Effect of Host Plants and Dietary Quercetin on Antioxidant Enzymes in Onion and Seedcorn Maggots, *Delia antiqua*¹ and *D. platura* (Diptera: Anthomyiidae)²

Yukio ISHIKAWA and Takashi KUBOTA

*Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

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Effect of host plants and dietary quercetin on antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPOX), was studied in the onion and seedcorn maggots, *Delia antiqua* and *D. platura*. SOD activity of the latter species was 1.7 times that of the former when the two species were grown on a synthetic diet devoid of plant secondary compounds. SOD activities of the two species feeding on respective host plants were 100-230% greater than those of larvae feeding on the synthetic diet. Synthetic diet containing up to 0.1% of quercetin, a prooxidant chemical present in onion bulbs, elicited no increase in SOD activity, though SOD grew ca. 2.5 times in both species when the concentration was raised to 1.0%. CAT levels were very high, being unaffected by the diet or the addition of quercetin in both species. Strong inhibition of GPOX was observed in onion maggots reared on onion and the synthetic diet containing 0.1% quercetin.

*Key words*: Delia antiqua, Delia platura, antioxidant enzymes, superoxide dismutase, insect-plant relationships

INTRODUCTION

Acquisition of aerobic metabolism was crucial for organisms on the earth, since the production of energy (as a form of ATP) was much more efficient than anaerobic metabolism. On the other hand, aerobic organisms must cope with toxicity of reactive oxygen species, i.e., O₂⁻, H₂O₂, H₂O₂ and ^1^O₂ (singlet oxygen), which lead to enzyme inactivation, DNA damage, lipid peroxidation etc. (HALLIWELL and GUTTERIDGE, 1985). All aerobic organisms thus developed antioxidants such as tocopherols, ascorbic acid and reduced glutathione (GSH), and antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPOX). The enzymes catalyse the following reactions:

SOD : \[2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2\]

CAT : \[2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2\]

GPOX : \[\text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{GSSG}\]

where \(\text{O}_2^-\), ROOH and GSSG indicate superoxide anion, organic hydroperoxide (or \(\text{H}_2\text{O}_2\)) and oxidized glutathione, respectively.

¹ *Delia* = *Hylophila*
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Recently, a number of plant secondary compounds have been shown to produce toxic oxygen species upon photoactivation (Joshi and Pathak, 1983) or through metabolism by diverting the electron flow to molecular oxygen (Hassan and Fridovich, 1979; Hodnick et al., 1986). SOD was induced by administration of redox active compounds in a bacterium, *Eschericia coli*, responding to the intracellular increase of O$_2^-$ which was produced by the latter mechanism (Hassan and Fridovich, 1979).

In the present study, SOD, CAT and GPOX activities of the onion and seedcorn maggots, *Delia antiqua* and *D. platura*, were investigated 1) to examine the effect of the host plant and its secondary compounds on antioxidant enzymes, 2) to compare the activities between a specialist feeder (onion maggot) and a generalist feeder (seedcorn maggot), and 3) to evaluate the effect of a prooxidant quercetin, known to be present in onion bulbs, on the antioxidant enzymes.

**MATERIALS AND METHODS**

*Chemicals.* Superoxide dismutase (SOD, from bovine erythrocytes), xanthine oxidase (XO), glutathione reductase (GR) and catalase (CAT) were obtained from Sigma. Nitroblue tetrazolium (NBT), diethylenetriamine pentaacetic acid (DETPAC), 1-chloro-2,4-dinitrobenzene (CDNB) and cumene hydroperoxide (CHP) were purchased from Nacalai Tesque. Nicotinamide adenine dinucleotide phosphate (NADPH) and xanthine were products of Khojin. Other chemicals used were commercial products of the highest purity available.

*Rearing of larvae.* To prevent exposure of larvae to plant secondary compounds, onion and seedcorn maggots were aseptically grown on a synthetic diet originally developed for *Drosophila melanogaster* (Erk and Sang, 1966). Newly laid eggs were disinfected by immersing in a 10% formalin solution for 1 hr, washed with sterile water and inoculated (20–30 eggs each) aseptically onto 10 ml of diet which had been prepared and autoclaved in 50 ml Erlenmyer flasks with silicon rubber caps. Almost all the eggs hatched a day after inoculation, and the larvae were allowed to grow for 7 more days at 23°C under 16L–8D. For comparison, the two species were reared conventionally on onion bulbs or soybeans, according to Matsumoto and Thorsteinson (1967) and Matsumoto and Sugiyama (1967), respectively. Larvae were also reared on another artificial diet mainly consisting of commercial rabbit food and defatted soybean flour (Ishikawa et al., 1983).

