Volatile blends released from plants may contain hundreds of different components. Some of them are characteristic for a plant family, but many compounds can be found in the blend that occur in many different and unrelated plants (Knudsen et al. 1993). The emission and composition of the volatile compounds emitted are dependent on internal factors of the plant, such as the phenological stage, as well as external factors including light, temperature, and humidity. There is also variation in chemistry between and even within the tissues of individual plants (Gouinguene and Turlings 2002, Dudareva et al. 2004), yet insects are able to use this information source to locate suitable host plants in the environment.

Plant volatiles play a major role in the behavioral responses that determine the performance, survival, and development of insects (Vet and Dicke 1992). Chemicals involved in conveying information in intraspecific interactions between organisms are known as semiochemicals (Howse et al. 1998). Herbivorous insects are known to use plant volatiles (kairomones) to locate host plants (Visser 1986), and the efficiency of attraction depends on the odor quality and/or the amount released (Tinzaara et al. 2002). Perception of these kairomones may provide relevant information to insects about the feeding suitability of the host or their potential as an oviposition site (Byers et al. 1989, Meiners and Hilker 2000, Hilker and Meiners 2002).

Information regarding the role of semiochemicals in interspecific interactions between the raspberry weevil Aegorhinus superciliosus Guérin (Coleoptera: Curculionidae), a native species from Chile and a serious pest in some fruit crops (Kuschel 1951, Aguilera 1995), have not been described yet. Although A. superciliosus is polyphagous, it prefers plant species belonging to the families Ericaceae and Rosaceae (Kuschel 1951, González 1989, Prado 1991). Larvae of the weevil cause severe damage to the roots of crops such as blueberry (Vaccinium corymbosum L.) and other berries (Rubus idaeus L., Ribes nigrum L., Fragaria ananassa Duch and Rubus strictureus Muell. et Lef.), being able to kill plants by consuming the below-ground vegetative parts (Kuschel 1951; Elgueta 1993; Aguilera 1988, 1995; Cisternas et al. 2000). Adult weevils are also active consumers of young leaves and blueberry fruits.
during the day, whereas at sunset, they walk down to the stems of the plant (Carrillo 1993, Aguilera 1995). The life cycle of *A. superciliosus* in southern Chile has been described by Aguilera (1988). There is much overlap between their different stages: (1) the females deposit fertilized eggs on the soil surface of crop fields (December to mid-March) when the plant is in the phenological stages of ripening blueberry; (2) larval stages develop from March to mid-December; (3) the pupal stage begins in September when the plant is in the phenological stage of full bloom and continues through early October; and (4) the adult emerges in September and copulates in January and February, depending on the temperature the copulation can begin in December.

In Chile, *A. superciliosus* is the main cause of *V. corymbosum* (Ericaceae) decline, and pesticides and biological control have not been successful in controlling this pest. Because of the problems associated with the use of synthetic insecticides, including environmental degradation, development of resistance, and residues on harvested fruit, there is a growing use of semiochemicals (ethologic control) in integrated pest management (IPM) (Getz and Gutierrez 1982, Jaffé et al. 1993). This approach has been useful for controlling some coleopteran pests (Vilela and Castro 1987, Cerda et al. 1999). The objectives of this study were to identify volatile chemicals, released from *V. corymbosum*, and to determine which seasonal phenological stage of *V. corymbosum* is attractive to *A. superciliosus*.

**Materials and Methods**

**Insects.** Male and female *A. superciliosus* adults were collected from an experimental blueberry plantation in Collipulli, La Araucanía, in southern Chile, between September 2006 and February 2007. Once transferred to the laboratory, individuals were maintained separately (males and females) in plastic boxes (10°C and 16-h light regimen) on blueberry leaves. Two hours before each behavioral assay, adult *A. superciliosus* were observed for 10 min, and those that were walking and active in that time were selected for olfactory experiments.

