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GUDBJORG ARADOTTIR (*) (**) - ANGELA KARP (*) - STEVE HANLEY (*) - IAN SHIELD (*)
CHRISTINE WOODCOCK (*) - SARAH DEWHIRST (*) - MATILDA COLLINS (**)
SIMON LEATHER (**) - RICHARD HARRINGTON (*)

HOST SELECTION OF THE GIANT WILLOW APHID (*TUBEROLACHNUS SALIGNUS*)

(*) *Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK;* richard.harrington@bbsrc.ac.uk
(**) *Imperial College London, Silwood Park Campus, Ascot, Berkshire, SL5 7PY, UK.*

Aradottir G., Karp A., Hanley S., Shield I., Woodcock C., Dewhirst S., Collins C., Leather S., Harrington R. – Host selection of the giant willow aphid *(Tuberolachnus salignus).*

The giant willow aphid [*Tuberolachnus salignus* (Gmelin)] has recently become noteworthy as a potential pest species due to the increased uptake of willow, its host-plant, for use in growing biomass for energy production. In this paper we describe host selection studies of *T. salignus* on short rotation coppice (SRC) willow varieties in laboratory bioassays and field experiments. In laboratory olfactometry tests, *T. salignus* was significantly attracted to certain SRC willow varieties, but not to others. Field trials during 2007 and 2008 showed that *T. salignus* infestation levels varied significantly on different SRC willow varieties and that levels are highest on the varieties to which they are most strongly attracted in the laboratory bioassays.

KEY WORDS: Willow, aphid, *Tuberolachnus salignus*, chemical ecology, host selection.

INTRODUCTION

Willow (*Salix* spp) grown as short rotation coppice (SRC) is one of the main biomass crops in the UK and has been identified as appropriate for the production of biomass energy in the Palearctic and North America due to its potential for rapid growth in temperate climates (KARP and SHIELD 2008).

Previous work has shown that willow plantings are beneficial to biodiversity. Bird diversity is high, generally exceeding that of agricultural land (VOLK *et al.,* 2006) and invertebrate surveys have also shown that butterfly abundance is higher in the margins of willow fields than around arable crops (HAUGHTON *et al.,* 2009).

There are a number of insect pests associated with willow trees. Among these is the giant willow aphid [*Tuberolachnus salignus* (Gmelin)], which has become more noticeable in willow plantings in recent years. *Tuberolachnus salignus* is a large aphid species, measuring up to 5,8 mm in length. It lives on stems and branches of various willow species, where it builds up large colonies and attracts insects, such as wasps, that feed on honeydew. *Tuberolachnus salignus* is found almost everywhere willows are planted, except for Australasia (BLACKMAN and EASTOP, 1994). Many aspects of the ecology of this aphid species and its interactions with host plants are poorly understood. The species is known to be anholocyclic, but where and how it overwinters is not known. Studies by COLLINS *et al.* (2001a, 2001b) showed that, in laboratory conditions, infestation by *T. salignus* could reduce above- and below-ground growth and the mass of existing wood tissue of willow. The presence of *T. salignus* on willow trees also increases photosynthetic rate, water use and nitrogen content in leaves (COLLINS, 2001; COLLINS *et al.,* 2001b).

The predicted expansion of land under SRC willow, as well as the possibility of springs and summers in the UK becoming warmer, has raised concerns that this aphid may become a serious pest on willow crops. There is therefore a need to understand the threat of this potential pest species by studying its biology and host selection behaviour, to inform the development of effective management strategies.

In this research, host selection studies of *T. salignus* on SRC willow varieties were undertaken to investigate the hypotheses that a) aphid behaviour is affected by chemical cues from the host and b) differences in host–aphid interactions have a basis in the genetics of the host. To address these hypotheses, laboratory bioassays and field experiments were carried out on the infestation pattern of *T. salignus* on different willow varieties.

METHODS

BIOASSAYS

Cultures of the giant willow aphid (*T. salignus*) were established in an insectary in October 2006. The aphids were collected from trees in the National Willow Collection at Rothamsted Research, Harpenden, UK, and reared on the willow variety 'Resolution' [(*Salix viminalis* L. x *viminalis* 'Jorrun') x (*S. viminalis* x *S. schwerinii* Wolf 'Bjorn')]. Aphids were kept at 18°C with a regime of 16 hours of light per 24 hours. Alate *T. salignus* which had been starved overnight were used for the bioassays.

