Project Title:	Vegetable Propagation : Evaluation of Novel Fungicides for Disease Control in Brassica Transplants
Project Number:	FV 235
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The results and conclusions in this report are based on a series of glasshouse experiments. The conditions under which the experiments were carried out and the results generated 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 to be used as the basis for commercial product recommendations.

It should also be noted that many of the products tested in this work are experimental in nature and under no circumstances should they be used commercially. If anyone is in doubt regarding the current approval status of a particular product they should either consult the manufacturer, check the status on an approved pesticide database or take independent advice from a BASIS qualified adviser.

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AUTHENTICATION

I declare that the 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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GROWER SUMMARY

Headlines

- Data on the safety of a range of novel, but currently experimental, fungicides on various brassica transplants (Cauliflower, B. sprouts, Cabbage & Cauliflower) has been gathered
- Information on the relative efficacy of various standard & novel fungicides against important fungal pathogens on brassicas has been generated
- The need for urgent clarification of the regulatory approval status of pesticides for use in vegetable propagation has been highlighted

Background and Expected Deliverables

Effective control of disease in the early stage of plant production is critical as this eliminates one of the primary sources of inoculum for subsequent epidemic development in the field. Vegetable transplant producers already have to rely on a narrow range of fungicides to achieve this and the ongoing EU Review Programme is likely to limit the available products still further. It is essential, therefore, that alternative broad spectrum (and highly specific) fungicides are found to maintain effective disease control in this highly specialised sector of the industry. An additional benefit is likely to be the opportunity to 'ring the changes' to minimise pathogen resistant strains developing; critical if the strobilurin analogues are to be used.

Diseases of primary concern in this respect are downy mildew (*Peronospora parasitica*) and wirestem (*Rhizoctonia solani*) though damping-off (*Pythium* spp.) and various leaf-spots e.g. ringspot (*Mycosphaerella brassicicola*) can also be of significance on occasions. Evaluation of strobilurin analogues e.g. azoxystrobin or Amistar and other fungicides not previously tested on brassica transplants will determine which products are safe for use on the various crops and what fungal targets they are active against. Ultimately, the project, once completed, will determine the most effective integrated programmes to effectively target the various pathogens, which occur either persistently or sporadically in brassica transplants.

The successful identification of new fungicides for use on vegetable transplants will be a major benefit to propagators and growers, assuming On- or Off-Label Approval can be secured. By reducing inoculum introduced into field crops it will delay epidemic development in the field. This, in turn, will hopefully assist in minimising fungicide inputs closer to harvest and assist in the industries aim of achieving minimal pesticide residues at harvest.

The expected deliverables from this project are:-

- An evaluation of the relative safety of a broad range of novel fungicides applied to cabbage, cauliflower, Brussels sprout & calabrese
- Identification of novel fungicides with activity against some of the key fungal pathogens which affect brassicas during the transplant period
- Comparison of various integrated programmes under realistic conditions on a commercial propagation unit
- Recommendations for the On- or Off-Label Approval of various products for use during the propagation phase of brassica production

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Summary of the Project and Main Conclusions to Date

Crop Safety : The project has evaluated approximately 25 novel fungicides on cauliflower, calabrese, cabbage & Brussels sprouts using 1/2Normal, Normal (N) and 2xNormal rates. None of the applied experimental fungicides, even at double the anticipated rates of application (i.e. 2 x N), caused severe economic damage to the plants. Some products evaluated caused a slight scorching e.g. tebuconazole (Folicur) whereas others eg pencycuron (Monceren) caused an unsightly deposit on the foliage.

Efficacy Testing: A selection of 10-12 fungicides were evaluated against downy mildew (*Peronospora parasitica*) and wirestem (*Rhizoctonia solani*), damping-off (*Pythium* spp.) and ringspot (*Mycosphaerella brassicae*). Each fungicide was applied at ½ Normal and Normal (N) rates of application.

(a) *P. parasitica*. Several fungicides including Ranman Twinpack, Tanos, Invader, Dithane & Shirlan proved to be moderately effective against the disease though none provided total control. It is considered that under commercial conditions where the entire crop is fungicide treated (i.e. no untreated controls) disease pressure is likely to be lower and overall control ought to be improved.

(b) *Rhizoctonia solani*. Those that did emerge succumbed, in many cases, to post-emergent damping-off or 'wirestem' symptoms. Some of the applied fungicides suppressed the *Rhizoctonia* infection though none prevented infection completely. Surprisingly, some of the applied fungicides appeared to adversely affect seedling emergence. Switch, Charisma & Amistar reduced emergence most noticeably and the differences recorded in assessments were statistically significant (Table 10) at the 1N application level. It should be noted however that the disease pressure in this experiment was probably much higher than that experienced in most commercial crops and as such was a very stern test for the products evaluated. The most effective control was provided by the current commercial standard product Basilex. Of the experimental fungicides only Amistar (azoxystrobin) provided a visible degree of disease suppression though the reduced emergence following treatment requires further investigation. Pencycuron (Monceren) which is approved for *Rhizoctonia* control in potato as a seed (tuber) treatment gave disappointing results in this study and this may relate to the specific strain (anastomosis group) of *R. solani* used for artificial inoculation. Whilst some of the other fungicides evaluated provided a slight reduction in disease incidence and/or severity none could be regarded as sufficiently effective for commercial use. It should be noted that in some situations eg bottom-rot control in glasshouse lettuce tolclofos-methyl (Basilex) has proved ineffective on some nurseries and resistance/insensitivity in the pathogen population is suspected. Alternative effective fungicides with different modes of action are therefore required to ensure continued control of this pathogen. Growers need also to be familiar with the recent restrictions imposed on Basilex, as an acetyl-cholinesterase inhibitor (i.e. an OP fungicide) that prevents hand-held or knapsack application due to operator safety concerns. It should not be used unless a remote gantry application system is available.

(c) *A Pythium sp.* The experiment was repeated (owing to first failure on cauliflowers) using the same methodology but using oilseed rape (marketed as 'cress'), which had also been shown to be highly susceptible in earlier pathogenicity tests. The second attempt was very successful and a high level of pre- & post-emergent damping-off was recorded in the untreated control. The standard product SL567A was highly effective in reducing infection by the introduced Pythium sp. However, it should be noted that other isolates of the same pathogen may be resistant to metalaxyl-M (mefanoxam). Filex (or Proplant) was totally ineffective in controlling or even suppressing the pathogen and, whilst not tested, is assumed to be resistant to this fungicide (see also HDC report PC 97a). Whilst none of the other experimental fungicides evaluated were as effective as SL567A most were more effective

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than Filex and provided some suppression of the disease. Those most worthy of further study from an efficacy standpoint were Electis, Invader, Ranman TP & Shirlan.

(d) *Mycosphaerella brassicola.* No results were possible owing to failure of disease to establish.

Financial Benefits

None at present.

Action Points for Growers

- Propagators need to be aware of the current situation regarding the approval of pesticides in brassica propagation under protection and the potential risk to their business should any issues arise

- Propagators need to liaise closely with their growers to agree effective crop protection programmes in advance of production.

- Communicate and liaise with other Members of the Plant Propagators Association to lobby effectively to ensure the urgent needs of this sector of the industry are delivered.

- Keep abreast of developments with this project, particularly the final 'commercial' study where integrated fungicide programmes will be evaluated on a propagation facility in Lincolnshire.

Special note
Be aware that many of the chemicals currently being evaluated in this study, including several of those that have been recommended as worth pursuing for SOLA applications, are not approved for commercial use on brassicas in propagation at the current time

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Science Section

Introduction

The introduction of new technology in recent years has allowed producers of module-raised brassicas to make significant improvements in terms of overall plant production. However, this has also brought about an increased risk of disease due to the overall intensification within centralised production units, often within the same area of intensive field production.

With the exception of organic plant raisers, most propagators rely heavily on pesticide application to minimise pest and disease problems and to improve the overall quality of plant material at point of sale. Their reliance on relatively few products, often with highly specific activity (e.g. against downy mildew), ensures a high level of selection pressure for tolerant or resistant strains of the fungus. Where pathogens are controlled only partially in propagation, the affected seedlings subsequently serve as primary 'infector' plants post-planting to spread the disease in the field if suitable weather conditions prevail at the time of planting. Poor control during propagation is likely to exacerbate the need for more intensive spray regimes post-planting and increases the potential for pesticide residues at harvest. This is certainly something the industry is striving to avoid as a result of consumer and lobby group pressure via the major multiple retailers. Delivery of healthy transplants ought therefore to be considered highly beneficial.

