Project Title: Vegetable Propagation : Evaluation of Novel Fungicides for Disease Control in Brassica Transplants

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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.

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

- Novel integrated fungicide programmes evaluated in Spring 2004 were found to be as, or more, effective than current commercial practice.
- Several promising novel fungicides have been found with potential for improving disease control (and therefore plant quality) in module-raised brassicas and at the same time assisting in anti-resistance strategies to safeguard their future use.
- Where relevant, approval for products will be sought by HDC in liaison with the Plant Propagators Association.

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 continues to limit the available products still further. It is essential, therefore, that both 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 site-specific fungicides, e.g. the strobilurin analogues, are to be used commercially.

Diseases of primary concern during the production of module-raised brassicas 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 various novel fungicides including strobilurin analogues e.g. azoxystrobin or Amistar 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 information so generated could be used to develop effective integrated programmes to successfully target the various pathogens, which occur either persistently or sporadically in brassica transplants.

The identification of a range of novel fungicides for use on vegetable transplants would be a major benefit to propagators and growers, assuming Approvals can be secured. By reducing inoculum introduced into field crops it would delay epidemic development in the field and, in turn, this would 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 have been:-

- 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 efficacy against some of the key fungal pathogens which affect brassicas during the transplant period
- A 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

Summary of the Project and Main Conclusions

Crop Safety : The project has evaluated 25 novel fungicides alongside current industry standards for crop safety (phytotoxicity) against specific cultivars of cauliflower, calabrese, cabbage & Brussels sprouts using 1/2Normal, Normal (N) and 2xNormal rates of application in fully replicated experiments. 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. The original aim was to short-list products for efficacy testing based on their relative crop safety. However, as very few of the products evaluated caused appreciable damage this was not possible. Instead, fungicides were selected, where possible, based on previous 'intelligence' following their use on other crops, data sourced from the scientific literature and on information gleaned from the manufacturers.

Efficacy Testing: A selection of 10-12 fungicides were selected to evaluate against each of 4 key target pathogens on brassica seedlings in fully replicated studies. The key targets were downy mildew (*Peronospora parasitica*) and wirestem (*Rhizoctonia solani*), though damping-off (*Pythium* spp.) and ringspot (*Mycosphaerella brassicae*) were also included in an attempt to gather additional data for the industry against these sporadic pathogens. Each fungicide was applied at ½ Normal and Normal (N) rates of application.

(a) P. parasitica was established on Cauliflower cv. Mayflower using a series of 'infector' plants sourced from a commercial crop. Disease pressure was exceptionally high in the trial crop and this provided a stern test for the experimental fungicides evaluated. Several fungicides including Ranman Twinpack, Tanos, Invader, Dithane & Shirlan proved to be moderately effective against the disease though none provided total control, even at full rates of application. However, it is considered that under commercial conditions where the entire crop is likely to be fungicide treated (i.e. no untreated controls) the actual disease pressure would be significantly lower than that experienced in the trial crop and therefore overall control ought to be improved. One or more of these products should be considered for On- or Off-Label approval on module-raised brassicas.

(b) Rhizoctonia solani was established on Cauliflower cv. Mayflower by incorporating the pathogen, raised in vermiculite culture, into the compost prior to tray filling and seeding. The pathogen successfully established on the emerging seedlings in the untreated control and this method presented an effective challenge for the fungicides being evaluated. Disease pressure was high and emergence of seedlings was significantly reduced in the inoculated control. 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 may require further investigation. Pencycuron (Monceren) which is approved for *Rhizoctonia* control in potato as a seed (tuber) treatment gave disappointing results in this study. It is considered that this may relate to the specific strain of *R. solani* used for artificial inoculation though this was not

investigated further at this stage. 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 urgently 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. The successful approval of Rhino (flutolanil) for use against *R. solani* on potatoes potentially provides a new opportunity for control of the same pathogen in other horticultural crops, including brassicas. Whilst no evidence of adverse reactions to its use in propagation (following its first use in the integrated programmes) were noted no efficacy data was generated specifically in this study due to the absence of wirestem in the trial. It is recommended that further work is initiated to validate the relative performance of flutolanil, azoxystrobin on comparison to the industry standard fungicide tolclofos-methyl (Basilex).

(c) A *Pythium* sp. was initially established in Cauliflower cv. Mayflower though unfortunately, it failed to infect the roots to produce visible symptoms of disease eg damping-off. This was in spite of earlier tests on this cv., which demonstrated pathogenicity. The experiment was therefore repeated 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 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. They could potentially be used as part of an integrated programme utilising different fungicides with contrasting modes of action to prevent control failure due to the development of resistant strains of the fungus. The apparent absence of a natural *Pythium* infection during the integrated study on a commercial nursery means that further performance data against this pathogen was not secured. However, where candidate products with known oomycete activity were applied no adverse crop safety reactions were observed and this is encouraging. It is recommended therefore, that where novel products are being considered for approval for d. mildew control consideration is also given to their potential application as compost drenches rather than just foliar sprays as this is likely to assist in minimising pre-and post-emergent damping-off by *Pythium* spp. The mode of action (systemic or otherwise) of the candidate products will be important in this regard if effective d. mildew control is to be achieved with compost drenches.

