Vegetable Consultancy Services Ltd

# FINAL CONTRACT REPORT

## To understand the interactions of leaf wax and downy mildew in onions

**FV 277**

**by**

***Steven J Roberts***

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| **Project Title:** | To understand the interactions of leaf wax and downy mildew in onions |
| **Project number:** | FV 277 |
| **Project leader:** | Dr Steven J Roberts<br>Vegetable Consultancy Services Ltd<br>The Finches, Cake Street<br>Old Buckenham<br>Attleborough<br>Norfolk NR17 1RU<br><br>Tel. 01926 498214<br>E-mail: s.roberts@planthealth.co.uk |
| **Report:** | Final Report |
| **Previous report:** | none |
| **Key staff:** | Dr Steven J Roberts (Plant Health Solutions): plant pathologist, experimental design and statistical analysis, report preparation.<br>Hugh Poths (VCS): project liaison, dissemination of project results.<br>Tom Will (VCS): project consultant. |
| **Location of project:** | Laboratory and glasshouse facilities at HDRA, Ryton Organic Gardens, Coventry. |
| **Project coordinator:** | P. Rayns, G's Growers Ltd, Hainey Farm, Barway, Ely, Cambs, CB7 5TZ. |
| **Date project commenced:** | 01 June 2005 |
| **Date completed:** | 31 March 2006 |
| **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 ............................................................ Date ...........................................

**Report authorised by:**

Tom Will
Consultant
VCS

Signature ............................................................ Date ...........................................

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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.

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# Grower Summary

## Headline

- In field and glasshouse experiments the removal of leaf wax gave increased infection by downy mildew.
- Assessing wetability with methanol-water mixes was the most repeatable and suitable method to routinely assess onion leaf wax properties (and therefore susceptibility to downy mildew).
- In glasshouse experiments, pre-treatment of leaves with two adjuvants appeared to reduce downy mildew infection and warrants further investigation.

## Background and expected deliverables

Despite advances in forecasting programs and recent fungicide approvals, downy mildew of onions remains a recurrent problem, demanding an intensive and expensive fungicide program, and, despite which, localised aggressive attacks still occur. A typical fungicide program costs £290/ha (total industry costs £2,610,000). 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 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 approved 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. There has therefore been a need to trial eradicant fungicides/bactericides that do not leave detectable residues in onions.

The leaf cuticle presents a plant’s first line of defence against infection by many pathogens. In onions and many other plant species it is covered with a water-repellent layer of wax. In order to initiate infection, fungal spores must first land on, and stick to the leaf, then penetrate the wax layer and cuticle beneath.

Many pesticides are formulated with wetting agents or mixed with adjuvants to stop the active ingredient simply running off and ensure it 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 could reduce a plant’s structural resistance to disease.

Previous HDC project FV 264 examined the efficacy of sterilants and novel products for the control of both fungal and bacterial onion foliage and bulb diseases. 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 was 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. Research in Australia supports this hypothesis (D. Metcalf, pers. comm.).

Altering spray programmes so that de-waxing of leaves is minimised could help a crop's natural defences, and increase the effectiveness of those sprays that are applied. In order to understand the effects of different sprays on leaf wax, we first need to have some way of measuring it. Therefore the aim of this project was to examine methods for measuring leaf wax/surface properties, as a first step towards understanding the inter-relationship between leaf wax and downy mildew infection and thereby help to identify spray programmes which minimise adverse effects on leaf wax and increase the level of disease control.

## Summary and main conclusions

A number of methods for assessing leaf wax and surface properties have been described. Some of these require expensive specialised equipment, or do not lend themselves to significant throughput of samples, or have associated health and safety issues. Three methods were considered appropriate for routine assessment of leaf surface properties: 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). These methods were compared by applying them to leaf samples obtained from a number of onion crops.

All of the methods for assessing leaf wax/surface properties provided useful results, and to some extent measured different aspects of the same thing. The three measurements of surface properties were significantly correlated (Table 1).

