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Effects of Cyanogenic Plants on Fitness in Two Host Strains of the Fall Armyworm (*Spodoptera frugiperda*)

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Abstract The generalist moth, *Spodoptera frugiperda* (J. E. Smith) consists of two genetic subgroups (host strains) that differ in their distribution among host plant species. The corn strain prefers crop plants such as corn, sorghum, and cotton, while the rice strain is found in small grasses such as *Cynodon* spp. and rice. Little is known about the physiological factors that drive this host preference. Here, we report a feeding study with natural host plants and an artificial diet containing cyanide. We found that corn, two *Cynodon* spp. (bermudagrass *C. dactylon* (L.) Persoon, 'NuMex Sahara', and stargrass *C. nlemfuensis* var. *nlemfuensis* Vanderyst, 'Florona'), and a hybrid between bermudagrass and stargrass, 'Tifton 85', exhibited differences in the concentration of the cyanogenic precursors or cyanogenic potential (HCNp) and the release of hydrogen cyanide per unit time or cyanogenic capacity (HCNc). Corn plants released low levels of hydrogen cyanide, while stargrass had greater HCNp/HCNc than bermudagrass and 'Tifton 85'. Feeding studies showed that corn strain larvae experienced higher mortality than the rice strain when fed stargrass or artificial diet supplemented with cyanide. Also, corn strain larvae excreted higher levels of cyanogenic compounds than the rice strain when fed *Cynodon* spp. These differences in excretion suggest potential disparities in cyanide metabolism between the two strains. We hypothesize that differences in the susceptibility to cyanide levels in various host plants could play a role in driving strain divergence and what appears to be the incipient speciation of this moth.

Key Words Life histories · Cyanogenic glycosides · Cyanogenesis · *Cynodon* spp. · Plant-insect interaction · *Spodoptera frugiperda*

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Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) is a polyphagous species that attacks important agricultural crops in semitropical and tropical regions (Sparks, 1979). Populations comprise two genetically differentiated strains with distinct host preference (Pashley et al., 1987; Pashley, 1988a; Lu and Adang, 1996; Nagoshi and Meagher, 2003). The rice strain is found primarily in grasses (*Cynodon* spp.) and rice (*Oryza sativa*) (Pashley, 1988a; Meagher et al., 2007), whereas the corn strain prefers corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], and cotton (*Gossypium hirsutum* L.) (Pashley, 1988a; Nagoshi et al., 2007). The full range of host preferences presently is unclear; plants consumed by larvae of *S. frugiperda* belong to 23 families constituting more than 80 plant species (Luginbill, 1928; Pashley, 1988b). Feeding studies have been performed only on a small subset of these host plants.

Many *Cynodon* grasses are cyanogenic (Georgiadis and McNaughton, 1988; Pederson and Brink, 1998; Mahmoodzadeh, 2010), whereas corn plants release low levels of hydrogen cyanide (Brünnich, 1903; Jones, 1998) upon tissue disruption. This raises the possibility that differential resistance to cyanogenic glycosides could be a factor in strain-specific host preference. Cyanogenic glycosides are defined as O-β-glycosides

of α-hydroxynitriles (cyanohydrins), biosynthetically derived from amino acids. They generally co-occur with β-glycosidases that can cleave the glycoside and release cyanide and carbonyl compounds upon damage to the plant. Both cyanide and carbonyl compounds are capable of conferring protection from herbivory and/or pathogens (Harborne, 1982). However, recent evidence has shown that high levels of cyanide (HCN) disrupt the enzymatic activity of polyphenol oxidase (PPO), enhancing the plant susceptibility to fungi (Lieberei, 1988; Ballhorn et al., 2010a; Ballhorn, 2011). Thus, there is a chemical trade-off for plants to resist herbivores or pathogens (Ballhorn et al., 2010a).

There have been several studies that compare the viability and development of the two strains when grown on different hosts (Pashley, 1988a; Quisenberry and Whitford, 1988; Whitford et al., 1988; Pashley et al., 1995; Meagher et al., 2007). In general, the rice strain tended to develop faster than the corn strain when fed on its preferred host, *C. dactylon*, (Whitford et al., 1988; Pashley et al., 1995; Meagher et al., 2007). Comparisons of the survivorship of the two strains on different *Cynodon* hosts were less consistent, with either no significant strain differences observed (Pashley et al., 1995; Meagher et al., 2007) or higher corn strain viability (Whitford et al., 1988). In particular, a past study from this laboratory found that stargrass, a *Cynodon* species frequently found in pastures, was a good host for the development of both strains, with high viability and developmental rates (Meagher et al., 2007).