Currently, brassica propagators rely on the use of fosetyl-al (Aliette) and propamocarb-HCl (Filex/Proplant) for downy mildew and *Pythium* control and either tolclofos-methyl (Basilex) or, until recently, quintozene (Terraclor) for the control of *Rhizoctonia* or 'wirestem'. For other leaf-spot pathogens propagators have tended to rely on fungicides already approved for use on field crops. However, these products are rarely approved for use under protection (due to the lack of a validated operator safety data package) and knowledge of their relative safety (phytotoxicity) when applied to brassica seedlings is not usually known. There is also concern in the industry that applications made during propagation phase of crop production may limit the total number of applications of specific products that can be applied post-planting. The EU pesticide Review programme is putting yet further pressure on pesticide availability in the horticultural sector and reliance on a limited range of chemicals is likely to exacerbate resistance development in pathogen populations.

There is therefore a need to balance the use of existing products with new safer, and hopefully broad-spectrum, products during propagation of brassica seedlings. This would assist in maintaining quality plant production, improve uniformity, reduce the risk of disease transfer to the field, delay the development of fungicide resistant strains and hopefully minimise the need for fungicide application close to maturity when the risk of residues in the harvested produce is at its greatest.

The primary aims of this project are to evaluate a broad array of new fungicides for their safety to various brassica crops (Cabbage, Brussels sprouts, Cauliflower & Calabrese), to determine their relative effectiveness against some of the key pathogens of importance on the crop and ultimately to investigate their optimum use in integrated disease control programmes to establish industry 'best practice'.

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Materials & Methods

(i) Phytotoxicity studies

Trial Design

A supply of the brassica seed listed below was sourced from commercial seed-houses.

- **Cabbage cv. Castello F1**
- **Brussels sprouts cv. Brilliant F1**
- **Cauliflower cv. Mexico F1**
- **Calabrese cv. Marathon F1**

Due to the large number of fungicides to be screened half the products were selected for an initial study during April-June 2002 the remaining chemicals being evaluated during July- August 2002 (See Tables 1-2 for further details). Seed of each of the 4 brassica types were sown into '345' module trays according to industry 'best practice' (White, *pers. com.)* using Levington F2 compost. At emergence of the first true leaf (50% expansion) the fungicides were applied at ½N, N and 2xN rates in 500 litres water/ha using an Oxford Precision sprayer with lance attachment operating at 2 bar pressure.

Table 1 : Standard & Experimental Fungicides evaluated for Crop Safety on a Range of Brassica Species. Phase I : April-June 2002

Crop Safety Study Experiment 1 [April – June 2002]	Rate of application (g or ml/litre)		
	½ N	N	2N
Tolclofos-methyl (Basilex)	1.0	2.0	4.0
Mancozeb (Dithane 945)	1.7	3.4	6.8
Pencycuron (Monceren Flowable)	2.0	4.0	8.0
Tebuconazole (Folicur)	1.0	2.0	4.0
Propamocarb-HCl (Filex)	0.5	1.0	2.0
Mefanoxam (SL567A)	0.16	0.32	0.65
Mefanoxam/mancozeb (Fubol Gold)	1.5	3.0	6.0
Fluazinam (Shirlan)	0.3	0.6	1.2
Dimethomorph/mancozeb (Invader)	5.0	10.0	20.0
Zoxamide/mancozeb (Electis 75 WG)	2.25	4.5	9.0
Fludioxinil/cyprodinil (Switch)	1.0	2.0	4.0
Difenoconazole (Plover)	0.3	0.6	1.2
Trichoderma spp. (Biomex)*	-	5.0	10.0

** applied at N & 2N rates only.*

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Table 2 : Standard & Experimental Fungicides evaluated for Crop Safety on a Range of Brassica Species. Phase II : July-August 2002

Crop Safety Study Experiment 2 [July – August 2002]	Rate of application		
	½ N	N	2N
Tolylfluanid (Elvaron Multi)	0.85	1.7	3.4
Triademefon (Bayleton)	0.5	1.0	2.0
Prochloraz-Mn (Octave)	1.0	2.0	4.0
Azoxystrobin (Amistar)	2.0	4.0	8.0
Pyraclostrobin/nicobifen (F516)	2.0	4.0	8.0
Famoxadone/flusilazole (Charisma)	3.0	6.0	12.0
Famoxadone/cymoxanil (Tanos)	1.4	2.8	5.6
Fosetyl-aluminium (Aliette)	10.0	20.0	40.0
Phosphonic acid (Omex DP98)	8.0	16.0	32.0
Pyrimethanil (Scala)	0.5	1.0	2.0
Epoxiconazole/pyraclostrobin (Opera)	2.0	4.0	8.0
Acibenzolar-S-methyl (Bion)	0.0125	0.025	0.05
Cyazofamid (Ranman Twinpack)	0.4+0.3	0.8+0.6	1.6+1.2
(Experimental fungicide) KIF 230	3.2	6.4	12.8

Crop Diary

Phase 1:	10 April 2002	Seed sown into module trays
	3 May 2002	Fungicides applied
	7 May 2002	Seedlings checked for phytotoxicity symptoms
	10 May 2002	Seedlings checked for phytotoxicity symptoms
	15 May 2002	Seedlings checked for phytotoxicity symptoms
	30 May 2002	Full crop safety assessment conducted
	31 May 2002	Experiment completed
Phase 2:	22 July 2002	Seed sown into module trays
	10 August 2002	Fungicides applied
	12 August 2002	Seedlings checked for phytotoxicity symptoms
	16 August 2002	Seedlings checked for phytotoxicity symptoms
	20 August 2002	Seedlings checked for phytotoxicity symptoms
	30 August 2002	Full crop safety assessment conducted
	31 August 2002	Experiment completed

At approximately 3-day intervals the seedlings were checked for the presence of scorch symptoms, other growth abnormalities relative to the untreated control and whether any unsightly chemical deposits were retained on the foliage following treatment. A full assessment of each product was made 2-3 weeks after the chemical application after which time each of the two phased experiments was terminated.

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(ii) Efficacy Studies

The efficacy experiments conducted against the 4 target pathogens were phased in an attempt to coincide with suitable weather conditions for successful establishment of the pathogens. At the same time, the aim was to spread the work more evenly over the growing cycle from a management perspective. Rather than relying on a natural infection each of the pathogens evaluated was introduced artificially either on 'infector' plants (e.g. non-culturable or obligate pathogens such as *Peronospora*), on agar culture (e.g. culturable or facultative pathogens e.g. *Pythium* spp., *Rhizoctonia*) or as dried leaf debris (e.g. *Mycosphaerella*). Each of the 4 pathogens was pre-inoculated onto the host to ensure viability and pathogenicity prior to commencing each experiment.

A short-list of fungicides with reported or expected activity against each of the 4 pathogens was selected (see Table 3) for detailed evaluation and applied to duplicate trays at two rates of application (½ N and N) with a '345' tray representing a single plot. Treatments were scheduled to be applied repeatedly (max. of 4 applications) at 7-14 day intervals though some flexibility was required depending on the disease pressure relative to the age of the seedlings. A representation of the trial layout for each of the efficacy studies is presented in Appendix 1.

Table 3 : Short-list of candidate fungicides selected for each efficacy evaluation against the 4 target pathogens

Peronospora parasitica	***Rhizoctonia solani***	***Pythium* spp.**	***Mycosphaerella brassicicola***
Untreated control	Uninoculated Control	Uninoculated control	Untreated control
Inoculated control	Inoculated Control	Inoculated control	Inoculated control
Amistar	Amistar	Amistar	Amistar
Dithane	Basilex (standard)	DP98	Scala
Electis	Biomex	Electis	Charisma
Elvaron Multi	Charisma	Filex (standard)	Elvaron Multi
F516	F516	F516	F516
Fubol Gold	Monceren	Invader	Folicur
Invader	Shirlan	Ranman TP	Octave
Ranman TP	Switch	Shirlan	Plover
Shirlan	-	SL567A	Shirlan
Tanos	-	Tanos	Switch

Trial design

(a) Peronospora parasitica

An isolate (or isolates) of the pathogen was secured on 'infector' plants from a commercial nursery in Lincolnshire. Pathogenicity on Cauliflower cv. Mayflower was demonstrated by raising young seedlings of this cv. in close proximity to the 'infector' plants under high relative humidity conditions and waiting for disease symptoms to be expressed.

