

PHC2018/03 – Final report on the assessment of the potential of the psyllid *Trioza anthrisci* to vector 'Candidatus Liberibacter solanacearum' (Lso) in Scotland

The aim of this project was to assess the distribution and population numbers of the psyllid *Trioza anthrisci* and its potential as a vector of 'Candidatus Liberibacter solanacearum' (Lso). Lso Haplotypes A and B are responsible for the economically damaging Zebra Chip disease on potato in New Zealand and USA, while Haplotype C causes stunted growth and discoloured leaves on carrot crops in Scandinavia and Northern Germany. Whilst other haplotypes have been discovered, the three identified above are currently considered to be those of most concern to agriculture in Scotland, with A and B listed as quarantine diseases which are not present in the EPPO region. Specimens of *T. anthrisci* carrying Lso Haplotype C have recently been discovered in the UK using the suction trap network. Individuals were caught in the Elgin trap, but the distribution and host plant in Scotland were not known. This species is relatively common in Scandinavia (where Lso haplotype C is present in carrot crops), although *Trioza apicalis* is considered the main vector species of Lso on commercial carrot in this region. *T. apicalis* is not believed to be widely distributed in the UK, and has not been confirmed as present in Scotland.

1a - Co-ordinated sampling by all teams (July-end August)

This focused on locating live individuals of *T. anthrisci* by sweep netting carrot crops and adjacent field borders in the main carrot growing areas, utilising local staff from Forest Research (FR), Hutton and SASA to sample an extensive area with minimal time away from base (fig 1). Teams conducted sampling over a six week period between July and early September 2018 at multiple sites between Edinburgh and Elgin (table 1). Several species of psyllid were found in both field borders and crops, but *T. anthrisci* was located in only one location in a carrot crop near Elgin by FR staff on 1 August 2018 (table 2), within 6 km of the original trapping. Identification of the psyllid was performed at SASA using classical taxonomy and molecular barcoding. Once the species was confirmed as *T. anthrisci*, follow-up sampling from two carrot crops near Elgin by SASA and Hutton staff collected sufficient individuals to set up colonies at two sites (SASA and Hutton, Invergowrie). It is of note that the carrot psyllid, *T. apicalis* (the main vector in Scandinavia, and known to be present in the U.K.) was not found during sampling.

Fig 1. Map of sites sampled for the presence of *T. anthrisci* July-September 2018 (red=positive)



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Table 1. Date, location and result of non-crop psyllid sampling by PHC teams between July and September 2018.

Date	Location	Sampling team	vegetation sampled	Psyllids found	<i>T. anthrisci</i> found
05/07/2018	Dronley, Angus	Hutton	cow parsley near woodland	N	N
12/07/2018	SASA, Midlothian	SASA	nettle, hawthorn	Y	N
17/07/2018	East Grange, Forfar	SASA	nettle	Y	N
17/07/2018	Tayport, Fife	SASA	nettle	Y	N
18/07/2018	Scone, East Perth	SASA	nettle	Y	N
18/07/2018	Hennhill, Perthshire	SASA	nettle	Y	N
19/07/2018	Newton, Elgin NJ15996380	FR	umbelliferous weeds	Y	N
2 x July 2018	Balruddery, Dundee NO30023281	Hutton	umbelliferous weeds in field margins	N	N
3 x July 2018	Moncreiffe Island, Perth NO120230	Hutton	umbelliferous weeds in woodland undergrowth	N	N
1/8/2018	Wester Manbeen, Elgin	FR	Nettles and umbelliferous weeds (2 sites)	N	N
02/08/2018	Tayport, Fife	SASA	nettle	Y	N
02/08/2018	Springfield, Fife	SASA	nettle	Y	N
14/08/2018	Insch NJ653276	Hutton	field margins	N	N
14/08/2018	Elgin NJ232639	Hutton	field margins	Y	N
14/08/2018	Wester Manbeen, Elgin	Hutton	field margins	N	N
15/08/2018	Tynninghame, East Lothian	SASA	field margins	Y	N
16/08/2018	Hennhill, Perthshire	SASA	nettle	Y	N
05/09/2018	Elgin NJ232639	Hutton	woodland near carrot rows	Y	N

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Table 2. Date, location and result of carrot crop psyllid sampling by PHC teams between July and September 2018.