**Table 1.** Correlation matrix for measures of leaf wax/surface properties.

| Method | Methanol -water | Contact angle | Crystal violet |
|---|---|---|---|
| Methanol-water | 1 | | |
| Contact angle | 0.745 | 1 | |
| Crystal violet | -0.827 | -0.768 | 1 |

degrees of freedom = 39

Crystal violet is perhaps most useful to indicate areas of leaf where wax has been damaged or removed, however it appears to lack discrimination with intact waxed surfaces. It was also messy and difficult to interpret by eye and required image analysis software to obtain useful data. Measurement of contact angles provided fine discrimination over a small scale, but it is probably most suited to laboratory application, requiring a macro-photography set-up and computerised image analysis. The methanol-water method provides a balance between the two methods and is easily interpreted, with values obtained directly. Although it was sometimes difficult to decide on a critical value, it appeared to have the highest level of repeatability amongst the three methods. The methanol-water method is therefore recommended as the most appropriate for routine use and could potentially be developed into a kit for use in the field or a basic field laboratory (e.g. kitchen).

Regardless of the method, comparisons of leaves from the same plant and parts of an individual leaf indicated, perhaps not surprisingly, that older leaves were more wettable and that leaf tips were more wettable than the 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. This observation supports the hypothesis that removal of wax increases susceptibility to downy mildew and suggests that the leaf wax /surface properties of different onion cultivars could provide an indication of their relative levels of field tolerance/resistance. However, it cannot be ruled out that the differences in leaf wax were a result of the downy mildew infection rather than the cause of the differences *per se*.

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, Figs. 1 and 2). However, the most surprising observation was that pre-treatment with two adjuvants inhibited downy mildew, reducing the effectiveness of the inoculum by 25 and 80 times.

We plan to extend this work over the next year by comparing the leaf wax characteristics of different onion cultivars and examining the effects of individual pesticide sprays on leaf wax. We will also evaluate leaf wax at key growth stages in fungicide efficacy trials, and try to gain some further insight into the surprising protectant effect of certain adjuvants.

## Financial benefits

As this project represented the initial phase of a planned two-year project, there are no direct financial benefits. It is anticipated that following a further year's work, growers will be able to use knowledge about the effects of different pesticide sprays on leaf wax to assist in the identification of more effective spray programmes which minimise damage to leaf wax

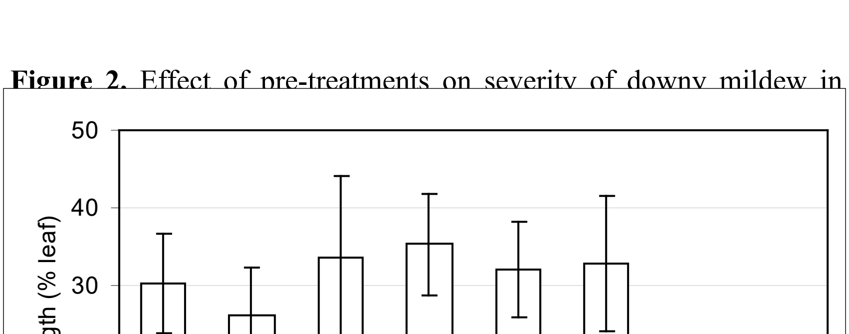
## Action points for growers

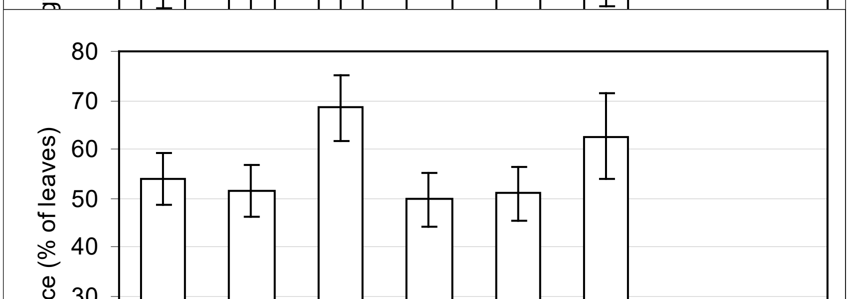
This project represents the initial phase of a planned two year project, therefore it would be premature to make firm recommendations at this stage.

- In the light of the data obtained to date, growers should consider the impact of spraying and other operations on leaf wax levels.

**Figure 1.** Effect of pre-treatments on incidence of downy mildew in onions. Abbreviations: Chl, chloroform; Fing, rubbing with a finger; H I, Headland Intake. The adjuvants (Bond, Headland Intake, LI-700, Silwet) and disinfectant (Jet 5) were sprayed; Chloroform was wiped on with a paper towel; finger involved removing wax by rubbing with a finger. Bars represent the combined results of three experiments, error bars represent approximate standard errors (non-linear model).

