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The effects of host plant on the coccinellid functional response: Is the conifer specialist *Aphidecta obliterata* (L.) (Coleoptera: Coccinellidae) better adapted to spruce than the generalist *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae)?

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ABSTRACT

Female *Aphidecta obliterata* consumed an average of 26.6 ± 5.8 *Elatobium abietinum* and males consumed an average of 19.9 ± 3.2 *Elatobium abietinum*, but there was no significant difference in the number consumed between the sexes. In Petri dish trials, the larval stages of *A. obliterata* and all stages of *Adalia bipunctata* appeared to exhibit a Type II response to prey density, although *A. obliterata* adults showed a more linear response to prey density. There was no significant difference between the functional responses of the 3rd instars of the two coccinellid species, but there was a significant difference between the responses shown by the 1st instars, with *A. obliterata* larvae consuming more than those of *A. bipunctata*, especially at low densities, suggesting that the two species respond differently to an increase in prey density.

At low prey densities, adults and 4th instars of both species exhibited a similar response to an increase in prey density but at higher densities the 4th instars and adult stages of *A. bipunctata* showed higher attack rates when compared with the same stages of *A. obliterata*.

Adult and 4th instar *A. obliterata* exhibited Type II functional responses on spruce sections. The 4th instar *A. obliterata* larva appeared to be a more effective predator than the adult of the species, and was more effective when compared with adult *A. bipunctata* at lower prey densities but *A. bipunctata* adults appeared to be a more effective predator at higher prey densities.

The host plant affected the rate of consumption by adult *A. obliterata* as adults on Sitka spruce sections consumed significantly higher numbers of aphids than their counterparts on Norway spruce. This was most noticeable at densities above 16 aphids.

The distributions of the two coccinellid species in the olfactometer were significantly affected by the presence of host plant material. *Aphidecta obliterata* adults were found in significantly higher numbers in the Sitka spruce chambers than the control chambers (those without plant material). *Adalia bipunctata* adults were found in significantly lower numbers in the Norway spruce chamber than the control chamber.

Although *A. bipunctata* has a higher level of voracity than *A. obliterata*, the latter is more adapted to the spruce environment and the boom and bust population dynamics of *E. abietinum*.

1. Introduction

The functional response of a predatory insect is the response in its consumption rate, which it shows to different prey densities. It can be used as an indicator of its likely effectiveness as a control agent (Houck and Strauss, 1985; Jervis and Kidd, 1996). Insect predators generally exhibit a Holling Type II response to increasing prey density (Hassell et al., 1977), where the consumption rate rises at a constantly decreasing rate up to a maximum, with increasing prey density (Jervis and Kidd, 1996). In a few cases, however, coccinellids have been found to produce a Type I (Leather and Owuor, 1996) or a Type III response (Hassell et al., 1977). A Type I response describes a linear increase in consumption rates towards a maximum, and a Type III response is where consumption rates accelerate at low prey densities, then follows a Type II response, giving a sigmoid response curve (Jervis and Kidd, 1996). Hemptinne et al. (1996) also found that the response can be markedly different between the sexes, with male two-spot ladybirds, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) consuming fewer aphids and showing no functional response, unlike their female counterparts.

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The substrate on which a natural enemy forages can also affect its searching behaviour (Carter et al., 1984). The Petri dish is often used as the arena in which functional responses are measured in the laboratory (e.g. Hassell et al., 1977; Leather and Owuor, 1996). This allows standardisation of results and thus facilitates comparison between studies. It may not however, be representative of what occurs in nature. For example, Everson (1980) found that the activity of the predatory mite, *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae) and its prey *Tetranychus urticae* (Koch) (Acarina: Tetranychidae) differed on a glass Petri dish compared with a bean leaf substrate. It is therefore preferable to extend such work to include measurements on the plant host.

The larch ladybird, *Aphidecta obliterata* (L.) is a conifer specialist (Wylie, 1958), and has commonly been reported to feed on *Adelges* spp. (Homoptera: Adelgidae) (Wylie, 1958; Parry, 1992). It has however, also been found in association with the green spruce aphid, *Elatobium abietinum* (Walker) (Homoptera: Aphididae) (Parry, 1992; Austarå et al., 1997, 1998; Day et al., 2006), a serious defoliating pest of commercial plantations of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Straw, 1995; Day et al., 1998; Straw et al., 2005).

This study aimed to expand the current knowledge of the functional response of *A. obliterata* to *E. abietinum*. Consumption rates of males and females were compared and the effects of host plant on the functional response investigated. Comparisons were also made between the functional responses in a Petri dish and on sections of host plant, for this coccinellid which is a conifer specialist (Wylie, 1958), and for *A. bipunctata*, which is a generalist. *Adalia bipunctata* was the most abundant coccinellid species found on Norway spruce (*Picea abies*) infested with *E. abietinum* at Silwood Park, Ascot (Leather and Owuor, 1996).

2. Materials and methods

2.1. Cultures

2.1.1. Aphids

Cultures of *E. abietinum* were reared on 2–3 years old potted Sitka spruce, which were kept outdoors at Silwood Park, Ascot. Additional cultures were reared on Sitka spruce branches cut from 24-year-old trees at a field site in Radnor Forest, Wales (Straw et al., 2006). These were kept in buckets of water at 15 ± 1 °C, 16:8 light:dark and ambient humidity.

Rhopalosiphum padi (L.) cultures were maintained on barley (*Hordeum vulgare* (L.)) grown in pots and kept in cages at 15 °C, 16:8 light:dark and ambient humidity.

2.1.2. Coccinellids

Cultures of *A. obliterata* and *A. bipunctata* were maintained separately at 15 ± 1 °C, 16:8 h light:dark and ambient humidity in 14 × 9.5 × 26.5 cm perspex boxes with muslin covered holes in the lid. Corrugated 9 cm diameter filter papers were added to provide a suitable egg laying surface. The coccinellids were fed every other day with an excess of a mixture of *E. abietinum* and *R. padi*, which is known to be a satisfactory alternative food source (Jones, 1998).

2.2. Aphidecta obliterata male versus female consumption

Adult *A. obliterata* were sexed using differences in the characteristics of the abdominal sternites, a method which is accepted to be accurate for coccinellids (Majerus and Kearns, 1989). An equal number of males and females were starved for six hours each day commencing at approximately 09:30.