(d) Mycosphaerella brassicicola was introduced into an experimental crop area of Cauliflower cv. Mayflower using infected, but dried, leaf debris, courtesy of Dr Roy Kennedy, HRI, Wellesbourne. Unfortunately, any spores liberated from the debris failed to infect the young plants and no lesions of ringspot developed in the untreated control plots. Further attempts at securing infection using fresh leaf debris and young infected plant material were also unsuccessful in establishing the disease in the trial area. No explanation can be found to account for the non-establishment of the pathogen on this occasion though it was presumed to be climatic factors at the time of the experiment rather than failure of the

inoculum itself or resistance in the host. No results for efficacy of the various products against this leaf pathogen were therefore gained during the initial phase of the study and leaf-spot infections in the final integrated programmes remained at negligible levels in the glasshouse and therefore further efficacy data was not generated.

Integrated Spray Programmes As a concluding component of this project a series of integrated, but experimental, spray programmes were formulated in conjunction with the Plant Propagators Association and Project Co-ordinator and evaluated alongside an untreated control and a standard commercial programme. The aim was to use a commercial module raised crop during a period when disease pressure, especially d. mildew, was likely to be high with spray programmes formulated to compare crop safety and efficacy of the various fungicides against a broad range of pathogens that may occur commercially during propagation e.g. downy mildew, ring spot, wirestem and damping-off caused by *Pythium* spp. The programmes were based primarily on a pre-emergence drench application followed by 3 foliar applications.

In the period when this final programme was carried out the only pathogen to develop at appreciable levels on the commercial trial site was downy mildew and a moderate-severe infection spread rapidly through all the trial plots to provide a relatively stern test for the fungicide programmes. All of the experimental integrated programmes performed as well, if not better than the standard commercial programme in controlling the d. mildew, although none eradicated it totally. No phytotoxic effects were observed following application of what were fairly intensive fungicide programmes. From this final study it is possible to conclude that there is considerable scope for improving the availability of fungicides for use on brassicas during propagation.

Financial Benefits

The project, which was designed to identify novel fungicides for disease control, has been successful though it is important now that the most effective (and safe) products identified are put forward for further studies via the HDC SOLA programme or via On-Label Approval to ensure a firm legal basis for product use.

There are currently no financial benefits to be gained by propagators and growers from this work. However, if any of the promising candidate fungicides are approved in the future this should benefit the industry by improving plant quality and reducing losses.

Action Points for Growers

- Be aware that many of the fungicides 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 in the UK at the current time.
- Propagators should review their current strategy for disease control in brassica modules and, on the basis of the work reported, and especially the final integrated study, consider the need for alternative fungicides to improve overall disease control in their crops.
- 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 should monitor crops for wirestem caused by *R. solani*. If symptoms of the disease are found, especially where Basilex has been applied, submit a sample for resistance testing to tolclofos-methyl.
- Keep abreast of other work on *Rhizoctonia*, especially the current DEFRA funded projects as early indications from this work suggest that the risk from this pathogen following the move to production in module trays is much reduced and there may be scope for reducing fungicide inputs in this area, assuming appropriate detection & monitoring techniques for the pathogen are available.
- Propagators would be advised to liase closely with their growers to agree effective crop protection programmes in advance of production as this would help minimise future problems, especially relating to fungicide resistance.
- Propagators and growers need to be aware of the impending changes (the phasing out) of the Long-Term Arrangements for Extension of Use which will potentially impact on this sector of the industry, and will need to liase with HDC.
- The brassica industry, via the Plant Propagators Association should re-open dialogue with HDC to investigate the possibility (assuming On-Label Approval is unlikely) of securing a number of new Specific Off-Label Approvals, where necessary through provision of residues data, for novel fungicides on module-raised brassicas.

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 'disease-free' transplants ought therefore to be considered highly beneficial.

Currently, brassica propagators rely largely 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 the 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 an increasingly limited range of chemicals is likely to further 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 have been 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 to recommend their optimum use in integrated disease control programmes to establish industry 'best practice'.

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.*

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.

(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.

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

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 (formerly at Warwick-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 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 again applied 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, Warwick-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

(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.**

Experiment 1

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

Experiment 2

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	
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 was undertaken in Spring 2004 on a commercial propagation facility in Lincolnshire. The primary aim was be to select the most effective products identified in the initial stages of the project for incorporation into a series of integrated fungicide programmes alongside an untreated control and a commercial standard programme. The performance of the various fungicide programmes against the predominant pathogens, which naturally occurred on the nursery, was assessed in a series of replicated trial plots.

This work, originally scheduled for Autumn/Winter 2003/4 was postponed following consultation with the Project Co-ordinator, Mr Roger White. The hot dry summer of 2003 had led to an unusually low level of many foliar and root diseases throughout the horticultural industry and this was particularly pertinent in respect of downy mildew infection. Following regular consultations with Roger White, and with the agreement of the HDC, it was therefore agreed to postpone the work until the spring of 2004 when environmental conditions were more conducive to disease development.

