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POTENTIAL IMPROVEMENT OF *LYMANTRIA DISPAR* L. MANAGEMENT BY QUERCETIN

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Abstract – *Lymantria dispar*, a polyphagous insect pest, copes with a wide variety of host-specific allelochemicals. Glutathione S-transferases (GST) are important for catalyzing detoxification in *L. dispar*. Larval mortality, GST activity in midgut tissue and mass of *L. dispar* with different trophic adaptations (originating from two forests with a suitable host, *Quercus robur*, and an unsuitable host, *Robinia pseudoacacia*), differed after feeding on quercetin supplemented diets (2% or 5% w/w). Quercetin inhibited GST most potently in oak forest larvae that were less adapted to flavonoids in their diet. The larvicidal effect of quercetin on *L. dispar* larvae depended on the host-use history. We believe this is important in strategies for sustainable control of insect pests.

Key words: Mortality; glutathione S-transferase; larval mass.

INTRODUCTION

The gypsy moth *Lymantria dispar* is a major polyphagous pest (more than 500 host plants) in forests, agroecosystems and urban environments (orchards, parks, tree rows etc.). Since last year, large green areas (171 914 ha) have been defoliated by *L. dispar* in the east and central parts of Serbia (Srbijašume, State Enterprise for Forest Management, Serbia). Population overcrowding and forest defoliation are at Europe's doorstep. *L. dispar* is a dangerous pest with eruptive population dynamics. The most susceptible to *L. dispar* overcrowding are oak forests. Gypsy moth defoliation results in loss of growth, mortality of oak species and shifts in species composition in mixed-oak forests (McGraw et al., 1990; Vanhanen et al., 2007). *L. dispar* attack in spring, when oak leaves contain small amounts of flavonoids, including quercetin (Salminen et al., 2004). A high population density of gypsy moth induces change in leaf chemistry during defoliation, i.e. high flavonoid content leads to suppression of *L. dispar* immune response and reduces the resistance to viruses (Martemyanov et al., 2012).

One of the very few species of plant that is an unsuitable host for gypsy moths is the locust tree, *Robinia pseudoacacia*, where populations are very rare, without overcrowding and the larvae are susceptible to pathogenic viruses (Sidor and Jodal, 1983; Barbosa and Krischik, 1987). Chromatographic separation of an ethanol extract of *R. pseudoacacia* leaves resulted in high amount of quercetin isolation (Nasir et al., 2005). In order to improve the effectiveness of pest management strategy, the use of flavonoids in combination with commercial long-lasting insecticides has been suggested (Mesbah et al., 2007). The toxicity of flavonoids originates from several different modes of action, including oxygen radical formation. Quercetin is one of many bioflavonoids present in several fruits and vegetables and has already been shown to affect insects, including life history traits of *L. dispar* (Perić Mataruga et al., 2001). Glutathione S-transferases (GST) (E.C.2.5.1.18) are a group of multifunctional detoxification enzymes with the capacity to bind many hydrophobic deterrent chemicals by catalyzing the conjugation with reduced glutathione (GSH). Populations of *L. dispar* originating from oak (*Quercus* population) and locust tree forests (*Robinia* population) were examined in our experiments. A unfamiliar host plant for *L. dispar*, *R. pseudoacacia* is a widespread species in its native habitat in southeastern North America. It was introduced to Europe in 1601 (Chapman, 1935). Today, it has spread throughout western, central, eastern and southern Europe and has become a major invasive species with significant impact on native plant communities. Despite the large number of polyphagous species of *Lepidoptera* and the long period after the introduction of *R. pseudoacacia* in Europe, so far only a few native lepidopteran species have adapted to it (Kulfan, 2012). The population of *L. dispar* in the *R. pseudoacacia* forest near Bačka Palanka (used in our experiments) has survived and adapted to unsuitable trophic conditions for more than 60 years (Sidor and Jodal, 1983).

In the present study, we examined the mortality, larval weight and GST activity in the midgut tissue of *L. dispar* originating from oak and locust tree forests, after feeding on diets supplemented with different concentrations of quercetin (more than ten times higher than found in plants – 2% and 5% w/w). Midgut tissue is the most important site for detoxification in pest insects.

