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ORIGINAL PAPER

# Blending of pheromone lures for two exotic European pest elaterid beetles

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**Abstract** Two exotic European click beetle species, *Agriotes obscurus* and *Agriotes lineatus,* were introduced into the lower Fraser valley of British Columbia over a century ago, and are now predominant pests of a number of arable crops. A semiochemical-based method of monitoring both species has been developed as a part of an integrated pest management plan, and there is interest in mass trapping with pheromones as a management tool. *A. obscurus* females produce primarily geranyl octanoate (G8) and geranyl hexanoate (G6), while *A. lineatus* females produce both G8 and geranyl butanoate (G4). The current studies examined the possibility of using a blend of G8, G6, and G4 components in a single lure to trap both species simultaneously. A blended G8, G6 and G4 lure in a 1:1:1 ratio was, on average, 1.42 times more attractive to *A. lineatus* males than standard *A. lineatus* pheromone lures, but caught only 0.24 times the number of *A. obscurus* in standard *A. obscurus* traps. Blended traps, therefore, are effective for monitoring and mass trapping of *A. lineatus*, but only for detection of *A. obscurus*.

**Keywords** Elateridae · *Agriotes lineatus* · *Agriotes obscurus* · Semiochemicals · Pheromone blends · Integrated pest management · Trapping

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## Introduction

Two species of click beetles, *Agriotes lineatus* L. and *Agriotes obscurus* L., (Coleoptera: Elateridae) were introduced to British Columbia (BC) from their native Europe in infested nursery stock and/or ship ballast in the late 1800s (Wilkinson 1963). Since then, both species have become established throughout the lower Fraser valley (LFV) in southwestern BC and in the northwestern counties of the state of Washington (Vernon and Päts 1997; Vernon et al. 2001; Lagasa et al. 2006). The larvae, commonly called wireworms, cause significant damage to many agricultural crops during their 4–5 year development cycle by feeding on seed (i.e. cereals and corn), causing cosmetic feeding damage to vegetable crops (i.e. carrots, rutabagas and potatoes) or as contaminants to small fruit such as strawberries in contact with the soil (Vernon et al. 2001; Vernon and van Herk 2012). Organochlorine, organophosphate and carbamate insecticides have traditionally been very effective at suppressing wireworm populations (Wilkinson et al. 1964, 1976). However, due to the de-registration of most of these products over the last two decades in Canada, dramatic increases in wireworm damage have recently been observed (Vernon and van Herk 2012), and few effective chemical control options are currently available in Canada. To address this problem, a number of alternative management approaches are being considered, including the use of semiochemicals to assist in the monitoring and control (i.e. mass trapping and mating disruption) of these species in BC and in Europe (Hicks and Blackshaw 2008; Sufyan et al. 2013; Vernon et al. 2014a, b).

European studies have indicated that both *A. lineatus* and *A. obscurus* produce a female sex pheromone composed of (*E*)-3,7-dimethyl-2,6-octadienyl esters: *A. lineatus* females produce primarily geranyl octanoate (abbreviated in this paper as G8) with geranyl butanoate (G4) as a trace component, while

*A. obscurus* females produce both geranyl octanoate and geranyl hexanoate (G6) (Borg-Karlson et al. 1988; Yatsynin et al. 1996; Tóth et al. 2003). Male *A. obscurus* respond to a combination of G8 and G6, while male *A. lineatus* require G8 plus the minor component G4 (Yatsynin et al. 1996; Toth et al. 2003), although in one study 1:1 was more effective than 10:1 ratios of G8 and G4 (Toth et al. 2008). Preliminary studies in the LFV have indicated that a bubble cap release device (Contech Enterprises Inc., Delta, BC, Canada) filled with a ratio of 1:1 G8 to G6 attracts *A. obscurus*, whereas a ratio of 9 or 10:1 G8 to G4 attracts *A. lineatus* (Vernon and Tóth 2007).

Because of the overlapping distributions of *A. lineatus* and *A. obscurus* in the LFV of BC (Vernon et al. 2001), a synthetic pheromone lure that could be used for monitoring and/or mass trapping of both species would be of economic and practical advantage in the development of lower risk IPM approaches. G6 is the main pheromone component of *A. obscurus*, but its presence does not repel sympatric males of *A. lineatus* (Siirde et al. 1993). Thus, a blended pheromone lure containing all three components (G8, G6 and G4) could potentially be used to attract both species to the same trap, provided the minor pheromone component of *A. lineatus* (G4) does not repel *A. obscurus*. Another consideration in the development of a multi-species trap for *A. lineatus* and *A. obscurus* is whether or not trap effectiveness will be consistent among populations occurring in different agricultural regions of the LFV, since differences in response to various ratios of key pheromone components (i.e. *A. sputator* and *A. obscurus*) have been observed in various regions of Europe and Russia (Yatsynin et al. 1996; Tóth et al. 2003). Since the introduction of *A. lineatus* and *A. obscurus* into the Pacific Northwest of North America is likely to have occurred in more than one location and from more than one European point of origin, it is possible that differences in response to individual, or multi-species lures may occur in different agricultural regions of at least the LFV.

The objectives of this study were to (1) identify optimal ratio(s) of pheromone components and lure quantity required to attract *A. lineatus* and *A. obscurus* males throughout the growing season, (2) establish if a blend of pheromone components from *A. lineatus* (G8 and G4) and *A. obscurus* (G8 and G6) can be used to attract males from both species to the same trap, and 3) determine if the response of *A. lineatus* and *A. obscurus* males to individual or candidate multi-species lures is consistent between key agricultural areas of the LFV of BC.