Starved adults were placed singly into 9cm diameter Petri dishes with Fluon® coated sides, containing 50 approximately 3rd instar *E. abietinum* aphids of a similar size. The reason for the approximation is that the size of each instar appeared to vary with the conditions under which they had been reared. Observation of aphids obtained from the potted Sitka spruce plants outside, compared with those reared on cut branches at 15 °C, 16:8 light:dark, was that the latter produced larger aphids than the former. Therefore, similar sized but not necessarily the same instar of aphids, were always selected for use. Each coccinellid was left to forage and feed for a period of 24 h before being removed. The number of aphids which had been consumed was then recorded. Aphids that showed no signs of attack were not included in this total. In all, 17 replicates for each sex were completed.

Adult aphids were not used, as in the work carried out by Leather and Owuor (1996), because these could produce young during the experimentation period which may have affected the results, and the approximately 3rd instars used, better represent the average individual size over the aphid population as a whole. It has been found that the early instars of coccinellid predators may experience difficulties when feeding on large aphids (Dixon, 1970).

Data were analysed using the Student's *t*-test.

2.2.1. Functional responses on the Petri dish surface

The functional responses of *A. obliterata* and *A. bipunctata* adults and 1st, 3rd and 4th instar larvae were measured at 15 °C, 16:8 h light:dark and ambient humidity, using densities of 1, 2, 4, 8, 16, 32, 64 and 128 *E. abietinum*. Second instar larvae were not used due to the fact that the starvation period induced early moulting to 3rd instar in a high proportion of cases. In the case of the adults, all were selected at random from the laboratory sample in order to accurately represent the sexes. An additional density of 200 aphids was used for the *A. obliterata* adults, but due to lack of appropriately aged aphids, not for the larvae, or for *A. bipunctata*.

All individuals were starved singly for 6 h prior to being placed into individual 9 cm diameter Petri dishes containing the relevant number of similar sized approximately 3rd instar *E. abietinum*. The coccinellids were allowed to feed for a 24 h period. The sides of all Petri dishes used were coated with Fluon® to prevent the aphids and coccinellids from crawling onto the roof of the dish.

Functional responses were determined as described below (Section 2.4). Differences in attack rates and handling times were analysed by fitting both species or life stages in a non-linear least squares regression (Section 2.3.1). Proportions of aphids consumed were analysed using ANOVA with binomial errors.

2.2.2. Functional responses on cut sections of spruce in a Petri dish

The same protocol was repeated with adults of both coccinellid species and 4th instar *A. obliterata*, using a cut 5 cm terminal shoot of Sitka spruce infested with 1, 2, 4, 8, 16, 32, 64 and 128 approximately 3rd instar *E. abietinum*. Uninfested spruce sections were placed into a 9 cm diameter Petri dish and the relevant number of aphids added. An hour later, any aphids which had not climbed onto the host plant were placed onto needles using a fine paintbrush if healthy looking or, if they appeared unhealthy (e.g. immobile), were removed and fresh aphids placed directly onto the host. A coccinellid was then placed onto each piece of spruce and a second Petri dish base was inverted and attached to the top. This was necessary because the lid sections were not tall enough and would have touched against the spruce needles. This experiment was repeated using 5 cm terminal shoots of Norway spruce, which is thought to be the original host of *E. abietinum* (Bejer-Peterson, 1962). This was done with adult *A. obliterata* only. Five replicates were carried out per treatment.

The predators were allowed to forage and feed for a 24 h period, after which they were removed and the remaining aphids carefully counted. The needle density was also recorded for each replicate.

Functional response was determined as described below (Section 2.3).

2.3. Type of functional response

Type of functional response was determined using a binomial logistic regression of proportion of prey eaten as a function of prey offered (Trexler et al. 1988; Juliano 2001) Eq. (1). Intercept (α), linear (β), quadratic (γ) and cubic (δ) coefficients were estimated as the sign of these coefficients is indicative of the type of functional response (Trexler et al. 1988; Pervez and Omkar, 2005). Type I responses are described by an intercept and constant positive slope (β). In Type II responses the linear coefficient (β) is < 0 and proportion of prey consumed declines monotonically with the initial number of prey offered. Type III responses are characterised by the linear coefficient (β) > 0 and the quadratic coefficient < 0. We fitted maximal models with linear, quadratic and cubic terms and simplified these in stepwise manner removing higher order coefficients where they were not significant. This allowed us to achieve a minimal adequate model, whereby coefficients could be inspected to infer the type of functional response occurring.

$$\mathrm{Na/N} \sim \alpha + \beta\mathrm{N} + \gamma\mathrm{N}^2 + \delta\mathrm{N}^3 + \epsilon \qquad (1)$$

2.3.1. Estimating parameters

Once the functional response was determined (Type II throughout) we used a linearization of Holling's disc equation (Holling, 1959) to find parameter estimates (Pervez and Omkar, 2005). Although this model usually characterises sampling with replacement (Fenlon and Faddy, 2006), we found it gave a good fit to our data with high r^2 values. A non-linear least squares model that assumes sampling without replacement (Rogers, 1972) was also fitted to the data but yielded parameter estimates for handling times < 0, which is not possible.

2.3.2. Comparing different predators

To compare the functional responses of different predator species or life stages, we used a non-linear least squared regression where two predators were fitted in the same model and the difference in attack rates and handling times were estimated. Detailed methods for this model are described in Pervez and Omkar (2005) and Juliano (2001), so we will not describe them again here. Initial parameter estimates supplied to the iterative model were the estimates from the Holling's disc equation (Section 2.3.1).

If the differences in parameter estimates are significantly different from zero, this indicates a difference in the functional response of the two predators.

