

# Field evaluation of 3-hydroxy-2-hexanone and ethanol as attractants for the cerambycid beetle pest of vineyards, *Xylotrechus arvicola*

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## Abstract

**BACKGROUND:** The beetle *Xylotrechus arvicola* (Coleoptera: Cerambycidae) is a serious pest of vineyards in the Iberian Peninsula. In previous work, the male beetles, but not females, were shown to produce (R)-3-hydroxy-2-hexanone, and female beetles were attracted to this compound in a laboratory bioassay. In this study, release rates of 3-hydroxy-2-hexanone from different dispensers were measured in the laboratory, and the attractiveness of these to *X. arvicola* adults was determined in trapping tests in three traditional wine-growing regions in Spain.

**RESULTS:** As a result of laboratory experiments, for field experiments 3-hydroxy-2-hexanone was formulated as 100  $\mu$ L in a polyethylene sachet (50 mm  $\times$  50 mm  $\times$  250  $\mu$ m), and ethanol was formulated as 1 mL in a polyethylene press-seal bag (76 mm  $\times$  57 mm  $\times$  50  $\mu$ m). Field catches were similar at all three study sites. Catches in traps baited with 3-hydroxy-2-hexanone alone were not significantly different from those in unbaited control traps, but catches in traps baited with 3-hydroxy-2-hexanone and ethanol in separate sachets, with 3-hydroxy-2-hexanone and ethanol in the same sachet or with ethanol alone were significantly greater than those in control traps. These results confirm that the beetles are attracted to ethanol, and the addition of 3-hydroxy-2-hexanone does not seem to make any difference.

**CONCLUSIONS:** Attraction of females for the male-produced compound (R)-3-hydroxy-2-hexanone has been observed in laboratory but not in field experiments. Traps baited with ethanol are highly attractive to both sexes of adults of *X. arvicola*, and these can be used for improved monitoring of the adult emergence and for population control by mass trapping.

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**Keywords:** *Xylotrechus arvicola*; Cerambycidae; (R)-3-hydroxy-2-hexanone; ethanol; panel traps; monitoring

## 1 INTRODUCTION

The beetle *Xylotrechus arvicola* (Olivier) (Coleoptera: Cerambycidae) is a serious pest of vineyards in the Duero Valley (Iberian Peninsula). *X. arvicola* has a great capacity to establish itself in new vineyards,<sup>1</sup> and larvae can spread fungi such as *Diplodia seriata* (De Not), *Eutypa lata* (Tul and Tul), *Phaeoacremonium aleophilum* (Gams, Crous, Wingf., Mugnai), *Phaeomoniella chlamydospora* (Crous and Gams) and *Formitiporia mediterranea* (Fisch) across the wood.<sup>2</sup>

After mating, females of *X. arvicola* lay their eggs in cracks or under the rhytidome in the wood of vines.<sup>3</sup> The eggs remain viable over a long period,<sup>4</sup> and the emerging larvae bore into the wood and make galleries inside the plant.<sup>5</sup> The stages of the pest most susceptible to intervention are adults, eggs and neonate larvae, but the latter are usually protected by the rhytidome.<sup>3</sup> Once inside the wood, the larvae are inaccessible to traditional foliar-applied chemicals that do not have systemic attributes.<sup>6</sup>

Insect sex pheromones are used in pest management for monitoring and control.<sup>7</sup> Monitoring of insect pests using pheromone traps can help pest surveillance and the forecasting of optimal timing for insecticide applications (e.g. Delisle *et al.*<sup>8</sup> and Boddum *et al.*<sup>9</sup>). Mass trapping using pheromone traps can reduce insect

pest populations, which can lead to a reduction in damage in field crops and stored products.<sup>7,10</sup> Male-produced aggregation pheromones have been identified for several *Xylotrechus* species, including *X. quadripes* (Chevrolat),<sup>11</sup> *X. pyrrhoderus* Bates,<sup>12</sup> *X. chinensis* (Chevrolat),<sup>13</sup> *Xylotrechus colonus* (Fabricius),<sup>14</sup> *X. nauticus* (Mannerheim)<sup>15</sup> and *X. rufilius* Bates.<sup>16</sup> 3-Hydroxy-2-hexanone, the corresponding 2,3-hexanediols and the homologous 8-carbon