This paper re-examines the issue of differential host plant usage by the two strains from the perspective of cyanide toxicity. The study was based on unexpected results from feeding studies that appeared to vary from past findings by this laboratory. It was found that a stargrass diet caused a substantial mortality in both strains, with significantly higher levels of larval and pupal mortality observed in the corn strain compared to the rice strain. The possibility that this might be due to high, but transient, levels of cyanogenic compounds produced in cut stargrass was examined. The ramifications of these findings to our understanding of strain-specific host preferences and to the interpretation of past feeding studies are discussed.

Methods and Materials

Plants and Insects Plants were cultivated in glass houses (5×8 m; Lord and Burnham, High Falls, NY, USA) under controlled temperature (22°C), humidity (80%) and photoperiod (14:10-h light/dark). 'Trucker's Favorite' corn and 'NuMex Sahara' bermudagrass plants grew from seeds, while stargrass (*C. nlemfuensis* Vanderyst var. *nlemfuensis* 'Florona') and 'Tifton 85' (a F_1 hybrid pentaploid between the bermudagrass PI 290884 (in the literature as 'Tifton 292') and the stargrass 'Tifton 68') (Burton, 2001) grew vegetatively from stolons in a 4:1 standard substrate (Metro mix 500) that was mixed with leveling sand. Plants were watered once a day and fertilized with 3.1 g/l Miracle Grow water soluble 24:8:16 once a week (The Scotts Company, LLC, Marysville, OH, USA). Two fall armyworm host strains were subjected to feeding experiments with corn and stargrass. The corn strain colony originated from collections of larvae made from corn at the University of Florida Dairy Research Unit, Hague, Alachua Co. FL, USA, while the rice strain colony originated from collections of larvae from pasture grasses at the University of Florida Range Cattle Research and Education Center, Ona, Hardee Co. FL, USA. These two host strains were identified by molecular markers (Levy et al., 2002) as modified by Nagoshi and Meagher (2003), and are currently maintained at the USDA, ARS, CMAVE Insect Behavior and Biocontrol Unit in Gainesville, FL, USA.

Larval Excretion and Adult Body Studies The excreta of 75 4th instars comprising 25 that were fed 'NuMex Sahara', 25 that were fed stargrass, and 25 that were fed 'Tifton 85' were collected after 3–5 h of feeding. Larvae were reared in plastic Sterilite tubs (Sterilite Corporation, Townsend, MA, USA) [26 (d) × 12.1 (h) cm] containing metal screens with a mesh size of 0.7 cm. Plant material was placed on top of the screen, and feces was collected on paper towels that lined the bottom of the tubs. The HCNp of the feces was determined as described below. Larvae were allowed to complete development on their host treatment and the emerged adult bodies were tested for cyanogenesis.

Cyanogenic Potential (HCNp) In order to measure the amount of cyanide released from a given tissue, or cyanogenic potential, the primary blades and the stems from corn, stargrass, 'NuMex Sahara' and 'Tifton 85' were assessed for HCNp during the winter of 2009. Feces from the insect trials, and eight adult moths fed on each diet, also were tested for HCNp.

Cyanogenic potential of plants was quantified by the Lambert procedure (Lambert et al., 1975) as modified by Brinker and Seigler (1989). The plant parts were ground to a fine powder in liquid nitrogen and added to a 10-ml vial containing 0.1 M phosphate buffer (pH 6.8). A 5-ml vial containing fresh 1 M NaOH solution then was placed inside the 10-ml vial, and the 10-ml vial was stoppered tightly and incubated at 37°C overnight. Samples were prepared quickly to avoid loss of HCN. During incubation, HCN released from plant tissue was trapped in the NaOH solution and formed NaCN. After incubation, cyanide was quantified spectrophotometrically (Biotek μQuant microplate, Rockville, MD, USA) using the reagents detailed in Brinker and Seigler (1989). The absorbance was measured at 580 nm. A cyanide standard curve was prepared with

0.02 M solution of NaCN in 0.1 N NaOH (0.980 g NaCN/L). The exact amount of cyanide in that solution was determined by a modified Liebig titration method as described by Brinker and Seigler (1989).

