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Project leaders:

Dr T M O'Neill	Dr G M McPherson
ADAS	HRI Stockbridge House
Arthur Rickwood	Cawood
Mepal, Ely	Selby
Cambridge	North Yorkshire
CB6 2BA	YO8 0TZ

Project co-ordinator:

Dr N O Dungey
Van Heyningen Bros Ltd
Toddington Lane
Littlehampton
West Sussex
BN17 7PP

Key workers:

Dr D Pattison, Dr D Teverson, Miss F Pomares, Mr N Hall (HRI)
Mr G Hilton, Mr D Pye (ADAS)

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The results and conclusions in this report are based on a series of experiments conducted over two seasons. The conditions under which the experiments were carried out, and the results obtained, have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations. Many of the chemicals used in the fungicide evaluation experiments at HRI Stockbridge House were experimental only and therefore currently are not approved for use on tomatoes in the UK.

Authentication

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

Dr G M McPherson
Research Plant Pathologist
HRI Stockbridge House

Signature .... Date 20.2.98

Dr T M O'Neill
Research Plant Pathologist
ADAS Arthur Rickwood

Signature T. M. O'Neill Date 12-2-98

Report authorised by:

Mr M R Bradley
Site Director
HRI Stockbridge House

Signature .... Date 16.2.98

Dr M Heath
Site Director
ADAS Arthur Rickwood

Signature M. C. Heath Date 13/2/98

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PRACTICAL SECTION FOR GROWERS

Background and Objectives

Botrytis is a major cause of plant death in many heated long-season tomato crops. Stem infection usually occurs at wound sites left after de-leafing or following leaf dieback. In recent years it has become evident that old fruit trusses are also an important infection site in crops affected by botrytis. The botrytis problem was particularly apparent in the late summer and autumn months of 1992 and 1993. As stems are layered it is thought that old fruit trusses are sometimes damaged when stems are lying on top of each other. Also, the fruit trusses are sometimes caught by picking-trolleys moving along the heating pipes. In both instances, if the fruit truss itself is damaged, or its attachment to the main stem is damaged, then fresh wound sites are created and these provide ideal entry points for botrytis. In other crops the layered stems are not well supported and fruit trusses may lie in water which has run from the rockwool slabs and pooled on the plastic. Wet fruit trusses are more likely to develop botrytis rot than dry ones. A few growers cut-off fruit trusses to avoid these problems. ADAS questionnaires in 1992 and 1993 indicated that stem botrytis was less damaging where old fruit trusses had been removed. Other growers apply protective fungicides to stems immediately before layering in an attempt to stop botrytis.

Unfortunately the fungicide used most widely for botrytis control in protected crops, iprodione (Rovral), is less effective than previously, due primarily to decreased sensitivity in populations of the fungal pathogen, *Botrytis cinerea*. Alternative products are available, eg Bravo 500 and Elvaron, but are protectant in action and less effective, particularly against leaf and stem infection.

The agrochemical manufacturers are currently developing a range of novel broad spectrum fungicides, some of which show good activity against *B. cinerea* in major crops eg vines. The last detailed fungicide evaluations for the control of botrytis in tomato were conducted in the late 1970s and whilst it is unlikely that products will be developed specifically for the tomato crop, it may be possible to work alongside the manufacturer to secure On-Label Approval following earlier approval on agricultural commodities or to secure a specific Off-Label Approval (SOLA) via the HDC sponsored SOLA programme.

The primary objectives of this project were:

(i) To provide information on treatments or practices which may reduce the occurrence and severity of grey mould in protected tomato crops and,

(ii) To identify novel fungicides with activity against the pathogen and which may be Approved either by the manufacturer (On-Label) or via the HDC sponsored SOLA programme (Off-Label).

Summary of Results

Over a period of two years (1995/96 to 1996/97) the effects of fruit truss removal and fungicide sprays after truss removal on stem rotting and plant death were investigated in commercial nurseries by ADAS. During 1995 experiments were carried out in a crop of Pronto in Essex and a crop of Solairo in Kent. In a season with high summer temperatures, the increase in botrytis stem rotting and plant death between June and November was low. At most assessments neither fruit truss removal nor fungicide treatment had significantly reduced these low levels of stem botrytis. Nevertheless, in both experiments, the increase in total number of botrytis stem lesions over the period of the trial appeared to support truss removal as a method of controlling stem botrytis. There was little difference between two methods of truss removal (pulling off or cutting off), but removal of trusses as they decayed appeared to be ineffective. Application of fungicides after removal of fruit trusses appeared to reduce stem botrytis in the experiment in Essex. In contrast, fungicide treatment appeared to increase stem botrytis in the experiment in Kent. Isolates of *B. cinerea* from both crops were sensitive to iprodione (Rovral) at the start of fungicide treatment. After application of three Rovral sprays (alternating with Bravo), all isolates from the Essex crop and most isolates from the Kent crop were still sensitive to iprodione.

In 1996 an experiment was carried out in a February-planted crop of Saporo in Norfolk. Botrytis occurred naturally in the crop with high incidences of leaf, fruit truss and stem wound infection following leaf removal. Stem lesions were first observed on 21 May and by the end of the season (10 September) the mean incidence of botrytis stem lesions on untreated plants (fruit trusses left on; no fungicides applied) was 58.9 lesions per plot (1.1 lesion per plant). This was reduced to 23.9 lesions per plot by, once every two weeks, pulling-off all fruit trusses which had begun to turn brown. Removal of all empty fruit trusses (green or brown) every two weeks reduced the number of stem lesions to 39.4 (pulling off) or 49.7 (cutting off). Application of fungicide sprays to wounds created by pulling-off fruit trusses resulted in a temporary reduction in stem botrytis compared with pulling-off fruit trusses and applying no fungicide. Leaving fruit trusses on and applying a fungicide spray every two weeks from 21 June reduced the number of attached fruit trusses developing botrytis.

Detailed examination of plants after fruit truss removal clearly showed that both pulling-off and cutting-off trusses prevented botrytis providing trusses were removed flush to the main stem. However, when a projecting stub was left, up to 39% of these sites became infected by botrytis, compared with rotting at less than 2% of sites where no stub was left. At sites of leaf removal, the incidence of stem botrytis was approximately three times more common where a stub was present than at sites where no stub was present. When empty fruit trusses were left on stems, 35% of them had turned brown by 10 September, 14% were obviously infected by botrytis and 10% had resulted in stem lesions.

At a further three grower sites located in Essex and Norfolk, the influence of fruit truss removal and method of truss removal on the occurrence of stem botrytis was investigated. At both Newport (cv. Solairo) and Clacton (cv. Pronto), the incidence of stem lesions was greatest where fruit trusses were left on and least where they were pulled off. At Stalham (cv. Solairo), the incidence of stem lesions was greatest where trusses were cut off and least where decayed trusses were pulled off. Low incidences of botrytis stem lesions at these sites appeared to be associated with treatments which resulted in few stubs. The proportion of empty fruit trusses left on plants which decayed and developed an associated botrytis stem rot ranged from 1.2% (Solairo at Stalham) to 5.7% (Solairo at Newport).

Over 200 small botrytis lesions on stems were treated with either a paste of Benlate plus Actipron, painted with vinegar, or left untreated. Both Benlate plus Actipron and vinegar significantly reduced the incidence of sporing lesions and the number of spreading lesions compared with lesions left untreated. Benlate plus Actipron appeared more effective than vinegar. The proportion of limited lesions which had developed into spreading lesions on untreated plants was 28.4% on 19 August; this was reduced to 23.0% by vinegar and to 12.7% by Benlate plus Actipron. No reduction in the incidence of girdling lesions or dead plants were detected.

At HRI Stockbridge House a number of novel fungicides with contrasting modes of action were evaluated for their efficacy against *B. cinerea* and crop safety following application to protected tomatoes. In 1995 five novel fungicides were selected (pyrimethanil, AgrEvo; cyprodinil, Ciba Agriculture; azoxystrobin, Zeneca; diethofencarb + carbendazim, Rhone-Poulenc and Bot 196, Bayer) and applied as HV sprays to run-off in a layered tomato crop (cv. Spectra) at 14 day intervals. Seven sprays were applied in total. To encourage grey mould development in the trial guard plants were artificially inoculated with a carbendazim and iprodione sensitive isolate of *B. cinerea* and conditions conducive to infection and subsequent disease development maintained. Subsequently, detached (*in vitro*) and attached (*in situ*) leaf bioassays and stem inoculations were carried out using the same isolate of *B. cinerea*.

Grey mould infection was slow to establish in the trial though leaf and stem infection did occur at moderate levels by crop termination in late November. All the fungicide treatments significantly reduced the incidence and severity of leaf and stem infection, including the standard treatments using iprodione (Rovral WP) and an integrated programme where chlorothalonil (Bravo 500), dichlofluanid (Elvaron), and iprodione (Rovral WP) were applied in an alternating programme.

