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Ascorbic and Uric Acid Responses to Xanthotoxin Ingestion in a Generalist and a Specialist Caterpillar

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For herbivorous insects, dietary sources of low molecular weight non-enzymatic antioxidants, such as ascorbic acid, may influence performance in the presence of phototoxic plant constituents. We examined responses of *Trichoplusia ni*, a broad generalist, and *Depressaria pastinacella*, a specialist on furanocoumarin-containing plants, to variation in dietary ascorbic acid availability in the presence and absence of xanthotoxin, a phototoxic furanocoumarin. In *T. ni*, dietary ascorbic acid significantly increased levels of this compound in body tissues (approximately 7-fold, 5-fold, and 8-fold in hemolymph, gut, and fat body, respectively). In the presence of xanthotoxin, however, the amount of ascorbic acid accumulated significantly decreased. This decrease was not due to antifeedant effects of xanthotoxin and may instead have resulted from depletion of ascorbic acid due to its radical scavenging activity. In contrast, ascorbic acid levels in *D. pastinacella* were less affected by variation in dietary levels of either xanthotoxin or ascorbic acid, although uric acid, another potential water-soluble nonenzymatic antioxidant, increased in response to dietary ascorbic acid, as it did in *T. ni*. Thus, for generalists, such as *T. ni*, that lack specialized detoxification mechanisms against phototoxins such as furanocoumarins, dietary ascorbic acid may play an important role in antioxidant defense, and, for caterpillars in general, uric acid may also contribute to antioxidant defenses. Arch. Insect Biochem. Physiol. 42:26–36, 1999. © 1999 Wiley-Liss, Inc.

Key words: ascorbic acid; antioxidant; furanocoumarins; generalist; specialist; uric acid

INTRODUCTION

According to Duffey and Stout (1996), dietary components should be classifed according to context, rather than structure, in large part because a "chemical, acting in a particular mode(s), is contingent upon insect, mixture and environment.... Context is an essential aspect of the impact of plant natural products in animal plant interactions." Among the modes of action of plant chemicals that may be contingent upon context is that of nutrient. Nutrient requirements vary enormously with taxon and uniformly designating a particular chemical as a nutrient ignores such taxonomic differences (Berenbaum, 1995).

Ascorbic acid is one such nutrient, charac-

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terized by a tremendous amount of variation in the degree to which it is required in the diet. Among mammals, adequate amounts can be synthesized by most species; humans, apes, monkeys, guinea pigs, and Indian fruit bats are conspicuous exceptions (Lehninger, 1970). Among insects, the status of ascorbic acid as an essential dietary nutrient is less clear. Early studies on herbivorous insects in three orders (including *Schistocerca gregaria*, *Bombyx mori,* and *Anthonomus grandis*) suggested that ascorbic acid is a universal dietary requirement among phytophages (Dadd, 1963).

Ascorbic acid fills many vital physiological functions in mammals, including enhancement of immune function and promotion of collagen synthesis, and may play similar roles in insects (Yasothornsrikul et al., 1997). Blood levels vary ontogenetically and are lowest just after a molt, suggesting a role for ascorbic acid in development (Dadd, 1963), or perhaps indicating that ascorbic acid is depleted while the insect is not actively feeding. One function that ascorbic acid may serve in organisms independent of taxon is as a water-soluble antioxidant. With its low redox potential, ascorbate is oxidized either by transfer of a single electron to generate ascorbic acid free radical or via transfer of two electrons to form dehydro-L-ascorbic acid. Thus, ascorbic acid is capable of reacting with a wide range of oxidants, including hydroxyl radical, singlet oxygen, superoxide anion, peroxyl radicals, and triplet carbonyl compounds (Felton 1995). Ascorbic acid is also important in maintaining other antioxidant systems in cells. For example, alpha-tocopheryl free radical is reduced by transfer of an electron from ascorbic acid, to regenerate alpha-tocopherol.

Among insects, phytophagous species may be particularly vulnerable to oxidative injury by virtue of their feeding habits. Photosynthesis itself is a source of active oxygen species (Dalton, 1995), so plant tissue feeders may well encounter some low level of oxidizing compounds as a normal component of feeding. Other sources of active oxygen species in plant tissues, which vary in abundance with plant identity, include photosensitizing secondary substances. Among these plant photosensitizers, found principally in the Apiaceae and Rutaceae, are the furanocoumarins (Berenbaum, 1991). These benz-2-pyrone compounds are capable of absorbing quanta of ultraviolet light energy to form an excited state molecule, which can react with ground state oxygen to generate active oxygen species, or with macromolecules, such as nucleic acids, directly via a free radical mechanism (Berenbaum, 1991, and references therein). Furanocoumarins are, thus, strong prooxidants and are generally toxic to consumers of plant tissues, including insects.

