Project Title:	Further investigations of onion downy mildew and leaf wax.
Project number:	FV 277a
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Report:	Final Report
Previous report:	None
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Location of project:	Laboratory, field and glasshouse facilities at HDRA, Ryton Organic Gardens, Coventry. Field sites in Lincs. and Norfolk.
Project coordinator:	P. Rayns, G's Growers Ltd, Hainey Farm, Barway, Ely, Cambs, CB7 5TZ.
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Keywords:	Field vegetables, fungicides, onions, *Allium cepa*, downy mildew, *Peronospora destructor*, disease, leaf wax

The results and conclusions in this report are based on experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

Authentication

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

Project leader:

Steven J Roberts
Project Leader
Plant Health Solutions

Signature ....

Report authorised by:

Tom Will
Consultant
VCS

Signature .... Date ....

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Grower Summary

Headline

- Onion cultivars differed in their leaf wax properties when measured with a new methanol-water 'test kit'; high leaf wax levels were associated with low levels of downy mildew for cultivars with the same level of tissue resistance. This kit is now being used in the HDC NIAB onion variety trials and can be used by growers.
- New onion cultivars from Bejo and Nickerson with claimed resistance to downy mildew showed negligible disease in the field.
- In a typical spray programme, herbicides had the biggest deleterious effect on leaf wax, suggesting that herbicide applications should be minimised in order to preserve leaf wax levels and enhance resistance to downy mildew.
- Two adjuvants (LI-700 and Silwet) have a protectant effect on downy mildew infection, but this effect is short-lived and unlikely to have any impact on disease levels in field crops.

Background and expected deliverables

Downy mildew of onions is caused by the Phycomycete 'fungus' *Peronospora destructor*. Despite advances in forecasting programs and recent fungicide approvals, the disease remains a continuing problem, resulting in an intensive and expensive fungicide program, and despite which, localised aggressive attacks still occur.

The leaf cuticle presents the primary barrier to infection for many plant pathogens. In onions, the leaf cuticle is covered with a (structured) layer of wax particles making it hydrophobic (i.e. water repellent). In order to initiate infection, fungal spores must first land on, and stick to the leaf and then penetrate the wax layer and the cuticle beneath.

Many pesticides are formulated with wetting agents or mixed with adjuvants in order that the chemical does not simply run off and sticks to the target. Necessarily these additives affect the surface properties of the plant (i.e. the wax layer) and by damaging the primary barrier to infection may reduce a plant's structural resistance to disease.

In a previous HDC project (FV 264, Roberts and Poths 2005) the efficacy of sterilants and novel products for the control of both fungal and bacterial onion diseases was examined. The results suggested that intensive fungicide spray programmes may not give the expected levels of disease control and that the cost of sprays may exceed the benefits in terms of reduced disease levels. Observations of the foliage during the trial suggested that this may be a result of de-waxing of mature leaves leading to increased susceptibility to disease at a period when the plant has declining natural resistance, particularly if cuticle penetrants are added. Un-published research on neck rot in Australia (pers. comm., D. Metcalf, DPIWE, Tasmania) supports this hypothesis.

In order to understand the effects of different sprays on leaf wax, it was first necessary to have some way of measuring it. So, as a first step towards understanding the inter-relationship between leaf wax and downy mildew infection, HDC project FV 277 (Roberts 2006) examined methods to assess leaf wax/surface properties and the effects of wax removal/adjuvants on downy mildew infection.

Samples of different varieties of bunching onions from the same field showed differences in leaf wax values which correlated with levels of downy mildew infection. In glasshouse experiments onion plants were treated with wetters/adjuvants, and in other ways, to manipulate wax levels. Chemical stripping of wax with chloroform or mechanical removal of wax tended to increase downy mildew (incidence and severity). However, the most surprising observation was that pre-treatment with two adjuvants (LI-700 and Silwet) appeared to inhibit downy mildew infection.

This project represents a continuation of the earlier project (FV 277) and also provides additional supporting data to work examining new products for downy mildew control (FV 189c, Richardson 2007).

The aim of this project was to increase understanding of the inter-relationship between pesticide sprays, leaf wax and downy mildew infection and thereby identify spray programmes which minimise adverse effects on leaf wax and increase the level of disease control. Specifically the objectives were to:

1. Compare the leaf wax / surface characteristics of different onion cultivars.
2. Examine the effects of individual pesticide sprays on leaf wax.
3. Evaluate leaf wax in fungicide efficacy trials (linked to FV 189c).
4. Investigate apparent protectant effect of certain adjuvants.

Summary and main conclusions

A leaf-wax 'test kit' was successfully used for routine assessment of onion leaf wax. The test kit could potentially be used in the field but would be most conveniently used in a kitchen or lab-type set up.

Twelve onion cultivars were grown in field plots and their leaf wax levels assessed at three key growth stages (6TL, early bulbing, late bulbing). The cultivars showed consistent differences in leaf wax levels. Wax levels were inversely correlated with levels of downy mildew for cultivars with similar levels of tissue resistance (Figure 1). New onion cultivars from Bejo and Nickerson with claimed resistance to downy mildew were included in the trial and showed negligible disease in the field.

Leaf wax levels in a crop receiving a standard spray programme were compared with an equivalent area of the same crop which did not received any sprays. Wax levels were greater in the un-sprayed area on four out of five assessment dates (Figure 2), but the differences were statistically significant only at the first assessment following several herbicide applications. Thus it would seem that repeated herbicide applications had a deleterious effect on leaf wax.

The 'test kit' was used to assess wax levels at key growth stages in each treatment in a fungicide trial (HDC FV 189c). The treatments did not give rise to any major differences in leaf wax levels (Figure 3), although levels tended to be higher in Agrowax treated plots (Treatment P) and lower in plots treated with Fandango (Treatment D) or an alternating treatment programme which included Folio Gold, Amistar, Invader, Bravo 500, Silwet L-77 (Treatment I).

The two adjuvants (LI-700 and Silwet) have a protectant effect on downy mildew infection, but this effect is short-lived and unlikely to have any impact on disease levels in field crops (Figure 4).

Financial benefits

This and the previous project on the interactions of leaf wax and downy mildew in onions has highlighted the importance of an integrated approach to pest, disease and weed management. The complexities of the interactions between crop protection products, the physical leaf surface, plant physiology and the target organisms means that great care is required to avoid adverse side-effects. For example, it is possible that direct-drilling of onions, which necessitates greater use of herbicides, leads to increased susceptibility to downy mildew at critical early stages in the development of disease epidemics, which in turn leads to a greater dependence on fungicides for successful cropping. Improved weed control with fewer, well-targeted herbicides could reduce the subsequent need for repeated fungicide sprays.

