Project Title	Is feeding by the conifer aphid *Cinara cupressivora* causing the browning seen in conifer hedges?
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Project leader:	Dr Jean Fitzgerald
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The results and conclusions in this report are based on a series of 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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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.

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Grower Summary

Headline

- Both trimming of Leyland and Lawson cypress hedges in autumn and presence of conifer aphids in May, have been linked with dieback.

Background and expected deliverables

Leyland and Lawson cypress are extensively used to make quick growing hedges. Regular trimming of the hedges is needed to avoid excessive growth. Brown patches (dieback) are becoming more common on trimmed hedges. Conifer aphids can cause browning on conifers by their feeding activity, but it is not clear if aphids are fully responsible for most of the damage seen or indeed which species are implicated. This research investigated the scale of the problem of browning in hedges and evaluated the association between plant damage and aphid populations.

The expected deliverables from this project were:

- An assessment of the incidence of dieback in conifer hedges
- An evaluation of the association between plant damage and aphid populations
- An understanding of the biology and behaviour of *C. cupressivora* in UK hedges
- Confirmation of the identity of any aphid species associated with plant damage

Summary of the project and main conclusions

In the first year of the project, a survey was conducted to gather information about possible links between where/how Leyland and Lawson cypress hedges are grown and managed, and the incidences of browning and dieback. Damage in 24% of samples of Leyland cypress sent to RHS was diagnosed as caused by aphids. Other significant contributors were honey fungus (18% of the samples) and physiological

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causes such as dry soil, dense planting of the hedge and excessive shoot pruning (14% of the samples).

In a hedge sampling programme in year 1, not all hedges that were reported as damaged showed signs of aphid presence on the plant material examined. Evidence that *Cinara cupressivora* was or had been present was seen in samples of plant material from only 11 of 21 locations sampled (52%).

In year 2, information from the RHS database on the identification of causes of damage to conifer hedges collected in 2007 was added to data obtained from 2005 and 2006. Only 5% of samples analysed in 2007 showed signs of *C. cupressivora* presence.

Experiments were undertaken on potted plants and on established hedges to clarify the biology and behaviour of *C. cupressivora* and the effects of aphid feeding on the occurrence of plant damage symptoms.

In assessments of hedges in 2007, most damaged branches with a firm (non-wilted) appearance were associated with aphid presence; brown wilted branches were not associated with aphid presence. Aphids were also found on green branches; it would be expected that these branches would become brown over time.

The biology of *C. cupressivora* was investigated on an infested hedge over a year. This case study showed that:

- *C. cupressivora* feed on the woody part of the branch with maximum numbers found 6-10 cm from the shoot tip.

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- alate (winged) aphids were present and flying early in May, with numbers decreasing later in the season.

- populations of apterous (non-winged) aphids were present on the hedge until early July, after which it became difficult to locate colonies of live aphids.

- since numbers of aphids on this hedge were very low in July it was not possible to determine if a second winged migratory phase occurred from June to August or if egg laying females and males occurred in autumn. However, no overwintering eggs of *C. cupressivora* or any live aphids were found in samples taken in January.

- by early May 2008 adult apterous and alate aphids plus nymphs were present in low numbers on the hedge.

In potted plant experiments with different varieties of conifer, there was evidence that aphid feeding caused browning damage on plants. In a mature hedge general signs of hedge yellowing were first seen in April, when adult apterous aphids and obvious honeydew were present; hedge browning was seen in areas with both low and high aphid numbers.

Samples of aphids collected during the surveys were identified as *Cinara cupressivora*.

Financial benefits

There are no immediate financial benefits to growers. However, this research has confirmed the link between conifer damage and aphid presence. An understanding of the biology and behaviour of *C. cupressivora* should enable growers to advise

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gardeners on sampling for this cause of conifer browning and suggest possible insecticidal solutions. This will increase customer confidence in the product.

Action points for growers

This project has outlined the biology of *C. cupressivora* on established hedges in UK and shown that aphid feeding is associated with some of the browning seen in conifer hedges. Aphids were first detected in April but were more numerous in May; this would be a good time to sample hedges to determine if aphids are present and to decide if an application of aphicide is required.

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Science Section

Introduction

Leyland cypress (× *Cupressocyparis leylandii*) and Lawson cypress (*Chamaecyparis lawsoniana*) are extensively used to make quick growing hedges that provide excellent backdrop for beds and borders as well as improving security and privacy. With the introduction of legislation (Anti-social Behaviour Act, 2003) and the frequent news coverage of issues associated with 'garden hedges', there is increased pressure for high level maintenance of evergreen hedges, by frequent trimming. Trimming is often required more than once a year to avoid excessive growth.

A major drawback of conifer hedges is the problem of brown patches (dieback) that can occur in place of the expected green foliage (Appendix photographs 1-3). This is thought to be a problem specific to mature hedges, and it appears that there are not only species specific differences but also cultivar differences in the occurrence of dieback. For example, Leyland cypress shows extensive browning on the golden foliar cultivar Castlewellan. RHS Advisory Service believes that many brown patches result from adverse growing conditions such as drought, frost or cold drying winds inhibiting regeneration after trimming (see advisory leaflet #0805).

Feeding damage caused by conifer aphid is also known to lead to browning of conifers. Recent work has suggested that the species formerly called *Cinara cupressi* (conifer aphid) is in fact a complex of four species (Watson *et al.*, 1999), three of which cannot easily be separated. Two of the species, *Cinara cupressi* and *C. cupressivora* both occur in UK. It is not clear if both species cause similar plant damage, but *C. cupressivora* is reported to be the most common in southern England (Murphy, CABI Bioscience, pers. comm.). Serious damage to commercial and ornamental plantings caused by *C. cupressivora* (recorded as *C. cupressi*) has been reported from southern and eastern Africa, Italy, Jordan, Yemen, Mauritius and

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Colombia (Inserra *et al.*, 1979; Mills, 1990; Mustafa. 1987; Ciesla, 1991; Murphy *et al.*, 1994; Binazzi *et al.*, 1998; Alleck & Seewooruthun, 2002).