The strobilurin fungicide from Zeneca (azoxystrobin) performed extremely well and provided excellent control of botrytis. Other fungicides showing good activity were pyrimethanil, diethofencarb + carbendazim and cyprodinil, though unfortunately the latter product produced phytotoxicity symptoms in the form of extensive leaf yellowing. Bot 196 provided some suppression of botrytis though compared to the other products in the trial it was inferior in this first years evaluation. The excellent control achieved with diethofencarb + carbendazim should be treated with some caution as it is uncertain which component in the fungicide mixture provided the most effective control, because the botrytis isolate introduced into the trial area was carbendazim sensitive.

In 1996, the most effective products from 1995 (pyrimethanil, AgrEvo; azoxystrobin, Zeneca; diethofencarb + carbendazim, Rhone-Poulenc) were re-evaluated in a tomato crop (cv. Liberto) alongside the standard products iprodione and an integrated programme of iprodione, chlorothalonil and dichlofluanid. In addition, an aerosol formulation of axaconazole + imazalil ('Scomrid Limb') and per-acetic acid (Jet 5) were also evaluated in this second years trial. Fungicides were applied HV to run-off at 14 day intervals with seven sprays applied in total.

To encourage grey mould development the fungus was again introduced artificially though on this occasion an MBC (eg carbendazim) resistant isolate, which also exhibited reduced sensitivity to iprodione, was used. As in 1995, the pathogen was slow to establish and disease levels in the trial area remained fairly low during the season even though the pathogen was repeatedly inoculated and conditions favourable for infection were maintained wherever possible.

Leaf infection by botrytis remained at negligible levels throughout the experimental period and there was no evidence of secondary spread from initial inoculum sources onto other leaves in the plots. A low level of stem infection did occur during the experimental period, occasional girdling lesions formed and a small number of plant losses occurred. Much of the stem infection observed appeared to originate from senescent trusses rather than from old leaf scar sites.

Neither the standard fungicide treatment nor the integrated programme reduced the level of stem infection significantly, perhaps not too surprising as the isolate introduced originally into the trial area was carbendazim-resistant and exhibited a reduced sensitivity to iprodione. Scala was disappointing, giving only a slight reduction in the mean number of stem lesions.

Both Amistar and Jonk gave a significant reduction in stem botrytis compared to the other treatments and this supports the results from 1995. Jet 5 was ineffective against botrytis in this evaluation.

An assessment of agronomic parameters during the time-course of the experiment indicated some variability in leaf length and leaf area between treatments. Plants treated with Amistar recorded the lowest leaf length and leaf area though not significantly so.

At termination the mean plant height was determined and again Amistar gave the lowest mean height. The cumulative yield for the trial in 1996 was lower than in 1995 and whilst there was some variability no significant differences between treatments were recorded.

Axaconazole + imazalil (Scomrid Limb aerosol) caused severe phytotoxicity on all tomato plant tissues with the exception of old stem tissues. Therefore, it could not easily be incorporated into the main trial alongside the other treatments, particularly as stem infection was uncommon in the early stages of the trial. It was necessary to conduct a separate evaluation using detached stem tissues to determine the efficacy of this fungicide. In the detached stem tissue study axaconazole + imazalil was very effective in preventing stem infection providing the fungicide was applied in advance of inoculation.

The results of this two-year study, whilst somewhat variable, has demonstrated the efficacy of azoxystrobin (Amistar), diethofencarb + carbendazim (Jonk) and latterly pyrimethanil (Scala), albeit in Year 1 only.

Jonk has still not been made available for use in the UK whereas both Amistar and Scala now have UK approval for use on cereals and apples in the UK respectively. It is recommended therefore that UK approval is sought for the use of one or more of these products at the earliest opportunity. This could potentially either be achieved as an On-Label use via the manufacturers or as an Off-Label use via the HDC funded SOLA programme.

Action Points for Growers

1. Minimise the risk of botrytis by maintaining effective hygiene standards in the crop.

2. During periods when the weather conditions are conducive to infection, make every effort to reduce the relative humidity by applying ventilation and pipe heat.

3. If old fruit trusses appear to be a major source of stem botrytis, consider regular removal of old trusses as they brown, to reduce the risk of stem botrytis.

4. Whatever method is used to remove trusses (pulling, secateurs or knives) ensure that no stub is left attached to the stem. The presence of a stub considerably increases the risk of botrytis developing at the site of truss removal.

5. Inspect crops regularly for botrytis stem lesions. Where low levels of infection are found consider treating them with vinegar.

6. Consider submitting a random selection of leaves or stem pieces affected by botrytis infection to your plant pathology laboratory to determine the sensitivity of the fungus to a range of fungicides.

7. When disease pressure increases, or after trimming operations in the crop, consider applying a protective fungicide programme according to normal commercial practice.

8. In view of the demonstrated efficacy of the novel botryticides evaluated, every effort should be made to ensure any necessary data is secured to allow either an On or Off-Label use of azoxystrobin (Amistar) or pyrimethanil (Scala)

Practical and Financial Benefit from the Study

Any information which provides an opportunity to reduce the number of plants or stems lost due to botrytis is extremely valuable. Reduction of stem botrytis may at the same time lead to an increased fruit quality by reducing botrytis 'ghost-spotting'.

Information on the extent of stem botrytis was collected by ADAS from 60 long-season tomato crops on 1992. 40 nurseries reported final plant losses to stem botrytis of greater than 5% and six nurseries had plant losses over 40%. It is estimated that annual financial losses of £0.5-1.0 million occur regularly in the UK.

The studies conducted here have highlighted some practices which potentially exacerbate stem/truss infection and recommend measures to minimise losses. Moreover, a number of novel fungicides with activity against this pathogen, and which appear safe to the crop, have been identified.

Assuming the necessary safety data can be secured and evaluated by the regulators, On- or Off-Label approval will hopefully be granted for one or more products in the future and this will assist greatly in developing an integrated strategy for botrytis control in the future.

It is also anticipated that because of the broad spectrum nature of azoxystrobin (Amistar) losses due to other diseases in tomatoes eg *Didymella,* powdery mildew, will be minimised.

EXPERIMENTAL SECTION

Introduction

Grey mould (*Botrytis cinerea*) is increasingly a major cause of stem rotting and plant death in some long-season protected tomato crops. In 1992 an ADAS survey on 60 long-season tomato crops indicated that 40 nurseries experienced >5% plant losses due to botrytis and six had losses of over 40%. It is estimated that if 5-10% plants were lost, even late in the season, financial losses to the industry could be as high as £1 M/annum. The disease often originates at wound sites left after de-leafing, where infection may develop to produce small lesions on layered stems or alternatively from infected leaf tissues following marginal/tip scorch. More recently, decaying fruit trusses (the stalks from which all fruit has been harvested) have been recognised as an important site at which some botrytis stem lesions originate. Some growers now pull or cut-off old fruit trusses in an attempt to prevent this infection route. ADAS questionnaires in 1992 and 1993 indicated that stem botrytis was less of a problem where old fruit trusses had been removed, but the effectiveness of this time-consuming operation has not been clearly demonstrated.

Many growers continue to apply fungicides to tomato plants, especially to the bundle of layered stems and to the lower halves of upright stems, in an attempt to reduce botrytis. Unfortunately, the fungicide most commonly used for botrytis control in protected crops, iprodione (as Rovral WP) is losing its effectiveness due primarily to decreased sensitivity in populations of *B. cinerea*. Alternative products (chlorothalonil, eg Bravo, and dichlofluanid, eg Elvaron) are few in number and generally afford only partial control of stem rotting. The last detailed experiment on fungicides for control of botrytis in tomato was conducted in the late 1970s. Fortunately, the agrochemical industry has recently developed a number of novel fungicides with contrasting modes of action to existing products and which have already been demonstrated to have activity against *B. cinerea* in major international crops such as vines. Whilst it is unlikely that any of these new products will be developed specifically for the tomato crop, it may be possible, assuming efficacy can be demonstrated, to secure data to encourage the manufacturers to support On-Label Approval. Alternatively, if manufacturer support is not available it may still be possible to generate a full residues data package to secure Specific Off-Label Approval (SOLA) to permit a products use on tomato.

The commercial objective of this project was to identify and develop treatments and/or practices which reduce stem rotting and plant death caused by botrytis.

The scientific targets of the work were to:

1. Determine the effect of fruit truss removal, method of removal, and fungicide treatment of fruit trusses and stem wounds (left after truss removal) on the incidence, severity and control of stem botrytis.