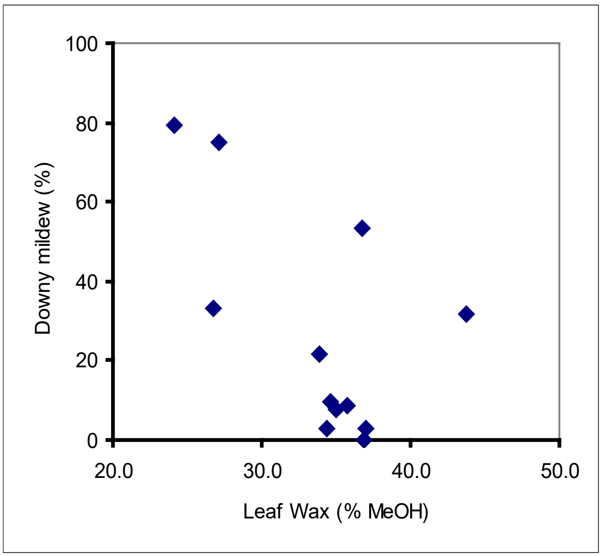

Figure 1. Relationship between downy mildew incidence and leaf wax levels in twelve onion cultivars.

Action points for growers

- Maintaining good levels of leaf wax will maximise field resistance to downy mildew; growers should be aware of the impact of any pesticide applications on leaf wax levels.
- Growers should consider selecting varieties with the highest levels of leaf wax to maximise ‘field’ resistance to downy mildew. Leaf wax will be assessed in 2007 HDC NIAB onion variety trials and reported to members.
- New resistant cultivars from Bejo and Nickerson appear to have high levels of tissue resistance to downy mildew.
- Growers should seek to minimise herbicide applications with fewer well targeted herbicides, in order to preserve leaf wax levels and enhance resistance to downy mildew.
- Growers can obtain and use the newly developed methanol/water leaf wax test kit from Steve Roberts at s.roberts@planthealth.co.uk.

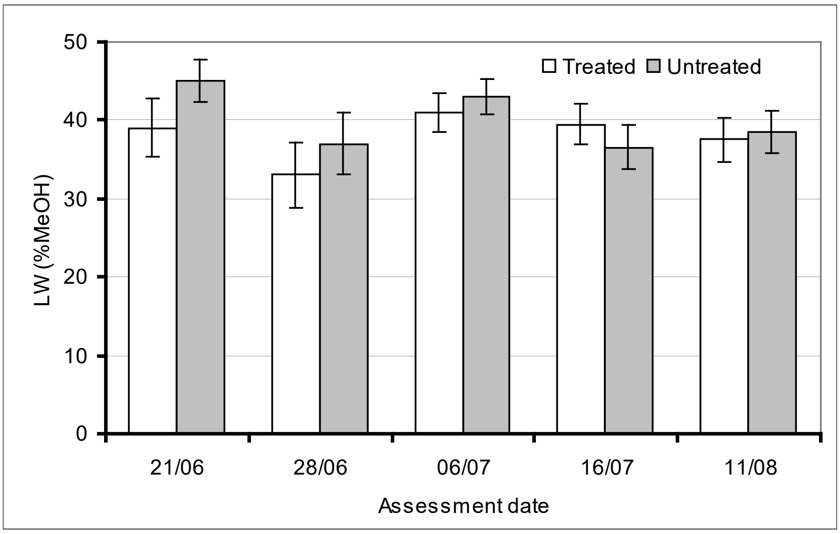

Figure 2. Leaf wax levels in treated and un-treated areas of an onion crop receiving a conventional spray programme. Error bars represent the approximate standard errors of the means.

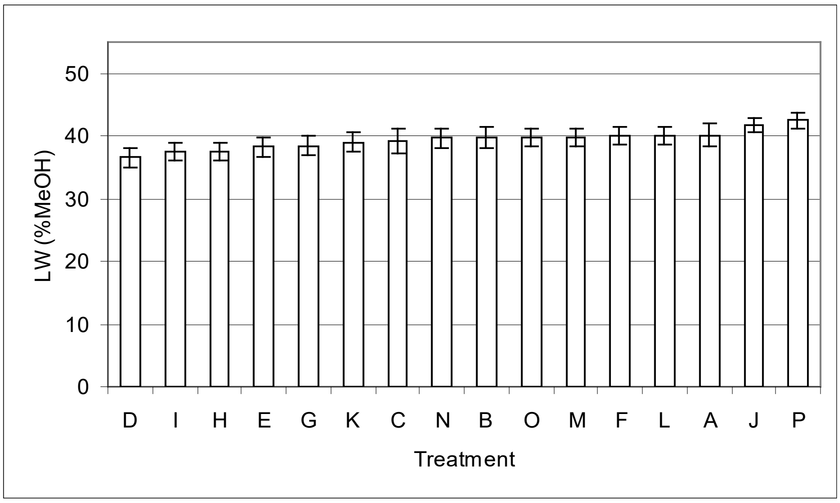

Figure 3. Mean leaf wax levels (LW) for each fungicide treatment in trials at two sites. For details of treatment codes see main text. Error bars represent approximate standard errors of the means.

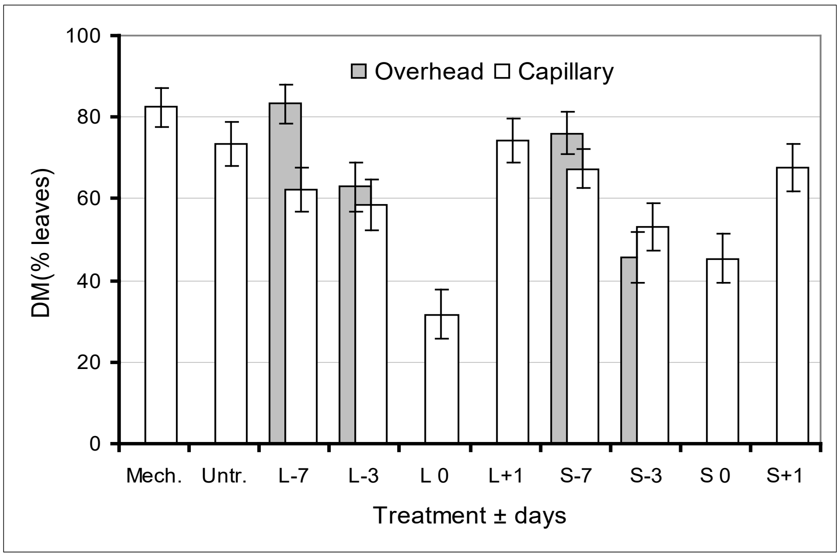

Figure 4. Effect of the adjuvants, LSI-700 (L) and Silwet L-77 (S), watering (overhead and capillary), and timing of application pre- and post-inoculation on downy mildew (DM) incidence in onions. Controls: Mech. – mechanical wax removal, Untr. – untreated. Error bars represent the approximate standard errors of the %.