A short-list of candidate fungicides was compiled using data generated in the earlier crop safety and efficacy studies, but also included additional new products recently made available. The final short-list of fungicides and proposed drench/spray schedule was agreed in advance of the trial with the Plant Propagators Association. Following a routine visit to the proposed Lincolnshire trial site in Spring 2004 low levels of d. mildew were observed on seedlings. The weather was conducive to disease development and a decision made to commence the final 'commercial' evaluation in this project.

Table 4 : Candidate fungicides selected for the evaluation of an integrated spray programme.

1st Application (post- sowing)	2nd Application (cotyledon stage)	3rd Application (7-10 days later)	4th Application (7-10 days later)
1. Basilex + Aliette*	Filex	Aliette	Aliette
2. Rhino	Filex	Aliette	Aliette
3. Amistar	Filex	Aliette	Aliette
4. Basilex	Amistar	Amistar	Ranman Twinpack
5. Basilex	Amistar	Invader	Ranman Twinpack
6. Basilex	Bravo	Amistar	Invader
7. Sl567A + Basilex	Amistar + Plover	Amistar + Plover	Ranman Twinpack
8. Sl567A + Rhino	Signum	Signum	Ranman Twinpack
9. Amistar + Rhino	Ranman Twinpack	Invader	Tanos
10. Amistar + Rhino	Fubol Gold + Plover	Signum	Invader
11. Biomax$	Biomax	Biomax	Biomax
12. Untreated Control	-	-	-

* Standard Commercial practice. $ Additional treatment requested by Plant Propagators Association.

Trial Design

Untreated Cauliflower cv. Fargo was sown using fully automated commercial equipment using conventional compost and '345' module trays. Four replicate trays/treatment were used, with each 'plot' consisting of a single tray. The first application was carried out 1 day post-sowing. The trial was randomised following the first treatment (see trial plan in Appendix 2). The second application was applied as soon as the seedlings had reached fully expanded cotyledon stage, with the last two applications made at 7-10 day intervals thereafter. All sprays were applied using an Oxford Precision Sprayer with a 3-nozzle boom attachment and operated at constant 2 Bar pressure. The products were applied using a fine nozzle (BCPC code F80/0.40/3 – Orange). The first application was applied as a drench in 500l water/ha, whilst subsequent foliage applications were made using 250l water/ha. Details of the spray timings are presented in the appropriate crop diary.

Specific pathogen inoculation procedures were not deployed in the trial (this was considered unacceptable on a commercial site) and instead considerable effort was made to undertake the trial in a commercial glasshouse in an intensive production area where other brassica modules were being raised at a time when natural disease pressure would be relatively high.

Disease assessments were carried out at regular intervals to record the incidence of any pathogens. Downy mildew predominated and unfortunately other brassica pathogens were either absent or remained at negligible levels during the study.

The following 0-3 Severity score was used to score downy mildew on the 30 April:

0 – No downy mildew present
1 - <10% of leaf area affected/plant
2 – 11-50% of leaf area affected/plant
3 - >50% of leaf area affected/plant

For statistical purposes the scales used for assessments (either 0-3, or 0-5) have been converted to a 0-100 severity scale using the following formula:

$$\frac{1(1)+2(2)+3(3)}{\text{No. of samples}} \times \frac{100}{3}$$

At the second disease assessment carried out on the 10 May the same 0-3 severity scale shown above was used, however the assessment focussed on the 1st and 2nd true leaves only. An overall tray severity score for downy mildew was also recorded using the following severity scale.

0 – No downy mildew
1 – Cotyledons still attached, low levels of chlorosis and limited infection on true leaves.
2 – Cotyledons still attached, but yellow, all true leaves affected with d. mildew.
3 – Cotyledons dead, plants all infected

A vigour score was also assigned to each tray using the following scale

0 – All plants dead
1 – Plants poor, distorted leaves, necrosis present
2 – Cotyledons mostly lost, plants smaller, vigour reduced
3 – Cotyledons present, true leaves growing well, colour good, vigour good.

The final disease assessment was carried out on the 19 May 2004. An assessment of d. mildew levels on the 1st and 2nd true leaves was carried out using the assessment scale shown above. An overall tray score using the scale shown below was also recorded:

0 - No d. mildew present on tray
1 – 1st true leaves only affected, older leaves unaffected
2 – 1st and 2nd true leaves affected with d. mildew, older leaves unaffected
3 – All 1st, 2nd and 3rd true leaves affected

Following the final disease assessment, 25 plants/tray were excised at compost level and returned to the laboratory to record fresh and dry weights.

During each assessment the plants were visually inspected for any sign of phytotoxic symptoms such as scorching, stunting or twisting. Details of any such symptoms, where present, were recorded and photographed.