2. Investigate the efficacy of a range of novel fungicides with reported activity against *B. cinerea* for the control of botrytis in tomato and their effect on crop growth as compared with the standard fungicide, iprodione (as Rovral WP) and a commonly used integrated programme comprising of alternating sprays of chlorothalonil (as Bravo 500), dichlofluanid (as Elvaron) and iprodione (as Rovral WP).

Materials and Methods

Details of Experiments 1-5 in commercial crops - 1996

Experiment:	1 Truss removal (replicated)	2 Truss removal (single plots)	3 Truss removal (single plots)	4 Truss removal (single plots)	5 Lesion paints (replicated)
Site	Walpole, Norfolk	Newport, Essex	Stalham, Norfolk	Clacton, Essex	Walpole, Norfolk
Cultivar	Saporo	Solairo	Solairo	Pronto	Saporo
Sown	16 Jan	2 Dec	5 Oct	15 Nov	16 Jan
Planted	14/15 Feb	29 Dec	28 Nov	12 Dec	14/15 Feb
Fungicide sprays	Elvaron 21 Jun Bravo 9 Jul Elvaron 23 Jul Rovral 6 Aug Bravo 20 Aug Rovral 3 Sep	Rovral 26 Apr Rovral 10 May Elvaron 14 Jun Rovral 5 Jul Thiovit 5 Aug Thiovit 16 Sep	Bavistin 2 Feb Elvaron 29 Feb Elvaron 29 Mar Rovral 24 Apr Elvaron 13 May Bravo 3 Jun Rovral 28 Jun Rovral 16 Jul Elvaron 10 Aug	Rovral 21 Jun Bravo 4 Jul Rovral 26 Jul	Nil
Disease assessments	16 Jul 19 Aug 23 Aug 10 Sep	30 Apr 29 May 18 Jul 15 Aug 23 Sep	17 May 16 Jul 13 Aug 2 Oct	23 Apr 29 May 18 Jul 15 Aug 8 Oct	21 May 6 May 21 Jun 9 Jul 16 Jul 23 Jul 6 Aug 19 Aug

All crops were grown on rockwool slabs utilising a run-to-waste system. The crop at Walpole was not layered.

Details of Experiment 6 at Stockbridge House - 1996

Site:	HRI Stockbridge House
Cultivar:	Liberto
Sowing date:	28 January 1996
Planting date:	2 April 1996
Fungicide timing:	1st spray 31 July 1996 2nd spray 14 August 3rd spray 28 August 4th spray 11 September 5th spray 25 September 6th spray 9 October 7th spray 30 October
Inoculation/Bioassay details:	Plants tagged 1 August 1996 Inoculation of tagged leaves 2 August Inoculation of tagged stems 8 August Inoculation of flowering trusses 13 August Inoculation of fruiting old trusses 21 August Re-inoculation of tagged leaves 19 September *In-situ* leaf bioassay 28 September Detached leaf bioassay 11 October Detached stem evaluation 11 October (Scomrid Limb only)
Crop safety assessments:	Phytotoxicity assessment 23 August Leaf length 17 October Leaf area 17 October
Disease assessments:	Botrytis assessment 6 August 28 September 15 November
Crop termination assessments:	15 November
Botrytis isolate used in inoculated studies (Experiment 6 only)	An isolate of *Botrytis cinerea,* insensitive to carbendazim and tolerant to iprodione at 2 mg/litre was selected for use in this study.

Experiment 5

1. Stem lesions left untreated.
2. Stem lesions painted with a paste of Benlate (2 g) and Actipron (3 ml).
3. Stem lesions painted with Sarsons Malt Vinegar (pH 2.6; 47.3 mg/ml acetic acid).

In experiment 1, fungicides were applied to treatments 5 and 6, as a high volume spray to run off, to stems on the same day that empty fruit trusses were removed. Products used were Elvaron at 1 g/litre, Bravo at 2.2 ml/litre and Rovral at 1 g/litre. A total of six sprays were applied (see Crop details).

In experiments 2-4, nursery staff applied their usual fungicide sprays programmes to all treatments.

In experiment 5, no fungicides were applied to plants, other than the paste and paint treatments to stem lesions which were applied to small (1-2 cm diameter) lesions as they occurred. The stems in this trial were examined every two weeks and all lesions found in treatments 2 and 3 were marked and treated.

Fruit truss removal by cutting at sites 3 and 4 was done by nursery staff using knives; at sites 1 and 2 it was done by ADAS staff using secateurs.

Experiment/Trial Design

Experiments 1 and 5 were of a randomised block design with 4 (experiment 1) or 3 (experiment 5) replicates. In both experiments, each plot consisted of a double row containing 60 plants. Treatments in experiments 2-4 were unreplicated and plots consisted of one or two double rows. Numbers of plants per treatment were 84 (experiment 2), 108 (experiment 3), and 152 (experiment 4). Experiment 6 was a randomised blocks design with 8 treatments x 3 replicate blocks. Each plot comprised a double row of layered tomato plants with a total of 18 plants per plot in six rockwool slabs. The crop was grown to a blueprint regime using a run-to-waste drip irrigation system. Plot area was 4.32 m^2.

Treatments

Experiment 1

1. Fruit trusses left on.
2. Fruit trusses pulled off.
3. Fruit trusses cut off (with secateurs).
4. Fruit trusses pulled off as they turned brown.
5. Fruit trusses left on; fungicide sprays applied to stems.
6. Fruit trusses pulled off; fungicide sprays applied to stem.

Experiment 2-4

1. Fruit trusses left on.
2. Fruit trusses pulled off.
3. Fruit trusses cut off.
4. Fruit trusses pulled off as they turned brown.

Experiment 6 (Stockbridge House)

1. Untreated control.

2. Iprodione (Rovral WP) applied HV to run-off at 1 kg/1000 litres water at 14 day intervals.

3. Integrated fungicide programme comprising HV sprays to run-off of chlorothalonil (Bravo 500), dichlofluanid (Elvaron), and iprodione (Rovral) at 14 day intervals at rates of 2.2 kg, 1 kg and 1 kg/1000 l water respectively.

4. Pyrimethanil (Scala) applied HV to run-off at 1.32 litres product/1000 litres water at 14 day intervals.

5. Azoxystrobin (Amistar) applied HV to run-off at 0.8 l product/1000 litres water at 14 day intervals.

6. Diethofencarb + carbendazim (Jonk) applied HV to run-off at 2 litres product/1000 litres water at 14 day intervals.

7. Axaconazole + imazalil (Scomrid Limb) applied as a spot treatment (rate unspecified) to protect all inoculated sites and subsequent infection areas at 14 day intervals.

8. Per-acetic acid (Jet 5) applied HV to run-off at 1 litre product/1000 litres water at 14 day intervals.

Disease assessments

Grower trials 1-5 were assessed on at least four occasions between May and September and the numbers of stem botrytis lesions and dead plants were counted. Experiment 5 was assessed every two weeks. A detailed final assessment was done prior to crop termination in experiments 1-4. Additional categories assessed at this final examination were:

- number of empty fruit trusses (ie no fruit left on them).
- number of brown fruit trusses (> 50% of truss length turned brown).
- number of fruit trusses obviously affected by botrytis (sporulation visible).
- number of fruit trusses affected by botrytis with an associated stem lesion.
- number of sites where a fruit truss has been removed and a projecting stub (> 2 mm) was left; the number of such sites affected by botrytis.
- number of sites where a fruit truss has been removed and no stub was left; the number of such sites affected by botrytis.
- number of sites where a leaf has been removed and a projecting stub was left; the number of such sites affected by botrytis.
- number of sites where a leaf has been removed and no stub was left; the number of such sites affected by botrytis.

In experiment 5, stem lesions were categorised as limited (confined to a node), spreading (extending more than 2 cm from a node) or girdling (extending around the stem completely and causing stem softening). The number of lesions with visible botrytis sporulation was also recorded.

A sample of 10 stem lesions was collected from the crop area adjacent to experiment 5 in early August and tested for resistance to benomyl (Benlate) and iprodione (Rovral), each at 2 and 20 mg/litre, by agar-plate mycelial growth tests.

In experiment 6 at Stockbridge House, regular assessments were made to determine the incidence of botrytis in the crop following the first fungicide applications in late July. Due to the low incidence of the disease, specific inoculation tests were conducted either *in vitro* or *in situ* to monitor fungicide performance. Finally detailed assessments of botrytis, particularly stem infection, was conducted in the crop following termination in mid-November.

In-situ Leaf Bioassay (Experiment 6)

After the fifth fungicide application on 25 September eight individual leaves of a similar age were selected in each plot and tagged. Each leaf was inoculated in five locations with the isolate of *B. cinerea*, which was insensitive to carbendazim and iprodione. Each leaf was subsequently enclosed in a plastic bag and incubated for 72-96 hours to aid infection and disease establishment. After this incubation period the polythene bags were removed and the individual lesions scored on a 0-3 severity scale from which a disease index could be calculated.