**Figure 2.** Effect of pre-treatments on severity of downy mildew in



# Science Section

## Introduction

Despite advances in forecasting programs and recent fungicide approvals, downy mildew of onions remains a recurrent problem, demanding an intensive and expensive fungicide program, and despite which localised epidemics still occur. A typical fungicide program costs £290/ha (total industry costs £2,610,000). 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 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; whereas public pressure is increasingly leading to demand for produce containing zero residues. There has therefore been a need to trial eradicant fungicides/bactericides that do not leave detectable residues in onions. 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.

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 plants structural resistance to disease. 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.

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 currently in progress 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, we first need to have some way of measuring it. So, as a first step towards understanding the inter-relationship between leaf wax and downy mildew infection this project examined methods for measuring leaf wax/surface properties and then carried out some initial (glasshouse) experiments to look at the relationship between leaf wax and downy mildew infection. The project had two specific objectives:

1. *Develop and validate methods for measuring leaf cuticle wax surface properties.*
2. *To test the hypothesis that removal of leaf wax increases the susceptibility of onions to downy mildew infection.*

A number of methods for assessing leaf wax and surface properties have been described. Some of these require expensive specialised equipment, or do not lend themselves to significant throughput of samples, or have associated health and safety issues. Three methods considered appropriate for routine assessment of leaf surface properties were compared: measurement of contact angles of water droplets (Beattie and Marcell 2002); measurement of wetability by methanol-water mixes (Wagner *et al.* 2003); and the qualitative crystal violet dip test (which is used to assess wax cover in peas prior to herbicide applications). These methods were compared.

Glasshouse inoculation experiments were done to test the hypothesis that removal of leaf wax increases the susceptibility of onions to downy mildew infection. Attempts were made to manipulate the leaf wax levels by treatment with several adjuvants and by other methods prior to spray inoculation with downy mildew spores.

## Materials and Methods

### Leaf samples

Leaf samples were collected from commercial onion crops of different ages, with different treatment histories and on a number of occasions. As a result of the relatively late start to the project, the majority of samples were collected from bunching onion crops. Samples were collected from the field in the form of whole plants so that they could be handled by the base without direct contact with the leaf surfaces, thereby avoiding damage. Plants were transported to the laboratory in polythene bags and stored in the fridge until assessment.

### Measurement methods

Detailed descriptions of each of the three methods examined are given in the relevant standard operating procedures (see Appendix) and so will not be given in detail here. However a brief description follows.

*Contact angle*

The procedure was based on that described by Beattie and Marcell (2002) . Sections of leaves were placed on horizontal surface and individual 10 µl droplets of de-ionised water placed on the surface using a pipette. A digital close-up photograph was taken of each drop, and the contact angle estimated using the open-source image analysis software ImageJ (Rasband, 2005) and Contact Angle plug-in.

*Wetability with methanol-water mixes*

The procedure was based on that described by Wagner, *et al.* (2003). 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.

*Crystal violet*

A test kit was obtained from BASF, UK. Leaf sections were dipped into an aqueous solution of crystal violet (1% w/v initially, later reduced to 0.5% w/v) for 10 s, then shaken to remove excess liquid. A digital close-up photograph was taken of each leaf section. The proportion of leaf area covered with crystal violet solution was estimated following cropping and transformation to grey-scale using the open-source image analysis software ImageJ (Rasband, 2005).

**Comparison of methods**

Leaf sections (10-15 cm long) were assessed by each of the three methods. By applying the methods in the order: contact angle, methanol-water, crystal violet; it was possible to assess each section with all three methods. Following photographing for contact angle measurements, water droplets were carefully blotted off using a torn-edge of paper towel. Leaf sections were then mounted for methanol-water, where care was taken to ensure only half of the section was wetted. Crystal violet assessment was then done on the other half of the leaf sections.

**Plants for inoculum production and inoculation**

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

**Maintenance and preparation of downy mildew inoculum**

The methods were based on those described by Gilles *et al.* (2004).

Onion plants showing visible downy mildew symptoms were collected from commercial fields on a number of occasions. These plants were then grown on in pots in the glasshouse and used to provide the initial inoculum.

In order to induce sporulation, infected plants which had passed through the latent period (i.e. at least 14 d since infection) were placed in a high-humidity tent in the glasshouse overnight. The tent consisted of polythene sheeting over a supporting framework on the glasshouse bench (which was covered with capillary matting). An ultrasonic mist unit which generates a fine mist/fog was placed in the tent. The tent was sealed up and the mist unit switched on at around 16:00 h; the tent was opened and the mist unit switched off the following morning at around 09:00 h. Downy mildew spores were then collected from the surface of leaves with visible sporulation using a vacuum device. The device consisted of two un-equal lengths of narrow-diameter glass tubes bent at 90° and inserted through holes in a rubber bung into a glass universal bottle. The longer (inlet) tube was inserted through the bung so that the end was just above the bottom of the bottle and the shorter (outlet) tube was inserted so that the end was just below the bottom of the bung. The outlet tube was connected to a vacuum pump via a flow regulator and a 0.4 µm air-filter (to protect the pump from contamination).

