Differential Feeding of Fall Armyworm (Lepidoptera: Noctuidae) Host Strains on Meridic and Natural Diets

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ABSTRACT Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is composed of two sympatric, morphologically identical host strains (corn and rice) that differ in their distribution on different host plants. This suggests possible strain specificity in the use of host plants. However, although feeding studies published since 1987 have reported such developmental differences, the results were often contradictory, making generalizations about strain-specific physiological traits problematic. Here, we tested whether more consistent results could be obtained using several genetically characterized colonies when assayed in the same laboratory. We also assessed whether a commonly used meridic diet was more favorable to one strain and the potential this might have on altering the behavior of artificially raised colonies. Corn and rice strain colonies were characterized by cytochrome oxidase I (COI) strain markers and were subjected to feeding studies using corn (*Zea mays* L.), stargrass (*Cynodon nlemfuensis* Vanderyst variety *nlemfuensis* 'Florona'), and a meridic pinto bean diet. In 2005 bioassays, all colonies developed best on corn, whereas the meridic and stargrass diets were associated with more pronounced strain differences. However, bioassays conducted in 2010 using different colonies showed fewer differences between host strains. The limitations of feeding bioassays and the COI marker to identify host strains and the potential for unintended selection of corn strain traits when using a meridic diet are discussed.

KEY WORDS *Spodoptera frugiperda*, larval feeding, host plant selection

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a migratory, polyphagous moth that attacks a wide variety of crops throughout the Western Hemisphere (Luginbill 1928, Sparks 1979). The species is composed of two sympatric, morphologically identical host strains that differ in their use of different host plants (Pashley et al. 1985, Pashley 1986). Corn strain populations are generally associated with large grasses, such as corn (*Zea mays* L.) and sorghum (*Sorghum* spp.), whereas the rice strain prefers small grasses, such as rice (*Oryza sativa* L.) and forage grasses (*Cynodon* spp.) (Pashley et al. 1985, Pashley 1986, Meagher and Nagoshi 2004, Nagoshi and Meagher 2004). The two strains were initially detected when host-associated electrophoretic differences were observed at five allozyme loci (Pashley 1986). Other strain-specific genetic markers have since been detected (Lu et al. 1992, Lu et al. 1994, Nagoshi 2010), with polymorphisms in the mitochondrial cytochrome oxidase I gene (COI) being among the most accurate and easiest to use (Ke and Pashley 1992, Levy et al. 2002, Meagher and Gallo-Meagher 2003, Nagoshi et al. 2006).

Efforts have been made to determine whether the strain-specific distribution on host plants is due, in part at least to differential adaptation to specific plants. The simple expectation was that each strain would be adapted to their preferred hosts upon which they might display higher viability and faster larval growth and developmental rates. Many such differences have been reported in controlled laboratory studies; however, the findings have been contradictory and difficult to reproduce. For example, one study involving colonies derived from Puerto Rico collections found that larvae of both strains attained similar larval and pupal weights when reared on rice but, corn strain larvae displayed a significantly faster rate of weight gain and development when fed corn (Pashley 1988). More efficient use of corn by the corn strain also was reported for a colony derived from Louisiana specimens based on larval weight gain and biomass consumption (Veenstra et al. 1995). However, feeding studies from another laboratory using different colonies from Louisiana and Mississippi failed to find strain differences in larval duration when grown on corn but did find that the rice strain developed faster than the

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corn strain on Bermuda grass [*C. dactylon* (L.) Persoon] or centipedegrass [*Eremochloa ophiuroides* (Munro) Hack], both rice-strain preferred hosts (Whitford et al. 1988). In comparison, our research using comparable methodologies and colonies derived from Florida populations found that rice strain larvae were larger and developed faster than the corn strain when grown on corn (Meagher et al. 2004).

Apparently, diet-based bioassays display substantial variation dependent on colony and laboratory, making their use as diagnostic indicators of fall armyworm strain identity problematic. Furthermore, this variability raises concerns about the validity of similar feeding protocols frequently used in host plant resistance or pesticide susceptibility bioassays, specifically whether the findings from any particular colony or laboratory can be generalized to extrapolate the behavior of wild populations. This led to recommendations for the standardization of the feeding bioassay for fall armyworm, most notably the use of molecular markers to confirm the strain identity of laboratory colonies (Pashley et al. 1987, Quisenberry and Whitford 1988). Although such actions have the potential to reduce the variability between studies, their effectiveness has yet to be demonstrated.