Science Section

Introduction

Downy mildew of onions is caused by the Phycomycete ‘fungus’ *Peronospora destructor*. Despite advances in forecasting programs and recent fungicide approvals, the disease remains a continuing problem, resulting in an intensive and expensive fungicide program, and despite which localised aggressive attacks still occur. A typical fungicide program costs £290/ha (total industry costs of over £2.5 million). Yield losses directly attributed to downy mildew can reach 30% but are more typically in the order of 10%. As every 1% loss in yield equates to £100/ha, total losses may amount to £1000/ha. However there may also be additional losses from rejected bulbs due to progressive downy mildew and/or secondary bacteria and in extreme circumstances the crop becomes unmarketable.

The rapid loss of active ingredients as EU directive 91/414 is implemented has led to the more frequent use of those fungicides which remain available. This reduced number of active ingredients is having an adverse effect on resistance management and increasing the likelihood of detectable residues. Growers generally utilise an intensive fungicide sequence commencing with protectant fungicides followed by protectant/systemic mixes once disease risk is perceived or predicted. Spray programmes tend to start at the 4-6 true leaf stage and continue to the harvest interval limits.

Several of the currently remaining fungicides are persistent and can leave residues (e.g. Iprodione, Chlorothalonil and Mancozeb); whereas public pressure is increasingly leading to demand for produce containing zero residues.

The leaf cuticle presents the primary barrier to infection for many plant pathogens. In many plant species the leaf cuticle is covered with a (structured) layer of wax particles making it hydrophobic (i.e. water repellent). In order to initiate infection, fungal spores must first land on, and stick to the leaf and then penetrate the wax layer and the cuticle beneath.

Many pesticides are formulated with wetting agents or mixed with adjuvants in order that the chemical does not simply run off and sticks to the target. Necessarily these additives affect the surface properties of the plant (i.e. the wax layer) and by damaging the primary barrier to infection may reduce a plant’s structural resistance to disease.

Neinhuis and Barthlott (Neinhuis and Barthlott 1997) discuss factors influencing the water-repellency of plant surfaces which is determined by the micro-structure of the leaf surface and the hydrophobicity of the cuticular wax. A method for assessing the ‘wetability’ of leaf surfaces using methanol-water mixes has been published (Wagner *et al.* 2003) and assessment of hydrophobicity by measurement of contact angles has been described by Beattie and Marcell (2002). These latter authors also discuss the relative merits of different methods for quantifying cuticular leaf wax and its properties.

In a previous HDC project (FV 264, Roberts and Poths 2005) the efficacy of sterilants and novel products for the control of both fungal and bacterial onion foliage and bulb diseases was examined. Three compounds were examined in the trials: grapefruit extract, Jet 5 and Vitafect and were applied alone or in addition to a standard spray programme. The results suggested that such intensive fungicide spray programmes may not give the expected levels of disease control and that the cost of sprays may exceed the benefits in terms of reduced disease levels. Observations of the foliage during the trial suggested that this may be a result of de-waxing of mature leaves leading to increased susceptibility to disease at a period when the plant has declining natural resistance, particularly if cuticle penetrants are added. Un-published research on neck rot in Australia (pers. comm., D. Metcalf, DPIWE, Tasmania) supports this hypothesis.

Altering the fungicide spray programmes to significantly reduce the number and complexity of sprays may reduce foliage de-waxing and maintain foliage integrity and a crop's natural defences. This in turn may reduce the total amount of fungicides applied and is likely to have real benefits in cost saving as well as reducing the risk of pesticide residues in the harvested crop.

In order to understand the effects of different sprays on leaf wax, it was first necessary to have some way of measuring it. So, as a first step towards understanding the inter-relationship between leaf wax and downy mildew infection, HDC project FV 277 (Roberts 2006) examined methods to assess leaf wax/surface properties and the effects of wax removal/adjuvants on downy mildew infection.

Three methods considered appropriate for routine assessment of leaf surface properties were compared: measurement of contact angles of water droplets; measurement of wetability by methanol-water mixes; and the qualitative crystal violet dip test (which is used to assess wax cover in peas prior to herbicide applications). Results from all three methods were significantly correlated, but both methanol and especially crystal violet lacked discrimination at the low (wettable) end of the scale (i.e. when contact angles were less than 90 to 100°). Nevertheless, from the practical point of view, the methanol method was selected as the easiest to interpret, most suitable for routine use and most appropriate to develop for use by growers.

Regardless of the method, comparisons of leaves from the same plant and parts of an individual leaf indicated that older leaves were more wettable than younger leaves, and that leaf tips were more wettable than leaf bases. Samples of different varieties of bunching onions from the same field showed differences in leaf wax values which correlated with levels of downy mildew infection.

In glasshouse experiments onion plants were treated with wetters/adjuvants, and in other ways, in an attempt to manipulate wax levels. The plants were then sprayed with downy mildew spores. Chemical stripping of wax with chloroform or physical removal of wax tended to increase downy mildew (incidence and severity). However, the most surprising observation was that pre-treatment with two adjuvants (LI-700 and Silwet) appeared to inhibit downy mildew infection, reducing the effectiveness of the inoculum by 25 and 80 times. It is possible that these effects are rather short-lived (adjuvants were applied only hours before spraying with downy mildew spores) as the effect (with LI-700) was not reported in the field trials done as part of HDC project FV 189b.

This project represents a continuation of the earlier project (FV 277) and will also provide additional supporting data to work examining new products for downy mildew control (FV 189c, Richardson 2007).

The aim of this project was to increase understanding of the inter-relationship between pesticide sprays, leaf wax and downy mildew infection and thereby identify spray programmes which minimise adverse effects on leaf wax and increase the level of disease control. Specifically the objectives were:

1. Compare the leaf wax / surface characteristics of different onion cultivars.
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Materials and Methods

Leaf Wax Measurement

The 'Methanol' method which had been selected and developed in the previous project (FV 277) was used for all measurements. A detailed description of the method is given in the standard operating procedure (see Appendix). The procedure was based on that described by Wagner, *et al.* (2003). Onion leaf sections were held at an angle of 25° to horizontal and droplets of increasing concentration of methanol-water mixes were dropped onto the surface in a standard way. The concentration at which the solution ceased to bead and run off was recorded as the critical value.