Very little work has been published on *C. cupressivora* or *C. cupressi* in UK. There is no published detailed information on its biology, distribution or damage symptoms. Strouts (2003) outlined the damage caused by conifer aphids in British gardens, Winter (1989) produced a general leaflet on conifer aphids for arboriculturalists, and Greig & Patch (2002) produced a leaflet on aphids on *Leylandii*.

The RHS Advisory Service reported that a significant proportion of samples sent from damaged conifer hedges could not be related to the presence of aphids (pers. comm. Guy Barter, Head of Advisory Service at the RHS). This lack of apparent relationship between aphid presence and hedge damage may be due to the biology of the aphid; aphids may move away from damaged plant material. Also some plant cultivars may show less damage in response to aphid feeding (e.g. Obiri *et al.*, 1994; Memmott *et al.*, 1995).

It has been suggested that browning damage is more common on trimmed hedges; the RHS describe damage caused by aphids on clipped hedges as "quite pronounced with the lower parts often more severely affected than the top", so it is possible that the damage seen is caused by interactions among different factors, *e.g.* aphid feeding and water stress.

The overall aim of this project was to determine if feeding by aphids is a major cause of the damage found in hedges of Leyland and Lawson cypress.

Summary of results from year 1

In the first year of the project we investigated the scale of the problem of browning in hedges in England. This was done by designing and administering a questionnaire which was used to build a picture of the conditions, *e.g.*, species, locality,

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management practices, climatic and soil conditions, presence of pests (in particular aphids) and diseases, etc., that may be linked with incidences of shoot browning.

Three hundred and sixteen respondents took part in the survey, the majority of which were members of the RHS living in the south-east of England. Out of the 23 commercial growers who are members of the Association of British Conifer Growers (ABCG) we achieved contact with 11, and, of those, six responded to the survey. Pests or diseases were only identified in 13% of the 316 surveyed cases, with six incidents of reported aphid damage, three of red spider mite and occasional cases of moths, juniper scale and honey fungus. In addition, two sites surveyed had had aphid damage confirmed by the RHS in 2004.

From the responses to the questionnaire the time of the appearance of dieback symptoms in 258 damaged hedges was collated and is shown in Figure 1. In general aphid numbers begin to increase with the onset of rapid growth of their host plants in spring. Feeding by conifer aphids could be responsible for the damage symptoms first seen in April (Figure 1). Reports have suggested that the aphid has an alate (winged) form that is produced from June to August and that egg laying females and males occur in autumn (Ciesla, 1991; Anon., 2003). Dispersal of the alate aphids in June-August onto new foliage could then be responsible for the damage symptoms first observed in September (Figure 1). Aspects of the biology and behaviour of *C. cupressivora* were investigated in Year 2 of this project and are discussed below.

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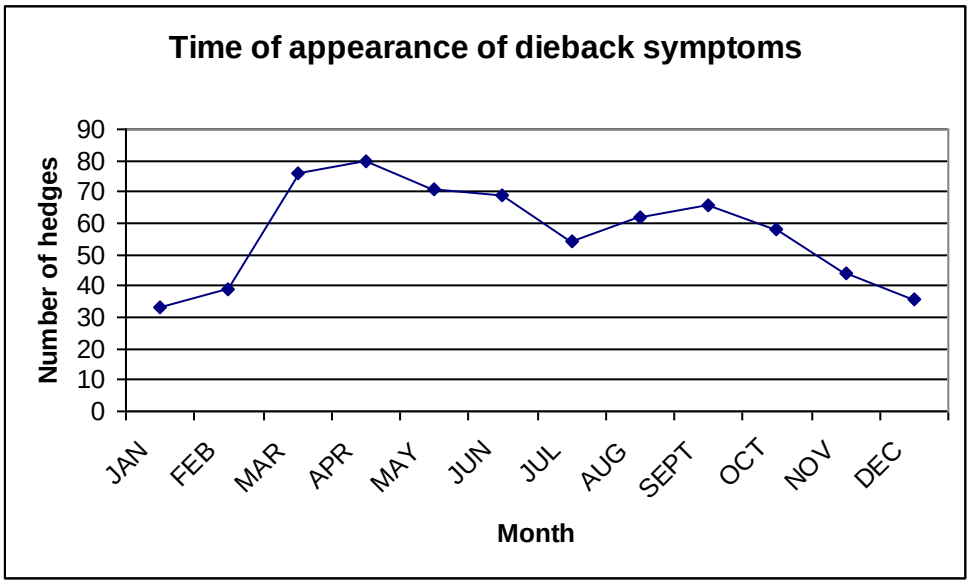

Figure 1: Monthly distribution of appearance of dieback symptoms in Leyland cypress hedges

In addition to the questionnaire and hedge survey, information from the RHS Advisory Service database for 2005 and 2006 relating to enquiries about browning and dieback in Leyland cypress hedges, was collated and summarised. A total of 78 inquiries relating to foliage browning and dieback in Leyland cypress were received. Overall, the major single identified cause of browning was Cypress aphid (24%) followed by honey fungus (18%) and 'physiological' issues, *e.g.* aftercare problems; dry soil; dense spacing of the hedge; excessive shoot pruning (14%). This suggests that although aphid feeding appears to be an important cause of the damage seen in Leyland cypress hedges, not all incidences of dieback could be explained by aphid presence; this is in line with results obtained from the questionnaire (above) and the hedge sampling programme undertaken in Year 1.

