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[The results and conclusions in this report are based on an investigation 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.

Cathryn Lambourne	
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Report authorised by:	
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Signature	Date

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#### **GROWER SUMMARY**

#### Headline

Survey results suggest that the highest risk of *Itersonilia* infection occurs during September and October in each year, with periods of high rainfall increasing the risk in crops at other times. It is often too late to take any action once infection is observed and crop losses can be high where environmental conditions are most conducive to pathogen development.

#### **Background**

In September 2009 a sample of dill with leaf-blight symptoms was received in the Plant Clinic at Stockbridge Technology Centre in North Yorkshire. The grower reported that several crops were similarly affected each year at around that time and that once the symptom was observed little or no control could be gained from applying fungicides. The affected crops were generally abandoned, particularly as the outdoor cropping season was drawing to a close.

Tests were carried out on the affected material, and a diagnosis of *Itersonilia* sp. was made. This fungal pathogen was later confirmed as the primary cause of infection following pathogenicity testing, when the symptoms were reproduced in healthy dill plants following inoculation with the isolated organism. The same diagnosis of *Itersonilia* sp. was later made on affected material supplied by the same grower to FERA. Additional work carried out by FERA later confirmed the pathogen as *Itersonilia perplexans*. Optimum conditions for the spread and development of this fungus are temperatures of 10-15°C and relative humidity >70%. Periods of heavy rainfall increase the risk of infection in susceptible crops.

As this was a newly recorded pathogen on dill in the UK some additional testing was carried out and the results were reported to the British Herb Trade Association (BHTA) at their meeting held at STC in March 2010. It was agreed that a better understanding of the incidence and severity of the leaf-blight pathogen on dill and other herbs was required to enable the BHTA to determine the potential impact of this new pathogen/host combination on the UK herb industry. Following low sample submission rates in 2010 few conclusions were able to be drawn regarding potential risk to UK crops; therefore, a continuation of the project into 2011 was agreed.

#### Summary

The extension of the project into an additional year has provided an opportunity to gather additional data on the incidence and severity of the Itersonilia sp. leaf blight pathogen in UK herb crops. The approach to the work carried out in 2011 was similar to that done in 2010.

With industry support, through HDC, information regarding the finding of *Itersonilia* perplexans as a new threat to UK herb crops was circulated more widely to all herb growers via a survey and sample request letter, which was sent out via the HDC and the BHTA.

A relatively low number of samples were received during the early part of the 2010 season; however a flurry of samples were received between September and November as the weather conditions became cooler and wetter providing a more conducive environment for the spread and development of leaf-blight problems. A similar pattern of low sample submissions was observed in 2011, again with more samples being received in September to November.

A total of 21 samples were received over the duration of the project. Samples of dill totalled 9, 7 of which were found to be infected with an *Itersonilia* sp. Eight coriander samples were received, of which four were found to be infected with an *Itersonilia* sp. A single sample of fennel was also received which was infected with an *Itersonilia* sp. Two parsley samples submitted with foliar symptoms were found to be infected with *Plasmopara umbelliferarum* (downy mildew). Unfortunately it was not possible to draw any conclusions on geographically high disease risk areas from the samples received as these came from relatively few growers overall.

Itersonilia pastinaceae is a recognized pathogen of parsnip (an umbelliferous species). Yet, I. perplexans is also known to infect a number of herb species, particularly in the Umbelliferae, e.g. coriander, parsley, cumin, anise, chervil, lovage and caraway, as well as members of the Asteraceae e.g. chrysanthemum, causing a petal blight symptom. The findings from the two years of this study suggest that the leaf-blight problem is predominantly a problem in dill crops, but it is also present in coriander, parsley and fennel. Several of the later received samples of dill and coriander were from protected crops, although these were not infected with Itersonilia spp. This supports the view that cool wet conditions are more conducive to infection development and spread of the disease whilst growing under protection would appear to provide a more controlled environment which reduces the risk of infection, presumably due to the drier conditions.

The fungus is spread primarily as a result of air-borne dispersal, though it may also be seed-borne and spread from susceptible weed species present in or around commercial crops. There may also be a risk from infected debris in soil and movement of spores on clothing etc., although there is no direct evidence for this.