On occasion, plants, animals, or feces that are cyanogenic can yield negative results in tests for cyanogens. The organism may lack cyanogenic glycosides, or may contain cyanogenic glycosides, but not the necessary β-glucosidases needed to release the cyanide. In the latter case, the addition of exogenous β-glucosidase is necessary to quantify the HCNp. Since the cyanogenic glycosides in *Cynodon* spp. are unidentified, a low specificity β-glucuronidase (β-glucuronidase Type H-1 from *Helix pomatia* Müller, Sigma Chemical Co., St. Louis, MO, USA), which releases cyanide from all cyanogenic glycosides, was added to all samples (Brimer et al., 1983; Jaroszewski et al., 2002, Hay-Roe, 2004). For analysis of the HCNp in excreta, we added 500 μl of β-glucuronidase (149 units/ml β-glucuronidase) after thawing the samples in 0.1 M phosphate buffer (pH 6.8). The homogenate then was incubated at 37°C, and HCNp was determined as described above. Eight adult moths fed on each diet were tested for cyanogenesis using samples containing 250 μl of added β-glucuronidase per sample.

Subsamples of ground plant tissue, adult moth bodies, or insect excreta prepared for the cyanogenesis analyses were dried separately at 65°C until they reached a constant dry weight. The dry weight was used to calculate the μg HCNp/g dry weight of plant tissue, adult body tissue, or dry feces. Three replicates each were performed for the ground plant tissue and the insect excreta.

Cyanogenic Capacity (HCNc) Plant tissues were assayed to determine the amount of hydrogen cyanide released per unit of time, a metric known as cyanogenic capacity (HCNc). We were interested only in relative comparisons of HCNc to rank the different plant hosts. Feigl-Anger cyanide test strips were used to quickly and economically provide qualitative information (Feigl and Anger, 1966). The primary blades of 'NuMex Sahara', 'Tifton 85', and stargrass were ground and placed in 5-cm vials with a test strip in each vial. The vial was closed immediately, and patterns of HCN released were tracked every hour for 7 h. Color changes in the test strips were ranked as 0, 1, 2, or 3, according to the intensity of the blue color. Three separate samples were analyzed per plant.

Feeding Experiments A subset of our laboratory colonies was raised on natural host plants for at least three generations before subjecting the neonates to feeding experiments. The corn strain was maintained on greenhouse-grown 'Truckers Favorite' corn, while the rice strain was maintained on greenhouse-grown 'Florona' stargrass. These cohorts were maintained in environmental chambers at 26°C, 75% humidity and a 14:10 h L:D photoperiod.

In total, 120 neonates derived from 30 females of both host strains were raised separately in 1-oz. acrylic SOLO cups with cardboard lids. Fresh food was provided every 8 h during the day. Caterpillars were checked for the presence of head capsule apolysis on a daily basis and the developmental time of each instar was recorded. Head capsule measurements were made with a Wild Heerbrugg M5A microscope and an ocular micrometer. The following formula was used to calculate growth rate:

$$\text{Growth rate} = (\ln(H_f) - \ln(H_i))/t,$$

where H_f is the measurement of the last instar head capsule width; H_i is the measurement of the 1st instar head capsule width; and t is the larval developmental time. The growth rate is approximately the relative size increase on any given day.

The day when pupae formed was recorded as the first day of pupation. Pupal development was complete when the adult moth emerged from the pupal case. Each pupa was weighed 2 d after pupation. Adult sex was recorded and wing length was measured from the base of the wing to the apex using a Vernier caliper (Caliper 2,000 mm) graduated to 0.1 mm. Mass measurements were made with an electronic balance accurate to the nearest 0.1 mg.

Tolerance to Sodium Cyanide In order to determine the toxic effects of cyanide, third, fourth, and fifth instars of the corn and the rice strains were reared on a pinto bean artificial diet (Guy et al., 1985) containing either 0.5% or 0.1% NaCN, which was replaced once a day. Control larvae were fed unamended pinto bean diet. Larvae were raised individually in 1-oz. cups, and body weight, larval developmental time, and mortality rates were recorded.