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compounds are common, and often the sole pheromone components for many species in the subfamily Cerambycinae.<sup>17</sup> 3-Hydroxy-2-hexanone has been used as bait in traps to catch multiple species of subfamily Cerambycinae.<sup>15,17–20</sup>

In previous work by the Natural Resources Institute (NRI) and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) during 2005–2007, *X. arvicola* male beetles, but not females, were shown to produce 3-hydroxy-2-hexanone.<sup>21</sup> The beetles produced exclusively the *R*-enantiomer, and rates of production of up to 68  $\mu\text{g h}^{-1}$  were recorded. The synthetic compound elicited electroantennographic (EAG) responses from receptors on the antennae of both males and females. In laboratory wind tunnel bioassays, females were significantly attracted to males and to (*R*)-3-hydroxy-2-hexanone or the racemic mixture, and this compound was assumed to be the major component of the sex/aggregation pheromone of this species. However, this attraction was not observed in preliminary trapping trials in the field in Spain. The aims of this work were to evaluate different types of dispenser for 3-hydroxy-2-hexanone in the laboratory, and to use these to carry out further field testing of this compound in Spanish vineyards.

## 2 MATERIALS AND METHODS

### 2.1 Chemicals

3-Hydroxy-2-hexanone was synthesised in the racemic form at the NRI by reaction of 1-hexyn-3-ol with mercuric oxide and boron trifluoride etherate in methanol, followed by acidic hydrolysis.<sup>22</sup>

For preparation of the *R*-enantiomer, the corresponding racemic acetate was hydrolysed with Amano AK lipase in phosphate buffer which selectively hydrolysed the *S*-enantiomer.<sup>23</sup> The remaining *R*-acetate was isolated by chromatography and converted to (*R*)-3-hydroxy-2-hexanone (96% enantiomeric excess) by careful hydrolysis with potassium carbonate in methanol.

### 2.2 Dispensers

The dispensers used in this work were press-seal, low-density polyethylene bags (76 mm  $\times$  57 mm  $\times$  50  $\mu\text{m}$  thick or 38 mm  $\times$  64 mm  $\times$  50  $\mu\text{m}$  thick; Transpack, Southampton, UK), sachets prepared by heat sealing low-density, polyethylene lay-flat tubing (50 mm width  $\times$  250  $\mu\text{m}$ , 120  $\mu\text{m}$  or 60  $\mu\text{m}$  thick; Transpack) or low-density polyethylene vials (30 mm  $\times$  15 mm  $\times$  1.5 mm thick; Fisher Scientific, Loughborough, UK). 3-Hydroxy-2-hexanone (100  $\mu\text{L}$ ) was impregnated onto a cellulose acetate cigarette filter (14 mm  $\times$  6 mm; Swan, High Wycombe, Bucks, UK) in all these dispensers.

The above dispensers were compared with those reported for other cerambycid beetles using pheromones dispensed from solutions in ethanol.<sup>24,25</sup> In initial laboratory work and field experiments carried out in 2011, a solution of 3-hydroxy-2-hexanone in ethanol (50 mg in 1 mL) was dispensed from press-seal, low-density polyethylene bags (76 mm  $\times$  57 mm  $\times$  50  $\mu\text{m}$  thick; Transpack). During 2012, the same solution was impregnated onto a cotton dental roll (a cylinder of purified and sterilised cotton wool; 35 mm  $\times$  8 mm; Kent Express Dental Supplies, Gillingham, Kent, UK) in the bag.

### 2.3 Release rates

Release rates of materials from dispensers were generally measured by weighing duplicate samples at intervals. Dispensers were maintained in a wind tunnel at 27 °C and 8 km  $\text{h}^{-1}$  wind speed, or in a laboratory fume hood at 20–22 °C.

For solutions of 3-hydroxy-2-hexanone in ethanol, a sample (10  $\mu\text{L}$ ) was withdrawn at intervals and added to a solution of decyl acetate in hexane (0.2 mg  $\text{mL}^{-1}$ ; 1 mL). The resulting solution was analysed by gas chromatography (GC) using a fused silica capillary column (30 m  $\times$  0.32 mm i.d.  $\times$  0.25  $\mu\text{m}$  film thickness) coated with polar DBWax (Agilent, Stockport, Cheshire, UK) with flame ionisation detection (FID; 250 °C). Injection was splitless (200 °C), the carrier gas was helium (2.4 mL  $\text{min}^{-1}$ ) and the oven temperature was programmed from 50 °C for 2 min and then at 10 °C  $\text{min}^{-1}$  to 240 °C. Data were captured and quantified using EZChrom Elite (Agilent).