The objectives of the current study were three-fold. The first objective was to test whether characterizing colonies by the COI strain markers improved the clarity and consistency of identifying strain-specific phenotypes using feeding bioassays. The second objective was to identify developmental traits sufficiently reproducible that they could potentially be used to test for the strain designation of a given colony. Analysis included a comparison of strain-specific feeding studies from different laboratories and using different colonies for three developmental variables. The third objective was to assess the developmental performance of each strain on the meridic diet (modified pinto bean) commonly used in fall armyworm artificial rearing protocols. Previous studies reported significant differences in growth rate and developmental time between strains, with the corn strain performing better than the rice strain (Quisenberry and Whitford 1988; Whitford et al. 1992; but see Whitford et al. 1988). If such differences can be confirmed, it would suggest that prolonged culturing on this diet would select for corn strain feeding characteristics.

Materials and Methods

Host Strain Colonies. Larvae for these bioassays were from several sources and were confirmed to carry the mitochondrial marker of either corn or rice strain (Meagher and Gallo-Meagher 2003, Nagoshi and Meagher 2003) (strain typing procedures are below). All colonies (except CS-Lab) originated from field-collected larvae. Unless otherwise stated, larvae completed development on and were subsequently reared on pinto bean diet (Guy et al. 1985, Stuhl et al. 2008). Only adults from laboratory pair matings that carried either corn or rice strain markers were used to develop the colonies.

The bioassays in 2005 used two corn strain (CS) and two rice strain (RS) colonies. CS-Lab larvae were from a laboratory colony reared on artificial diet that originated from individuals received from USDA–ARS, Tifton, GA (sent by J. Carpenter). This colony had been in the laboratory for several years and had introductions of field insects before we received individuals. CS-JS05 (generation at first testing was F_4) larvae were from a merged colony collected from sweet corn in Miami-Dade Co., FL, from two different sites in October and November 2004. Larvae from the two colonies were merged in February 2005 to form the colony. RS-Ona03 (F_{13}) larvae were from individuals collected from various forage grasses at the Range Cattle Research and Extension Center, Ona, Hardee Co., FL, in May 2003. This colony was originally reared on two grasses [Bermuda grass and stargrass, *C. nlemfuensis* Vanderyst variety *nlemfuensis* 'Florona'], and on pinto bean diet. The grass and pinto bean diet cultures were merged in May 2004 and became the RS-Ona03 colony. RS-MS04 (F_8) larvae were from individuals collected from Bermuda grass in Washington Co., MS (collected by J. Adamczyk) in August 2004. The colony was developed from parents carrying both the mitochondrial and genomic marker for rice strain (Nagoshi and Meagher 2003). Bioassays in 2010 used CS-DRU08 (F_{19}), derived from individuals collected from field corn at the University of Florida Dairy Research Unit, Hague, Alachua Co., FL, in May 2008. RS-Ona05 (F_{69}) was an extension of the RS-Ona03 colony but with additional insects collected from pasture grasses in 2004 and 2005. The added insects were pair mated, and only progeny from rice strain parents were added to RS-Ona05. Finally, RS-Chief09 (F_6) was from larvae collected in a 'Tifton 85' Bermuda grass pasture in Levy Co., FL, in August 2009.

Plant leaves (corn, 'Truckers Favorite', or Florona stargrass) for the bioassays were grown in 550-ml pots in a greenhouse at ambient temperature (22–40°C) and were fertilized weekly with Miracle-Gro (Marysville, OH) 15–30–15 plant food. Florona stargrass is a long-lived, persistent perennial grass similar to Bermuda grass types that was observed growing in Ona in 1973 (Mislevy et al. 1989, 1993). Previous research showed this grass to be an excellent host for fall armyworm (Meagher et al. 2007).

DNA Preparation. The host strain of each colony was determined using the following methods. Individual specimens were homogenized in 4 ml of phosphate-buffered saline (20 mM sodium phosphate and 150 mM NaCl, pH 8.0) in a 15-ml test tube by using a tissue homogenizer (PRO Scientific Inc., Oxford, CT). Cells and tissue were pelleted by centrifugation at 6000 *g* for 5 min at room temperature. The pellet was resuspended in 800 μl of cell lysis buffer (0.2 M sucrose, 0.1 M Tris-HCl at pH 8.0, 0.05 M EDTA, and 0.5% sodium dodecyl sulfate), transferred to a 1.5- or 2.0-ml microcentrifuge tube, and incubated at 55°C for 5 min. Proteins were precipitated by the addition of 100 μl of 8 M potassium acetate. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Orange, CA) and processed according to manufactur-

er's instructions. The DNA preparation was increased to a final volume of 40 μl with distilled water. Each polymerase chain reaction (PCR) reaction required 1 μl of the DNA preparation (≈0.02 μg).

