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Larvae of the fall webworm, *Hyphantria cunea*, inhibit cyanogenesis in *Prunus serotina*

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SUMMARY

The larvae of the fall webworm, *Hyphantria cunea* (Dru.), though vulnerable to cyanide poisoning, consume the cyanogenic leaves of black cherry, *Prunus serotina*, without apparent harm. The cyanide contents of leaves, defensive regurgitant, the bolus, and frass were assayed by ion chromatography to determine the fate of the toxin in the caterpillar. Leaves collected in September, when the caterpillars were feeding, contained 1592±276 p.p.m. cyanide. Samples of dried frass obtained from caterpillars fed these leaves yielded 2868±552 p.p.m. cyanide. Frass extracted directly in NaOH yielded approximately five percent of the cyanide obtained from frass ground in buffer and distilled in Warburg flasks, indicating that cyanogenesis is largely inhibited as the bolus traverses the gut. This inhibition is attributable to the ability of the caterpillar to maintain a foregut environment in the presence of the bolus that is sufficiently alkaline to suppress the conversion of the plant cyanogen to cyanide. Although a number of caterpillars feed without harm on cyanogenic plants, this it the first shown to inhibit cyanogenesis in this manner.

Key words: *Hyphantria cunea*, fall webworm, cyanide, *Prunus serotina*, black cherry, Arctiidae, foregut, pH.

INTRODUCTION

The leaves of the black cherry tree (*Prunus serotina*) contain the cyanogenic glucoside prunasin. When a leaf is crushed by a herbivore, prunasin is cleaved by β-glycosidase, yielding α-hydroxynitrile (mandelonitrile), which then dissociates spontaneously or in the presence of a second enzyme to benzaldehyde and hydrogen cyanide (HCN). Cyanide causes respiratory failure by interacting with the terminal oxidase in the mitochondrial respiration chain. Although there have been numerous studies of cyanogenesis in the leaves, fruits and seeds of black cherry [Hu and Poulton (Hu and Poulton, 1999) and references therein], the fate of the cyanogenic leaf of cherry as it passes though the digestive tract of invertebrate herbivores has been studied only in the eastern tent caterpillar, *Malacosma americanum* (Fitzgerald et al., 2002). Tent caterpillars eclose from an overwintered egg mass just as the leaves of black cherry are unfolding in the spring. The young leaves have the highest cyanide potential (HCN-p) and the caterpillars prefer them to aged leaves (Fitzgerald, 1995; Fitzgerald et al., 2002). Cyanogenesis occurs in the foregut of the caterpillar but the insect is immune to the toxin. The toxin appears in the caterpillar's regurgitant and it has been suggested that the caterpillars may seek out the most cyanogenic leaves to enhance the defensive value of the regurgitant (Peterson et al., 1987). The ultimate fate of cyanide in the tent caterpillar is unknown, but significantly decreasing quantities are found in the bolus as it passes along the digestive tract, and the fecal pellets are largely devoid of the chemical (Fitzgerald et al., 2002).

The fall webworm, *Hyphantria cunea*, is an ecological equivalent of the eastern tent caterpillar, eclosing from eggs laid on the leaves of cherry in early July, a month or more after the last of the tent caterpillars has finished the feeding phase of its life cycle. Webworms feed until mid-September, then pupate in the soil. Although previous studies indicate that the cyanogenic potential of cherry leaves declines precipitously as they age (Smeathers et al., 1973), preliminary studies indicated that even the senescent leaves that mature webworms consume in September have a significant HCN-p. The present study was undertaken to determine the amount of the toxin in the caterpillar's diet. The cyanide content of the caterpillar's defensive regurgitant, gut bolus, and fecal pellets (frass) were measured to determine the fate of cyanide as it passes though the alimentary tract of the insect. The effect of gut pH on cyanogenesis was also assessed.

MATERIALS AND METHODS

Study site

Leaves of *P. serotina* and *H. cunea* caterpillars used in this study were collected in Cortland County, NY, USA. The insect has only a single generation each year, and four field seasons (2002, 2005–2007) were required to complete the study.

Analytical procedures

Cyanide assays

Samples were prepared for analysis using techniques modified from Brinker and Seigler (Brinker and Seigler, 1992). Samples of leaf and insect material were ground in a micro tissue-grinder in approximately 0.5 ml of 0.1 mol l^{-1} phosphate buffer ($NaH_2PO_4.H_2O$, pH 6.8), chilled in an ice bath to approximately 4°C unless otherwise noted. Material was ground until fully homogenized, typically in 5–10 s. The buffer containing the sample material was immediately transferred to the outer chamber of an 18 ml Warburg flask, the center well of which was preloaded with 0.2–0.3 ml of 1 mol l^{-1} NaOH. The sealed flask was placed in an oven and the material incubated at 35°C for a minimum of 18 h. HCN volatilizing from the sampled material was trapped in the center well as CN^-. At the end of the incubation period, the contents of the center well were drawn off and stored in a sealed

microcapsule at 2°C. The rationale for this procedure and a comparison of the method with that involving freezing samples in liquid nitrogen is given in Fitzgerald et al. (Fitzgerald et al., 2002).

Samples were analyzed with a Dionex Ion Chromatograph (Sunnyvale, CA, USA) with a GP 50 gradient pump and ED 40 electrochemical detector. The machine was fitted with a 4 mm AG9-HC guard column and a 250 mm×4 mm AS7 analytical column. The eluent consisted of 41 g $NaC_2H_3O_2$, 5 ml ethylenediamine, and 16.5 ml of 6.0 mol l^{-1} NaOH per liter, delivered at a flow rate of 1.0 ml min^{-1}. 25 μl of the NaOH center-well-solution were injected into the apparatus. CN^- eluted at approximately seven minutes (Fig. 1). A standard curve with known concentrations of CN^- was prepared prior to each run.