#### **Financial Benefits**

The scope of this study was to gather information on behalf of the UK herb industry. No financial benefits have resulted from this work to-date, though a better understanding of the cause of crop loss due to leaf blight could ultimately have a significant economic benefit to the industry.

#### **Action Points**

- Herb growers should remain vigilant and become familiar with the host range of the basidiomycete pathogen genus *Itersonilia* i.e. dill, parsley, fennel, coriander, cumin, caraway and perhaps tarragon and chamomile. Particular attention should be paid to crops during or following periods of high rainfall.
- Where leaf blight is found in herb crops, samples should be submitted to a diagnostic plant clinic to check for the presence of *Itersonilia* spp.
- As Itersonilia spp. are known to be seed-borne, seed of known susceptible herb crops should be submitted to an appropriate diagnostic clinic to check for seedborne infection.
- Where possible plants affected by leaf-blight should be removed as soon as possible following detection to minimize risk of spread.
- No detailed information on the efficacy and crop safety of fungicides approved for use on herb crops is known at this time. However, some relevant information may be gleaned from approvals on parsnip crops where *I. pastinaceae* is a significant problem. In addition to this, it is presumed that broad spectrum fungicides such as the strobilurin fungicides e.g. Amistar (azoxystrobin) have some activity against the

fungus, though crop safety of each herb species would need to be evaluated in addition to the approved status on specific herb crops prior to use.

### **SCIENCE SECTION**

#### Introduction

Leaf blight symptoms had been observed on both dill and coriander crops since 2003 (Peter Knight, pers. comm.), though the primary cause of such symptoms has not previously been elucidated. Samples of dill with severe leaf blight symptoms were received in the Stockbridge Technology Centre (STC) Plant Clinic in September 2009. An *Itersonilia* sp. was recovered and diagnosed as the likely cause of the leaf blight. This was later confirmed by pathogenicity testing. Confirmation of this initial diagnosis was subsequently provided by FERA, who carried out molecular tests and identified the organism as *Itersonilia* perplexans. Additional samples, received from other growers were also found to be infected with an *Itersonilia* sp. and also assumed to be *I. perplexans*.



Figure 1. Typical leaf-blight symptoms on dill.

Although *Itersonilia* sp. is a well recognised pathogen in small a number of other crops in the UK and overseas, no records of this pathogen/host combination had been previously

reported in the UK, although records of this leaf blight pathogen on dill had been reported in Australia (Aldaoud 2009) and in Europe (Rodeva 2009).

Itersonilia is a member of the basidiomycete family of fungi. Fungi in this group produce specialist spores called ballistospores which are actively propelled into the air by the fungus under suitable environmental conditions to infect new material on wind currents. The related pathogen I. pastinaceae is known to be seed-borne in parsnip and whilst it is not yet proven in herbs, it is possible that the fungus may also be seed transmitted in herb crops such as dill. During this investigation we carried out tests on the weed species groundsel (Senecio vulgaris) which was exhibiting leaf-blight symptoms and confirmed the presence of an Itersonilia spp. However, the pathogen was not detected on a number of other weeds examined at other times in the study. It is important to note though that pathogenicity tests were not conducted on groundsel to determine if this was a primary pathogen on this weed and it is possible that it was present as a saprophyte. However, the fact that the organism could be found on groundsel suggests that it may act as a source of inoculum and this may be important. There are two important plant pathogenic members of the Itersonilia genus; I. perplexans and I. pastinaceae. These organisms have been reported as pathogens on several economically important crops including parsnips, chrysanthemum, gerbera, parsley, parsnip and carrot.

Early symptoms on dill are described as small grey/green spots on the leaves. Infection is reported to progress quickly to cause death of leaflets and eventually to the complete collapse of the plant when conditions are optimal for the pathogen. Development and spread of infection is favoured by high rainfall periods producing high RH (>70%) and cool temperatures (10-15°C).

Contact with several growers, where the pathogen was confirmed on susceptible dill crops, suggested that it was causing almost complete collapse of the crop and was therefore incurring significant costs in terms of lost income. Following an initial survey of UK herb crops in 2010, the BHTA requested a continuation of the project into the 2011 season to determine the incidence and severity of *Itersonilia* infection on herbs. It was hoped that this information would provide herb growers with a greater understanding of the infection and allow them to determine whether further work to investigate possible control measures for *Itersonilia* infection might be required in the future.