*Enzyme source.* About 80 mg of 3rd instar larvae of the two species were homogenized in 1 ml of 50 mm potassium phosphate buffer, pH 7.0 with a glass-class homogenizer. The homogenate was centrifuged at 16,000×g for 20 min, and the supernatant under a thin lipid layer was collected. This preparation was used for SOD, GPOX and GST measurement. For CAT extraction, a non-ionic detergent, Triton X-100 was added to the homogenizing buffer at 1%.

*Superoxide dismutase (SOD) activity.* SOD activity was measured by the method of Oberley and Spitz (1984), and expressed in units g protein as defined by McCord and Fridovich (1969). In brief, xanthine and xanthine oxidase were used to generate O$_2^-$ at a constant rate, which immediately reacted with NBT to form a compound having absorption maximum at 560 nm. The percentage inhibition of absorbance change caused by SOD was converted to units using a calibration curve constructed with bovine SOD as the standard. The final assay mixture contained 1 mm DETAPAC,
1 unit CAT, 56 μM NBT, 0.153 mM xanthine and buffered with 50 mM potassium phosphate at pH 7.0. Amount of xanthine oxidase was adjusted to obtain absorbance change of 0.020–0.025/min at 25°C in the absence of SOD.

Protein assay. Protein concentration was determined by a method modified from LOWRY et al. (PETERSON, 1977) using bovine serum albumin as the standard. Photometric measurements were made on a UV-visible recording spectrophotometer (Shimadzu, UV-160A).

Activity staining of superoxide dismutase on polyacrylamide gels. Samples of 16,000×g supernatant of the crude homogenate each containing 100 μg protein were subjected to 7% polyacrylamide gel disc-electrophoresis, and stained for SOD activity by the method of BRAUCHAMP and FRIDOVICH (1971). The gels were stained until maximum contrast between violet background and colorless bands showing SOD activity was reached.

Catalase activity. Catalase activity was measured by the method of AEBI (1984). Decomposition of H2O2 was monitored as a decrease of absorbance at 240 nm (ε240 = 0.0394 μM⁻¹ cm⁻¹). Assay was done in a 50 mM potassium phosphate buffer, pH 7.0 and initial concentration of H2O2 was adjusted to give about 0.5 optical units. One unit of CAT was defined as the amount that decomposed 1 μmol H2O2/min at pH 7.0 and 25°C.

Glutathione peroxidase (GPOX) and glutathione S-transferase activities (GST). GPOX activity was measured by the third method of WENDEL (1981) with cumene hydroperoxide as substrate. GSSG produced by GPOX was reduced by exogenous GR with concomitant consumption of NADPH, the decrease of which was monitored at 366 nm. One unit of GPOX was defined as the amount that catalysed the absorbance change of 0.001/min.

GST activity was measured at 25°C by using 44 mM sodium phosphate buffer, pH 6.5 with CDNB as substrate (KUBOTA and ISHIKAWA, 1990). One unit of GST was defined as the amount that formed 1 μmol/min of GSH-CDNB conjugate under the conditions described above.

Estimation of quercetin concentration in onion bulbs and soybeans. Methanol extracts from onion bulb (150 g) and soybeans (40 g) were made conventionally. The extracts were applied to silica gel TLC plates and developed with a mixture of benzene–pyridine–formic acid (72: 18: 10). Spots of quercetin (Rf 0.24) were scanned with a chromatodensitometer (Shimadzu, CS-910) at 315 nm, and quantified using pure quercetin as the standard.

