Field investigation of mating behaviour of *Agrilus cyanescens* and *Agrilus subcinctus*

Jonathan P. Lelito, Michael J. Domingue, Ivich Fraser, Victor C. Mastro, James H. Tumlinson, Thomas C. Baker

Abstract—We examined mate-finding behaviour in *Agrilus subcinctus* Gory (Coleoptera: Buprestidae) and *Agrilus cyanescens* Ratzeburg (Coleoptera: Buprestidae) in ash (*Fraxinus* L. (Oleaceae)) stands in the Great Lakes region of the United States of America. Dead specimens of both species were affixed to ash foliage to serve as models to test visual stimulation of aerial attraction of males. The models were washed in dichloromethane to remove cuticular lipids or were left unwashed, to test for the presence of close-range pheromonal cues. Males of both species located females *via* a visually guided aerial approach from up to 1 m above females, similar to the behaviour of *Agrilus planipennis* Fairmaire. When male *A. subcinctus* or *A. cyanescens* made contact with females, contact sex pheromones mediated subsequent copulation behaviour. During their visually mediated airborne descent and landing, male *A. cyanescens* landed directly on female models; whereas, male *A. subcinctus* landed significantly more often on the leaf surface supporting a model. Male *A. subcintus* performed precopulatory pounce behaviour on female models after assessing surface chemical cues with their antennae. Male *A. cyanescens* landed upon a variety of models, but after landing, rejected a significant proportion of models lacking female *A. cyanescens* cuticular lipids.

Résumé—Nous étudions le comportement de recherche de partenaire chez *Agrilus subcinctus* Gory et *A grilus cyanescens* Ratzeburg (Coleoptera : Buprestidae) dans des peuplements de frênes (*Fraxinus* L. (Oleaceae)) dans la région des Grands Lacs aux États-Unis. Nous avons fixé des spécimens morts des deux espèces à du feuillage de frênes pour servir de modèles afin de vérifier la présence de stimulation visuelle dans l'attraction aérienne des mâles. Les modèles ont été lavés au dichlorométhane afin de retirer les lipides de la cuticule ou alors laissés sans lavage, afin de vérifier la présence de signaux de phéromones à courte distance. Les mâles des deux espèces retrouvent les femelles par une approche aérienne à orientation visuelle jusqu'à une distance de 1 m au-dessus des femelles, un comportement semblable à celui d'*A grilus planipennis* Fairmaire. Une fois que le mâle d'*A. subcintus* ou d'*A. cyanescens* a fait contact avec la femelle, les phéromones sexuelles de contact élicitent le comportement d'accouplement subséquent. Lors de leur descente aérienne à vue et leur atterrissage, les mâles d'*A. cyanescens* se posent directement sur les modèles femelles, alors que les mâles d'*A. subcinctus* atterrissent significativement plus souvent sur la surface de la feuille qui porte le modèle. Dans leur comportement pré-copulatoire, les mâles d'*A. subcinctus* se précipitent sur les modèles de femelles après avoir évalué les signaux chimiques de surface avec leurs antennes. Les mâles d'*A. cyanescens* se posent sur une variété de modèles, mais après s'être posés, ils rejettent une proportion significative des modèles qui ne portent pas les lipides cuticulaires d'*A. cyanescens*.

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Introduction

Experimental studies of mate-finding behaviour of buprestid beetles (Coleoptera: Buprestidae) under field conditions are rare. Lelito *et al.* (2007) demonstrated the primary use of visual cues by males of one species to locate females from about 1 m away. In males of another species, Gwynne and Rentz (1983) demonstrated the involvement of visually mediated attraction to man-made objects. Carlson and Knight (1969) suggested that buprestids, particularly species of *Agrilus* L., first locate host plants through olfactory cues or other means and then locate conspecifics by visual, vibratory, or tactile cues. Dunn and Potter (1988) observed greater attraction of male *Agrilus bilineatus* (Weber) to cages containing females than to cages containing only host logs, suggesting the use of female host-feeding volatiles or a female-produced pheromone to locate females. No long-range pheromones have been identified in buprestids. A contact sex pheromone, however, is involved in emerald ash borer (*Agrilus planipennis* Fairmaire) mating behaviour (Lelito *et al.* 2007, 2009; Silk *et al.* 2009). Application of synthetic pheromone components to solvent-washed female models caused emerald ash borer males to attempt to copulate with the models (Lelito *et al.* 2009; Silk *et al.* 2009).