Measurement of regurgitant and gut pH

The pH of the caterpillar's regurgitant and gut were measured with a 100 μm-diameter Beetrode® NMPH1 pH electrode and a 450 μm-diameter Dri-Ref® 450 reference electrode. A Bee-Cal® compensator was used to produce standard pH readings on an Orion® 301 pH meter. All electrode components were manufactured by World Precision Instruments, Sarasota, FL, USA. For gut and regurgitant pH, the meter was calibrated prior to each measurement with Hydrion® buffer (Micro Essentials Laboratory, Inc., Brooklyn, NY, USA) with a pH of 12.0±0.02 at 25°C. Meter accuracy was monitored with Hydrion pH 10 and 11 buffers.

Experimental procedures

Effect of experimental procedures on the HCN-p of leaves

Initial studies were conducted to determine if either the method of handling leaf samples or the feeding damage to leaves inflicted by caterpillars during the course of the studies affected the results. In mid June 2002, five branch tips bearing 4–5 terminal leaves each were cut from a *P. serotina* tree and placed individually in water-piks. The samples were brought indoors and immediately prepared for distillation by cutting a section (3.5±0.1 mg) from one randomly chosen leaf from each branch. The samples were ground and distilled in Warburg flasks. A second sample was taken from each of the previously sampled leaves after the branches had been in the water-piks for 24 h. These samples (4.0±0.9 mg) were prepared and distilled in the same manner as described for freshly picked leaves. The cyanide content of the fresh leaves and leaves held in water-piks for 24 h was determined and statistically compared.

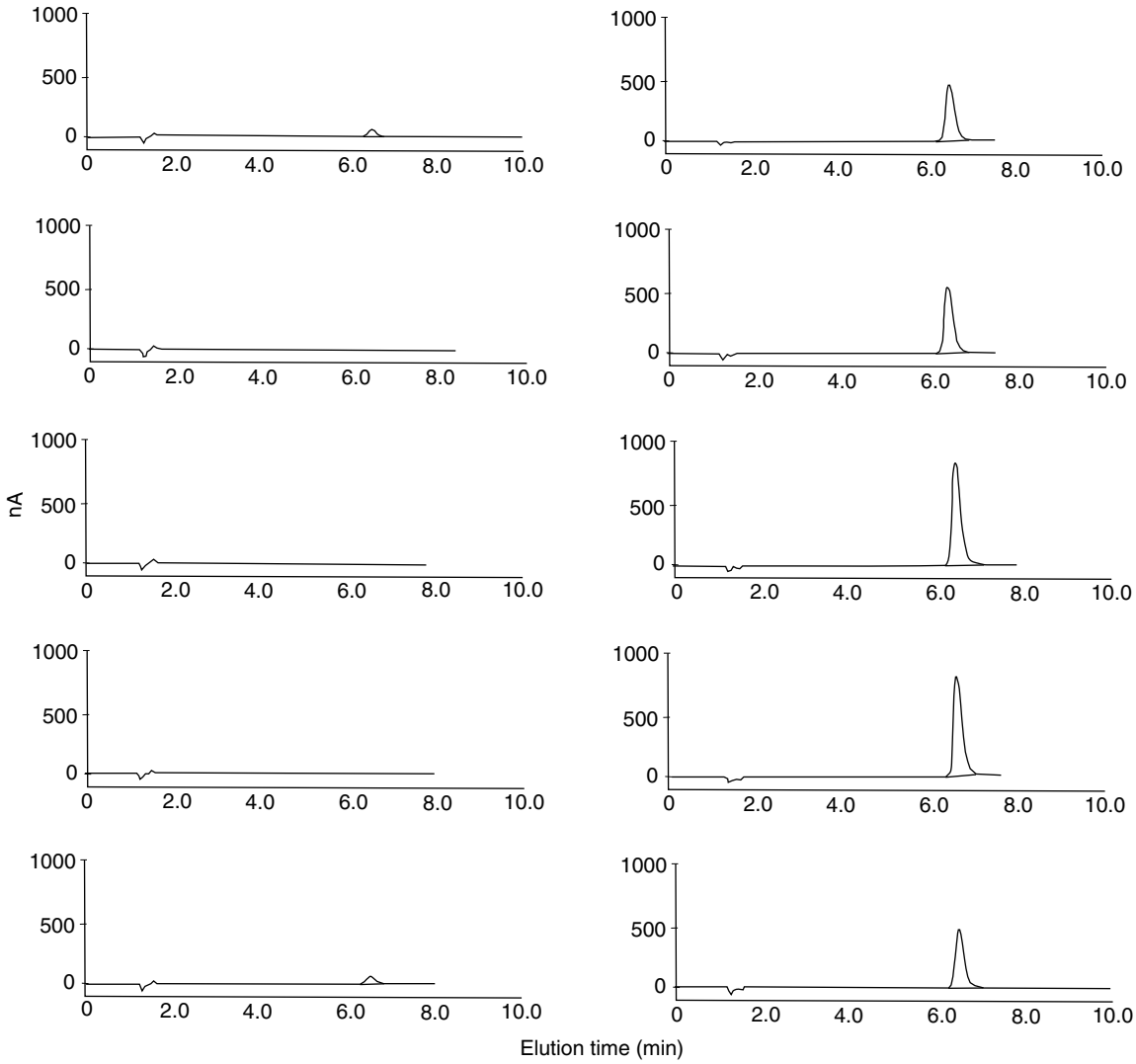

Fig. 1. Ion chromatographs of samples of the frass of *H. cunea* extracted directly in NaOH (left) and ground in buffer and distilled (right). CN^- eluted at approximately 7 min.