## Methods and materials

### Pheromone lure evaluation

Five field experiments investigating the attractiveness of various *A. lineatus* and *A. obscurus* lures were conducted between 2001 and 2002 in three regions of the LFV of BC: Agassiz in the east; Cloverdale in the central west; and Delta in the west. Selected fields had high *Agriotes* spp. populations, but differed in species composition. Relative abundance of the two *Agriotes* species in these fields was determined by pheromone trap placements in previous years (R.S. Vernon, unpublished data). All experiments used Vernon beetle traps (VBTs) (Vernon and Tóth 2007) baited with one or more bubble cap lure (Contech Enterprises Inc.) arranged in randomized complete block designs. Standard component ratios for each of the three compounds tested (G8, G6 and G4), as well as additional formulated load weights in bubble caps are provided in Table 1.

Studies conducted in 2001 used newly purchased VBTs. In 2002 studies, all traps were washed three times and contained no detectable odour prior to use. During trap deployment or trap inspections, unbaited, baited and blended *A. lineatus* and *A. obscurus* component traps were handled sequentially and with changes in gloves to avoid between-treatment contamination. Traps were generally inspected every 7–14 days, at which time all insects captured were removed and elaterids identified to species and sex. Traps remained in the same locations throughout the duration of the study.

Experiments (Exp.) 1 and 2 tested the effect of doubling the number of standard *A. lineatus* (G8, G4) and *A. obscurus* (G8, G6) lures on trap efficacy, the response of each species to traps with both *A. lineatus* and *A. obscurus* standard lures, and their response to a single-blended lure containing all three components (G8, G6 and G4) at two sites (Table 1). Unbaited traps served as controls. Exp. 1 was conducted in Agassiz in a fallowed 1 ha field between 9 April and 12 June, 2001, with the predominant species being *A. obscurus*. The seven treatment traps were placed 12 m apart in four parallel rows (blocks) spaced 20 m apart. Exp. 2 was conducted in Cloverdale in a field of pasture from 5 April until the field was ploughed on 30 May 2001. Both *A. obscurus* and *A. lineatus* were previously found to be present in similar numbers in this field (R.S. Vernon, unpublished data). The seven treatment traps were placed 20 m apart in four parallel rows (blocks) spaced 30 m apart.

Exp. 3 tested the response of *A. lineatus* to modifications in the G8:G4 component ratio of the standard *A. lineatus* and blended lures in 2002 (Table 1). The study was conducted in a bare headland area along one edge of a potato field in Delta over two periods: 28 April–15 May, and 7-16 June. Traps were temporarily removed for field planting between 15 May and 7 June. Based on earlier studies, the species composition in the area was expected to be mixed, but with predominantly *A. lineatus*. The five treatments were replicated eight times, with traps placed 12 m apart, and with 15 m between replicates.

**Table 1** List of treatments in each experiment, testing the effect of lure composition, load weight and quantity on *Agriotes lineatus* (AL) and *A. obscurus* (AO) populations (one lure was used per trap unless otherwise noted)

| Exp. no. | Treatment | Component ratio | | | Load weight (mg) | Description |
|---|---|---|---|---|---|---|
| | | G8 | G6 | G4 | | |
| 1 (Agassiz) and 2 (Cloverdale) | 1 | 1 | 1 | 1 | 160 | Standard G8,G6,G4 blend lure[c] |
| | 2 | 9 | 0 | 1 | 160 | Standard AL[a] + standard AO[b] lure |
| | | 1 | 1 | 0 | 160 | |
| | 3 | 1 | 1 | 0 | 2 lures × 160 | 2 standard AO lures |
| | 4 | 1 | 1 | 0 | 160 | Standard AO lure[b] |
| | 5 | 9 | 0 | 1 | 2 lures × 160 | 2 standard AL lures |
| | 6 | 9 | 0 | 1 | 160 | Standard AL lure[a] |
| | 7 | 0 | 0 | 0 | 0 | Blank control |
| 3 (Delta) | 1 | 1 | 1 | 1 | 156 | Standard G8,G6,G4 blend lure[c] |
| | 2 | 1 | 0 | 1 | 156 | Minus AO compound G6 |
| | 3 | 1 | 0 | 5 | 156 | Minus AO compound G6 + increased AL compound G4 |
| | 4 | 9 | 0 | 1 | 156 | Standard AL lure[a] |
| | 5 | 0 | 0 | 0 | 0 | Blank control |
| 4 (Agassiz) and 5 (Delta) | 1 | 1 | 1 | 1 | 156 | Standard G8,G6,G4 blend lure[c] |
| | 2 | 3 | 3 | 1 | 312 | AO components increased |
| | 3 | 9 | 9 | 1 | 312 | AO components increased |
| | 4 | 1 | 1 | 0 | 156 | Standard AO lure[b] |
| | 5 | 9 | 0 | 1 | 156 | Standard AL lure[a] |
| | 6 | 0 | 0 | 0 | 0 | Blank control |

[a] Standard AL lure released at 0.25 mg/day at 20 °C

[b] Standard AO lure released at 0.20 mg/day at 20 °C

[c] Standard blend lure released at 0.60 mg/day at 20 °C

Exp. 4 and 5 examined the response of *Agriotes* spp. to modifications in the blended lure component ratio (Table 1). Exp. 4 was conducted between 1 May and 11 June, 2002, at two locations within a 2 ha field in Agassiz where the population was predominantly *A. obscurus*. Six treatments were replicated four times in a freshly ploughed section of the field, and in an adjacent unploughed pasture, with traps and replicate blocks being a minimum of 12 m apart.