Here, through extensive field observations and experimentation, we report on the mate-finding behaviour of two species of *Agrilus*, *A. subcinctus* Gory (a native North American species) and *A. cyanescens* Ratzeburg (a Eurasian species introduced to North America) that use species of ash (*Fraxinus* L. (Oleaceae)) and honeysuckle (*Lonicera* L. (Caprifoliaceae)), respectively, as host plants (Jendek and Grebennikov 2009; Petrice *et al.* 2009). *Agrilus cyanescens* was first recorded in North America in 1922 (Jendek and Grebennikov 2009). Adults of both species fly in early summer in ash stands in the Great Lakes region of the United States of America (Lelito *et al.* 2008).

We tested three hypotheses to determine the roles of visual and chemical cues in mate-finding and assessment behaviour of these two species. Our first hypothesis was that dead males and females would attract conspecific male *A. subcinctus* and *A. cyanescens* in a manner similar to that observed in *A. planipennis* (Lelito *et al.* 2008). For *A. cyanescens*, we further hypothesized that visually similar heterospecific insects might also elicit some or all of the mate-finding behaviour elicited in male *A. cyanescens* by conspecifics. Finally, our third hypothesis was that male *A. cyanescens* utilize cuticular hydrocarbons as close-range sex pheromones to promote or inhibit copulation when a female or male is encountered, as has been demonstrated for *A. planipennis* (Lelito *et al.* 2009).

Materials and methods

Insects used in bioassays

Individual *A. subcinctus* that were used as lures were captured by hand from the foliage of infested ash trees in Brighton (approximately 42°32′N, 83°47′W) and Pinckney (approximately 42°27′N, 83°57′W), Michigan, in May 2007, placed into separate glass vials for transport to the laboratory, and then separated by gender with the aid of a binocular microscope. Gender identification was made by holding a specimen with forceps and applying gentle pressure to the lateral portions of the abdomen to cause extension of the genitalia. Male *A. subcinctus* also have tell-tale green iridescence on the frons, a narrower abdomen, and are usually smaller than females. Specimens were only used if gender identification did not damage them. Otherwise, specimens of each gender were killed by freezing and then randomly assigned to one of two treatments: (1) washed for 5 min by gentle agitation in a 5 mL vial containing 1 mL dichloromethane, or (2) unwashed. Beetles were placed in vials segregated by treatment and gender and stored in a freezer (−20 °C) until use in experiments. All experiments involving *A. subcinctus* were conducted from 0800 to 1400 eastern standard time (EST) between 24 May and 6 June 2007.

Individual *A. cyanescens* were captured by hand from the foliage of infested honeysuckle bushes near Pinckney, Michigan, in May 2008, and separated by gender as described above.

The beetles were then placed into gender-segregated vials, frozen for 1 h, pinned through the elytra with a size 0 steel insect pin, and either gently washed for 5 min in dichloromethane and dried for 30 min, or left unwashed but similarly allowed to stand for 30 min. All pinned insects were stored separately by gender and wash treatment in a freezer (−20 °C) until use in experiments. All experiments involving *A. cyanescens* were conducted between 0900 and 1400 EST between 29 May and 23 June 2008.

Experiment 1: Response of *A. subcinctus* males to dead conspecifics

To create the lure setups for this experiment, we harvested undamaged ash leaves from epicormic shoots on ash tree stumps and cut each leaf such that only the lowest pair of leaflets remained attached to the petiole; this two-leaflet ash substrate was then placed into a plant wick with fresh water. We placed two equally spaced drops of Tangle-Trap (The Tanglefoot Company, Grand Rapids, Michgan) onto the mid-vein of each of the two ash leaflets. We then affixed one dead *A. subcinctus* to each of the Tangle-Trap droplets for a total of four beetles per setup. Each of these setups contained one individual randomly selected from each of the following four treatments: (1) unwashed female, (2) unwashed male, (3) washed female, and 4) washed male. Finally, we pinned each leaf-plus-beetle-lure setup to a live ash twig adjacent to a leaf and observed the behaviour of wild *A. subcinctus* individuals. We used each setup only once for 2 h. The mean length in millimeters (± SE) of the dead beetle lures marginally differed between males (3.84 ± 0.05) and females (3.95 ± 0.05) (*t* test, $t = 1.757$, df = 78, $P = 0.083$).