**Table 1.** Summary of pre-treatments applied and downy mildew inoculum concentrations used in glasshouse inoculation experiments

| Pre-treatment[1] | Experiment no. | | |
|---|---|---|---|
| | 844 | 846 | 848 |
| None | ✓ | ✓ | ✓ |
| Bond (0.14%) | ✓ | ✓ | ✓ |
| Chloroform | ✓ | ✓ | ✗ |
| Finger | ✓ | ✓ | ✓ |
| Headland Intake (0.5%) | ✓ | ✓ | ✓ |
| Jet 5 (0.8%) | ✗ | ✗ | ✓ |
| LI-700 (0.5%) | ✓ | ✓ | ✓ |
| Silwet (0.15%) | ✓ | ✓ | ✓ |
| No. of pre-treatments[2] | 1 (0.5 d) | 3 (10, 4, 0.5 d) | 3 (7, 3, 0.5 d) |
| Inocula (spores/ml) | $1.3 \times 10^4$ | $3.3 \times 10^3$ | $6.5 \times 10^4$, $4.8 \times 10^3$, $1.6 \times 10^3$ |

[1] Product concentration in parentheses. The adjuvants (Bond, Headland Intake, LI-700, Silwet) and disinfectant (Jet 5) were sprayed; Chloroform was wiped on with a paper towel; finger involved removing wax by rubbing with a finger.
[2] Time before inoculation in parentheses.

Following collection, spores were either used to inoculate plants the same day or stored in the fridge or freezer for subsequent use.

### Inoculation

Spores were suspended in distilled water and then diluted to obtain appropriate concentrations. The numbers of spores in inocula were estimated by directly counting the number of spores in a 10 µl drop using a microscope. Plants were sprayed with inocula using a deVilbiss atomiser, using 9-10 ml of inoculum for seven pots of (3-4) plants.

### Experimental treatments

Three separate inoculation experiments were done. Batches of onion plants were subjected to seven different pre-treatments (Table 1). The treatments consisted of spraying the plants on one or more occasions with the authorised adjuvants (Bond, Headland Intake, LI-700, Silwet) and disinfectant (Jet 5) at the recommended concentrations, rubbing leaves with a finger to remove/damage leaf wax, and gentle wiping with a chloroform impregnated paper towel. Six of the treatments were the same in all three experiments, but in the third experiment chloroform was replaced with Jet 5.

### Disease assessments

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. > 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), in addition for a subset of plants/pots in each treatment the lesion length and the leaf length was recorded and used to estimate the percentage leaf length affected (severity). The density of sporulation was also recorded on a categorical (0-4) scale.

### Data and Statistical analysis

Data for the leaf-wax assessments were recorded in Excel™ spreadsheets, and summarised, correlations coefficients calculated, and repeatability estimated using Genstat (Payne *et al.* 2003).

Data from each disease assessment were recorded in Excel™ spreadsheets. Data for disease incidence, severity and sporulation density were analysed using the generalised linear modelling (GLM) procedures of Genstat (Payne *et al.* 2003). For disease incidence data, the model was specified with binomial error distribution and a complementary log-log link-function; for severity data (proportion of leaf length covered by lesions), the model was specified with binomial error distribution and logit link function. The sporulation density scores were analysed as ordinal categorical data using a proportional odds model as suggested by McCullagh & Nelder (1989).

In each case, a series of nested models was fitted to the data 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.

## Results

### Comparison of methods

**Table 2.** Correlation matrix for measures of leaf wax/surface properties.

| Method | Methanol -water | Contact angle | Crystal violet |
|---|---|---|---|
| Methanol-water | 1 | | |
| Contact angle | 0.745 | 1 | |
| Crystal violet | -0.827 | -0.768 | 1 |

degrees of freedom = 39

All three measurements of surface properties were significantly correlated (Table 2, Figure 1), although both methanol and especially crystal violet seemed to lack discrimination at the low (wettable) end of the scale (i.e. contact angles < 100°).