In early September 2002, six branch tips, each bearing 4–5 terminal leaves, were cut from six different cherry trees. The branches were immediately placed in separate water-piks and a sample (3.9±0.3 mg) taken for distillation from a randomly selected leaf on each branch as described above. A fine pin was placed in the petiole of each of the sampled leaves to mark it and each branch was placed in a cage with 20 fall webworms, which were allowed to feed *ad libitum* overnight. The next morning at 09.00 h, a second sample (3.8±0.3 mg) was cut from each of the marked leaves and prepared for distillation. All of the marked leaves had significant feeding damage from the caterpillars. Both sets of samples were analyzed for cyanide content and compared statistically to determine if feeding altered the cyanogenic potential of the leaves.

HCN-p of leaves and the cyanide content of the regurgitant, the bolus and frass

Studies were conducted to determine the cyanide content of regurgitant, the bolus and frass derived from leaves of known HCN-p. In 2002, six whole field colonies were brought into the laboratory and placed separately on freshly cut, undamaged branches of *P. serotina* obtained from six different trees. The colonies were allowed to feed overnight. At 07.00 h the next morning, a section was cut from a leaf that the caterpillars had fed on overnight from each of the six branches. These samples were immediately prepared for distillation as described above. At the same time, caterpillars were selected at random from each colony and stored in a freezer at –23°C. These caterpillars were subsequently analyzed to determine the cyanide content of the boluses of the foregut (N=12) and midgut (N=9). In preparing gut bolus samples for distillation, the gut contents were dissected from frozen caterpillars on a cold table and transferred before thawing to the tissue grinder.

A 20 μl sample of regurgitant was collected from caterpillars from each of the six colonies at 07.00 h. To collect the regurgitant, a caterpillar was quickly pulled from the nest, causing it to express a droplet from its mouth. The droplet was drawn by capillary action into a micropipette. Some caterpillars produced little or no regurgitant and it was necessary to collect droplets from several caterpillars from each colony to obtain a single 20 μl sample. Because the purpose of this study was to assess the defensive value of the droplet at the time it was produced, 20 μl samples were injected directly into microvials filled with 0.3 ml of 1 mol l^{-1} NaOH. Centrifuged (8422 ***g***, 10 min) samples of this solution were then injected into the chromatograph to determine their cyanide content.

Each of the six colonies produced copious quantities of frass overnight, and samples of this material were collected from paper sheets placed under the branches after the pellets had dried, then stored at –23°C. Fifty-seven separate analyses of these dry pellets were undertaken to determine their cyanide content. The mean mass of pellets analyzed was 1.0±0.4 mg. For 24 of these analyses, the mean cyanide content of three pellets ground together was determined. For 10 of these analyses, the mean cyanide content of two pellets ground together was determined. For the remaining 23 analyses, single pellets were analyzed.

Components of the 2002 study were replicated during subsequent field seasons. Five field colonies collected in early September 2005 were brought into the laboratory and each was allowed to feed overnight on a branch of *P. serotina*. Branches were collected from five different trees. The next morning at 08.00 h, two leaf samples were taken from each of three trees and one each from the other two trees to determine their cyanide content. Samples were taken from near the tips of branches. Four fecal pellets (1.4±0.1 mg) that had fallen onto paper sheets placed under each colony were collected at random when dried, then analyzed individually as described above to determine their cyanide content. The procedure used in 2002 to determine the cyanide content of the defensive regurgitant was also replicated in September 2007 by collecting 10 samples (5 μl) of regurgitant from 6th and 7th instar caterpillars that had just fed.

Analysis of data derived from the above studies indicated that the webworm was inhibiting cyanogenesis, leading to additional studies in 2006 and 2007. A colony of webworms developing on *P. serotina* was collected from the field in late August 2006 and maintained overnight on branches in the laboratory. The next morning, following overnight feeding, 10 samples of three pellets of frass that had fallen out of the branch overnight were collected. Half of these samples were distilled in Warburg flasks. The other five samples were ground in microvials containing NaOH, then centrifuged and the supernatant analyzed for cyanide. The combined boluses of the foreguts and midguts of eight caterpillars were removed from caterpillars killed in a freezer, then dissected while still frozen on a cold table. The boluses were ground in microvials containing NaOH. The formed fecal pellets found in the rectums of seven caterpillars were removed and treated in a similar fashion. These bolus and fecal pellet samples were centrifuged (8422 ***g***, 10 min) and the supernatant analyzed for cyanide.

To determine if the addition of β-glycosidase to frass during the distillation process would increase the yield of cyanide, seven samples of pellets were obtained from caterpillars from different colonies and ground separately with a mortar and pestle. From each of these samples, 3 μg quantities were distilled in either standard buffer or in buffer to which 5–10 units of β-glycosidase were added (Sigma-Aldrich product number G4511).

