Antennae of *Cryptorhynchus lapathi* (Coleoptera: Curculionidae) detect two pheromone components of coniferophagous bark beetles in the stems of *Salix sitchensis* and *Salix scouleriana* (Salicaceae)

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Several scolytid beetles (Coleoptera: Scolytidae) produce conophthorin, (*E*)-7-methyl-1,6-dioxaspiro[4.5]decane, and use it as an aggregation or antiaggregation pheromone or competition-mediating synomone (Francke *et al.* 1979; Kohnle *et al.* 1992; Birgersson *et al.* 1995; Pierce *et al.* 1995; de Groot *et al.* 1998; Dallara *et al.* 2000; Rappaport *et al.* 2000). Predators or associates of these beetles may use conophthorin as a host- or habitat-finding kairomone (Kohnle *et al.* 1992). Other conifer-infesting scolytid and predator species use chalcogran, 2-ethyl-1,6-dioxaspiro[4.4]nonane, a semiochemical with functions similar to those of conophthorin (Francke *et al.* 1977; Heuer and Vité 1984; Baader 1989; Byers 1993; Byers *et al.* 1989, 2000).

Recently, conophthorin has been found in several north-temperate angiosperm trees in the genera *Betula* L. (Betulaceae), *Populus* L. (Salicaceae), *Acer* L. (Aceraceae), and *Quercus* L. (Fagaceae) (Francke *et al.* 1995; Byers *et al.* 1998; Huber *et al.* 1999). Several coniferophagous bark beetles and two woodborers, *Monochamus* Dejean spp. (Coleoptera: Cerambycidae), have been shown to be repelled by conophthorin (Huber *et al.* 1999; Zhang *et al.* 2001; Morewood *et al.* 2003; Zhang and Schlyter 2003), presumably as an avoidance response to non-hosts. Because the presence of conophthorin in angiosperm trees presumably predates its occurrence in coniferophagous scolytids, its role as a synomone in bark beetles has been postulated to be a form of chemical Batesian mimicry (Huber *et al.* 1999). Chalcogran has not been identified in angiosperm trees to date but has been found in five species of tropical orchid, once in conjunction with (*E*)- and (*Z*)-conophthorin (Kaiser 1991, 1993).

We report the discovery of both of these compounds in the stem volatiles of willow (*Salix* L. spp.; Salicaceae), as detected by antennae of the poplar and willow borer *Cryptorhynchus lapathi* (L.) (Coleoptera: Curculionidae).

Salix spp. stems approximately 3 cm in diameter were collected in mid-November 1999 and cut into approximately 25-cm lengths. They were aerated (Rudinsky 1974) for 6 days in a plastic chamber fitted with a water aspirator. Volatiles were captured on Porapak-Q (Byrne *et al.* 1975), eluted with 4 mL of distilled pentane, and subjected to gas chromatographic – electroantennographic detection analysis (Arn *et al.* 1975) using both male and female *C. lapathi* antennae. Captured volatiles (1 µL) were injected splitless into a Hewlett Packard 5890 gas chromatograph (injector port 250 °C, detector port 260 °C, temperature held for 1 min at 50 °C then increased by 10 °C/min to 280 °C) fitted with a DB-5 fused silica column (30 m × 0.32 mm i.d., J&W Scientific, Folsom, California), then passed over an antenna or a flame ionization detector. Antennally active compounds were identified by gas chromatography – mass spectrometry (Varian Saturn 2000 Ion Trap, DB-5 column as above, electron impact mode) and confirmed by coelution and mass spectra with synthetic standards.

Electroantennographic detection traces disclosed a strong antennal response to (*E*)-conophthorin and a weaker response to (*E*)- and (*Z*)-chalcogran (Fig. 1). The responses occurred repeatedly in different antennae. Conophthorin constituted 0.05% of the volatiles, and the two isomers of chalcogran (*E*- and *Z*-), 0.07% and 0.09%. Too little material was present to

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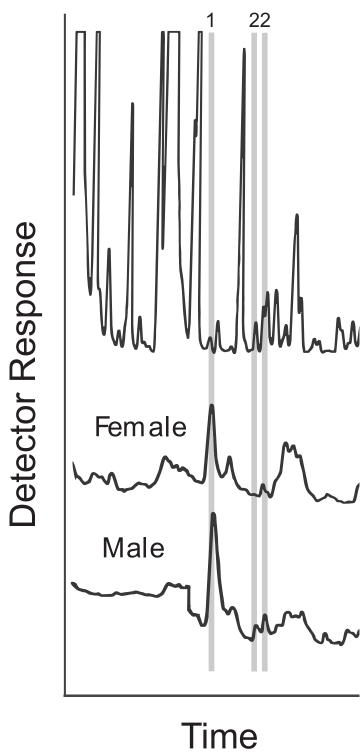
Fig. 1. Representative flame ionization detection (upper) and gas chromatographic – electroantennographic detection (lower) traces for *Salix* spp. stem volatiles against female and male *Cryptorhynchus lapathi* antennae. Identified peaks are (1) (*E*)-conophthorin and (2) (*E*)- and (*Z*)-chalcogran, respectively.



determine the enantiomeric composition of these compounds.

Because the original willow stems collected in November had no leaves and could not be identified to species, we repeated aerations in November 2004 using verified *Salix sitchensis* Sanson *ex* Bong. and *Salix scouleriana* Barratt *ex* Hook. Based on gas chromatography – mass spectrometry comparisons with authentic standards, (*E*)-conophthorin and (*E*)- and (*Z*)-chalcogran were present in both species, but not in a solvent control.

These findings extend the presence of conophthorin into a new angiosperm genus and document for the first time the occurrence of chalcogran in a dicotyledonous species. The consistent strong responses of antennae of both sexes to conophthorin, despite its being a minor volatile component, suggest that conophthorin may be behaviorally important in *C. lapathi*, as it is for numerous bark beetles.

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