In year 1, plant material from damaged and healthy hedges was examined to determine if there was an association between aphid occurrence and plant damage. In the samples taken in May from Surrey, in four out of five damaged hedges there was evidence that *Cinara cupressivora* was or had been present (*exuviae* – cast

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skins, and honeydew were detected), with live aphids present at two sites (Appendix photographs 4-6). The remainder of locations sampled (one damaged and two recorded as healthy) showed no evidence that aphids had been present.

In the Kent locations sampled in June and July 2006, aphids were present in three out of seven sites in an area close to the damaged patch. *Exuviae* (cast skins/moults) and honeydew were present on samples close to the damaged patch from these three, plus one additional location. In the damaged area, aphids were found in two locations, *exuviae* in one and honeydew in one. Thus aphids were present in the damaged area and the area close to the damaged part in two locations. Four out of seven locations visited in Kent showed signs of aphid presence.

Postal samples were received in late July and early August from Cornwall (2 samples), Essex (2 samples), Leicestershire (2 samples), Lincolnshire (1 sample) and Pembrokeshire (2 samples). Only three locations showed evidence of aphid presence and no live aphids were found in any samples; *exuviae* were found in the damaged area and the area close to the damage from all three locations. This was also the case where plant material was separated depending on its proximity to the damaged areas (Kent and postal samples). In a total of 16 samples, evidence of aphid presence was seen in seven samples of vegetation close to the damaged area and in five samples from the dead area. It is apparent that on the plant material examined, *Cinara cupressivora*, where present, was at very low densities.

It is evident from these results that not all hedges that were reported as damaged showed signs of aphid presence on the plant material examined; from a total of 21 locations inspected only 11 showed signs of aphid presence (52%).

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Research undertaken in year 2

Objective 1. Undertake a survey of the incidence of browning in Leyland and Lawson hedges

Additional data were obtained from the RHS on causes of dieback identified in samples sent to them in 2007 and results are shown in Table 1. The first column shows the results of inquiries received by the Pathology/Entomology group at RHS and covers all conifer species; aphids were not associated with damage in any of the samples received and the majority of the damage seen was diagnosed as having been caused by fungal pathogens. The Advisory Service also separately identified causes of damage in *Cupressocyparis*, where there was only one case of damage caused by aphid feeding and in *Cupressus* where there were 7. Thus, in 2007 only 5% of damaged samples received had signs of *C. cupressivora* presence; most cases of damage resulted from environmental or physiological causes. Table 2 shows the results from the Year 1 analysis for comparison. It was apparent that fewer cases of damage caused by aphids were reported in 2007 than in 2005/06. Fungal pathogens appeared to be related to many of the damaged samples acquired by the RHS.

Table 1: Causes of dieback in conifer hedges identified in 169 samples received by the RHS Advisory Service in 2007

Likely cause of dieback	'conifers'	*Cupresocyparis*	*Cupressus*	% of samples
Cypress aphid	0	1	7	5
Honey fungus	15	7	2	14
Physiological	6	17	3	15
Environmental stress	0	11	10	12
Pestalotia-related disease	15	5	1	12
Phytophora	4	0	2	4
Pythium	9	0	1	6
Other	3	5	1	5
Undiagnosed	8	2	0	6
Insufficient sample size	31	0	3	22
Total	91	48	30	

Table 2: Causes of dieback in Leyland cypress hedges identified in 78 samples received by the RHS Advisory Service in 2005 and 2006

Likely cause of dieback	No. of samples	Percentage of samples
Cypress aphid	19	24
Honey fungus	14	18
Physiological (dense spacing, drought, extensive pruning)	11	14
Pestalotia-related disease	7	9
Juniper scale (*Carulaspis juniperi*)	2	3
Undiagnosed	16	21
Insufficient sample size	9	12
Total	78	

Objective 2. Determine if there is an association between plant damage and aphid populations

Further hedge sites were examined in 2007 to assess associations between plant damage and aphid presence.

Methods

Twelve sites in Kent were assessed in July and early August 2007. In sites where there was browning of the hedges, areas of both brown and green vegetation were assessed; hedges without brown areas were also included. In total 33 sample areas were assessed. Twenty branches in each area (five branches at 75, 100, 125 and 200 cm above ground level) were assessed and classed as having no live aphids and no signs of aphids, no live aphids but with signs of aphid presence (*exuviae*,

honeydew and/or black sooty mould), 1-10 live aphids, 10-20 live aphids or 20+ live aphids. Branches from both green and brown areas were sampled where hedge damage was seen. Hedge colour was recorded as green, green/brown, brown with the branches having a firm appearance, brown with the branches having a wilted appearance. Branches were also assessed from eight additional sites which had either been visited, or had had samples sent in by owners. All branches (209 in total) were assessed for colour using the same categories as above. The numbers of aphid *exuviae* and/or live aphids present were counted under a stereo microscope.

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Results

Of the 33 sample areas assessed, 21 were green, two were green/brown, six were brown with the branches having a firm appearance and four were brown with the branches having a wilted appearance. Only one of the areas assessed had any live aphids, but there were signs of previous aphid infestation in many areas. The brown areas that had a firm appearance were associated with aphid presence (Table 3); in all but one of six cases, at least 75% of branches assessed showed signs of aphid presence (Table 4). The brown areas with a wilted appearance were not associated with aphid presence. Aphid presence was also recorded from green vegetation in 13 of 21 areas.