Crop Diary

6 April 2004	48 trays of cauliflower seed cv. Fargo sown
7 April 2004	1st Application of fungicides
21 April 2004	2nd Application of fungicides
30 April 2004	Disease assessment carried out.
30 April 2004	3rd Application of fungicides
10 May 2004	Disease and vigour assessment carried out
10 May 2004	4th Application of fungicides
19 May 2004	Final disease assessment carried out, trial terminated.

Statistical Analysis

Where appropriate data collected from each phase of the project was subjected to statistical analysis using ARM 7 trial management software (Gylling Data Management Inc).

Quality Assurance

The study described was undertaken in accordance with the guidelines for Official Recognition of Efficacy Testing Organisations.

Certificate No.: ORETO 110
Date of Issue : 3 May 2001 Expiry Date : 31 March 2006

Results

(i) Crop Safety or Phytotoxicity Screen (2002-2003)

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 the study 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).

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

Surprisingly perhaps, but none of the symptoms observed during the dual-phased phytotoxicity study were considered sufficiently severe 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 (2003)

(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 though 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 as high or 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 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 gave 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 at this assessment stage 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 appeared to increase 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

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*

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.*

(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% of seedlings 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 that there is anecdotal observations in other crops e.g. lettuce to suggest that insensitive and/or resistant strains of the fungus may be present in the pathogen population). 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 evaluated 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)*

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 seasonal time-frame. 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.

In this repeat study, the introduced *Pythium* sp. established very effectively and aerial mycelium was clearly visible on the trays of seedlings 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 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 disease control failure. As Invader, Ranman TP, Electis & Shirlan also provided a good-moderate suppression of damping-off in this study they were taken forward for consideration as possible alternative products for use in integrated disease control strategies in brassicas. Ultimately it was not possible to include them all in the final integrated programmes and for a variety of reasons only the former two products, in conjunction with SL567A, were evaluated in the final commercial study.

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.

[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.*

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 following application of the various fungicides throughout the study.

(iii) Integrated Programmes (2004)

The integrated programmes phase of this work was delayed but finally carried out during Spring 2004 on a commercial propagation nursery in Lincolnshire when environmental conditions and disease (inoculum) pressure were judged to warrant the trial commencing. The work had been originally scheduled for completion during Autumn/Winter 2003 but was postponed on the advice of the Plant Propagators Association due to unusually low disease pressure following the hot dry summer of 2003.

Fungicides used in the integrated programmes were chosen as a result of earlier work carried out in Phases I and II of this project, along with other products recently introduced onto the market that had not been evaluated in the earlier 'screening' studies.

The trial plots were located in a commercial Brassica propagation glasshouse where natural disease pressure was present as artificial introduction by inoculation of specific pathogens was not deemed appropriate. The trial area was monitored and assessed for a range of propagation pathogens e.g. d. mildew, *Rhizoctonia* wire stem, *Pythium* damping-off, and leaf spots such as *Mycosphaerella brassicicola* (ring spot) or *Alternaria*.

Germination of the seedlings was extremely good and no evidence of damping-off was observed in the experimental plots. The integrated spray programmes were initiated as pre-germination drenches followed by 3 foliar applications post-germination to mirror current commercial practice. Infection of the seedlings in the untreated control plots with d. mildew was first seen on the 30 April and a detailed disease assessment was conducted at this time (Table 14).

Table 14. Evaluation of Integrated Spray Programmes in Brassica Modules - An assessment of d. mildew - 30 April 2004.

Treatment	**D. mildew index (0-100)**	**% Control (relative to the untreated)**
1. Basilex+Aliette/Filex/Aliette/Aliette	4.17[b]	91.5
2. Rhino/Filex/Aliette/Aliette	8.33[b]	83.1
3.Amistar/Filex/Aliette/Aliette	0.83[b]	98.3
4. Basilex/Amistar/Amistar/Ranman TP	10.00[b]	79.7
5. Basilex/Amistar/Invader/Ranman TP	23.33[b]	52.6
6. Basilex/Bravo/Amistar/Invader	20.83[b]	57.6
7. SL567+Basilex/Amistar+Plover/ Amistar+Plover/Ranman TP	15.00[b]	69.5
8.SL567+Rhino/Signum/Signum/Ranman TP	10.83[b]	78.0
9. Amistar+Rhino/Ranman TP/ Invader/Tanos	20.00[b]	59.3
10. Amistar+Rhino/Fubol Gold+Plover/ Signum/Invader	3.33[b]	93.2
11. Biomax/Biomax/Biomax/Biomax	14.17[b]	71.2
12 Untreated control	49.17[a]	-
LSD (P = 0.05)	16.25	-
Standard Deviation	11.25	-
Coefficient of Variation	75.03	-

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

All the fungicide programmes resulted in a significant reduction in the incidence of d. mildew relative to the untreated control. Very low levels of d. mildew were observed

with treatment programmes1, 3 & 10 in particular and these all provided over 90% disease suppression. No other pathogens were recorded at this assessment date.