Measuring the contact angle provided the most objective and quantitative values. Due to the small drop size it can pick up small scale differences and avoid areas of mechanical damage, but conversely this means that several measurements are needed to provide overall assessment of a leaf section.

Using the methanol-water mixes was the most straightforward with a single overall value obtained for each leaf section, and effectively integrating small-scale variations. However it was sometimes difficult to decide on a cut-off point, leading to an element of subjectivity.

The crystal violet method was messy and it was difficult to get consistent and reliable results. It was also prone to error caused by handling of samples and results were very dependent on how and to what extent the leaf is shaken to remove excess liquid. Results were also difficult to assess visually, requiring photography and image analysis software.

Assessing the repeatability of each of the methods was problematical, due to the nature of the data (percentages and angles) and the larger number of data units for the contact angles. Thus in order to make comparisons data from all of the methods were assumed to follow a normal distribution, and analysis was restricted to only the mid-sections of leaves. On this basis, wetting with methanol-water mixes was the most repeatable method (lowest between plants within sample variance and lowest residual variance, see Table 3) followed by contact angle, with crystal violet the least repeatable.

**Table 3.** Comparison of variance components for each of the onion leaf wax measurement methods. Smaller values indicate greater repeatability.

| Source | Method | | |
|---|---|---|---|
| | Crystal violet | Methanol-water | Contact angle |
| Between plants | 253 | 63 | 756 |
| Between leaves | 918 | 30 | 215 |

Regardless of the method, comparisons of leaves from the same plant and parts of an individual leaf indicated, perhaps not surprisingly, that older leaves tended to be more wettable and that leaf tips were more wettable than the bases. There appeared to be no relationship with the number of previous pesticide sprays applied.

On one occasion, samples of different varieties of bunching onions with different levels of downy mildew infection were obtained from the same field. The leaf wax measurements showed the same trend in values as infection, the lowest values (most wettable) had the highest levels of downy mildew (Table 4).

**Table 4.** Association between downy mildew levels and leaf wax properties for three bunching onion varieties growing in the same field.

| Sample | Downy mildew score (0-4) | Methanol-water | Crystal violet | Contact angle |
|---|---|---|---|---|
| 1 | 0.5 | 36 | 18 | 127 |
| 2 | 3 | 27 | 55 | 100 |
| 3 | 4 | 8 | 71 | 103 |

### Inoculation studies

Data from all three experiments was combined for analysis. Analyses of deviance indicated that treatments had a significant effect on both downy mildew incidence (proportion of leaves affected, Table 5), severity (proportion of leaf length affected, Table 6), and sporulation density (0-4 score, Table 7). The combined results for all three experiments are summarised in Figure 2 and Figure 3 and Table 8. Chloroform, finger and Jet 5 pre-treated leaves tended to have the highest levels of disease indicated by either disease incidence, lesion length or sporulation score. Chloroform and Jet 5 had the highest incidence, chloroform and finger had the highest severity and finger had the highest sporulation density, although in most cases these effects were not statistically significant. The most striking effect, however, was the significantly lower (almost absence of) disease (by all measures) in the Silwet and LI-700 treated leaves.