In-vitro (Detached) Leaf Bioassay (Experiment 6)

After the sixth fungicide application on 9 October eight individual leaves of a similar age were selected from each plot. Two agar plugs of *B. cinerea* (carbendazim and iprodione insensitive) were placed on each leaf and they were subsequently enclosed in humid chambers for a period of 72-96 hours. After this period the lesion diameter, where present, was measured.

Crop Termination Assessments (Experiment 6)

At crop termination in mid November each of the inoculated plants (9/plot) were assessed in detail.

Leaves were examined individually for botrytis infection and scored according to the following scale:

0 = No grey mould.

1 = Grey mould on occasional leaflets, petiole unaffected.

2 = Grey mould progressing into petiole.

3 = Grey mould infection progressing from petiole into main stem.

On each plot the basal 10 nodes on 9 plants were examined in detail and scored for botrytis infection using a 0-3 scale where:

0 = No infection.

1 = Slight lesion progressing into stem.

2 = Established lesion on stem affecting ca. 50% stem circumference.

3 = Severe botrytis lesions causing stem girdling.

Agronomic Assessments (Experiment 6)

Following each spray application the crop was examined for phytotoxicity symptoms and where present symptoms were recorded.

After the sixth fungicide application the leaf length and leaf area were calculated by measuring eight randomly selected leaves/plot of a similar age and position in the crop canopy.

At termination the plant height was determined on 9 plants per plot.

Inoculation of Tagged Plants (Experiment 6)

Leaves: Eight fully expanded leaves (1 per tagged plant) were tagged and inoculated by placing 4 droplets of a macerated suspension of *B. cinerea* onto each leaf. The leaves were subsequently enclosed in polythene bags for 72-96 hours to aid infection and establishment.

Stems A length of stem containing a minimum of eight nodes was selected on each of eight plants/plot and four leaves removed to create 'fresh' wound sites. Onto each leaf scar a droplet of a *B. cinerea* suspension was applied (4.1×10^5 cfu/ml) after 1 hour, 6 hours, 24 hours and 30 hours, after which time they were wrapped in Parafilm for a minimum of 72-96 hours.

Trusses Eight flowering trusses, eight fruiting trusses and four spent trusses were tagged (1 type per tagged plant) and inoculated by spraying with a *B. cinerea* suspension comprising 3.0×10^4 spores/ml. After inoculation inoculated trusses were enclosed in polythene bags for a minimum of 72-96 hours.

Detached stem inoculation for evaluation of 'Scomrid Limb' (Experiment 6)

A series of 0.5 m stem segments were taken from an unsprayed tomato crop at Stockbridge House and placed in humid chambers. Five leaves/stem section were removed to create five wounds and these were inoculated with a *B. cinerea* suspension. The stem pieces were either pre-treated immediately after leaf removal (pre-inoculation) with Scomrid Limb or it was applied following inoculation at the first sign of symptom development. Other treatments were included as controls. On half of the inoculated stems the wound sites were wrapped with Parafilm, the others remained exposed.

Treatments were:

1. Untreated, uninoculated control.
2. Untreated, inoculated control.
3. Inoculated, Scomrid Limb applied at first sign of lesion development.
4. Inoculated, Scomrid Limb applied before inoculation.
5. Scomrid Limb applied, uninoculated.

Yield Records (Experiment 6)

Yield data was recorded following fruit harvests three times per week, throughout the experimental period.

Statistical Analysis

Results from all experiments were analysed using an Analysis of Variance (ANOVA). Data was transformed where necessary (see results tables for details) and significance indicated either by:

NS	No significant difference
*	Significant at the 95% probability level
**	Significant at the 99% probability level
***	Significant at the 99.9% probability level

Or by comparing treatments using the LSD (Least Significant Difference) figure for significant effects at the 5% probability level.

Archiving

Experiments 1-5: The raw data and a copy of the final report from these trials will be stored at ADAS Arthur Rickwood, Mepal, Ely, Cambs, for a minimum period of 5 years.

Experiment 6: The raw data and a copy of the final report from this trial will be stored in a secure archive facility at HRI Stockbridge House for a minimum period of 5 years. If an On-Label Approval is secured during this period the data will be retained so long as the product concerned is authorised in the Community. Access to the data can only be made via the designated archivist.

Official Recognition and Quality Assurance

Experiments 1-5: These trials were conducted in accordance with ADAS Good Field Practice guidelines.

Experiment 6: This trial was conducted in accordance with the draft guidelines for Official Recognition of Efficacy Testing Organisations as outlined by PSD (PSD ref 2400/2996). A specific quality assurance audit was not undertaken in the trial.

Results

1996

(See also Annual Report for 1995).

Experiment 1

Botrytis stem rot

Botrytis leaf rot was first observed in April and by 21 May stem lesions were evident. Stem lesions occurred at sites of leaf removal, at sites of fruit truss removal and following dieback of leaves and fruit trusses which were not removed. When assessed on 19 August, the mean incidence of botrytis stem lesions per plot on untreated plants was 40.7. This figure was reduced by all treatments, except for cutting-off fruit trusses (Table 1). Fungicide treatment did not reduce the number of lesions showing botrytis sporulation. At the final assessment on 10 September, the mean incidence of botrytis stem lesions on untreated plants was 58.9 per plot (Table 2). This was reduced to 23.9 per plot where all decaying fruit trusses present had been pulled-off as they occurred. Removal of all empty fruit trusses (green or brown) once every two weeks reduced the number of stem lesions to 39.4 (pulling off) or 49.7 (cutting off). Application of a fungicide after each occurrence of fruit truss removal did not result in a further reduction in stem botrytis. Leaving empty fruit trusses on plants and applying a fungicide spray every two weeks did not reduce stem botrytis. Where a protruding stub was left on the stem after removal of fruit trusses, the resultant incidence of stem botrytis was considerably greater than in comparable treatments where no stub was left (Table 3). Removal of trusses by cutting-off with secateurs was most damaging in this respect, resulting in both the largest number of stubs (18.8% of sites) and the greatest proportion of stubs (31.3%) to develop botrytis.

Removal of decaying fruit trusses by pulling them off was most effective in preventing stem rot from stub infection. The effect of leaving a stub was highly significant (Table 4). A greater incidence of stem botrytis was also found at leaf removal sites where a stub was left, compared with sites where no stub was left (Table 5). An assessment on 23 August of the lowest three fruit truss positions showed that removal of decaying fruit trusses as they occurred was highly effective in preventing stem botrytis from this source. The incidence of sites with stem rot was reduced from 16.8% to 0.2% (Table 6). Leaving empty fruit trusses attached to stems and applying fungicide sprays to them appeared to give a small reduction in stem rot. The importance of decaying fruit trusses as a source of stem rot was confirmed on 10 September 1996 when a detailed assessment of fruit truss browning, infection by botrytis and associated stem rot was made (Table 7). Where all trusses were left on stems and no fungicides were applied, the proportions turned brown, infected by botrytis and developing an associated stem rot were 25.3, 14.2 and 9.6% respectively. All methods of fruit truss removal reduced the incidence of brown fruit trusses to less than 10%, the incidence of infected trusses to less than 3% and the incidence of stem rot to less than 1%. A programme of six fungicide sprays applied to stems from 21 June to 3 September had reduced the number of attached fruit trusses developing botrytis by 25% when assessed on 23 August (Table 6). However, no effect was detected at the final assessment on 10 September (Table 7). There was a slight reduction in the incidence of stem lesions following application of fungicides to wound sites after truss removal (Table 1) at an assessment on 19 August, but no effect was detected at the end of the experiment (Table 2). Dead plants were first observed on 16 July and by 10 September 6.3% of plants in untreated rows were dead. None of the treatments resulted in a significant reduction in the incidence of plant death (Table 8).

Fruit truss decay

The incidence of brown fruit trusses, infected fruit trusses and fruit trusses with associated stem rot was made at the final assessments in late September/early October (Table 11). The occurrence of fruit trusses infected by botrytis and of stem rotting was not obviously associated with the incidence of fruit truss browning. Fruit truss browning ranged from 14.8% of trusses (Pronto at Clacton) to 27.7% (Solairo at Stalham). Stem rotting associated with fruit truss dieback ranged from 1.2% (Solairo at Stalham) to 5.7% (Solairo at Newport).

Experiment 5

Botrytis stem lesions were first observed on 21 May and the incidence of lesions increased steadily with time. By 23 August over 200 lesions had been treated with vinegar or Benlate plus Actipron.

