Structure elucidation of semiochemicals related to: *Polygraphus poligraphus*, *Polygraphus punctifrons*, *Trioza apicalis*, *Whittleia retiella*, *Neodiprion edulicolus*, *Neodiprion scutellatus*, *Neodiprion knereri* and *Neodiprion virginianus*

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Abstract

Pest insects can have adverse and damaging impacts on agricultural production, the natural environment, and our lifestyles. They may cause problems by damaging forest and food production. To cope with these problems, many industries use pesticides. However, pesticides are detrimental for the environment and produce considerable damage to ecosystems. Pesticides can be harmful to non-target species; they pollute air, water and soil; and can also have considerable effects on natural biological equilibria. A more environmentally friendly form of pest management is thus called for, and one such alternative to pesticides is the use of semiochemicals, chemical substances that insects use for communication. Semiochemicals can be used to interfere with this communication by, for example, attracting the pests to traps to either kill or estimate the population size of the pest. By using species-specific communication, one can direct the effort towards only the insect one wants to influence. The method is very effective, which means that it can also be used for conservation purposes to investigate the occurrence of very rare and red-listed insect species.

The focus of this thesis is on purification methods, the separation of stereoisomers, analysis and identification, structure elucidation, the synthesis of identified compounds, and the evaluation of identified compounds in field trials. The thesis is composed of four parts:

The first part deals with a collection of volatile compounds emitted from different bark beetles, which are pest insects of Norway spruce (*Picea abies*), causing significant economic damage in different areas of Sweden. We followed chemicals by collecting all volatile compounds produced by male *Polygraphus poligraphus*, *Polygraphus punctifrons* and by Norway spruce using solid phase micro-extraction. The collected samples were analysed by gas chromatography and mass spectrometry. We found that single males of *P. poligraphus* emitted (−)-(*R*)-terpinen-4-ol in high optical purity (>96.3% ee) but also that the

optical purity decreases after the beetles have mated (down to 67.7% ee). In the field evaluation for *P. poligraphus*, more optically pure (−)-(*R*)-terpinen-4-ol increased the catches compared to when lower optical purity was used (50% ee). Thus, the content of (+)-(*S*)-terpinen-4-ol decreased the catches. Also the percentage of males was higher when (−)-(*R*)-terpinen-4-ol was used alone, compared to when (−)-(*R*)-terpinen-4-ol was combined with frontalin. The addition of frontalin seems to lower the proportion of males.
A combination of (+)-(1*R*, 2*S*)-grandisol and (−)-(*R*)-terpinen-4-ol was identified for the first time as the pheromone components of *P. punctifrons*. The activity of the synthetic references was verified by field assays (not pure enantiomers of grandisol). From field tests, a synergistic effect of *rac*-grandisol together with (−)-(*R*)-terpinen-4-ol was noted as the combination increased the number of catches; in contrast to the effect of *rac*-grandisol only in the trap.

The second part of the thesis is related to the separation and purification of bioactive compounds using preparative gas chromatography (Prep-GC). Purification and structure elucidation was done for an unknown bioactive compound in leaves from carrots (*Daucus carota*). The compound is active on the carrot psyllid, *Trioza apicalis* (Homoptera: Psylloidea) antennae, as recorded by GC-SSR; this species is one of the major pests for carrots in Northern Europe. The purification part of this study was done with Prep-GC, and further analysis was performed with infrared spectroscopy, gas- and liquid-chromatography coupled to mass spectrometry, and by nuclear magnetic resonance. The bioactive compound was identified as sesquisabinene B, which was confirmed by comparing with data from the literature.

The third part deals with the analysis and identification of pheromones of the rare insect salt marsh bag moth species *Whittleia retiella*. Identifying the pheromone opens up the possibility of monitoring this rare and threatened species to survey its distribution and habitat requirements in order to use appropriate conservation methods. Two compounds produced by *W. retiella* females elicited electrophysiological responses from the antennae of males. The major compound was identified as the novel (2*S*)-butan-2-yl (5*Z*)-dec-5-enoate and the activity was confirmed by field bioassay. The minor compound was suggested to be the novel propan-2-yl (5*Z*)-dec-5-enoate or propan-2-yl (4*Z*)-dec-4-enoate. The identity of this minor component needs to be confirmed by further field tests using both compounds, as only propan-2-yl (5*Z*)-dec-5-enoate was used in this work.

The final part is about pine sawflies, which are a family of insects that in some cases can be severe defoliators of pine trees. They store long chained secondary alcohols in the female body as a pheromone precursor, which is esterified to the sex pheromone when released from the body. Female extracts of eight *Neodiprion* species were purified by solid phase extraction and analysed using gas chromatography and mass spectrometry. Female extracts of *N. excitans, N. autmnalis, N. pinusrigidae* and *N. fabricii* contained no alcohol in our analysis, probably due to the small amount of extracted females. However, a combination of 3-methyl-2-pentadecanol and 3,7-dimethyl-2-pentadecanol were identified as pheromone precursors of *N. edulicolus* females. The identification was confirmed by comparing retention time and mass spectra with synthetic references. 3-methyl-2-pentadecanol and an unidentified alcohol X were isolated as pheromone precursors of *N. scutellatus* females. X was also found in the female extract of *N. knereri* and *N. virginianus* but the alcohol X is still unidentified.

Keywords: Pheromone, semiochemicals, bark beetles, pest, *Polygraphus poligraphus, Polygraphus punctifrons, Trioza apicalis, Whittleia retiella*, *Neodiprion* spp., analysis.