Plant material was obtained from the National Willow Collection at Rothamsted Research. The list of accessions used in testing is shown in Table 1. The non-host species poplar, variety 'Unal', was also included as a control.

Olfactometer tests were performed in the laboratory using a Linear Track Olfactometer (LTO) modified from the original design (SAKUMA and FUKAMI, 1985), and six willow genotypes were initially chosen for use in the bioassays. The treatments were placed in glass jars out of sight of the olfactometer. Using airflow of 3 l/min and a plant mass of 30±2 g (stem and leaf), aphids were

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Table 1 – Willow varieties used for bioassays and their parentage.

Variety name	Parentage
Bowles Hybrid	*S. viminalis* L.
Discovery	*S. schwerinii* Wolf 'K3 Hilliers' x (*S. viminalis* L. x *S. schwerinii* Wolf 'Bjorn')
Tordis	*S. viminalis* L. x *S. schwerinii* Wolf
Stott 10	*S. burjatica* Nasarov x *S. viminalis* L.
Baldwin	*S. triandra* L.
S. gilgiana	*S. gilgiana* Seemen
Unal (poplar)	*Populus trichocarpa* L. x *P. deltoides* Bartr.Ex Marsh.

observed for ten minutes climbing up a vertical wire to a T-junction with a horizontal wire, leading to a choice of two directions. Only responding aphids were counted and the decision at the T-junction was recorded. To avoid bias, the direction from which the odour was presented was alternated.

Each of the different plants was tested against a control of a Whatman filter paper moistened with distilled water. To test *T. salignus* attraction to conspecifics, ten apterae were tested against the same control. The data were analysed using a one-tailed chi-square probability test for distribution.

FIELD TRIAL

The field trial comprised six SRC willow varieties (Table 2), replicated six times in plots of 40 plants (10x4), using a 6x6 Latin square design, and a plot size of 4.5x4.8 m.

The willow material was obtained from the National Willow Collection at Rothamsted Research. Cuttings for the trial were taken on 19th and 30th January 2007 and stored in a cold room at -4°C. Before planting, the cuttings were soaked in water for 24 hours at room temperature. The field trial was planted on 2nd April 2007, in Highfield IV at Rothamsted Research, and cut back in March 2008 after the first year of growth, in accordance with standard management methods for SRC willows.

Measurements of aphid infestation were recorded using seven size classes (Table 3). Two trees from each plot were assessed.

The infestation level data were analysed using Anova, testing for differences in aphid infestation level between varieties, and for each date separately, in Genstat 10 (PAYNE *et al.,* 2007).

RESULTS

Out of the alatae placed in the holding pot of the LTO, 61.7% (n=474) responded. *Tuberolachnus salignus* showed a significant positive response to 'Bowles Hybrid' and 'Discovery', but no willow variety elicited a negative response from the aphids (Table 4). When testing for responses to poplar, a non-host plant, the results were not significant, with half going towards the plant odour and half going to the control. The alatae did not show an attraction to the apterous aphids.

In contrast to results found when 'Bowles Hybrid' was tested against the control, when the aphids were given the choice between 'Bowles Hybrid' and 'Baldwin' in the LTO (Table 5), they did not show a significant response to 'Bowles Hybrid'.

Preliminary analysis on results from field trials during 2007 and 2008 have shown that *T. salignus* infestation levels vary significantly on different SRC willow varieties when infestation is at its peak density (18 September 07, P=0.005; 28 October 08 P=0.007) and that levels were

Table 2 – Willow varieties planted in field trial and their parentage.

Variety name	Parentage
Bowles Hybrid	*S. viminalis* L.
Resolution	(*S. viminalis* L. x *S. viminalis* L. 'Jorrun') x (*S. viminalis* L. x *S. schwerinii* Wolf 'Bjorn')
Tora	*S. viminalis* L. x *S. schwerinii* Wolf
Tordis	*S. viminalis* L. x *S. schwerinii* Wolf
Q83	*S. triandra* L. x *S. viminalis* L.
Baldwin	*S. triandra* L.

Table 3 – Size classes used to measure area of aphid colonies on willow trees in the field.