In order to determine possible habituation to high cyanide concentration, three replicates of 20 newly-molted fourth instars of both strains were fed a 0.5% NaCN-containing pinto bean diet, with replacement of the cyanide-containing diet every 8 hr. When larvae began to molt to the fifth instar, they were transferred to 1% NaCN-containing diet that was replaced every 3 hr during the day (Brattsten et al., 1983).

Statistical Analysis Two-way analysis of variance was used to analyze the feeding experiment, the HCNp in plants, and the HCNp in insect excreta. A Tukey test was used to compare differences between different categories of a discrete variable. Head capsule and HCN measurements were transformed to a natural logarithm to alleviate skewness. A regression analysis was performed to correlate pupal weight and adult size. Data collected in the test of tolerance to NaCN-containing diet were compared to the control pinto bean diet data with the Student paired *t*-test. We used SYSTAT v. 12 (SYSTAT Software, Inc. San Jose, CA, USA) to perform statistical analysis.

Results

Cyanogenic Potential (HCNp) in the Primary Host Plants of Fall Armyworm Each species of plant used in this study had a distinct HCNp. The highest concentration of cyanide was observed in stargrass (1.54 mg HCN/g dry leaf tissue). This level was significantly higher ($F_{3, 40}$=348.2, P<0.001) than the levels found in the bermudagrass line 'NuMex Sahara' (0.16 mg HCN/g dry leaf tissue) or in the hybrid 'Tifton 85' (0.10 mg HCN/g of dry leaf tissue) (Fig. 1). In contrast, corn plants had low HCNp (0.01 mg HCN/g dry leaf tissue) (Fig. 1). Cyanogenic potentials in leaves were higher than in stems for both'NuMex Sahara' and 'Tifton 85' (P<0.05). HCNp in corn leaves and stems and in stargrass leaves and stems were not significantly different (P >0.05) (Fig. 1).

Cyanogenic Capacity (HCNc) in Grasses To estimate the temporal pattern of cyanide release from cut grasses, we determined cyanogenic capacity (HCNc). HCNc is a measure of the release of hydrogen cyanide per unit time (Alonso-Amellot and Oliveros-Bastidas, 2005; Ballhorn et al., 2005, 2010b). Large amounts of cyanide were released from stargrass within seconds after tissue injury, and the cyanide slowly dissipated and became undetectable after 8 hr (Table 1). A similar result was obtained with 'NuMex Sahara', but with lower levels of cyanide released during the first 5 hr. 'Tifton 85' had the lowest HCNc, with detectable cyanide only in the first hour after tissue injury (Table 1). This analysis defined the frequency with which host plant clippings had to be replaced in feeding studies in order to maintain exposure to plant cyanogenic compounds.

Feeding Experiments Laboratory feeding studies were used to compare mortality and developmental growth rates of the two host strains fed either corn or stargrass (Table 2). Both strains had <10% mortality when grown on corn. In comparison, using stargrass as a host resulted in high mortality, with the mortality level for corn strain larvae (77%) significantly higher than that for rice strain larvae (50%) (Z=-2.14, P<0.05). Larvae fed stargrass became dark, lethargic, and flaccid before dying. Pupae developed through the third day of pupation, but then became darker and dried out; some pupae were malformed. The difference in mortality between strains was limited to the larval stage. The few corn strain individuals that emerged to adulthood (N=7) were morphologically normal.

Despite the higher mortality, corn strain larvae had higher growth rates (1.06±0.02 mm/day) (mean ± SE) than rice strain larvae (1.00±0.02 mm/day) ($F_{1,107}$=8.3, P<0.005), with both strains displaying higher growth rates on corn (1.08±0.02 mm/day) than on stargrass (0.98±0.02 mm/day) ($F_{1, 107}$=18.8, P<0.001). Corn strain larvae (20.4±0.5 days) took longer to develop than rice strain larvae (17.6±0.3 days) ($F_{1, 89}$=10.8, P=0.001), no matter which diet was consumed. However, larvae of either strain feeding on corn (20.3± 0.3 days) took 4 days longer to develop than those feeding on stargrass (16.1±0.3 days) ($F_{1, 89}$=59.3, P<0.001). Pupal developmental time in the corn strain (12.0±0.2 days) was 1 day longer compared to the rice strain (11.2±0.2 days) ($F_{1, 74}$=4.7, P=0.03) regardless of diet. When both strains were fed corn they had a longer pupation time than when fed stargrass ($F_{1, 74}$=17.3, P<0.0001). A regression analysis showed that adult size was positively correlated with differences in pupal size in both host strains (P <0.001). The few surviving corn strain adults that had been fed stargrass had significantly longer wings than their rice strain counterparts (P=0.02), while no significant adult size difference was observed when both strains were fed corn (Table 2).