For three replicates of the experiment, an observer actively recorded *A. subcinctus* behaviour. Observers used a sweep net to capture samples of *A. subcinctus* that responded to the various models to determine gender. In seven replicates, approach and behaviour of individual *A. subcinctus* were recorded during the 2 h observation period using a video camera (Model DCR-TRV350, Sony Corporation of America, New York City) on a tripod focused on the lure setup.

Flight behaviour toward models and behavioural interactions close to models were scored for videotaped and directly observed beetles. Beetles were scored on whether or not they executed a "direct" airborne landing onto a model from 0.3 to 1 m above the model, in a manner similar to the "paratrooper copulations" described for *A. planipennis* males onto conspecific models (Lelito *et al.* 2007). Beetles that landed on the leaf surface adjacent to models rather than directly upon them were scored as having performed an "indirect" approach. Indirect approaches were further scored as to whether a responding beetle "pounced" onto a model from the leaf surface to initiate a copulation attempt or "antennated" the model and then flew off without mounting. The frequency of these behaviours, as well as the duration of copulatory pair formations or antennation behavior, was noted. The term "copulation" refers to the period a wild beetle remained mounted on a model, during which intermittent attempts at aedeagus insertion were often observed.

Experiment 2: Response of *A. cyanescens* males to dead conspecifics

Each of 10 replicates of this experiment were performed as follows: one dead *A. cyanescens* from each of the four treatments (male and female, washed and unwashed) was pinned to the mid-vein of a honeysuckle leaf in random order at a height of about 1.5 m. Pinned beetles were separated from each other by 10–15 cm. The mean length (mm) of the females (6.89 ± 0.07) was greater than the males (6.35 ± 0.07) (*t* test, $t = 6.35$, df = 38, $P < 0.0001$). The pinned beetles were observed for 1 h, during which time the number of approaching *A. cyanescens* and the number and duration of copulation attempts with each of the pinned beetles were recorded. At the end of each hour of observation, the pinned beetles were removed and new lures were pinned on a honeysuckle bush located at least 100 m from the first to prevent the accumulation of possible positional biases and to ensure relative consistency of the condition of beetles and the chemical nature of the cuticle. In addition, we performed six replicates of this experiment using nonhost blackberry (*Rubus* L. (Rosaceae)) and oleaster (*Elaeagnus* L. (Elaeagnaceae)) plants growing 2–4 m (mean of 2.32 m) from infested honeysuckle bushes. Each replicate of the videotaped observations was performed on a different day and with a fresh set of lures. We recorded the frequency of direct landings on the pinned models, as well as the duration of subsequent copulation-related behaviour. Data were recorded in a similar fashion in all subsequent experiments involving *A. cyanescens*.

Experiment 3: Response of *A. cyanescens* males to dead heterospecifics

To further understand the visual mate-finding process in *A. cyanescens,* we videotaped the responses of individuals to the following dead heterospecific insects or body parts: (1) an intact green immigrant leaf weevil, *Polydrusus sericeus* (Schaller) (Coleoptera: Curculionidae); (2) an intact male six-spotted tiger beetle, *Cicindela sexguttata* F. (Coleoptera: Carabidae); (3) a single elytron of a male *C. sexguttata*; (4) a head of a male *C. sexguttata*; (5) the dorsal thorax and abdomen (wings and legs removed) of a bluebottle fly, *Calliphora* Robineau-Desvoidy (Diptera: Calliphoridae); (6) an intact wood boring beetle, *Brachys ovatus* (Weber) (Coleoptera: Buprestidae); and (7) an intact male emerald ash borer. We placed one of each of the heterospecific lures onto an individual leaf of an *A. cyanescens*-infested honeysuckle bush and recorded the behaviour of approaching wild *A. cyanescens* for approximately 1 h. This was repeated four times with lures rearranged each time to prevent development of positional biases.

Experiment 4: Response of *A. cyanescens* males to conspecific and *C. sexguttata* lures with and without cuticular washing and reapplication of wash from *A. cyanescens*.