Loss of HCN to atmosphere while feeding

A study was conducted to obtain an estimate of the fraction of HCN lost to the atmosphere while leaves were being consumed by caterpillars. Ten 6th instar webworm caterpillars were housed in a sealed chamber (8 cm long × 2 cm diameter) with a half of a leaf of *P. serotina* of known mass. A tube leading into the chamber carried an air stream to the bottom of the chamber, and another tube at the top carried the air to a capture chamber of the same dimensions. Air entering the capture chamber was directed by a tube to the bottom of the chamber and bubbled through 10 ml of 1 mol l^{-1} NaOH. Glass beads were used to cause the rising air to take a circuitous route through the NaOH, increasing the capture rate. Air exited the chamber through an opening at the top. The air flow rate was adjusted to 2–3 ml per min. The caterpillars were allowed to feed on the leaf for 24 h, during which the entire leaf was consumed. The NaOH was retrieved and analyzed for cyanide as described above. At the same time as this study was being conducted, effluent from the other half of the leaf was collected using an identical apparatus except that the feeding chamber was used as an extraction chamber. The extraction chamber was immersed in a water bath at 35°C. The purpose of this was to determine the total HCN-p of the leaf. In preparation, the leaf section was placed in the bottom of the extraction chamber and the chamber immersed in liquid nitrogen. The frozen leaf was then ground to a powder. Approximately 1 ml of phosphate buffer was added and the chamber immediately sealed. An air stream, adjusted to the same rate as that used in the chamber housing the caterpillars, was then passed though the chamber housing the leaf and then into the capture chamber for 24 h. The process was replicated five times with leaves from different trees and different groups of caterpillars.

pH of leaves, regurgitant and the bolus

Approximately 0.25 g of a *P. serotina* leaf collected in September was ground with a mortar and pestle in 10 ml of distilled water. The pH of the slurry was then measured to determine the pH of the leaf tissue. Five leaves obtained from the same tree were measured in this way. The pH of the regurgitant was determined immediately after 6th or 7th instar caterpillars were fed the leaves of this same tree and compared to the pH of the regurgitant of the same caterpillars starved for 24 h. To obtain regurgitant, a caterpillar was held between the thumb and forefinger while the opening of a 5 μl micropipette was placed against its mouthparts. This caused the caterpillar to regurgitate and the material to be pulled into the pipette by capillary action. The collected regurgitant was immediately injected into a 2 mm-long glass sample tube glued horizontally to the top of a 30 mm-long glass tube of the same diameter forming a 'T', the base of which was fixed to a wooden block to secure it in an upright position. The sample tube had an external diameter of 1.4 mm and an internal diameter of 0.9 mm. The pH of the regurgitant was measured by inserting the pH probe into one end of the sample tube and the reference electrode into the other. The sample tube was observed at the low power of a dissecting microscope to facilitate the placement of the pH electrodes. Using this procedure, it was possible to measure the pH of samples with a volume of approximately 2 μl. Samples of regurgitant were drawn from 22 caterpillars that had been starved for 24 h and from 22 caterpillars that had just fed to repletion.

The pH of the contents of the foregut, midgut and hindgut were measured for 6th or 7th instar caterpillars given constant access to host leaves. Caterpillars were killed by placing them in a freezer for 15 min, then dissected to reveal the entire extent of the gut. Hemolymph was blotted away and the gut probed with the electrodes under a dissecting microscope. Since the pH of the gut varies along its extent (Dow, 1992) maximum values were recorded for each compartment of the tract. The pH of the guts of 10 caterpillars was measured.

Effect of gut pH on cyanogenesis

The influence of pH on cyanogenesis was investigated by following the same procedures outlined above to determine the HCN-p of leaves except that the pH of the buffer was varied. Leaf samples were ground and distilled in pH 7, 8, 9, 10 and 11 Hydrion® buffers. Five 3–4 mg samples were cut from adjacent sites on the same leaf, then each was ground and distilled in one of the buffers. A total of three leaves was treated in this manner.

Rate of food intake

To obtain an index of the rate of food intake, six 6th or 7th instar caterpillars that had been deprived of food for 24 h were weighed and then presented with a host leaf in separate containers and allowed to feed *ad libitum* at room temperature (initial mass 0.160±0.012 g). The duration of the feeding bout of each caterpillar was determined by direct observation. The mass gained by each caterpillar was determined immediately after it finished feeding. In addition, five 6th or 7th instar caterpillars were each housed with a host leaf and video recorded at 1 frame s^{-1} for an average of 10.7±2.4 h to obtain a record of the frequency and duration of feeding bouts of individuals allowed to feed *ad libitum* over extended periods. Caterpillars were maintained under a 14 h:10 h L:D photoperiod regime at room temperature. Red light was used during the scotophase to illuminate the foraging arena.

Extent of food processing

The fecal pellets of webworms fed *P. serotina* leaves range from black though brown to bright green, suggesting that the extent to which food is processed as it transits the digestive tract varies. Pellets of these varying colors were rehydrated and their contents compared with respect to apparent degradation. The cyanide contents of 42 green pellets were also compared to that of 23 dark (brown to black) pellets.

Susceptibility of caterpillars to cyanide poisoning

The susceptibility of fall webworms to cyanide poisoning was determined by placing single caterpillars in a chamber filled with the fumes of cyanide liberated by grinding approximately 0.5 g of the young leaves of black cherry in 2 ml of pH 6.8 phosphate buffer (Fitzgerald et al., 2002). The insects were observed for one hour and symptoms of cyanide poisoning recorded.

Statistics

Statistical analyses as detailed below were carried out with ProStat (Poly Software International, Pearl River, NY, USA) and SigmaStat statistical software (Systat Software Inc., Chicago, IL, USA). All values are given as the mean ± s.e.m.

RESULTS

Effect of experimental procedures on the HCN-p of leaves

The mean cyanide content of leaves freshly collected in mid June 2002 (2697±289 p.p.m.) was not significantly different from the cyanide content of the same leaves after they were maintained in water-piks for 24 h (2637±177 p.p.m.) (*t*-test, t=0.372, P=0.7289). The cyanide content of leaves freshly picked in September 2002 (1894±141 p.p.m.) was not significantly different from the cyanide content of the same leaves sampled after caterpillars had fed on them overnight (2022±156 p.p.m.) (*t*-test, t=–0.92, P=0.41). Thus, the procedure employed in collecting and preparing leaves for analysis is not likely to have affected their cyanide content. The cyanide content of leaves sampled in June was significantly greater than that in September (*t*-test, t=–2.49, P=0.04), consistent with previous observations that the HCN-p of *P. serotina* declines as the season advances (Smeathers et al., 1973).