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Seed of the same cv. were then sown in module compost using‘345’ trays (44 in total) using automated equipment at Westhorpe Flowers & Plants Ltd, Lincolnshire and then returned to STC Ltd for trial purposes. Once the seedlings had emerged and the cotyledons fully expanded the ‘infector’ plants with *P. parasitica* were introduced into the experimental area to enable air-borne dissemination of the pathogen. At the onset of disease symptoms on the untreated control plants the first fungicide application was made with repeat applications scheduled for 7-10 day intervals. However, due to exceptionally high disease pressure, the second application was applied much earlier than scheduled to try and maintain effective control of the disease. Details of the spray timing are presented in the appropriate crop diary. Disease assessments were made at regular intervals to assess the performance of the various applied fungicides. Initially (22nd May), the % infection/tray was estimated visually using the following scale:-

0 = 0% infection of seedlings
1 = 1-10% seedlings with d. mildew
2 = 11-25% seedlings with d. mildew
3 = 26-50% seedlings with d. mildew
4 = 51-100% seedlings with d. mildew

Ten seedlings/tray were then randomly selected and the number of plants infected with mildew and the incidence and severity of sporulation determined using the scale outlined below:-

0 = No visible sign of infection in the tray
1 = Slight necrosis/yellowing of the cotyledons, trace level of sporulation
2 = Moderate necrosis/yellowing of the cotyledons, sporulation found readily
3 = Severe infection with d. mildew. Extensive yellowing of cotyledons and sporulation abundant. Cotyledons dying on some plants

After a further 2 weeks (12th June) a further disease assessment was conducted both on the whole trays and on the 1st true leaf of 10 randomly selected but individual plants using the following severity scales:-

Whole tray assessment

0 = No visible sign of d. mildew infection
1 = <1% plants affected with d. mildew/tray
2 = 2-5% plants affected with d. mildew/tray
3 = 6-10% plants affected with d. mildew/tray
4 = 11-20% plants affected with d. mildew/tray
5 = >20% plants affected with d. mildew/tray

1st true leaf assessment

0 = No infection sites/leaf
1 = 1-10 infection sites/leaf
2 = 11-25 infection sites/leaf
3 = 26-50 infection sites/leaf
4 = 51-100 infection sites/leaf
5 = > 100 infection sites/leaf

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Finally, all the seedlings in each tray were excised at soil level (345 seedlings/tray) and weighed to provide a fresh weight/plot. The seedlings were subsequently oven-dried for 48 hrs and then re-weighed to determine the dry weight/plot. The experiment was subsequently terminated.

(b) Rhizoctonia solani

An isolate of *R. solani* was secured from the STC culture collection having previously been isolated from a Plant Clinic sample. Pathogenicity to brassica seedlings was assured in artificial inoculation studies prior to commencing the replicated trial. The pathogen was bulked up in a vermiculite culture and incubated for 10 days. It was then mixed with F2 compost (50:50 ratio) before filling the module trays. It should be noted that because of the compost incorporation technique for this specific pathogen the automated seed sowing equipment at Westhorpe Flowers Ltd could not be used due to the inherent risk of contaminating subsequent batches of plants destined for commercial production. Instead the Cauliflower seed were sown by hand at STC Ltd. Fungicide treatments were applied to the trays within 24 hours of sowing as a HV spray application using an Oxford Precision sprayer with boom attachment operating at 2 bars. As this fungus has the potential to affect the seedlings either pre-or post-emergence germination counts (i.e. total number of seedlings/tray) and subjective seedling vigour assessments (see scale below) were used as a measure of the pathogenicity of the fungus and efficacy of the applied fungicides at different time intervals between the fungicide applications. Finally, the fresh & dry weights of 50 seedlings/tray taken at random provided a quantitative measure of seedling vigour in each treatment.

Vigour score

0 = Most seedlings emerged but weak, dying or dead
1 = Most seedlings weak and many with unexpanded cotyledons, poor colour
2 = Emerging seedlings slightly weaker than best with reduction in leaf expansion
3 = Most seedlings emerged, good leaf expansion, good colour and plants strong

(c) Pythium spp.

A series of isolates of *Pythium* spp. were secured from Dr T Pettitt, HRI Wellesbourne and pathogenicity to young brassica seedlings pre-determined in artificial inoculation studies. Seed of Cauliflower cv. Mayflower were then sown in module compost using '345' trays (44 in total) using automated equipment at Westhorpe Flowers Ltd and returned to STC Ltd for experimental purposes. The *Pythium* spp., bulked up in agar culture, were macerated and then applied to the compost surface as a drench application pre-emergent. However, for some unaccountable reason, the introduced *Pythium* spp. failed to infect the root tissues in the module compost even though earlier pathogenicity tests had proved positive. After repeated inoculation attempts with alternative isolates of *Pythium* spp. and after application of the first fungicide treatment the experiment was terminated.

In a bid to secure data against this sporadic pathogen a further replicated study was established but on this occasion using commercial 'cress' (oil-seed rape) seed as the brassica host. An alternative, and highly pathogenic, isolate of *Pythium* recently recovered from infected sunflower roots, was used in the repeat study. Artificial

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inoculation tests demonstrated this organism to be highly pathogenic to 'cress' (OSR) prior to onset of the replicated study. The *Pythium* sp. was bulked up on agar as previously and evenly incorporated into the compost prior to seed sowing. Fungicide treatments were applied as previously at approximate weekly intervals and disease assessments carried out as soon as symptoms of the disease appeared. Initial assessments focused on the incidence and severity of mycelium of the introduced *Pythium* sp. though as the disease progressed an assessment of the percentage of plants damping-off was recorded.

(d) Mycosphaerella brassicicola

Infected leaf debris was secured from Dr R Kennedy, HRI, Wellesbourne for this study together with fresh leaf material with ringspot symptoms and an agar culture containing the fungus. Cauliflower seed cv. Mayflower was sown at Westhorpe Flowers Ltd and returned to STC Ltd for germination and emergence. At 100% emergence (cotyledon stage) the dried leaf debris and fresh infected leaf tissues were placed evenly around the trial area in an attempt to establish infection. In addition agar plugs containing the fungus were placed directly onto the brassica seedling leaf tissues and incubated under high RH conditions. Unfortunately, after repeated attempts at establishing infection the trial was terminated unsuccessfully and no data was gathered against this sporadic pathogen.

Crop Diary

(a) Peronospora parasitica

28 April 2003	Cauliflower seed cv. Mayflower sown
1 May 2003	Trays collected and returned to STC Ltd
7 May 2003	Seedlings emerged, cotyledons expanded
9 May 2003	'Infector' plants with d. mildew introduced into trial area
19 May 2003	D. mildew observed on cotyledons of untreated control plants
19 May 2003	1st fungicide application
22 May 2003	Disease assessment
23 May 2003	2nd fungicide application
12 June 2003	Disease assessment
13 June 2003	3rd fungicide application
27 June 2003	4th fungicide application
10 July 2003	Disease assessment
11 July 2003	Trial completed

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(b) Rhizoctonia solani

6 February 2003	*Rhizoctonia* culture established in vermiculite
18 February 2003	*Rhizoctonia* culture incorporated into module compost
19 February 2003	Cauliflower seed cv. Mayflower sown in F2 compost in '345' module trays at STC Ltd
20 February 2003	1st fungicide application
27 February 2003	Seedlings germinated & cotyledons expanded in inoculated control. Delayed emergence evident in inoculated control.
3 March 2003	2nd fungicide application
4 March 2003	Germination/emergence count & disease assessment
11 March 2003	Repeat emergence count & disease assessment
13 March 2003	3rd fungicide application
18 March 2003	Fresh & dry weight determination
19 March 2003	Trial completed

(c) Pythium spp.

