.
Unix (cyprodinil)	66.6	19.4	67.8	56.8	Feint chlorosis, distortion on Enigma.
Systhane (myclobutanil)	38.8	11.7	45.9	55.5	Variable. Enigma stunted chlorotic.
Opus (epoxiconazole)	13.5	10.4	10.8	12.3	Leaves curled, shrivelled, plants dying.
Bayfidan (triademenol)	32.8	35.1	28.8	32.5	Leaves mottled, plants stunted.
Amistar (azoxystrobin)	73.6	63.3	66.8	51.5	Healthy.
Stroby (kresoxim-methyl)	72.9	24.0	73.5	77.8	Healthy.
Milsana ('plant extract')	79.0	61.6	44.0	49.8	Healthy.

* Plants dead.

Table 10: Relative efficacy of various fungicide treatments against *B. cinerea*, cause of grey mould and *M. melonis*, cause of black stem rot, in *in vitro* (detached leaf) bioassays.

Treatment	Mean Diameter of *Botrytis* Leaf Lesions* (mm)	Mean Diameter of *Mycosphaerella* Leaf Lesions* (mm)
Water control	27.0	43.2
Fungaflor (imazalil)	13.3	10.5
Rubigan (fenarimol)	4.0	9.3
Nimrod (bupirimate)	8.2	36.8
Folicur (tebuconazole)	4.0	4.0
Rocket (triflumizole)	33.0	7.7
Genie (flusilazole)	4.0	4.0
Aura (fenpropimorph)	4.0	20.0
Topas (penconazole)	40.8	26.7
Dorado (pyrifenox)	6.8	30.0
Calixin (tridemorph)	4.0	23.2
Alto 100 (cyproconazole)	4.0	4.0
Unix (cyprodinil)	4.0	11.8
Systhane (myclobutanil)	4.0	4.0
Opus (epoxiconazole)	4.0	4.0
Bayfidan (triademenol)	8.3	4.0
Amistar (azoxystrobin)	4.0	23.5
Stroby (kresoxim-methyl)	37.2	20.7
Milsana ('plant extract')	17.8	23.3

* Mean of 2 isolates each inoculated onto 3 replicate detached leaves.

Discussion

The level of fungicide resistance detected in the first screen conducted during 1997 was surprisingly low given the increasing 'anecdotal' reports of poor performance received from commercial nurseries over recent years. It is possible of course that the move to mildew tolerant cultivars has eased the pressure on fungicide use and this in time may have allowed more sensitive strains of *S. fuliginea* to re-establish in the population.

The fungicides commonly used on cucumber have previously been considered to fall into two distinct classes of fungicide. Imazalil (Fungaflor) and fenarimol (Rubigan) have both been regarded as triazole or 'DMI' fungicides and therefore it would reasonably have been expected that the relative sensitivity of *S. fuliginea* populations would have been similar. Yet, the results generated in this first year's evaluation would appear to suggest that whilst there is a high, though variable, degree of tolerance to fenarimol (Rubigan), only one isolate exhibited cross-resistance to imazalil (Fungaflor).

Interestingly, the one isolate (cpm/19/97) which showed significant growth following treatment of the leaf discs with imazalil (Fungaflor) also exhibited a degree of insensitivity to both fenarimol (Rubigan) and bupirimate (Nimrod). It should be considered that the poor fungicide performance may be related to inefficient spray application rather than widespread resistance to fungicides. This aspect is worthy of further study.

Recently, the commercial mildew fungicides used on cucumber have been re-assigned to different fungicide groups (see Pesticide Register 11th Edition, 1997) as follows:

imazalil (Fungaflor) - triazole
bupirimate (Nimrod) - pyrimidine
fenarimol (Rubigan) - pyrimidinyl carbinol

On the basis of this, the results obtained during the resistance screening conducted during 1997 are not too surprising. Further information has been requested to try and clarify this new fungicide classification as it could have significant implications with regard to strategies for resistance management of powdery mildew in the future. (See 'Stop Press' at the back of this report).

Clearly, further resistance testing will be necessary during 1998 to examine this aspect further.

In the preliminary efficacy screen a moderate level of mildew developed on the control discs treated with water whereas those treated with fungicides exhibited a significantly reduced infection level in all cases. The standard fungicides whilst variable, were in line with results of the resistance testing. Indeed, in most cases mildew was totally absent on the leaf discs. Unfortunately however, the complete absence of mildew on many of the treated leaf discs prevented any comparative evaluation to be carried out. Of particular note however, was the fact that several of the applied chemicals caused a marked yellowing or premature senescence of the leaf discs indicating a high risk of phytotoxicity and this was further confirmed in the phytotoxicity studies conducted on the young plants.

Detailed phytotoxicity evaluations conducted on a range of 4 different cultivars (Pyralis, Enigma, Jessica and Bronco) revealed that several of the fungicides, particularly the triazoles and morpholines were phytotoxic to young cucumber plants. Tebuconazole (Folicur) was particularly damaging and in some cases caused plant death. There was, however, a surprising difference between the different triazole products and some (eg. triflumizole 'Rocket') were not phytotoxic on any of the cultivars tested. This is particularly unfortunate for whilst triflumizole is approved for use in other EU member states it appears unlikely that it will be progressed for a UK registration due primarily to the high cost of registration data requirements set by the regulatory authority, PSD. The novel strobilurin fungicides (azoxystrobin and kresoxim-methyl) appeared to cause few phytotoxicity problems and are certainly worthy of further evaluation. Only where kresoxim-methyl was used on the mildew tolerant cv. Enigma did there appear to be the possibility of some stunting of the plants.