### 2.4 Experimental vineyards

Field trapping tests were conducted in three experimental vineyards with Protected Denomination of Origin (PDO), which is a certification to distinguish quality food products of a particular region (UE Reg. No. 1151/2012 published on 21 November 2012), called 'Ribera Del Duero', located in Peñafiel (Valladolid), 'Toro', located in El Pego (Zamora), and 'Tierra de León', located in Gordocillo (León). The vineyards were chosen on the basis of the presence of *X. arvicola* damage such as larval galleries inside the plants and exit holes of *X. arvicola* adults on trunks and branches. These vineyards were planted uniformly with the same *Vitis vinifera* Tempranillo variety. Vines were spaced 3  $\times$  1.5 m in 'Ribera Del Duero' and 'Tierra de León', and 3.5  $\times$  3.5 m in 'Toro'. Vineyards were surrounded by other vineyards. More details of the experimental vineyards are shown in Table 1.

### 2.5 Traps, lures and experimental design

The traps used were the CROSSTRAP<sup>®</sup> design (Econex, Murcia, Spain). This trap is made of two black panels of Correx sheet (80 cm  $\times$  30 cm) held at right angles. Unless otherwise stated, the panels were coated with Fluon (DYNEON<sup>™</sup>; 3M<sup>™</sup>, Berkshire, UK), as recommended by Graham *et al.*<sup>26</sup> The lures were attached to the trap at the midway point, and insects were trapped in a receiver at the base. The collected insects were identified and sexed in the laboratory, according to the description of Moreno.<sup>27</sup> In all experimental vineyards, there were four replicates of each treatment in a randomised complete block design, including an unbaited control. Traps were spaced at 18 m intervals, approximately 1.5 m above ground level, and treatments were randomised within blocks. Traps were visited every 2–3 days, when trapped beetles were counted and removed. Lures were renewed every 10 days. To avoid spatial effects, the traps were moved on one position every 10 days.

Three sets of treatments were evaluated during 2011 and 2012, and these are shown in Table 2. During 2011, racemic and (*R*)-3-hydroxy-2-hexanone dispensed neat in sachets were compared with an ethanolic solution of the racemic compound in a press-seal bag. Traps with and without Fluon coating were compared to confirm the value of this treatment. In 2012, racemic 3-hydroxy-2-hexanone in sachets was compared with the racemic compound in ethanol solution in press-seal bags and with ethanol alone. In a final experiment during 2012 at 'Tierra de León', catches with ethanol alone were compared with those in unbaited traps.

### 2.6 Statistical analysis

For statistical analyses, total numbers of beetles trapped were transformed to  $\log(x + 1)$  to normalise the variances and subjected to analysis of variance (ANOVA). Differences between means were

**Table 1.** Details of experimental vineyards with PDO

	'Ribera Del Duero'	'Toro'	'Tierra de León'
Location	Peñañiel	El Pego	Gordoncillo
Province	Valladolid	Zamora	León
Coordinates	41° 35' 39.1" N, 4° 05' 19.1" W	41° 20' 26.4" N, 5° 25' 51.8" W	42° 08' 14.9" N, 5° 25' 41.6" W
Height above sea level (m)	754	697	747
Annual average temperature (°C)	11	12.5	11.7
Average rainfall (mm)	450	375	500
Soils	Alluvial sand and clay soils in low areas, limestone soils and chalk in high areas	Calcareous soils formed by sandstone, clay and limestone sediments	Calcareous soils, low in minerals and poor in organic matter
Training system of vines	Bilateral cordon	Bush vines	Bilateral cordon
Training system characteristics	Spur pruning over two arms per trunk (1 m)	Spur pruning over 4–5 branches per trunk (0.5 m)	Spur pruning over two arms per trunk (1 m)
<i>Vitis vinifera</i> variety	'Tempranillo'	'Tempranillo'	'Tempranillo'
Age (years)	25	50	18

**Table 2.** Details of experiments and treatments trapping *X. arvicola* during two seasons at three vineyards with PDO in Spain