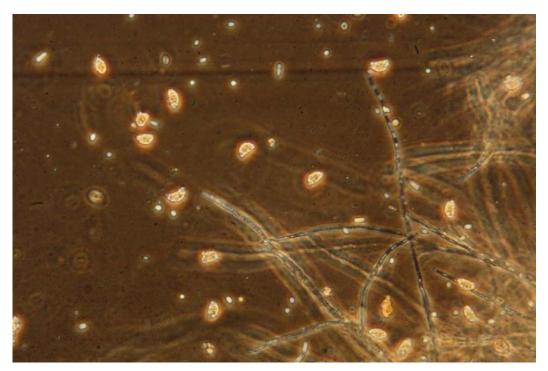


Figure 2. Mycelium and ballistospores (x 100) of *Itersonilia* sp.

#### Materials and methods

## Information and sample collection

Growers were contacted by letter via the HDC and BHTA and requested to monitor all umbelliferous herb crops e.g. dill, coriander, parsley, fennel, cumin, etc. for signs of leaf blight or similar symptoms during the season. It was also requested that they monitor weed species in close proximity to the crops for foliar symptoms as it is possible that some weed species may provide a source of inoculum (spores) for transfer to herb crops. Additional reminders were posted on the HDC and BHTA websites during the autumn to prompt growers to send in samples.

Information on the pathogen gathered in previous years indicated that the most likely time for infection in outdoor crops was September/October as environmental conditions prevalent at that time of year were most conducive to the spread and development of infection i.e. during cool, damp weather.

#### Sample handling

When samples of infected leaf material were received they were booked into the STC Plant Clinic. Samples were examined using low and high power microscopy to determine whether any spores consistent with *Itersonilia* spp. were present. Diagnostic tests were then initiated e.g. aseptic direct isolations on to potato dextrose agar and also selective suspended tissue tests<sup>1</sup> which involve attaching plant tissues to the lid of a petri-dish using double sided tape or Vaseline and placing over artificial growth media (PDA or similar). This method is particularly suited to basidiomycete fungi as ballistospores are shot onto the agar and resulting isolations are often free from other contaminating fungi or bacteria that might be present in the plant tissues. The isolation tests were checked after approximately 48 hours for the presence of mycelium and spores consistent with *Itersonilia* spp. A report on the findings was sent back to the originator of the sample and details were retained for inclusion in the project report. However, isolation of the fungus is not a measure of its pathogenicity.

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<sup>&</sup>lt;sup>1</sup> The suspended tissue test is a selective isolation technique that relies on the ability of basidiomycete fungi to actively shoot ballistospores from infected tissues. The method, which relies on attaching tissue pieces to the agar plate lid, prevents growth of faster growing saprophytic fungi and bacteria, allowing selective growth of the slower-growing *Itersonilia* species.

#### Fungicide in-vitro screening

A small-scale fungicide screening experiment was designed and carried out using a range of products already approved for use on outdoor dill, the aim being to explore which products might have efficacy against *Itersonilia* spp.

Approved fungicides were added to Potato Dextrose Agar (PDA – a standard laboratory agar for the growth of fungi) at the label rate (Table 1). Triplicate plates of each fungicide amended agar were used and an un-amended (control) test was run alongside. Following pouring and drying of the plates a 5mm agar plug from an actively growing culture of *Itersonilia* isolated from dill was attached to the lid of the test plates using double-sided sticky tape. Plates were incubated at 24°C for 48 hours to allow spores liberated from the agar plug of *Itersonilia* to germinate and produce small colonies on the agar below. Plates were assessed for the presence or absence of germinating spores following incubation as a measure of fungicide activity against spore germination and survival.

