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RESPONSE OF THE ANTIOXIDATIVE DEFENCE TO FLAVONOID QUERCETIN IN TWO POPULATIONS OF *LYMANTRIA DISPAR* L.

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Lymantria dispar L. a polyphagous herbivore is the most dangerous insect pest of forest and fruit trees. Its host range is estimated at more than 500 plant species from 73 families (Lance, 1983, Liebhold et al., 1995). The locust tree *Robinia pseudoacaccia* is a plant that the gypsy moth avoids as a food (Barbosa and Krischik, 1987). Locust tree leaves contain large quantities of alkaloids and flavonoids (Bagrbosa & Krischik 1987). Some of them may have toxic and prooxidant effects (Hodnick et al., 1986). Gypsy moth populations in locust tree forest are rare (Jankovic 1958). The ingestion of oxidizable flavonoids can exacerbate oxidative stress in herbivorous insects (Ahmad, 1992; Felton and Summers, 1995; Pardini, 1995). The flavonoid quercetin used in this experiment was chosen as test prooxidant plant allelochemical. Upon insect ingestion quercetin is metabollicaly activated by one-electron oxidation to a free radical (o-semiquinone) which in turn reacts with O_2 (oxygen) to generate $O_2^{\cdot-}$ (superoxide anion radical) and consequently H_2O_2 (hydrogen peroxide) and OH (hydroxyl radical) resulting in numerous destructive reactions in insect cell (Hodnick et al.,1986; Hodnick et al.,1989).

The cellular antioxidative defense of herbivorous insects includes the enzymes (superoxide dismutase-SOD, catalase-CAT, glutathione-S-transferase-GST, glutathione reductase GR, ascorbat peroxidase and dehydroascorbate reductase) and antioxidants (e.g. ascorbic acid, glutathione and α-tocopherol) that protect cells from oxidative stress (Ahmad 1992; Felton and Summers 1995; Pardini 1995). Considering that the gypsy moth population is present in the Bagremara (our experimental population) for more than 50 years (Sidor & Jodal, 1983), it is to a certain extent adapted to a locust-tree leaves diet (Peric-Mataruga et al., 1997; Lazarevic et al., 2002). Our previous results have shown that locust tree leaf diet lead to on increase in GST and SOD activities and GSH content as well as to a decrease in CAT activity in the midgut tissue. Fifty-year adaptation of the gypsy moth population to the unfavourable host plant in the locust tree forest have resulted in the changes of antioxidative defence (Peric-

Mataruga et al., 1997). The adaptive changes of the constitutive expressi of the activities of antioxidative enzymes of the pest insects are very important component of their susceptibilty to insecticides (Gordon 1961).

The aim of this research was investigating the effects of artificial diet supplemented with the flavonoid quercetin (1.5%w/w) on the level of midgut tissue antioxidative defence: the activity of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione reductase (GR) and glutathione content (GSH) in the 4th instar of the gypsy moth originating from oak and locust tree forest.

Materials and Methods

Egg masses of *Lymantria dispar L.* were collected from two localities (oak forest – "Bogovadja", locust-tree forest – "Bagremara"). During the winter the egg masses were kept at 4°C until May when they were transferred to a constant temperature of 23°C to hatch. After hatching the gypsy moth caterpillars were divided into the following four experimental groups:

- OC- caterpillars from the oak forest fed arteficial diet without quercetin
- OQ- caterpillars from the oak forest fed arteficial diet supplemented with quercetin (1.5% w/w).
- LC- caterpillars from the locust-tree forest fed arteficial diet without quercetin
- LQ- caterpillars from the locust-tree forest fed arteficial diet supplemented with quercetin (1.5% w/w).

The caterpillars (4th instar) were reared in plastic containers (2dl) at 23 °C and fed standard artificial diet for gypsy moth (O' Dell et al., 1985) with or without quercetin (3,3',4',5,7-pentaxydroxyflavone, Sigma Chemicals Co., St Louis, Misouri). After the caterpillars were sacrificed the midguts were dissected on ice, washed several times with ice-cold physiological saline solution (0.9 % NaCl), midgut peritrophic membrane with content was removed and midgut were rinsed again with ice-cold physiological saline solution again. Midguts of 7-10 larvae were pooled by weight and homogenized in 0.25 M sucrose, 0.05 M Tris-HCl, 1mM EDTA pH=7.4 buffer (1:10 w/v) according to Rossi et al. (1983), and sonicated according to Takeda et al. (1982). For determination of the total amount of glutathione, part of the sonicated homogenate used to precipitate proteins with 5% sulpho-salicylic acid and the total amount of glutathione was measured after centrifugation at 5000 rpm for 10 min. The rest of the sonicated homogenate was centrifuged at 10500g for 90 min and the activities of SOD, CAT, GST and GR were determined in the supernatant.