**Population Fluctuation of *A. superciliosus.** Adults of *A. superciliosus* were collected following the methodology proposed by Ruesink and Kogan (1982), which consisted of visual searches in a certain time using relative capture methods (Metcalf and Luckmann 1982). Field captures of weevils by hand were carried out during the seasonal emergence period, from 21 September 2006 to 8 February 2007. The number of individuals *A. superciliosus* captured was recorded daily, between 1200 and 1700 hours, and the collected weevils were taken to the laboratory to maintain the colony. An effort unit index was used to calculate the catch per unit effort (insect person/h/ha) to account for variation in the number of workers (between four and six) collecting insects (Speight et al. 1999) on multiple transects. Depending of the numbers of the workers, each one walked 2–3 ha in 5 h to cover 12 ha. A graph of the insect population fluctuation was constructed to determine the maximum emergence of *A. superciliosus*.

**Volatile Compound Trapping Procedures.** Plant volatiles were collected from an experimental blueberry plantation in Collipulli, (southern Chile), during 24 h (at 1 liter/min) by enclosing an individual branch of *V. corymbosum* cultivar Bluecrop (15–30 cm tall) in a 900-ml Pyrex glass chamber (6 cm ID and 30 cm high). Depending of the phenological stage, the branch included flowers and mature and immature fruits. Volatiles were adsorbed on 100 mg Porapak Q columns (80–100 mesh; Waters Associates), previously cleaned with 1 ml of redistilled diethyl ether (GC grade; Merck, Darmstadt, Germany), and conditioned at 150°C for 2 h in a stream of nitrogen (70 ml/min). The entrainment was performed by using a positive/negative pressure air system (Agegopoulos et al. 1999). The air was dried and purified by passage through activated 5-Å molecular sieves and then charcoal, and finally drawn through the glass chamber. Volatiles were extracted from the Porapak Q by elution with 1 ml of redistilled hexane (GC-MS grade; Optima Scientific, Darmstadt, Germany), which was concentrated to 100 μl under a flow of nitrogen. Odor sources from which collections were made included six phenological stages of blueberry: full bloom, fruit set, blue-pink, immature-mature fruits, mature fruits (stages of intermediate development), and postharvest.

**Analysis of Volatile Compounds by GC-MS.** The volatile compounds (1.0 μl) were analyzed using a gas chromatograph (model Focus; Thermo Electron, Waltham, MA) coupled to a mass spectrometer (model DSQ; Thermo Electron) equipped with a HP-Ultra 1 capillary column (25 m, 0.2 mm, 0.33 μm). Helium was used as the gas carrier, with a flow rate of 1.5 ml/min. Mass spectrum acquisition was performed in the mass range from 35 to 500 m/z. Ionization was performed by electron impact at 70 eV with an ion source at 200°C. The GC oven was programmed to remain at 40°C for 1 min and increased at 5°C/min to 260°C and held for 5 min. The interface temperature was programmed at 250°C. Volatiles were identified by comparing gas-liquid chromatography (GLC) Kovats indices (KIs) and mass spectra (MS) with the corresponding commercial standards (Sigma Aldrich, St. Louis, MO). The experimental KIs were compared with theoretical KIs from synthetic standards compounds and reported in the Pherobase database. The resulting spectra were compared with a library database by a reverse search technique (Tapia et al. 2007). Calibration curves based on peak area ratio were constructed using standards and docosane as an internal standard for quantification of each volatile compound identified from the samples of *V. corymbosum*. Standards used as a stimulus in the olfactometer were purchased from Aldrich Chemical (99% purity) and diluted in hexane before use.

**Olfactometer Bioassays.** The behavioral response of *A. superciliosus* was evaluated by testing a blueberry volatile extract, 15 synthetic compounds identified in extract (fruit set and blue pink) at four different con-
centrations (0.1, 1, 10, and 100 µg/ml), and an artificial mixture representing blueberry odor. Bioassays were performed in a four-arm olfactometer adapted from the design of Vet et al. (1983), but this type of olfactometer was enlarged (40 by 40 by 2.5 cm). The observation arena was divided into four arm zones and one indifferent zone in the center and designated the decision zone. One A. superciliosus was enclosed in an observation quadratic arena permeated by charcoal filtered air (800 ml/min) coming from each of its four stretched-out corners and drawn out through a hole above its center. Insects of both sexes were used. Olfactometric bioassays were carried out between 1000 and 1800 hours (photophase) at 22°C and 80 lux. Two lines of air coming from each stimulus treatment were connected in opposite corners of the arena; the other two lines released air as a control treatment (Tapia et al. 2007). The treatment chemicals (odors of plant extracts or synthetic compounds) were applied onto Whatman N filter paper (2 cm²) and placed into the glass tubes (10.0 cm high; 1.0 cm ID) connected to each olfactometer arm. The time that each A. superciliosus spent in each arm of the olfactometer was recorded during 20 min with the olfactometer being rotated 90° every minute to minimize directional bias. Five minutes before starting the experiment, the test specimen of A. superciliosus was placed in the olfactometer for acclimation. Each treatment was replicated 10 times. A different individual was used in each separate replicate of the experiments.