28 April 2003	Cauliflower seed cv. Mayflower sown into module compost in '345' module trays at Westhorpe Flowers Ltd
1 May 2003	Trays collected & returned to STC Ltd
1 May 2003	*Pythium* sp. bulked up on an agar medium
7 May 2003	*Pythium* sp. inoculated into trial
9 May 2003	1st fungicide application
19 May 2003	Repeat inoculation of *Pythium* sp. into the trial area.
19 May 2003	Visual check for phytotoxicity from applied treatments
24 May 2003	Inoculated seedlings checked in laboratory. Infection not occurring, trial terminated

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20 July 2003	*Pythium* sp. pathogenic to commercial 'cress' (oil-seed rape or OSR) seed bulked up
29 July 2003	Seed of OSR sown high density into seed trays and *Pythium* sp. inoculum applied
30 July 2003	1st fungicide application
6 August 2003	Disease assessment
7 August 2003	2nd fungicide application
11 August 2003	Disease assessment
15 August 2003	Trial completed

(d) Mycosphaerella brassicicola

13 February 2003	Cauliflower seed cv. Mayflower sown in module compost in '345 module trays at Westhorpe Flowers Ltd
18 February 2003	Trays collected and returned to STC Ltd
19 February 2003	1st fungicide treatment applied
24 February 2003	Seedlings germinated & cotyledons expanded
27 February 2003	Inoculum of *M. brassicicola* applied throughout the trial area
3 March 2003	2nd fungicide application
7 March 2003	Pathogen re-inoculation
11 March 2003	

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20 March 2003	3rd fungicide application
31 March 2003	Disease assessment & re-inoculation of *M. brassicicola*
10 April 2003	Disease assessment
17 April 2003	Trial terminated

(iii) Evaluation of integrated fungicide programmes – A Commercial Study

The final work in this project will be undertaken in Autumn-Winter 2003/4 on a commercial propagation facility in Lincolnshire. The aim will be to select the most effective products identified in the initial stages of the project and to incorporate and use them in a series of integrated fungicide programmes alongside a commercial standard programme. Their performance against the predominant pathogens, which naturally occur on the nursery, will be assessed using replicated trial plots.

Results

(i) Crop Safety (Phytotoxicity) Screen

The crop safety screen was undertaken in two phases to allow a greater number of chemicals to be tested. In all, some 45 fungicides were evaluated at 3 different rates (1/2 N, 1N and 2N) for their relative safety to 4 different brassica seedlings (Cauliflower, Cabbage, Brussels sprouts and Calabrese) at the first true leaf stage.

Surprisingly, very few observations of crop damage were made during this work and where adverse symptoms were recorded they tended to be transient; the plants growing away from the symptoms fairly quickly. Where adverse effects were noted during routine evaluation & assessment of the plants these were recorded (Table 4A & 4B).

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Table 4A : Records of phytotoxicity and related symptoms in the various brassicas following application of a range of experimental fungicides - Phase I

Date	Product	Active Ingredient	Rate of application	Comments
Phase 1 (sown on 10th April 2002, applications made on 3 May 2002)				
7 May	-	-	-	No phytotoxicity symptoms seen
10 May	Folicur	tebuconazole	1N	Very slight scorch on Calabrese only. Not evident at 2N
15 May	-	-	-	No phytotoxicity symptoms seen
30 May (final assessment)	Monceren	Pencycuron	2N	Visible reddish deposit on leaf surface, especially at 2N rate. Residue not washed off during routine irrigation requirements for crop
	Folicur	tebuconazole	2N & 1N	Occasional seedlings with 'cupping' of individual leaves and marginal necrosis of leaf 4
	Folicur	tebuconazole	1N	Slight scorch symptom on leaf 3
	Folicur	tebuconazole	½ N	Slight hint of similar damage to that at 1N

Table 4B : Records of phytotoxicity and related symptoms in the various brassicas following application of a range of experimental fungicides - Phase II

Date	Product	Active Ingredient	Rate of application	Comments
Phase 2 (sown on 22 July 2002, applications made on 10th August 2002)				
12 August	-	-	-	No phytotoxicity symptoms seen
16 August	Scala	pyrimethanil	2N	Slight leaf distortion in cauliflower, leaf cupping in B. sprout*
	Scala	pyrimethanil	1N	Slight leaf cupping in B. sprout*
	Charisma	famoxadone + flusilazole	2N	Very slight scorching of the leaf margins
20 August[1]	Charisma	famoxadone + flusilazole	2N	Slight stunting of the plants
30 August	Charisma	famoxadone + flusilazole	2N	Slight stunting of the plants

** Similar symptom also recorded on control plots*

[1] generalised yellowing of leaves on many treatments but control plots equally affected and therefore not considered significant with respect to treatment applications

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None of the symptoms observed during the dual-phased phytotoxicity study were considered sufficient to warrant using the results as part of a selection process for screening fungicides for further efficacy investigation. It therefore proved necessary to select candidate products for further testing using knowledge of their likely activity from the scientific & commercial literature.

(ii) Efficacy Evaluations

(a) Peronospora parasitica

Following the introduction of 'infector' plants into the trial area on 9 May the disease established successfully. After 10 days incubation d. mildew was observed to be present on the under-surface of occasional cotyledons in the untreated control plots. The scheduled fungicide programmes commenced immediately on 19 May. Within 24 hours cotyledon infection of seedlings was extremely severe and infection pressure was regarded to be very high. This was considered to be due, in part, to favourable climatic conditions during May 2003. As a result of the severe disease pressure it was decided to reduce the interval between the initial scheduled spray applications in a bid to maintain effective disease control with the various experimental (and standard) treatments. Details of the precise spray timings are provided in the crop diary.

An assessment of the disease on 22 May (3 days after the first fungicide application) using a disease severity scale, based on the incidence of sporulating lesions in each tray, highlighted the severity of the disease. In the untreated control approximately 20% of the seedlings were infected and perhaps disappointingly none of the fungicides prevented infection altogether. The most promising fungicides at this early stage in the trial were Invader, Ranman TP, Shirlan and Dithane, all of which showed in the region of 50% reduction in seedling infection as compared with the untreated control. (Table 5). Some of the applied fungicides were ineffective and in plots treated with Amistar, Elvaron Multi & Electis the level of seedling infection was higher than in the untreated control. The mediocre level of control achieved with Fubol Gold suggests perhaps that one or more strains of the fungus present in the population exhibited resistance to the metalaxyl-M component in the formulated product.

A more detailed assessment based on infection level of 10 randomly selected seedlings/tray supported this initial result. The most effective treatments (at 1N rate of application) were Ranman (97% control), Shirlan (87% control), Dithane (75% control), Invader (56% control) and Tanos (50% control). The least effective products at this stage were Electis, Elvaron Multi and Amistar all of which appeared to retain infection levels as high or higher than the untreated control (Table 6).

By 12 June (2 fungicide applications made) the pathogen had caused considerable yellowing of the cotyledons on infected seedlings. The first true leaf, which was now fully emerged, exhibited the early signs of d. mildew infection and a marked speck necrosis was evident on seedlings in some trays. An overall assessment of mildew/tray and a further detailed assessment of 10 seedlings/tray selected randomly was made at this time. Infection levels in the untreated control had increased considerably and several of the treatments (on a whole tray basis) appeared visibly much healthier. Products identified as providing the most effective control at this

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stage in the trial were Amistar, Fubol Gold, Ranman TP & Tanos (Table 7). This was mirrored closely in the detailed assessment of 10 randomly selected seedlings where again Amistar, Fubol Gold, Ranman TP & Tanos yielded the lowest Disease Index (DI) relative to the untreated control (Table 8) though it should be noted that the differences observed in this assessment were not significant at the 5% level of probability. The least effective products in this assessment were Shirlan, Invader & Dithane surprisingly.