Exp. 5 was conducted between 28 April and 16 June in Delta at the opposite end of the same field used in Exp. 3. Traps were placed 12 m apart along the fallowed field edges, and the six treatments were replicated eight times with a minimum of 12 m between replicates. The traps were temporarily removed for field planting between 15 May and 3 June, 2002.

## Regional pheromone lure evaluation

The efficacy of traps baited with standard *A. obscurus* or *A. lineatus* lures relative to the standard G8, G6 and G4 (1:1:1) blend lure was evaluated in 19 strawberry fields situated throughout the LFV in 2002. In each field, all 3 trap types were established roughly midway along each of the 4 field edges and in mid-field. Traps along field edges were placed 10 m into the field, with approx. 5 m between traps situated within strawberry rows, and all traps in each replicate were equidistant from the field edge. Traps in the middle of the field were also 5 m apart in different rows, but with the middle trap in the middle row of the field. Traps were positioned at random at each of the 5 replicate sites. Trapping generally began early in April and was terminated in late June or July at which time carabid (*Pterostichus melanarius*) predation inside the traps became severe.

## Statistical analysis

The number of beetles of each species collected from each trap was converted to the mean number of beetles caught per day and analysed by ANOVA and paired and unpaired Student's t tests. Means were separated by the Tukey–Kramer HSD procedure ($\alpha = 0.05$). Normality was assessed with the UNIVARIATE procedure, and data were square-root or log transformed where necessary. All

statistical analyses were carried out using SAS version 9.3 (SAS Institute Inc.).

To test for any differences occurring among lures over time in each trial, the relative catch of beetles during the approximate first month of trapping was analysed separately from the remaining period of trapping and the results compared for Exp. 1–3 and 5 (date breakdown shown in Figs. 1, 2, 3 and 5). In each case, there was no significant difference in the number of beetles caught per day ($P > 0.1$), and so data were combined for subsequent analyses. Data for the two separate fields involved in Exp. 4 were analysed separately.

With respect to the regional pheromone trap study, the number of male *A. obscurus* and *A. lineatus* beetles caught per day in traps baited with standard *A. obscurus*, standard *A. lineatus*, and standard blend lures was compared with ANOVA using data from all 19 strawberry fields pooled together, with field and lure type as main effects (Table 2). Separate analyses were conducted for *A. obscurus* and *A. lineatus* beetles. Model LS mean estimates for beetle catch per trap per day were compared between the blend and either standard *A. obscurus* or standard *A. lineatus* lures for *A. obscurus* and *A. lineatus* beetles, respectively, using *t* tests. Per day male *A. obscurus* and *A. lineatus* beetle catches in standard *A. obscurus*, standard *A. lineatus* and blend-baited traps in the three Agassiz studies (Figs. 1, 4) were compared together in a separate analysis similar to the studies done in the commercial fields.

## Results

### Experiment 1

In Agassiz, the various treatments captured 1,159 *A. obscurus* (1,024 males and 135 females) and 11 *A. lineatus* males in 2001. Analyzing trap catch over time showed that male catches early in the season were not significantly different than later in the season (mean = 11.9 beetles/day from 3 April–1 May; mean = 16.9 beetles/day from 1 May–12 June; $F = 1.96$, $df = 1.48$, $P = 0.17$). There were significant differences between treatments for both collection periods ($F = 30.67$, $df = 6.18$, $P < 0.0001$; $F = 9.06$, $df = 6.18$, $P = 0.0001$, respectively). For both periods, and for the combined data, traps baited with one or two standard *A. obscurus* lures collected more males than all other traps ($F = 14.82$, $df = 6.18$, $P < 0.0001$) (Fig. 1). Two standard *A. obscurus* lures did not appear to confer a significant advantage over single lures in trapping males overall. Traps containing the standard G8, G6 and G4 blend lure (1:1:1), paired standard *A. obscurus* and *A. lineatus* lures, or standard *A. lineatus* lures alone caught very few male *A. obscurus* (Fig. 1).

Female *A. obscurus* were also captured, although the female catch rate over the trapping period was significantly lower than that of males ($t = 3.67$, $df = 27$, $P = 0.0011$) (Fig. 1). Analyzing trap catch over time showed that catches of females were not significantly different early versus later in the season (mean = 1.61 beetles/day for 3 April–1 May; mean = 2.21 beetles/day for 1 May–12 June; $F = 2.58$, $df = 1.48$, $P = 0.11$). There were significant differences between treatments for both collection periods ($F = 7.24$, $df = 6.18$, $P = 0.0005$; $F = 6.62$, $df = 6.18$, $P = 0.0008$, respectively). For both periods and for the combined data, unbaited traps and those baited with one or two standard *A. obscurus* lures collected similar numbers of females, with the single standard *A. obscurus* lure collecting significantly more females than all lures containing the *A. lineatus*-specific geranyl butanoate (G4) component ($F = 8.89$, $df = 6.18$, $P = 0.0001$) (Fig. 1). The data do not suggest that females are significantly attracted to their own pheromone, but do suggest that some avoidance of females to the G4 component may have occurred.

### Experiment 2

In the Cloverdale trial, both *A. lineatus* (1,047 males and 3 females) and *A. obscurus* (800 males and 2 females) were captured in high numbers in 2001. There was no significant difference in the number of males trapped per day between the two species ($t = 1.00$, $df = 54$, $P = 0.33$).