Despite the general toxicity of furanocoumarins, herbivorous insects display a range of tolerance to these compounds. *Trichoplusia ni* (Lepidoptera: Noctuidae), the cabbage looper, is a generalized feeder that rarely encounters these compounds. Ahmad et al. (1987) reported adverse effects of consumption of furanocoumarins in this species at concentrations of 0.0004%, although other studies (Zangerl, 1990; McCloud and Berenbaum, 1994) demonstrated tolerance of concentrations of 0.1% or greater. In contrast, *Depressaria pastinacella* (Lepidoptera: Oecophoridae) is an oligophagous species that feeds exclusively on the furanocoumarin-rich reproductive tissues of two genera of Apiaceae, *Pastinaca* and *Heracleum*. This species, which is capable of high rates of cytochrome P450-based furanocoumarin metabolism, is capable of ingesting up to 2% of its body weight in furanocoumarins each day (Zangerl and Berenbaum, 1991; Zangerl et al., 1997). We undertook this study to determine whether furanocoumarin ingestion, in the presence and absence of photoactivating ultraviolet light, in these two differentially adapted species affects the disposition of ascorbic acid and whether dietary supplementation with ascorbic acid can ameliorate the adverse effects of furanocoumarin exposure.

MATERIALS AND METHODS

Larvae of the cabbage looper, *T. ni*, and the parsnip webworm, *D. pastinacella*, were reared on a semiartificial diet (Nitao and Berenbaum, 1988) for colony maintenance; adults of both species had free access to 10% honey solution. Eggs that were laid during one night were considered a cohort and the hatched larvae were placed singly into diet cups for all experimental procedures. *D. pastinacella* larvae used in all experiments were collected as eggs from wild parsnip plants in Champaign County, Illinois, and *T. ni* were

from a laboratory colony, initiated with wild-caught larvae from Champaign County, Illinois, and maintained by the Department of Entomology, UIUC. Ingredients for preparation of artificial diet were obtained locally or from Sigma (St. Louis, MO) or U.S. Biochemical (Cleveland, OH). The ascorbic acid-free diet was prepared by reconstruction of the Vanderzant vitamin mixture for insects with the omission of ascorbic acid.

Sequestration of Dietary Ascorbic Acid

To determine the fate of ingested ascorbic acid in the two species of caterpillars, an ultimate instar feeding study was conducted. After reaching the last instar, newly ecdysed fifth (*T. ni*) or sixth (*D. pastinacella*) instars were transferred to the four experimental diets. These diets were made with and without xanthotoxin at a concentration of 0.1% wet weight in the case of *T. ni* and 0.2% wet weight in the case of *D. pastinacella,* and with and without ascorbic acid at a concentration of 0.88%, the normal concentration in artificial diets for both of these species (Nitao and Berenbaum, 1988; Zangerl, 1990). Species differences in furanocoumarin exposure were incorporated into the experimental design so as to allow exposure of each species to a concentration within its range of tolerance. All larvae were exposed to supplemental UVB radiation to increase the prooxidant potential of dietary components. The diet cups with the larvae were placed under a bank of two Westinghouse FS-72 fluorescent UVB bulbs, emitting approximately 360 W/cm^2 UVB radiation, as measured with a UVX light meter (UVX Corp., San Gabriel, CA). The UVB light was supplemented throughout the experiment with two Sylvania Cool-white fluorescent bulbs, which produce no UVB. The experiments were carried out at 27°C under long day conditions (16:8 L:D).

Larvae were allowed to feed for 36 h (*T. ni*) and 92 h (*D. pastinacella*), respectively, after which time tissue samples were taken. Hemolymph samples were collected by cutting a proleg; hemolymph flowing freely from the wound without external pressure was collected with a micropipette into dry-ice chilled eppendorf tubes. The entire gut was removed and washed twice, after removal of its contents and Malpighian tubules, in ice-chilled phosphate buffer (pH 7.8). The fat body attached to the cuticle was freed from silk glands and remaining tracheae and washed twice in ice-chilled phosphate buffer. Gut and fat body were blotted by drawing them repeatedly across tissue paper and were lyophilized afterwards. Finally, all tissues were individually frozen at –80°C and kept until they were assayed.

Gut and fat body samples were weighed and homogenized with a tissue tearor (Biospec Products, Inc., Bartlesville, OK) prior to analysis. Tissue samples (gut, fat body, and hemolymph) were then extracted in 1 ml of lithium carbonate (1%). To each sample, 0.5 ml chloroform was added; after chloroform addition, samples were centrifuged for 2–3 min at 12,000*g* and aliquots from the same supernatants were used for the analyses of both uric acid (Van Handel, 1975) and ascorbic acid (Omaye et al., 1979). For ascorbic acid analysis, 0.5 ml of supernatant was added to 0.5 ml of ice-cold 10% trichloroacetic acid, mixed thoroughly, and centrifuged for 5 min (12,200*g*). In order to form the 2, 4-dinitrophenylhydrazone, 0.5 ml of supernatant was mixed with 0.1 ml of DTC (thiourea, $CuSO_4.5H_20$, 2,4-dinitrophenylhydrazine in 9N H_2SO_4; Omaye et al. 1979) and incubated for 3 h at 37°C. The method of Omaye et al. (1979) determines the total ascorbic acid content in its oxidized forms of dehydroascorbic acid and diketogulonic acid, by the subsequent formation of a hydrazone from these products. To minimize interference from possible non-ascorbic acid chromogens, we used thiourea to produce a mildly reducing medium and incubated at 37°C for 3 h, as recommended by Omaye et al. (1979). After incubation, the test tube was removed from the water bath and placed into ice water; 0.75 ml of ice-cold H_2SO_4 (65%) was added, and the solution mixed well. Solutions were allowed to stand at room temperature for an additional 30 min, after which time absorbance was determined at 520 nm.