When assessed on 16 July, sporing botrytis was evident on 34.3% of lesions left untreated. The incidence of botrytis sporulation was lower on lesions previously treated with vinegar (12%) or Benlate + Actipron (1.1%). At this stage very few lesions, either treated or untreated, had progressed into spreading or girdling lesions (Table 12). Five weeks later (19 August) over 30% of untreated lesions had progressed into spreading or girdling lesions (Table 13). Treatment with Benlate and Actipron was more effective than treatment with vinegar in reducing the incidence of spreading and sporing lesions (Table 13).

The incidence of dead plants was relatively low (3.8% in untreated plots on 10 September) and no significant differences between treatments were detected (Table 14).

Experiments 2-4

Botrytis stem rot

The development of botrytis stem lesions and plant death is shown for all three sites in Table 9. Botrytis stem rot occurred first in experiment 3 (Stalham) and developed steadily throughout the season. In experiment 4 (Clacton), the disease remained at a low incidence until September. In experiments 2 and 4 (Newport and Clacton) the greatest incidence of botrytis stem lesions at the final assessment occurred where fruit trusses were left on. Cutting-off trusses (with a knife) and pulling-off trusses as they decayed appeared to be the most effective methods of preventing botrytis stem rot from truss dieback at both these sites. At Stalham, cutting-off trusses appeared to have reduced botrytis stem rot at the assessment on 13 August, but a high incidence of stem rot subsequently developed in this treatment.

A more detailed assessment of the origins of stem botrytis towards the end of cropping (Table 10) revealed that high incidences of botrytis stem rot were generally associated with (a) treatments that left a protruding stub and (b) decay of fruit trusses left attached to the stem. At Newport, there was a relatively high incidence of stem rot associated with stubs left after cutting-off trusses, and a high incidence of rot where trusses were left attached to the stem. At Stalham there was a relatively high incidence of botrytis stem rot associated with stubs following both pulling-off and cutting-off of trusses. There was also a relatively low incidence of fruit truss decay where trusses were left attached to stems. Overall, none of the treatments at this site resulted in a marked reduction in the incidence of stem botrytis or plant death. Pulling-off of trusses appeared to increase the incidence of plant death. At Clacton, there was little stem rot originating from fruit truss stubs, the majority originating from decay of fruit trusses left attached to the stem.

Fungicide resistance

B. cinerea isolated from leaves collected at Walpole (site of experiments 1 and 5) was found to be resistant to benomyl at both 2 and 20 mg/litre; it was resistant to iprodione at 2 mg/litre and sensitive at 20 mg/litre.

Experiment 6 (Stockbridge House)

At the commencement of the experimental treatments in the established tomato crop there was little evidence of grey mould in the crop. A series of artificial inoculation treatments were introduced immediately after the first spray application to a) establish the pathogen, *B. cinerea*, in the crop and b) to attempt to learn more about the disease etiology.

The initial leaf infection was successful in establishing infection on the leaves though unfortunately a change to hot weather conditions prevented further development of the disease. Following inoculation of stem wounds, flowering, fruiting and spent trusses, botrytis lesions did not develop. Throughout the experimental period there appeared to be no correlation between inoculation of the 'tagged' infection sites, and subsequent botrytis occurence in the crop.

Due to the low incidence of botrytis in the crop by late September (after five sprays had been applied) an *in situ* study was conducted to determine the efficacy of the applied fungicide treatments (Table 15). All the fungicides, with the exception of per-acetic acid reduced the severity of botrytis lesions which developed. Azoxystrobin (Amistar) and diethofencarb + carbendazim (Jonk) performed most effectively. It should be noted that due to the absence of distinct botrytis stem lesions in the crop by this stage 'Scomrid Limb' had not been applied. In any case, applied as a spot treatment, it is unlikely to have reduced botrytis infection where applied directly to the leaves. Also, where the product was applied to plant trusses other than the older stem tissues, phytotoxicity symptoms developed within 24 hours. Symptoms took the form of a dryish scorched or burned appearance with plant tissues, including flowering trusses, shrivelling up.

Following application of the sixth spray application on 9 October untreated leaves (treatment 1) and treated leaves (treatments 2-6, 8) were removed, returned to the laboratory and inoculated with *B. cinerea* in an *in vitro* study. After incubation the developing leaf lesions were measured (Table 16). Again all the fungicide treatments applied reduced the development of botrytis lesions though Jet 5 was particularly poor in this evaluation. The most effective treatments were diethofencarb + carbendazim (Jonk), the integrated programme (Bravo 500, Elvaron and Rovral) and azoxystrobin (Amistar). Rovral and Scala (pyrimethanil) were only partially effective in this evaluation.

Due to the lack of distinct botrytis stem lesions in the crop it was necessary to conduct an *in vitro* test to determine the performance of the Scomrid Limb spot treatment aerosol. Detached stem segments were artificially inoculated with *B. cinerea* either immediately before or after the Scomrid Limb treatment had been applied (Table 17). A high number of botrytis stem lesions developed following artificial inoculation in the untreated stems and the infection potential was enhanced slightly by wrapping the wounds with Parafilm to maintain conditions conducive to infection. No lesions developed in the uninoculated controls and 'Scomrid Limb' caused no visible phytotoxicity to the old stem segments used in the study. Where the fungicide was used as a spot treatment on wounded nodes prior to inoculation with *B. cinerea* the product was extremely effective reducing the infection level from 68% to 0%. However, where the treatment was applied to established infections on these detached stem sections it was less effective reducing the level of infection from 68 to 44% only (NS).

Throughout the duration of the experiment the crop was monitored regularly for symptoms of phytotoxicity, though with the exception of the initial aerosol treatment, none was visible. To support this an interim agronomic measurement was undertaken in mid October to determine whether any of the applied treatments had adversely affected the crop. Measurements of leaf length and leaf area were taken, the latter using a Planimeter housed in the laboratory (Table 18). Whilst differences in both leaf length and leaf area occurred they were not significantly different from the untreated.

Azoxystrobin (Amistar) in this second year's evaluation gave the lowest leaf length and leaf area measurements and this will need further monitoring should the product be approved on the crop in the future. Pyrimethanil (Scala) which was reported to show some phytotoxicity in the first years evaluation caused no detrimental effect in 1996.

The crop was finally terminated on 15 November 1996 at which time a series of detailed assessments were made on individual plants in each plot. Only trace levels of leaf infection remained apparent in the crop and therefore these results have not been tabulated. A low-moderate level of stem infection by *B. cinerea* did occur however, and these results are presented (Table 19). In the untreated control a mean number of 1.6 botrytis stem infections/plant occurred. Neither Rovral nor the integrated programme comprising Bravo 500, Elvaron and Rovral had an appreciable effect on reducing the stem lesion number of severity. Pyrimethanil (Scala) was also disappointing, at best only reducing the stem lesion incidence marginally. However, whilst no treatments eliminated the incidence of stem lesions completely, both azoxystrobin (Amistar) and diethofencarb + carbendazim (Jonk) reduced the infection significantly. Jet 5 was ineffective in this evaluation. As indicated earlier, however, even though detailed examination of all the tagged inoculation sites was made there appeared to be no good correlation between inoculation and subsequent botrytis development and there are clearly other over-riding factors which have influenced botrytis infection and severity on this occasion.

As the plants were removed the mean plant height was determined for each treatment (Table 20). These results were non-significant though Amistar and Scala treated plants were marginally shorter than the control plants.

Finally, yield data collected during the experimental period is presented in Table 21. Whilst there was some variability between treatments there were no significant effects. It is worthwhile noting however that the lowest yielding treatments in this evaluation were following treatments with pyrimethanil (Scala), diethofencarb + carbendazim (Jonk) and azoxystrobin (Amistar).