Treatments with one or two standard *A. lineatus* lures, paired *A. lineatus* and *A. obscurus* lures, and standard G8, G6 and G4 blend lures captured significantly more male *A. lineatus* than one or two standard *A. obscurus* lures or the blank control traps ($F = 45.53$, $df = 6.18$, $P < 0.0001$) (Fig. 2). This was observed for both the early (5–27 April) and later (27 April–30 May) trapping periods ($F = 9.79$, $df = 6.18$, $P < 0.0001$; $F = 34.88$, $df = 6.18$, $P < 0.0001$; respectively) (Fig. 2). There was a significantly higher overall *A. lineatus* trap catch per day during the second trapping period (early: 12.2/day; late: 23.6/day; $F = 21.04$, $df = 1.48$, $P < 0.0001$). This is likely due to the later emergence of *A. lineatus* relative to *A. obscurus* observed in this study as well as in earlier surveys in the LFV (Vernon et al. 2001).

There was no significant difference in the overall trap catch of *A. obscurus* per day between the two trapping periods (early: 13.1/day; late: 15.6/day; $F = 0.86$, $df = 1.48$, $P = 0.36$). *A. obscurus* catch was the highest in traps with one or two *A. obscurus* lures ($F = 28.63$, $df = 6.18$, $P < 0.0001$), which was true for both the early and later trapping periods ($F = 3.94$, $df = 6.18$, $P = 0.011$; $F = 53.66$, $df = 6.18$, $P < 0.0001$; respectively) (Fig. 2), and was also observed in the concurrent Exp. 1 Agassiz trial (Fig. 1). In contrast to Exp. 1 (fallowed field), however,

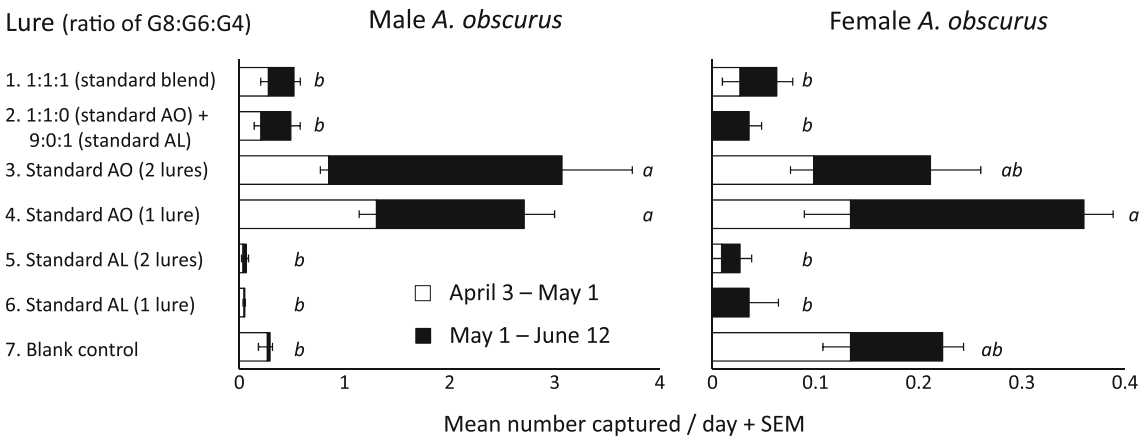


**Fig. 1** Response of male and female *A. obscurus* beetles to different pheromone lures in a fallow field in Agassiz in 2001 (Exp. 1). *Bars with different letters* differ significantly ($P < 0.05$) (Note that the scale differs between sexes)

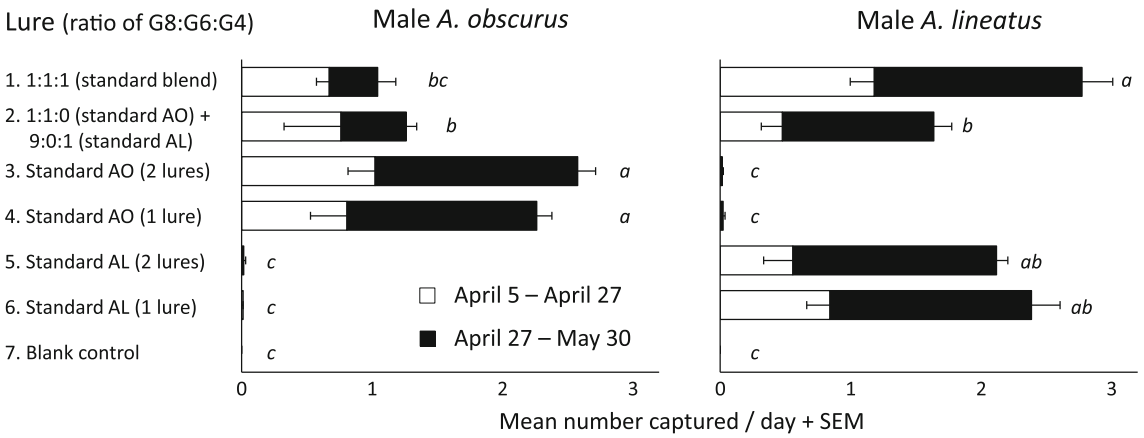


**Fig. 2** Response of male *A. lineatus* and *A. obscurus* beetles to different pheromone lures in a field of pasture in Cloverdale in 2001 (Exp. 2). *Bars with different letters* differ significantly ($P < 0.05$)