After determining that *C. sexguttata* elytra could be used to elicit the approach behaviour preceding mating attempts by male *A. cyanescens*, another experiment was performed to determine whether additional aspects of the mating sequence could be induced by applying cuticular surface compounds of *A. cyanescens* onto *C. sexguttata* elytra. We reapplied dichloromethane cuticular washes of male and female *A. cyanescens* to heterospecific *C. sexguttata* lures as potential contact cues. A wash was prepared from each gender of *A. cyanescens* by placing 10 *A. cyanescens* of the same gender into 150 μL of dichloromethane in a 4 mL glass vial, and gently agitating the vial for 5 min. There were eight lure treatments: (1–4) one for each of the four *A. cyanescens* treatments (washed and unwashed, male and female), as described previously; (5) an unwashed *C. sexguttata* elytron; (6) a *C. sexguttata* elytron washed in dichloromethane; (7) a *C. sexguttata* elytron washed in dichloromethane, dried for 30 min and then treated with 10 μL of the female *A. cyanescens* wash; and (8) a *C. sexguttata* elytron washed in dichloromethane, dried for 30 min, then treated with 10 μL of the male *A. cyanescens* wash.

Six replicates of this experiment were performed on infested honeysuckle bushes and six on blackberry and oleaster plants at a mean distance of 2.05 m from the nearest honeysuckle bush. Each replicate consisted of 1 h of observations of eight lure treatments using the same spacing between lures, placement guidelines, and periodic rearrangement as above. Fresh lures were prepared for each observation period and were discarded afterward.

Statistical analyses

Behavioural choices among males were observed as numbers of responses to different available models. In such cases a log-linear maximum likelihood was fit to the data using the PROC CATMOD feature of SAS version 9.2 (SAS Institute Inc. 2006), assuming a multinomial model in which responses are distributed over unordered treatments. When appropriate, experimental factors such as sex of models and cuticular washing were tested in a manner analogous to factorial ANOVA.

In one case for the *A. subcinctus* experiment, the data were assumed to fit a binomial distribution concerning the probability of pouncing during an indirect approach given different model characteristics. A logit link function was fit to the data, using PROC GENMOD in SAS, to model the effect of sex and cuticular washing on this behaviour. Wald χ^2 tests were computed and evaluated to test for significant effects of these factors.

In many cases we performed an ANOVA for assessing differences in the duration of behaviour such as copulation-related contact with, or antennation of, models. In most cases assumptions of normality (PROC UNIVARIATE, Wilks' normality, $\alpha = 0.05$) and heterogeneity of variance (PROC DISCRIM Bartlett's likelihood ratio test, $\alpha = 0.05$) across treatments were violated. Thus Friedman's nonparametric test was performed using rank-transformed data. Depending on the experiments, host *versus* nonhost plant location, cuticular washing, reapplication of cuticular washes, species, and sex of models were tested as possible factors affecting behaviour. When appropriate, post hoc comparisons were made using the Tukey–Kramer adjustment.

Results

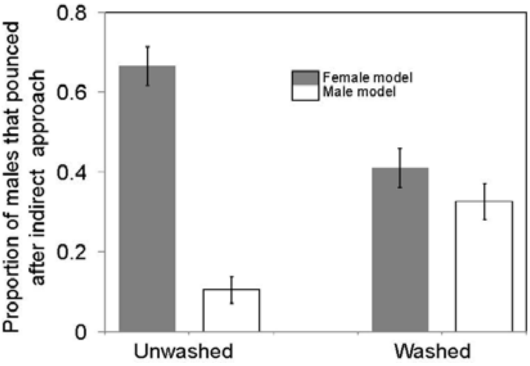
Experiment 1: Response of *A. subcinctus* males to dead conspecifics

A total of 448 indirect or direct flight approaches toward the models were observed and nearly all of these were likely to have been males. Of the nine wild *A. subcinctus* captured after performing direct "paratrooper copulation" attempts on these models, all were identified as males. Furthermore, 91% of beetles that were captured after an indirect leaf landing plus pounce behaviour were males (30 males out of 33 *A. subcinctus* netted).

There were no significant differences in choices made among the four types of models when attempting a paratrooper approach ($\chi^2 = 2.19$, df = 3, $P = 0.53$), which ranged from 23% to 27% for each model. However, across the different types of lures, 86% of approaches were indirect. Thus, if flight approach type is included in the multinomial model, there are no significant differences with respect to "sex" ($\chi^2 = 1.38$, df = 1, $P = 0.24$) and cuticular "washing" ($\chi^2 = 0.12$, df = 1, $P = 0.73$), but a strongly significant effect of indirect *versus* direct "approach" ($\chi^2 = 174$, df = 1, $P < 0.0001$). None of the possible interactions between sex, washing, and approach were significant at $\alpha = 0.05$ if included in the model (details not shown).