Table 3: **Effect of leaving a stub at fruit truss removal on tomato stem botrytis - Norfolk, 10 September 1996.**

Method of fruit truss removal	No. fruit trusses removed /plot	Stub left			No stub left		
		No. sites assessed	Stem rot		No. sites assessed	Stem rot	
			No.	(%)		No.	(%)
1. Untreated	0.0	0.0	0.0	-	0.0	-	-
2. Pulled off	316.3	47.3	10.5	(22.2)	268.5	5.3	(1.9)
3. Cut off	331.1	62.3	19.5	(31.3)	268.8	5.8	(2.2)
4. Decaying off	40.1	9.0	1.5	(16.7)	35.8	1.5	(4.0)
5. Leaf on + fungicides	0.0	0.0	-	-	0.0	-	
6. Pulled off + fungicides	318.6	45.8	10.0	(21.9)	272.8	6.3	(2.3)
Significance SED (15 df)				(*) (3.92)			(NS) (-)

Table 4: **Mean effect of leaving a stub at fruit truss removal on tomato stem botrytis - Norfolk, 10 September 1996.**

Treatment	Mean % fruit truss sites affected by botrytis
Stub left	31.3
No stub	2.7
Significance SED	*** 2.91

Table 1: **Effect of fruit truss removal and fungicide sprays on stem botrytis - Norfolk, 19 August 1996.**

Truss removal treatment	Mean no. lesions/row	% of lesions		Mean no. plants/row alive[a]
		Sporing	Girdling	
1. Untreated	40.7	72.9	5.2	59.0
2. Pulled off	34.7	71.5	6.2	58.0
3. Cut off	42.0	63.8	3.9	58.3
4. Decaying off[b]	25.5	63.9	7.6	59.5
5. Leaf on + fungicides	27.0	70.9	0.0	60.0
6. Pulled off + fungicides	26.2	64.7	9.5	58.5
Significance	*	NS	NS	NS
SED (15 df)	5.44	(-)	(-)	(-)

[a] 60 plants/row

[b] Fruit trusses pulled off as they turned brown

Table 2: **Origin of tomato stem botrytis and effect of fruit truss removal and fungicide sprays on disease control - Norfolk, 10 September 1996.**

Method of fruit truss removal	Mean no. dead plants per plot[a]	Mean No. botrytis stem lesions/plot originating from:				
		Decaying fruit trusses	Sites of fruit truss removal	Sites of leaf removal	Unknown	Total no. of lesions
1. Untreated	3.8	37.5	0.0	21.1	0.3	58.9
2. Pulled off	3.8	0.8	17.3	19.8	1.5	39.4
3. Cut off	3.3	0.0	30.1	19.3	0.3	49.7
4. Decaying off[b]	2.5	1.3	6.5	15.8	0.3	23.9
5. Leaf on + fungicides	1.0	43.0	0.0	20.6	0.8	64.4
6. Pulled off + fungicides	3.0	0.0	20.1	19.6	0.5	40.2
Significance	NS	***	***	NS	*	***
SED (15 df)	(-)	4.92	2.56	(-)	0.34	6.08

[a] 60 plants/row

[b] Fruit trusses pulled off as they turned brown

Table 5: Occurrence of tomato stem botrytis at leaf scar sites - Norfolk, 10 September 1996.

Method of fruit truss removal	Mean no. botrytis lesions/plot			
	Leaf site		Unknown origin	Total[a] leaf and unknown
	Stub present	No stub		
1. Untreated	16.3	4.8	0.3	21.3
2. Pulled off	15.3	4.5	1.5	21.3
3. Cut off	15.0	4.3	0.3	19.5
4. Decaying off	12.8	3.0	0.3	16.0
5. Leaf on + fungicides	14.8	5.8	0.8	21.3
6. Pulled off + fungicides	14.8	4.8	0.5	20.0
Significance	NS	NS	*	NS
SED (15 df)	-	-	0.34	-

[a] Excluding lesions originating from fruit trusses.

Table 6: Effect of fungicides on decay of fruit trusses - Norfolk, 23 August 1996.

Treatment	Total number trusses assessed	Mean brown	% fruit with botrytis	Trusses[a] causing stem rot
1. Untreated	174.8	46.4	27.7	16.8
2. Pulled off	-	-	-	-
3. Cut off	-	-	-	-
4. Decaying off	133.8	17.7	3.2	0.2
5. Leaf on + fungicides	179.3	44.9	25.0	12.7
6. Pulled off + fungicides	-	-	-	-
Significance	-	**	**	**
SED (15 df)	-	4.77	2.73	1.56

[a] Based on assessment of basal three trusses on every plant.

Table 7: Development of tomato stem botrytis from decaying fruit trusses - Norfolk, 10 September 1996.

Method of fruit truss removal	Mean no. empty fruit trusses/plot						
	Total	Brown		Sporing botrytis		Caused stem rot	
		No.	(%)	No.	(%)	No.	(%)
1. Untreated	392	99.0	(24.7)	55.8	(14.1)	37.5	(9.5)
2. Pulled off	148	10.0	(6.8)	2.8	(1.8)	0.8	(0.5)
3. Cut off	155	9.8	(6.4)	2.5	(1.7)	0.0	(0.0)
4. Decaying off	365	32.8	(9.0)	10.0	(2.8)	1.3	(0.3)
5. Leaf on + fungicides	392	87.5	(22.6)	62.0	(16.0)	43.0	(11.1)
6. Pulled off + fungicides	153	9.5	(6.3)	2.5	(1.6)	0.0	(0.0)
Significance SED (15 df)			(***) (2.62)		(***) (1.89)		(***) (1.44)

Table 8: Effect of fruit truss removal and fungicide sprays on plant death - Norfolk, 1996.

Treatment	Mean no. dead plants/row (60 plants)			
	16 July	6 August	19 August	10 September
1. Untreated	0.3	0.8	1.0	3.8
2. Pulled off	0.8	2.3	2.0	3.8
3. Cut off	0.5	1.8	1.8	3.3
4. Decaying off	0.0	0.5	0.5	2.5
5. Leaf on + fungicides	0.0	0.0	0.0	1.0
6. Pulled off + fungicides	0.5	1.3	1.5	3.0
Significance SED (15 df)	- -	- -	NS -	NS -

Table 9: Development of botrytis stem rot and plant death at three grower sites - 1996.

Site & Treatment	Cumulative no. of botrytis stem lesions				Cumulative no. of dead plants			
Newport (Exp. 2)	29 May	18 Jul	13 Aug	23 Sep	29 May	18 Jul	13 Aug	23 Sep
1. Left on	0	10	28	82	0	0	0	13
2. Pulled off	0	14	34	39	0	0	16	18
3. Cut off	0	7	18	27	0	0	8	11
4. Decayed off	0	0	13	28	0	1	10	11
Stalham (Exp. 3)	17 May	16 Jul	13 Aug	2 Oct	17 May	16 Jul	13 Aug	2 Oct
1. Left on	1	2	9	40	1	2	2	12
2. Pulled off	0	3	11	47	1	3	12	30
3. Cut off	1	0	1	70	1	3	3	8
4. Decayed off	0	0	4	33	1	2	6	12
Clacton (Exp. 4)	29 May	18 Jul	15 Aug	8 Oct	29 May	18 Jul	15 Aug	8 Oct
1. Left on	0	2	3	64	0	0	2	4
2. Pulled off	0	3	6	13	0	0	1	5
3. Cut off	0	6	4	7	0	0	4	8
4. Decayed off	0	3	3	8	0	0	0	2

The number of plants per treatment were 84, 108 and 152 at Newport, Stalham and Clacton respectively.

Table 10: Observations on the effect of tomato fruit truss removal at three grower sites - 1996.

Site, treatment & date assessed	No. plants alive (all assessed)	% dead plants	No. botrytis stem lesions at different sites on live plants						Total no. botrytis stem lesions	No. botrytis lesions/ live plant
			Truss site - stub	Truss site - no stub	Leaf site - stub	Leaf site - no stub	Decaying trusses	Unknown		
Newport (23 Sept)										
1. Left on	71	15.5	-	-	6	1	70	5	82	1.2
2. Pulled off	73	13.1	5	2	6	1	9	4	27	0.4
3. Cut off	66	21.4	12	2	7	1	11	6	39	0.6
4. Decayed off	73	13.1	4	1	4	2	14	3	28	0.4
Stalham (2 Oct)										
1. Left on	96	11.1	-	-	7	2	28	3	40	0.4
2. Pulled off	78	27.8	17	7	4	1	16	2	47	0.6
3. Cut off	100	7.4	26	12	6	0	22	4	70	0.7
4. Decayed off	96	12.9	6	1	3	1	19	3	33	0.3
Clacton (8 Oct)										
1. Left on	148	2.6	-	-	5	1	56	2	64	0.4
2. Pulled off	144	5.3	5	1	1	0	0	0	7	0.1
3. Cut off	147	3.3	6	2	3	1	0	1	13	0.1
4. Decayed off	150	1.3	4	1	2	0	0	1	8	0.1

The varieties were Solairo (Newport and Stalham) and Pronto (Clacton).
Planting dates were 29 December (Newport), 2 February (Stalham) and 12 December (Clacton).

Table 11: Incidence of tomato fruit truss decay and associated stem botrytis at three grower sites - 1996.