Preparation of Test kit

A 'test kit' consisting of a ready-prepared series of methanol-water solutions in dropper bottles, together with an 'angle-finder' was put together and distributed to field trials officers. As the dropper bottles produced a drop size which is larger than that used in the experimental set-up used in the previous project, results for the two systems were compared.

Comparison of cultivars

Seeds of 12 different onion cultivars (see Table 3), selected to represent different types and perceived susceptibilities to down mildew, were obtained from their respective suppliers. Plants were raised from seed sown on 27 March in '308' module trays of Bulrush Modular Organic compost. Trays were maintained in a glasshouse with the following set day/night temperatures: minimum 17/14°C and venting at 21/17°C. Watering was by overhead sprinkler irrigation. Plants received a liquid feed (Nugro) and were moved outside to harden off on 09 May and transplanted into plots by hand on 17 May.

Each cultivar was planted in a single plot on the field trial area at Ryton Organic Gardens. Each plot consisted of one bed (1.8 m) of 4 rows by 2.5 m. Plants were spaced approx 6.7 cm apart within the rows to give 15 per m of row and a total of 148 plants per plot/cultivar. Plots were weeded by hand and irrigated as necessary according to normal management practices at Ryton.

At key growth stages (6 TL, 09 June; early bulbing, 07 Jul; late bulbing, 03 Aug) five or six typical plants were sampled from each plot. To avoid damaging the leaf surfaces, roots were cut just below the soil surface with a trowel and whole plants gently transferred to large polythene bags by holding the leaf tips or bulb base. The leaf wax/surface properties of the middle 10-15 cm of up to two leaves (5, 5 and 9, 9/10 and 13/14 at each assessment date respectively) was measured using the "methanol" method (see above).

In addition to the leaf wax measurements originally planned, levels of natural infection with downy mildew (incidence) were also recorded.

Effect of standard programme on leaf wax

A 14.6 ha commercial crop of cv. Red Baron was direct-drilled in Thetford, Norfolk, on 20 March and treated/sprayed with pesticides according to normal practice by the grower. An area of the crop (10 m x 12 m) was left un-sprayed throughout the season. This area was hand-weeded to ensure that that crop growth and micro-climate were not affected (which could have an impact on leaf wax development), whilst at the same time minimising mechanical damage.

Plants from both areas were sampled on five occasions throughout the growing season (from 21 June to 11 Aug). Samples were collected from three to four locations in each of the sprayed/unsprayed areas at each assessment. To avoid damaging the leaf surfaces, roots were cut just below the soil surface with a trowel and whole plants gently transferred to large polythene bags by holding the leaf tips or bulb base. The leaf wax/surface properties of the middle 10-15 cm of up to two leaves at each assessment date was measured using the "methanol" method.

Detailed records of pesticide applications were maintained.

Fungicide trials

Leaf wax evaluation field kits and instruction sheets were prepared for use by technical officers involved in monitoring of fungicide efficacy trials done at two sites (Lincs. and Norfolk) as part of HDC project FV 189c (Richardson 2007). To ensure consistency between sites, the use of the kits was demonstrated to trials officers.

The treatments applied are summarised in Table 1. Full details of the trials can be found in the report (FV 189c, Richardson 2007). The trials examined the efficacy of a number of individual compounds. mixtures and alternating programmes against downy mildew, and consisted of three replicate plots of 16 different treatments at each of two sites.

At key growth stages (~6 TL; early bulbing; late bulbing) five plants were sampled from each treatment. To avoid damaging the leaf surfaces, roots were cut just below the soil surface with a trowel and whole plants gently transferred to large polythene bags by holding the leaf tips or bulb base. The leaf wax/surface properties of the middle 10-15 cm of up to two leaves on each plant was measured using the "methanol" method (see above).

Protectant effect of certain adjuvants

Downy mildew inoculum was produced and maintained, and conidia harvested as described in the previous project (FV 277, Roberts 2006)

Onion plants of cv. Red Barron were used for both maintenance of inoculum and inoculations. Plants were raised in the glasshouse from seed sown in compost in P40 trays and then potted on into 7 cm pots (3-4 plants per pot) .

Table 1. Summary of treatments applied in onion fungicide spray trials at two sites (HDC project FV 189c)

Code	Treatment
A	Water control
B	Mechanically de-waxed control
C	Dithane NT
D	UK958 (Fandango)
E	EXP 11120A (Infinito)
F	A13978D
G	A4111B
H	Folio Gold and Amistar *alternating with* Invader and Bravo 500
I	Folio Gold, Amistar and Silwet L-77 *alternating with* Invader, Bravo 500 and Silwet L-77
J	Folio Gold and Grevit *alternating with* Invader and Grevit
K	KIF 230, CERF025 and Amistar *alternating with* Amistar, Invader and Bravo 500
L	Sonata and Amistar *alternating with* Invader and Bravo 500
M	Dithane NT and Bravo 500
N	Dithane NT and Bravo 500 *followed by* Folio Gold and Invader
O	Silwet L-77
P	Agrowax

For further details of application timings and product rates see HDC report FV 189c.

Batches of plants were sprayed with either Silwet L-77 (0.15% v/v) or LI-700 (0.5% v/v) at seven and three days prior to, on the day of, and one day after inoculation with downy mildew conidia. Of the plants sprayed prior to inoculation, half were maintained with sub-irrigation via capillary matting. and half were maintained with overhead sprinkler irrigation. Additional batches of plants were left untreated as controls, or wax was mechanically removed by gentle rubbing by hand just prior to inoculation.

Plants were inoculated in late afternoon by spraying with a suspension of downy mildew conidia prepared in distilled water. Spraying was done using a DeVilbiss atomiser. The numbers of conidia in the inoculum was estimated by direct counting of the number of spores in a 10 µl drop using a light microscope and dark-field illumination. Following inoculation, plants were placed in a humid tent overnight to maintain close to 100% RH and encourage germination of conidia. Plants were removed from the humid tent the following morning and maintained in the glasshouse on capillary matting for a further 14 d, when disease levels were assessed.

Disease assessment

Foliar symptoms of downy mildew are difficult to determine in the absence of sporulation. Therefore, prior to disease assessment, inoculated plants which had passed through the latent period (i.e. $\geq$ 14 d after inoculation) were placed in the humid tent overnight to induce sporulation. Disease assessments were then done on the basis of visible downy mildew sporulation. The presence/absence of sporulating downy mildew lesions was recorded for each leaf on each plant (disease incidence).

Microscopic investigations

A. Plants were sprayed with the adjuvants as above or left un-sprayed. After the spray had dried, leaves were removed and placed horizontally in a humid chamber. Drops of spore suspension were then placed on the leaf surfaces and left to incubate at room temperature (approx. 20°C). Sections of leaves where the drops had been placed were then excised and observed microscopically.