PCR Analysis and Cloning. PCR amplification of the mitochondrial *COI* gene was performed in a 30-μl reaction mix containing 3 μl of 10× manufacturer's reaction buffer, 1 μl of 10 mM dNTP, 0.5 μl of 20 μM primer mix, 1 μl of DNA template (0.05–0.5 μg), and 0.5 U of *Taq*DNA polymerase (New England Biolabs, Ipswich, MA). The thermocycling program was 94°C (1 min), followed by 33 cycles of 92°C (30 s), 56°C (45 s), 72°C (45 s), and a final segment of 72°C for 3 min. Typically 96 PCR amplifications were performed at the same time by using either 0.2-ml tube strips or 96-well microtiter plates. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Amplification of the *COI* region used the primer pair *COI-893 F* (5′-CACGAGCATATTTTACATCWGCA-3′) and *COI-1303R* (5′-CAGGATAGTCAGAATATC-GACG-3′) to produce a 410-bp fragment.

For fragment isolations 6 μl of 6× gel loading buffer was added to each amplification reaction, and the entire sample was run on a 1.8% agarose horizontal gel containing GelRed (Biotium, Hayward, CA) in 0.5× Tris-borate buffer (45 mM Tris base, 45 mM boric acid, and 1 mM EDTA, pH 8.0). Fragments were visualized on a long-wave UV light box and cut out from the gel. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research) according to manufacturer's instructions. The isolated fragments were analyzed by DNA sequencing performed by Northwoods DNA, Inc. (Bemidji, MN) or the University of Florida Interdisciplinary Center for Biotechnology Research (Gainesville, FL). All other DNA sequences were obtained from GenBank (National Center for Biotechnology Information, Bethesda, MD). DNA comparisons, alignments, and restriction site mapping were performed using the DS Gene program (Accelrys, San Diego, CA).

Feeding Bioassays. The bioassays in 2005 used pinto bean diet, corn leaves, or stargrass leaves as host material; the 2010 bioassays compared feeding results from pinto bean diet and stargrass. Neonate larvae (<24 h old) were individually placed in 29.6-ml portion cups (Jet Plastica Industries, Hatfield, PA). Larvae in the pinto bean diet treatment were weighed on day 9 and then were observed daily after 15 d for pupal formation. For the plant foliage treatments, extra leaves were placed in the cups on day 3. On day 5, the old leaves were removed, and new leaves were added on day 7. Larvae were weighed on day 9. New leaves were then added daily until pupal formation. Pupae were weighed, and larval duration (in days) from neonate to pupa was recorded. Thirty cups were placed in a tray in an incubator at 23.9 ± 2°C with a photoperiod of 14:10 (L:D) h. Larval weight (milligrams ± SEM), larval duration (days ± SEM), pupal weight (milligrams ± SEM), and sex were recorded for all surviving individuals in the tray, and each tray was considered a replication. Setup survival was measured by dividing the number of live larvae weighed on day 9 by 30; developmental survival was measured by dividing the number of adults by the number of larvae weighed on day 9. Factors affecting setup survival included neonate age (how many hours neonates were alive before placing on host material), neonate handling, and host suitability. Host suitability was the main factor affecting developmental survival.

Statistics. The factorial experiments in 2005 and 2010 used a randomized complete block design to compare variation among colonies, host material, and sex for larval weight, larval duration, and pupal weight (PROC MIXED, SAS 9.2, SAS Institute 2008 using $\log_{10}$-transformed data). Replication was the random variable (Littell et al. 1996). The number of replicates varied in 2005 (CS-JS05 corn 6, pinto bean 4, stargrass 5; CS-Lab corn 5, pinto bean and stargrass 4; RS-MS04 corn and pinto bean 4, stargrass 8; RS-Ona03 corn and pinto bean 5, stargrass 6). CS-DRU08 and RS-Ona05 had five replicates each for pinto bean and stargrass, whereas RS-Chief09 had four replicates each for the two diets in the 2010 bioassays. All interactions among main factors were tested, and when the colony × host interaction was significant, differences among colonies were tested for each host. Treatment means were separated using simple effect differences of the least square means (LSMEANS). Nontransformed means ± SEM are shown in the text and tables. Analysis of variance (ANOVA) of square root-transformed data (PROC Mixed, SAS 9.2, SAS Institute 2008) was used to examine variation among colonies and between host strains for setup and developmental survival.