**Table 1.** Products and rates used in the *in-vitro* fungicide screening study

Product	Active ingredient	Crop application	Amount added to
		rate/ha	100ml agar
Untreated control	-	-	-
Amistar	azoxystrobin	11	0.2ml
Serenade	Bacillus subtilis	101	2ml
Signum	pyraclostrobin +	1.5kg	0.3g
	boscalid		
Invader	dimethomorph +	2kg	0.4g
	mancozeb		
Teldor	fenhexamid	1.5kg	0.3g
Aliette 80WG	fosetyl-aluminium	2kg	0.4g
Previcur Energy	fosetyl-al +	2.51	0.5ml
	propamocarb HCl		
Rovral WG	iprodione	78g/100l	0.78g
Fubol Gold	mancozeb +	1.9kg	0.38g
	metalaxyl-M		
SL567A	metalaxyl-M	0.12l	0.024ml
Croptex Fungex	cupric ammonium	125ml/100l	1.25ml
	carbonate		
Croptex i ungex	·	1201111/1001	1.201111

## Isolate speciation

Following the first UK confirmation of *Itersonilia* sp. on dill (Sept 2009), mycologists at FERA subsequently carried out molecular tests to confirmed the isolate as *Itersonilia perplexans*. During this study we requested the same species ID be carried out on the isolates of *Itersonilia* that had been obtained from samples of dill, fennel and coriander during the season.

#### Results

#### Sample collection

A total of 21 samples of plant material with leaf-blight symptoms were received between September and November 2011.

Tests were carried out on all received samples with the following findings.

**Table 2.** Details of the samples received and the outcome of laboratory tests.

Crop	No. of samples	Month received	Itersonilia spp.
	received	(2011)	detected +/-
Dill	1	September	+
Coriander	1	September	-
Dill	1	September	+
Coriander	1	September	-
Dill	2	October	both +
Dill	1	November	+
Coriander	1	November	+
Fennel	1	November	+
Dill	2	November	+ (1 only)
Coriander	2	November	both +
Curly parsley	1	November	-
Flat parsley	1	November	-
Coriander	1	November	+
Weeds in dill	1	November	-
Dill	2	November	-
Coriander	2	November	-

All of the samples of the *Itersonilia* infected crops (i.e. dill, coriander and fennel) samples were received from September onwards and this corresponds with the periods of cool weather with high humidity which are most conducive to disease development and spread. This is particularly relevant with regard to weather conditions experienced in 2011 which were drier than average. Some of the latest samples received were collected from protected crops and although some very slight leaf necrosis was observed they were free

from *Itersonilia*. This supports the information that cool wet conditions are required for disease spread and symptom development.

Little information regarding the cultivars of dill was provided by growers, although information provided by the Project co-ordinator suggests that whilst many growers are growing the cultivar Dukat, other cultivars have become available e.g. Moulton more recently and are proving popular with growers. No clear conclusions regarding potential susceptibility of cultivars based on 2011 samples. Samples received during the project originated from 6 growers only, and unfortunately this means that it is not possible to get a clear indication of the geographical spread and hence disease risk in certain areas.

The findings of *Itersonilia* on both coriander and fennel are interesting, and came from the same grower in the south-east of England. We confirmed the infection on a fennel sample from the same site in the 2010 survey. Although the infection is likely to cause yellowing and decay of the foliage, it is unknown at this stage whether there may possibly be lesions formed on developing bulbs, in a similar way to *I. pastinaceae* affecting parsnip roots. However, if the fern is to be used for oil or seed production the presence of the pathogen has more relevance. Also, it is important to consider that fennel may harbour the pathogen when planting close to more susceptible foliar crops e.g. dill and coriander.

## Fungicide screening work

Although the approach to this 'extra-curricular' part of the study took a rather simplistic approach some interesting results were obtained (Table 2).

**Table 3.** Results of the fungicide screening work investigating the potential efficacy of various products against *Itersonilia*.

Product	Growth of <i>Itersonilia</i> ✓/X		
	Replicate 1	Replicate 2	Replicate 3
Control	✓	✓	✓
Amistar	X	Χ	X
Serenade	X	X	X
Signum	Χ	X	X
Invader	X	Χ	X
Teldor	✓ Trace	X	X
Aliette 80WG	X	X	X
Previcur Energy	✓	✓	✓
Rovral WG	X	X	X
Fubol Gold	X	X	X
SL567A	✓	Χ	✓
Croptex Fungex	X	X	X

Although the results should not necessarily be relied upon for in-crop control of this pathogen, they do suggest that, *in-vitro*; at least, some products do appear to have activity on spore germination and survival. This information may assist growers in choosing particular products for small-scale on-site experimentation for control. More importantly it provides a good indication that further *in-vivo* work with fungicides and bio-control products could be highly relevant and important.