B. Drops (10µl) of Silwet and LI-700 solutions and distilled water were placed on clean microscope slides and allowed to dry. Drops (10µl) of aqueous suspensions of downy mildew conidia were then placed on the same places on the slides. These were then incubated in a humid chamber and observed microscopically at intervals over a period of several hours.

C. Equal volumes (5 µl) of wetter solutions or distilled water and spore suspensions were mixed together on clean microscope slides and then incubated in a humid chamber in the dark and observed microscopically at intervals over a period of several hours.

Data and Statistical analysis

Data for the leaf-wax assessments were recorded in Excel™ spreadsheets, and then summarised and analysed using the generalised linear modelling (GLM) procedures of Genstat (Payne *et al.* 2005). Prior to analysis, the critical values of methanol concentration were re-scaled to proportions of the maximum possible value. The model was specified with binomial error distribution and a probit link function.

For each data set, a series of nested models was fitted and used to generate an accumulated analysis of deviance. This was then used to assess the relative importance of terms in the model on the basis of mean deviance ratios, as suggested by McCullagh & Nelder (1989). Estimates of means and their standard errors were obtained using the PREDICT directive of Genstat, with standard errors based on the residual mean deviance for the appropriate model stratum.

Effects of standard programme on leaf wax

In order to investigate the effects of the different spray histories on leaf wax levels throughout the season, a number of variables were calculated which were considered to have potential as predictors of leaf wax levels: e.g. numbers of, and cumulative numbers of, spray applications, herbicides, fungicides, insecticides, other sprays, since previous recording date, and in the two weeks prior to recording. The predictive value of these variables was assessed using the RSEARCH procedure of Genstat (Payne *et al.* 2005), models were selected using the Akaike information criterion (Aic).

Results

Comparison of cultivars

There were significant effects of leaf age, cultivar and leaf number (Table 2) with leaf age having the biggest effect. The effect of leaf number is probably an artefact of the precise timing of sampling. There were clear differences in leaf wax levels between cultivars (Table 3) which could be divided into three distinct groups. Most cvs. were in the middle group with average (age-adjusted values of 34-37%), one cv. was higher (Barito 44%), three cvs. lower (Sturon, VCS, Wellington, 24-27%).

There were large differences in the incidence of downy mildew between cultivars (Table **3**) (although some caution should be attached to this as plots were not replicated), with the newly released resistant cultivars (Santaro, NIZ 37-1001, BGS 237) showing negligible or no disease. These cultivars were also the latest to fallover. A plot of downy mildew levels against leaf wax (Figure 1) indicated a correlation between low leaf wax levels and high downy mildew but with cultivars falling into two distinct groups. It seems likely that these two groups represent different levels of tissue resistance which effectively modify the impact of leaf wax levels. However it should be noted that despite having lowest leaf wax levels and highest levels of DM cv. VCS gave the highest yield and largest bulbs.

During the trial many cultivars suffered severe infection with *Stemphylium* and this appeared to be a major cause of premature leaf loss.

Effect of pesticide sprays on leaf wax

The spray history of the treated area is shown in Table 4.

Separate analysis of the effect of treatment (i.e. sprayed v. un-sprayed) at each recording date, indicated that although the mean wax levels were lower in the treated area at four out of the five assessments (Figure 2), this was statistically significant only at the first recording date (21 July).

Table 2. Accumulated analysis of deviance for comparison of leaf wax levels between cultivars.

Change	d.f.[1]	Deviance	Mean deviance	Deviance ratio	
Age	1	22.14	22.14	134.23	*
CV	11	13.79	1.25	7.60	*
Age.CV	11	2.25	0.20	1.24	
Leaf	4	4.42	1.11	6.70	*
Age.Leaf	3	1.05	0.35	2.12	
CV.Leaf	22	6.10	0.28	1.68	
Samp	167	24.94	0.15	0.91	
Residual	73	12.04	0.17		
Total	292	86.74	0.30		

[1] Degrees of freedom.
* Indicates terms considered significant.

Table 3. Summary of mean leaf wax levels (critical % methanol) for each cultivar, together with natural incidence of downy mildew (DM). Values were obtained as predictions from a fitted model[1]

Cultivar	Supplier	Leaf Wax[2] (% MeOH) Mean	s.e.[3]	DM (%)
Arthur	Steve Howe Seeds	35.0	2.0	8
Barito	Nickerson	43.8	1.4	32
BGS 237 F1	Bejo	36.9	1.8	0
Hyfort	Bejo	35.8	1.9	9
Hytech	Bejo	33.8	1.9	21
NIZ 37-1001	Nickerson	34.3	1.9	3
Red Baron	Bejo	36.7	1.9	53
Santaro	Nickerson	37.0	1.9	3
Sprinter	Syngenta	34.7	2.0	10
Sturon	HDRA	27.2	2.3	75
VCS 12	VCS	24.1	2.0	79
Wellington	Syngenta	26.7	2.0	33

[1] Fitted terms: Age, CV, Leaf
[2] Larger values = more leaf wax
[3] Standard error.

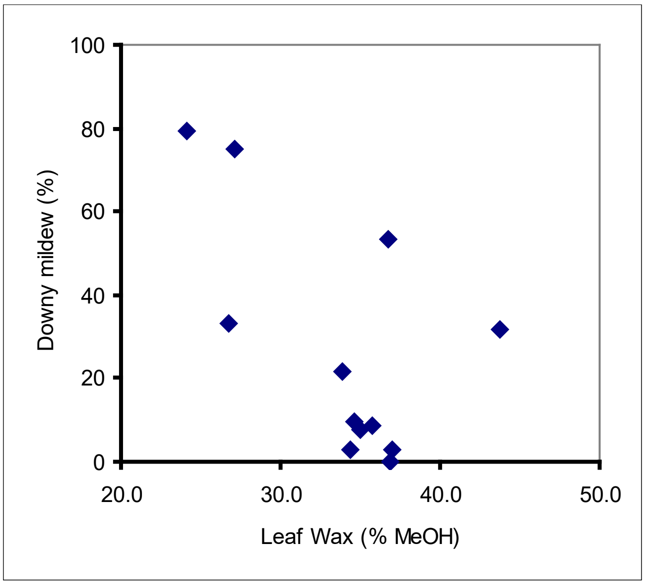

Figure 1. Relationship between downy mildew incidence and leaf wax levels in twelve onion cultivars.

The stepwise multiple regression analysis of factors influencing leaf wax levels was problematical due to the high degree of correlation between some of variables. Therefore separate analyses were done for sets of mutually exclusive variables. The RSEARCH procedure in Genstat attempts to find the best 1, 2, 3, 4…etc. term models based on minimising the selection criterion (Aic) and also provides significance levels for each term in the model. A detailed summary of the best 1, 2, 3, and 4 term models for each of three sets of variables is given in Appendix I.