Larval Excretion Studies Fecal matter from larvae feeding on cyanogenic grasses was collected and examined for cyanogenic potential per fecal weight (μg HCNp/g). There was no significant interaction between host strain and plant host (ANOVA, P=0.46), so comparisons are shown between host strains and among grass diets. Corn strain larvae excreted significantly higher levels of cyanogenic compounds than rice strain larvae (Fig. 2a). Roughly equal amounts of cyanogenic compounds were excreted when the corn and rice strains fed on stargrass and 'Tifton 85',

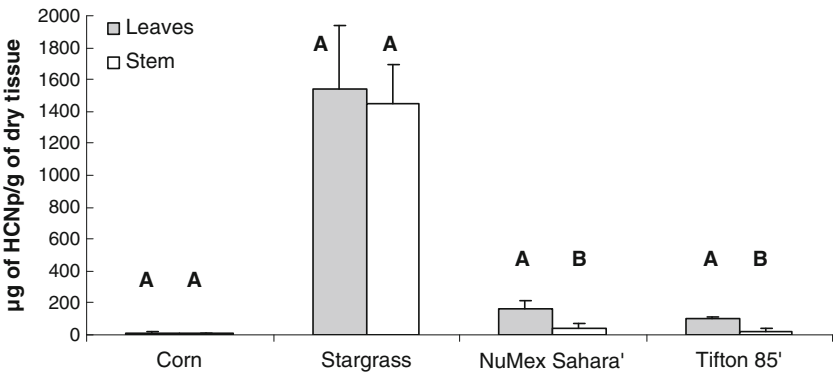

Fig. 1 Spectrophotometric measurement of HCNp from corn, stargrass, 'NuMex Sahara', and 'Tifton 85' leaves and stems. Letters indicate significant differences between plant parts according to *post hoc* analysis (Tukey's HSD P< 0.05). Bars indicate standard error. Mean of three replicates

Table 1 Cyanogenic capacity (HCNc) of injured *Cynodon* spp. plant tissue monitored over an hourly basis with Feigl-Anger strips

Cynodon spp.	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h
Stargrass	3	3	2	2	2	1	1	1	0
'NuMex Sahara'	2	2	1	1	1	0	0	0	0
'Tifton 85'	1	0	0	0	0	0	0	0	0

Progress of hydrolysis: no reaction (0), trace (1), present (2), extreme (3)

and levels for both strains were significantly higher than levels in excretions from larvae consuming the 'NuMex Sahara' diet (Fig. 2b).

Adult moths from both strains were acyanogenic regardless of diet. The addition of β-glucuronidase did not alter the results.

Tolerance to Sodium Cyanide To test more directly whether the two strains differed in their tolerance to cyanide toxicity, feeding studies were performed in which inorganic cyanide (NaCN) was added to an artificial diet. Cyanide concentrations of 0.05% and 0.1% NaCN did not increase larval mortality, but did retard larval development time for both strains. Corn and rice strain 5th instars took a longer time to develop when feeding on either the 0.05% NaCN-diet (corn strain: *t*-test df=25, t=-2.2, P =0.04; rice strain: *t*-test df=28, t=-10.4, P <0.001), or the 0.1% NaCN-diet (corn strain: *t*-test: df=24, t=-3.262, P=0.003; rice strain: *t*-test df=29, t=-8.3, P <0.001) compared to larvae of each strain feeding on the control diet (Table 3). A similar delay in rice strain larval development was reached in the 4th instar for both the 0.05% NaCN-diet (*t*-test df=29, t=-2.8, P=0.009) and 0.1% NaCN-diet (*t*-test df=29, t=-10.0, P<0.001) (Table 3). Neither NaCN treatment produced a significant change in corn strain larval weight. A significant reduction in rice strain larval weight was observed for 3rd instars feeding on 0.1%NaCN (*t*-test df=29, t=2.1, P=0.04) and 5th instars on both NaCN concentrations (0.05%NaCN: *t*-test df=28, t=3.4, P<0.002; 0.1%NaCN: *t*-test df=29, t=4.9, P<0.001) (Table 3).