Data Analyses. The time insects spent in stimulus and control areas of the olfactometer was compared through nonparametric statistics with a Friedman test \( P \leq 0.05 \). Furthermore, an olfactometric-preference index (OPI), where \( OPI = 2T/(T+C) \) (Kogan 1972), was calculated to reflect the effect of the stimulus on the behavior of the insect. The OPI values ranged from 0 to 2, with \( OPI = 1 \) indicating no olfactometric preference for the control or treatment odor source, \( OPI > 1 \) indicating preference for treatment odor source, and \( OPI < 1 \) indicating preference for control odor source. OPI values were compared using a Kruskal-Wallis test (a nonparametric one-way analysis based on rank), and groups were separated using the Conover-Inman test \( P \leq 0.05 \) (Conover 1999).

Results

Population Fluctuation. Figure 1 shows the population fluctuation of A. superciliosus observed in Collipulli, La Araucanía, in southern Chile, from September 2006 to January 2007. The population peak was recorded on 13 November 2006, with a catch per unit effort of 2.07 insect/person/h/ha. A total of 744 A. superciliosus were captured in 12 ha during observation of blueberry fields by six workers during 5 h. All weevils were collected from the foliage of blueberry plants.

Identification of Volatile Compounds. The analysis of volatiles from V. corymbosum was focused on the blue pink and fruit set phenological stages because (1) the attack of A. superciliosus occurs during the blue pink phenological stage of the blueberry, (2) the maximum A. superciliosus population was observed during these phenological stages, and (3) the respective volatile extracts elicited the maximum attraction response from A. superciliosus (Fig. 3).

Qualitative and quantitative differences were observed between the analyzed phenological stages (Table 1). The fruit set and blue pink phenological stages released 6 and 16 different compounds, respectively. Blue pink showed a higher diversity of chemical groups than the fruit set stage, showing the presence
Table 1. Compounds released from different fruit ripening stages of highbush blueberry (V. corymbosum L.) and their olfactometric effect on A. superciliosus

<table>
<thead>
<tr>
<th>Compounds (reliability of identificationa)</th>
<th>Area (%)</th>
<th>IK exp. b</th>
<th>IK Lib. c</th>
<th>Concentration (ng/μl)</th>
<th>Biological activity on A. superciliosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenes</td>
<td></td>
<td>Fruit set</td>
<td>Blue pink</td>
<td>Fruit set</td>
<td>Blue pink</td>
</tr>
<tr>
<td>a-Pinene (1)</td>
<td></td>
<td>20.54</td>
<td>20.16</td>
<td>930</td>
<td>934</td>
</tr>
<tr>
<td>Sabinene (1)</td>
<td></td>
<td>6.39</td>
<td>2.73</td>
<td>968</td>
<td>968</td>
</tr>
<tr>
<td>β-Pinene (1)</td>
<td></td>
<td>47.45</td>
<td>8.08</td>
<td>1,015</td>
<td>1,015</td>
</tr>
<tr>
<td>Eucalyptol (1)</td>
<td></td>
<td>19.01</td>
<td>0.92</td>
<td>1,017</td>
<td>1,017</td>
</tr>
<tr>
<td>Limonene (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkanes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonane (1)</td>
<td>1.5</td>
<td>900</td>
<td>900</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Decane (1)</td>
<td>3.32</td>
<td>997</td>
<td>1,000</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Undecane (1)</td>
<td>3.28</td>
<td>1,098</td>
<td>1,100</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Dodecane (1)</td>
<td>1.67</td>
<td>1,200</td>
<td>1,200</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Carbonic compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Nonanone (1)</td>
<td>9.62</td>
<td>1,070</td>
<td>1,070</td>
<td>—</td>
<td>0.009</td>
</tr>
<tr>
<td>(Z)-3-Hexenyl butyrate (2)</td>
<td>4.37</td>
<td>1,168</td>
<td>1,168</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>4-Ethyl acetoephone (1)</td>
<td>7.29</td>
<td>1,230</td>
<td>1,230</td>
<td>—</td>
<td>0.06</td>
</tr>
<tr>
<td>Aromatic compound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Ethyl benzaldehyde (3)</td>
<td>2.44</td>
<td>1,128</td>
<td>—</td>
<td>—</td>
<td>0.01</td>
</tr>
<tr>
<td>Green leaf volatile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Z)-3-Hexen-1-ol (1)</td>
<td>17.94</td>
<td>839</td>
<td>837</td>
<td>—</td>
<td>1.42</td>
</tr>
<tr>
<td>(Z)-3-Hexenyl acetate (1)</td>
<td>17.21</td>
<td>986</td>
<td>988</td>
<td>—</td>
<td>0.19</td>
</tr>
</tbody>
</table>