Results

Larval Growth and Development. The 2005 feeding bioassays showed significant colony x host interactions for all variables (larval weight: $F = 9.3$; df = 6, 89; $P < 0.0001$; larval duration: $F = 15.6$; df = 6, 89; $P < 0.0001$; pupal weight: $F = 6.9$; df = 6, 89; $P < 0.0001$); therefore, results were analyzed for host differences by colony (Tables 1–3, rows) and for colony differences by host (Tables 1–3, columns). Differences between males and females were only significant with pupal weight ($F = 5.8$; df = 1, 89; $P = 0.0183$).

All colonies developed better on corn based on measurements of larval weight and larval duration (Tables 1 and 2), with no statistical differences observed between strains (corn strain, 58.6 ± 4.3 mg; rice strain, 61.1 ± 3.0 mg; $F = 0.33$; df = 1, 11; $P = 0.5750$). Average 9-d larval weight was 59.7 ± 2.7 mg for development on corn compared with 15.9 ± 1.9 and 21.7 ± 1.8 mg on pinto bean diet or stargrass, respectively. Larvae developing on corn pupated on average 3–5 d earlier than with the other two hosts (corn, 17.4 ± 0.1 d; stargrass, 20.7 ± 0.3 d; pinto bean diet, 22.5 ± 0.5 d). Larvae that fed on corn produced intermediate-sized pupae (Table 3), and male corn strain pupae were heavier (208.3 ± 5.6 mg) than rice strain pupae (190.3 ± 5.0 mg) ($P = 0.0133$).

Colony and strain differences were more pronounced with larvae grown on pinto bean diet or

Table 1. Larval weights (milligrams) of fall armyworm larvae from four different laboratory colonies on Truckers Favorite corn, Florona stargrass, and pinto bean diet in bioassays completed in 2005

Colony	Corn	Stargrass	Pinto bean	*F*	df	*P*
CS-Lab	64.5 ± 8.6aA	13.3 ± 1.6cB	31.0 ± 2.7bA	21.8	2, 6	0.0018
CS-JS05	53.6 ± 3.1aA	15.8 ± 1.0bAB	14.6 ± 2.7bB	27.1	2, 7	0.0005
RS-Ona03	63.4 ± 5.1aA	26.3 ± 3.7bA	10.9 ± 2.5cB	15.1	2, 8	0.0019
RS-MS04	58.2 ± 2.1aA	26.1 ± 3.7bA	8.3 ± 2.2cB	16.4	2, 6	0.0037
F	0.4	2.8	6.3			
df	3, 11	3, 12	3, 9			
P	0.7819	0.0869	0.0140			

Means ± SE within rows followed by the same lowercase letter are not significantly different; means within columns followed by the same uppercase letter are not significantly different.

stargrass. The CS-Lab colony displayed the highest larval and pupal weights and shorter larval duration on pinto bean diet, all significantly higher than that observed with either rice strain colony (Tables 1–3). In comparison, CS-JS05 gave intermediate results. Larval weights from both corn strain colonies were on average 2 times larger (corn strain, 22.8 ± 2.8 mg; rice strain, 9.8 ± 1.7 mg; $F = 13.1$; df = 1, 9; $P = 0.0055$), larval duration almost 4 d earlier (corn strain, 20.5 ± 0.5 d; rice strain, 24.2 ± 0.6 d; $F = 25.2$; df = 1, 9; $P = 0.0007$), and pupal weights heavier than the rice strain colonies (Table 3). These relationships were reversed on stargrass, where the rice strain larvae were heavier (rice strain, 26.2 ± 2.6 mg; corn strain, 14.7 ± 0.9 mg; $F = 7.9$; df = 1, 12; $P = 0.0157$) and larval duration was quicker (rice strain, 19.5 ± 0.3 d; corn strain, 22.4 ± 0.3 d; $F = 22.4$; df = 1, 12; $P = 0.0005$). Larvae from RS-MS04 and RS-Ona03 were on average 11 mg heavier, and pupation occurred 3 d earlier than with either corn strain colony. However, this quicker developmental rate was not associated with heavier pupae because no strain differences were observed in pupal weight for larvae fed stargrass (both males and females $P > 0.12$).