A second disease assessment was carried out on the 10 May. Ten plants/tray were assessed for the severity of d. mildew on the 1st and 2nd true leaf. An overall tray severity score for d. mildew was recorded and also a vigour score (Table 15).

Table 15. Evaluation of Integrated Spray Programmes. Assessment of d. mildew and vigour carried out on 10 May 2004.

Treatment	D. mildew severity on 1st TL (0-100 index)	D. mildew severity on 2nd TL (0-100 index)	Overall Plot score (0-100 index)	Vigour (0-100 index)
1. Basilex+Aliette/Filex/Aliette/Aliette	50.8^{c}	25.8^{bc}	41.7^{c}	91.7^{a}
2. Rhino/Filex/Aliette/Aliette	56.7^{c}	28.3^{bc}	62.5^{bc}	83.3^{a}
3.Amistar/Filex/Aliette/Aliette	47.5^{c}	19.2^{c}	41.7^{c}	75.0^{ab}
4. Basilex/Amistar/Amistar/Ranman TP	60.8^{bc}	25.0^{bc}	54.2^{bc}	75.0^{ab}
5. Basilex/Amistar/Invader/Ranman TP	76.7^{ab}	38.3^{ab}	76.3^{ab}	66.7^{ab}
6. Basilex/Bravo/Amistar/Invader	79.2^{ab}	42.5^{ab}	91.7^{a}	50.0^{bc}
7. SL567+Basilex/Amistar+Plover/ Amistar+Plover/Ranman TP	46.7^{c}	27.5^{bc}	41.7^{c}	91.7^{a}
8.SL567+Rhino/Signum/Signum/Ranman TP	53.3^{c}	29.2^{bc}	41.7^{c}	83.3^{a}
9. Amistar+Rhino/Ranman TP/ Invader/Tanos	68.3^{abc}	35.8^{bc}	58.3^{bc}	83.3^{a}
10. Amistar+Rhino/Fubol Gold+Plover/ Signum/Invader	56.7^{c}	30.0^{bc}	50.0^{c}	100.0^{a}
11. Biomax/Biomax/Biomax/Biomax	80.8^{a}	40.8^{ab}	100.0^{a}	33.3^{c}
12 Untreated control	84.2^{a}	52.5^{a}	100.0^{a}	33.3^{c}
LSD (P = 0.05)	13.99	10.97	18.06	21.36
Standard Deviation	9.69	7.60	12.51	14.79
Coefficient of Variation	15.27	23.09	19.76	20.48

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

By this second disease assessment the levels of d. mildew had increased dramatically. The severity of the disease was higher on the 1st true leaf than on the second and, in both instances, the highest disease severity was on the untreated control plots. Most of the fungicide programmes significantly reduced the incidence and severity of d. mildew relative to the untreated control (T12). Only treatments 5, 6, 9 (1st true leaf only) & 11 performed poorly at this stage. An important effect of some of the more successful spray programmes was the retention of the cotyledons. Although these were infected with d. mildew in all treatment regimes, the treatments in many cases did control the infection enough to allow them to remain on the plants, albeit in a chlorosed state. Figure 1 (overleaf) demonstrates the retention of the yellow cotyledons on the seedling from Treatment 10, the cotyledons are absent on the untreated seedlings.

Figure 1. Comparison of untreated seedling and an integrated spray programme (T10) with respect to retention of cotyledons.

The final disease assessment was carried out on the 19 May. Many of the plants had reached the 5 true leaf stage, and it was evident that as the plants matured the level of d. mildew on the newly produced leaves was very low. The plants were becoming more able to withstand leaf infection by *P. parasitica* as they became older and there are a no. of possible explanations for this. During this final assessment the d. mildew infection level on the 2nd and 3rd true leaves was assessed and an overall disease severity score per tray was given.

Table 16. Evaluation of Integrated Spray Programmes. Assessment of d. mildew and plant vigour carried out on 19 May 2004.

Treatment	D. mildew severity on 2nd TL (0-100 index)	D. mildew severity on 3rd TL (0-100 index)	Overall Plot score for d. mildew (0-100 index)
1. Basilex+Aliette/Filex/Aliette/Aliette	45.8^{ab}	12.5^{ab}	75.0^{a}
2. Rhino/Filex/Aliette/Aliette	49.2^{ab}	15.0^{ab}	83.3^{a}
3.Amistar/Filex/Aliette/Aliette	52.5^{ab}	19.2^{a}	87.5^{a}
4. Basilex/Amistar/Amistar/Ranman TP	35.8^{ab}	1.7^{b}	70.8^{a}
5. Basilex/Amistar/Invader/Ranman TP	36.7^{ab}	6.7^{ab}	70.8^{a}
6. Basilex/Bravo/Amistar/Invader	44.2^{ab}	2.5^{b}	70.8^{a}
7. SL567+Basilex/Amistar+Plover/ Amistar+Plover/Ranman TP	40.8^{ab}	5.8^{ab}	75.0^{a}
8.SL567+Rhino/Signum/Signum/Ranman TP	30.3^{b}	0^{b}	58.3^{a}
9. Amistar+Rhino/Ranman TP/ Invader/Tanos	40.8^{ab}	5.8^{ab}	79.2^{a}
10. Amistar+Rhino/Fubol Gold+Plover/ Signum/Invader	38.3^{ab}	5.8^{ab}	66.7^{a}
11. Biomax/Biomax/Biomax/Biomax	58.3^{a}	7.5^{ab}	87.5^{a}
12 Untreated control	59.2^{a}	5.8^{ab}	79.2^{a}
LSD (P = 0.05)	15.66	9.59	17.20
Standard Deviation	10.84	6.64	11.91
Coefficient of Variation	24.48	90.19	15.81