Uric acid was measured because it has been suggested to function as a water-soluble antioxidant in insects (Larson, 1988). For uric acid analysis (van Handel, 1975), 0.2 ml supernatant was brought to 2 ml with distilled water. One ml of reagent (Na_2CO_3, glycine and $CuSO_4.5H_20$) was added to each sample. To start the reaction, neocuproine (0.05 ml) was added and the solution mixed well; absorbances were determined at 450 nm. Because ascorbic acid interferes in the

uric acid assay (25 μg showing the same OD as 0.9 μg uric acid), uric acid data were corrected according to the ascorbic acid content in the same sample.

Differences in uric acid and ascorbic acid concentrations among treatments were evaluated by three-way analysis of variance (ANOVA), with xanthotoxin (presence and absence), ascorbic acid (presence and absence), and day of analysis as main effects. All but one set of analyses were based on samples of nine or ten individuals per treatment (variation in sample size was due to problems in sample handling). The ascorbic acid analyses of parsnip webworms were based on 13 or 14 samples per treatment (again, variation in sample size was due to problems in sample handling).

Gravimetric Performance

In order to separate effects of feeding deterrency and toxicity of furanocoumarins on caterpillars, a gravimetric analysis of performance was conducted. After reaching the last instar, newly ecdysed fifth (*T. ni*) or sixth (*D. pastinacella*) instars were transferred to the experimental setups. Larvae were given artificial diet with or without ascorbic acid, as described, in the presence of supplemental UVB radiation at 27°C under long day conditions (16:8 L:D), simulating more natural conditions. UV-free treatments were not incorporated into the design because UV-radiation did not appear to substantially affect the tissue distribution pattern of ascorbic acid in our earlier experiment (see results). After feeding for 36 h (*T. ni*) and 92 h (*D. pastinacella*), larvae, frass, and remaining uneaten diet were collected, oven-dried to a constant weight at 70°C for 3 days, and weighed.

Analysis of covariance (ANCOVA) was used to test main effects and interaction effects of xanthotoxin and ascorbic acid on final larval mass and consumption, with initial mass as the covariate; growth was evaluated with the amount of food consumed and with absorption as the covariates (Raubenheimer and Simpson, 1992).

RESULTS

Sequestration of Ascorbic Acid

We examined the effects of dietary xanthotoxin and ascorbic acid on the tissue contents of ascorbic acid in hemolymph, gut, and fat body of *T. ni* and *D. pastinacella* under two light regimes in order to determine if furanocoumarin ingestion affected sequestration and distribution of nonenzymatic antioxidants. Ascorbic acid was found in all tissues examined in both species. Ascorbic acid was detected in both species of larvae feeding on diet containing no ascorbic acid; these low but detectable levels may well be reserve accumulations carried over from previous stages. The hierarchy in the amount of ascorbic acid was gut > hemolymph > fat body for both species, although the significance of differences among tissue types varied with species.

In webworms, levels of ascorbic acid in all tissues increased significantly in larvae fed diets containing ascorbic acid (all main effects $P < 0.02$). Hemolymph, gut, and fat body levels increased 44%, 90%, and 1.5-fold, respectively, in the absence of UV and 24%, 22%, and 3-fold, respectively, in the presence of UV. Only in the fat body was there a significant interaction effect of dietary ascorbic acid and xanthotoxin on tissue levels of ascorbic acid (Fig. 1) such that ascorbic acid levels increased with ingestion of xanthotoxin if ascorbic acid was also in the diet but declined if ascorbic acid was not present. Uric acid levels also increased significantly in all tissues in response to dietary ascorbic acid (Fig. 2, all main effect $P < 0.02$). In hemolymph, gut, and fat body, uric acid increased 32, 66, and 16%, respectively, in the absence of UV and 1.5-fold, 47%, and 27%, respectively, in the presence of UV. In addition, there was a significant main effect of dietary xanthotoxin on hemolymph levels of uric acid in the presence of UV; xanthotoxin-fed larvae contained 48% less uric acid (main effect $P < 0.0001$).

In the cabbage looper, there was an overall main effect of ascorbic acid ingestion on ascorbic acid content of all tissues irrespective of UV treatment (all main effect $P < 0.0001$), with dietary ascorbic acid consistently increasing hemoymph, gut, and fat body ascorbic acid levels by 7.4-, 5.2, and 7.9-fold, respectively, in the absence of UV and 6.7-, 4-, and 8-fold, respectively, in the presence of UV (Fig. 3). Although there was a main effect of xanthotoxin ingestion on ascorbic acid levels in all three tissues when UV was absent (all main effect $P < 0.0001$), this effect was manifested only when ascorbic acid was present in the diet (Fig 3.). The extent to which ascorbic acid