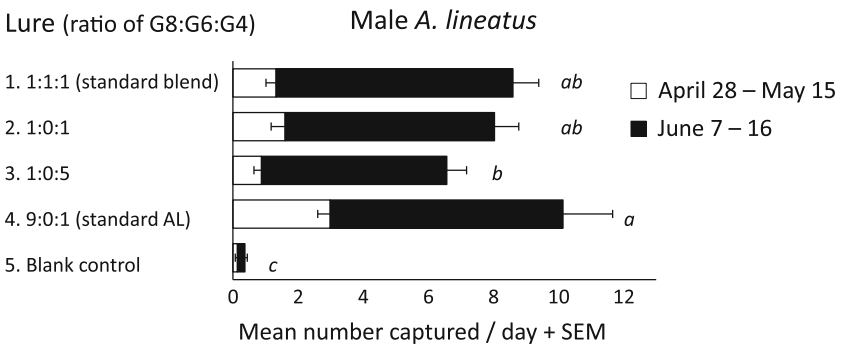


**Fig. 3** Response of male *A. lineatus* beetles to different pheromone lures in Delta in 2002 (Exp. 3). *Bars with different letters* differ significantly ($P < 0.05$)

traps with paired *A. lineatus* and *A. obscurus* lures or standard G8, G6 and G4 blend lures in Exp. 2 (grassy field) caught male *A. obscurus* intermediate in number between traps with the standard *A. obscurus* lures and *A. lineatus* lures (Fig. 2).

## Experiment 3

Contrary to earlier studies which showed that both *A. lineatus* and *A. obscurus* were present in high numbers in the study area (Vernon et al. 2001), pheromone traps in the Delta

**Table 2** Daily *Agriotes obscurus* (AO) and *A. lineatus* (AL) catches in Vernon Beetle Traps baited with standard and blend lures in commercial strawberry fields situated throughout the Fraser valley of British Columbia

| Region | Field | Mean (SEM) male *A. obscurus* beetle catch/trap/day | | | | Mean (SEM) male *A. lineatus* beetle catch/trap/day | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Standard AO | Blend | Blend:AO | All AO collected over period | Standard AL | Blend | Blend: AL | All AL collected over period |
| Delta | Gil1 | 0.47 (0.05) | 0.10 (0.02) | 0.21 | 268 | 1.42 (0.15) | 0.94 (0.14) | 0.66 | 1,040 |
| Delta | Gil2 | 0.38 (0.13) | 0.08 (0.03) | 0.22 | 235 | 0.84 (0.10) | 0.60 (0.12) | 0.71 | 755 |
| Delta | Sf2b | 0.36 (0.07) | 0.06 (0.00)* | 0.16 | 209 | 0.97 (0.10) | 1.13 (0.15) | 1.17 | 1,035 |
| Surrey | Baha2 | 0.98 (0.08) | 0.42 (0.09) | 0.43 | 744 | 1.59 (0.36) | 2.61 (0.47) | 1.64 | 2,231 |
| Surrey | Baha3 | 0.86 (0.10) | 0.13 (0.04)* | 0.16 | 483 | 1.98 (0.19) | 2.56 (0.31) | 1.29 | 2,097 |
| S. Aldergrove | Bj1 | 1.20 (0.17) | 0.30 (0.08)* | 0.25 | 737 | 0.32 (0.11) | 0.87 (0.21) | 2.73 | 565 |
| S. Aldergrove | Gd4 | 1.29 (0.19) | 0.22 (0.04)* | 0.17 | 752 | 0.61 (0.11) | 0.54 (0.20) | 0.89 | 579 |
| N. Aldergrove | Gv | 0.44 (0.09) | 0.07 (0.02) | 0.17 | 247 | 1.23 (0.37) | 1.14 (0.26) | 0.93 | 1,140 |
| N. Aldergrove | Jo5 | 1.01 (0.23) | 0.37 (0.08) | 0.36 | 621 | 0.18 (0.06) | 0.27 (0.08) | 1.52 | 202 |
| N. Aldergrove | Jo6 | 0.47 (0.14) | 0.10 (0.03) | 0.21 | 282 | 0.27 (0.10) | 0.42 (0.09) | 1.57 | 347 |
| N. Aldergrove | Jo7 | 1.18 (0.14) | 0.30 (0.03) | 0.26 | 758 | 0.12 (0.06) | 0.48 (0.09) | 3.91 | 308 |
| N. Aldergrove | Jo8 | 1.10 (0.15) | 0.22 (0.03)* | 0.20 | 700 | 0.14 (0.04) | 0.24 (0.06) | 1.71 | 197 |
| N. Aldergrove | Pan1 | 0.38 (0.16) | 0.08 (0.02) | 0.20 | 224 | 0.07 (0.01) | 0.22 (0.03) | 2.94 | 150 |
| N. Aldergrove | Pan2 | 1.69 (0.70) | 0.31 (0.11)* | 0.18 | 875 | 0.35 (0.15) | 0.43 (0.16) | 1.24 | 329 |
| Abbotsford | Jk3 | 0.80 (0.07) | 0.16 (0.05)* | 0.20 | 444 | 1.01 (0.19) | 0.80 (0.17) | 0.80 | 841 |
| Abbotsford | Ra2 | 0.33 (0.10) | 0.14 (0.05) | 0.42 | 162 | 0.20 (0.08) | 0.18 (0.10) | 0.89 | 138 |
| Chilliwack | Sah2 | 9.93 (4.86) | 2.81 (1.50)* | 0.28 | 3,263 | 1.79 (0.16) | 1.32 (0.12) | 0.74 | 917 |
| Chilliwack | Sh7 | 2.22 (0.48) | 0.44 (0.12) | 0.20 | 987 | 0.82 (0.13) | 0.75 (0.16) | 0.92 | 624 |
| Chilliwack | Sh9 | 1.59 (0.43) | 0.38 (0.15)* | 0.24 | 981 | 1.69 (0.29) | 1.20 (0.24) | 0.71 | 1,448 |
| | | | Mean (SD) ratio | 0.24 (0.08) | | | Mean (SD) ratio | 1.42 (0.88) | |
| ANOVA statistics | | | | | | | | | |
| Field | | $F = 18.22$, $df = 18.153$, $P < 0.0001$ | | | | $F = 25.72$, df = 18.153, $P < 0.0001$ | | | |
| Lure | | $F = 243.31$, $df = 1.153$, $P < 0.0001$ | | | | $F = 1.78$, $df = 1.153$, $P = 0.18$ | | | |
| Field*lure | | $F = 0.85$, $df = 18.153$, $P = 0.64$ | | | | $F = 1.95$, $df = 18.153$, $P = 0.016$ | | | |
| Model $R^2$ | | 0.79 | | | | 0.77 | | | |