Year	PDO name – location (province)	Treatments <sup>a</sup>
2011	'Ribera Del Duero' – Peñañiel (Valladolid) 'Toro' – El Pego (Zamora)	3-Hydroxy-2-hexanone in sachet 3-Hydroxy-2-hexanone in sachet (trap without Fluon) Unbaited control 3-Hydroxy-2-hexanone in ethanol solution in press-seal bag ( <i>R</i> )-3-Hydroxy-2-hexanone in sachet
2012	'Ribera Del Duero' – Peñañiel (Valladolid), 'Toro' – El Pego (Zamora) 'Tierra de León' – Gordoncillo (León)	3-Hydroxy-2-hexanone in sachet 3-Hydroxy-2-hexanone in sachet + ethanol in press-seal bag <sup>b</sup> Unbaited control 3-Hydroxy-2-hexanone in ethanol solution in press-seal bag <sup>b</sup> Ethanol in press-seal bag <sup>b</sup>
2012	'Tierra de León' – Gordoncillo (León)	A3: ethanol in press-seal bag <sup>b</sup> B3: ethanol in press-seal bag <sup>b</sup> C3: ethanol in press-seal bag <sup>b</sup> D3: ethanol in press-seal bag <sup>b</sup> E3: ethanol in press-seal bag <sup>b</sup>

<sup>a</sup> Unless otherwise stated, 3-hydroxy-2-hexanone is the racemic mixture.

<sup>b</sup> Solution impregnated on cotton dental roll.

tested for significance by the least significant difference (LSD) test. The level of significance considered was  $P < 0.05$  in all cases.

### 3 RESULTS

#### 3.1 Release rates

Release rate data are summarised in Table 3. Release rates of 3-hydroxy-2-hexanone from press-seal bags (76 mm × 57 mm × 50 µm; 86.6 cm<sup>2</sup>) and heat-sealed sachets (50 mm × 50 mm × 120 µm; 50 cm<sup>2</sup>) were rapid at 17.9 and 11.4 mg day<sup>-1</sup> respectively. Release from a thinner 60 µm sachet was even more rapid at 19.4 mg day<sup>-1</sup>.

Reducing the size of the dispensers reduced the release rate as expected. A smaller press-seal bag (38 mm × 64 mm × 50 µm; 48.6 cm<sup>2</sup>) released at a rate of 11.5 mg day<sup>-1</sup>, and half-size sachets (25 mm × 50 mm × 120 µm; 25 cm<sup>2</sup>) at approximately 7 mg day<sup>-1</sup>. Increasing the length of the sachet from 50 to 90 mm increased the release rate from 6.5 to 7.1 mg day<sup>-1</sup>. Increasing the thickness of the sachet reduced the release rate as expected, with a 50 mm × 50 mm × 250 µm sachet releasing at a rate of 4.4 mg day<sup>-1</sup>.

Release of 3-hydroxy-2-hexanone from a polyethylene vial was much slower. There was no release for the first 3 days as the material passed through the walls, but thereafter release was uniform at 0.23 mg day<sup>-1</sup>.

Release of ethanol from a press-seal bag (76 mm × 57 mm × 50 µm thick) was linear at 38.6 mg day<sup>-1</sup> at 20–22 °C. Release from all these 'reservoir-type' dispensers was zero order and continued until the contents were exhausted.

When a solution of 3-hydroxy-2-hexanone (50 mg) in ethanol (1 mL) was formulated in a press-seal bag (76 mm × 57 mm × 50 µm), the ethanol was released at 86 mg day<sup>-1</sup> at 27 °C. Analysis of the solution remaining at daily intervals showed that the concentration of 3-hydroxy-2-hexanone remained essentially the same [50.0 ± 1.2 (SE) mg mL<sup>-1</sup> at day 0, 47.0 ± 1.8 mg mL<sup>-1</sup> at day 7,  $N = 3$ ], indicating that this compound was released in proportion, i.e. at 5.7 mg day<sup>-1</sup>.