2.4. Responses to olfactory cues from plants

The olfactometer used for this experiment was based on an adaptation of a design given by Douloumpaka and Van Emden (2003), by P. Vamvatsikos (personal communication), for use with parasitoids (Fig. 1). It was further adapted for this study so that the bottom of the internal surface of the tubes leading from the central arena to the sample chambers were flush with the floor of the central arena. The central arena was constructed from a solid piece of hard, clear plastic. The chambers and connecting tubes were made from sections of clear nylon tubing with internal diameter of the central arena of 49 mm and height 27.5 mm. A 6 mm diameter section of tube (54 mm in length) was fitted flush with the inner surface of the top of the central arena, through which air was drawn by a suction pump. The internal opening was covered with muslin to prevent insects or debris entering this tube. The plant sample chambers had an internal diameter of 24 mm and were 95 mm in length with tubes connecting the central arena to the sample chambers ending flush with the central arena (internal diameter 10 mm, 45 mm in length, 10 mm of which protruded into the sample chambers). These tubes were flush with the base of the central arena, so that insects could walk straight into them from the central arena. Air was drawn through each of the chambers via an end filter containing activated charcoal. The end filters were constructed with vertically positioned tube sections measuring 29 mm in length, and with an internal diameter of 24 mm. They were connected to the sample chambers with a 22 mm long section of tube, which had an internal diameter of 6 mm. A piece of muslin covered the end to prevent loss of the charcoal into the chamber, and to prevent the coccinellids from entering these tubes.

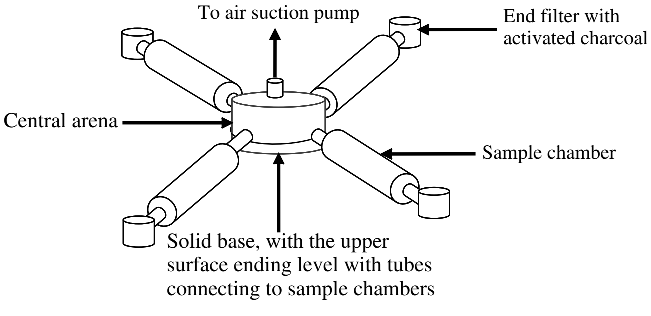

Fig. 1. The design of the four-way olfactometer used in the experiment.

Approximately the same volume of plant material was placed into three of the four olfactometer chambers. Sitka spruce was placed into one chamber, Norway spruce into another and barley (*Hordeum vulgare* (L.)) into the third. A group of 20 coccinellid adults of one species, randomly selected from the laboratory cultures to reflect the sex ratio of the population, was placed into the central arena. The position of each coccinellid was noted every 2 h until 8 h had passed. Individuals remaining in the central arena were not counted. Twenty replicates were carried out using groups of *A. obliterata* adults and 15 replicates using groups of *A. bipunctata* adults. All chambers were cleaned and the plant material renewed between each of the replicates.

A Pearson's X^2 test was carried out to determine whether the distributions of the coccinellids were affected by olfactory cues. Student's *t*-tests were carried out to determine differences in the responses of the two coccinellid species used. This could be done because these variables are independent (unlike chamber type). To investigate the effect of treatment types within species, data were analysed using a generalised linear model with binomial errors, and treatment contrasts performed using the statistical package, 'S-Plus' (Version 6.0.2, Insightful Corp.). Turlings et al. (2004) suggest that when analysing multiple choices log linear models should be used. The analysis was thus also repeated using this approach.

2.5. Coccinellid weights

Larger coccinellids generally consume more aphids than smaller ones (Hodek, 1973). Therefore, it was of relevance to measure the weights of males and females of both species of coccinellids. Ten individuals of each sex and species were weighed by placing adults singly into small plastic tubes which were then placed onto a micro-balance (Sartorius micro PRO 11, accurate to 0.002 mg, Sartorius GMBH, Gottingen, Germany). Confining the insects in tubes prevented inaccuracies due to movement of the coccinellids during the weighing process. Data were analysed using the Student's *t*-test.

3. Results

3.1. Male versus female consumption

Female *A. obliterata* consumed an average of 26.6 ± 5.8 (1SE) *E. abietinum* and males consumed an average of 19.9 ± 3.2 (1SE) *E.*

abietinum. There was no significant difference in the number consumed between the sexes ($t = 1.57$, df = 32, $p = 0.12$).

3.2. Functional responses on the Petri dish surface

The larval stages of *A. obliterata* and all stages of *A. bipunctata* show a Type II response to the density of approximately 3rd instar *E. abietinum* prey (Table 2 and Figs. 2 and 3, respectively). *Aphidecta obliterata* adults however, showed a more linear response to prey density (Fig. 2).

There was no significant difference between the functional responses of the 3rd instars of the two coccinellid species ($F = 0.08$, df = 1.76). There was, however, a significant difference between the responses shown by the 1st instars ($F = 22.51$, df = 1.76, $p < 0.0001$), with *A. obliterata* larvae consuming more than those of *A. bipunctata*, especially at low densities (Fig. 4). The interaction between density and species was significant ($F = 5.34$, df = 1.76, $p = 0.02$), suggesting that the two species respond differently to an increase in prey density.

At low prey densities (<20 aphids per dish), adults and 4th instars of both species exhibited a similar response to an increase in prey density (Fig. 5), but at densities of 32 aphids per dish and above, the 4th instars and adult stages of *A. bipunctata* showed slightly higher attack rates when compared with the same stages of *A. obliterata* (Tables 3a,b and 5 and Fig. 5). There was a significant difference between the two species in the proportion of aphids eaten by adults ($F = 38.9$, df = 1.76, $p < 0.001$) and 4th instars ($F = 46.2$, df = 1.76, $p < 0.001$).

The 4th instars of both species also consumed significantly more aphids than their respective adults over prey densities of 32–128 aphids and attack rates were significantly higher (Table 5 and Fig. 5) ($F = 10.4$, df = 1.76, $p = 0.002$ for *A. obliterata*; $F = 4.03$, df = 1.76, $p = 0.05$ for *A. bipunctata*).