Table 3: Colour of hedge and associated presence or absence of aphids recorded in assessments of 33 areas of hedge at 12 different sites in 2007

Indications of aphid presence	Hedge colour			
	Green	Green/Brown	Brown (firm appearance)	Brown (wilted appearance)
No	8	0	0	4
Yes	13	2	6	0

Table 4: Hedge colour related to the number of branches in a sample of 20 inspected from 33 areas of hedge at 12 different sites in 2007 with and without signs of aphid presence

Number of branches with indications of aphid presence	Hedge colour			
	Green	Green/Brown	Brown (firm appearance)	Brown (wilted appearance)
0 (No aphids)	8	0	0	4
1 - 5	4	0	1	0
6 – 10	4	1	0	0
11 – 15	2	0	1	0
16 - 20	3	1	4	0

The number of aphids and *exuviae* found on branches with different levels and types of browning is shown in Figure 2. Approximately 75% and 90% of branches

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assessed in the 'green/brown' and 'brown with a firm appearance' categories respectively were associated with some signs of aphid presence. In general, less than 50% of the branches in the green areas assessed showed signs of aphid presence. Medium to high levels of infestation were seen for less than 15% of the green branches. As there is a time delay in branch browning after aphid feeding it is not surprising that aphids were present on some green branches.

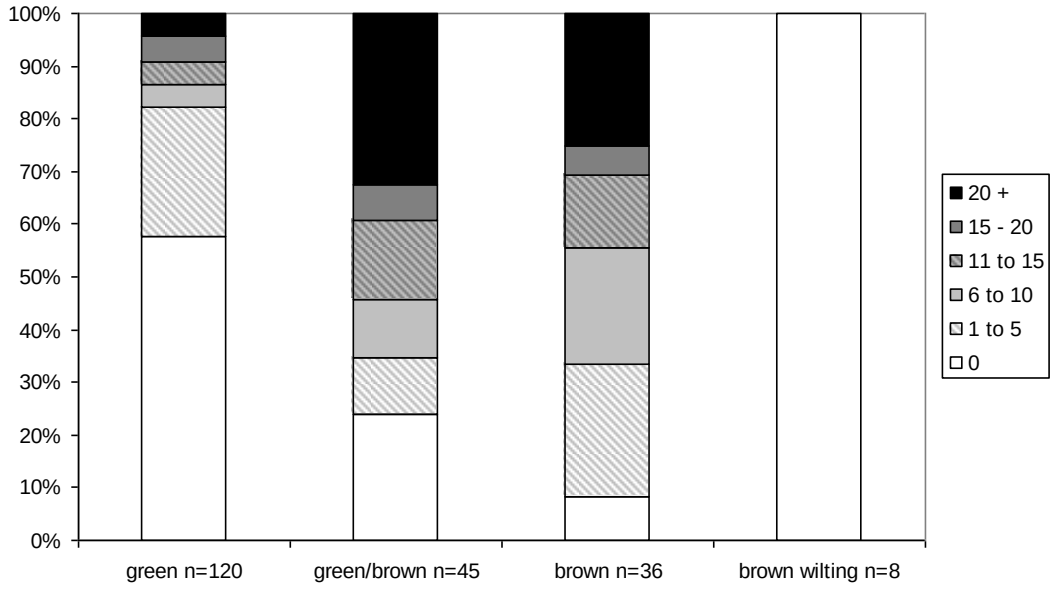

Figure 2: The number of aphids and *exuviae* found on branches with different levels and types of browning (the number of branches assessed in each category is shown as n=x)

Discussion

This survey enabled us to relate aphid population density to damage on the infested hedges; most damaged branches with a firm appearance were associated with aphid presence, but brown wilted branches were not associated with aphid presence. Aphids were also found on green branches; it would be expected that these branches would become brown over time. The biology of the aphid over a season on a mature hedge was evaluated in the case study in Objective 4; on this hedge damage symptoms progressed along the hedge over the summer season and damage was greater on the warmer southern side of the hedge.

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Objective 3. Determine if feeding by Cinara cupressivora results in the typical browning or 'die back' seen on Leyland and Lawson hedges

Experiments were undertaken on potted plants and on a mature hedge at EMR. Aphid infested branches (approximately 25 cm in length) were collected from an established conifer hedge in Barming, Kent, and used to inoculate the experimental plants.

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Experiment 1 - potted plant experiment

Methods

Green *C. leylandii* in individual pots were used for this experiment. The plants were approx. 1m tall with open branching. The plants were placed in two gauze-houses (ten in each) standing in grey plastic trays. A "safety net" of horticultural fleece was erected above the trays and at the level of soil in the pots (Appendix photograph 7) to prevent aphids from falling from the plants into the water. The plants in one gauze house were inoculated on 2 June with 2-3 infested branches per plant; the plants in the second gauzehouse were not infested with aphids. A second inoculation was made in the first gauzehouse on 21 June. On 11 July, all branches on each plant were assessed for aphids or signs of aphids.

Results

No aphids had established on any of the treated plants, and the control plants were also aphid free.

Experiment 2 - mature hedge experiment

Methods

An established conifer hedge at East Malling Research was inspected for the presence of conifer aphids in four marked areas. The centre of each assessment area was at a height of 1.5 m and the areas were at least 5 m apart horizontally. Twenty branches up to 30 cm in length were assessed within 50 cm of the central point. All areas were free from aphids. The hedge was then inoculated with aphids by placing infested branches containing at least 50 conifer aphids in each of two of the marked areas on 14 June. The remaining two areas were not inoculated. On 11 July, 20 branches in each area were assessed for aphids or signs of aphid presence (*exuviae*, honeydew or sooty mould).

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Results

There were no signs of live aphids in either of the infested areas, although in one of the areas a parasitised winged conifer aphid was recorded.

Discussion

We were unable to establish colonies of *C. cupressivora* on plants in these experiments. It is not clear why this was the case. Researchers who have worked with this aphid species have suggested that it is sometimes difficult to move aphids successfully from plants from one provenance to plants from another (Sean Murphy, CABI, pers comm.). It is possible that the open structure of the potted plants did not favour aphid colony development. Predators such as spiders were seen on the mature hedge; it is possible that the aphids were attacked by predators and parasitoids. Since no aphid establishment occurred and no damage was seen on the plants, damage assessments were done on plants as part of Objective 2 above and Objective 4 below.