a The reliability of the identification is indicated by the following letters: 1, mass spectrum, Kovats indices and standard; 2, mass spectrum and Kovats indices in agreement with corresponding data in the literature; 3, mass spectrum and standard. 
b Kovats indices experimental. 
c Kovats indices library. 
ND, not detected.

The population fluctuation exhibited by A. superciliosus adult in the south of Chile during 2006–2007 supports the findings of Aguilera (1988, 1995) that A. superciliosus has one generation per year. A. superciliosus was observed feeding on blueberry fruit and leaves during this study, and identification of the cues that attract this weevil to its host plants will contribute to the development of a monitoring system.

This study showed that volatiles released from V. corymbosum at different phenological stages have qualitative differences in the mixture of chemicals, in such a way that A. superciliosus may be able to discriminate between volatiles from each mixture.

Our olfactometer results showed that the maximum attractant response was elicited when the plant was in blue pink phenological stage, coinciding with the maximum population peak of A. superciliosus. These results are supported by Hardee et al. (1996) and Kalinova et al. (2000), who showed that different curculionid species in the genus Anthonomus were attracted to volatiles from their host plants. The adult weevils used those chemical signals for olfactory discrimination during host-searching behavior (Kalinova et al. 2000).
Our results did not show any significant behavioral difference between sexes in olfactometric bioassays when *A. superciliosus* was stimulated with volatile extracts (results not shown). This agrees with Váldes-Rodríguez et al. (2004), who has indicated that males and females of the curculionid *Scyphophorus acupunc-

![Graph showing average time spent by *A. superciliosus* in olfactometer arms with different odor sources.](image1)

**Fig. 2.** Average time spent (±SE) by *A. superciliosus* in olfactometer arms containing a clean air control or plant volatiles from different stages of *V. corymbosum* (*n* = 10). Bars within a stage with the same letter are not significantly different based on the Wilcoxon test (*P* > 0.05).

![Graph showing average OPI value of *A. superciliosus* in olfactometer tests comparing clean air and odor from different phenological stages of *V. corymbosum*.](image2)

**Fig. 3.** Average OPI value (±SE) of *A. superciliosus* in olfactometer tests comparing clean air and odor from different phenological stages of *V. corymbosum*. An OPI = 1 indicates no olfactometric preference for the control or treatment odor source. OPI > 1 indicates preference for treatment odor source, and OPI < 1 indicates preference for control odor source. Means followed by the same letter are not significantly different from each other (*P* > 0.05) based on the Kruskall-Wallis test followed by Conover-Inman test. *Significant differences between treatment and control (*P* < 0.05) based on Friedman test followed by Conover-Inman test.
Gyllenhal were similarly attracted toward volatile released from the leaves of its host-plant *Agave fourcroydes* Lem. However, some synthetic compounds elicited a different behavioral response between male and female as it occurs with *Coleomegilla maculata* (Degeer) and *Chrysoperla carnea* (Stephens) (Zhu et al. 1999).