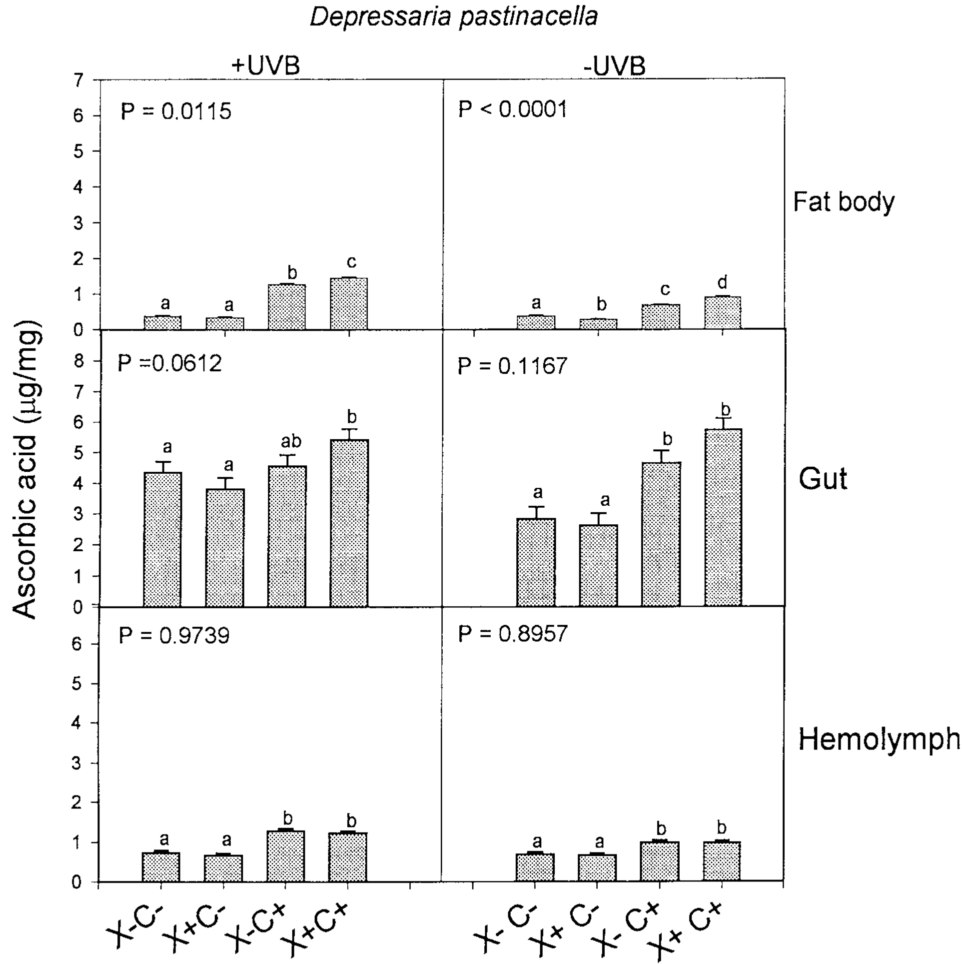

Fig. 1. Effects of dietary xanthotoxin and ascorbic acid on content of ascorbic acid in various tissues of *Depressaria pastinacella* with and without supplemental UVB-radiation. Values are least square means (± S.E.). Bars sharing the same letters are not significantly different from one another (t-test, $P > 0.05$). The P value in each graph is for the interaction effect from 3-way ANOVA of xanthotoxin and ascorbic acid.

accumulated in these tissues was jointly affected by xanthotoxin and ascorbic acid in the diet. When ascorbic acid was consumed in the presence of xanthotoxin, significantly less ascorbic acid was accumulated (t-test, $P < 0.0001$, Fig. 3). In the presence of UV, larval uric acid content of these tissues was affected by diet; dietary ascorbic acid reduced uric acid content by 18% in the gut (main effect $P < 0.0001$) and by 38% in the fat body (main effect $P = 0.0004$) while it increased uric acid levels by 27% in the hemolymph (main effect $P = 0.0176$). In the absence of UV, dietary ascorbic increased uric acid by 21.3% in fat body (main effect $P = 0.038$) and by 28.6% in gut (main effect $P < 0.0001$). Also in the absence of UV, dietary xanthotoxin decreased uric acid in hemolymph by 40.5% (main effect $P = 0.0001$). In the presence of UV, xanthotoxin caused a 36% drop in uric acid in the hemolymph (main effect $P < 0.0001$).

The suppressive effect of xanthotoxin ingestion on accumulation of ascorbic acid could have resulted from the use of ascorbic acid to counteract toxic molecules or could simply reflect the feeding deterrency of xanthotoxin (Berdegué et al., 1997); in the presence of a feeding deterrent, less diet is ingested and, consequently, accumulation of ascorbic acid could be negatively affected. Accordingly, we undertook a series of gravimetric studies, to tease apart behavioral and physiological contributions to ascorbic acid accumulation.