The analyses indicated that leaf age had biggest effect on leaf wax levels; it was highly significant and always the most significant term regardless of the number of other terms included. Overall the models providing the best explanation of leaf wax levels were the two term models which include a leaf age term and either the number of other compounds or number of herbicides applied since planting / previous recording:

$$\mathrm{Probit}(LW/50) = 1.58 - 0.31Age - 0.17Oth$$

$$\mathrm{Probit}(LW/50) = 1.57 - 0.31Age - 0.06Herb$$

where *LW* is the critical leaf wax value (% methanol), *Age* is the relative leaf age (Growth Stage minus leaf number) and *Herb, Oth* are the number of herbicides, other products (i.e. not herbicides, insecticides or fungicides) applied since planting or previous recording date, respectively.

Fungicide trials

The accumulated analysis of deviance is shown in Table 5**.** The relative size of the mean deviance values gives an indication of the importance of each term in the model. It is clear that Site and leaf Age had the biggest effects on leaf wax levels: values were greater at Sleaford than at East Harling and declined with leaf age. Comparison of Treatment with the Age.Site.Treat interaction term indicates that overall there was no significant effect of Treatment on leaf wax levels in the trials. However, Treatment P (Agrowax) had a tendency to higher levels and Treatments D and I lower levels (Figure 3).

Protectant effect of certain adjuvants

The accumulated analysis of deviance is shown in Table 6 and indicated highly significant effects of Treatments and Timing, and smaller effects of watering and a Timing.Watering interaction The results are summarised in the bar chart in Figure 4. Treatment with either Silwet L-77 or LSI-700 gave a significant reduction in the level of downy mildew. The effect was greatest when the adjuvants were applied on the same day as inoculation and decreased as the interval between treatment and inoculation increased. Applying the treatment one day after inoculation had little effect, the effect was also reduced by overhead irrigation between treatment and inoculation.

Microscopic observations did not reveal any structural differences in conidia amongst the treatments.

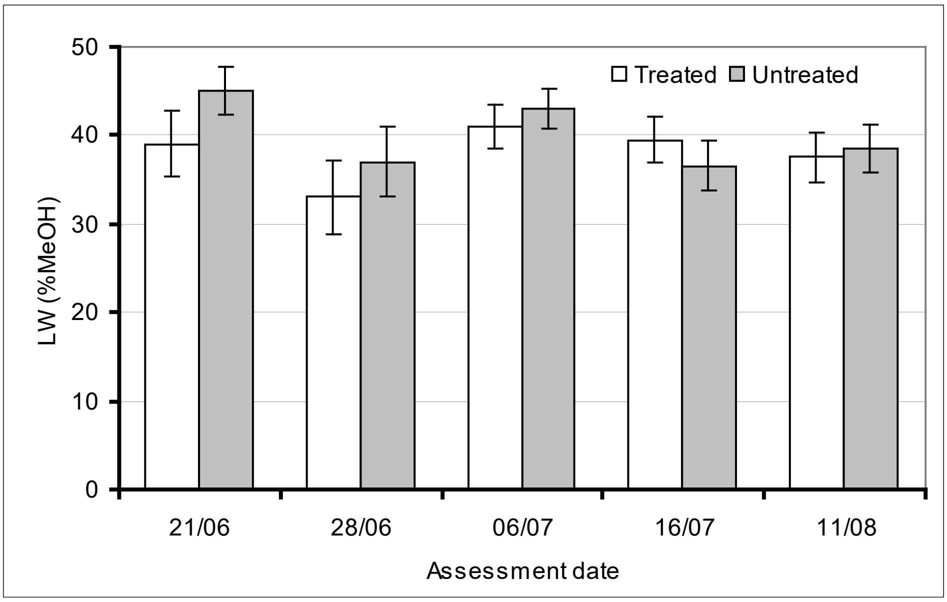

Figure 2. Leaf wax levels in treated and un-treated areas of an onion crop receiving an conventional spray programme. Error bars represent the approximate standard errors of the means.

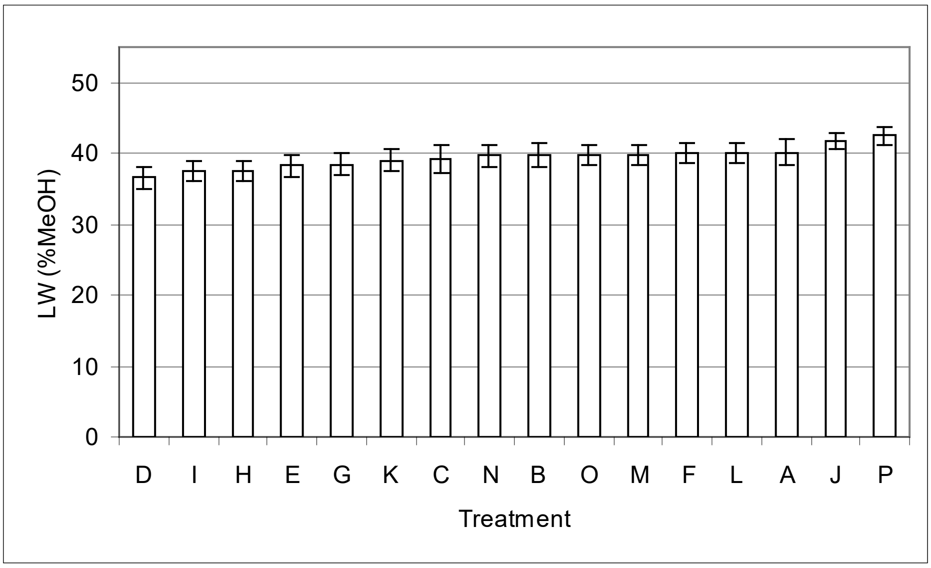

Figure 3. Mean leaf wax levels (LW) for each fungicide treatment in trials at two sites. For details of treatment codes see Table 1. Error bars represent approximate standard errors of the means.

Table 4. Conventional spray programme in treated area, recording dates and approximate growth stages.