Substantial mortality was observed at higher concentrations of NaCN. In the habituation study, 58% of corn strain larvae died by the 5th instar on a 0.5% NaCN-diet. The larvae showed evidence of poisoning, such as flaccidness and regurgitation. An additional 19% of the surviving larvae died within 2 days of exposure to the 1% NaCN diet producing a total mortality of 77% by the end of the habituation experiment (Table 4). Only 13 corn strain adults (8 females and 5 males) survived the experiment. Dissection of the dead larvae showed necrosis of the crop and the anterior midgut and a peculiar overgrowth of the salivary glands. In contrast, 80% of the rice strain specimens survived to eclosion. Both strains produced significantly smaller pupae on the high NaCN-diet (Table 4).

Discussion

Corn and stargrass leaves have very different levels of cyanogenic compounds, suggesting that cyanide toxicity may explain elevated fall armyworm mortality when the latter is used as a host plant. Differences in the ability to metabolize or eliminate cyanide may be the physiological basis for the plant host biases exhibited by fall armyworm

Table 2 Percent mortality at different stages of development and adult wing lengths for corn strain (CS) and rice strain (RS) of *Spodoptera frugiperda*. Insects were fed corn or stargrass

Fall armyworm stage	Diet			
	Corn		Stargrass	
	CS($\bar{x} \pm SE$)	RS($\bar{x} \pm SE$)	CS($\bar{x} \pm SE$)	RS($\bar{x} \pm SE$)
Larval mortality (%)	6 [a]	3[a]	47[b]	20[c]
Pupal mortality (%)	0	0	30	30
Total mortality (%)	6[a]	3[a]	77[b]	50[c]
Adult wing length (cm)	1.51±0.01[a]	1.52±0.01[a]	1.50±0.03[a]	1.40±0.02[b]

*Mean comparisons are within strain. Letters indicate significant differences calculated by a *post hoc* (Tukey's HSD; P<0.001) after ANOVA test. Percentage mortality within a column followed by the same letter are not significant different (P>0.05; Z-test)

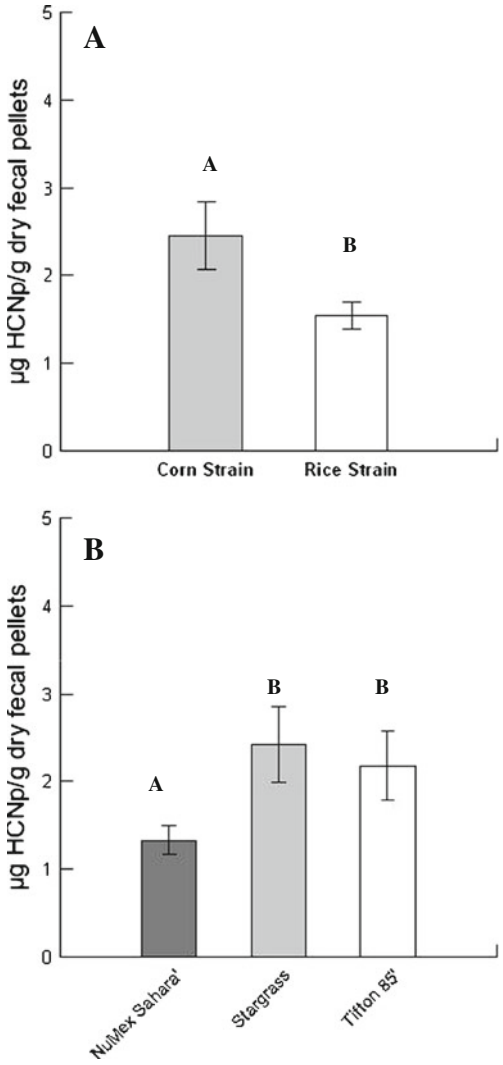

Fig. 2 Spectrophotometric measurement of HCNp from dry fecal pellets of corn and rice strain larvae fed'NuMex Sahara', stargrass, and 'Tifton 85'. **a** shows HCNp for corn strain vs. rice strain ($F_{1,18}$= 6.9, P=0.02). **b** shows HCNp for both strains fed each grass type separately ($F_{2,18}$=3.7, P<0.05). Letters indicate significant differences. Bars indicate standard error. Mean of three replicates