Site, treatment & date assessed	No. plants assessed	No. fruit trusses present	Fruit truss decay		
			% brown	% infected by botrytis	% with stem rot
Newport (23 Sept)					
1. Left on	71	1,228	15.6	7.9	5.7
2. Pulled off	73	621	6.8	3.4	1.4
3. Cut off	66	601	5.9	4.5	1.8
4. Decayed off	73	923	6.9	4.6	1.5
Stalham (2 Oct)					
1. Left on	96	2,429	27.7	3.2	1.2
2. Pulled off	78	1,466	14.3	2.3	1.1
3. Cut off	100	1,750	12.7	2.2	1.3
4. Decayed off	96	2,170	26.7	2.2	0.9
Clacton (8 Oct)					
1. Left on	148	2,930	14.8	3.2	1.9
2. Pulled off	144	1,066	0.2	0.1	0.0
3. Cut off	147	1,191	0.6	0.2	0.0
4. Decayed off	150	1,530	0.2	0.0	0.0

The varieties were Solairo (Newport and Stalham) and Pronto (Clacton).
Planting dates were 29 December (Newport), 2 February (Stalham) and 12 December (Clacton).

Table 12: **Effect of paint treatments on tomato stem botrytis - Norfolk, 16 July 1996.**

Treatment	Mean no. treated lesions per plot	Mean % of treated lesions			
		Limited	Spreading	Girdling	Sporing
1. Untreated	55.7	98.8	1.2	0.0	34.3
2. Benlate + Actipron	68.0	96.8	2.4	0.8	1.1
3. Vinegar	53.7	97.2	2.8	0.0	12.0
Significance	NS	NS	NS	NS	NS
SED (4 df)	-	-	-	-	-

Table 13: **Effect of paint treatments on stem botrytis - Norfolk, 19 August 1996.**

Treatment	Mean no. treated lesions per plot	Mean % of treated lesions			
		Limited	Spreading	Girdling	Sporing
1. Untreated	117.7	69.3	28.4	2.4	27.9
2. Benlate + Actipron	147.3	83.3	12.7	3.9	8.7
3. Vinegar	129.7	75.2	23.0	1.8	20.3
Significance	NS	0.08	*	NS	**
SED (4 df)	-	4.51	4.21	-	3.05

Table 14: **Effect of paint treatments on plant death - Norfolk, 1996.**

Treatment	Mean no. dead plants/row			
	16 July	6 August	19 August	10 September
1. Untreated	0.0	1.0	0.7	2.3
2. Benlate + Actipron	0.3	1.7	2.3	5.0
3. Vinegar	0.7	0.7	1.3	2.0
Significance	NS	-	NS	-
SED (4 df)	-	-	-	-

Table 15: ***In situ* leaf bioassay to evaluate fungicides for the control of *B. cinerea* conducted after the fifth spray application on 28 September 1996.**

Treatments	Index of Botrytis Leaf Infection (0-100)
Untreated control	56.6 (58.0)
Rovral WP	20.0 (25.0)
Integrated programme	30.0 (24.7)
Scala	33.3 (34.5)
Amistar	16.7 (15.8)
Jonk	16.7 (18.3)
Scomrid Limb aerosol*	63.3 (56.9)
Jet 5	79.9 (83.4)
Significance	(***)
SED (11 df)	(11.67)
LSD (5%)	(25.68)

* Equivalent to the untreated as no treatments applied to the crop at this assessment date.

Figures in parentheses refer to means adjusted to account for non-orthogonal variation.

Table 16: **Detached leaf bioassay conducted after the sixth spray to evaluate the efficacy of fungicide treatments against *B. cinerea* on 11 October 1996.**

Treatments	Mean Lesion Diameter (mm)
Untreated control	7.8 (2.1)
Rovral WP	4.5 (1.2)
Integrated programme	0.8 (0.4)
Scala	5.7 (1.6)
Amistar	2.3 (1.0)
Jonk	0.0 (0.0)
Scomrid Limb aerosol*	- (-)
Jet 5	8.3 (2.0)
Significance	(*)
SED (90 df)	(0.60)
LSD (5%)	(0.99)

Figures in parentheses refer to square root transformation of mean values.
SED and significance levels are approximate due to data not being fully normally distributed.

* This treatment was not included, as the aerosol treatment was not applied to leaf tissue.

Table 17: ***In vitro*** **assessment of the efficacy of 'Scomrid Limb' aerosol using detached stem segments artificially inoculated with *B. cinerea.***

Treatments	% Nodes with *B. cinerea* lesions (31.10.96)	
	- Parafilm	+ Parafilm*
Uninoculated control	0.0	0.0
Uninoculated control + Scomrid Limb aerosol	-	0.0Δ
Inoculated control	62.0	68.0
Inoculated, pre-treatment with Scomrid Limb aerosol	-	0.0
Inoculated, post-treatment with Scomrid Limb aerosol	-	44.0

There are no significant differences between either of the inoculated control values, nor between the inoculated control and post-treatment with Scomrid Limb values.

* Parafilm was used to wrap around the node following inoculation to aid infection.

Δ No symptoms of phytotoxicity were recorded with this treatment.

Table 18: Assessment of mean leaf area and leaf length on 17 October 1996.

Treatments	Mean Leaf Length (cm)	Mean Leaf Area (cm^2)
Unsprayed control	36.9 (37.6)	231.8 (241.0)
Rovral	37.5 (37.1)	288.9 (286.4)
Integrated programme	37.9 (37.7)	268.7 (265.8)
Scala	37.0 (37.1)	239.1 (235.5)
Amistar	35.3 (35.9)	213.1 (222.3)
Jonk	36.9 (36.4)	250.6 (248.1)
Scomrid Limb aerosol	37.6 (37.4)	256.5 (253.5)
Jet 5	37.4 (37.5)	259.8 (256.2)
Significance	(NS)	(NS)
SED (11 df)	(-)	(-)
LSD (5%)	(-)	(-)

Figures in parentheses refer to means adjusted to account for non-orthogonal variation.

Table 19: Assessment of mean number of stem lesions of *B. cinerea* at crop termination on 15 November 1996.

Treatments	Mean No. of Stem Lesions/Plant	Index of Stem Lesions Severity (0-100)
Unsprayed control	1.6 (1.6)	6.6 (6.5)
Rovral	1.4 (1.4)	6.4 (6.2)
Integrated programme	1.8 (2.0)	8.1 (8.8)
Scala	1.2 (1.2)	7.3 (7.2)
Amistar	0.6 (0.6)	3.0 (2.7)
Jonk	0.6 (0.6)	2.3 (2.1)
Scomrid Limb aerosol*	- (-)	- (-)
Jet 5	1.7 (1.6)	9.8 (9.5)
Significance	(NS)	(NS)
SED (9 df)	(-)	(-)
LSD (5%)	(-)	(-)

* Assessment of this treatment not carried out as no treatments applied.

Figures in parenthesis refer to means adjusted to account for non-orthogonal variation.

Table 20: Mean plant height at termination of the experiment on 15 November 1996.

Treatments	Plant Height (m)
Unsprayed control	7.8 (7.8)
Rovral WP	7.9 (8.0)
Integrated programme	7.8 (7.8)
Scala	7.7 (7.7)
Amistar	7.7 (7.6)
Jonk	8.0 (8.0)
Scomrid Limb aerosol*	- (-)
Jet 5	8.0 (7.9)
Significance	(NS)
SED (9 df)	(-)
LSD (5%)	(-)

* Assessment of this treatment not carried out as no treatments applied.

Figures in parentheses refer to means adjusted to account for non-orthogonal variation.

Table 21: Mean marketable yield of tomato fruit during the experimental period.

Treatments	Marketable Yield (kg/plot)	Marketable Yield (kg/m^2)
Unsprayed control	99.5	23.1
Rovral	98.6	22.8
Integrated programme	98.8	22.8
Scala	95.8	22.2
Amistar	97.9	22.7
Jonk	96.6	22.4
Scomrid Limb aerosol*	103.8	24.0
Jet 5	101.3	23.4
Significance	NS	NS
SED (11 df)	-	-
LSD (5%)	-	-

* This treatment was effectively untreated as the aerosol treatment was not applied.

Discussion

Experiments 1-5 (1996)

Decaying fruit trusses left attached to stems were confirmed as an important source of stem botrytis. In a crop of Saporo with severe botrytis, 17% of fruit trusses were associated with botrytis stem rot by 23 August. In three other crops where botrytis was less common 1.2 and 5.7 % of fruit trusses were associated with botrytis stem rot by late September.

Assuming 20 empty fruit trusses per plant and an even distribution of rotting fruit trusses amongst plants, 5% of fruit trusses with an associated botrytis stem rot is equivalent to one botrytis stem lesion on every plant. Empty fruit trusses will become an increasingly important source of botrytis stem rot as a season progresses and the number and age of empty trusses increases. Control of botrytis infection on fruit trusses will be particularly important in long-season crops and nurseries where fruit truss infection occurs early in a season. Further work is needed to investigate the biology of fruit truss rotting and factors which may influence the susceptibility of empty fruit trusses to infection by *B. cinerea* (eg variety, age and physiological state (colour) of fruit truss).