The proportion of *A. subcinctus* males that pounced on a dead conspecific after landing differed among the lure treatments (Fig. 1). These differences were marked by significant interaction effect in sex and sex × washing. Thus, unwashed females were most likely to be pounced upon after an indirect approach; whereas, unwashed males were least likely to be pounced upon.

Fig. 1. Proportions of wild male *Agrilus subcinctus* that pounced on and attempted to copulate with a model after an indirect approach. Mean proportion and one standard error bar are depicted. The overall effect of cuticular washing on the likelihood of pouncing is not significant ($\chi^2 = 0.5$, df = 1, $P = 0.48$), but the effects of sex ($\chi^2 = 26.7$, df = 1, $P < 0.0001$) and the interaction of sex and cuticular washing were highly significant ($\chi^2 = 46.1$, df = 1, $P < 0.0001$).

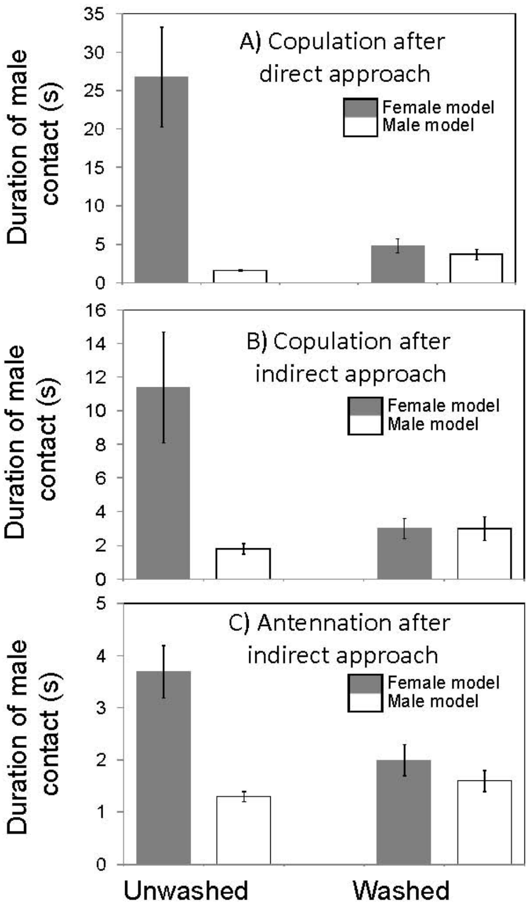


There were similar patterns of differences among the sex and cuticular wash treatments with respect to copulation duration after direct or indirect approaches, and antennation duration after indirect approaches (Fig. 2). For each of these classes of behaviour, longer durations were dedicated to responses toward unwashed females than to other treatments if compared individually ($\alpha = 0.05$, Tukey's adjustment). Unwashed males elicited the shortest durations of each class of behaviour. The duration of copulation behaviour on unwashed males after direct approach was significantly shorter than that on both washed treatments ($\alpha = 0.05$, Tukey's adjustment).

Experiment 2: Response of *A. cyanescens* males to dead conspecifics

All approaches by wild *A. cyanescens* toward models involved direct aerial landings onto the backs of models, followed by copulation attempts. There were 269 observations

Fig. 2. Means and standard errors for time spent by wild *Agilus subcinctus* in response to washed or unwashed male or female *A. subcinctus* models (results of 2 × 2 ANOVA using Friedman's ranking procedure). Copulatory pair formation after a direct flight approach onto the top of a model (n = 63), sex (F = 27.2, df = 1,59, $P < 0.0001$), washing (F = 0.07, df = 1,59, P = 0.79), and sex × washing (F = 19.9, df = 1,59, $P < 0.0001$) (A). Copulatory pair formation after pouncing onto a model following an indirect approach (n = 147), sex (F = 17.7, df = 1,143, $P < 0.0001$), washing (F = 6.38, df = 1,143, P = 0.013), and sex × washing (F = 17.5, df = 1,143, $P < 0.0001$) (B). Antennation of a model before flying off as part of an indirect approach (n = 238), sex (F = 39.2, df = 1,234, $P < 0.0001$), washing (F = 7.99, df = 1,234, P = 0.005), and the sex × washing interaction (F = 13.2, df = 1,234, $P < 0.0001$) (C).