HCN-p of leaves and the cyanide content of the frass, regurgitant and the bolus

Wet leaf samples collected from branches fed on by fall webworms in the laboratory in the fall of 2002 contained 1522±234 p.p.m. cyanide. Dry fecal pellets collected from caterpillars feeding on these same leaves contained 2433±265 p.p.m. Wet leaf samples collected in the fall of 2005 contained 1592±276 p.p.m. cyanide. Dry fecal pellets collected from larvae that had fed on these leaves contained 2868±552 p.p.m. cyanide. There was no significant difference in the amount of cyanide recovered when pellets were distilled with buffer alone or buffer to which β-glycosidase was added (ANOVA, F=0.11, P=0.74). Fecal pellets ground in buffer and distilled in Warburg flasks in 2006 yielded 2262±360 p.p.m. cyanide. Fecal pellets collected at the same time from this same colony but extracted directly in NaOH yielded 117±75 p.p.m. cyanide (Fig. 1).

Regurgitant collected in NaOH in 2002 from caterpillars following an overnight bout of feeding yielded 10±4 p.p.m. cyanide. Regurgitant collected in the same manner in 2007 from caterpillars that had just fed yielded 8±2 p.p.m. cyanide.

The foregut and midgut boluses of caterpillars distilled in buffer in 2002 contained 141±25 p.p.m. (N=12) and 137±38 p.p.m. (N=9)

cyanide, respectively. Of the eight foregut and midgut boluses of caterpillars extracted directly in NaOH in 2006, two had no detectable cyanide and six had 0.4±0.1 p.p.m. Six of seven hindgut boluses extracted directly in NaOH contained 44±18 p.p.m. cyanide.

Loss of HCN to atmosphere while feeding

When caterpillars were fed leaves in enclosed chambers, 10.4±1.1% of the HCN-p of the leaves was lost to the atmosphere. Simultaneous attack by 10 caterpillars left the partially consumed leaves with many tattered edges, and loss to the atmosphere is likely to be largely attributable to HCN escaping from these damaged surfaces.

pH of leaves, regurgitant and the bolus

The pH of five leaves from a *P. serotina* tree measured in September was 5.9±0.1. The pH of the regurgitant of caterpillars starved for 24 h was 12.0±0.1 while that of these same caterpillars allowed to feed to repletion on leaves of this tree was 12.0±0.0. The maximum pH of the bolus of caterpillars allowed to feed to repletion was 11.8±0.1 for the foregut, 11.0±0.1 for the midgut and 10.2±0.1 for the hindgut. The three values are significantly different from each other (Kruskal-Wallis ANOVA, H=25 and SNK subtest P>0.05). There was no significant difference between the pH of regurgitant collected from either starved or fed caterpillars and that of the foregut bolus (ANOVA, F=0.75, P=0.48).

Effect of gut pH on cyanogenesis

Cyanogenesis was greatest when leaf tissue was ground and distilled in buffer at pH 7–8 (Fig. 2). Cyanogenesis declined slightly at pH 9, and markedly at pH 10, to approximately 18% of its value at pH 7. No cyanogenesis occurred at pH 11.

Rate of food intake

Six caterpillars starved for 24 h and then allowed to feed to repletion fed for 46.8±4.7 min before resting. Mass gained from this single bout of feeding was 6.4±0.5 mg, a percentage mass gain of 4.0±0.3%. Five caterpillars allowed to feed *ad libitum* for 10.7±2.4 h had 49.6±11.9 discrete bouts of feeding during this period. These bouts of feeding, averaging 7.0±0.3 min, were alternated with resting periods averaging 5.6±0.2 min.

Extent of food processing

In one randomly chosen sample of 100 pellets, 61% were green and the remainder black to dark brown. Green pellets contained significantly more cyanide (2546±304 p.p.m.) than dark pellets (1342±370 p.p.m.) (Mann–Whitney Rank Sum Test, T=515, P<0.001). When rehydrated, dried pellets were shown to consist of fragments approximately 0.25 mm wide by 0.5–2 mm in length.

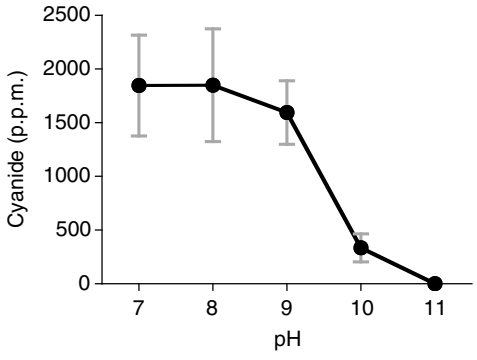

Fig. 2. Cyanide liberated from ground leaf tissue of *P. serotina* distilled in buffers of varying pH.

Susceptibility of caterpillars to cyanide poisoning

All four caterpillars subjected to the fumes of cyanide succumbed to the poison. The first indication of poisoning – regurgitation – occurred about two minutes after the caterpillars were placed in the chamber. After approximately 5 min, there was pronounced trembling of the thoracic prolegs accompanied by trembling of the head, followed, a few minutes later, by trembling of the abdominal prolegs. 10–12 min after being placed in the chamber, the caterpillars doubled over, in some cases so far as to bring the head into contact with the last set of abdominal prolegs. Thereafter, the caterpillars remained motionless. The caterpillars were removed from the chambers after one hour and observed over the next 24 h. Two showed no recovery while the other two showed partial recovery but were permanently injured and eventually died.