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

This final assessment was undertaken 9 days after the final spray application. Surprisingly perhaps only one treatment (T8) resulted in significantly lower levels of disease on the 2nd true leaf as compared to the unsprayed control. However, treatments 4, 6 and 8 did provide generally good control of the infection and limited movement up onto the 3rd true leaf in the crop. Generally disease levels were lower on the 3rd true leaf than the 2nd, although somewhat surprisingly, treatments 1, 2 and 3, all of which received Aliette in the last two spray applications resulted in the highest levels of infection on the 3rd true leaf and this suggests that such repeat treatment with the same fungicide is not good practice. Where alternative fungicides were used in an integrated strategy e.g. T8 control of the disease was much improved. The results for the overall tray scores for this assessment tended to 'mirror' the individual leaf disease assessment though no significant differences were observed.

Following the final disease assessment 25 plants/tray were excised at compost level and returned to the laboratory to measure the fresh and dry weights (Table 17).

Table 17. Evaluation of Integrated Spray Programmes. Measurement of fresh and dried weights of seedlings on 20 May 2004.

Treatment	Mean fresh weight/plot (g)	Mean dried weight/plot (g)	Mean fresh wt (as a % of control)	Mean dry wt (as a % of control)
1. Basilex+Aliette/Filex/Aliette/Aliette	23.9^{ab}	3.4^{ab}	108.3^{ab}	112.2^{ab}
2. Rhino/Filex/Aliette/Aliette	24.7^{ab}	3.3^{ab}	111.8^{ab}	111.2^{ab}
3.Amistar/Filex/Aliette/Aliette	24.5^{ab}	3.4^{ab}	111.0^{ab}	113.9^{ab}
4. Basilex/Amistar/Amistar/Ranman TP	23.5^{ab}	3.3^{ab}	106.3^{ab}	109.1^{ab}
5. Basilex/Amistar/Invader/Ranman TP	22.4^{ab}	3.1^{ab}	101.5^{ab}	101.9^{ab}
6. Basilex/Bravo/Amistar/Invader	22.1^{ab}	3.0^{ab}	100.1^{ab}	100.2^{ab}
7. SL567+Basilex/Amistar+Plover/ Amistar+Plover/Ranman TP	25.6^{a}	3.6^{a}	116.2^{a}	119.7^{a}
8.SL567+Rhino/Signum/Signum/Ranman TP	23.6^{ab}	3.4^{ab}	107.1^{ab}	112.8^{ab}
9. Amistar+Rhino/Ranman TP/ Invader/Tanos	24.1^{ab}	3.4^{ab}	109.1^{ab}	112.4^{ab}
10. Amistar+Rhino/Fubol Gold+Plover/ Signum/Invader	26.3^{a}	3.7^{a}	119.4^{a}	124.8^{a}
11. Biomax/Biomax/Biomax/Biomax	19.7^{b}	2.8^{b}	89.4^{b}	92.1^{b}
12 Untreated control	22.1^{ab}	3.0^{ab}	100^{ab}	100^{ab}
LSD (P = 0.05)	3.23	0.47	14.65	15.49
Standard Deviation	2.24	0.32	10.15	10.73
Coefficient of Variation	9.51	9.83	9.51	9.83

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

The fresh and dry weight analyses of the seedlings correlates fairly well with the overall incidence of d. mildew in the experiment. Those treatments providing the most effective control of the disease e.g. T10 providing the highest fresh and dry weight analysis. Whilst those providing the poorest disease control e.g. T11 (Biomax) tended to have the lowest fresh & dry weights. Unfortunately, variability between the different replicates was too high and the differences measured and tabulated here were not significantly different (P=0.05).

No other pathogens were recorded on the trial plots throughout the period of the trial.

Finally, and encouragingly, there was no evidence or symptoms of phytotoxicity following application of any of the integrated spray programmes applied under commercial conditions in this final study.

Discussion

The initial aim of this project (Phase I) was to screen a wide range of novel fungicides for crop safety and, on this basis, to generate a short-list of products for further performance or efficacy testing. However, this was thwarted by the surprising absence of gross phytotoxicity symptoms on any of the 4 brassica species (Cauliflower, Calabrese, Cabbage & Brussels sprouts) 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 only one cultivar of each brassica species was included and specific varietal reactions may occur. This would need to be considered prior to widespread use, subject to the appropriate regulatory approval. Also, it must be considered 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 a different effect on the relative crop safety of the applied products. It is encouraging however that in the final integrated programmes where a selection of first choice products were applied as drench applications pre-emergent no adverse phytotoxic reactions were observed. Yet. if such use of specific products is to be pursued further then it would still 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 reported, 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 into the Phase II efficacy evaluations. This provided further opportunity to evaluate crop safety under different climatic factors and, in some cases, using different application methods e.g. drench applications for *Rhizoctonia* & *Pythium* control.