Objective 4. Understand the biology and behaviour of C. cupressivora in UK hedges

Two approaches were used to investigate the biology of *C. cupressivora* on Leyland hedges; a detailed case study of a hedge that was naturally infested with *C. cupressivora*, and inoculation of Leyland and Lawson cypress plants held in controlled conditions. The case study was also used to gain extra information on the association between aphid presence and conifer browning.

1. Case Study

Methods

A hedge that was showing signs of dieback, and that was found to have an aphid infestation was used in this case study (Appendix photograph 10). The owner was

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asked to complete the questionnaire used in the survey undertaken in year 1 to give us information on the hedge and its management.

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Owner responses to standard questionnaire:

Location: Barming, Maidstone, Kent

Hedge Age: More than 15 years

Approximate length: 20 m

Approximate height: 3.5 m

Approximate width: 1 m

Planting density: 0.7 m

Direction: North-West/South-East

The hedge is not close to a wall, fence or barrier, but is less than 3 m from a road on the south-western side.

Planted on clay soil, but not waterlogged in the winter, although frequently dry in the summer (no additional water is given).

Hedge is trimmed back to the previous trimming (removing approx 10-20 cm) annually in the winter, usually Dec-Feb. It was trimmed late in 2007 in April. A powered hedge trimmer is generally used.

The hedge is not given hedge fertiliser, fungicide or insecticide.

The hedge was inspected regularly from April to July to follow the aphid population development on the hedge. For assessment the hedge was separated into 10 areas with 2-3 trees per area and assessments were made on both sides of the hedge in each area. The hedge was assessed on the 9-10, 18, 30-31 May, 13 June, 16 July, 26 October 2007 and 28 January, 28 February, 17 March, 1 and 17, 29 April and 7 May 2008. Assessments were undertaken to determine where *C. cupressivora* are most often found on the branches (Appendix photograph 11), to assess the distribution of the aphids vertically and horizontally within the hedge, to study the

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population structure of the aphid through time and to determine how conifer browning damage is associated with this pest.

Assessment on 9-10 May 2007: an assessment of aphid numbers was made in a vertical transect of the hedge; single branches from 25 cm to 175 cm above the ground were sampled at 25 cm intervals along the branch. A horizontal transect was also done in the central section of each of the areas 1-10, which were 150 cm apart and 150 cm above ground level. A 30 cm x 30 cm assessment of 5 areas of the hedge was done on 9 May at the same height as the horizontal transect and the number of winged aphids on the external tips of the hedge was counted.

Assessment on 18 May 2007: an assessment was made of aphid numbers on branch samples at each of 100, 125 and 150 cm above ground surface in each of the 10 areas on the south side of the hedge. An aphid infested branch, 30 cm in length, was also collected from the hedge. This was washed in 70% ethanol in the laboratory to remove all aphids, and the aphid population structure was recorded.

Assessment on 30-31 May 2007: Five branches at each of 75, 100, 125 and 150 cm above ground were inspected and each branch was assigned to one of five categories:

0 (clean) - clean and no signs of aphids

0 (signs) - signs of aphids (e.g. honeydew, black sooty mould, *exuviae*)

low - 0-10 aphids

medium - 11-20 aphids

high - 20+ aphids

Green branches in all areas of the hedge were assessed, as were brown patches in area 1 (on both the north and south sides). Two aphid infested branches, 30 cm in length, were also taken from the hedge and washed in 70% ethanol as described above.

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Assessment on 13 June 2007: This was as for the previous assessment. Two aphid infested branches, 30 cm in length, were taken from the hedge and were washed in 70% ethanol as described above.

Assessments on 16 July 2007; 26 October 2007; 28 January 2008; 28 February 2008

Twenty branches were assessed as described previously. On 28 January, five branches were also collected and taken to the laboratory to determine if aphid eggs were present.

Assessments on 17 March, 1 April, 17 April, 29 April and 7 May 2008

Tap samples were done to dislodge any insects in the hedge (5 taps over a white tray, 25 × 30 cm, placed in the hedge) in each of the 10 areas of the hedge, at 1.25 m above ground level and on the lowest branches. A visual infestation assessment was also done on 17 March and 29 April.

Results

General signs of hedge yellowing were first seen on 18 April 2007, when adult apterous aphids and obvious honeydew were present; March 2007 had been warm with 9 days when the temperature was above 14 °C. On 1 May winged aphids were also seen.

The assessments showed that the greenest parts of the branches were generally in the first 10 cm from the growing tip; from 15 cm into the hedge the branches become mostly woody. The highest numbers of aphids were found within the green areas of the branch when assessed on 9-10 May 2007, although they were feeding on the main stems which were often woody. In the seven branches assessed in the vertical transect totals of 48, 104 and 27 aphids were recorded at 0-5, 6-10 and

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11-15 cm along the branch from its tip respectively. The total of the 10 branches assessed in the horizontal transect had 22, 87 and 55 aphids at 0-5, 6-10, 11-15 cm along the branch from the tip respectively. These assessments showed that the aphids were present at all heights along the hedge, although branches with aphids present had been assessed in preference to branches with no aphids at this time. Winged aphids were seen in low numbers on the branches during the assessment; they could be seen resting on the external tips of the branches, with a mean of 5.4 winged aphids in each area. The morning of the 9 May was dull. However, as the aphids were not present on the 10 May, the assumption was that they had flown and dispersed during the brighter afternoon of the 9 May.

Aphids were again found along the length of the hedge on 18 May. There was a mean of 17 aphids per branch for the 32 samples. By 31 May there was a difference in the incidence of damage between the south and the north side of the hedge (Figure 3). At this time the brown areas of the hedge did not support any aphid populations and there was no detectable difference between incidence of damage in assessment areas with low and high aphid numbers. On average there was a mean of 11 aphids (+/- s.e. = 1.4) per branch for the 30 samples.