RESULTS

Growth of the onion and seedcorn maggots on synthetic diet

Both onion and seedcorn maggots grew normally and pupated on the synthetic diet under aseptic conditions, though the pupation rate was somewhat reduced in the seedcorn maggot (Table 1). Larval period and weight and the size of pupae obtained were comparable to those reared on a conventional artificial diet, on which stock cultures had been maintained in our laboratory for several years. The growth of onion maggots was also comparable to those reared on onion bulbs (Table 1).
Table 1. Growth of *Delia antiqua* and *D. platura* on a synthetic diet,a a conventional artificial dietb and onion bulbs

<table>
<thead>
<tr>
<th></th>
<th>Onion maggot</th>
<th>Seedcorn maggot</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Synthetic</td>
<td>Conventional</td>
</tr>
<tr>
<td></td>
<td>dietc</td>
<td>Artificial</td>
</tr>
<tr>
<td></td>
<td>Onion</td>
<td>bulbs</td>
</tr>
<tr>
<td>Pupation rate (%)</td>
<td>66.7</td>
<td>66.4</td>
</tr>
<tr>
<td>Pupal weight (mg)</td>
<td>12.2±1.2</td>
<td>12.0±2.1</td>
</tr>
<tr>
<td>Pupal length (mm)</td>
<td>6.1±0.4</td>
<td>6.1±0.4</td>
</tr>
<tr>
<td>Pupal width (mm)</td>
<td>2.0±0.1</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>Larval period (days)</td>
<td>12.7±1.4</td>
<td>11.5±1.3</td>
</tr>
</tbody>
</table>

a A holidic diet developed for *Drosophila* (Erk and Sang, 1966). Larvae were reared aseptically.
b An oligidic diet developed for *Delia* stock cultures.
c From Ishikawa et al. (1983) except for larval period.

Table 2. Activities (units·mg⁻¹ protein) of antioxidant enzymes and glutathione S-transferase in *D. antiqua*b

<table>
<thead>
<tr>
<th>Dietb</th>
<th>Superoxide dismutase</th>
<th>Catalase</th>
<th>Glutathione peroxidasec</th>
<th>Glutathione S-transferasec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onion</td>
<td>4.90±0.73c</td>
<td>185.0±49.5a</td>
<td>5.7±2.1a</td>
<td>0.23±0.04b</td>
</tr>
<tr>
<td>Synthetic</td>
<td>1.48±0.15a</td>
<td>159.1±14.5a</td>
<td>11.6±3.2b</td>
<td>0.32±0.13c</td>
</tr>
<tr>
<td>+0.01 % quercetin</td>
<td>1.22±0.18a</td>
<td>170.6±19.1a</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>+0.1 % quercetin</td>
<td>1.37±0.20a</td>
<td>173.6±19.8a</td>
<td>5.6±0.9a</td>
<td>0.12±0.02a</td>
</tr>
<tr>
<td>+1.0 % quercetin</td>
<td>3.42±0.58b</td>
<td>151.5±18.4a</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>+onion MeOH extract</td>
<td>2.44±0.64b</td>
<td>178.2±31.2a</td>
<td>6.2±1.5a</td>
<td>0.09±0.01a</td>
</tr>
</tbody>
</table>

a Refer to the text for the definition of unit for each activity. Means±S.D. from two experiments each with three replicates. --- not determined.
Means in the same column followed by the same letter are not significantly different by Duncan's multiple range test at 5% level.
b Reared aseptically except for onion.
c GPOX activity toward CHP. GST activity toward CDNB.

Table 3. Activities (units·mg⁻¹ protein) of superoxide dismutase and catalase in *D. platura*b

<table>
<thead>
<tr>
<th>Dietb</th>
<th>Superoxide dismutase</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean seeds</td>
<td>5.00±1.29b</td>
<td>277.9±41.9a</td>
</tr>
<tr>
<td>Synthetic</td>
<td>2.48±0.53a</td>
<td>248.2±42.6a</td>
</tr>
<tr>
<td>+0.01 % quercetin</td>
<td>2.17±0.54a</td>
<td>217.8±51.8a</td>
</tr>
<tr>
<td>+0.1 % quercetin</td>
<td>2.70±0.69a</td>
<td>204.8±23.6a</td>
</tr>
<tr>
<td>+1.0 % quercetin</td>
<td>6.13±1.40c</td>
<td>228.4±41.1a</td>
</tr>
</tbody>
</table>

a See Table 2 footnotes.
b Reared aseptically except for soybean seeds.
SOD activities of the maggots reared on synthetic diet and on host plants

SOD activity of the seedcorn maggot was 1.7 times that of the onion maggot when both were reared on the synthetic diet. When the two species were grown on their natural host plants (sprouting soybeans and onion bulbs, respectively), SOD activities were 100% and 230% greater than those of the counterparts reared on the synthetic diet, respectively, reaching a similar level (Tables 2 and 3). Increase of SOD activities is indicative of intracellular increase of O$_2^-$ and suggests that the host plants impose oxidative stress on these maggots.