Viability. There were no significant interactions with setup or developmental survival among host, colony, or strain ($P > 0.05$). Fewer neonates placed into cups survived 9 d when given stargrass (82.0 ± 2.7%) than when given corn (90.3 ± 2.8%), whereas pinto bean diet was intermediate (88.0 ± 2.3%) ($F = 3.8$; df = 2, 41; $P = 0.0314$). CS-Lab larvae (94.9 ± 2.2%) had higher setup survival than RS-Ona03 (85.8 ± 3.1%), RS-MS04 (85.8 ± 3.0%), or CS-JS05 larvae (80.7 ± 3.3%) ($F = 4.2$; df = 3, 41; $P = 0.0116$). There was no difference in setup survival between the corn strain (87.3 ± 2.4%) and the rice strain (85.8 ± 2.1%) ($F = 0.33$; df = 1, 41; $P = 0.5680$). Developmental survival among hosts and among colonies was not significantly different ($P > 0.12$). When survival for the corn strain and rice strain colonies were compared, more corn strain larvae (86.5 ± 1.7%) survived than rice strain larvae (78.9 ± 2.4) ($F = 4.7$; df = 1, 41; $P = 0.0367$).

2010 Studies. These studies were repeated, in part to test whether the strain-specific differences in development on pinto bean diet and stargrass could be reproduced with a different set of colonies, in this case one corn strain colony and two rice strain lines that differed in length of time in culture. Within-colony variability tended to be substantially higher than observed in 2005. Unlike the 2005 results, none of the colony × host interactions was significant and sex as a variable was not significant for any parameter.

RS-Chief09 performed most consistently with the 2005 bioassay results, displaying a pattern similar to that of RS-Ona03 and RS-MS04. On the pinto bean diet, RS-Chief09 larvae were, on average, 6 mg smaller than the corn strain colony (CS-DRU08), with pupation occurring 1 d later and pupal weight 60 mg lighter, although only pupal weight was statistically significant (Table 4). CS-DRU08 performed differently than the 2005 corn strain colonies in that there was no difference in larval weight or larval duration between the pinto bean and stargrass hosts. However, pupal weight on stargrass was significantly lower than on pinto bean diet as had occurred in 2005. The greatest departure from the 2005 results was shown by RS-Ona05. The developmental pattern for these larvae was more similar to that of CS-DRU08 than RS-Chief09 (Table 4). Setup and developmental survival was similar among

Table 2. Larval duration (days) of fall armyworm larvae from four different laboratory colonies on Truckers Favorite corn, Florona stargrass, and pinto bean diet in bioassays completed in 2005

Colony	Corn	Stargrass	Pinto bean	*F*	df	*P*
CS-Lab	17.4 ± 0.3aA	23.0 ± 0.5cB	19.3 ± 0.3bA	34.7	2, 6	0.0005
CS-JS05	17.5 ± 0.2aA	22.0 ± 0.3bB	21.7 ± 0.6bB	35.2	2, 7	0.0002
RS-Ona03	17.3 ± 0.2aA	19.3 ± 0.5aA	24.4 ± 1.1bC	16.4	2, 8	0.0015
RS-MS04	17.1 ± 0.1aA	19.7 ± 0.5bA	24.0 ± 0.5cBC	19.3	2, 6	0.0024
F	0.3	8.0	10.4			
df	3, 11	3, 12	3, 9			
P	0.8266	0.0034	0.0028			

Means ± SE within rows followed by the same lowercase letter are not significantly different; means within columns followed by the same uppercase letter are not significantly different.

Table 3. Weights (milligrams) of male and female fall armyworm pupae that developed from four different laboratory colonies on Truckers Favorite corn, Florona stargrass, and pinto bean diet in bioassays completed in 2005