* Beetle catch in blend traps differ significantly ($P < 0.05$) from catch in standard AO traps (based on model LSMean calculations)

trial in 2002 trapped >99 % *A. lineatus* (3,058 males and 66 females). There was a significantly higher trapping rate per day for the second (7–16 June) than first (28 April–15 May) trapping period (early: 55.4/day; late: 214.3/day; $F = 71.71$, $df = 1.74$, $P < 0.0001$). Significant differences between treatments were observed during both trapping periods (early: $F = 15.75$, $df = 4.28$, $P < 0.0001$; late: $F = 14.48$, $df = 4.28$, $P < 0.0001$), with all treatments with lures catching significantly higher numbers than the blank control (Fig. 3). During the first trapping period, traps with the standard *A. lineatus* lure also collected significantly higher numbers than those with other blends ($P < 0.05$; Fig. 3), but there was no significant difference between baited treatments during the second period (Fig. 3). Combining the trapping data over both periods also indicated significant differences between treatments ($F = 29.15$, $df = 1.74$, $P < 0.0001$) (Fig. 3), with the standard *A. lineatus* lure, the standard G8, G6 and G4 blend lure, and the 1:1 G8:G4 lure all catching significantly more males than the blank control traps (Fig. 3). Catch of males in traps with an elevated level of geranyl butanoate (1:5 ratio of G8:G4) was intermediate and differed significantly from the standard *A. lineatus* lure and blank control (Fig. 3).

## Experiment 4

Click beetles captured in the Agassiz trial in 2002 were predominantly *A. obscurus* (6,990 males and 178 females), with only a few *A. lineatus* males captured ($N = 45$). Analysis of variance confirmed that the number of male beetles taken in traps was significantly affected by habitat (grassy field: $N = 4{,}871$; fallow field: $N = 2119$; $F = 37.83$, $df = 1.42$, $P < 0.0001$), pheromone lure ($F = 60.49$, $df = 6.42$,

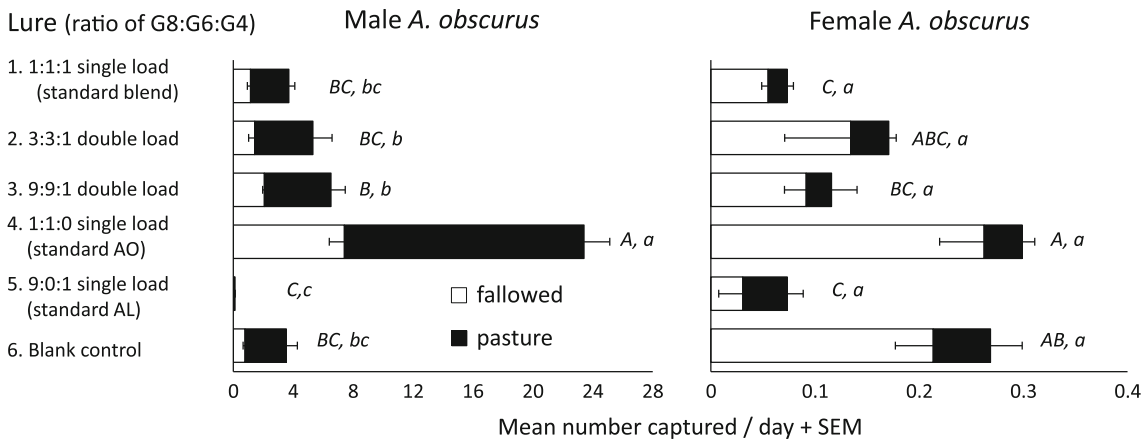


**Fig. 4** Response of male and female *A. obscurus* beetles to different pheromone lures in Agassiz in 2002 (Exp. 4). The *dark bars* represent the mean catch in a field of pasture, and the *white bars* represent the mean catch in an adjacent fallowed field. *Bars with different letters* indicate that the total catch for each treatment differs significantly ($P < 0.05$). (Note that the scale differs between sexes)

$P < 0.0001$), and the interaction between these variables ($F = 8.06$, $df = 6.42$, $P < 0.0001$). As a result, the analysis was repeated separately for the two habitat types, which indicated that in both situations, the standard *A. obscurus* lure was more attractive to *A. obscurus* males than all other treatments, and that the standard *A. lineatus* lure was least attractive (grassy field: $F = 45.49$, $df = 6.18$, $P < 0.0001$; fallow field: $F = 47.1$, $df = 6.18$, $P < 0.0001$) (Fig. 4). It is interesting that relative to the low response to the standard *A. lineatus* lure (containing G8 and G4), lures that also contained the *A. obscurus*-specific G6 component did appear to have an elevated catch rate (sometimes significantly so) of *A. obscurus* males. On the other hand, lures that contained the standard *A. obscurus* G8 and G6 components as well as the minor *A. lineatus* G4 component (Fig. 4) had a significantly reduced catch relative to the standard *A. obscurus* lure (Fig. 4). This was also observed in Experiments 1 and 2.