Date	Products applied	GS (TL)
20/03/06	Crop drilled	
05/04/06	Ramrod Flowable, Stomp 400 SC	
26/04/06	Ramrod Flowable, Stomp 400 SC	
06/05/06	Alpha Chlorpyriphos	
13/05/06	Tortril	
16/05/06	Mg Sulphate, Mn Sulphate, Activator 90	
03/06/06	Totril, Starane 2	
12/06/06	Totril, Fortrol	
21/06/06	**Leaf wax recorded**	6
27/06/06	Aramo, Dithane DF, Invader, Mn Sulphate	
28/06/06	**Leaf wax recorded**	7
29/06/06	Totril	
06/07/06	**Leaf wax recorded**	8
07/07/06	Folio Gold, Invader, Decis, Bortrac	
16/07/06	**Leaf wax recorded**	8.5
17/07/06	Amistar, Invader, Liquid Copper, Decis	
01/08/06	Invader, Decis Protech	
11/08/06	**Leaf wax recorded**	9
11/08/06	Amistar, Invader, Decis	
22/08/06	Folio Gold, Invader, Liquid Copper	
27/08/06	Source	

Table 5. Analysis of deviance for the effect of treatment on onion leaf wax levels in fungicide spray trials at two field sites.

Change	d.f.	deviance	mean deviance	deviance ratio	
Site	1	8.59	8.59	47.98	*
Age	1	11.03	11.03	61.66	*
Treatment	15	4.59	0.31	1.71	
Age.Treat	15	2.62	0.17	0.97	
Site.Treat	15	2.44	0.16	0.91	
Age.Site	1	0.37	0.37	2.06	
Age.Site.Treat	15	11.81	0.79	4.40	
Residual	720	128.84	0.18		
Total	783	170.29	0.22		

[1] Degrees of freedom.
* Indicates terms considered significant.

Table 6. Analysis of deviance for the effect adjuvants (LI-700 and Silwet L-77), their application timing, and watering system on downy mildew in onions

Change	d.f.[1]	deviance	mean deviance	deviance ratio	approx. χ^2 pr.[2]
Timing	3	22.75	7.58	7.58	<.001
Treatment	3	43.61	14.54	14.54	<.001
Water	1	3.78	3.78	3.78	0.052
Timing.Water	1	3.95	3.95	3.95	0.047
Treat.Water	1	3.38	3.38	3.38	0.066
Timing.Treat	3	4.70	1.57	1.57	0.195
Residual	43	42.44	0.99		
Total	55	124.63	2.27		

[1] Degrees of freedom.
[2] χ^2 probability, values ≤ 0.05 are considered significant

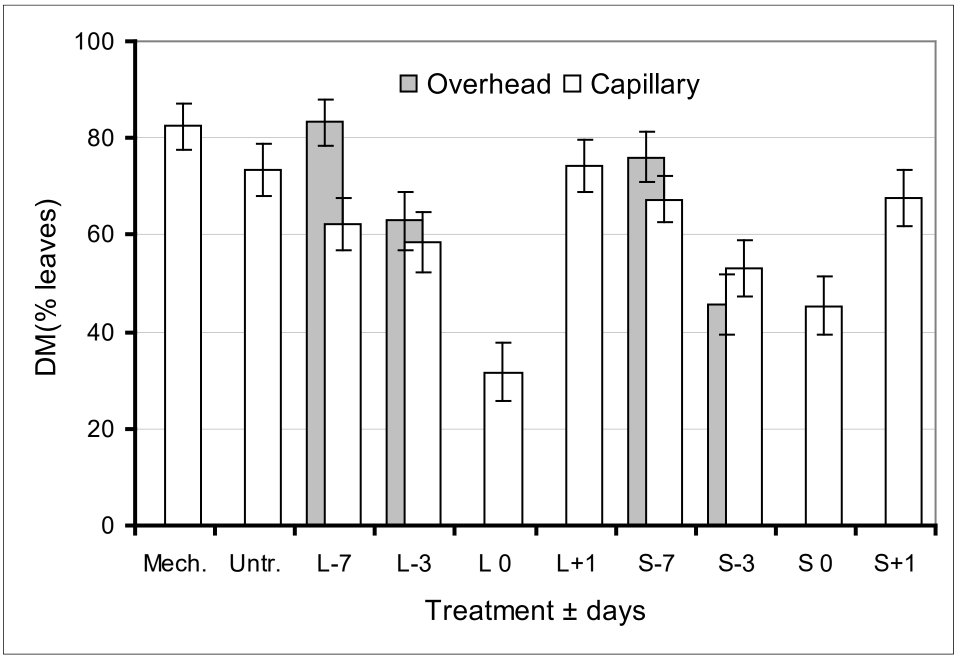

Figure 4. Effect of the adjuvants, LSI-700 (L) and Silwet L-77 (S), watering (overhead and capillary), and timing of application pre- and post- inoculation on downy mildew (DM) incidence in onions. Controls: Mech. – mechanical wax removal, Untr. – untreated. Error bars represent the approximate standard errors of the %.

Discussion

The methanol-water method for assessing leaf wax/surface properties developed in the previous project (HDC FV 277, Roberts 2006) was successfully applied in three trials which examined differences in leaf wax between onion cultivars, the effects of a conventional spray programme on leaf levels, and the effects of different fungicides.

The comparison of cultivars confirmed the preliminary observations of the previous project, that there are differences in leaf wax levels between cultivars and that these differences give rise to different levels of DM in the field. It was also clear that there are different levels of tissue resistance amongst cultivars and that both tissue resistance and wax levels interact to determine susceptibility to DM in the field.

The comparison of wax levels in a crop receiving a conventional spray programme and an un-sprayed area of the same crop showed a significant difference only at the first assessment; this assessment, at the six true leaf stage, followed several herbicide applications. This difference was visually apparent in the field, with the un-sprayed area appearing to be greener and healthier. Later in the season, although there was a general tendency for higher leaf wax levels in the unsprayed area, the differences were not statistically significant. The greater variability in values obtained in the sprayed area is probably a reflection of variation in the distribution of pesticides both within and between individual leaves and plants, and may have contributed to a lack of significant differences. Thus it would appear that the greatest impact on leaf wax levels is from herbicides and that the benefits (in terms of pest and disease control) of later fungicide and insecticide applications may outweigh any negative impacts on leaf wax levels. Clearly growers should minimise herbicide applications in order to avoid adverse effects on leaf wax.

In the fungicide trials, there were no major, consistent effects of treatments on leaf wax levels. Most of the treatments gave similar overall leaf wax levels. However, and perhaps not surprisingly, the Agrowax treatment gave the highest leaf wax values and two treatments, Fandango and an alternating treatment programme which included Folio Gold, Amistar, Invader, Bravo 500 , Silwet L-77 gave the lowest values.

The un-expected results with the adjuvants LI-700 Silwet L-77 obtained in the previous project were confirmed. Both compounds gave significant reductions in the incidence of DM when applied before inoculation with DM conidia. The results indicated that the effect was a short-lived (≤ 3 days) protectant effect and was reduced by overhead irrigation (i.e. would not be rain-fast). Given the short-term nature of the effect it is perhaps not surprising that neither compound has had any impact on disease levels in the field trials conducted in HDC projects FV189b and 189c. The precise mechanism for the effect remains to be elucidated.