(26.9/h) of direct approaches to models on honeysuckle and 61 (10.2/h) on nonhost plants. Examining the distribution of flights to the models using the log linear choice analysis, there was a significant effect of sex but not washing or sex × washing interaction in host and nonhost observations (Table 1). In both locations there were more flight attempts to females in comparison to the males (57% in hosts, 64% in nonhosts).

Data from host and nonhost plants were combined to perform an ANOVA of ranked copulation durations. A comparison of treatment means depicting this interaction is shown in Figure 3. Unwashed females elicited the longest copulation durations, unwashed males the shortest, and washed treatments of either sex were intermediate. Interactions involving host *versus* nonhost location, sex, and washing were also considered, but were not included in the final model because they were never significant at α = 0.05 and there is little *a priori* expectation that host-plant location would affect copulation duration.

Experiment 3: Response of *A. cyanescens* males to dead heterospecifics

A total of 86 wild male *A. cyanescens* performed direct aerial landings onto the tops of heterospecific lures in the field before flying away without sustained attempts at copulation. Numbers of flights by males onto models were *P. sericeus*, 1; whole *C. sexguttata*, 19; *C. sexguttata* elytra, 42; *C. sexguttata* heads, 7; *B. ovatus*, 3; *A. planipennis*, 14; and *Calliphora*, 0. This distribution is statistically significantly different from that expected if the probabilities of selecting each model were equivalent (χ^2 = 115, df = 6, $P < 0.0001$).

Experiment 4: Response of *A. cyanescens* males to conspecific and *C. sexguttata* lures with and without cuticular washing and reapplication of wash from *A. cyanescens*.

We observed 120 direct aerial landings onto models (20 per period) on host (honeysuckle) plants and 81 (13.5 per period) on nonhost plants. There were no significant differences in the proportion of flights to the eight types of *A. cyanescens* and *C. sexguttata* lures when the models were placed on honeysuckle, (χ^2 =

Table 1. Analysis of the effect of sex, cuticular washing, and their interaction on the likelihood of flight of adult male *Agrilus subcinctus* and *A. cyanescens* to lures placed on host (*Lonicera*) and nonhost (*Rubus* and *Elaeagnus*) plants.

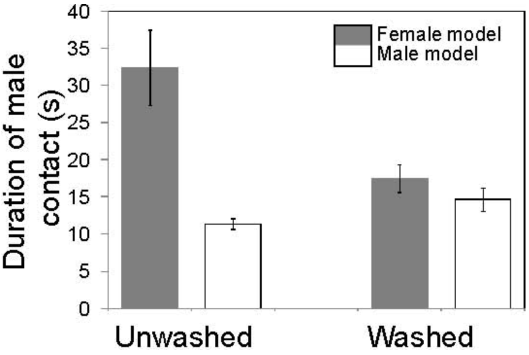
	χ^2	df	*P*
Host plants			
Sex	5.67	1	0.017
Washing	0.15	1	0.698
Sex × washing	0.36	1	0.549
Nonhost plants			
Sex	4.65	1	0.031
Washing	0.06	1	0.806
Sex × washing	0.19	1	0.662

Note: A log-linear model using maximum likelihood estimation of parameters was employed. Separate analyses were performed for the host and nonhost locations.

3.55, df = 7, $P = 0.83$) or nonhosts ($\chi^2 = 2.42$, df = 7, $P = 0.93$).

Unwashed *A. cyanescens* females, as well as *C. sexguttata* elytra, dosed with cuticular extract from *A. cyanescens* females elicited copulation durations of similar length, which were significantly longer than the durations evoked by the other models (Fig. 4). Male mating behaviour, including aedeagus extension and prolonged copulation attempts, were strongly evoked by *C. sexguttata* elytra dosed with cuticular extract (Fig. 5).

Fig. 3. Means and standard errors of times spent by wild *Agrilus cyanescens* in copulatory pair formation after a direct flight landing onto washed or unwashed male or female *A. cyanescens* models ($n = 329$). ANOVA was performed on rank-transformed data (Friedman) sex ($F = 22.32$, df = 1,325, $P < 0.0001$), washing ($F = 0.85$, df = 1,325, $P = 0.36$) sex × washing ($F = 5.03$, df = 1,325, $P = 0.026$), and host *versus* nonhost location ($F = 0.54$, df = 1,325, $P = 0.46$) (not depicted on the graph).