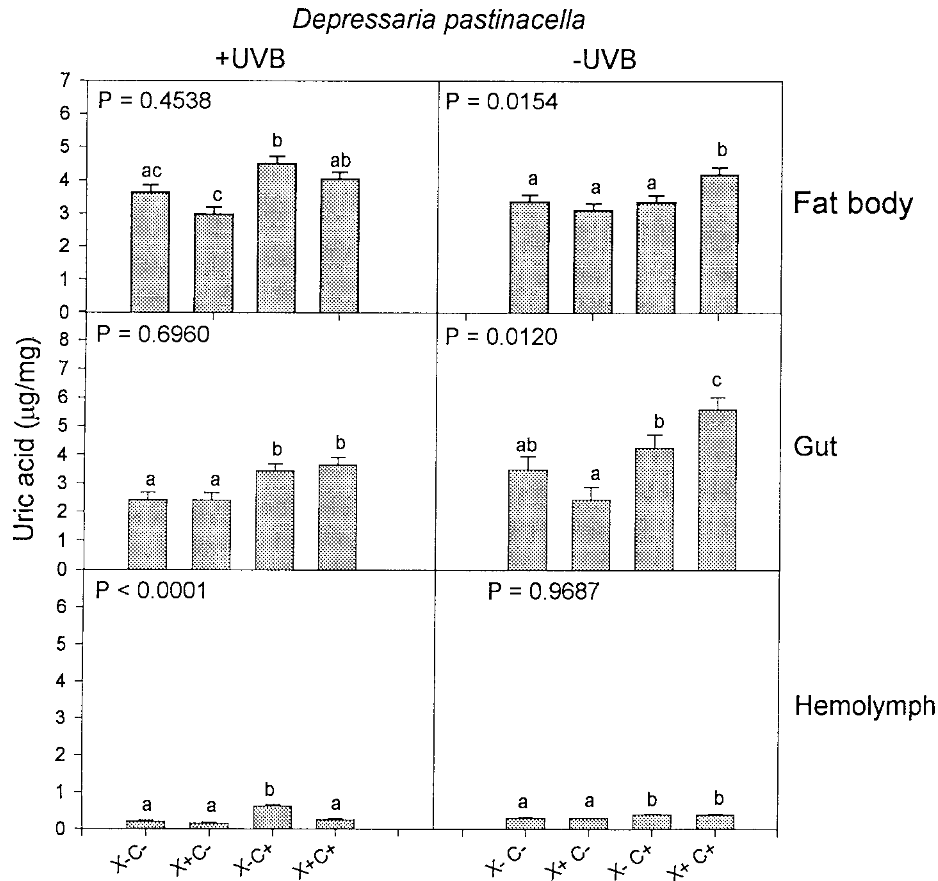

Fig. 2. Effects of dietary xanthotoxin and ascorbic acid on content of uric acid in various tissues of *Depressaria pastinacella* with and without supplemental UVB-radiation. Values are least square means (± S.E.). Bars sharing the same letters are not significantly different from one another (t-test, $P > 0.05$). The P value in each graph is for the interaction effect from 3-way ANOVA of xanthotoxin and ascorbic acid.

Gravimetric Estimates of Performance

Analysis of covariance was used to test main and interaction effects of xanthotoxin and ascorbic acid on final mass, consumption, and mass gain of *T. ni* and *D. pastinacella*. Final mass and consumption were evaluated with initial mass as the covariate, and mass gain was evaluated first with consumption as a covariate, and second with absorption as the covariate. In some of the experiments the slope of the regression differed among treatments, as evidenced by a significant interaction between the covariates (consumption and absorption) and treatment. Accordingly, ANOVA was conducted for *T. ni* with the covariates (consumption and absorption) nested within treatments.

In *T. ni*, mass gain, with either consumption as covariate or absorption as covariate, was affected by both dietary xanthotoxin and ascorbic acid; each significantly decreased conversion of food into body mass (P = 0.0001, Table 1A). Dietary xanthotoxin did not affect consumption rate (P = 0.75, as a main effect); however, when larvae consumed ascorbic acid-containing diet in the presence of xanthotoxin, the consumption rate was elevated by approximately 3-fold compared to a diet containing xanthotoxin but lacking ascorbic acid (P = 0.0001, Table 1B).

In *D. pastinacella*, dietary xanthotoxin and dietary ascorbic acid did not affect consumption rate (P = 0.44 for xanthotoxin and P = 0.89 for ascorbic acid, as main effects); however, consumption was reduced by xanthotoxin in diet without ascorbic acid and was increased when larvae were feeding on diet containing ascorbic acid, as indicated by a significant interaction effect of dietary ascorbic acid and xanthotoxin (P < 0.0101, Table 1B). Ascorbic acid