Where the experiment was repeated on slightly older plants the severity of plant damage was reduced. It is possible therefore that in established crops those products causing phytotoxicity symptoms on young plants may be less damaging. Also, information on appropriate rates of use on cucurbits was not available for many of the products and therefore the use pattern selected may have been too high in some cases. It is possible that, in some cases, lower rates of use may be effective in suppressing mildew whilst at the same time minimising the risk of phytotoxicity. It is recommended that this aspect is further examined during 1998 studies.

Finally, where the fungicides were evaluated in *in vitro* bioassay tests against *Mycosphaerella* and *Botrytis* some interesting results emerged. Firstly, the standard commercial fungicides were all partially effective in suppressing the development of these pathogens. Fenarimol (Rubigan) and bupirimate (Nimrod) were particularly effective against *B. cinerea*, whereas fenarimol (Rubigan) and imazalil (Fungaflor) were most effective against *M. melonis*. Several of the experimental products evaluated showed marked activity against both pathogens, whereas others were only effective against one or other of these important stem infecting fungi. Clearly the selection of candidate fungicides for commercial evaluation must take this into account during 1998.

Conclusions

- A range of isolates of powdery mildew (*S. fuliginea*) were collected from commercial nurseries for use in resistance tests.

- An *in vitro* resistance bioassay was devised to determine the relative performance of the commercially available fungicides, Rubigan, Fungaflor and Nimrod.

- Of the isolates tested several exhibited reduced sensitivity to fenarimol (Rubigan) one isolate showed resistance to the triazole fungicide imazalil (Fungaflor). A few isolates appeared to exhibit a reduced sensitivity to the pyrimidine fungicide bupirimate (Nimrod). Only one isolate exhibited tolerance to all three of the fungicides tested.

- An *in vitro* bioassay was devised to measure the relative performance of a range of novel mildew fungicides alongside the commercially available products.

- Several of the novel fungicides provided excellent efficacy against *S. fuliginea* in the *in vitro* screen. The most effective products were Folicur, Rocket, Genie, Aura, Dorado, Calixin, Alto 100, Unix, Systhane, Opus, Bayfidan, Amistar and Stroby.

- Several of the novel fungicides, particularly the triazoles and morpholines, were phytotoxic at the rates tested, causing variable symptoms from leaf distortion, leaf scorch to stunting of the plants.

- An *in vitro* bioassay was devised to determine the relative efficacy of the novel experimental fungicides against both *B. cinerea* and *M. melonis.*

Several of the experimental fungicides exhibited good activity against *B. cinerea* and/or *M. melonis* in *in vitro* tests in the laboratory.

Recommendations for Further Work

1. A short-list of candidate fungicides should be drawn up from the preliminary screening studies and incorporated in a replicated study on a commercial crop to further measure the performance and safety of the novel fungicides as compared with commercial standard products.

2. Further resistance testing should be conducted during 1998 to determine the extent of insensitive isolates in a different season.

3. The primary efficacy screening of fungicides conducted in 1997 should be extended to:

   (i) evaluate other novel mildew fungicides which were previously unavailable. eg. KWG 4168 'Spiroxamine' (Bayer AG), DE795 'Quinoxyfen' (Dow Elanco), DPX JE874 'Famoxadone' (Du Pont), and 35-35 'Mepanipyram' (Shionogi).

   (ii) evaluate reduced rates of those products tested, particularly where phytotoxicity symptoms were observed.

4. The phytotoxicity screening of fungicides should be extended to:

   (i) evaluate reduced rates to minimise crop damage (and to relate this to the rates needed for effective control in bioassay tests).

   (ii) evaluate the crop safety of novel fungicides not previously tested.

5. The *in vitro* bioassays against *B. cinerea* and *M. melonis* should be further refined and repeated using different isolates of the fungi.

6. Further clarification should be sought regarding the current fungicide groupings of the commercially available mildew fungicides. Where necessary advice regarding resistance strategies should be modified accordingly. (See 'Stop Press' on page 35).

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'Stop Press'

Whilst this report was being prepared clarification was sought regarding the re-classification of fungicides currently approved for use on cucumbers in the UK.

The following information has been provided:

"The chemical nomenclature in the 11th Edition of the Pesticide Manual has been confirmed as correct".

"DMI fungicides include a number of different chemical structures including triazoles, imidazoles, pyrimidinyl carbinols and pyrimidines. The common feature of these molecules is a heterocyclic nitrogen atom so positioned that it interacts with the sixth co-ordination position of the haem iron so blocking entry of the oxygen-atom and inhibiting oxidative demethylation of the sterol substrate. Bupirimate (Nimrod) is a pyrimidine but does not have the same chemical structure as fenarimol (Rubigan), and has no effect on the demethylase as far as I know".

"...There are several mechanisms of resistance to DMIs and although there is cross resistance; resistance factors may be different in different isolates. So I would suggest that you assay both imazalil (Fungaflor) and fenarimol (Rubigan) in your sensitivity tests. Nevertheless, there is no need to change advice to farmers with regard to the anti-resistance strategy used to minimise the risk of resistance".

(Dr D Holloman, IACR - Long Ashton, May 1998)