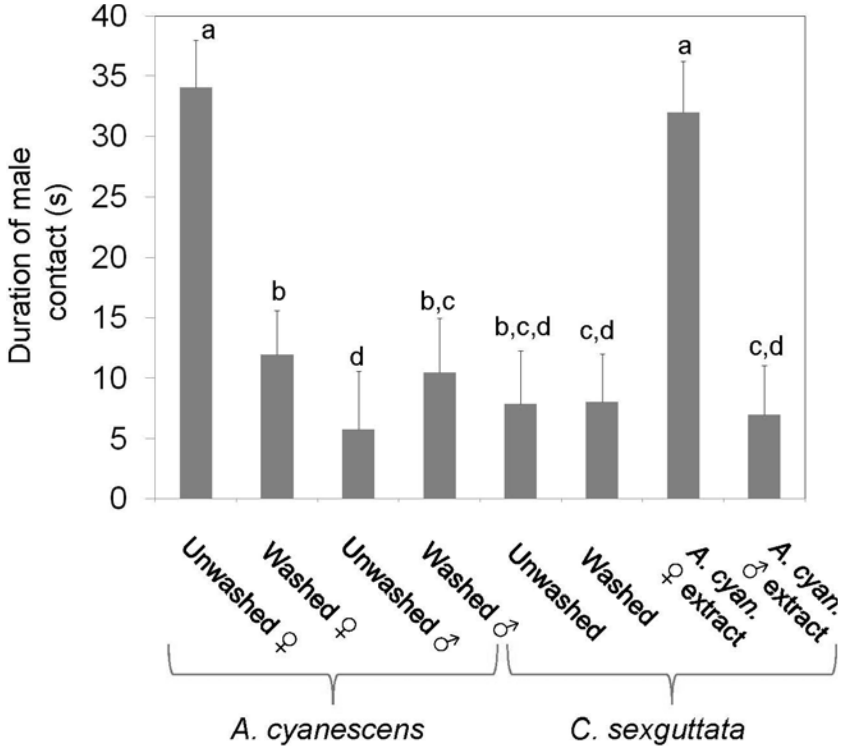


Discussion

These results demonstrate that mate location and assessment by male *A. subcinctus* and *A. cyanescens* are mediated by visual cues and contact pheromones, respectively, which is similar to what has been recorded for *A. planipennis*, the invasive pest of ash. Flying male *A. subcinctus* and *A. cyanescens* located and descended on female models *via* a visually guided aerial approach from up to 1 m above a female, similar to behaviour recorded for *A. planipennis* (Lelito *et al.* 2007, 2008). Dead female conspecifics with intact cuticular hydrocarbons ("unwashed") evoked more prolonged contact and copulatory behaviour from male *A. subcinctus* and *A. cyanescens* than dead female conspecifics from which the cuticular hydrocarbons had been removed ("washed") (Figs. 1, 2, 4). Moreover, males of both species flew away from unwashed dead males significantly faster than they did from washed dead males (Figs. 1, 2A, 4). These results are again similar to those of *A. planipennis* that demonstrated that the presence of contact sex pheromones causes males to increase their persistence in copulating with females (Lelito *et al.* 2007, 2009; Silk *et al.* 2009) and deter their efforts to copulate with other males (Lelito *et al.* 2007) after contact.

One difference in their visually mediated airborne descent and landings is that male *A. cyanescens* always landed directly on female models; whereas, male *A. subcinctus* landed significantly more often on the leaf surface immediately adjacent to female models, and then performed antennal assessment of chemical cues followed by pounce behaviour onto the models. Again, the persistence of contact by a male with a model following the pounce was increased significantly when female cuticular compounds were present, indicating the presence of a female-produced contact sex pheromone. Another difference between *A. cyanescens* and the other two species is that male *A. cyanescens* approached and landed onto conspecific female models more frequently than onto conspecific male models. This may be because the female models were nearly 10% longer than the males.

Fig. 4. Means and standard errors of time spent by wild *Agrilus cyanescens* in copulatory pair formation after a direct flight approach to washed or unwashed male or female *A. cyanescens* models, washed or unwashed *Cicindela sexguttata* elytra, or *C. sexguttata* elytra dosed with male or female cuticular extract (n = 201). ANOVA was performed on rank-transformed data. Copulation durations evoked by some of the different types of lures were significantly different (F = 26.5, df = 7,192, $P < 0.0001$). Different letters indicate treatments that are significantly different (Tukey's, α = 0.05). Host *versus* nonhost data are pooled across lure types. There was no significant host *versus* nonhost location effect (F = 2.48, df = 1,192, P = 0.12).