DISCUSSION

While the seasonal decline in the HCN-p of the cherry leaves assayed in this study is consistent with the results of a previous study undertaken in Kentucky (Smeathers et al., 1973), the trees sampled did not decline as precipitously. Smeathers et al. reported a mean HCN-p of 2472 p.p.m. for *P. serotina* leaves at the beginning of the growing season in mid-April but their data indicate that the mean HCN-p of leaves declined to less than 1000 p.p.m. by mid May and to less than 500 p.p.m. by September (Smeathers et al., 1973). Fitzgerald et al. found a mean HCN-p of 3032 p.p.m. for new, unfolding, tip leaves collected from the Cortland area in June of 2001 (Fitzgerald et al., 2002) but they did not measure the HCN-p of fall leaves. Peterson et al. (Peterson et al., 1987) reported that the tip leaves of black cherry sampled in May and June on Long Island, NY, USA had a HCN-p of 1845 p.p.m. Santamour measured cyanide in black cherry from June to September in Washington, DC, USA and reported a mean HCN-p of 2644 p.p.m. (Santamour, 1998). Thus, these previous reports indicate that different populations of *P. serotina* trees vary significantly in both the maximum HCN-p and the rate at which cyanogenic potential declines as the season progresses. Indeed, leaves collected from *P. serotina* trees on 2 August 2002 from nearby Tompkins County in conjunction with another study contained only 1068±169 p.p.m. cyanide (T.D.F., personal observation).

Wheeler and Bennington found that feeding by the larvae of *Agraulis vanillae* on the sun-exposed leaves of *Passiflora incarnata* resulted in a decrease in the HCN-p of the leaves, while the cyanogenic potential of both damaged and intact shaded leaves did not differ (Wheeler and Bennington, 2001). They attributed the decline in HCN-p in sun-exposed leaves to an overall deterioration of the leaf and possible autotoxic effects of released cyanide following herbivory. The present study shows that damage inflicted on host foliage due to feeding by the webworm under laboratory conditions did not induce a short-term localized change in the cyanogenic potential of the plant and is consistent with the results of other studies of the influence of herbivory on the HCN-p of leaves. Gleadow and Woodrow found no difference in the concentration of cyanogenic glucosides in the intact and mechanically wounded leaves of *Eucalyptus cladocalyx* (Gleadow and Woodrow, 2000). In their study of the bird cherry-oat aphid, *Rhopalosiphum padi*, Leszczyński et al. found that sustained

feeding by the insect on *Prunus padus* had no significant effect on the cyanogenic glycoside content of the leaves (Leszczyński et al., 2003).

The present study shows that the webworm is vulnerable to cyanide poisoning yet feeds with no apparent ill effects on cherry leaves having significant HCN-p. Three mechanisms that enable caterpillars to feed on cyanogenic plants without harm have been described. Rhodanese (thiosulfate sulfur transferase) is widely distributed in insects and may be used by some species to catalyze the conversion of HCN to thiocyanate (Conn, 1979). Long and Brattsten (Long and Brattsten, 1982) and Beesley et al. (Beesley et al., 1985), however, assayed for rhodanese in 55 species of insects and found that it occurs at much lower levels than in vertebrates and suggested that it plays a minor role in cyanide detoxification in insects. Witthohn and Naumann (Witthohn and Naumann, 1987) and Meyers and Ahmad (Meyers and Ahmad, 1991) conducted studies suggesting that detoxification of cyanide by L-3-cyanoalanine synthase may be the primary mechanism employed by insects. In the southern army worm, *Spodoptera eridania*, and the cabbage looper, *Trichoplusia ni*, L-3-cyanoalanine synthase is associated with the mitochondria (Meyers and Ahmad, 1991). Engler et al. reported that *Heliconius sara* possesses an enzyme system that enables the insect to convert a cyclopentenyl cyanogen derived from host *Passiflora* leaves to a compound in which a thiol group replaces the cyanogen's nitrile group (Engler et al., 2000). This not only precludes the production of cyanide but also allows the released nitrogen to be metabolized to useful compounds.

None of these previously described mechanisms accounts for the ability of the webworm to feed on cherry. Two interrelated factors enable the webworm to process cyanogenic leaves without succumbing to the toxin: high foregut alkalinity and a capacious foregut. Cyanogenesis in crushed leaves of *P. serotina* is strongly inhibited at pH 10 and completely inhibited at pH 11 (Fig. 2). The maintenance of gut alkalinity in excess of these values in the presence of the bolus largely suppresses cyanogenesis. This is supported by the relatively small quantities of cyanide recovered from NaOH extracts of regurgitant and the boluses of the fore and midguts compared to the amounts in the ingested leaf. Moreover, frass extracted directly in NaOH yielded only 5% of the cyanide obtained from distilled frass, indicating that the cyanogen survives gut transit (Fig. 1). The presence of significantly greater quantities of cyanide in the hindgut bolus compared with the anterior gut compartments is consistent with the lower pH of this compartment.