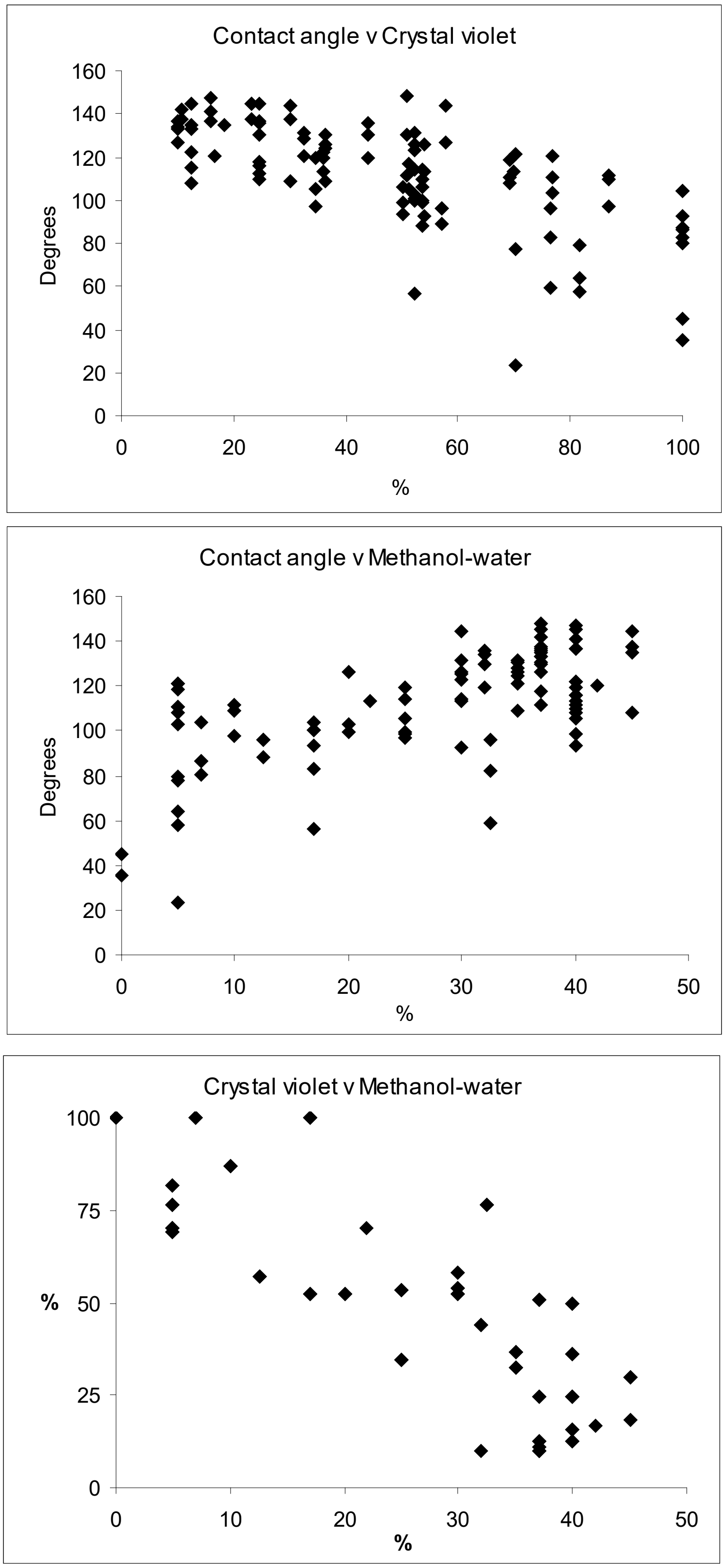



**Figure 1.** Relationships of measurements of onion leaf wax/surface properties made with different methods. Each value represents an individual leaf section.

## Discussion

All of the methods for assessing leaf wax/surface properties provided useful results, and to some extent measured different aspects of the same thing. Crystal violet is perhaps most useful to indicate areas of leaf where wax has been damaged or removed, however it appears to lack discrimination with intact waxed surfaces. It was also difficult to interpret by eye and required image analysis software to obtain useful data. Measurement of contact angles provided fine discrimination over a small scale, but it is probably most suited to laboratory application, requiring a macro-photography set-up and computerised image analysis. The methanol-water method provides a balance between the two methods and is easily interpreted, with values obtained directly. Although it was sometimes difficult to decide on a critical value, it appeared to have the highest level of repeatability amongst the three methods. The methanol-water method is therefore suggested as most appropriate for routine use and could potentially be developed into a kit for use in the field or a basic field laboratory (e.g. kitchen).

The observation of an association between mildew levels and wetability in bunching onions in the same field, supports the hypothesis that removal of wax increases susceptibility to downy mildew. The three crops had a similar spray treatment history so it seems likely that the differences were a result of inherent differences amongst the cultivars. This suggests that the leaf wax /surface properties of different onion cultivars could provide an indication of their relative levels of field tolerance/resistance. However, it cannot be ruled out that the differences in leaf wax were a result of the downy mildew infection rather than the cause of the differences in downy mildew.

The inoculation studies provided some un-expected results and demonstrated that the relationships among leaf wax, surface properties and downy mildew infection are not straightforward. Both physical removal (i.e. by gentle rubbing of leaves with a finger) and chemical removal (with chloroform) had a tendency to give increased levels of downy mildew. However it was difficult to apply either treatment consistently, and this is probably the reason for the lack of statistical significance. In the case of chloroform, there were also problems with phyto-toxicity, so that some of the inoculated leaves had died before disease assessment and were excluded from the analysis. The Silwet treated leaves were visibly the most wettable and, when sprayed over the plants, the inoculum produced an even film of liquid, it was a great surprise therefore when at disease assessment disease symptoms were almost absent. Silwet and LI-700 treatment both tended to give the most wettable leaves, but also had the lowest levels of disease. At present we can only presume that this apparent protectant effect is the result of either toxicity to inhibition of downy mildew spores by the compounds.