On 13 June aphids were recorded in all areas of the hedge, but were still more prevalent on the south side. Aphid numbers on 26 branches sampled across the south side of the hedge were 16 (+/- s.e. 3.2). By 16 July few live aphids were found on the branches sampled. However, there were signs of previous aphid presence on almost all branches assessed.

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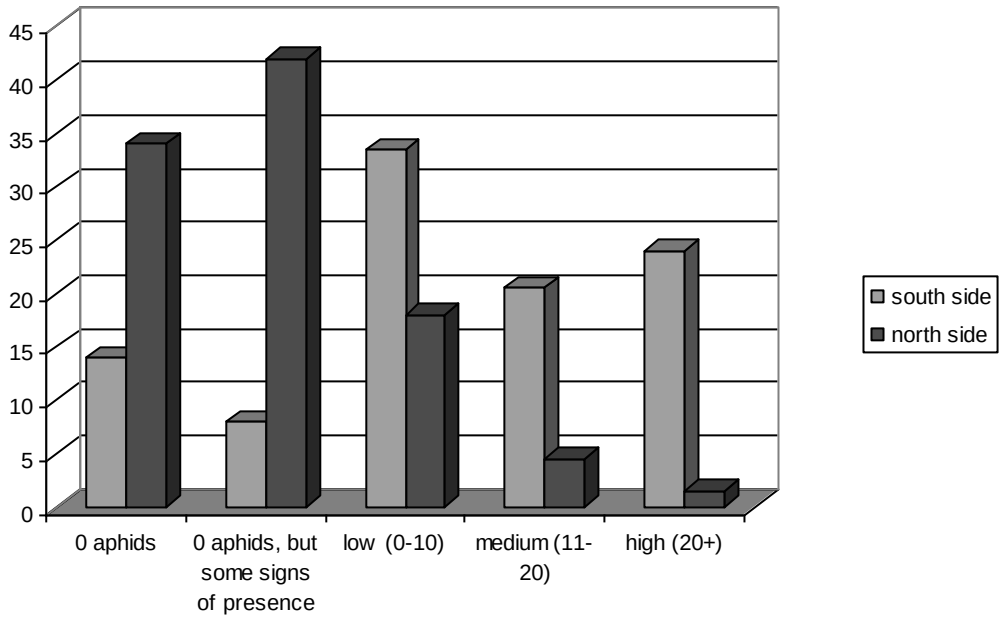

Figure 3: Percentage of branches with aphid levels in each category (200 branches assessed on each side) on 30-31 May 2007

No aphid eggs were found on the branches sampled on 28 January 2008, although signs of previous aphid infestation from 2007 were still clearly visible. No live aphids were found in any of the samples taken on 28 February or 17 March 2008.

No aphids were found in any of the tap samples taken on either the upper or lower branches on 17 or 29 April or in associated visual assessments of the trees. No live aphids were found in assessments of two other sites in the same locality on 11 and 29 April; *exuviae* from the previous year were still visible as were empty parasitised aphids from which the parasitoid had emerged.

In samples taken on 7 May one winged aphid was found. There were no aphids in samples taken from the two other local hedges that had been sampled on 11 and 29 April. However, on a hedge adjacent to the case study hedge 7 apterous adults, 1 nymph with wing pads and one alate *C. cupressivora* were recorded.

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When the aphid population structure was assessed in the laboratory over time all nymphal stages and adults were found, of both alate (winged) and apterous (non-winged) forms. Alate *C. cupressivora* have an additional 4th nymphal instar during development; developing wing pads can be seen under the microscope on 3rd and 4th instar nymphs but not on 1st and 2nd instars. The population structure of aphids on the hedge changed over time (Table 5). Alate aphids were produced in May; 24% of aphids collected on 18 May were alates. This agrees with the timing of flight recorded in the visual assessment on 9-10 May above. Numbers of alate aphids present were very low on the subsequent assessment dates.

Table 5: Population structure of aphids from conifer branches sampled on 18, 30 May and 13 June 2007; percentage of total collected on each date in each category

Date	Total aphids	Apterous or alate		Apterous aphids		Alate aphids		
		Instar 1	Instar 2	Instar 3	Adult	Instar 3	Instar 4	Adult
18.05.07	86	23	16	27	9	1	17	6
30.05.07	57	23	49	16	7	4	0	2
13.06.07	90	44	31	19	4	0	0	1

2. Inoculation of Leyland and Lawson cypress plants held in controlled conditions

Methods

Four *Cupressocyparis leylandii* plants and individual plants of *Cupressocyparis leylandii* 'Castlewellan Gold', *Chamaecyparis lawsoniana* 'Pembury Blue' and *Chamaecyparis lawsoniana* 'Broomhill Gold' were purchased from local nurseries that had not used insecticides in their compost. These plants were inoculated with aphids on 22 May 2007 by placing aphid infested branches collected from the case study site in Kent on the plants. Plants were held at 20°C in long-day length 14:10 light:dark conditions. Detailed assessments of the aphid populations on the plants were carried out on 20 July, 24 August and 23 October 2007 and 14 March 2008.

Results

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On 14 June the plants were inspected and aphids could be seen on some branches of all varieties. Populations were highest on the 'Castlewellan Gold' and aphids had started to drop from the branches, perhaps due to overcrowding or to a natural migration phase at this time. All dropped aphids, approximately 60 in total, were apterous (non-winged) adults.

'Castlewellan Gold' - By 20 July numbers of aphids had decreased from the numbers recorded on 14 June, with a total of 39 non-winged adults, 1 winged adult and 93 nymphs on the plant. By 24 August only four branches contained any live aphids, with only nine aphids recorded in total. At this time the plant was showing signs of browning damage. On 23 October there were few live aphids present, and browning was obvious especially on the lower branches (Appendix photograph 8). An assessment of each individual branch (including the smaller branches at the bottom) was done. Infestation levels were classed as previously having been low, medium or high and the branch colour was recorded. Brown branches were associated with previously high numbers of aphids, whereas green branches had had no aphids present.