#### Isolate speciation

FERA were not able to carry out molecular speciation of the 3 isolates provided to them and it has therefore not been possible to confirm that all isolates detected were *I. perplexans*.



**Figure 3.** Leaf blight symptoms confirmed as being caused by *Itersonilia* sp. on fennel.





Figure 4. Leaf blight symptoms confirmed as being caused by *Itersonilia* sp. on coriander.

#### Discussion

The results of the survey of UK herb crops gathered during the 2011 season supported our initial knowledge of this leaf-blight pathogen. The majority of the confirmed *Itersonilia* sp. infections resulted from samples received from September through to November, and this supports observations seen during the 2010 survey. This suggests that this is a particularly high risk period for growers. No samples were received earlier than September during 2011 and this may well be linked to the very dry spring and summer experienced over most of England in this year. The infection process would appear to require lower temperatures and high levels of humidity to allow spore germination and complete the infection process from plant-to-plant.

Once again no pot-grown dill samples were received in 2011 and this may suggest that the different environmental conditions experienced by these crops is far less conducive to the development and spread of infection, being a more controlled, drier and warmer regime.

The very small-scale fungicide and biocontrol amended agar testing which was carried out in 2011, although not fully conclusive, does provide a strong indication that some products could have useful activity against *Itersonilia* spp. These data could be used to steer follow-up *in-vivo* trials to develop new on- and/or off- label approvals for disease control. Obviously crop safety and harvest intervals need to be considered carefully for all crops treated. In the absence of follow-up work the data is still highly relevant to help growers minimize infection risk from this pathogen.

The very low number of samples received would suggest either that the drier environmental conditions experienced during the 2011 growing season have been less conducive to the development of leaf blight symptoms in susceptible crops, or that growers are more aware of the symptoms on crops and are able to diagnose the disease themselves. It may also be possible that as the leaf blight often coincides with the closing of the outdoor season many growers may be attributing slight infections to general senescence in the crop and not considering it as significant. Alternatively, growers may be using an effective spray programme which is keeping infection down to low/acceptable levels i.e. is not causing economic losses in the crop.

#### Conclusions

The information gathered on the incidence and severity of *Itersonilia* in UK herbs during the 2011 season does provide further evidence that some growers are experiencing problems with the pathogen, but that their experiences are variable and generally dependent on weather conditions in their location and drainage within the crops. When *Itersonilia* infection occurs it can be in localised patches or, in some cases, it may affect the whole crop when weather conditions are conducive to spread. In these situations serious financial losses may occur.

The number of respondents to the various requests for samples and feedback on growers' experiences of the leaf-blight problem in crops was low in both years of the survey and this has again made it very difficult for the project team to draw clear conclusions and to make recommendations regarding the potential need for further work. For example it is not clear whether existing fungicide strategies are able to control severe infections with this pathogen at present. The small-scale fungicide screening work carried out in this study does suggest that a range of already approved products may have some good activity, therefore if a lack of good disease control is being experienced further work may be needed to investigate effective spray programmes and regimes to optimise their use. Alternatively, growers may in reality be protecting crops or controlling early infections with the fungicide treatments at their disposal and this may explain the lack of samples received during the two years of this study.

#### **Knowledge and Technology Transfer**

Update supplied to Tom Davies for BHTA meeting

#### References

Aldaoud R, et al, (2009) First record of *Itersonilia perplexans* on Anethum graveolens in Australia, Australian Plant Disease Notes, 4, 60-61.

Rodeva R, et al (2009) First evidence of *Itersonilia perplexans* on dill in Bulgaria, Scientific works of the Lithuanian Institute of Hort. and Lithuanian University of Ag. Sodininkysté ir Daržininkysté.

# Acknowledgements

STC would like to thank all the growers who sent in samples or provided information for the 2011 survey.