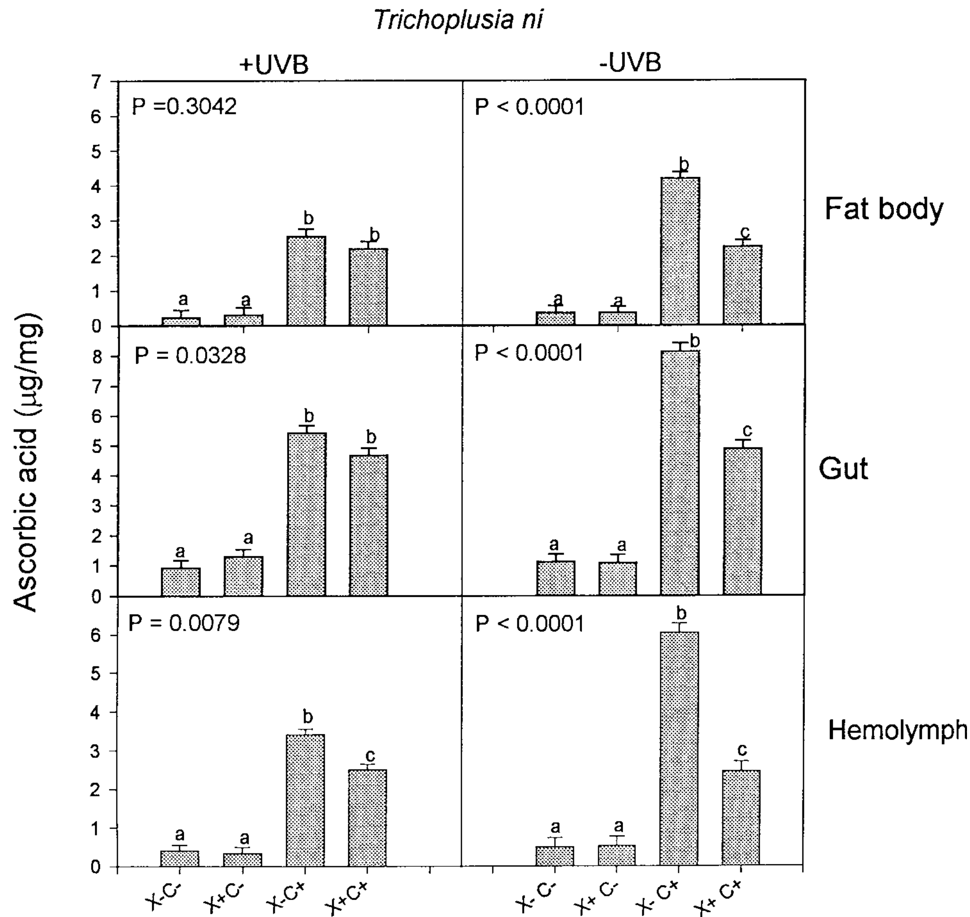

Fig. 3. Effects of dietary xanthotoxin and ascorbic acid on content of ascorbic acid in various tissues of *Trichoplusia ni* with and without supplemental UVB-radiation. Values are least square means (± S.E.). Bars sharing the same letters are not significantly different from one another (*t*-test, $P > 0.05$). The P value in each graph is for the interaction effect from 3-way ANOVA of xanthotoxin and ascorbic acid.

in the diet significantly reduced mass gain with absorption as a covariate ($P = 0.0023$, Table 1A), while xanthotoxin in the diet significantly increased this parameter ($P = 0.0102$) as main effects.

DISCUSSION

The presence of higher levels of ascorbic acid in gut than in hemolymph and fat body tissues in both *T. ni* and *D. pastinacella* is consistent with a function associated with food processing. In a study of ascorbic acid distribution in *Manduca sexta* larvae, Kramer et al. (1981) also found higher levels of ascorbic acid in gut tissues than in fat body. In our study, we examined the possibility that nonenzymatic antioxidants, such as ascorbic acid and uric acid (Souza et al. 1997), are potential defenses for insect herbivores feeding on phototoxic compounds in the presence of UVB-radiation.

We found that in *T. ni* the tissue level of ascorbic acid was significantly reduced in the presence of xanthotoxin. This effect was manifested despite the fact that consumption of diets containing both xanthotoxin and ascorbic acid was increased. The fact that ascorbic acid levels in the tissues were depleted in the absence of an antifeedant effect of xanthotoxin suggests that ascorbic acid might function as an antioxidant, possibly being depleted as it scavenges radicals. Such an interpretation, however, must be considered in the context of the limitations of the analytical methods used. The method of Omaye et al. (1979) estimates ascorbic acid in the oxidized form, so the possibility exists that some ascorbic