Area	Area mid-point
no infestation	0
≤ 1 cm^2	0.5
> 1 cm^2, ≤ 8 cm^2	4.5
> 8 cm^2, ≤ 27 cm^2	17.5
> 27 cm^2, ≤ 64 cm^2	45.5
> 64 cm^2, ≤ 125 cm^2	94.5
> 125 cm^2, ≤ 216cm^2	170.5

Table 5 – Results from Linear Track Olfactometer bioassay testing 'Bowles Hybrid' against 'Baldwin'. Number of starved alatae choosing willow or control: n, total number tested; pobs, proportion observed going towards the willow odour; chi square; d.f., degrees of freedom and *P*-value.

Bowles Hybrid	Baldwin	n	pobs	χ^2	d.f.	P
42	28	70	0.60	1.40	1	0.24

Table 4 – Results from Linear Track Olfactometer bioassays testing different willows against the control. Number of starved alatae choosing willow or control: n, total number tested; pobs, proportion observed going towards the willow odour; chi square; df, degrees of freedom and *P*-value.

Varietyname	Willow	Control	n	pobs	χ^2	d.f.	P
Discovery	43	18	61	0.70	5.12	1	0.02
Bowles Hybrid	28	10	38	0.74	4.26	1	0.04
Tordis	24	18	42	0.57	0.43	1	0.51
Stott 10	18	23	41	0.44	0.30	1	0.58
Baldwin	20	27	47	0.43	0.52	1	0.47
Salix gilgiana	28	41	69	0.41	1.22	1	0.27
Poplar	14	14	28	0.50	0.00	1	1.00
10 aphids	30	48	78	0.38	2.08	1	0.15

highest on the varieties which give the highest positive response in the laboratory bioassays. This work needs further substantiation. The infestation peaked just over a month earlier in 2007 than in 2008.

DISCUSSION

The results confirm the hypothesis that *T. salignus* is affected by the chemical cues from their host plant, as they respond differently to the odour of different willow varieties in the olfactometer. *Salix viminalis* 'Bowles Hybrid', which has been shown to be susceptible to *T. salignus* infestation (COLLINS, 2001), had a significant positive response from the aphids in the olfactometer. COLLINS (2001) tested the variety Q83, a *S. triandra* L. x *S. viminalis* hybrid, and found it to be a poor quality food plant for *T. salignus*, in an experiment comparing six willow varieties. Here, a pure *S. triandra* variety ('Baldwin') was tested, but the aphids were not significantly repelled by this species in the olfactometer. The four most attractive willow varieties to *T. salignus* all have *S. viminalis* in their parentage. The most attractive were the pure *S. viminalis* 'Bowles Hybrid' and 'Discovery'. The least attractive varieties were 'Baldwin', a pure *S. triandra*, and *S. gilgiana* Seemen, but there was no significant difference between aphids going to the control over the odour of these three willows. *Salix triandra* has recently been shown to be genetically distant from other species of the subgenus *Salix* (TRYBUSH *et al.,* 2008). This is an interesting finding as *S. triandra* seems to have a low number of associated pests (COLLINS *et al.,* 2001a; TOPP *et al.,* 2002; HJALTEN *et al.,* 2007). The unattractiveness of this species has been attributed to its secondary compound composition, as it contains phenolic glucosides which can be detrimental to herbivores (LINDROTH, 1988; KOLEHMAINEN *et al.,* 1995). Based on the genetic diversity studies of willows, *S. gilgiana* is closely related to *S. purpurea* L. (TRYBUSH *et al.,* 2008), which has been shown to be resistant to several insects in America (NORDMAN *et al.,* 2005).

Tuberolachnus salignus did not show a significant attraction to 'Bowles Hybrid' when tested against 'Baldwin', a less attractive willow variety. The fact that 'Baldwin' was not significantly repellent to the aphids when tested on its own may explain the lack of a clear difference in responses to these two varieties when tested together. When *T. salignus* was presented with a choice between a control and 10 conspecific apterae, no significant preference was detected. This is surprising given that the species is entirely parthenogenetic, with no requirement to find a mate. Also, landing on a plant with a pre-existing colony could mean competition for resources.

There was a significant difference in infestation densities on the six willow varieties in the field trial for both field seasons 2007 and 2008. COLLINS (2001) showed that *T. salignus* has different reproductive output on different willow hosts. This raises the question whether the observed differences in infestation densities on different willow varieties are due to pre- or post-alighting behaviour by the aphids, or to a combination of both.

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