host strains. There are precedents for this mechanism of conferring host plant specificity. Cyanogenesis is a chemical defense mechanism of plants against herbivores, with cyanide inhibiting the electron transport chain in the cell respiratory pathway (Solomonson, 1974). However, some insects and other organisms have adapted to this toxicity and are able to overcome it by sequestration, synthesis, metabolic detoxification, excretion, or regurgitation of the cyanogenic compounds and/or cyanide (Conn, 1981; Nahrstedt, 1988; Jones, 1988; Zagrobelny et al., 2004). Although the function of cyanogenesis in interactions with generalist and specialist herbivores is not fully understood (Gleadow and Woodrow, 2002), there is evidence that plant selection by generalists differs from specialists due to cyanogenic features of the host plant (Engler-Chaouat and Gilbert, 2007; Ballhorn et al., 2010b).

Evidence that the two fall armyworm strains examined here differ in their response to cyanide exposure is based on two main results. The corn strain has significantly higher mortality levels when fed inorganic cyanide than the rice strain. The relative tolerance of the rice strain to cyanide toxicity is consistent with its preference for *Cynodon* species (Pashley, 1988a; Meagher et al., 2007) that produce substantial concentrations of cyanogenic compounds. The second observation is the statistically significant difference in cyanide levels in the fecal excretions of corn strain larvae when grown on cyanogenic grasses (Fig. 2). This indicates that there are physiological differences between strains for the processing, elimination, or tolerance of ingested cyanogenic compounds.

These results appear to contradict an earlier study from our laboratory that did not demonstrate increased mortality, or any aberrations in the development of fall armyworm, when grown on a stargrass diet (Meagher et al., 2007). We believe this apparent conflict is due to methodological differences in the two studies. In the previous study, plant cuttings used for diet, including stargrass, were replaced every 1–2 days. Given the lability of the cyanogenic compounds in cut plant tissue (Table 1), the availability of cyanide in the diets in our earlier study was probably intermittent at best. In the current study, the stargrass was replaced with fresh cuttings every 8 hr, thereby providing a more consistent exposure to cyanide.

In addition, Meagher et al. (2007) reared larvae in a chamber where the plant cuttings were placed on a water-saturated filter paper (to prolong the quality of the plant material). Because cyanide is water-soluble, this procedure had the potential to leach cyanide from the diets, thereby reducing the levels ingested. To avoid such leaching in this study, rearing containers were kept dry, and fresh plant cuttings were added more frequently. Cyanogenesis in stargrass may also vary substantially, depending on a number of environmental factors (Aguilera et al., 1982a,b, 1984). In particular, seasonal variation in cyanide concentration is a common trait of cyanogenic plants (Cooper-Driver et al., 1977; Kaplan et al., 1983; Jones and Rammani, 1985; Gebrehiwot and Beuselinck, 2001; Hay-Roe and Nation, 2007).

Finally, the young leaves of cyanogenic plants generally contain larger quantities of cyanide than mature leaves and stems (Conn, 1981; Goodger et al., 2007; Hay-Roe and Nation, 2007; Ballhorn et al., 2008). This phenomenon has been associated with higher concentrations of soluble protein in young leaves, compared to mature leaves (Ballhorn et al., 2008). In our present study, we found that leaves and stems of stargrass did not differ in their HCNp. Aguilera et al. (1985) reported that under certain conditions, related to

Table 3 Life history parameters at different stage of development of corn strain (CS) and rice strain (RS) of *Spodoptera frugiperda.* Insects were fed a control pinto bean diet, or the same diet amended with 0.05% or 0.1% NaCN

Fall armyworm stage	Control diet		0.05% NaCN		0.1% NaCN	
	CS($\bar{x} \pm SE$)	RS($\bar{x} \pm SE$)	CS($\bar{x} \pm SE$)	RS($\bar{x} \pm SE$)	CS($\bar{x} \pm SE$)	RS($\bar{x} \pm SE$)
3rd instar						
LDT (days)	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0
Larval weight (mg)	54.0±1.9	50.8±2.4	47.3±2.6	51.2±2.0	53.2±2.4	43.6±2.4*
4th instar						
LDT (days)	3.0±0.2	2.3±0.1	2.9±0.2	2.5±0.1*	2.8±0.1	3.3±0.1**
Larval weight (mg)	142.5±3.7	128.5±3.1	133.8±4.4	132.9±3.4	134.3±4.3	134.2±2.7
5th instar						
LDT (days)	6.0±0.3	5.3±0.1	6.4±0.2*	6.2±0.1**	6.6±0.2*	6.0±0.03**
Larval weight (mg)	239.2±5.0	225.2±3.0	240.1±5.0	209.3±3.6*	230.9±3.7	203.3±3.7**