MATERIALS AND METHODS

Larvae were hatched from egg masses sampled from two different populations referred to as *Quercus* (an oak forest) and *Robinia* (a locust forest) populations. After hatching, the larvae were divided into six experimental groups: (i) *Quercus* larvae fed on the control diet; (ii) *Quercus* larvae that were given the same diet supplemented with quercetin – 3,3',4',5,7-pentaxydroxyflavone, (2% w/w); (iii) *Quercus* larvae fed on the quercetin supplemented diet (5% w/w); (iv) *Robinia* larvae fed on the control diet; (v) *Robinia* larvae fed on the quercetin-supplemented diet (2% w/w); and (vi) *Robinia* larvae fed on the quercetin-supplemented diet (5% w/w). Ten larvae (4th instar) were reared in Petri dishes (2 dl), at 23°C, L16:D8 regime, and killed on the third day after molting into the fourth instar. The mortality was observed daily and recorded using the formula (Abbott 1924): $P_r = (P_o - P_c \backslash 100 - P_c) \times 100$, where P_r = corrected mortality %; P_o = observed mortality %; P_c = control mortality %.

Larvae were weighed individually to the accuracy of 0.0001 g, and the average body mass was calculated for each group. After the larvae were killed, their midguts were dissected on ice and washed several times with ice-cold physiological saline for insects. The midgut peritrophic membrane with content was removed and the tissue rinsed with ice-cold physiological saline again. Midgut tissues were biochemically processed and GST activity was analyzed according to Habig et al. (1974).

Two-way ANOVA models were applied on logarithmically transformed values of traits and post-hoc multiple range test (Fisher's LSD).

RESULTS

L. dispar larval mortality was 11.4% in the *Quercus* population and 2.5% in the *Robinia* population when larvae were given 2% quercetin-supplemented diet. With 5% of quercetin in the diet, the mortality values were 39.6% in the *Quercus* population and 15.2% in the *Robinia* population (Fig. 1). Pronounced differences were observed in constitutive GST activity between the control larvae from the *Quercus* and *Robinia* populations (P<0.001). As dietary quercetin was increased, midgut GST activity gradually decreased in the larvae from both populations; there were significant differences among all groups of larvae from

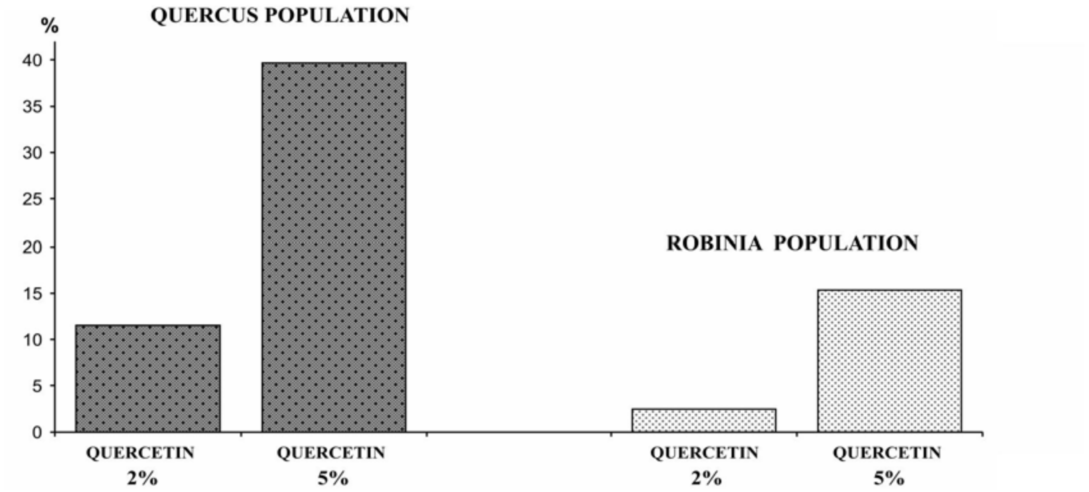

Fig. 1 - Effect of quercetin-supplemented diets (2% and 5% w/w) on the mortality of *L. dispar* larvae, n = 300.

