Summary

Throughout the whole of Europe, the genus *Aesculus* trees are annually inhabited by an invasive pest – the horse-chestnut leaf miner (*Cameraria ohridella*). As a result of the feeding of the larvae, a round dark-brown damage called mines develops, which gradually occupies an increasingly larger surface of the leaves. An important role in the interaction between the insects and the host plant is played by volatile compounds (SZAKIEL 2015). Emitted by chestnut trees, they can act as a lure or a deterrent to *C. ohridella* moth and thus affect the degree of tree colonization. In addition, the profile of the chestnut leaf volatile compounds changes as a result of feeding of the horse-chestnut larvae (JOHNE 2006 a). Another group of chemical compounds that play a crucial role in the interaction between the pest and the host plant are trypsin inhibitors with antinutritional activity. They reduce the activity of the digestive enzyme – trypsin. It competes with proteins to bind trypsin and therefore renders it unavailable to bind with proteins for the digestive process. As a result, protease inhibitors that interfere with the digestive activity indicate an antinutritional effect (KATOCH et al. 2014, GADGE et al. 2015).

The aim of this study was to compare the profile of volatile compounds emitted by *Aesculus* trees differing in susceptibility to horse-chestnut leaf miner, as well as examining whether the profile of leaf volatiles changes due to the plant colonization and pest feeding. Another aim of the study was to examine the activity of trypsin inhibitors in chestnut leaves during the growing season in connection with colonization and pest feeding.

For this purpose, a profile of volatile leaf compounds of seven chestnut specimens, differing in susceptibility to horse-chestnut varieties, was determined. Furthermore, the changes in the volatile compound profile due to leaf colonization and the onset of the feeding of the pest larvae were analyzed. The activity of trypsin inhibitors was determined as well. The *Aesculus* individuals covered in the study are the susceptible: *Ae. turbinata*, *Ae. flava*, *Ae. hippocastanum* (two genotypes: intensively damaged and less susceptible) and the resistant *Ae.* × *neglecta*, *Ae. glabra*, *Ae. parviflora*. These trees grow in the Adam Mickiewicz University Botanical Garden and at the campus of the University of Life Sciences in Poznan. In the growing season between May and September, the fully developed leaf blades were collected every 14 days over a three-year span (2014, 2015, 2016). The volatile compound profile was determined by the GC-MS method (SERMAKKANI and THANGAPANDIAN 2012), whereas the activity of trypsin inhibitors was determined by the spectrophotometric method (CASARETTO and CORCUERA 1998). In order to conduct the statistical analysis of

volatile compounds profile Andrews curve method and principal component analysis (PCA) were adopted. The results of trypsin inhibitor activity were statistically analyzed by using analysis of variance in the system with repeated measurements (KHATTREE and NAIK 2002).

The influence of air temperature on the feeding intensity of *Aesculus* trees was noticed. The greatest damage to chestnut leaves was observed in 2014, when the average air temperature was the highest of all the analyzed years. The most damaged chestnut among the specimens from the Botanical Garden was the Japanese horse-chestnut (*Ae. turbinata*). The more resistant individual of the horse-chestnut, growing on the University of Life Sciences campus in Poznan, was damaged only in the first year of the study. The qualitative and quantitative profile of the volatile compounds of resistant leaves of the resistant *Ae. glabra* and *Ae. parviflora* was similar in all years. However, the susceptible *Ae. turbinata* differed in its volatile compounds profile from the resistant chestnuts. The examined horse-chestnut trees had a similar qualitative and quantitative profile of the volatile compounds only in one growing season (2014). In the subsequent years (2015, 2016), these trees were characterized by a different profile of the volatile compounds. These differences coincided with the cease of a chestnut tree being colonized by the horse-chestnut leaf miner. As a result of the colonization and the onset of the leaf miner feeding on the leaves of all susceptible chestnuts, the leaves were found to contain other volatile compounds than those present in the undamaged leaves, before plant colonization. These compounds constituted approx. 50% of the volatile compound profile of each of the colonized specimens.

In the trees in the Botanical Garden, the lowest activity of trypsin inhibitors was found in the leaves resistant to *C. ohridella*. *Ae. glabra* and *Ae. parviflora*, and the highest in the ones susceptible to *Ae. turbinata*. An increase in the activity of these peptides was noticed in the susceptible chestnut tree after feeding in each generation of the pest. This increase resulted in a short-term inhibition of foraging and new damage from occurring. The activity of trypsin inhibitors in horse-chestnut leaves was similar. In 2014 and 2015 it was higher in the more resistant individual, whereas in 2016 in the more susceptible *Ae. hippocastanum*. The changes in the activity of trypsin inhibitors in the susceptible chestnut trees were associated with the intensity of pest feeding on the leaves.