Conclusions

- A leaf-wax 'test kit' was successfully used for routine assessment of onion leaf wax.
- Onion cultivars showed consistent differences in leaf wax levels.
- Wax levels correlated with levels of downy mildew for cultivars with similar levels of tissue resistance.
- Growers should consider selecting varieties with the highest levels of leaf wax to maximise 'field' resistance to downy mildew.
- New onion cultivars from Bejo and Nickerson with claimed resistance to downy mildew had showed no or little disease in the field.
- Repeated herbicide applications had a deleterious effect on leaf wax.
- Growers should seek to minimise herbicide applications in order to preserve leaf wax levels and enhance resistance to downy mildew.
- The different fungicide treatments in project FV 189c did not give rise to any major differences in leaf wax levels, although levels tended to higher in Agrowax treated plots and lower in plots treated with Fandango (Treatment D) or an alternating treatment programme which included Folio Gold, Amistar, Invader, Bravo 500 , Silwet L-77 (Treatment I).
- The two adjuvants (LI-700 and Silwet) have a protectant effect on downy mildew infection, but this effect is short-lived and unlikely to have any impact on disease levels in field crops.

Recommendations for further work

- Assessment of leaf wax could be provide useful information in any future onion variety trials.

Acknowledgements

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Appendix I

Output from RSEARCH procedure

Appendix Table 1a. Significance of terms in the best four one, two, three and four term models for the effect of spray history on leaf wax levels, identified by the RSEARCH procedure of Genstat. Variable subset: number since start or previous recording.

Aic[1]	C_p[2]	d.f.[3]	Leaf Age	Number since start/previous recording				
				Apps.	Herb.	Fung.	Insect.	Others
Best subsets with one term								
88.4	8.4	2	<0.001	-	-	-	-	-
115.5	35.5	2	-	-	-	-	0.265	-
116.9	36.9	2	-	-	-	0.581	-	-
117.0	37.0	2	-	-	-	-	-	0.625
Best subsets with Two terms								
84.9	4.9	3	<0.001	-	-	-	-	0.024
85.6	5.6	3	<0.001	-	0.036	-	-	-
87.3	7.3	3	<0.001	0.095	-	-	-	-
90.3	10.3	3	<0.001	-	-	0.778	-	-
Best subsets with Three terms								
85.7	5.7	4	<0.001	-	-	0.285	-	0.013
86.7	6.7	4	<0.001	0.023	-	0.114	-	-
86.8	6.8	4	<0.001	-	0.73	-	-	0.362
86.8	6.8	4	<0.001	0.734	-	-	-	0.120
*Best subsets with f*our terms								
83.1	3.1	5	<0.001	0.012	0.006	-	0.014	-
83.7	3.7	5	<0.001	-	0.002	0.016	0.022	-
84.2	4.2	5	<0.001	0.002	-	0.010	0.036	-
85.7	5.7	5	<0.001	-	-	0.084	0.164	0.005

[1] Akaike information criterion.
[2] Mallow's C_p statistic.
[3] Degrees of freedom.

Appendix Table 1b. Significance of terms in the best four one, two, three and four term models for the effect of spray history on leaf wax levels, identified by the RSEARCH procedure of Genstat. Variable subset cumulative number since planting.

Aic[1]	C_p[2]	d.f.[3]	Leaf Age	Cumulative number of				
				Apps.	Herb.	Fung.	Insect.	Other
Best subsets with one term								
88.4	8.4	2	<0.001	-	-	-	-	-
116.6	36.6	2	-	-	-	0.484	-	-
116.6	36.6	2	-	-	-	-	0.495	-
116.7	36.7	2	-	-	-	-	-	0.506
Best subsets with two terms								
90.0	10.0	3	<0.001	-	0.547	-	-	-
90.0	10.0	3	<0.001	-	-	-	0.552	-
90.2	10.2	3	<0.001	-	-	-	-	0.699
90.2	10.2	3	<0.001	0.754	-	-	-	-
Best subsets with three terms								
88.5	8.5	4	<0.001	-	-	0.073	0.062	-
89.0	9.0	4	<0.001	-	0.1	-	0.101	-
89.0	9.0	4	<0.001	0.101	0.086	-	-	-
89.0	9.0	4	<0.001	0.102	-	-	0.087	-
Best subsets with four terms								
84.7	4.7	5	<0.001	0.008	0.006	0.014	-	-
89.3	9.3	5	<0.001	-	-	0.186	0.08	0.305
89.4	9.4	5	<0.001	-	0.31	0.215	0.107	-
89.5	9.5	5	<0.001	0.344	-	0.232	0.092	-

[1] Akaike information criterion.
[2] Mallow's C_p statistic.
[3] Degrees of freedom.

Appendix Table 1c. Significance of terms in the best four one, two, three and four term models for the effect of spray history on leaf wax levels, identified by the RSEARCH procedure of Genstat. Variable subset: number in the two weeks prior to recording.

Aic[1]	C_p[2]	d.f.[3]	Leaf Age	Number in the previous 2 weeks				
				Apps.	Herb.	Fung.	Insect.	Other
Best subsets with one term								
88.4	8.4	2	<0.001	-	-	-	-	-
117.0	37.0	2	-	-	-	-	0.631	-
117.1	37.1	2	-	-	-	0.701	-	-
117.2	37.2	2	-	-	-	-	-	0.816
Best subsets with two terms								
88.1	8.1	3	<0.001	-	0.154	-	-	-
89.4	9.4	3	<0.001	-	-	0.342	-	-
90.1	10.1	3	<0.001	-	-	-	-	0.639
90.2	10.2	3	<0.001	0.73	-	-	-	-
Best subsets with three terms								
88.7	8.7	4	<0.001	0.246	0.072	-	-	-
89.8	9.8	4	<0.001	-	0.227	0.554	-	-
89.9	9.9	4	<0.001	-	0.145	-	0.666	-
90.1	10.1	4	<0.001	-	0.173	-	-	0.79
Best subsets with four terms								
89.1	9.1	5	<0.001	0.102	0.03	-	0.217	-
89.7	9.7	5	<0.001	0.166	0.092	0.333	-	-
90.2	10.2	5	<0.001	0.196	0.061	-	-	0.522
91.8	11.8	5	<0.001	-	0.376	0.687	0.978	-

[1] Akaike information criterion.
[2] Mallow's C_p statistic.
[3] Degrees of freedom.

Standard Operating Procedures

Number	Title	Filename
04-016	Leaf wax – methanol-water test kit	04-016v1-0 Leaf Wax test kit.pdf