#### **APPENDICES**

# Appendix 1 – Sample request letter

BHTA Members
UK Herb Growers

September 2011

Dear all

#### HDC Project FV 381a - Surveying leaf-blight (Itersonilia spp.) in herb crops

As many of you will be aware, samples of UK field dill with a leaf-blight symptom were diagnosed as being infected with *Itersonilia* sp. at STC in Sept/Oct 2009. This was subsequently confirmed as *I. perplexans* by Fera. Although the *Itersonilia* fungus is a well recognised pathogen in other crops e.g. parsnip and chrysanthemum it has not previously been recorded in the UK on this host. It has since also been confirmed on UK coriander.

So far, whilst these findings are few in number, they have caused significant crop losses to at least one UK grower. The HDC funded work to carry out a survey of herb samples during 2010 to investigate the incidence and severity of infection. Sample numbers received were relatively low and following dissemination of the findings at the BHTA meeting on 12th October it was felt that a second round of the survey in 2011 would provide a clearer picture of the problem to umbelliferous (and possibly asteraceae) herb crops in the UK.

I would therefore like to invite you to take part in this national survey of UK protected and field herbs to enable STC to meet this goal. You can help by monitoring your umbelliferous herb crops (dill, coriander, fennel, cumin, parsley, anise, chervil, caraway and lovage) for any signs of leaf-blight (see the attached sheet for pictures). However, it may also be worth checking on any umbelliferous weeds that may be present around field/site margins e.g. cow parsley and also weeds of the Asteraceae e.g. dandelion, groundsel, mayweed, thistles, as these may be providing a 'green bridge' for the leaf infection. As the fungus can also be pathogenic on members of the Asteraceae (previously the Compositae) family you should also check these crops e.g. tarragon, chicory, globe artichoke etc. if grown and submit samples if required.

I have enclosed a sample submission form with details of how to package and send samples to me at the STC plant clinic. Samples received will be examined and tested for *Itersonilia* free of charge. Results from these samples will be forwarded to you by email in the first instance, but will also feed into the project anonymously to allow us to build a complete picture of the problem. Some of the gathered information should be available by October – hopefully in time for the next BHTA technical meeting. The full project results and report will be available in December 2011.

Many thanks in anticipation of your help.

Yours sincerely

Cathryn Lambourne

Plant Pathologist

Stockbridge Technology Centre Ltd.

# Stockbridge Technology Centre PLANT CLINIC SERVICES – Herb Survey work FV 381a

Name	
Company	
Address	
Postcode	
Phone	
Mobile	
Email	

The Sample	
Plant type - Genus,	
species, variety/cultivar /	
common name	
Distribution of the	
problem	
(% of crop affected)	
Source of plant material	
e.g. cutting, seed, weed	
Where is the crop	
grown? e.g. protected,	
shaded, outdoors etc.	
What are the	
symptoms?	
Briefly describe the	
problem as you see it.	
Have you seen this	
symptom in previous	
years? Please give	
details	
Are nearby crops	
affected if susceptible?	
e.g. either on your site or	
neighboring fields	
Previous cropping	
history? Can you give	
details of the previous 2	
crops if field grown.	
Weather conditions –	
can you provide brief	
details of prevalent	
conditions immediately	
prior to seeing the	

problem, especially temps	
& rainfall?	
Fungicides applied –	
and impression of the	
level of control seen?	

# Sample selection

On dill the leaf-blight symptoms appear as shown below. However, on other crops the symptoms may vary slightly, possibly including stem lesions or smaller more discrete spots.



Please feel free to send more than one sample over the growing period as the problem may show up on crops at different times of year (it was first seen in September on dill). Also remember to check umbelliferous weeds around the field margin if crop plants are affected.

#### Sending the samples

Samples can be sent by post, courier or dropped off directly at Stockbridge Technology Centre. In all cases it is important that samples arrive in excellent condition to enable accurate diagnosis, therefore please:

 Select plant material immediately before sending to the clinic; if there is any delay then store in a cool place out of sunlight.

- Wrap the plant material in newspaper or paper towel and pack in a strong padded envelope or box. If sending in a box, then pad-out with newspaper to restrict movement.
- Where possible pack the samples to avoid soil/compost contamination of the foliage.
- Ensure all samples are clearly labelled.
- Complete the Plant Clinic form, place it in an envelope and send it with your sample.

# DO NOT SEND SAMPLES ON A FRIDAY UNLESS THEY ARE SURE TO ARRIVE BEFORE THE LABORATORY CLOSES AT 5PM.

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