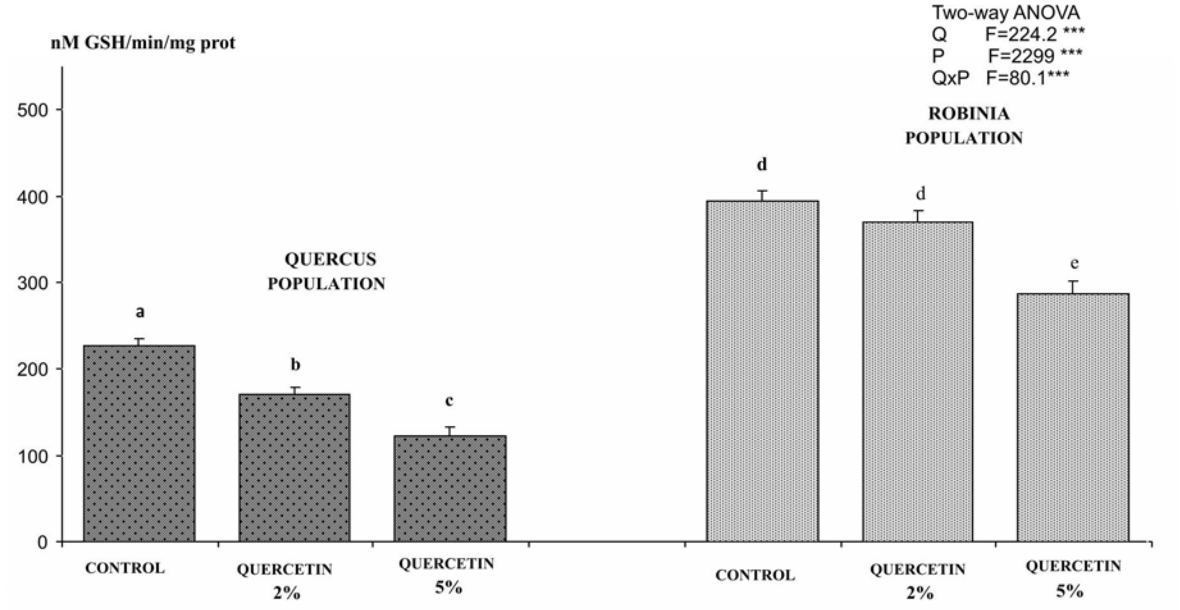

Fig. 2 - Effect of dietary quercetin (2% and 5% w/w) on GST activity in midgut tissue of the 4th instar *L. dispar*. The bars represent the means (±SE), n = 50. The statistical significance of population origin, quercetin concentration and their interaction were tested by two-way ANOVA (F values are presented), and post hoc Fisher's multiple range test; the means marked with different letters are significantly different at P<0.001***.

the *Quercus* population, while a significant difference was recorded between the control and the group fed on the 5% quercetin supplemented diet in the *Robinia* population (Fig. 2). Two-way ANOVA confirmed the the effect of population origin, concentration of quercetin and strong interaction between both factors (P<0.001).

The quercetin treatment induced a decrease in larval masses in both populations of *L. dispar*, ex-