It should be noted that in all the inoculation studies, necessarily the plants had been grown and maintained in the glasshouse, both before and after pre-treatment, watering was done by sub-irrigation on capillary matting, and a maximum of only three treatments had been applied. We may consider that these results may not be representative of what may occur in the field situation. Thus if any of the adjuvants had an adverse effect on leaf wax, the absence of overhead watering and weathering effects may have meant that the wax layer remained relatively intact, compared to what may be expected in a field situation. We might therefore expect that effects of treatments which affect the wax would be greater in the field than that observed in these glasshouse experiments.

**Table 5.** Analysis of deviance for the proportion of downy mildew infected leaves (incidence).

| Source | d.f. | deviance | mean deviance | deviance ratio | |
|---|---|---|---|---|---|
| Experiment | 2 | 47.3 | 23.6 | 12.4 | * |
| Treatment | 7 | 215.8 | 30.8 | 16.2 | * |
| Exp.Treat | 11 | 44.4 | 4.0 | 2.1 | |
| Dose | 1 | 99.6 | 99.6 | 52.2 | * |
| Dose.Treat | 6 | 10.7 | 1.8 | 0.9 | |
| Residual | 98 | 187.1 | 1.9 | | |
| Total | 125 | 604.9 | 4.8 | | |

* indicates terms considered significant

**Table 6.** Analysis of deviance for the proportion of leaf length covered with downy mildew (severity).

| Source | d.f. | deviance | mean deviance | deviance ratio | |
|---|---|---|---|---|---|
| Experiment | 2 | 43.2 | 21.6 | 80.7 | * |
| Treatment | 7 | 38.5 | 5.5 | 20.6 | * |
| Exp.Treat | 11 | 5.9 | 0.5 | 2.0 | |
| Residual | 287 | 76.9 | 0.3 | | |
| Total | 307 | 164.4 | 0.5 | | |

* indicates terms considered significant

**Table 7.** Analysis of deviance for the sporulation density score.

| Source | d.f. | deviance | mean deviance | deviance ratio | |
|---|---|---|---|---|---|
| Experiment | 2 | 89.6 | 44.8 | 44.8 | * |
| Treatment | 7 | 115.0 | 16.4 | 16.4 | * |
| Exp.Treat | 11 | 20.9 | 1.9 | 1.9 | |
| Residual | 60 | 68.8 | 1.1 | | |
| Total | 80 | 294.4 | 3.7 | | |

* indicates terms considered significant

**Table 8.** Probabilities of leaves being in a particular sporulation score category. Values represent the combined results from three experiments.

| | Pre-treatment[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Score | None | Bond | Chl | Finger | H I | Jet 5 | LI-700 | Silwet |
| 0 | 0.18 | 0.16 | 0.10 | 0.10 | 0.10 | 0.11 | 0.81 | 0.81 |
| 1 | 0.11 | 0.11 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.08 |
| 2 | 0.21 | 0.21 | 0.17 | 0.17 | 0.17 | 0.18 | 0.06 | 0.06 |
| 3 | 0.29 | 0.30 | 0.33 | 0.33 | 0.33 | 0.33 | 0.04 | 0.03 |
| 4 | 0.20 | 0.22 | 0.33 | 0.34 | 0.34 | 0.31 | 0.01 | 0.01 |

[1] Abbreviations: Chl, Chloroform, H I, Headland Intake. The adjuvants (Bond, Headland Intake, LI-700, Silwet) and disinfectant (Jet 5) were sprayed; Chloroform was wiped on with a paper towel; finger involved removing wax by rubbing with a finger.

## Conclusions

- The methanol-water method is the best method for routine assessment of onion leaf wax /surface properties.
- Removal of leaf wax tended to give increased levels of downy mildew.
- Two adjuvants (LI-700 and Silwet) appeared to have a protectant effect on downy mildew infection.

## Recommendations for further work

- The methanol-water method should be used to assess the effects of different pesticides on leaf wax/surface properties in the field.
- A comparison should be made of the leaf wax/surface properties of a range of cultivars.
- Confirm the apparent protectant effect of the two adjuvants and investigate the mechanism.