A change to less favourable weather during mid-late June appeared to check disease development and further detailed disease assessments were not possible, especially as most of the infected cotyledons shrivelled and abscised. The earlier differences between treatments, in terms of leaf necrosis of the 1st true leaf, persisted and there appeared to be subtle differences in seedling size and overall vigour. It was therefore decided to terminate the experiment by undertaking a destructive fresh & dry weight assessment of all the seedlings in each tray. This data is presented as an overall measure of seedling vigour during the experiment and hopefully reflects the performance of the individual products in terms of maintaining photosynthetic function of the cotyledons and 1st true leaf of the seedling brassicas (Table 9). Several of the applied fungicides increased seedling fresh & dry weight though interestingly, these same treatments did not necessarily correlate well with those that were recorded as providing the most effective control of d. mildew. This suggests perhaps that there might be other factors involved other than straightforward control of the introduced d. mildew and this is supported by the lack of statistically significant differences in the data (P=0.05).

Table 5 : Assessment of % plant infection with downy mildew/tray on 22 May 2003

Treatment	**% plant infection 22 May 2003 (Based on 0-4 severity scale) [1]**	
	½ Normal*	**Normal***
Untreated control	1.8 [a]	1.8 [a]
Amistar	2.5 [a]	2.5 [a]
Dithane 945	2.0 [a]	1.0 [a]
Electis	2.0 [a]	3.0 [a]
Elvaron Multi	2.5 [a]	3.0 [a]
F516	2.0 [a]	2.0 [a]
Fubol Gold	2.5 [a]	1.5 [a]
Invader	2.0 [a]	1.0 [a]
Ranman TP	0.5 [a]	1.0 [a]
Shirlan	1.0 [a]	1.0 [a]
Tanos	1.5 [a]	2.0 [a]
LSD (P=0.05)	1.10	1.18
Standard Deviation	0.50	0.53
Coefficient of Variation	26.85	29.57

* Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)

[1] Assessment based on overall disease level on 345 seedlings/tray

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Table 6 : Visual assessment of d. mildew severity and estimate of Disease Index on 22 May 2003

Treatment@	Disease severity Score (0-3 scale)		Disease Index[1] (0-100)	
	½ Normal	Normal	½ Normal*	Normal*
Untreated control	1.6	1.6	51.7 [ab]	51.7 [ab]
Amistar	1.3	1.8	41.7 [ab]	59.9 [ab]
Dithane 945	1.1	0.4	36.6 [ab]	13.3 [ef]
Electis	1.9	2.2	61.6 [a]	71.6 [a]
Elvaron Multi	2.0	2.1	65.0 [a]	69.9 [a]
F516	1.3	1.4	41.6 [ab]	44.9 [bc]
Fubol Gold	1.3	1.8	66.6 [a]	30.0 [cd]
Invader	1.2	0.7	40.0 [ab]	23.3 [cde]
Ranman TP	0.3	0.	10.0 [b]	1.7 [e]
Shirlan	0.6	0.2	20.0 [ab]	6.7 [de]
Tanos	0.7	0.8	21.7 [ab]	26.7 [cd]
LSD (P=0.05)	-	-	27.79	16.10
Standard Deviation	-	-	12.47	7.23
Coefficient of Variation	-	-	30.07	19.89

** Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*
@ Mean of 2 trays assessed for each rate of application of the various products.
[1]*Disease Index calculated from the Disease Severity Score using the formula outlined in the Materials & Methods section of the report.*

Table 7 : Assessment of % plant infection with downy mildew/tray on 12 June 2003

Treatment	% plant infection 12 June 2003 [1] (Based on 0-5 severity scale)	
	½ Normal*	Normal*
Untreated control	2.5[a]	2.5[ab]
Amistar	1.5[a]	1.0[c]
Dithane	2.0[a]	2.0[abc]
Electis	1.5[a]	1.5[bc]
Elvaron Multi	2.0[a]	1.5[bc]
F516	1.5[a]	1.5[bc]
Fubol Gold	1.5[a]	1.0[c]
Invader	1.5[a]	2.0[abc]
Ranman TP	2.0[a]	1.0[c]
Shirlan	2.0[a]	3.0[a]
Tanos	1.5[a]	1.0[c]
LSD (P=0.05)	1.78	0.79
Standard Deviation	0.80	0.36
Coefficient of Variation	45.0	21.8

*****Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*
[1] *assessment based on overall disease level per 345 seedlings/tray*

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Table 8 : Severity of d. mildew on the first true leaf on 12 June 2003

Treatment	Disease severity Score [1] (0-3 scale)		Disease Index (0-100)	
	½ Normal	Normal	½ Normal *	Normal *
Untreated control	2.6	2.6	51.0[a]	51.0[a]
Amistar	1.5	1.1	30.0[a]	21.0[a]
Dithane	2.0	1.9	40.0[a]	37.0[a]
Electis	1.3	1.7	26.0[a]	34.0[a]
Elvaron Multi	1.8	1.4	35.0[a]	27.0[a]
F516	1.6	1.4	32.0[a]	28.0[a]
Fubol Gold	1.6	1.2	31.0[a]	24.0[a]
Invader	1.8	1.9	36.0[a]	39.0[a]
Ranman TP	2.4	1.3	48.0[a]	26.0[a]
Shirlan	1.9	2.6	37.0[a]	52.0[a]
Tanos	1.6	1.0	31.0[a]	20.0[a]
LSD (P=0.05)	-	-	31.33	17.74
Standard Deviation	-	-	14.06	7.96
Coefficient of Variation	-	-	38.97	24.39

** Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*

[1] *Mean of 2 trays assessed*

Table 9 : Assessment of fresh and dry mean weight of seedlings (g) [1] in each treatment

Treatment	Fresh weight (g)		Dry weight (g)	
	½ Normal*	Normal*	½ Normal*	Normal*
Untreated control*	146.3[a]		29.4[a]	
Amistar	168.3[a]	149.1[a]	34.6[a]	29.6[a]
Dithane	144.4[a]	160.7[a]	29.5[a]	29.3[a]
Electis	164.2[a]	177.1[a]	32.7[a]	33.3[a]
Elvaron Multi	146.5[a]	125.5[a]	29.1[a]	26.4[a]
F516	178.8[a]	180.3[a]	34.2[a]	34.8[a]
Fubol Gold	160.6[a]	146.1[a]	33.1[a]	28.4[a]
Invader	186.5[a]	194.2[a]	33.6[a]	34.3[a]
Ranman TP	182.4[a]	160.8[a]	31.8[a]	27.6[a]
Shirlan	155.2[a]	153.0[a]	29.5[a]	30.0[a]
Tanos	172.4[a]	182.1[a]	31.6[a]	33.4[a]
LSD (P=0.05)	45.81	41.95	6.18	6.69
Standard Deviation	20.56	18.83	2.77	3.00
Coefficient of Variation	12.53	11.67	8.73	9.82

** Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*

[1] *All 345 seedlings/tray excised at compost level and weighed then oven dried and re-weighed.*

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(b) Rhizoctonia solani

Following incorporation of the *Rhizoctonia* fungus into the compost prior to sowing on 19 February it quickly became apparent that emergence of the Cauliflower seed cv. Mayflower was adversely affected, relative to the uninoculated control. Whilst the uninoculated control seedlings had emerged successfully by 4 March (97% emergence) germination and emergence of the inoculated control seedlings was reduced considerably (73% emergence) by comparison (Table 10). The most effective treatments, in terms of improved emergence, were Basilex (87% emergence) and Monceren (86% emergence). None of the other experimental fungicides improved emergence relative to the inoculated control and in several cases eg Switch, Charisma, Amistar, F516 & Biomex seedling emergence was worse than the inoculated control, though these differences were not, in all cases, statistically significant. Where the number of dead seedlings were counted post-emergence some 13% were lost in the inoculated control (assumes 100% emergence in the uninoculated '345' module trays). Interestingly, the only treatment to significantly reduce the number of dead plants at this stage was Amistar, yet this same fungicide appeared to adversely affect emergence relative to the control.

By 11 March emergence in the inoculated control and in some of the experimental plots had improved slightly (Table 11) though the overall results of repeat assessments were similar, in most respects, to that conducted on 4 March. Basilex continued to perform effectively (though note comments regarding the potential for insensitive/resistant strains of the fungus elsewhere in this report). Monceren whilst effective in the early stages failed to maintain its efficacy and a large proportion of the emerged seedlings subsequently died. None of the remaining experimental treatments provided effective control relative to the standard Basilex and a large no. of the seedlings in each treatment died as a direct result of *Rhizoctonia* infection.