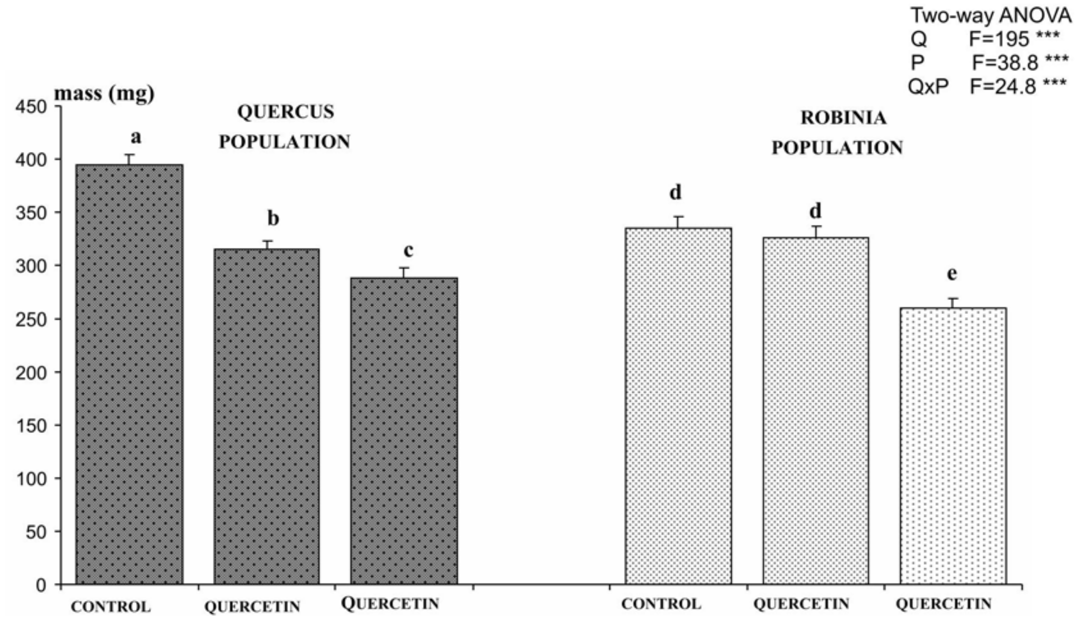

Fig. 2 - Effect of dietary quercetin (2% and 5% w/w) on mass of the 4th instar *L. dispar*. All abbreviations are the same as for Fig. 2.

cept on the control and the group of larvae fed on 2% quercetin-supplemented diet in the *Robinia* population (Fig. 3). This indicates less susceptibility to quercetin treatment in larvae originating from the locust tree forest. Two-way ANOVA confirmed significant interaction between quercetin treatment and population origin, and considerable effects of population origin and quercetin concentration separately (P<0.001).

DISCUSSION

Our previous investigations on host-associated *L. dispar* populations from oak and locust tree forests revealed local differentiation among physiological, behavioral and fitness traits (Perić Mataruga et al., 1994; Mrdaković et al., 2013). The ubiquitous flavonoid quercetin is an example of a prooxidant plant flavonoid. Sivori et al. (1999) also found that quercetin was recognized as toxic to *Triatoma infestans* by inhibiting GST. Our studies revealed differences in the susceptibility to quercetin between two locally adapted populations of gypsy moth. Thus, mortality was higher in the *Quercus* population than in the *Robinia* population of *L. dispar* at both quercetin concentrations. We previously found that *Robinia* larvae began to die with a higher concentration of quercetin (1% w/w) and in later instars than *Quercus* larvae (Perić Mataruga et al., 2001). The susceptibility of the gypsy moth to the prooxidative effects of quercetin depends on a local population's adaptation to food containing flavonoids (Perić Mataruga et al., 1997; Pritsos et al., 1988). The higher constitutive GST activity in the *Robinia* midgut tissue than in the *Quercus* group could explain the greater potential of the gypsy moth from the *Robinia* population to survive higher dietary quercetin concentrations compared to the *Quercus* larvae. Thus, GST activity in the *Robinia* population decreased significantly only with 5% quercetin-supplemented diet, whereas GST activity in the *Quercus* population declined significantly at both concentrations of quercetin. It is possible that this was one of the main reasons for the larvicidal effects of quercetin. In addition, maintaining gut conditions and synthesizing the enzymes necessary to minimize the negative effects of toxic substances reallocates resources to energy metabolism and consequently may lead to decreased larval mass and greater mortality. Mass was significantly lower in the *Quercus* population fed on both quercetin concentrations. However, larvae masses in the *Robinia* population decreased only at higher quercetin concentration.

As an inhibitor of GST, quercetin might suitably be included in IPM (Integrated Pest Management) programs aimed at preventing or postponing resistance in serious pest insects like the *L. dispar*, in comparison to currently used insecticides. Quercetin is particularly interesting because it is safe for other organisms such as vertebrates including humans. The main commercial insecticides for *L. dispar* are based on microbial toxins. As quercetin showed an immunosuppressive effect in *L. dispar* (Martemyanov et al., 2012), it might be useful in synergistic combination with insecticides used in pest management. We also suggest that this would be an eco-friendly improvement that would address the trophic adaptations of local insect populations in insect pest management strategies and could enhance pest management efficiency.

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