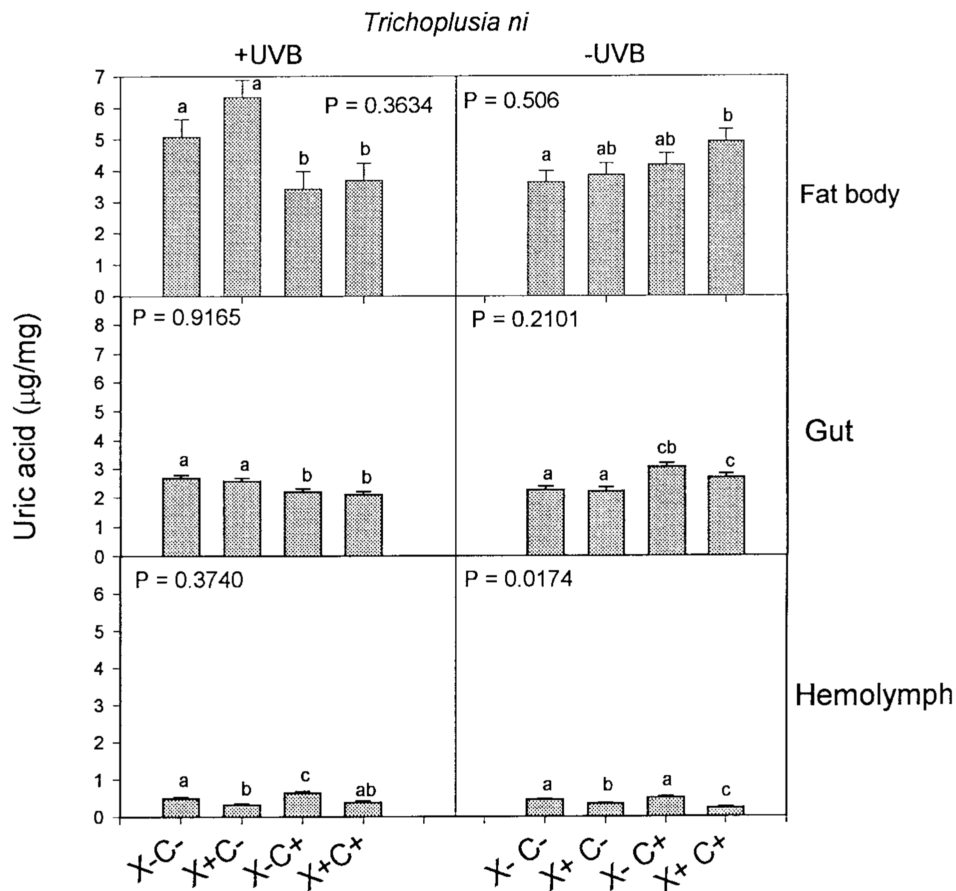

Fig. 4. Effects of dietary xanthotoxin and ascorbic acid on content of uric acid in various tissues of *Trichoplusia ni* with and without supplemental UVB-radiation. Values are least square means (± S.E.). Bars sharing the same letters are not significantly different from one another (*t*-test, $P > 0.05$). The *P* value in each graph is for the interaction effect from 3-way ANOVA of xanthotoxin and ascorbic acid.

acid is converted into a form that is not detectable by the method.

Relying on dietary sources of nonenzymatic antioxidants for amelioration of oxidative stress is consistent with the status of *T. ni* as a generalized feeder. As a generalist, it potentially can encounter a broad variety of photooxidative compounds in its diets. Indeed, within the family Rutaceae, no fewer than five biosynthetically distinct classes of photosensitizers have been reported (Downum, 1991). Despite the structural diversity of the photosensitizers, all share a similar mode of action in that they typically generate the same range of active oxygen species (including singlet oxygen, hydroxyl radical, and superoxide anion) (Downum, 1991). *T. ni* and *D. pastinacella* have comparable levels of superoxide dismutase (SOD) activity in the presence of xanthotoxin, that is, 2.59 SOD U mg^{-1} min^{-1} in *T. ni* and 2.98 SOD U mg^{-1} min^{-1} in *D. pastinacella* (Lee and Berenbaum, 1989, 1990). However, *T. ni* displays relatively low levels of cytochrome P450-mediated metabolism of furanocoumarins such as xanthotoxin, in comparison with specialists such as the parsnip webworm (Lee and Berenbaum, 1989). Moreover, ascorbic acid is present in abundant supply in foliage of a wide range of species and thus is likely to be readily available to cabbage loopers as a ready-made antioxidant (Jones and Hughes, 1983). Therefore, for a generalist, mechanisms of tolerance that target the common products of photosensitization may be more effective defenses than elaborating specific mechanisms of detoxification for a broad diversity of structural types of photosensitizers.

As a specialist herbivore, *D. pastinacella* is restricted to feeding on the flowers and fruits of

TABLE 1. Main and Interaction Effects (From two-way ANOVA) of Dietary Xanthotoxin (X) and Ascorbic Acid (C) on Gravimetric Estimates of Performance in *Trichoplusia ni* and *Depressaria pastinacella**

	T. ni	*D. pastinacella*
A. Main effects of diet components		
Diet component	Mass gain with consumption as covariate	
–C	52.58 ± 2.45 (15)	15.56 ± 0.57 (19)
+C	44.12 ± 3.01 (14)	15.46 ± 0.68 (15)
Main effect	$P = 0.0001$	$P = 0.91$
–X	52.74 ± 1.16 (16)	14.80 ± 0.69 (14)
+X	43.96 ± 3.71 (13)	16.24 ± 0.55 (20)
Main effect	$P = 0.0001$	$P = 0.11$
Diet component	Mass gain with absorption as covariate	
–C	63.00 ± 3.48 (15)	16.73 ± 0.53 (19)
+C	49.35 ± 3.02 (14)	13.93 ± 0.63 (15)
Main effect	$P = 0.0001$	$P = 0.0023$
–X	60.52 ± 2.14 (16)	14.21 ± 0.64 (14)
+X	51.82 ± 4.08 (13)	16.45 ± 0.51 (20)
Main effect	$P = 0.0001$	$P = 0.0102$
B. Significant interaction effects of dietary components		
Diet component	Consumption	
–C–X	153.86 ± 8.32[a] (8)	98.23 ± 8.30[a] (9)
–C+X	65.18 ± 9.25[b] (7)	67.16 ± 7.87[bc] (10)
+C–X	178.73 ± 9.55[a] (8)	72.88 ± 10.28[ac] (5)
+C+X	260.91 ± 9.99[c] (6)	90.15 ± 7.27[a] (10)