Impregnating the ethanolic solution of 3-hydroxy-2-hexanone on a cotton dental roll caused a slight but not significant reduction in release rate ( $N = 4$ ;  $t = 1.56$ ,  $df = 4$ ,  $P = 0.08$ ). These results

**Table 3.** Release rates of 3-hydroxy-2-hexanone and ethanol from different dispensers in the laboratory

Compound	Dispenser	Dimensions	Temperature (°C)	Rate (mg day <sup>-1</sup> ) <sup>a</sup>
3-Hydroxy-2-hexanone	Press-seal bag	76 mm × 57 mm × 50 μm	27	17.9 ± 0.6
	Press-seal bag	38 mm × 64 mm × 50 μm	27	11.5 ± 0.2
	Sachet	50 mm × 50 mm × 60 μm	27	19.4 ± 0.3
	Sachet	50 mm × 50 mm × 120 μm	27	11.4 ± 0.2
	Sachet	50 mm × 50 mm × 250 μm	27	4.4 ± 0.04
	Sachet	25 mm × 50 mm × 120 μm	27	6.5 ± 0.1
	Vial	30 mm × 15 mm × 1.5 mm	27	0.2 ± 0.0
	Press-seal bag (50 mg mL <sup>-1</sup> in ethanol)	76 mm × 57 mm × 50 μm	27	5.7 ± 0.4 <sup>b</sup>
Ethanol	Press-seal bag	76 mm × 57 mm × 50 μm	27	86.0 ± 6.1 <sup>b</sup>
	Press-seal bag	76 mm × 57 mm × 50 μm	20–22	38.6 ± 3.0

<sup>a</sup> Mean of two replicates ± ranges.

<sup>b</sup> Mean of three replicates ± ranges.

were obtained by heat-sealing the press-seal bags. Without heat sealing, release rates sometimes differed markedly between otherwise identical dispensers, probably because of difficulties in reliably sealing the bags containing the solution. Impregnating the solution on a dental roll gave consistent release rates without the need for heat sealing.

In view of these laboratory results, for field trials 3-hydroxy-2-hexanone was formulated as 100 μL in a heat-sealed, thick polyethylene sachet (50 mm × 50 mm × 250 μm), and ethanol was formulated as 1 mL in a press-seal bag (76 mm × 57 mm × 50 μm). The dispensers were renewed every 10 days in field experiments, as described in Section 2.

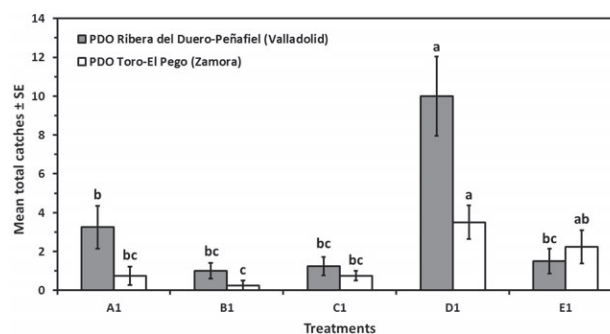
### 3.2 Field trial 2011

Catches of *X. arvicola* beetles in traps (Fig. 1) baited with 3-hydroxy-2-hexanone alone dispensed from polyethylene sachets either in the racemic form (treatments A1 and B1) or as the *R*-enantiomer (E1) were not significantly different ( $P > 0.05$ ) from those in control traps at 'Ribera del Duero'. However, catches in traps baited with 3-hydroxy-2-hexanone formulated as a solution in ethanol in press-seal bags (D1) were significantly higher. Catches in traps without Fluon coating (B1) were significantly lower than those in comparable traps coated with Fluon (A1). Results were similar at 'Toro' (Fig. 1), but catches were lower and differences between the treatments were not so marked. Catches were 50:50 male:female at 'Ribera del Duero' and 53:47 at 'Toro' (Fig. 1).

### 3.3 Field trial 2012

The results of the first experiment were similar at all three sites, and the combined results are shown in Fig. 2. Catches of *X. arvicola* beetles in traps baited with 3-hydroxy-2-hexanone alone (A2) were not significantly different from those in unbaited control traps (C2). Catches in traps baited with 3-hydroxy-2-hexanone and ethanol in separate sachets (B2), with 3-hydroxy-2-hexanone and ethanol in the same sachet (D2) or with ethanol alone (E2) were significantly greater ( $P < 0.05$ ) than those in control traps (C2). These results confirm that the beetles are attracted to ethanol (E2), and the addition of 3-hydroxy-2-hexanone (B2 and D2) does not seem to make any difference.