Table 1
A comparison between the weights of adult males and females of the coccinellids, *Aphidecta obliterata* and *Adalia bipunctata*

	Fresh weight (live) (mg)	
	♀	♂
A. obliterata	9.2 ± 0.2 ($n = 10$)	7.9 ± 0.1 ($n = 10$)
A. bipunctata	12.7 ± 0.4 ($n = 10$)	10.0 ± 0.3 ($n = 10$)

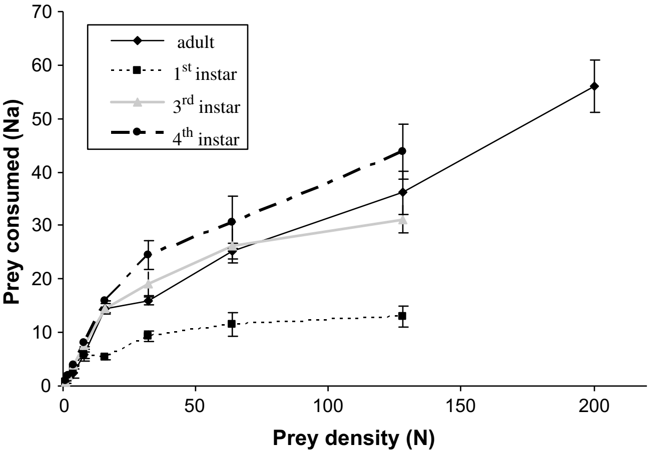

Fig. 2. The functional response of *Aphidecta obliterata* feeding on 3rd instar *Elatobium abietinum* at 15 °C (error bars show ± 1SE).

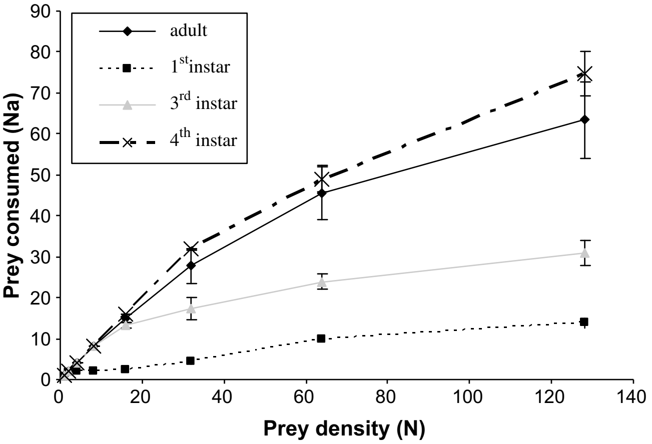

Fig. 3. The functional response of *Adalia bipunctata* feeding on 3rd instar *Elatobium abietinum* at 15 °C (error bars show ± 1SE).

3.3. Functional responses on cut sections of spruce in a Petri dish

Adult and 4th instar *A. obliterata* exhibited Type II functional responses on spruce sections (Tables 3b and 4 and Fig. 6) The re-

Table 2
Estimates of coefficients in a binomial logistic regression of proportion of prey eaten on total prey for *Aphidecta obliterata* and *Adalia bipunctata* at 15 °C

Type of functional response	*Aphidecta*				*Adalia*			
	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*
Intercept	1.676000	0.353300	4.743	<0.001	0.265200	0.320300	0.828	0.408
Linear	−0.143500	0.027830	−5.157	<0.001	−0.141300	0.028560	−4.945	<0.001
Quadratic	0.002063	0.000054	3.826	<0.001	0.002518	0.000574	4.385	<0.001
Cubic	0.000009	0.000003	−3.408	<0.001	−0.000012	0.000003	−4.190	<0.001
Intercept	4.650000	0.751800	6.185	<0.001	5.244000	0.806200	6.504	<0.001
Linear	−0.211100	0.046520	−4.537	<0.001	−0.265900	0.050180	−5.299	<0.001
Quadratic	0.002847	0.000793	3.592	<0.001	0.003810	0.000852	4.474	<0.001
Cubic	−0.000012	0.000004	−3.256	0.001	−0.000017	0.000004	−4.167	<0.001
Intercept	4.389000	0.425700	10.309	<0.001	11.417535	2.621984	4.355	<0.001
Linear	−0.102800	0.011360	−9.048	<0.001	−0.233278	0.061789	−3.775	<0.001
Quadratic	0.000496	0.000065	7.641	<0.001	0.001146	0.000323	3.547	<0.001
Cubic	—	—	—	—	—	—	—	—
Intercept	1.298000	0.137000	9.470	<0.001	3.850000	0.446200	8.646	<0.001
Linear	−0.030000	0.003112	−9.630	<0.001	−0.063180	0.012100	−5.223000	<0.001
Quadratic	0.000092	0.000013	7.266	<0.001	0.000257	0.000070	3.694000	<0.001
Cubic	—	—	—	—	—	—	—	—

All linear terms are negative and all quadratic terms positive indicating type II functional responses. Dashes occur where cubic terms were non-significant and removed from the model.

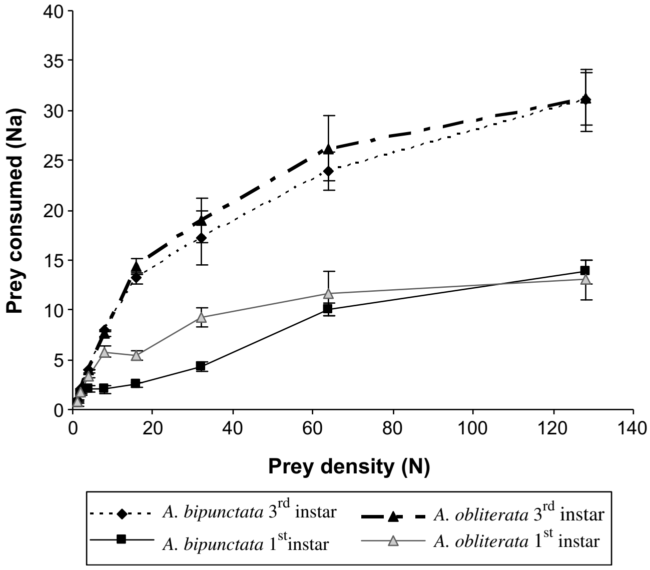

Fig. 4. A comparison of the functional responses of 1st and 3rd instar *Aphidecta obliterata* and *Adalia bipunctata* feeding on 3rd instar *Elatobium abietinum* at 15 °C (error bars show ± 1SE).