Table 10 : Assessment of *Rhizoctonia* Infection on 4 March 2003

Treatment	No. of Seedlings Emerged [1]		Seedling Vigour (0-3) [1]		No. of Dead Seedlings[1]	
	½ Normal*	Normal*	½ Normal*	Normal*	½ Normal*	Normal*
Uninoculated Control	335.5 ^{a}	335.5 ^{a}	3.0 ^{a}	3.0 ^{a}	0.0 ^{f}	0.0 ^{c}
Inoculated Control	232.5 ^{abc}	232.3 ^{cd}	0.92 ^{c}	0.92 ^{c}	43.5 ^{abc}	43.5 ^{ab}
Amistar	235.0 ^{abc}	184.0 ^{e}	1.3 ^{bc}	1.0 ^{c}	31.5 ^{cd}	13.5 ^{c}
Basilex	304.0 ^{ab}	300.5 ^{b}	1.5 ^{b}	1.5 ^{b}	16.0 ^{e}	39.5 ^{ab}
Biomex	256.5 ^{abc}	215.5 ^{de}	1.0 ^{bc}	1.0 ^{c}	45.0 ^{abc}	59.5 ^{a}
Charisma	183.0 ^{c}	190.0 ^{e}	1.0 ^{bc}	0.8 ^{cd}	23.0 ^{de}	46.0 ^{ab}
F516	250.5 ^{abc}	201.0 ^{de}	1.0 ^{bc}	1.0 ^{c}	48.5 ^{abc}	39.0 ^{ab}
Monceren	240.0 ^{abc}	298.5 ^{b}	0.8 ^{c}	1.0 ^{c}	58.5 ^{a}	41.0 ^{ab}
Shirlan	262.0 ^{abc}	257.5 ^{c}	1.0 ^{bc}	1.0 ^{c}	36.0 ^{bcd}	36.5 ^{ab}
Switch	201.0 ^{bc}	147.5 ^{f}	1.0 ^{bc}	0.5 ^{d}	52.5 ^{ab}	30.0 ^{b}
LSD (P=0.05)	65.70	29.24	0.33	0.26	11.72	16.08
Standard Deviation	29.04	12.92	0.15	0.11	5.18	7.11
Coefficient of Variation	11.62	5.47	11.88	9.77	14.54	20.29

*****Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*

[1] *Based on a standard tray size of 345 cells (seedlings)*

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Table 11 : Assessment of *Rhizoctonia* on 11 March 2003

Treatment	No. of Seedlings Emerged[1]		Seedling Vigour (0-3) [1]		No. of Dead Seedlings[1]	
	½ Normal*	Normal*	½ Normal*	Normal*	½ Normal*	Normal*
Uninoculated Control	335.5 [a]	335.5 [a]	3.0 [a]	3.0 [a]	0.0 [e]	0.0 [d]
Inoculated Control	249.5 [a]	249.5 [abc]	1.7 [b]	1.7 [bc]	74.4 [b]	74.4 [abc]
Amistar	239.0 [a]	209.5 [abc]	1.5 [b]	1.8 [bc]	34.0 [d]	42.5 [c]
Basilex	308.5 [a]	312.0 [ab]	2.0 [b]	2.0 [b]	8.5 [e]	11.0 [d]
Biomex	266.0 [a]	239.5[abc]	1.8 [b]	1.0 [d]	61.0 [bc]	98.0 [a]
Charisma	205.5 [a]	204.0 [bc]	1.0 [bc]	1.0 [d]	54.5 [c]	72.5 [abc]
F516	266.0 [a]	229.0 [abc]	1.3 [bc]	1.0 [d]	71.0 [bc]	84.0 [ab]
Monceren	264.0 [a]	263.0 [abc]	1.5 [b]	2.0 [b]	91.5 [a]	59.0 [bc]
Shirlan	285.0 [a]	225.5 [abc]	1.8 [b]	1.3 [cd]	64.5 [bc]	72.5 [abc]
Switch	236.5 [a]	180.0 [c]	0.5 [c]	1.0 [d]	89.5 [a]	72.5 [abc]
LSD (P=0.05)	77.03	74.60	0.66	0.41	13.11	22.11
Standard Deviation	34.06	32.98	0.29	0.18	5.80	9.78
Coefficient of Variation	12.82	13.47	18.28	11.64	10.56	16.67

*****Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*
[1] Based on a standard tray size of 345 cells (seedlings)

(c) *Pythium* spp.

The initial replicated study in the series of efficacy trials was terminated prematurely after the first fungicide application as it became evident from monitoring the seedling roots that the introduced pathogen had not established in the trial area, even after repeated inoculation events. The precise reason for this is unclear, especially as earlier *in vivo* tests had demonstrated that the isolates were pathogenic on the various brassicas, including Cauliflower cv. Mayflower. It was, however, encouraging to note that a visual assessment for phytotoxicity symptoms 10 days after fungicide application was negative.

In order to secure some efficacy data against this sporadic, but occasionally troublesome, pathogen a further replicated study was designed and scheduled into the trials programme. A different brassica sp. ('cress' or oilseed rape) was used as the host plant and a different, highly pathogenic, *Pythium* sp. was used to inoculate the trial area. This host-pathogen combination allowed a more rapid trial to be conducted within a tight time-frame for inclusion of the results in this report. More importantly, it allowed decisions in time for the final stage (Phase III) of the project using a series of integrated programmes on a commercial nursery during Autumn-Winter 2003/4.

In this repeat study, the introduced *Pythium* sp. established very effectively and aerial myceliuim was clearly visible on the trays of 'cress' by 6 August, some 7 days after inoculation. An assessment of mycelial development showed a highly significant difference between the uninoculated control plots (10% mycelial cover[1]) and the inoculated control plots (55% mycelial cover). Several of the experimental treatments

[1] *This low level of mycelial cover in the uninoculated control plot suggests there may have been slight contamination by the introduced Pythium during routine operations in the trial area.*

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were effective in reducing mycelial development especially SL567A (0%), Shirlan (8%), Electis (8%) and F516 (9%). Amistar, Invader, Ranman TP & Tanos also provided a moderate suppression of mycelial development in this study (Table 12).

By 11 August, classic damping-off symptoms were apparent in the inoculated control plots and an assessment on this date showed that over 50% of the seedlings in the inoculated control plots were lost due to the disease (Table 13). This provided a stern test for the experimental fungicides under evaluation. SL567A provided an exceptional level of control of the introduced *Pythium* sp., though earlier comments regarding the potential for tolerant/resistant strains of this fungus are still valid. This means that effective control with this fungicide cannot necessarily be assured in all cases and a strategy utilising at least two products with contrasting modes of action would provide a better level of 'insurance' against control failure. As Electis, Invader, Ranman TP & Shirlan provided a good-moderate suppression of damping-off in this study they should be considered as possible alternative products for use in integrated disease control strategies in brassicas, subject to the necessary approval process.

Table 12 : Evaluation of Novel Fungicides for the Control of damping-off caused by *Pythium* spp. Assessment of mycelial development on 6 August.