At 'Tierra de León', the final week of the experiment was run with treatments A3, B3 and D3 replaced by sachets of ethanol only,



**Figure 1.** Mean total catches of *X. arvicola* beetles at 'Ribera del Duero' – Peñafiel (Valladolid) and 'Toro' – El Pego (Zamora) during 2011 (4 June–4 July). Treatments – A1: 3-hydroxy-2-hexanone in sachet; B1: 3-hydroxy-2-hexanone in sachet (trap without Fluon); C1: unbaited control; D1: 3-hydroxy-2-hexanone in ethanol solution in press-seal bag; E1: (*R*)-3-hydroxy-2-hexanone in sachet. For each location, means with different letters are significantly different at  $P < 0.05$  after ANOVA and LSD test ('Ribera del Duero':  $F = 15.19$ ,  $df = 4, 12$ ,  $P < 0.001$ ; 'Toro':  $F = 3.82$ ,  $df = 4, 12$ ,  $P = 0.03$ ).

as in E3. High catches of *X. arvicola* were obtained in the baited traps during 22–28 June 2012, and these were very significantly greater than catches in the control traps by a simple *t*-test on untransformed data (Fig. 3).

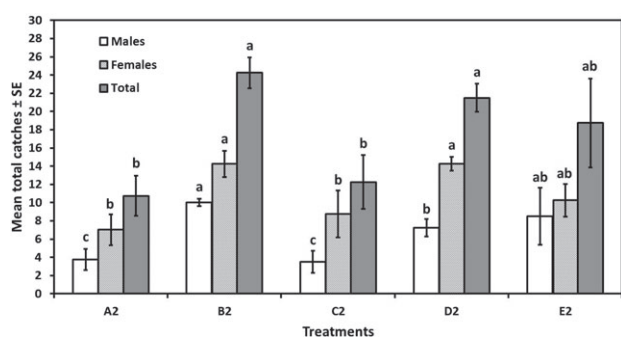
## 4 DISCUSSION

3-Hydroxy-2-hexanone is produced by males of many cerambycid species, particularly those in the Cerambycinae subfamily, and in many of these it has been shown to function as a sex/aggregation pheromone.<sup>17</sup>

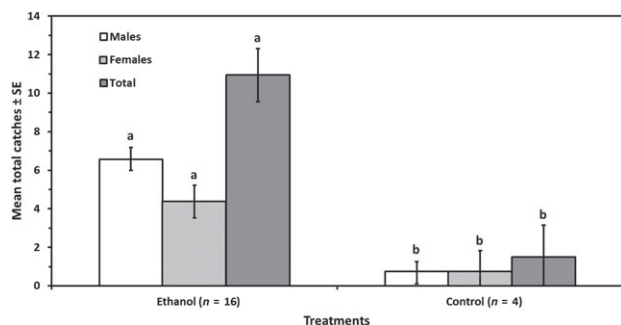
In order to evaluate a potential pheromone in the field effectively, it is essential to use dispensers releasing the compound at an appropriate rate as uniformly as possible for the duration of the experiment. Several slow-release formulations of this candidate pheromone component were evaluated in the laboratory, and a thick-walled polyethylene sachet made from heat-sealed, lay-flat tubing was shown to be a suitable dispenser with a zero-order release rate of 4.4 mg day<sup>-1</sup>, comparable with the rate of production by a male *X. arvicola* beetle of 68 μg h<sup>-1</sup> (1.6 mg day<sup>-1</sup>).<sup>21</sup>

The dispensing system used in many other studies of pheromones of cerambycid beetles (e.g. Ray *et al.*<sup>24</sup> and Rodstein





**Figure 2.** Mean total catches of *X. arvicola* beetles at 'Ribera del Duero' – Peñafiel (Valladolid), 'Toro' – El Pego (Zamora) and 'Tierra de León' – Gordoncillo (León) during 2012 (18 May–18 June 2012). Treatments – A2: 3-hydroxy-2-hexanone in sachet; B2: 3-hydroxy-2-hexanone in sachet + ethanol in press-seal bag; C2: unbaited control; D2: 3-hydroxy-2-hexanone in ethanol solution in press-seal bag; E2: ethanol in press-seal bag. For each category, means with different letters are significantly different ( $P < 0.05$ ) after ANOVA and LSD test (males:  $F = 4.58$ ,  $df = 4, 44$ ,  $P = 0.003$ ; females:  $F = 5.31$ ,  $df = 4, 44$ ,  $P < 0.001$ ; combined:  $F = 6.86$ ,  $df = 4, 44$ ,  $P < 0.001$ ).