*Values (mg) are least square means ± S.E.; least square means for the interactions that differ by a *posteriori* *t*-test are indicated by different letters. Sample sizes are indicated in parentheses.

a small number of plants in the family Apiaceae. Our experiments showed no significant effect of xanthotoxin on antioxidant levels in the tissues. Zangerl et al. (1997) analyzed flowers and fruits of *Pastinaca sativa*, the main host plant of the parsnip webworm, with respect to both primary and secondary metabolites and found ascorbic acid levels of 0.53 ± 0.44 μg mg^{-1} for fruits and 0.29 ± 0.15 μg mg^{-1} for buds (dry weights). In contrast, foliage of parsnips contains on average approximately ten times the ascorbic acid levels found in buds (Zangerl and Berenbaum, 1998). Artificial diets, containing 8.82 μg mg^{-1} ascorbic acid, resemble the higher foliar content of ascorbic acid, relative to flowers and seeds (Jones and Hughes, 1983). Due to its highly specialized feeding behavior, the parsnip webworm is unlikely to encounter high levels of ascorbic acid in its natural diet and might thus not be dependent upon sequestration of ascorbic acid for antioxidant functions. As well, there may be a reduced need for dietary antioxidants in this species, inasmuch as rates of metabolic detoxification of furanocoumarins are extremely high (up to 300 times faster than those displayed by *T. ni*; Lee and Berenbaum, 1990). Oxidative stress levels may thus be concomitantly lower.

There is some indication, however, that webworms as well as cabbage loopers may rely on other sources of water-soluble nonenzymatic antioxidants. Uric acid is one candidate. In hemolymph, dietary ascorbic acid is associated with increased levels of urate in both *T. ni* ($P = 0.0176$) and *D. pastinacella* ($P = 0.0001$). Humans, who as a species lack the ability to synthesize ascorbic acid, also lack the enzyme uricase and selectively resorb urate from the kidneys. Plasma levels are extremely high (2–7.5 mg/100 ml in blood, compared with prosimians, with less than 0.5 mg). Urate may have replaced "some of the antioxidant functions of ascorbic acid during primate evolution" (Ames et al., 1981) and may serve a similar function in caterpillars. Urate may actually "protect" what little ascorbic acid is sequestered from the diet, particularly where fat-soluble antioxidants may be less effective.

The role of ascorbic acid and uric acid as antioxidants is a very complex one, and according to the existing literature their functions are not entirely clear. Davies et al. (1986) presented evidence that urate protects ascorbic acid from oxidation in human blood by forming stable complexes with iron ions. Simic and Jovanovic (1989) and Maples and Mason (1988) claim that

the uric acid radical can be reduced by ascorbic acid, since the redox potential of uric acid at the physiological pH 7 is considerably higher than that of ascorbic acid. Souza et al. (1997) demonstrated greatly increased urate concentrations in the hemolymph of *Rhodnius prolixus* following a blood meal, consistent with antioxidant protection of urate. The role of urate as an antioxidant in another insect, *Drosophila melanogaster*, has already been demonstrated by the sensitivity of urate-null mutants to experimentally induced oxidative stress (Hilliker et al., 1992). However, Ogihara et al. (1995) and Ames et al. (1981) suggested that a decrease of uric acid indicates detoxification reactions since uric acid acts as a sacrificial radical scavenger. They demonstrated a simultaneous increase of allantoin, which is one of the resultant products of oxidized uric acid.

It is unlikely that nonenzymatic molecules with antioxidant activity act independently. Rather, vitamins may work in concert to produce optimal protection against oxidative stress (Ahmad, 1995). In human plasma, ascorbic acid and uric acid are among the most widely cited forms of water-soluble antioxidants and appear to serve as the main defense against oxidizing species in the aqueous phase (Ames et al., 1981). It is becoming increasingly clear that oxidative stress imposes significant pressure upon insect herbivores and that a diversity of antioxidants exists (Felton, 1995). There are, however, certain situations where specific dietary antioxidants also express a prooxidative effect (Podmore et al. 1998), so their usefulness as antioxidants is partly reduced by autoxidation and generation of oxygen radicals. This is part of the difficulty in assessing the antioxidant functions of uric acid and ascorbic acid. The broad diet of *T. ni* requires this species to tolerate a variety of allelochemicals and thus might necessitate a generalist approach to coping with photooxidative molecules in its diet. In contrast, with its extremely narrow diet, *D. pastinacella* predictably encounters high levels of a single class of photosensitizer and is well suited to detoxify xanthotoxin with its high constitutive, as well as inducible, levels of P450-mediated metabolic activity. The degree to which phytophagous insects rely on dietary sources of antioxidants may thus depend not only on the frequency with which they encounter prooxidant phytochemicals but also on the reliability with which they encounter substantial levels of dietary antioxidants such as ascorbic acid.

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