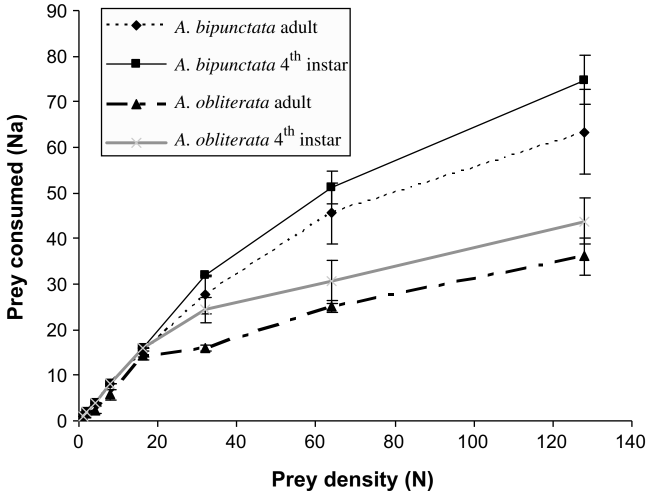

Fig. 5. A comparison of the functional responses of adult and 4th instar *Aphidecta obliterata* and *Adalia bipunctata* feeding on 3rd instar *Elatobium abietinum* at 15 °C (error bars show ± 1SE).

Table 3a

Estimates of functional response parameters from linearization of Holling's Type II model. Attack rate (*a*) and handling time (Th) in h

Stage	*a*	Th	r^2 at $p < 0.001$
Aphidecta			
1st instar	0.045	2.230	0.916
3rd instar	0.042	0.435	0.998
4th instar	0.042	0.243	0.990
Adult	0.040	0.996	0.849
Adalia			
1st instar	0.050	6.753	0.476
3rd instar	0.043	0.504	0.998
4th instar	0.042	0.062	1.000
Adult	0.042	0.165	0.999

Table 3b

Estimates of functional response parameters from linearization of Holling's Type II model

Stage	*a*	Th	r^2 at $p < 0.001$
Aphidecta			
Petri	0.041	1.478	0.820
Sitka	0.040	1.693	0.800
Adalia			
Petri	0.042	0.165	0.998
Sitka	0.041	0.926	0.901

Attack rate (*a*) and handling time (Th) in hours of *Aphidecta obliterata* and *Adalia bipunctata* in Petri dishes and on Sitka spruce.

sponse of adult *A. bipunctata* also conformed with a Type II response, analysis showing that there was no significant difference in the number of aphids consumed between the two highest densities ($t = 0.72$, df = 8, $p = 0.49$ ns). The 4th instar *A. obliterata* larva appeared to be a more effective predator than the adult of the species, and was more effective when compared with adult *A. bipunctata* at prey densities of 16 and 32 aphids per 5 cm section of host plant. *Adalia bipunctata* adults however, were more effective predator at higher prey densities (Table 4 and Fig. 6).

There were significant differences between the number of aphids consumed by adult *A. bipunctata* compared with adult *A. obliterata* ($F = 24.3$, df = 1.76, $p < 0.001$) and 4th instar compared with adult *A. obliterata* ($F = 4.02$, df = 1.76, $p = 0.05$).

The host plant itself also appeared to affect the rate of consumption by adult *A. obliterata*. The adults on the Sitka spruce sections consumed significantly higher numbers of aphids than their counterparts on Norway spruce ($F = 10.04$, df = 1.76, $p = 0.002$). This was most noticeable at densities above 16 aphids (Fig. 6). The number of needles per 5 cm section was significantly different between the two host species ($t = 3.05$, df = 48, $p = 0.004$), with an average of 81.0 ± 2.3 for Norway spruce and 90.8 ± 2.2 for Sitka spruce.

3.4. Comparisons between functional responses on the Petri dish surface and on cut sections of spruce

There was no significant difference between the prey consumed by *A. obliterata* adults on the host plant compared with the number consumed in the Petri dish ($F = 1.16$, df = 1.76, $p = 0.28$ ns). In the case of *A. bipunctata*, however, there was a significant difference between the two types of arena ($F = 8.88$, df = 1.76, $p = 0.004$). This was due mainly to the relatively lower number of aphids consumed on the host plant at the highest density (Fig. 7). Attack rate and handling time estimates on the two food types were, however, not significantly different (Tables 6a,b)

There was no significant difference in the functional response of the 4th instar *A. obliterata* larvae between the Petri dish and host plant experiments (Tables 6a,b and Fig. 8), ($F = 0.22$, df = 1.76, $p = 0.64$ ns).

3.5. Responses to olfactory cues from plants

The distributions of the two coccinellid species in the olfactometer after 8 h were significantly different from that expected if there was no influence from the presence of the plant material (i.e. if individuals were equally distributed between chamber) ($X^2 = 24.7$, df = 3 $p < 0.01$). There were also differences between the two species. Significantly higher proportions of *A. bipunctata* than *A. obliterata* adults were found in the control chamber ($t = 3.82$, df = 33, $p < 0.001$) (Fig. 9). There were no significant differences in the proportions found between any of the other treatments (Sitka spruce: $t = 0.43$, df = 33, $p = 0.67$ ns; Norway spruce: $t = 1.81$, df = 33, $p = 0.08$ ns; barley: $t = 1.47$, df = 33, $p = 0.15$).

The within species contrasts revealed that *A. obliterata* adults were found in significantly higher numbers in the Sitka spruce

Table 4
Estimates of coefficients in a binomial logistic regression of proportion of prey eaten on total prey for *Aphidecta obliterata* and *Adalia bipunctata* at 15 °C in Petri dishes or on Sitka spruce

	Aphidecta				*Adalia*			
	Estimate	SE	*t*	*p*	Estimate	SE	*t*	*p*
Petri								
Intercept	1.590000	0.230700	6.891	<0.001	3.858000	0.446200	8.65	<0.001
Linear	−0.045400	0.007386	−6.148	<0.001	−0.063180	0.012100	−5.22	<0.001
Quadratic	0.000201	0.000046	4.347	<0.001	0.000257	0.000070	3.69	<0.001
Cubic	–	–	–	–	–	–	–	–
Sitka								
Intercept	1.190000	0.216700	5.492	<0.001	1.859000	0.396400	4.690	<0.001
Linear	−0.030230	0.006812	−4.438	<0.001	−0.077690	0.029660	−2.619	0.008
Quadratic	0.000091	0.000043	2.111	0.035	0.001579	0.000561	2.814	0.005
Cubic	–	–	–	–	−0.000009	0.000003	−3.177	0.001

All linear terms are negative and all quadratic terms positive indicating Type II functional responses. Dashes occur where cubic terms were non-significant and removed from the model.