**Figure 3.** Mean total catches of *X. arvicola* beetles at 'Tierra de León' – Gordoncillo (León) during 2012 (18–28 June) in traps baited with ethanol alone or in unbaited control traps. Means with different letters are significantly different at  $P < 0.001$  after  $t$ -test (males:  $t = 6.75$ ,  $df = 16$ ,  $P < 0.001$ ; females:  $t = 4.91$ ,  $df = 12$ ,  $P < 0.001$ ; combined:  $t = 8.30$ ,  $df = 12$ ,  $P < 0.001$ ).

*et al.*<sup>25</sup>) was included in these studies. This consisted of the pheromone dissolved in ethanol in a press-seal polyethylene bag. The release rate of the pheromone from these systems has not previously been reported because the concomitant release of ethanol makes it difficult to measure by weight loss and also potentially affects the adsorptive properties of adsorbents used in dynamic headspace trapping. Here the release rate was calculated by assaying the concentration of 3-hydroxy-2-hexanone in the ethanol solution remaining by gas chromatography after various intervals of exposure and by determining the amount of ethanol remaining. Rates for the ethanol and 3-hydroxy-2-hexanone were 86 and 5.7 mg day<sup>-1</sup> respectively at 27 °C, the latter being of a similar order to that from the polyethylene sachet. It was also found that release rates from the press-seal bags containing the solutions were very erratic, probably because of difficulties in reliably sealing the bags. Impregnating the solution on a dental roll in the bag gave more consistent release rates, as reported recently by Hanks and Millar,<sup>17</sup> and the studies here showed there was no significant effect on the release rate of ethanol.

Field trapping tests carried out over two seasons at three sites in Spain failed to demonstrate any attraction of *X. arvicola* beetles to 3-hydroxy-2-hexanone, but significant numbers

of beetles were attracted to the compound in ethanol and to ethanol alone. Lack of attraction of *X. arvicola* adults to traps baited with (*R*)-3-hydroxy-2-hexanone was unexpected, considering that it is produced in large quantities by the male beetles, and that some attraction had been observed in laboratory bioassays.<sup>21</sup> This may be because other pheromone components are required, although the corresponding 2,3-hexanediols or the 8-carbon homologues could not be detected in volatile collections from males (González-Núñez M and Hall DR, unpublished data). Other, unrelated compounds may be required to synergise the male-produced compound, as reported for *Callidiellum rufipenne* (Motschulsky) (Coleoptera: Cerambycidae), the males of which also produce (*R*)-3-hydroxy-2-hexanone.<sup>28</sup> It may be that the pheromone is only attractive in the presence of key host-plant volatiles, although in other cerambycid species where the attractiveness of the pheromone is synergised by host-plant volatiles the pheromone alone shows significant attraction (e.g. Pajares *et al.*<sup>29</sup> and Collignon *et al.*<sup>30</sup>). Other examples of this lack of attractiveness of compounds produced by male cerambycid beetles, particularly 3-hydroxy-2-hexanone, have been reported recently by Hanks and Millar.<sup>17</sup>

Attraction of beetles to ethanol is by no means unprecedented, and other cerambycids have been attracted by combinations of pheromones and host-plant volatiles, including ethanol.<sup>31–34</sup> *Xylotrechus* species are reported to be attracted to stressed and weakened plants,<sup>35</sup> and it is likely that these would produce ethanol. Ethanol emissions have been shown to increase in trees after a stress event,<sup>36–39</sup> and thus some cerambycids probably use ethanol to locate stressed host trees, even in the absence of pheromone signals.<sup>33</sup>

The field experiments also confirmed that black panel traps are suitable for capture of *X. arvicola*. Rodríguez-González *et al.*<sup>40</sup> evaluated different trap types for capture of *X. arvicola* adults, and showed that the black panel trap was significantly better than the other two types of trap tested, delta and screen adhesive. Coating the panels with Fluon also improved catches, as reported by Graham *et al.*<sup>26</sup>

## 5 CONCLUSIONS

Although in previous studies females showed attraction by 3-hydroxy-2-hexanone in wind tunnel tests, to date no attraction has been demonstrated in field experiments. However, it has been shown that black panel traps baited with ethanol are highly attractive to both sexes of adults of *X. arvicola*, and these traps can be used for improved monitoring of the emergence of the adult beetles and perhaps even for controlling them.

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