Fruit truss removal was found to reduce the occurrence of botrytis stem rot in a crop where a severe botrytis epidemic developed. The incidence of botrytis stem lesions was reduced by up to 60% when decaying fruit trusses were pulled off, compared with leaving them attached to stems.

Different methods of fruit truss removal differed in their effectiveness in reducing stem botrytis. Regularly pulling-off fruit trusses as they turned brown was most effective. Pulling-off or cutting-off all fruit trusses every 2 weeks was less effective. Cutting-off fruit trusses with secateurs was the least effective treatment. Detailed examination of stems at positions where fruit trusses had been removed revealed an association between occurrence of truss stubs and development of stem botrytis. Cutting of trusses with secateurs left the greatest number of stubs (19% of sites) and a high proportion of them (31%) developed botrytis. Pulling-off naturally senescing trusses left relatively few stubs and few of them (17%) developed botrytis. It would appear that pulling-off decaying fruit trusses was the most effective treatment in reducing stem botrytis because it left the least number of stubs for *B. cinerea* to colonise. It is suggested that fruit truss (and leaf) stubs are a particularly favourable site for botrytis development because of the presence of damaged or senescent tissue. Possibly fruit truss removal with secateurs was particularly ineffective in reducing botrytis in part because of the crushing damage caused to fruit truss stubs.

Although removal of fruit trusses as they begin to turn brown was found to be the most effective method of preventing stem botrytis, it is increasingly difficult in practice to find and remove decaying trusses in long-season layered crops, as the bundle of layered stems increases. Regular removal of old fruit trusses, from early in the season is probably a more practical option. Whenever truss removal is done, it is recommended that care is taken to ensure no stubs are left.

A programme of six fungicide sprays applied to stems from 21 June to 3 September reduced the number of attached fruit trusses developing botrytis by 25% at an interim assessment on 23 August (Table 6), although no difference was detected at a final assessment on 10 September. Similarly, application of fungicides to stem wounds after fruit truss removal appeared to result in a 25% reduction in number of stem lesions in August (Table 1), but no difference was detected at the end of the trial. The difficulty in achieving good spray coverage of stems and fruit trusses, because of shading by leaves, may have explained in part this relatively poor control. Also, isolates of *B. cinerea* taken from this crop in August were found to be resistant to iprodione at 2 mg/l, suggesting that the two Rovral sprays in the programme may have given reduced control.

Observation of fruit truss decay indicates that a high incidence of fruit truss browning does not necessarily result in a high incidence of fruit truss and stem botrytis. The incidence of brown fruit trusses in early October at Clacton and Stalham was 14.8 and 27.7% respectively, while the incidence of fruit trusses visibly infected by botrytis was 3.2% in both crops. At Walpole (where botrytis was severe), 24.7% of old fruit trusses were brown and more than 14% were visibly infected by botrytis. These observations suggest that the botrytis inoculum level in a green house may be important in determining the proportion of old fruit truss which become infected by botrytis.

Both vinegar and Benlate + Actipron when applied to small (non-girdling) stem lesions reduced subsequent sporulation on these sites. A reduction in sporulation may reduce epidemic development in a crop. Probably more important is the observation that Benlate + Actipron also significantly reduced the number of limited lesions developing into spreading or girdling lesions (46% reduction between May and August). Vinegar had a smaller effect (20% reduction). These results indicate that treatment of botrytis stem lesions when they are small should delay the rate at which lesions develop to cause stem girdling and plant death, and consequently help to maintain crop yield.

Experiment 6 (1996)

Whilst much effort was expended in the 1996 evaluation to ensure botrytis established successfully in the trial area, these efforts were largely unsuccessful. Numerous repeated, artificial inoculation studies were conducted during the time course of the study yet very few of the tagged inoculations led to visible stem lesions. This result is not too dissimilar to the experience in the experiment conducted during 1995. It would appear that there are other over-riding factors influencing the development of this disease and further studies to elucidate conditions and mechanisms for infection with this pathogen would be well worthwhile.

Fortunately, results for the *in situ* and detached leaf studies yielded useful information in the relative performance of the fungicides evaluated and the results, in general, conformed to those generated in 1995. Interestingly, whilst the incidence of stem infection in 1996 was lower than in 1995 the overall performance of the fungicides was poorer. This can, to some extent, be accounted for by the isolate of *B. cinerea* used which was insensitive to carbendazim and moderately tolerant to iprodione. However, there should be no cross-resistance to other fungicide groups and the performance of pyrimethanil, azoxystrobin and diethofencarb should not have been adversely affected.

In general the fungicides most effective in the 1995 evaluation performed best in 1996 and no marked phytotoxicity symptoms were recorded.

In view of the results obtained in this two year study and in recognition of the difficulties controlling botrytis currently being encountered commercially it is recommended that one or more fungicides be pursued for On or Off-Label Approval.

Diethofencarb + carbendazim (Jonk) whilst promising, does not currently have a UK approval on any edible crop and therefore cannot be progressed further at this stage without manufacturer support. Both pyrimethanil (Scala) and azoxystrobin (Amistar) now have an approved use in the UK and these appear to be the most promising candidate materials against botrytis from both these trials and others conducted elsewhere. It should be noted that whilst pyrimethanil will be largely specific against botrytis azoxystrobin is a 'broad-spectrum' protectant fungicide and is likely to be active against other target pathogens such as powdery mildew, *Cladosporium*, *Didymella* and *Phytophthora* blight in the tomato crop. This latter product should be given priority if only one fungicide is to be pursued.

Discussions are now underway with the manufacturers and with appropriate support it is anticipated that On or Off-Label Approval for one or more of these products will be forthcoming in due course.

Finally, the apparent lack of development of stem disease following artificial inoculation on wounded stems or trusses in the crop *in situ* warrants further investigation. In some crops (eg strawberries) *B. cinerea* can reside in a latent form though whether a similar phase occurs in tomato is uncertain. It is recommended that further studies should be undertaken with this important pathogen in the tomato crop to further elucidate the disease etiology. This work, if supported financially by MAFF, would provide effective underpinning 'strategic' science for the more applied HDC projects.

Conclusions

1. Empty fruit trusses left attached to stems can be an important infection route leading to stem botrytis.

2. Removal of old fruit trusses reduced stem botrytis originating by this route. Regular crop inspection and pulling-off of all fruit trusses which had begun to turn brown was the most effective method.

3. Truss removal must be done flush to the main stem to be effective. When a projecting stub was left at fruit truss removal, up to 39% of these stubs became infected by botrytis. Under some circumstance routine truss removal could increase the incidence of stem botrytis.

4. Stem botrytis can also arise at sites of leaf removal. The incidence of stem botrytis was three times more common where a stub was left at leaf removal, compared with sites where leaves were removed flush to the main stem.

5. Application of six high-volume fungicide sprays at 2-week intervals from 21 June, to old fruit trusses, resulted in a 25% reduction in the incidence of fruit trusses with associated stem botrytis on 23 August, but no effect was detected at the end of the trial.

6. Application of six high-volume fungicide sprays at 2-week intervals from 21 June, to stem wounds left after pulling-off fruit trusses, resulted in a 25% reduction in the number of stem botrytis lesions on 19 August, but no effect was detected at the end of the trial.

7. Treatment of small botrytis lesions on stems with Benlate + Actipron or vinegar reduced subsequent spore production from these lesions.

8. Treatment of small botrytis lesions on stems with Benlate + Actipron or vinegar reduced the rate at which lesions progressed into spreading and girdling lesions, where the disease is more likely to cause yield loss. Benlate + Actipron was more effective than vinegar.

9. Artificial inoculation of *B. cinerea* was largely unsuccessful in establishing stem infection in a layered experimental tomato crop.

10. Novel fungicides with contrasting modes of action successfully reduced the low incidence of botrytis stem lesions which occurred in the crop at termination.

11. None of the fungicides evaluated caused visible phytotoxicity symptoms when applied repeatedly to an experimental crop of tomatoes.

12. Control of botrytis was relatively poor using Rovral where an isolate with reduced sensitivity to iprodione was used.

13. Further studies are required to evaluate programmed sprays to determine the most effective methods to integrate the novel chemicals into existing spray programmes without increasing the risk of resistance development and at the same time maintaining efficacy.

14. Important base-line sensitivity studies are required for *B. cinerea* and it is recommended that this should form the basis of a routine monitoring programme for fungicide resistance.

15. On or Off-Label Approval should be sought for the use of pyrimethanil (Scala) and azoxystrobin (Amistar) for use on tomatoes in the UK. Discussions are underway with the manufacturers in this respect.

16. The relative lack of stem lesion development following extensive inoculation procedures has undoubtedly revealed a gap in our knowledge of this ubiquitous pathogen. Fundamental studies are required to determine the potential latency of botrytis in the tomato crop and the influence of different isolates for causing aggressive lesions.

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