Asterisks indicate significant differences from the control calculated by *t*-test * $P<0.05$, ** $P<0.001$. (LDT larval developmental time)

the amount of fertilizer and the testing period after plant fertilization, cyanide concentration in leaves and stems of *C. nlemfuensis* does not vary. Therefore, comparisons of feeding studies, even from the same laboratory, could be problematic if the potential variability in cyanide levels is not considered.

Cyanide poisoning in larvae generally was characterized by flaccidness in the body and regurgitation behavior, with dead larvae displaying crop necrosis and overgrowth of the salivary glands. Similar phenotypes including flaccid bodies and dried, dark and lacerate pupae, also were observed in the dead larvae and pupae that had been fed the stargrass diet. These observations suggest that cyanide poisoning may account in part for the reduced viability observed on stargrass. We also note that sublethal levels of NaCN in the diet produce significant delays in developmental time and larval weight (Table 3). Previous research showed that pinto bean artificial diet slows larval and pupal developmental time and that rice strain fed on this diet experienced lower growth rates than the corn strain (Quisenberry and Whitford, 1988; Hay-Roe, personal observations), suggesting that this variability is related to the pinto bean diet.

Table 4 Pupal weight and percent mortality after the habituation test of corn (CS) and rice strain (RS) of *Spodoptera frugiperda.* Insects were fed a control pinto bean diet, or the same diet amended from 0.5% to 1% NaCN

	Habituation			
	Control		1%	
	CS($\bar{x} \pm SE$)	RS($\bar{x} \pm SE$)	CS($\bar{x} \pm SE$)	RS($\bar{x} \pm SE$)
Pupal weight (mg)	214.2±4.1	201.4±2.5	168.2±7.2**	153.1±3.0**
Mortality (%)	0	2	77 **	20 **

Asterisks indicate significant differences from the control calculated by *t*-test ** $P<0.001$. Mortality rates differences were calculated by *Z*-test, $P<0.001$

The observation that the two strains differ in the amount of cyanide excreted while feeding on stargrass is intriguing but difficult to interpret (Fig. 2). The data indicate that the corn-strain is more efficient at eliminating cyanide, yet it is also the most susceptible to its toxic effects. These results suggest that excretion is not the primary mechanism used by either strain to detoxify cyanogenic compounds and that the strain difference observed may simply reflect the higher sensitivity of the corn strain to the toxic effects. In other words, the higher HCN levels in the corn strain feces may be an incidental consequence of physiological abnormalities caused by cyanide toxicity. The possibility that the metabolism of the toxic cyanogenic compounds involves insect digestive enzymes or formamide hydrolyase produced by pathogens of cyanogenic plants (Fry and Munch, 1975) cannot be discarded.

Adult moths were acyanogenic in both host strains, and this suggests that cyanogenic glycosides are not sequestered by the larvae and are not used as a protective mechanism by fall armyworm adults, as occurs in some other Lepidoptera (Franzl and Naumann, 1985; Zagrobelny et al., 2004). The larvae do regurgitate gut contents as a defensive mechanism against predators, but regurgitates from fall armyworm fed cyanogenic compounds were not analyzed for cyanogenesis.

In summary, evidence is presented that plant cyanogenic glycosides might play a significant role in defining the host range of the two fall armyworm strains. Differences in cyanide levels were observed in the host plants, and the two strains differed in their sensitivity to cyanogenic compounds and in their capacity to metabolize and/or eliminate these compounds. Host plant specificity could be the force driving the divergence and incipient speciation of the two host strains.

Our analysis of cyanogenic capacity has clarified factors in the variable and often contradictory results reported in previous feeding studies. The way that plant material is

prepared and the frequency with which new material is added to the diet can significantly affect the levels of cyanide exposure in feeding studies, with potential to confound experimental observations of developmental rate, mortality, and larval behavior.

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