Table 5
Differences in attack rate and handling time between adult and final instars of *Aphidecta obliterata* and *Adalia bipunctata* in Petris

Comparing different life stage within a species	*D* (*a*)	*D* (Th)
Differences between *Aphidecta* 1st and 4th instar	0.0138 (*p* = 0.0108)	1.9220 (ns)
Differences between *Adalia* 1st and 4th instar	0.0552 (*p* < 0.001)	0.00449 (ns)

There are significant differences in attack rates but not handling times.
Significant *p*-values in parentheses (ns indicates no significant difference).

Table 6a
Differences in attack rate between adult *Aphidecta obliterata* and *Adalia bipunctata* feeding in Petris dishes or on sitka spruce

	Aphidecta Petri	*Aphidecta* Sitka	*Adalia* Petri	*Adalia* Sitka
Attack rate				
Aphidecta Petri		0.0082 (ns)	0.0041 (ns)	0.0046 (ns)
Aphidecta Sitka			0.0040 (ns)	0.0127 (ns)
Adalia Petri				0.0088 (ns)
Adalia Sitka				

Significant *p*-values in parentheses (ns indicates no significant difference).

Table 6b
Differences in handling time (hours) between *Aphidecta obliterata* and *Adalia bipunctata* feeding in Petri dishes or on Sitka spruce

	Aphidecta Petri	*Aphidecta* Sitka	*Adalia* Petri	*Adalia* Sitka
Handling time				
Aphidecta Petri		0.3752 (ns)	0.2880 (ns)	0.7177 (ns)
Aphidecta Sitka			0.6633 (*p* = 0.0477)	1.0929 (ns)
Adalia Petri				0.4296 (ns)
Adalia Sitka				

Significant *p*-values in parentheses (ns indicates no significant difference).

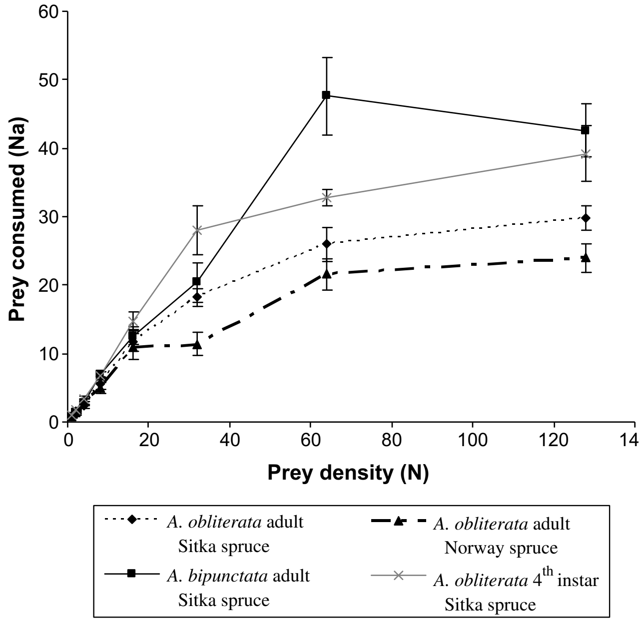

Fig. 6. A comparison of the functional responses of *Aphidecta obliterata* and *Adalia bipunctata* on 5 cm sections of host plant at 15 °C, infested with 3rd instar *Elatobium abietinum* (error bars show ± 1SE).

chambers than the control chambers ($p = 0.002$) (Fig. 9), but that the numbers found in the other chambers were not significantly different from the control (Norway spruce versus control, $p = 0.12$ ns; barley versus control, $p = 0.58$ ns). There was also no significant difference between the numbers found in the two spruce chambers ($p = 0.10$ ns).

Adalia bipunctata adults were found in significantly lower numbers in the Norway spruce chamber than the control chamber ($p = 0.0003$) or the Sitka spruce chamber ($p = 0.005$), and in significantly lower numbers in the barley chamber than the control chamber ($p < 0.0001$) (Fig. 9). There was no significant difference between the number of *A. bipunctata* found in the Sitka spruce or control chamber.

3.6. Coccinellid weights

Females weighed significantly more than males ($t = 11.03$, df = 38, $p < 0.001$) and *A. bipunctata* weighed significantly more than *A. obliterata* individuals ($t = 11.0$, df = 38, $p < 0.001$) (Table 1).

4. Discussion

4.1. Aphidecta obliterata male versus female consumption

Male coccinellids are generally smaller than their female counterparts (Hodek, 1973; Majerus and Kearns, 1989). This holds true for *A. obliterata*, the males of whom were found to weigh 14% less on average, than the females. Therefore, it would be expected that males would consume fewer aphids per day. The result of this experiment showed that although the males did consume fewer aphids on average, the difference was not significant.

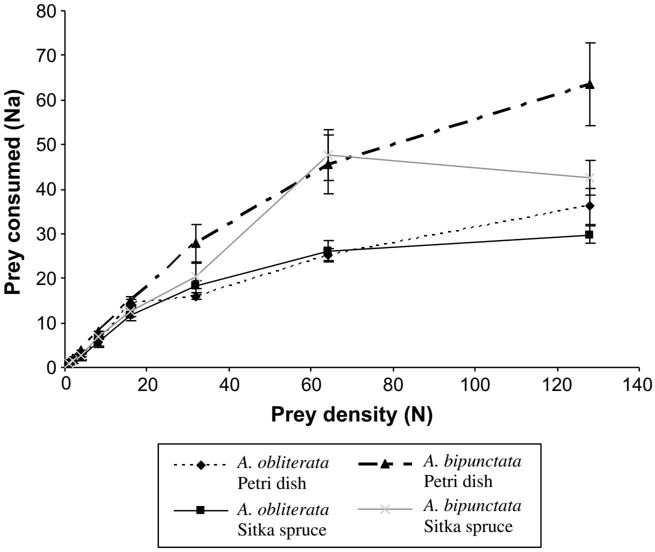

Fig. 7. A comparison of the functional responses of adult *Aphidecta obliterata* and *Adalia bipunctata* in a Petri dish and on the host plant at 15 °C (error bars show ± 1SE).