Treatment	**% mycelial development[1]**	
	½ Normal*	**Normal***
Uninoculated control	10.0 [b]	10.0 [c]
Inoculated control	55.0 [a]	55.0 [ab]
Amistar	27.5 [ab]	20.0 [bc]
DP98	50.0 [ab]	45.0 [abc]
Electis	7.5 [b]	7.5 [c]
Filex	40.0 [ab]	62.5 [a]
F516	40.0 [ab]	8.5 [c]
Invader	30.0 [ab]	15.0 [bc]
Ranman TP	32.5 [ab]	27.5 [abc]
Shirlan	35.0 [ab]	7.5 [c]
SL567A	40.0 [ab]	0.0 [c]
Tanos	7.5 [b]	25.0 [abc]
LSD (P=0.05)	24.35	25.85
Standard Deviation	11.06	11.75
Coefficient of Variation	35.40	49.72

*****Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*

[1] *Assessment based on the actual percentage of the tray surface affected by mycelium of Pythium.*

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Table 13 : Evaluation of Novel Fungicides for the Control of damping-off caused by *Pythium* spp. Assessment of percentage damping-off on 11 August

Treatment	% damping-off	
	½ Normal*	Normal*
Uninoculated control	5.0 [c]	5.0 [c]
Inoculated control	50.0 [a]	50.0 [a]
Amistar	25.0 [bc]	42.5 [ab]
DP98	50.0 [a]	27.5 [abc]
Electis	25.0 [bc]	17.5 [bc]
Filex	32.5 [ab]	42.5 [ab]
F516	35.0 [ab]	35.0 [ab]
Invader	35.0 [ab]	20.0 [bc]
Ranman TP	17.5 [bc]	17.5 [bc]
Shirlan	32.5 [ab]	25.0 [abc]
SL567A	25.0 [bc]	5.0 [c]
Tanos	52.5 [a]	35.0 [ab]
LSD (P=0.05)	14.01	16.41
Standard Deviation	6.37	7.46
Coefficient of Variation	19.84	27.74

*****Means followed by the same letter do not differ significantly (P=0.05, Student-Newman-Keuls)*

(d) *Mycosphaerella brassicicola*

A fully replicated trial was undertaken with a number of strobilurin, triazole and other different mode of action fungicides to secure efficacy data against this air-borne (ascospore) and trash-borne pathogen. Unfortunately, a concerted effort to establish the disease during the time course of conventional brassica propagation was unsuccessful. As a result no efficacy data was generated against this target. However, the various fungicides were applied as per the original schedule and it is particularly encouraging to note that phytotoxicity symptoms were not evident on the young Cauliflower seedlings cv. Mayflower throughout the study.

(iii) Integrated Programmes

This phase of the work programme is scheduled to be undertaken during Autumn-Winter 2003/4 the results of which will be included in the final report for the project.

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Discussion

The initial aim of this project to screen a wide range of novel fungicides for crop safety and to generate a short-list of products for further performance or efficacy testing was thwarted by the surprising absence of gross phytotoxicity symptoms on any of the 4 brassica species evaluated, even at twice the normal (2N) rate of application. Whilst this presented a problem with respect to progressing to the next stage of the project it is a positive outcome for the industry as it potentially allows use of a wider range of fungicides on the crop during propagation than perhaps originally anticipated. It must be noted however, that in the initial crop safety studies the various products were applied as HV sprays at the cotyledon-1st true leaf stage. Some of the products evaluated, especially for the control of *Rhizoctonia* and/or *Pythium* may need to be applied earlier either as a compost incorporation or drench application post-sowing. This method of application, targeted at protection of the germinating seedlings, may have an effect on the relative crop safety of the applied products. If such use of a specific product is to be pursued further then it would be advisable to conduct follow-up tests to be assured of continued crop safety prior to widespread commercial adoption.

In the specific crop safety studies conducted, where seedling damage was recorded, it tended to be transient and the seedlings quickly grew away from the symptoms. Of course the study was undertaken during a single season at one UK site only and different climatic factors could potentially produce different results. For this reason, it is always advisable for growers to test treat a small batch of plants, preferably on a range of cultivars, when using a new product for the first time.

Due to the lack of differentiating factors a larger number of products than originally anticipated were taken forward to Phase II efficacy evaluations. This provided a further opportunity to evaluate crop safety under different climatic factors and, in some cases, using different application methods eg *Rhizoctonia* & *Pythium* studies.

In the initial *Pythium* study, whilst the pathogen inoculations were unsuccessful, an early application of the various products was applied as the seedlings were germinating (50% emergence). It is encouraging to note that at ½ N and 1N rates of application no visible differences between the treatments were present. In the *Rhizoctonia* study, the various products short-listed were applied even earlier (1 day post-sowing). Relative to the inoculated control some of the experimental products, most notably Switch, Charisma & Amistar did appear to suppress emergence and this may signify some form of phytotoxic reaction. It is recommended that this aspect is investigated more thoroughly in advance of any future SOLA applications, assuming promising candidate products can be identified and pursued successfully.

In the Phase II efficacy evaluation component of the project the most difficult challenge proved to be establishing infection with the respective pathogens and significantly more effort was devoted to this task than anticipated. This unfortunately delayed the original schedule for the project as the work was required to be done during as near-optimum conditions climatically as possible. It is important to note that climatic factors have a significant bearing on the infection process of many fungi, including those investigated in this project. The high temperatures experienced during 2003 were certainly less than conducive to optimum development of pathogens such

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as *P. parasitica* and *Pythium* spp. and this may account for some of the problems experienced during the time course of this project.

For the d. mildew evaluation it was fortunate that a temporary change to cool wet weather during May aided disease development though the ferocity of the ensuing infection was perhaps surprising. It certainly presented a formidable challenge for the fungicides being evaluated. Whilst a number of the products with moderate activity against *P. parasitica* were identified it is considered that they may perform more effectively under lower disease pressure as a component of an integrated strategy especially where they are applied prophylactically in advance of pathogen establishment. This aspect will be evaluated further in Phase III of the project where a series of integrated programmes will be compared against a standard commercial programme on a commercial propagation facility during Autumn-Winter 2003/4.

For the *Rhizoctonia* study the pathogen established well and created an excellent challenge by which to measure the performance of the various fungicides. With the exception of the standard organo-phosphate product Basilex and, to a lesser extent, Monceren no new promising active substances for *Rhizoctonia* control were identified. Given the recent restrictions imposed on the use of Basilex which prevents hand-held application (to ensure operator safety) and the anecdotal, but worrying, reports of crop failure and the threat of resistance development following its use in protected lettuce it is imperative that alternative effective fungicides are found to counter this aggressive pathogen. Monceren, whilst showing some control of *Rhizoctonia*, proved to be less effective than the standard product. It is reported elsewhere that this fungicide is less effective against some strains (anastomosis groups) of the fungus and the poorer response observed here may be a reflection of this. The strains of *R. solani* prevalent on Brassica species are not currently known though a new DEFRA funded project (HH3214TFV) is currently investigating this aspect. In this study, the problem of a visible residue from the current formulation of Monceren is likely to present a further problem. It is perhaps advisable therefore to look for further novel chemistry rather than trying to extend the use of this, albeit mediocre, product. In this regard, one new fungicide with reported activity against *Rhizoctonia* has been launched during 2003 as Rhino in the UK by Certis. Based on the novel active substance flutolanil, it is marketed as a seed tuber treatment in potato against black scurf caused by *R. solani*. Unfortunately, this product was not made available for the early screening phase of this project. However, the manufacturers are now eager to extend product approvals into other UK crops and vegetable propagation may be an area of interest. It is therefore recommended, due largely to the fact that no other promising candidates have been found, that this product is included in Phase III studies in Autumn 2003 to validate integrated programmes under commercial conditions.

Pythium spp. as pathogens of brassica seedlings in modules appears to be a relatively new phenomenon and is restricted to relatively few vegetable propagation facilities in the UK. The precise species of *Pythium* involved and the relative pathogenicity of each is not known. This aspect warrants independent investigation as fungi in this genus have the potential to be very damaging to young seedlings of many species, especially those raised intensively. It is recommended that a separate project be established aimed at determining the prevalence and pathogenicity of this genus in the various brassica spp raised commercially in intensive propagation facilities. Similar

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studies have already been undertaken successfully on both bedding plants (HDC PC 98a) and in hydroponic crops of tomato & cucumber (HDC PC 98b).

Like many other fungi, *Pythium* spp have the propensity to develop insensitivity (resistance) to fungicides, especially those regarded as single, as opposed to multi, -site inhibitors. In the study conducted here, the *Pythium* sp. finally used (originally isolated from the roots of sunflower), was successful in gaining data on fungicide efficacy. It proved to be highly sensitive to metalaxyl-M (mefanoxam) and, as a result, was highly effective in controlling the disease. Evidence elsewhere suggests that other *Pythium* spp. (or strains of the same species) reported as pathogenic on brassica species in propagation may be highly tolerant and/or resistant to the same fungicide. It is recommended therefore that as part of any future project on brassicas the relative sensitivity of *Pythium* spp. to oomycete fungicides is determined.