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 applied in this experiment, 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 efficacy evaluation component of the project (Phase II) the most difficult challenge proved to be establishing infection with the respective pathogens and significantly more effort was devoted to this task than originally 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 as *P. parasitica* and *Pythium* spp. and this may account for some of the difficulties 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 severity 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 likely to be experienced in commercial propagation nurseries; especially where they are applied prophylactically in advance of pathogen establishment as a component of an integrated strategy. This aspect was further evaluated in Phase III of the project at which time a series of integrated programmes were compared against a standard commercial programme on a commercial propagation facility.

For the *Rhizoctonia* study conducted in Phase II of the project 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 have not been studied in detail previously though a new DEFRA funded project (HH3214TFV) conducted by CSL in conjunction with STC and the brassica industry is currently investigating this aspect.

In the same Phase II 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 product. In this regard, one new fungicide with reported activity against *Rhizoctonia* was 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 included in the early stages of the project as information relating to its potential activity against

R. solani was not widely available due to commercial confidentiality. However, good activity against *R. solani* has now been demonstrated in other horticultural crops (McPherson, pers comm) and the manufacturers are looking to potentially extend the current approval from potatoes and vegetable propagation may be an area of interest. Due largely to the fact that no other candidate products were found in the early screening stages of the project flutolanil (Rhino) was included in the integrated Phase III studies aimed at validating fungicide programmes under commercial conditions in Spring 2004. Unfortunately the absence of *Rhizoctonia* in this final evaluation means that efficacy data on brassica modules is lacking and it is recommended that further work be undertaken in this respect. On a more positive note, there appeared to be no phytotoxic reactions following its application to brassica seedlings in this experiment.

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 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, this product (SL567A) 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 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. Finally it is important to consider that, because of the need to target both root and leaf infecting oomycete fungi, the application method and timing of application of the various products may be important.

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.

The final study (Phase III), which was conducted after a slight delay, in Spring 2004 evaluated a series of integrated spray programmes for their efficacy in controlling the predominant root and foliar pathogens that occur in module-raised brassicas. The study was conducted on a commercial propagation facility in Lincolnshire where typical disease pressure was present and pathogens were not artificially introduced into the trial area.

No evidence of the 'damping-off' pathogens *Pythium* spp. or *R. solani* was seen during this trial period. As discussed previously, *Pythium* spp. is a relatively new phenomenon, and seems to be restricted to low numbers of propagators at this time. The majority of propagators are well aware of the risks to young seedlings from this pathogen and maintain high levels of hygiene involving tray washing and glasshouse cleanliness to inhibit the spread of this organism. A similar picture has been seen with *Rhizoctonia solani* in propagation. Initial unpublished results from a current DEFRA study (HH3214TFV) indicates that *R. solani* is seldom found in propagation. There are a no. of reasons for this including a switch to modular production of plants, the move out of the soil reducing the risk from this soil-borne pathogen; the use of module trays themselves which, in comparison to a seed-bed, provides a physical barrier between the individual plants to prevent lateral spread of the pathogen through the growing medium and the increased diligence regarding nursery hygiene which further reduces inoculum pressure more generally. The tray cleaning aspect of this process is of particular importance with *R. solani* as plants are supplied to growers in the modular trays. These trays are used with mechanical planters in the field and then stacked at the side of the field prior to being returned to the propagator. During this time they can easily become infected with propagules of *R. solani* (or *Pythium* spp.) from blown or rain splashed dust or soil particles. The trays used in this trial had been processed and treated in the same fashion as other trays used by this propagator.

No leaf spot pathogens developed in the trial area, or on other plants in the glasshouse during the time of this trial. Low levels of ring spot (*Mycosphaerella brassicicola*) had been seen at the propagation facility immediately prior to commencement of the trial, but either inoculum levels had declined by the time this trial was *in situ* or

alternatively the environmental conditions were not conducive to infection and symptom expression at this time.

A moderate-severe infection of *P. parasitica* (d. mildew) did infect the plots in the trial area and a significant reduction of the disease was seen with all the treatments following the first two applications of the spray programmes. A number of the integrated programmes (T1, 2, 3 and 10) had a marked effect in reducing the severity of the infection at that time. Interestingly treatment programmes 1, 2 and 3 all seemed to be reducing the mildew well until the final assessment (which was carried out 9 days after the last fungicide application) when they were out-performed by other programmes e.g. programmes 4, 7, 8 and 9.