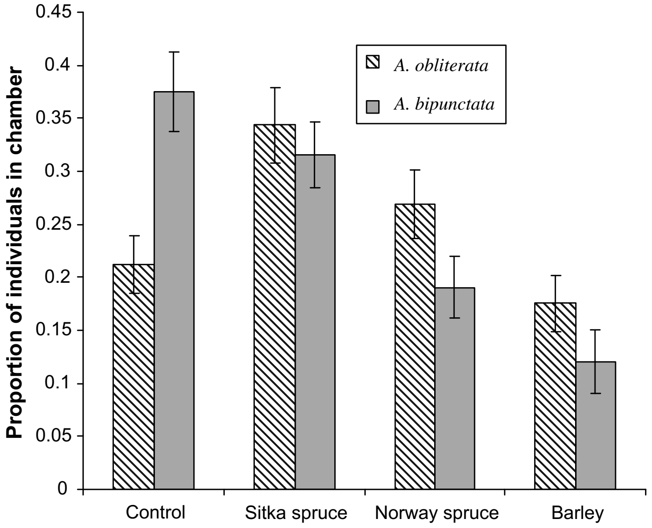

Fig. 9. The average distributions of *Aphidecta obliterata* and *Adalia bipunctata* adults in a four-way olfactometer containing plant material (error bars show ± 1SE).

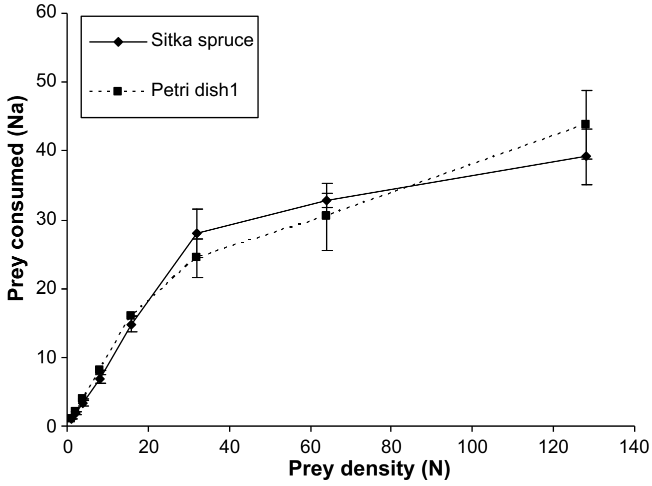

Fig. 8. A comparison of the functional response of 4th instar *Aphidecta obliterata* larvae in a Petri dish and on a section of host plant at 15 °C (error bars show ± 1SE).

These findings contrast with the study carried out by Hemptinne et al. (1996) on *A. bipunctata*, who found that at a density of 5 aphids in a 9 cm Petri dish, the daily intake by males was approximately half that of females, and that at 20 aphids per dish, the difference was even greater, with females consuming approximately four times that of the males per day. The proposed explanation for the difference was that females had a higher energy requirement due to egg production and the fact that they exhibited significantly higher activity levels.

It was noticeable from observations of the ladybird cultures maintained for this study, that adult female *A. obliterata* appeared much less active than the adult males, when kept under the same conditions. It is possible, therefore, that there is a lower or no difference in energy requirements between the male and female *A. obliterata.* This could explain the lack of significant difference in aphid consumption between the two sexes. However, it is also possible that the 25% difference found in average consumption rates between the two sexes in this experiment may be indicative of a real difference, which would become significant if an increased number of individuals were assessed.

4.2. Functional responses on the Petri dish surface

Counts of aphids on Sitka spruce trees in Radnor Forest indicated *E. abietinum* densities of between 0 and 140 aphids per 3 cm section of shoot (Straw et al., 2006). The densities used in this study are therefore representative of *E. abietinum* populations in the field, although the age-class structure was not.

Both coccinellid species exhibited a Type II functional response when feeding on approximately 3rd instar *E. abietinum*, although consumption rates differed between species and instars. The 1st instars of *A. bipunctata* consumed significantly fewer prey than those of *A. obliterata*, while the opposite was seen in the 4th instar and adult stages, where significantly higher numbers were consumed by *A. bipunctata*. The 4th instar and adult consumption generally accounts for a very high proportion of the overall prey consumed during the life of a coccinellid (Hodek, 1973). This suggests that *A. bipunctata* could consume a greater number of aphids over its lifetime, which would, in theory, make it a better control agent. The markedly higher consumption rates of *A. obliterata* 1st instars at low prey densities however, compared with *A. bipunctata*, suggests that the survival rates of early instar *A. obliterata* are likely to be better at lower prey densities, since increased mortality rates are associated with lower consumption rates (Dixon, 2000). This could therefore have a significant positive impact on the overall fitness of *A. obliterata*.

4.3. Functional responses on sections of the host plant

4.3.1. Petri dish surface versus cut spruce section

The advantage of carrying out a functional response study on the Petri dish surface, rather than on a section of spruce, is that it diminishes the number of extraneous variables which may affect the results in unpredictable ways. It also allows for comparison with previous standard Petri dish studies. However, there is no guarantee that a predator will exhibit the same response in its natural environment, and it is the realised functional response in the natural environment which is more important in relation to biological control.

It is interesting, therefore, to consider how well the standard Petri dish method of measuring the functional response of an insect compares with the more realistic scenario produced when using a section of the host plant as an arena. Several factors alter when using the host plant. One is that the search area increases dramatically in the case of spruce because the predator must search each needle individually. Another difference to consider is that the behaviour of prey may be altered by the addition of the host plant. On a bean leaf, the phytophagous mite, *T. urticae* was inactive, while on a glass Petri dish it was active (Everson, 1980).

Feeding aphids also need to remove their stylet from the plant before escaping and can only do so by walking, or flying in the case of alates. Some species, including *E. abietinum* and the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), exhibit a dropping response to disturbance (Lowe and Taylor, 1964; Timms, 1999; Braendle and Weisser, 2001; Day et al., 2006). The host plant does have the advantage of shelter and an aphid may be able to escape predation by hiding in a location which the predator is too large to fit into. This behaviour was observed in the cereal aphid, *Sitobion avenae* (L.) (Homoptera: Aphididae), individuals of which hid in the leaf–stem junction of wheat seedlings (Timms, 1999).