Date	Location	Sampling team	Psyllids found	<i>T. anthrisci</i> found
12/07/2018	SASA, Midlothian	SASA	Y	N
17/07/2018	Tayport, Fife	SASA	Y	N
17/07/2018	Springfield, Fife	SASA	Y	N
18/07/2018	Scone, East Perth	SASA	Y	N
18/07/2018	Hennhill, Perthshire	SASA	Y	N
18/07/2018	Milnathort, Kinross	SASA	Y	N
01/08/2018	Wester Manbeen, Elgin (2 sites)	FR	Y	1
08/08/2018	Wester Manbeen, Elgin	SASA	Y	8
17/08/2018	Wester Manbeen, Elgin	Hutton	Y	2
21/08/2018	Forfar NO442442	Hutton	N	N
21/08/2018	Kirriemuir NO392524 NO408550 NO409554	Hutton	N	N
23/08/2018	Wester Manbeen, Elgin	Hutton	Y	5
28/08/2018	Wester Manbeen, Elgin	Hutton	Y	N
29/08/2018	Wester Manbeen, Elgin	Hutton	Y	4

2a – Development and maintenance of colonies for transmission studies (August – December)

This focused on developing rearing techniques to allow colonies of *T. anthrisci* to be maintained for future transmission studies. *T. anthrisci* collected during sampling as part of objective 1a were reared on cow parsley (*Anthriscus sylvestris*) and carrot plants in cages within CE insectaries at SASA and Hutton respectively (fig 2). Temperatures were maintained at 18 °C and day length at 16:8 light:dark. Teams communicated regularly by E-mail to compare rearing methods, and the SASA team visited Hutton on 16th November to observe their rearing facilities and discuss progress. *T. anthrisci* colonies declined and were mostly lost by late December 2018. A very small number of individuals remained in culture at SASA by January 2019,

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and immature life stages and eggs were observed in the cages by mid-February so it is hoped that this culture will continue. Teams concluded during a 'lessons learned' discussion at the training event in January that a larger initial population of *T. anthrisci* may be more likely to establish (recommending that at least 20-30 individuals are used to form founding colony), and that the cow parsley appears to be a suitable host plant, allowing the species to complete a full life cycle. Furthermore we now have a better understanding of the geographic location of this species and when and where to sample to collect live individuals of this species. This will aid in the set-up of further cultured colonies during the upcoming field season. 16 individuals of *T. anthrisci* collected from the Elgin area were tested for Lso via real-time PCR, 100 % of specimens were positive for Lso. Positive samples were then amplified using PCR and 3 ribosomal gene regions were sequenced to determine the Lso haplotype. All psyllids harboured the carrot associated haplotype C (average $C_t = 33.17$).

Fig 2. Mating pair of *Trioza anthrisci* on cow parsley at SASA rearing facility.



3a – Training Workshop

Objective 3a identified a requirement for a training workshop to ensure adequate skills were available within the PHC in the event of an outbreak. A training programme was developed (appendix 1) with modules on basic identification of

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psyllid species, molecular identification, awareness of species of plant health concern, an update on developments, collection and rearing. This workshop was held at SASA on 22-23 January 2019, with seven attendees from FR, Hutton and SASA, all of whom had entomology related specialisms within their own field (table 3). Training expertise was provided by SASA staff and David Ouvrard from NHM (attendees and trainers pictured in fig 3). Positive feedback was received from attendees who reported that the specific training objectives had been met, and beyond this many noted that learning the taxonomy of a new group of insects had helped them to refresh their general scientific expertise.

Table 3. List of tutors and trainees attending the psyllid training event held at SASA on 22/23 January 2019.

Name	Organisation	Tutor/attendee
David Ouvrard	NHM	Tutor
Mairi Carnegie	SASA	Tutor
Jason Sumner-Kalkun	SASA	Tutor
Alison Karley	Hutton	Attendee
Gaynor Malloch	Hutton	Attendee
Jenni Stockan	Hutton	Attendee
Carolyn Mitchell	Hutton	Attendee
Katy Dainton	FR	Attendee
Fiona Highet	SASA	Attendee
Yvonne Arnsdorf	SASA	Attendee

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Fig 3. Attendees and tutors from the psyllid workshop 22-23 January 2019.



Conclusions and summary

The project has allowed the PHC to better understand the distribution and number of *T. anthrisci* populations in carrot growing areas to better inform assessment of the risk of Lso transmission to crops in Scotland. A small population of this species has been identified in the North East, detected on carrot crops around Elgin during August. No further populations were found on carrot crops further south or on wild plants at any sampling site across Scotland, so the population may be relatively small and isolated. Although found on carrots, individuals collected were reared successfully on cow parsley, which is commonly found in Scotland around

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hedgerows and verges. It is therefore possible that the distribution is wider than that observed during sampling, but that difficulties in inspecting and sampling of plants contributed to the lack of detection. This wider distribution, especially in the north of Scotland, could have important implications for high health potato seed production in this region.

This project has allowed staff working within the PHC to develop new networks and work in partnership, sampling a more extensive area and sharing risk by working together. The objectives have been met and a colony of *T. anthrisci* is now in culture at SASA, which can be optimised through lessons learned during this study and potentially used for Lso transmission studies in future projects. Plant Health Centre capability has been increased, improving preparedness in the event of an outbreak.

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