Analysis of variance confirmed that the number of females taken in traps in Exp. 4 was significantly affected by habitat, but opposite to that observed with males (grassy field: $N = 38$ females; fallow field: $N = 140$ females; $F = 34.36$, $df = 1.42$, $P < 0.0001$), and that pheromone lure response ($F = 4.65$, $df = 6.42$, $P = 0.001$), and the interaction between these variables ($F = 3.53$, $df = 6.42$, $P = 0.0064$) were significant. Repeating the analysis separately for the two habitat types indicated that in the grassy field, there were no significant differences in catch of *A. obscurus* females between treatments ($F = 0.47$, $df = 6.18$, $P = 0.82$), but in the fallowed field, significantly more beetles were caught in traps baited with a standard *A. obscurus* lure or blank control than the standard *A. lineatus* lure or the 1:1:1 (G8, G6, G4) blend ($F = 7.01$, $df = 6.18$, $P = 0.0006$) (Fig. 4). This trend was also observed in the fallowed field in Exp. 1 (Fig. 1), again suggesting that *A. obscurus* females are avoiding some traps with the G4 component.

## Experiment 5

In the same field as Exp. 3 in Delta, pheromone traps in Exp. 5 also trapped primarily *A. lineatus* males ($N = 5{,}470$). Significantly, more beetles were collected per day during the second (3–16 June) than the first (28 April–15 May) trapping period (157.5 vs. 77.3, respectively; $F = 16.74$, $df = 1.89$, $P < 0.0001$). For both trapping periods, significantly more male *A. lineatus* were collected in all traps containing *A. lineatus*-specific compounds (G8 and G4) than the standard *A. obscurus* lure or blank control (first period: $F = 17.64$, $df = 5.35$, $P < 0.0001$; second period: $F = 11.51$, $df = 5.35$, $P < 0.0001$; combined: $F = 22.06$, $df = 5.35$, $P < 0.0001$) (Fig. 5). Similar to Exp. 3, the standard *A. lineatus* lure was significantly more attractive than other blends (except the 3:3:1 double loaded blend in Exp. 5) early in the season, while all pheromone lures containing G8 and G4 (with or without G6) components elicited an equivalent response later in the season (Fig. 5).

Catches of *A. obscurus* ($N = 22$) and female *A. lineatus* ($N = 26$) in Delta were too low to analyse statistically.

## Regional pheromone trap comparison

The mean catches of *A. obscurus* and *A. lineatus* males per trap per day in standard *A. obscurus* and *A. lineatus* traps, or in standard G8, G6 and G4 blend traps in 19 strawberry fields across the LFV are shown in Table 2. The number of *A. obscurus* collected per trap per day in standard *A. obscurus* versus blend traps varied between fields (min. 0.33, 0.06, respectively; max. 9.93, 2.81, respectively), with generally more *A. obscurus* captured towards the eastern end of the LFV (Chilliwack; Table 2). ANOVA indicated that both field and lure type, but not their

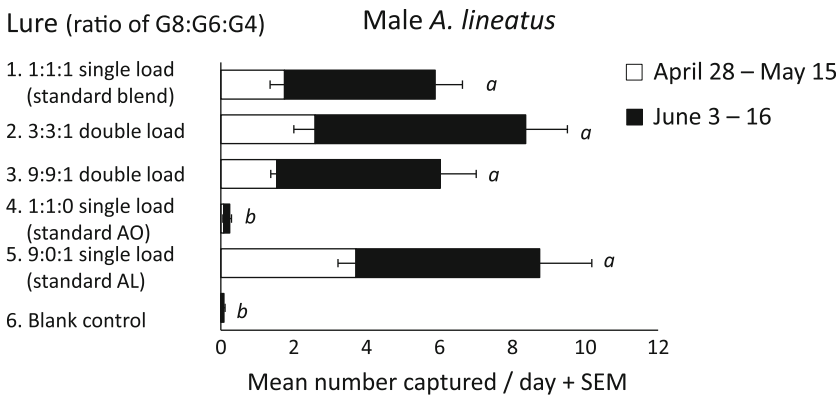


**Fig. 5** Response of male *A. lineatus* beetles to different pheromone lures in Delta in 2002 (Exp. 5). *Bars with different letters* differ significantly (<0.05)

interaction, were the significant factors explaining variability in trap catches (Table 2). The ratio of male *A. obscurus* beetles collected in blend versus standard *A. obscurus* traps in the 19 fields ranged from 0.16 to 0.43 (mean = 0.24, SD = 0.08; Table 2). In the three separate Agassiz pheromone studies (Exp. 1, 4), also located at the eastern end of the LFV (near to Chilliwack), the mean cumulative *A. obscurus* males taken per study in blend traps plus standard *A. obscurus* traps (mean = 1,627; range 436–3,044) were similar to catches in the Chilliwack fields (Table 2). In these Agassiz studies, the LS mean (SEM) daily catch in standard *A. obscurus*, and blend traps were 8.26 (0.48) and 1.32 (0.48), respectively, with the ratio of catch in blend vs standard AO traps in the Agassiz studies ranging from 0.15 to 0.20 (mean = 0.16).