It was noted that the disease progression onto the new seedling leaves slowed down by the time the plants reached the 3-4th true leaf stage in all of the treatments including in the untreated control trays. This is relatively standard for this disease under commercial conditions (Mr R White, pers. comm.) and can potentially be explained by a number of factors including the development of systemic acquired resistance, an increased cuticle thickness and maturation of the waxy leaf surface and general plant resistance with increased age. However, in situations where the initial infection is very severe, young unprotected seedlings may not survive, or their development may be suppressed, and this can increase the time that these plants may have to remain in propagation before reaching point of sale size. It is also pertinent herre that in Cauliflower & Calabrese at least the d. mildew pathogen can cause a sever curd/floret infection and this is considered to result from a systemic invasion of the host, possibly during the seedling stage of growth. Whilst the precise conditions under which such infections occur are not known it is important to maintain good control of the disease at this early stage to minimise the risk of curd and/or floret infection occurring later in the production cycle.

Many of the spray programmes evaluated here attempted to compare alternative strategies for dealing with a range of pathogens in the hope of increasing the fungicide 'armoury' for disease control in propagation. This final phase has demonstrated that all of the programmes resulted in healthier and stronger plants than those left untreated, and that the majority of the treatments (with the exception of T11) were at least as good as, if not better than, the current standard commercial programme (T1). Although this single replicated experiment has not provided an overly stern test in terms of trialling the programmes, and the novel products, against a full range of pathogens that may occur in module-raised brassica propagation, it has demonstrated their crop safety when used as tank mixes or as independent components of integrated programmes. It has also validated their efficacy against the most prevalent, and therefore most important, disease in the crop.

Whilst not within the immediate scope of this project, it is imperative that the industry secures further 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. Further, proposed changes to the Long-Term Arrangements for Extension of Use could soon compound this further and the industry, through HDC, need to grasp this opportunity to finally clarify their position to ensure appropriate SOLA's are made available for an effective range of fungicides in the propagation stage of production both outdoors and under protection.

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 future residue 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 entitled :- *"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*
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 have continued to show interest in the proposal, to date, funds have not been made available for this work to be undertaken and the industry

continues to be left in a difficult position with respect to crop protection measures on crops in propagation. It is therefore recommended that HDC, on behalf of the brassica propagators, and in collaboration with the Plant Propagators Association, urgently re-evaluate their current position, relative to impending changes in the Long-Term Arrangements. The goal must be to ensure appropriate fungicides, as required by this sector of the industry, are made available where necessary via individual SOLA applications.

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 conclusions can now be drawn from the project:-

- Seedlings of Cauliflower, Calabrese, B. sprouts & Cabbage are surprisingly resilient to a broad range of fungicide applications and the risk of crop damage (phytotoxicity) from their application appears relatively small. This potentially widens the choice of products that can be used during propagation subject to efficacy and subsequent approval via the regulatory process.
- Several novel fungicides have been identified from the work that have shown moderate-good activity against the key pathogen *P. parasitica*, cause of downy mildew. A number of these should be put forward for approval on module-raised brassicas via the HDC funded SOLA programme
- No immediate alternative effective products have been found to supplement or replace tolclofos-methyl (Basilex) for the control of wirestem caused by *Rhizoctonia solani,* though the merit of applying routine 'prophylactic' applications against this pathogen may be in doubt and the results form the ongoing DEFRA study will be interesting in this respect. However, the lack of alternative effective products continues to be of concern, especially from an anti-resistance standpoint. The recent approval (on potato) of flutolanil (Rhino) by Certis is worthy of further investigation in this regard and efforts should be made to secure efficacy and residues data in support of an On- or Off-Label approval on module-raised brassicas (outdoor & protected cultivation) as this could potentially fill a gap in the armoury.
- 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 especially where future approval could potentially allow early pre-emergent drench applications of specific products.
- No efficacy data was gathered against the incidental pathogen *Mycosphaerella* though it is anticipated that products with known activity against the pathogen applied in the field can be extended to permit use under protection, including during propagation. The crop safety evaluations conducted on protected module-raised brassicas in this project will be important in this regard.
- 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 continue to be encouraged to be responsive to the needs of the industry in this respect and assist the industry in clarification of the issue. The impending changes to the Long-Term Arrangements for Extension of Use (to allow harmonisation with the rest of the EU) provides an excellent opportunity to finally resolve this issue. The Plant Propagators Association will need to be pro-active in this regard and work closely with the NFU & HDC to ensure their needs are met.

Technology Transfer

Direct grower contact via the grower coordinator through the Plant Propagators Association, and through one-to-one contact by project staff at STC.

Throughout this period the regulatory position has been in a ‘state of flux’ and most enquiries have related to the legal status of specific crop uses and this continues to require urgent clarification.

References

Dring, D M (1961) Studies on *Mycosphaerella brassicicola* (Duby) Oudem. Transactions of the British Mycological Society **44** (2), 253-264

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Appendices

Appendix I : Experimental layout for each of the studies described
Appendix II : Experimental layout for Phase III study.

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.

Appendix II

Trial Plan for Fungicide Evaluation (Phase III)