'Broomhill Gold' - By 20 July there were nine infested areas present, and a total of 117 aphids (ranging from 2 to 42 per infested area). On 24 August there were ten colonies, five of which had between 40-50 aphids; there were in excess of 350 aphids present on the plant. By 23 October there were only four aphids present, 1 adult and 3 nymphs. Of the 20 branches assessed 3 showed no previous signs of aphid presence and had no damage, 9 branches had previously had low aphid numbers of which 5 were green and 4 were showing damage symptoms, 6 had previously had medium infestation levels and 2 had previously had high aphid populations and all were showing damage symptoms (Appendix photograph 9).

'Pembury Blue' - On 20 July only 2 adults were found on this plant. On 24 August aphids were only found on one branch, however by 23 October, when 22

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branches were assessed, five branches showed browning, generally at the base of the branch. Of these branches, four had either a high infestation of live aphids (20+ aphids) or *exuviae*, indicative of a previously high aphid infestation. Only 1 of these branches had a low number (4) of aphids. Of the green branches, 6 had no aphids, 9 had low numbers of aphids, and 1 had 11 aphids (medium infestation level).

Cupressocyparis leylandii – The aphid populations developed differently on the four plants inoculated with aphids, but on 20 July all trees had some aphid colonies present. The maximum number of aphids recorded on a plant was in excess of 50. Aphids had also fallen or dropped from the plants; 68 aphids were found under one plant. By 24 August no aphids were visible on the plants and branches that had supported aphid colonies were showing damage symptoms. Branches that had no signs of previous aphid infestation remained green. No live aphids were found in subsequent assessments.

On 14 March 2008, aphids were visible only on one *Cupressocyparis leylandii* plant. This was destructively sampled and aphids counted under a microscope. There were live aphids on 5 branches and signs of dead aphids and *exuviae* on 32 branches. Only 14 branches had no signs of aphids and the majority of these were in the upper half of the tree. There were no signs of any eggs or hatched eggs. Many aphids were observed to have dropped from the branches onto the surface below; it was not clear when this had occurred.

Discussion

The case study showed that *C. cupressivora* feed on the woody part of the branch with maximum numbers found 6–10 cm from the shoot tip. *Cinara cupressivora* has a relatively short rostrum (an external part of the mouthparts) and in aphids this is

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generally associated with being able to feed at locations on the plant where the bark is thin (*e.g.* Carter & Maslen, 1982).

Alate aphids were present and flying early in May, with numbers decreasing later in the season. Populations of apterous aphids were present on the hedge until early July, after which it became difficult to locate colonies of live aphids; *exuviae* and honeydew were still apparent on the branches that had previously been infested. It is not clear what caused the population crash at this time. In other aphid species reductions in aphid populations can be due to the poor nutritional state of the plant. However, it could also be a result of predation or parasitism; spiders were often seen in the branch assessments on the case study hedge (and on other hedges sampled) and some parasitised aphids were also seen. Numbers of aphids were affected by temperature, with higher populations found on the south side of the hedge. Since numbers of aphids on the hedge were very low in July it was not possible to determine if a second winged migratory phase occurred from June to August or if egg-laying females and males occurred in autumn as suggested by Ciesla, 1991, and Anon., 2003. However, no overwintering eggs of *C. cupressivora* were found in the sample taken on 28 January. It is possible that if eggs were present they were distributed in low numbers across the hedge and were not detected in the sampling. No overwintering aphids were detected either, so the biology of *C. cupressivora* during the winter is still not clear. By early May 2008 adult apterous and alate aphids plus nymphs were present in low numbers on the hedge; this is in line with the sampling results from 2006.

In the potted plant experiments there was evidence that aphid feeding caused browning damage on the plants. There appeared to be a difference in susceptibility to *C. cupressivora* in the conifer varieties used in the experiment, but since only individual plants of three varieties were used this would need further investigation. Anecdotal evidence suggests that 'Castlewellan Gold' is particularly susceptible to aphid damage (Roger Ward, pers comm.).

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Objective 5. Confirm the identity of any aphid species associated with plant damage

Recent work has suggested that the species formerly called *Cinara cupressi* (conifer aphid) is in fact a complex of four species (Watson *et al.*, 1999), three of which cannot easily be separated. Two of the species, *Cinara cupressi* and *C. cupressivora* both occur in UK. Using the criteria outlined in Watson *et al.* (1999), samples of aphids collected during the surveys done in this project were identified as *Cinara cupressivora*.

Overall Conclusions

- Analysis of the responses to the survey undertaken in year 1 suggests that, of the factors that were analysable, only trimming the hedges in the autumn months (predominantly October) was significantly linked with the occurrence of foliage dieback in Leyland cypress

- Questionnaire respondents were unable to provide detailed information about the occurrence of possible pests or diseases on hedges affected by dieback and it was thus not possible to draw any conclusions on the impact of these factors

- Analysis of the causes of foliage dieback in Leyland cypress hedges in the 78 samples sent to the RHS Advisory Service in 2005 and 2006 determined that in 24% of the samples damage could be attributed to aphids. Other contributors were honey fungus (in 18% of the samples) and physiological causes, *e.g.* dry soil; dense spacing of the hedge; excessive shoot pruning (in 14% of the samples). Only 5% of samples analysed in 2007 showed signs of *C. cupressivora* presence

- In the hedge sampling programme undertaken in year 1, not all hedges that were reported as damaged showed signs of aphid presence on the plant

material examined. Evidence that *Cinara cupressivora* was or may have been present was seen in samples of plant material from only 11 of 21 locations sampled (52%)