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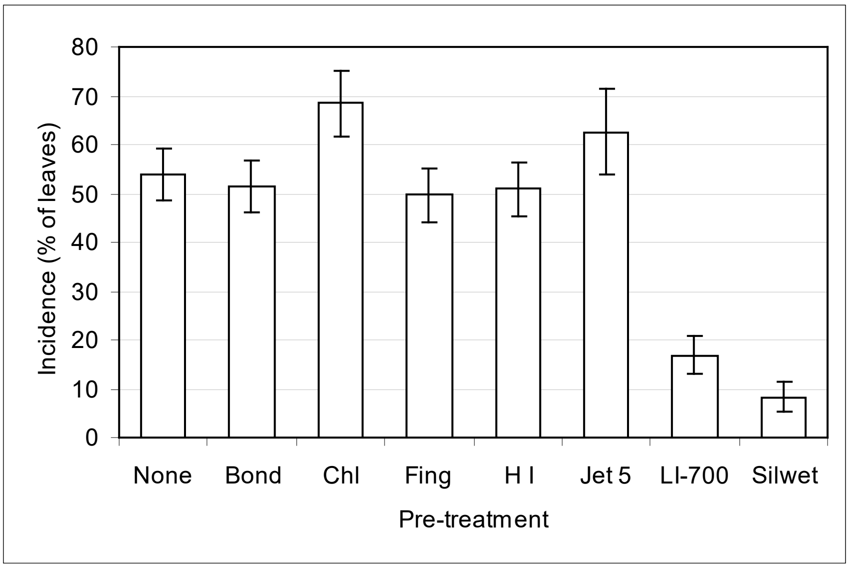


**Figure 2.** Effect of pre-treatments on incidence of downy mildew in onions. Abbreviations: Chl, chloroform; Fing, finger; H I, Headland Intake. The adjuvants (Bond, Headland Intake, LI-700, Silwet) and disinfectant (Jet 5) were sprayed; Chloroform was wiped on with a paper towel; finger involved removing wax by rubbing with a finger. Bars represent the combined results of three experiments, error bars represent approximate standard errors (non-linear model).

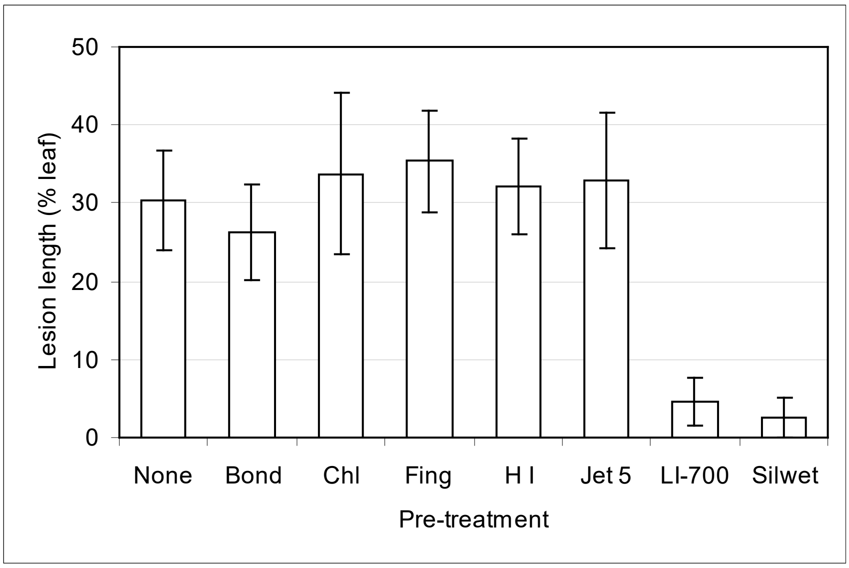


**Figure 3.** Effect of pre-treatments on severity of downy mildew in onions. Abbreviations: Chl, chloroform; Fing, finger; H I, Headland Intake. The adjuvants (Bond, Headland Intake, LI-700, Silwet) and disinfectant (Jet 5) were sprayed; Chloroform was wiped on with a paper towel; finger involved removing wax by rubbing with a finger. Bars represent the combined results of three experiments, error bars represent approximate standard errors (non-linear model).

# Appendix

## Standard Operating Procedures

| Number | Title | Filename |
|---|---|---|
| 04-013 | Leaf wax - Contact angle | 04-013v1-0 Contact angle.pdf |
| 04-014 | Leaf wax – Wetability with methanol-water mixes | 04-014v1-0 Methanol-water.pdf |
| 04-015 | Leaf wax – Crystal violet test | 04-015v1-0 Crystal violet.pdf |