Foregut capacity coupled with a relative low rate of ingestion may enable the webworm to maintain an alkaline gut in the presence of the bolus. The foregut of the webworm constitutes 49% of the total length of the alimentary tract, proportionately the longest of any of 33 species of caterpillars measured by Grant (Grant, 2006); mean=19%. By contrast, the foregut of the eastern tent caterpillar constitutes 21% of the total gut length (Grant, 2006). Following a single bout of feeding, the foregut of the tent caterpillar is fully packed with leaf fragments to the extent that the bolus strains its capacity (Snodgrass, 1922). The pH of the foregut bolus of the tent caterpillar is between 6 and 7 (T.D.F., unpublished data) and both the regurgitant (Peterson et al., 1987) and the foregut bolus exude the strong odor of benzaldehyde and contain levels of cyanide consistent with unrestrained cyanogenesis (Fitzgerald et al., 2002). The present study shows that during the 6th and 7th larval stadia, webworms alternate brief periods of feeding and rest. Despite the near constant intake of food, observations of starved caterpillars allowed to feed to repletion indicate that the amount ingested during these feeding bouts is small. Thus, the capacious foregut of the caterpillars is much more loosely packed with food than that of the tent caterpillar, the bolus is watery and it bears no trace odor of benzaldehyde. The production of large quantities of green fecal pellets also indicates rapid passage of the bolus through the webworm's alimentary tract. The presence of nearly twice as much cyanide in green pellets than in dark pellets suggests that the former move through the gut more rapidly and may be less subjected to digestive processes that deplete the cyanogen or its enzymes.

There are few data regarding the amount of cyanide occurring in the fecal pellets of insects that feed on cyanogenic plants. Fitzgerald et al. (Fitzgerald et al., 2002) found that the frass of eastern tent caterpillars fed the highly cyanogenic young leaves of *P. serotina* contained 63–85 p.p.m. cyanide. Alonso-Amelot et al. determined that the frass of *Helioconius erato* and *Spodoptera frugiperda* fed the cyanogenic plant *Passiflora capsularis* contained an average of approximately 500 and 1000 p.p.m., respectively, and that, as in the case of the tent caterpillar, most of the cyanide did not survive gut transit (Alonso-Amelot et al., 2006). Although the frass of the webworm contains relatively large quantities of cyanide compared with these species, the present study indicates that a significant proportion of the HCN-p of the leaf is not recoverable as cyanide in the caterpillar's frass. *P. serotina* leaves collected in late summer lost an average of 58% of their mass when oven dried. The aeration study showed that an average of 10.5% of the HCN-p of the leaf is lost to the atmosphere as the caterpillars feed. Schroeder and Malmer found that the webworm feeding on *P. serotina* had an assimilation of efficiency of 38% (Schroeder and Malmer, 1980). When loss of mass due to assimilation and drying and loss of HCN to the atmosphere are considered collectively, dry fecal pellets of webworms would be expected to have approximately 3.5 times as much cyanide per unit mass as the leaf tissue they ingest if all of the ingested cyanogen were transferred to the frass. The frass of caterpillars fed leaves collected in 2002 with a mean HCN-p of 1522 p.p.m. had 45.6% of the expected value while those fed leaves collected in 2005 with a mean HCN-p of 1592 p.p.m. had 51.5%. Although some cyanogensis occurs in the hindgut, this loss may be largely attributable to the destruction of prunasin since the addition of β-glucosidase to the Erlenmeyer flasks during the distillation process did not significantly increase the yield of cyanide.

Alkalinization of the midgut in larval Lepidoptera is due to the secretion of K^+ in the gut lumen by epithelial goblet cells (Dow, 1984; Dow, 1992; Moffett and Koch, 1992). As Dow (Dow, 1992) noted, the development of microprobes for measuring pH revealed that the midguts of caterpillars are markedly more alkaline than earlier reports suggested. Thus, while Berenbaum's (Berenbaum, 1980) survey of the literature indicated that the midgut pH of 60 species of caterpillars ranged from 7.0 to 10.3, with the majority <9.0, measurements with microelectrodes showed that caterpillar midguts have the highest pH values of any biological system. Dow (Dow, 1981) recorded a peak pH of 12.0 in the midgut fluids of the death's head hawkmoth, *Acherontia atropos*, while Schultz and Lechowicz recorded pH values as high as 12.4 in the midgut of the gypsy moth, *Lymantria dispar* (Schultz and Lechowicz, 1986). Since the midgut is the site of digestion, interest in the effect of gut pH on the bolus has focused almost exclusively on this compartment of the alimentary tract and there are few data on the pH of the foregut. pH values reported for the webworm in the present study appear to be the highest yet recorded for the

regurgitant and foregut fluids of any insect. As in this study, Krishnan et al. found that pH of the foregut of the last instar larva of the beetle *Leptinotarsa decemlineata* (7.0) exceeded that of the midgut (5.4–6.3) (Krishnan et al., 2007) but did not speculate on the physiological basis for this. In the webworm, lower midgut alkalinity may be a consequence of both the digestive process and food packing, although neither of these possibilities was explored in the present study.

It has been proposed that the high midgut pH of caterpillars may allow them to tolerate plant allelochemicals that can interfere with digestion (Berenbaum, 1980; Govenor et al., 1997) and facilitate the extraction of nutrients from plant material (Felton and Duffey, 1991). In the webworm, the maintenance of a highly alkaline foregut environment inhibits cyanogenesis at the point where food particles first enter the caterpillar's body and allows the caterpillar to feed on an otherwise toxic plant. However, with 400 recorded host plant species (Wagner, 2005), the webworm has one of the widest host ranges of any caterpillar, and the significance of high foregut alkalinity in this broader context is unknown. In itself, it is unlikely to account for the insects' broad food base. Schultz and Lechowicz (Schultz and Lechowicz, 1986) and Appel and Maines (Appel and Maines, 1995) reported a mean pH of 7.8 for the empty foregut of the caterpillars of *L. dispar*, a generalist caterpillar with an even larger host range than the webworm (Wagner, 2005).

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REFERENCES

Alonso-Amelot, M. E., Ávila-Núñez, L. J., Duarte, L. and Oliveros-Bastidas, A. (2006). Hydrogen cyanide release during feeding of generalist and specialist lepidopteran larvae on a cyanogenic plant, *Passiflora capsularis. Physiol. Entomol.* **31**, 307-315.