- Not all damage symptoms seen on the vegetation at sites where aphids were present appeared to be caused by aphid feeding

- We were unable to establish colonies of *C. cupressivora* on potted plants or a mature hedge in experiments designed to demonstrate the effects of feeding damage by *C. cupressivora*. Reasons for this are unclear

- In assessments of hedges in 2007, most damaged branches with a firm (non-wilted) appearance were associated with aphid presence; brown wilted branches were not associated with aphid presence. Aphids were also found on green branches; it would be expected that these branches would become brown over time

- The biology of *C. cupressivora* was investigated on an infested hedge over a year. This case study showed that:

    - *C. cupressivora* feed on the woody part of the branch with maximum numbers found 6-10 cm from the shoot tip
    - Alate aphids were present and flying early in May, with numbers decreasing later in the season
    - Populations of apterous aphids were present on the hedge until early July, after which it became difficult to locate colonies of live aphids
    - Since numbers of aphids on the case study hedge were very low in July it was not possible to determine if a second winged migratory phase occurred from June to August or if egg laying females and males

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occurred in autumn. However, no overwintering eggs of *C. cupressivora* or any live aphids were found in samples taken in January

- By early May 2008, adult apterous and alate aphids plus nymphs were present in low numbers on the hedge

- In the case study hedge general signs of hedge yellowing were first seen in April, when adult apterous aphids and obvious honeydew were present; hedge browning was seen in areas with both low and high aphid numbers

- In potted plant experiments with different varieties of conifer, there was evidence that aphid feeding caused browning damage on the plants

- Samples of aphids collected during the surveys were identified as *Cinara cupressivora*

Technology transfer

Olga Grant (2007). Hardy Nursery Stock Research at East Malling Research. Oral presentation to the Hardy Nursery Stock technical committee of the Horticultural Trades Association.

Jean Fitzgerald (2007). Oral presentation of results of conifer aphid project to Kent Horticultural Discussion Group.

Olga Grant and Jean Fitzgerald. (2007). Browning disorders in ornamental conifers. Article for *HDC News.*

References

Alleck, M. & Seewooruthun, S.I. (2001). *Cinara cupressivora*, a pest of cypress: some aspects of its biology and the assessment of its damage. *Revue Agricole et Sucriere de L'Ile Maurice*, 80/81 (3/1-3) 17-28

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Anonymous (2003). Cypress aphid, *Cinara cupressi*. *Forest Research Entomology Branch Advisory Note*, May

Binazzi A., Covassi M.V. & Roversi P.F. (1998). Role of *Cinara cupressi* and other sap-sucking insects in the decay of the common cypress (*Cupressus sempervirens*). *Annali*, 47, 55-66

Carter, C.I. & Maslen, N.R. (1982). Conifer lachnids. *Forestry Commission Bulletin 58*

Ciesla, W.M. (1991). Cypress aphid, *Cinara cupressi*, a new pest of conifers in eastern and southern Africa. *FAO Plant Protection Bulletin*, 39, 82-93

Grieg B. & Patch D. (2002). Leylandii – are they dying? *Tree damage Alert 75*. AAIS.

Inserra R.N., Luisi N. & Vovlas N. (1979). The role of infestations by the aphid *Cinara cupressi* in cypress decline. *Informatore Fitopatologico*, 29, 7-11

Memmott J., Day R.K. & Godfray H.C.J. (1995). Intraspecific variation in host plant quality: the aphid *Cinara cupressi* on the Mexican cypress, *Cupressus lusitanica*. *Ecological Entomology*, 20, 153-158

Mills, N.J. (1990). Biological control of forest aphid pests in Africa. *Bulletin of Entomological Research*, 80, 31-36

Murphy S.T., Chilima C.Z., Cross A.E., Abraham Y.J., Kairo M.T., Allard G.B. & Day R.K. (1994). Exotic conifer aphids in Africa: ecology and biological control. In Leather S.R., Watt A.D., Mills N.J. & Walters K.F.A. (eds) Individuals, populations and patterns in ecology. *Intercept*, pp 233-242

Mustafa T.M. (1987). Reproductive biology and population studies of cypress aphid *Cinara cupressi* and pine aphid *C. maritimae*. *Dirasat*, 14, 99-105

Obiri J.F. 1994. Variation of cypress aphid (*Cinara cupressi*) attack on the family Cupressaceae. *Commonwealth Forestry Review*, 73, 43-46

Strouts R.G. (2003). Major diseases and pests of conifers in British gardens. *Acta Horticulturae*, 615, 297-301

Watson G.W., Voegtlin D.J., Murphy S.T. & Foottit R.G. (1999). Biogeography of the *Cinara cupressi* complex on Cupressaceae, with description of a pest

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species introduced into Africa. *Bulletin of Entomological Research*, 89, 271-283

Winter, T.G. (1989). Cypress and juniper aphids. D.O.E. Arboricultural Advisory and Information Service, *Arboriculture Research Note 80*

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Appendix (Photographs)

Appendix Photographs 1-3: Examples of damaged hedges

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Appendix Photograph 4: *Cinara cupressivora* on conifer sample

Appendix Photograph 5: Evidence that aphids have been present on the sample; cast skin and sooty mould growing on honeydew

Appendix Photograph 6: Evidence that aphids have been present on the sample; cast skins

Appendix Photograph 7: One-year-old plants in gauze house inoculated with *C. cupressivora*

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Objective 4: Damage symptoms seen on potted plants previously innoculated with aphids in assessments on 23-24 October 2007

Appendix Photograph 8: Lower branch browning of *Cupressocyparis leylandii* 'Castlewellan Gold'

Appendix Photograph 9: Lower branch browning of *Chamaecyparis lawsoniana* Broomhill Gold

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Objective 4: Case study hedge

Appendix Photograph 10

Objective 4: High aphid population density on case study hedge

Appendix Photograph 11

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