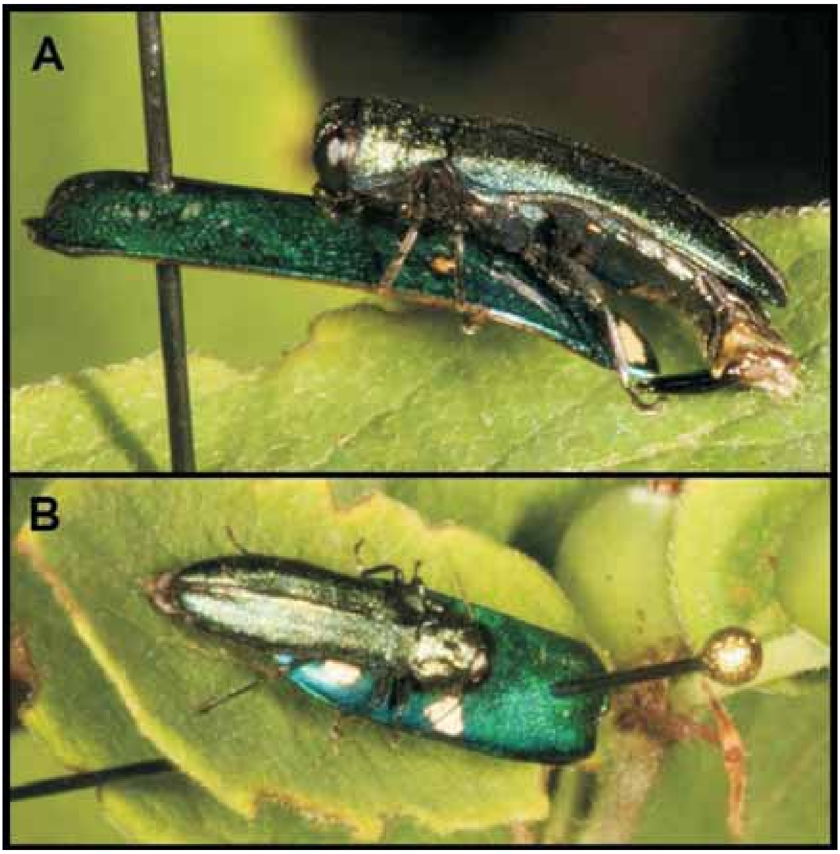


Male *A. subcinctus* and *A. cyanescens* conducted aerial approaches to other insect species as well, especially if the size and colour of those insects were similar to those of the approaching males. This was illustrated most strikingly in our results with *A. cyanescens*, whose males were attracted most strongly to single elytra from the tiger beetle species, *C. sexguttata* (Fig. 5). When these elytra had a solvent extract from female *A. cyanescens* applied to them, males were more likely to remain in contact and attempt to copulate with them. The tendency for male *A. cyanescens* to approach and land on any like-coloured insect in our study is consistent with the high rate of capture of *A. cyanescens* on visual-lure traps that used dead *A. planipennis* beetles as models, reported by Lelito *et al.* (2008).

The similarities between the mate location and assessment systems of *A. subcinctus*, *A. cyanescens*, and *A. planipennis* raises the possibility that many other *Agrilus* species may use similar systems in which males visually locate and descend upon females basking on upper surfaces of leaves, and then assess their pheromonal composition by contact chemosensory means. Similar behaviour has recently been observed for European *Agrilus* species that feed on oak (*Quercus* L. (Fagaceae)) (Domingue *et al.* 2011) and that could pose a threat to North American oaks should they expand their ranges.

For all *Agrilus* species, host volatiles likely play an initial role in attracting adults of both sexes to stressed trees. Field trapping has provided evidence of this: the addition of various plant-extracted oils to monitoring traps for

Fig. 5. A live male *Agrilus cyanescens* attempting copulation with a *Cicindela sexguttata* elytron treated with female *A. cyanescens* cuticular extract, lateral (A) and dorsal view (B).



detecting *A. planipennis* resulted in significant increases in trap capture (Crook *et al.* 2008; Lelito *et al.* 2009). Further experiments aimed at clarifying the role of tree volatiles in increasing capture rates of *Agrilus* beetles should be helpful in improving strategies for the detection and monitoring of *Agrilus* species, many of which pose serious threats to tree species in North America.

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