SOD activity staining of gels

Figure 1 clearly shows that SOD activities of the two host-reared maggots are higher than respective counterparts reared on the synthetic diet. It is also confirmed that basal SOD activity is higher in the seedcorn maggot than in the onion maggot (Fig. 1-b, f). Furthermore, inhibition of SOD activity by 1 mM cyanide suggests that this SOD activity is due to cytosolic Cu/Zn SOD rather than mitochondrial Mn SOD (Halliwell and Gutteridge, 1985). In the onion-reared onion maggot, two additional faint bands were observed (Fig. 1-d). Whether these are newly induced SOD isozymes or mere artifacts produced by limited proteolysis of the main SOD remains unknown.

Effect of onion methanol extract on SOD activities

To test whether the cause of increase in SOD activity in the onion-reared larvae is the presence of plant secondary compounds, methanol extract of onion bulbs was incorporated into the synthetic diet. The synthetic diet containing the extract at a concentration equivalent to onion tissue elevated SOD activity of onion maggot significantly, but it was still about half of that of the onion-reared counterpart (Table 2). These values, however, cannot be compared directly since onion-reared larvae were grown non-aseptically and thus may have been under stress from microbial products.

![Superoxide dismutase (SOD) activities of onion maggot (a-d) and seedcorn maggot (e-h) reared on synthetic diet (S) and their host plants, onion bulb, Allium cepa (A) and soybean, Glycine max (G). CN^-: 1 mM potassium cyanide. Arrows indicate the positions of SOD.](image-url)
Effect of quercetin on SOD activities

Concentration of free quercetin in the whole onion bulb (variety: Sapporo-ki) was estimated as 0.01% (wt./fresh wt.). Free quercetin was not detected in the soybean seed extract. Effect of this compound on antioxidant enzymes was tested since it is known as a prooxidant, a compound that produces reactive oxygen species when ingested by organisms (Hodonick et al., 1986). As shown in Table 2, addition of up to 0.1% quercetin did not show any significant increase of SOD activity in the onion maggot. Addition of 1.0% quercetin, however, resulted in 2.3-fold increase. Similar results were obtained for the seedcorn maggot: no effect up to 0.1%, and 2.5-fold increase at 1.0% (Table 3).

Catalase activity

Host plants or quercetin had no effect on CAT activity: CAT activities in all treatments in each species were at a similar level. CAT activities, however, were about 1.5-fold greater in the seedcorn maggot than in the onion maggot (Tables 2 and 3).

Glutathione peroxidase and glutathione S-transferase activities of the onion maggot

The GPOX activities in the onion maggot were strongly inhibited in the larvae reared on onion and the synthetic diet with methanol extract of onion bulbs. Similar level of inhibition was caused by dietary 0.1% quercetin (Table 2). GST activities were measured additionally because there is evidence that GPOX activity is largely contributed by GST isozyme(s) in this species (Kubota and Ishikawa, 1990). GST activities were inhibited to a similar extent as GPOX in the onion maggots reared on onion, synthetic diet with onion methanol extract and the diet with 0.1% quercetin (Table 2).

Effect of dietary quercetin on the larval growth

The onion and seedcorn maggots were quite tolerant of quercetin and their growth on the synthetic diet was not obviously affected by this compound in concentrations up to 1%. Rather, mean weights of the onion maggot reared on the synthetic diet with three levels of quercetin content tended to be greater, though not significantly, than the control (Table 4).

<table>
<thead>
<tr>
<th>Table 4. Effect of dietary quercetin on the larval growth of D. antiqua and D. platura*</th>
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<tbody>
<tr>
<td>Diet</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Synthetic diet</td>
</tr>
<tr>
<td>+0.01% quercetin</td>
</tr>
<tr>
<td>+0.1% quercetin</td>
</tr>
<tr>
<td>+1.0% quercetin</td>
</tr>
</tbody>
</table>

\(^a\) Reared aseptically.

\(^b\) Measured 8 days after the inoculation of eggs. Means±S.D. were obtained from at least 20 measurements.

\(^a\) Reared aseptically.

Means in the same column are not significantly different by ANOVA.
DISCUSSION

Synthetic diet supported the growth of the onion and seedcorn maggots under aseptic conditions, showing that basic nutrient requirements of Delia are similar to that of Drosophila. Since synthetic diet did not contain any plant secondary substances (Erk and Sang, 1966), oxidative and xenobiotic stress should be in the lowest level and thus suitable for assessing the effect of plant secondary compounds on antioxidant enzyme activities.