SOD activity was determined according to Misra and Fridovich (1972). The method includes monitoring the degree of inhibition of adrenaline autooxidation in an alkaline medium in the presence of SOD. The enzyme unit was defined as the amount of enzyme inhibiting 50% of the control reaction and was expressed per mg protein.

CAT activity was determined by monitoring spectrophotometrically the degradation of a standard concentration of hydrogen-peroxide (Beutler, 1982) and was expressed as nmol H_2O_2 /min /mg protein.

Habig's method (Habig et al., 1974) was used for determining GST activity. The unit is defined as nmol GSH /min/ mg protein.

GR activity was measured according to Glatzle et al., 1974, by monitoring spectrophotometrically changes of the amount of NADPH consumed for the reduction of a standard amount of oxidized glutathione (GSSG). The activity was expressed as nmol NADPH /min/ mg protein. All enzyme assays were performed at 30°C. The total amount of glutathione both oxidized and reduced was measured according to Griffith 1980 and was expressed per g wet midgut mass.

The statistical significance of the results was estimated by analysis of variance (Sokal and Rohlf, 1981).

Results

Activity of the superoxide dismutase in the midgut tissue of the larvae fed artificial diet supplemented with quercetin (OQ and LQ) was higher than in the control groups (OC and LC) (Table 1). Two way ANOVA confirmed significant effect of quercetin in a diet for the SOD activity (Table 2). This difference was more expressed in a oak population which were more sensitive to nutritional stress. SOD activity was higher in a control group from locust tree (LC) than in a control group from oak population (CO) (Table 1).

An artificial diet with quercetin was associated with a decrease of the gluathione-S-transferase activity regardless of the population origine (Table 1.). Two-way ANOVA revealed significant population and significant host-plant effects of the diet suplemented with quercetin on the GST activity in the midgut tissue of the gypsy moth caterpillars (Table 2.). The effect is more pronounced in oak adapted than in locust tree adapted population. Both populations showed the trend of elevated total amount of glutathione in the midgut tissue as a response to quercetin supplemented arteficial diet (but with no significance) (Table 1.).

Quercetin in the arteficial diet did not change activity of the catalase and glutathione reductase in the midgut tissue of the larvae of bouth populations.

Table 1. Activity of antioxidative defence enzymes and amount of glutathione in the midgut tissue of 4th instar gypsy moth larvae originating from different populations and fed artificial diet supplemented with quercetin (1.5% w/w quercetin)

OC- caterpillars from the oak forest fed arteficial diet without quercetin
OQ- caterpillars from the oak forest fed arteficial diet supplemented with quercetin (1.5% w/w)
LC- caterpillars from the locust-tree forest fed arteficial diet without quercetin
LQ- caterpillars from the locust-tree forest fed arteficial diet supplemented with quercetin (1.5% w/w)

	OC	OQ	LC	LQ
SOD	10.77 ± 1.46	21.39 ± 1.78	16.01 ± 1.21	21.37 ± 1.49
CAT	88.9 ± 25.8	120.17 ± 20.0	99.91 ± 13.3	84.67 ± 1.65
GST	29.77 ± 2.17	17.62 ± 4.26	13.17 ± 3.65	10.46 ± 0.6
GR	1.35 ± 0.275	1.79 ± 0.29	1.73 ± 0.625	1.45 ± 0.425
GSH	0.83 ± 0.08	0.93 ± 0.12	0.73 ± 0.068	0.95 ± 0.065

Discussion

Due to its poliphagous nature, the gypsy moth is exposed to a variety of allelochemicals, some of which has prooxidant effect. The preference of gypsy moth caterpillars for host plants correlates negatively with the presence of flavonoids and alkaloids (Barbosa and Krischik 1987).

Upon insect ingestion quercetin can be metabolically activated by one-electron reduction to generate free radical species, which can further react with molecular oxygen to form the oxygen radical, superoxide (Hodnick et al., 1989).