The number of *A. lineatus* per trap per day collected in standard *A. lineatus* versus blend traps also varied between fields (min. 0.07, 0.18, respectively; max. 1.98, 2.61, respectively), with no obvious bias in numbers occurring across the various regions of the LFV (Table 2). ANOVA indicated that field but not lure type was a significant factor explaining variability in trap catches (Table 2). The ratio of male *A. lineatus* beetles collected in blend vs standard *A. lineatus* traps varied considerably, ranging from 0.66 to 3.91 (mean = 1.42, SD = 0.88; Table 2), and accounts for the weakly significant interaction effect between the two factors (Table 2). In the three separate Agassiz pheromone studies (Exp. 1, 4), the cumulative number of *A. lineatus* males taken per study in blend traps plus standard *A. lineatus* traps ranged from only 2–16, which were considerably lower than in the other LFV fields.

## Discussion

These results confirm that *A. obscurus* and *A. lineatus*, introduced to the lower Fraser Valley about a century ago, can be captured using semiochemical lures similar to those tested in Europe (Yatsynin et al. 1996; Tóth et al. 2003, 2008; Toth 2013). In Europe, a combination of G8 and G6 loaded in closed polyethylene vials in 2:1, 1:1 or 1:2 ratios was equally effective at capturing *A. obscurus* males (Tóth et al. 2003). In the current and earlier LFV studies (Vernon and Tóth 2007), the highest captures of *A. obscurus* occurred with lures containing a 1:1 ratio of G8:G6, and baiting with 2 lures did not significantly improve catch. Trapping efficacy was reduced significantly, however, when lures also contained the *A. lineatus*-specific compound, G4. In fact, numbers of *A. obscurus* trapped in the standard G8, G6 and G4 blend (1:1:1 ratios) and in traps with blends containing elevated rates of G8 and G6 relative to G4 (3:3:1 and 9:9:1) were not significantly different from numbers in the unbaited traps (Figs. 1, 2 and 4). Despite this significant drop in attraction, the presence of the G4 component did not completely prevent *A. obscurus* from entering the blend-baited traps, and the trapping rate in the standard blend traps gave, on average, 24 % (range: 16–43 %) of the catch in standard *A. obscurus* traps in the 19 strawberry fields monitored in 2002 (Table 2). Therefore, standard blend traps would be adequate if simple *A. obscurus* detection is the objective of monitoring, but would not be preferred if optimal trapping is the goal, as in IPM monitoring programmes or for mass trapping. In additional studies, we have determined that standard *A. obscurus* traps presented in dense arrays in grassy habitats (3 m trap spacings) will capture 85.6 % of mark-released *A. obscurus* males (Vernon et al. 2014a, b), which would be greatly reduced with standard blend traps.

For *A. lineatus*, Tóth et al. (2003) determined that the optimal blend for male *A. lineatus* capture in Europe was G8:G4 in a 100:3–10:3 ratio, although in parts of Europe captures were higher with a 1:1 than 10:1 ratio (Tóth et al. 2008). In our studies, G8:G4 in a 9:1 ratio consistently provided high levels of male attraction (Figs. 2, 3 and 5), which was not improved when two lures were presented (Fig. 2). Similar results were obtained in earlier trials (Vernon and Tóth 2007). The presence of the *A. obscurus*-specific compound, G6, in the standard G8, G6 and G4 blend and in other blends (3:3:1 and 9:9:1) had no significant effect on total *A. lineatus* attraction over the course of the

trapping studies. However, the standard *A. lineatus* lure did perform better than the standard blend lure early in the season in Exps. 3 and 5. The equivalent overall performance of the standard *lineatus* and blend lures shows that *A. lineatus* males are not behaviourally affected by, or cannot antennally detect the presence of G6. Geranyl hexanoate is not produced by *A. lineatus*, and it is unknown if *A. lineatus* can detect this compound. The lack of behavioural response to G6 indicates that a blended pheromone lure could be used to monitor *A. lineatus* without significantly reducing the capture rate, and in fact, the standard blend lure outperformed the standard *A. lineatus* lure in 10 of the 19 strawberry field studies, and did not differ significantly in any (overall ratio of standard blend to *A. lineatus* lure, 1.42:1, ranging from 0.66:1 to 3.91:1, Table 2). In addition to monitoring, standard blend lures would also be effective in a mass trapping programme to reduce *A. lineatus* males. We have determined that standard *A. lineatus* traps presented in dense arrays in grassy habitats will capture 77.8 % of mark-released *A. lineatus* males (Vernon et al. 2014a, b), which should also be achievable with standard blend traps. However, where mass trapping for both *A. lineatus* and *A. obscurus* males is the objective, standard *A. obscurus* lures would still have to be used, but blend lures may have an advantage over standard *A. lineatus* lures in both the total number of *A. lineatus* captured and the removal of some additional *A. obscurus* males (according to Table 2 data).

The results of these field experiments (Figs. 1, 2, 3, 4, and 5) as well as the strawberry field survey data (Table 2) showed that there were no consistent regional differences in the ratios of *A. obscurus* or *A. lineatus* males caught in blend traps versus their standard pheromone traps (Table 2). In addition, there did not appear to be significant differences in the response of *A. obscurus* or *A. lineatus* to their individual pheromone lures when presented in different regions of the LFV. These data suggest that differences in response of each species to ratios of their key pheromone components are not occurring in the LFV, as has been reported in regions of Europe and Russia (Yatsynin et al. 1996; Tóth et al. 2003).

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