Volatile compounds identified from *V. corymbosum* belong to five different categories: (1) green leaf volatiles, C-6 compounds arising from enzymatic transformations of linolenic and linoleic acids (Hatanaka 1993); (2) common terpenoid plant constituents; (3) alkanes commonly present in plant epicuticular waxes; (4) carbonilic compounds, such as acetates, aldehydes, and ketones; and (5) aromatic compounds. The present composition of blueberry volatiles differs from those given in an earlier report by Liburd (2004). In the earlier study, the investigator identified volatile compounds from ripening blueberries using the solid phase microextraction technique.

In our evaluations of 15 synthetic compounds and a mixture of synthetic volatiles representing blueberry,
most of the individual compounds identified were inactive in the olfactometer. The results showed that 2-nonanone, eucalyptol, 4-ethyl benzaldehyde, and R- and S-limonene elicited a significant attractant response ($P < 0.05$) from the weevil in at least one of the concentrations. The attractant activity shown by 2-nonanone coincides with the report by Wakefield et al. (2005), describing it as highly attractive for the foreign grain beetle *Ahasverus advena* (Cucujidae). Limonene has been reported as attractant for some insects (Ibrahim 2001) or as repellent (Pureswaran et al. 2004; Tapia et al. 2007). Moreover, limonene has been mentioned as a component of the aggregation pheromone of the curculionid *Conotrachelus nenuphar* (Herbst) (Leskey et al. 2001, Pinerò and Prokopy 2003) and as kairomone and component of the aggregation pheromone of scolytids (Rudinsky et al. 1977, Blight et al. 1980), and it has been applied in traps for monitoring the curculionid *Hyllobius pales* L. (Siegfried 1987). 4-Ethyl benzaldehyde has been described to enhance the attractive response on *Sitophilus granarius* L. (Curculionidae) in pitfall bioassays, which was confirmed by electroantennography (EAG) bioassays (Collins et al. 2007).

Only females of *A. superciliosus* were attracted when they were stimulated with eucalyptol at 1 $\mu$g/ml (Fig. 5). The female *A. superciliosus* may use eucalyptol as a foraging kairomone to locate suitable hosts for feeding and oviposition (Ruther et al. 2002). Eucalyptol has been described as attractant for several insects belonging to different orders such as Coleoptera, Lepidoptera, Diptera, Hymenoptera, and Thysanoptera. Ndiege et al. (1996) reported that eucalyptol was attractive to *Cosmopolites sordidus* Germar, a pest of banana cultivars, but was also toxic to *Sitophilus granarius* L. (Kordali et al. 2006).

All the compounds that elicited biological activity showed a dose-dependent effect on behavior (Fig. 4). Attractants can function as repellents when the concentration exceeds a certain threshold (Bernays and Chapman 1994). Dose-dependent response of *Musca domestica* to aggregation pheromone has also been observed, where low and intermediate doses are highly attractive, but high doses caused aversion (Jiang et al. 2002). Oviposition bioassays with pheromones components of *M. domestica* showed a dose-dependent relationship, where oviposition rates increased with dose up to a point, and thereafter decreased (Jiang et al. 2002). Several bark beetle species also showed dose-dependent response to aggregation pheromones released by conspecifics (Raffa 2001).
response over a broad range of release rates must be expected by individuals of species that breed in adjacent areas, reflecting the relative probabilities of mating opportunities and levels of interspecific competition (Byers 1989). Behavioral variation in responses observed in some experiments, e.g., 2-nonenone and S-limonene (Fig. 4), could be explained because the experiments were conducted at different times of year, resulting from seasonal shifts in insect behavior. Similar behavior was reported by Roitberg et al. (1992) for the parasitic wasp, Leptopilina heterotoma (Thomson) (Hymenoptera: Eucoilidae).

Some of the volatiles extracted from V. corymbosum volatiles could be used as an attractive source for A. superciliosus in integrated pest management programs (IPMs). For example, these volatiles could be used to monitor temporal oscillation of A. superciliosus and in aggregation or mating disruption or lure-kill strategies in association with pesticides (Rodriguez and Niemeyer 2005). Future work should aim to (1) determine the electrophysiological response of A. superciliosus to individual odors and combinations of multiple odors released by V. corymbosum and (2) develop a kairomonal lure for monitoring A. superciliosus under field conditions. This information will provide an understanding of the role of volatiles in mediating the host selection and acceptance process of the weevil Aegorhinus superciliosus and will provide an essential component of an effective IPM program for this damaging insect.

Acknowledgments

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