The comparisons between the Petri dish and spruce section experiments showed that there was surprisingly little difference between the functional responses measured despite some of the potential advantages to be gained from the presence of the plant discussed above. These results allow greater confidence to be placed upon the results gained from Petri dish experiments and are an important step on the way to linking field and laboratory studies.

The performance of the two coccinellid species on the host plant was also interesting. The 4th instars and adults of the generalist coccinellid, *A. bipunctata* performed better than the conifer specialist, *A. obliterata* in the Petri dish experiments, but may have been expected to perform less well, on the conifer host. However, this was not apparent from the results in this study. Although *A. bipunctata* is not a conifer specialist and is not generally associated with conifer plantations, it was the most abundant coccinellid predator found on Norway spruce in an urban area (Leather and Owuor, 1996) which suggests that it is well adapted to searching on needles.

4.3.2. Sitka versus Norway spruce

The reason for the significantly lower aphid consumption by *A. obliterata* adults on Norway spruce compared with Sitka spruce is not obvious. Vohland (1996) found that older larvae and adults of the coccinellid *Scymus nigrinus* Kugelann spent a higher proportion of time searching for prey in those parts of the canopy of Scots pine (*Pinus sylvestris* (L.)) which had higher needle densities. His results do not imply that the coccinellids showed a preference for higher needle density *per se* because these were also the areas of highest prey density. The needle counts on the shoot sections used in this study, showed that the Norway spruce had a lower needle density, on average, than Sitka spruce and so the lower consumption rate was not due to an increase in the search area. A more detailed study of the interactions between the predator, prey and the host plant would be required in order to determine why this result occurred. It could be due to altered behaviour of the aphid on a different host. Wainhouse et al. (1998) reported that two provenances of Sitka spruce differed in the concentration of resin present in their needles, and that *E. abietinum* was more restless on and was more likely to leave the provenance which contained the most resin. Epicuticular wax thickness (Lamb et al., 1993) and chemical composition (Jackson and Dixon, 1996) have been shown to affect aphid distribution and preference on plants and may also have an effect on predators (Rutledge et al., 2003; Chang et al., 2006).

Another possibility is that the prey may be less favourable nutritionally on one of the hosts. The quality of a host plant has been shown to affect the functional response of, for example, the big-eyed bug, *Geocoris punctipes* (Say) (Heteroptera: Geocoridae), which consumed fewer pea aphids on plants which had pods compared with those which did not (Eubanks and Denno, 2000). It has also been found that *A. obliterata* feeding on the adelgid *Gilletteella* (*Adelges*) *cooleyi* (Gillette), weighed less than those feeding on *E. abietinum* on Sitka spruce (Parry, 1992). It was suggested that this was due to the woolly covering present on the adelgid. It could however, have been a combination of a host plant as well as prey mediated effect.

4.4. Responses to olfactory cues from plants

The behaviour of the coccinellid itself can also be mediated by chemical signals which it receives from prey species or from the host plant (Zhu et al., 1999; Dixon, 2000). The results of the olfactometer experiment revealed that there were differences between the responses of the two coccinellid species to olfactory cues from the plant species tested. *Aphidecta obliterata* tended to enter chambers which contained Sitka spruce more than the control chamber but showed no such preference for Norway spruce or barley. This is interesting in the light of the lower consumption rates of this coccinellid on Norway compared with Sitka spruce and suggests that there may be some plant-mediated differences in the behaviour of *A. obliterata*, favouring the currently predominant species over the original host tree. Plant volatiles have indeed been shown to affect insect colonisation in the field (Pettersson et al., 1999). The lack of significant difference when comparing the numbers of *A. obliterata* found in the two spruce chambers, however, implies that any such difference due to olfactory cues alone, is likely to be subtle.

The significantly lower numbers of *A. bipunctata* which entered the barley chamber compared with the control is also interesting. During much of the season, the barley plants suffered from mildew. Although this did not prevent *R. padi* cultures from successfully growing on the plants, and the coccinellids ate aphids grown on the affected plants, it is possible that given a choice, the coccinellids would tend to avoid plants or aphids tainted with plant mildew, which would give rise to the response seen in the olfactometer experiment. *Adalia bipunctata* can be found on grasses in nature (Leather et al., 1999) and this result was not expected. Considering that *A. obliterata* is a conifer specialist, and *A. bipunctata* is a generalist coccinellid, it was expected in fact, that *A. bipunctata* would exhibit greater preference for the barley chamber than *A. obliterata* and that it would certainly have selected it over the control chamber.

4.5. Coccinellid weights

Both coccinellid species used in this study exhibited the usual difference in weight between the sexes, with females tending to be heavier. This concurs with other studies and fits in with the fact that males have been found to eat less (Hodek, 1973; Hemptinne et al., 1996), although the male *A. obliterata* did not consume significantly fewer aphids in the experiment carried out as part of this study.

Adalia bipunctata is a larger species than *A. obliterata* and weighs significantly more. This fact, and the higher consumption rates found in the functional response experiments, suggest that it also has a higher dietary requirement, with each individual consuming more aphids in its lifetime than *A. obliterata*. Thus, it would appear that this would make it a more effective natural control agent for *E. abietinum*, but it is only part of the story. A second characteristic associated with a good natural enemy is its numerical response to prey availability. This can be achieved by altering the reproductive rate to suit the prey density or by the aggregation of adults in areas of high prey numbers, or by a combination of these processes (Crawley, 1975).

A coccinellid which requires more prey to reach maturity and begin reproduction is likely to take longer and produce fewer individuals in times of prey scarcity, so may actually be less successful as a control agent than a species whose tactic was to produce higher numbers of smaller adults. When considering the case presented by a generally low density prey such as *E. abietinum*, it would seem that a smaller, less voracious coccinellid, such as *A. obliterata* may be more suited for long term control, although it may not provide high enough levels of predation in outbreak years. A predator with higher consumption rates would be more effective in the short term and in outbreak years, but is more likely to reduce aphid populations to levels which can no longer support the predator, and thus become a victim of the boom and bust cycle seen in other predator–prey relationships (Hodek, 1973; Jervis and Kidd, 1996).

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