ABSTRACT

Assessing the detoxification efficiency of insects should take into account their previous experiences, such as their history of exposure to metals or flavonoids present in their food. Variations in metal contamination, especially in areas degraded by smelting activity, can have long-term implications for their impact and accumulation in the host plants of some insect species. These species, under the influence of metals, may differ in their flavonoid content. Both allelochemicals, such as flavonoids, and metals such as cadmium and zinc, can be used to defend against herbivores. Another common stressor can be the short-term unavailability of food, which can be important in the colonization of subsequent host plants. Some of these host plants may be important crops. I chose the alfalfa ladybug *Subcoccinella vigintiquatuorpunctata* as my research subject, as it can cause damage to alfalfa (*Medicago sativa*) crops. At the beginning of the growing season, it can colonize other plants, such as the inflated catchfly (*Silene vulgaris*). Its resistant ecotypes can colonize areas with high metal contamination, such as the area around the disused Szopienice steelworks in Katowice. Alfalfa ladybugs were collected during two months. Larvae were collected in June, and adults in July, from three study sites, located 450 m, 250 m, and 50 m from the disused emitter, respectively. At the same time, shoots of the inflated catchfly were also collected from these sites. Adults were then reared for 5 days on the leaves of the inflated catchfly; some were subjected to a 1- or 2-day starvation period, or not. The remaining individuals were transferred to *M. sativa* seedlings and cultured similarly, then subjected to the same starvation periods as for the inflated catchfly. Flavonoid content was measured in the above-ground shoots of the inflated catchfly plants. The concentrations of the metals cadmium and zinc were determined in the above-ground shoots of the inflated catchfly plants and in the bodies of adult alfalfa moths.

For these sites, a gradient in metal concentrations was previously detected in the shoots of the inflated catchfly. This situation allowed for the assessment of the efficiency of defence systems primarily involved in reducing antioxidant stress in ladybugs when feeding on plants with varying metal contamination and flavonoid levels, which were transferred to the cultivated plant alfalfa, and whether they additionally responded to a short period of starvation. The concentrations of the metals cadmium and zinc were determined in the above-ground shoots of the inflated catchfly and in the bodies of adult alfalfa beetles. In the bodies of *S. vigintiquatuorpunctata* larvae and adults, the following measurements were performed: the concentration of reduced glutathione using a spectrofluorometer, and the following spectrophotometric determinations were performed: total protein concentration using the

Bradford method, glutathione S-transferase (GST) activity using CDNB, total antioxidant capacity (TAC) using the ABTS+ radical, malondialdehyde (MDA) concentration, the ratio of reduced to oxidized thiols (RSH/RSSR), and γ-glutamyl transpeptidase (GGT) activity (only in larvae). In the bodies of the adults of *S. vigintiquatuorpunctata* grown on above-ground shoots of the inflated catchfly *S. vulgaris* (from sites I and II, untreated or subjected to a 1-day and 2-day period of starvation), the following measurements were performed: the concentrations of energy substrates (carbohydrates, protein, lipids, glycogen).

A concentration gradient was confirmed when considering metal concentration in plant shoots depending on their location from the disused smelter; higher concentrations were found in plants from sites closer to the disused emitter. In the case of flavonoids, the differences were independent of the metal gradient presented. Although such differentiation did not occur in insects feeding on plants from different locations, a dominance of different antioxidant defence components was observed between ladybugs feeding on shoots of the inflated catchfly from different locations in terms of the utilization of small-molecule antioxidants such as glutathione or protein thiols. This differentiation appears to be maintained also in individuals transferred to the next host plant, alfalfa. This indicates that a prior history of exposure to food, particularly with varying metal content, may be important for the insects' subsequent detoxification efficiency.

Keywords: *Subcoccinella vigintiquatuorpunctata*, starvation, cadmium, zinc, flavonoids, detoxification