Effect of quercetin was apparent only at 1.0%, a 100-fold concentration of that found in the whole onion bulb (Table 2). However, Herrmann (1976) reported that quercetin, partly as 4'-glucoside, was concentrated in the inner and outer epidermis of the scales at high concentrations (1.1–2.4% wt./fresh wt.) but absent in mesophyll. This may allow the maggot to feed on this compound at concentrations high enough for SOD induction. Quercetin may be one of the prooxidants responsible for the induction of SOD during the onion maggot consumption of onion tissue.

In the black swallowtail butterfly, two-fold increase of SOD was induced by feeding host plant treated with 2.0% quercetin (Pristos et al., 1988b). Thus high concentration of quercetin seems to be necessary for the induction of SOD in insects in general. However, many plants have been reported to contain very high levels of quercetin (Herrmann, 1976).

Although dietary quercetin did increase SOD activity in the seedcorn maggot (Table 3), quercetin was not found in the soybean seeds. The cause of SOD induction in the soybean-reared seedcorn maggot, therefore, should be attributed to prooxidant(s) other than quercetin, the identities of which are unknown at present.

GPOX activity toward organic hydroperoxides such as CHP is contributed by two discrete activities, Se-dependent and Se-independent GPOX. In mammals, the latter activity is now generally recognized as a function of GST isozyme(s) (Mannervik and Danielson, 1988). In the onion maggot, a purified GST was shown to express GPOX activity toward CHP. Moreover, Se-dependent GPOX measured with H$_2$O$_2$ as substrate was below the detection limit in this species (Kubota and Ishikawa, 1990). The GPOX activity shown in Table 2, therefore, must mostly be contributed by GST. The results of GST measurements with CDNB as substrate were consistent with this premise (Table 2). Since quercetin is known as a potent inhibitor of GST (IC$_{50}=1.0 \times 10^{-5}$ M, Das et al., 1984), GPOX (largely GST) may have been inhibited by endogenous quercetin, which was incorporated in the larval tissue.

In some insects e.g., Trichoplusia ni, quercetin exerts a strong antifeeding and toxic effect, virtually leading to inhibition of growth and finally death at relatively low concentrations (Ahmad et al., 1987). The tolerance of Delia maggots against quercetin indicates that these species possess capabilities to alleviate quercetin toxicity. Little is known about these mechanisms at present. The very high level of SOD induced by this compound suggests that SOD is one of the mechanisms giving protection against quercetin toxicity in these species.

Krieger et al. (1971) showed that cytochrome P-450 dependent monooxygenase (MFO) activities were higher in polyphagous species compared with oligo- or monophagous species in 35 lepidopteran species. This result was taken as evidence for counter-adaptation of herbivores to plant defenses: "Polyphagous insects, because of the diversity of diet, must be prepared to counter a wider range of potentially toxic
plant secondary compounds, whereas monophagous insects save expenditure of energy by restricting their countermeasure to one or a few toxins” (KRIEGER et al., 1971). Many attempts thus have been made to clarify the relationships between insects’ feeding habits and other detoxifying enzymes (e.g., GST, hydrolases and esterases), and the induction mechanisms of these enzymes by plant secondary compounds.

Effect of plant secondary compounds on antioxidant enzymes, however, has drawn little attention until AHMAD et al. (1987) and PRISTOS et al. (1988a, b) investigated antioxidant enzyme activities of three herbivorous insects with different feeding habits. These studies revealed that SOD activities were higher in the species which have higher possibility to ingest prooxidants.

SOD and CAT levels were 1.5–1.7 times higher in general in the seedcorn maggot than in the onion maggot, except for SOD activities of the larvae reared on their respective host plants (Tables 2 and 3). This may indicate that seedcorn maggot, a generalist feeder which may encounter prooxidants, is always keeping SOD and CAT activities at relatively high levels in preparation for occasional high oxidative stress. This observation may favor the hypothesis of KRIEGER et al. (1971). However, further studies on other detoxifying enzymes are needed for conclusion. No induction of CAT was observed in both species. However, the CAT activities were among the highest of the insects reported to date (AHMAD et al., 1987; PRISTOS et al., 1988a, b). Because of these high activity levels, there might have been no need for further increase. This possibility, however, awaits more detailed investigations.

REFERENCES


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