Our results show that both oak and locust tree caterpillars fed on diet supplemented with quercetin have higher SOD activity than control groups (Table 1.). SOD is an antioxidative enzyme that catalyzes the dismutation of superoxide radical to hydrogen peroxide (Fridovich 1978). It is intersting that SOD activity in the midgut tissue was higher in a control group from locust tree population than in a control group from oak forest (Table 1). Superoxide dismutase is one of the most important components of the antioxidative defence against prooxidant effects of quercetin (Pritsos et al., 1988). This high constitutive SOD activity explains a potential of the gypsy

Table 2. The two-way analysis of variance (ANOVA) for the impact of population origin
– P and types of diet (artificial diet and artificial diet supplemented with quercetin)
– D on the levels of components of antioxidative defence in the midgut tissue of 4th instar gypsy moth

		P	D	PxH	Error
	df	1	1	1	17
SOD	MS	0.027	0.29	0.026	0.0093
	F	2.9	31.06 ***	2.84	
	df	1	1	1	17
CAT	MS	0.0017	0.0091	0.0855	0.043
	F	0.038	0.21	1.96	
	df	1	1	1	14
GST	MS	0.17	0.24	0.02	0.0111
	F	15.69***	21.37***	1.84	
	df	1	1	1	14
GR	MS	0.017	0.00027	0.0126	0.042
	F	0.401	0.0064	0.3	
	df	1	1	1	17
GSH	MS	0.0011	0.029	0.0083	0.0108
	F	0.105	2.712	0.767	

*P<0.05; **P<0.01; ***P<0.001;

moth population from the locust forest to survive at higher quercetin concentration in the artificial diet than oak population (Peric-Mataruga et al., 2001). As the gypsy moth had inhabited the locust tree forest for more than fifty years it is likely that a trophic process occured which is also manifested in the rise in SOD activity. The dismutation reaction catalysed by SOD results in the production of toxic H_2O_2 (Fridovich 1978). H_2O_2 is scavenged by another antioxidant enzyme catalase (Ahmad et al., 1987). Since there was no changes in CAT activity in the midgut of the treated groups of the gypsy moth, toxic effect of quercetine can be attributed to H_2O_2 mediated effect.

Our results showed that glutathion-S-transferase activity decrease if caterpillars from both populations fed diet supplemented with quercetin (Table 1). In insects GST is inportant in metabolic detoxification of

insecticides (Yu 1996), of allelochemicals from host plants (Yu 1993), protect insects from the toxic effects of active oxygen species (Parkes et al., 1993, Zaman et al. 1994, Hodnick et al., 1996) and for the turning on the detoxifying enzymes enhancing the defense machinery, speeding the development of resistance to insecticides (Hinkle et al, 1995, Carlini et al., 1995). The natural flavonoids such as quercetin and gossypol are capable of inhibiting GST (Wood et al., 1990). That is one more fact that can explaine toxic effect of quercetin on the gypsy moth larvae.

Our results show the trend of increas in the amount of GSH in the midgut tissue of the caterpillars which were fed diet suplemented with quercetin (Table 1). Reduced glutathione can also react passively as an antioxidant and can restitute enzymes by reduction of oxidized SH groups (Jocelson, 1962).

It is well known that feeding on certain host plants can alter the susceptibility of the herbivore to insecticides (Berry et al., 1980). The herbivorous insects metabolize and detoxify insecticides using the same enzymes that are involved in the metabolism of ingested plant allelochemicals (Brattsten 1979). Induction of a detoxification and antioxidative enzyme system as a result of feeding on particular host plants can alter to susceptibility to insecticides (Berry et al., 1980).

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Summary

RESPONSE OF THE ANTIOXIDATIVE DEFENCE TO FLAVONOID QUERCETIN IN TWO POPULATIONS OF *LYMANTRIA DISPAR* L.

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The gypsy moth caterpillars used in this experiment were originating from two populations (oak and locust tree forest) which were differently adapted to toxic effects of quercetin supplemented in artificial diet. The responses of 4th instar *Lymantria dispar* L. to artificial diet with quercetin (1.5% w/w) were monitored at the level of antioxidative defence in the midgut tissue: the activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione reductase (GR) and glutathione content (GSH). Regardless of population origin activity of SOD was higher in the caterpillars if fed diet with quercetin than in a control group. In average SOD and CAT activities were higher in the population from locust tree forest than oak forest population. An artificial diet with quercetin led to a decrease of GST activity in both populations. The diet with quercetin did not affect activity of CAT and GR.