It was encouraging in the efficacy study reported that a number of alternative active substances, with contrasting modes of action, were also found to be moderately effective against the pathogen. It should therefore be possible to develop an integrated strategy using oomycete fungicides to effectively target root infecting oomycete fungi e.g. *Pythium* spp. and the air-borne d. mildew fungus *P. parasitica*. Incidental control of white blister caused by *Albugo candida* could also reasonably be expected from such a fungicide programme. It will be important to evaluate oomycete fungicides applied at different timings i.e. post-seeding and post-emergence to effectively target both pathogen groups.

Where *Mycosphaerella brassicicola* was introduced repeatedly the lack of infection was disappointing, though the precise reason for this is unclear. It is generally regarded that ascospores, released from old infected leaf and other plant debris, are primarily responsible for the mass occurrence of the disease in intensive brassica production areas (Dring, 1961). It must therefore be assumed that either the conditions for ascospore release from the applied debris (and plant material) was unfavourable, that conditions for spore germination and infection were unsuitable or that the selected host (Cauliflower cv. Mayflower) was tolerant to infection. Whatever the cause it meant that efficacy data with the various applied fungicides could not be gathered. Fortunately perhaps, whilst this disease is of considerable significance on commercial crops of B. sprout & cabbage in particular, it is a more cosmetic problem on Cauliflower and Calabrese (unless very severe) as generally the harvested product i.e. the curd is unaffected. In the production of brassica modules the problem tends to occur primarily in areas where commercial brassicas are grown intensively in the immediate vicinity e.g. Lincolnshire, and hence a high inoculum potential. It is largely regarded to be a cosmetic problem during propagation affecting the overall appearance of seedlings at point of sale/dispatch. However, occurrence of the pathogen so early in the production cycle could potentially exacerbate epidemic development of the disease on the more critical crops of B. sprout & cabbage & spring greens and should therefore be avoided wherever possible.

A final study (Phase III) conducted in Autumn-Winter 2003/4 will evaluate a series of integrated spray programmes on a commercial propagation facility in Lincolnshire. Here the study will rely on the natural invasion of fungal spores, as it is inappropriate to introduce aggressive pathogens onto commercial premises. From the results of this final study it is hoped that, with the assistance of the project co-ordinators, recommendations for approval of various products will be made.

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Whilst not within the scope of this project, it is imperative that the industry secures clarification regarding the Approval status of both existing and novel products used during propagation. This effort will need to clearly define what products can legally be used under protection and consider what the relationship is with respect to pre-and post-planting application timings on the different and, at times, complex array of brassica species grown. The pesticide review process, through EU Directive 91/414 has had the unfortunate consequence of highlighting various ambiguities with respect to On-Label authorisations, which, in effect, has severely restricted the legal use of crop protection products on brassica modules in protected cultivation.

To permit use under protection it is imperative that the manufacturers have demonstrated operator safety. Providing there is at least one protected crop use On-Label it is reasonable to assume that an operator safety data package exists and could therefore be accessed for the benefit of other uses, subject to the necessary agreement from the manufacturers.

The risk of residues at harvest of brassica crops is likely to be greatest on short-term crops where the product is applied closer to crop maturity rather than on the more conventional crops of B. sprout, cabbage, cauliflower etc. Therefore any studies should logically be conducted on crops with a short production period to demonstrate a 'worst case scenario'. For protectant products the residue risk from treating such crops during the propagation period is negligible because none of the leaves treated during this period will persist through to harvest. As the products are not mobile through the plant, i.e. they are not systemic; the residues cannot be transferred to the new growth. For systemic products the active substance, including potential metabolites, may be translocated through the plant and may potentially persist through to harvest. Here, residue data would be required to demonstrate consumer safety though again it would be logical to apply a 'worst case scenario' across the brassica group as a whole as any risk, relative to treatment of field crops closer to harvest, must be minimal.

A project proposal with the title:

*"To **Ex**amine **T**he **R**isk from **A**pproved **P**esticides applied in Propagation **O**nly on Residue **L**evels at harvest and potential for **A**mendments to the Long-**T**erm **E**xtension of Use Arrangements"*

aimed at addressing the issue of pesticide residues in propagation by extrapolation, was prepared by STC Ltd in conjunction with CSL and submitted to DEFRA in March 2002. The primary objectives of the study were to:-

i. To establish, develop and refine a series of residue models for transplanted crops in a broad generic study based on 'worst case' principles, to validate underlying assumptions regarding plant development and residue persistence and to demonstrate robustness of the models under a range of edaphic and climatic conditions
ii. To determine whether there are any inherent differences in pesticide deposition, distribution, metabolism and ultimately residue risk in a range of crop sub-species where plant habit is similar during propagation e.g. Brassicas such as cauliflower, cabbage, b. sprouts, calabrese and Chinese cabbage

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iii. To use the validated residue models as a tool to investigate opportunities for broad extrapolation of pesticide use during propagation of various crops via the Long Term Arrangements for Extension of Use

Unfortunately, however, whilst DEFRA has shown some interest in the proposal, to date, funds have not been made available for this work to be undertaken and the industry is therefore left in a difficult position with respect to crop protection measures on crops in propagation.

Conclusions

Gathering data in this project has proved much more difficult than originally anticipated, primarily because climatic factors during the time course of the study have not been particularly conducive to disease development. However, the following preliminary conclusions can be drawn from the work conducted to date:-

- Seedlings of Cauliflower, Calabrese, B. sprouts & Cabbage are surprisingly resilient to a broad range of pesticide application and the risk of crop damage (phytotoxicity) from application appears relatively small. This potentially widens the choice of products which can be used during propagation subject to efficacy and subsequent approval via the regulatory process.
- Several novel fungicides have been identified from the work to date with moderate activity against the key pathogen *P. parasitica*, cause of downy mildew, and these warrant further investigation as a component of integrated programmes under commercial conditions.
- No alternative effective products have been found to supplement or replace Basilex for the control of wirestem caused by *Rhizoctonia solani*. This continues to present a significant gap in the armoury, though the recent approval (on potato) of flutolanil (RhiNo) by Certis is worthy of further investigation as the specific active substance was not available at the commencement of the project.
- Several products were identified with moderate-good activity against *Pythium* and it is anticipated that incidental suppression and/or control of this disease could be achieved with fungicides applied primarily for d. mildew control.
- No efficacy data was gathered against the incidental pathogen *Mycosphaerella* though it is hoped that some information will be generated during the final phase of the project when a series of integrated fungicide programmes will be evaluated on a commercial propagation facility in Lincolnshire.
- The significant problem regarding the legal status of fungicide use in brassicas during propagation has been identified and highlighted in this report following discussion with the industry through the Plant Propagators Association and through involvement in the HDC GAP analysis initiative. This is now a highly significant issue as ‘harmonisation’ through EU Directive 91/414 impacts on the vegetable industry and further efforts must be made to clarify and improve the approval status of pesticides in this specialist sector of the industry. The UK Pesticide Safety Directorate must be encouraged to be more responsive to the needs of the industry in this respect and assist the industry in clarification of the issue.

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Technology Transfer

It is too early to provide much in the way of technology transfer from this project as use of any novel fungicides will require further work to secure On- or Off-Label Approval via the HDC-funded SOLA programme.

Perhaps the most important issue of relevance at this stage is that of the approval status of existing products used by propagators of Cauliflower, Cabbage, B. sprouts, Cabbage & other specialist 'edible' commodities. There is clearly considerable confusion in the regulatory position on specific crops and with specific products and it is possible that some growers are in breach of the current legal position. Vegetable propagators are urged to liase with colleagues, where possible through the Plant Propagators Association, to put pressure on the UK regulators (PSD) to ensure the industry is fully compliant with UK & EU legislation regarding pesticide use.

References

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Appendices

Appendix I : Experimental layout for each of the studies described

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Appendix 1: Example of the experimental layout for the crop safety and efficacy trials.

Inoc control	
	Uninoc control
	Inoc control
Uninoc control	

Each rectangle represents 1 '345' module propagation tray. All trays were arranged raised on wooden battens to assist air circulation and to prevent rooting into the substrate below.

Duplicate trays/chemical/rate were used throughout.

Trays in each particular experiment were randomised following the first spray application.

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