Appel, H. M. and Maines, L. W. (1995).The influence of host plant on gut conditions on gypsy moth (*Lymantria dispar*) caterpillars. *J. Insect Physiol.* **41**, 241-246.

Beesley, S. G., Compton, S. G. and Jones, D. A. (1985). Rhodanese in insects. *J. Chem. Ecol.* **11**, 45-50.

Berenbaum, M. R. (1980). Adaptive significance of midgut pH in larval lepidoptera. *Am. Nat. Sci.* **115**, 138-146.

Brinker, A. M. and Seigler, D. S. (1992). Determination of cyanide and cyanogenic glycosides from plants. In *Plant Toxin Analysis* (ed. H. F. Linskens and J. F. Jackson), pp. 360-381. Berlin: Springer-Verlag.

Conn, E. E. (1979). Cyanide and cyanogenic glycosides. In *Herbivores: Their Interaction with Secondary Plant Metabolites* (ed. G. A. Rosenthal and D. H. Janzen), pp. 387-412. New York: Academic Press.

Dow, J. A. T. (1984). Extremely high pH in biological systems: a model for carbonate transport. *Am. J. Physiol.* **246**, R633-R636.

Dow, J. A. T. (1992). pH gradients in lepidopteran midgut. *J. Exp. Biol.* **172**, 355-375.

Engler, H. S., Spencer, K. C. and Gilbert, L. E. (2000). Preventing cyanide release from leaves. *Nature* **406**, 144-145.

Felton, G. W. and Duffey, S. S. (1991). Reassessment of the role of gut alkalinity and detergency in insect herbivory. *J. Chem. Ecol.* **17**, 1821-1836.

Fitzgerald, T. D. (1995). *The Tent Caterpillars*. Ithaca, NY: Cornell University Press.

Fitzgerald, T. D., Jeffers, P. M. and Mantella, D. (2002). Depletion of host derived cyanide in the gut of the eastern tent caterpillar, *Malacosoma americanum*. *J. Chem. Ecol.* **28**, 257-268.

Gleadow, R. M. and Woodrow, I. E. (2000). Temporal and spatial variation in cyanogenic glycosides in *Eucalyptus cladocalyx*. *Tree Phys.* **20**, 591-598.

Govenor, H. L., Schultz, J. C. and Appel, H. M. (1997). Impact of dietary allelochemicals on gypsy moth (*Lymantria dispar*) caterpillars: importance of midgut alkalinity. *J. Insect Physiol.* **12**, 1169-1175.

Grant, J. B. (2006). Diversification of gut morphology in caterpillars is associated with defensive behavior.*J. Exp. Biol.* **209**, 3018-3024.

Hu, Z. and Poulton, J. E. (1999). Molecular analysis of (R)-(+) mandelonitrile lyase microheterogenity in black cherry. *Plant Physiol.* **119**, 1535-1546.

Krishnan, N., Kodrík, D., Turanli, F. and Sehnal, F. (2007). Stage-specific distribution of oxidative radicals and antioxidant enzymes in the midgut of *Leptinotarsa decemlineata*. *J. Insect Physiol.* **53**, 67-74.

Leszczyński, B., Józwiak, B., Urbańska, A. and Dixon, A. F. G. (2003). Does cyanogenesis influence host alternation of bird cherry-oat aphid? *Electr. J. Pol. Agric. Univ.* **6**.

Long, K. Y. and Brattsten, L. B. (1982). Is rhodanese important in the detoxification of dietary cyanide in southern armyworm (*Spodoptera eridania* Cramer) larvae? *Insect Biochem.* **12**, 367-375.

Meyers, D. M. and Ahmad, S. (1991). Link between L-3-cyanoalanine synthase activity and differential cyanide sensitivity of insects. *Biochem. Biophys. Acta* **1075**, 195-197.

Moffett, D. F. and Koch, A. (1992). The insect goblet cell: a problem in functional cytoarchitecture. *News Physiol. Sci.* **7**, 19-23.

Peterson, S. C., Johnson, N. D. and Leguyader, J. L. (1987). Defensive regurgitation of allelochemicals derived from host cyanogenesis by eastern tent caterpillars. *Ecology* **68**, 1268-1272.

Santamour, F. S., Jr (1998). Amygdalin in *Prunus* leaves. *Phytochemistry* **47**, 1537-1538.

Schroeder, L. A. and Malmer, M. (1980). Dry matter, energy and nitrogen conversion by Lepidoptera and Hymenoptera larvae fed leaves of black cherry. *Oecologia*. **45**, 63-71.

Schultz, J. C. and Lechowicz, M. J. (1986). Host plant, larval age, and feeding behavior influence midgut pH in the gypsy moth (*Lymantria dispar*). *Oecologia* **71**, 133-137.

Smeathers, D. M., Gray, E. and James, J. H. (1973). Hydrocyanic acid potential of black cherry leaves as influenced by aging and drying. *Agron. J.* **65**, 775-777.

Snodgrass, R. E. (1922). The tent caterpillar. *Ann. Rep. Smithson. Inst.* **1922**, 329-362.

Wagner, D. L. (2005). *Caterpillars of Eastern North America: A Guide to Identification and Natural History*. Princeton, NJ: Princeton University Press.

Wheeler, H. C. and Bennington, C. C. (2001). Effects of light availability on herbivory and defense chemistry in *Passiflora incarnata*. *Fla. Sci.* **64**, 237-245.

Witthohn, K. and Naumann, C. M. (1987). Cyanogenesis – a general phenomenon in the Lepidoptera? *J. Chem. Ecol.* **13**, 1789-1809.