Colony	Corn	Stargrass	Pinto bean	*F*	df	*P*
Females						
CS-Lab	204.4 ± 5.4bA	162.3 ± 15.0cA	272.3 ± 9.3aA	23.3	2, 6	0.0015
CS-JS05	189.0 ± 6.3bA	152.9 ± 8.8cA	233.8 ± 6.2aB	51.9	2, 7	<0.0001
RS-Ona03	191.4 ± 7.7aA	176.8 ± 5.9aA	195.5 ± 10.1aC	2.2	2, 8	0.1793
RS-MS04	176.4 ± 8.1aA	167.0 ± 10.5aA	203.4 ± 8.0aC	2.8	2, 6	0.1384
F	2.4	1.2	16.8			
df	3, 11	3, 12	3, 9			
P	0.1230	0.3386	0.0005			
Males						
CS-Lab	222.4 ± 3.0bA	187.4 ± 14.8cA	242.3 ± 6.4aA	18.5	2, 6	0.0027
CS-JS05	196.6 ± 6.8bB	155.2 ± 4.0cA	238.2 ± 4.2aB	66.8	2, 7	<0.0001
RS-Ona03	194.6 ± 3.2aB	178.2 ± 5.4bA	200.2 ± 4.9aC	6.2	2, 8	0.0239
RS-MS04	185.0 ± 10.8aB	175.7 ± 10.9aA	210.9 ± 7.4aC	2.4	2, 6	0.1706
F	5.4	2.3	31.7			
df	3, 11	3, 12	3, 9			
P	0.0158	0.1260	<0.0001			

Means ± SE within rows followed by the same lowercase letter are not significantly different; means within columns followed by the same uppercase letter are not significantly different.

colonies and between hosts ($P > 0.08$). Setup survival averaged 90.0 ± 1.4%, whereas developmental survival was 84.5 ± 1.9%.

Strain Differences Among Different Colonies and Laboratories. Despite the variability in collection and strain identification method used among studies (Table 5), certain trends were sufficiently consistent to suggest that they may represent reproducible strain-specific traits. This was most apparent with the pinto bean diet where all the significant differences observed (in 25 of 30 measurements) were consistent with faster development of corn strain larvae than rice strain (heavier larval weight, shorter larval duration, and heavier pupal weight) (Table 6). On a corn diet the two strains show either equal developmental rate or, with one exception (Meagher et al. 2004), evidence of faster corn strain development. This is consistent with the corn strain population, on average, being better adapted to its preferred host.

Greater variability was observed with studies using a rice strain host as the feeding material, though this may reflect the variety of host plants used in different studies (Table 5). Even here there seems to be a trend consistent with better adaptation of the rice strain to its preferred host. With respect to larval weight and larval duration, whenever statistically significant differences were found, they tended to be indicative of faster rice strain development with heavier larvae and shorter larval duration. However, this relationship was not observed for pupal weight, because all studies showed either no statistical difference or heavier corn strain pupae.

Inconsistencies between studies were most pronounced when comparing the development of the two strains on different hosts (Table 7). Contradictory results were found in all three developmental parameters tested, making broad generalizations problematic. This variability could reflect difficulties in diet preparation, particularly with that involving live plant material.

Discussion

COI Characterization of Colony Strain Identity Did Not Improve Bioassay Consistency. The two morphologically identical fall armyworm strains have been reported to differ in a number of physiological and behavioral characteristics (Pashley 1988, Yu 1992, Veenstra et al. 1995, Lima and McNeil 2009, Groot et al. 2010). Therefore, the validity of many laboratory studies with fall armyworm will depend upon the accuracy and integrity of the colonies used with respect to strain identity. Even relatively minor contamination of a colony with the inappropriate strain could

Table 4. Feeding results of fall armyworm larvae from three colonies on pinto bean diet and Florona stargrass completed in 2010

Colony/host/strain	Larval wt	Larval duration	Pupal wt
CS-DRU08	16.3 ± 2.3a	23.5 ± 0.6a	201.7 ± 18.4a
RS-Ona05	14.7 ± 2.6a	22.4 ± 0.7a	187.4 ± 14.2a
RS-Chief09	10.3 ± 1.4a	24.2 ± 0.7a	154.9 ± 13.7b
	$F = 2.0$; df = 2, 18; $P = 0.1700$	$F = 1.5$; df = 2, 14; $P = 0.2607$	$F = 15.7$; df = 2, 18; $P < 0.0001$
Corn strain	16.3 ± 2.3a	23.5 ± 0.6a	201.7 ± 18.4a
Rice strain	12.7 ± 1.6a	23.3 ± 0.5a	173.0 ± 10.4b
	$F = 2.7$; df = 1, 18; $P = 0.1210$	$F = 1.8$; df = 1, 14; $P = 0.1981$	$F = 160.3$; df = 1, 18; $P < 0.0001$
Pinto bean	13.9 ± 2.4a	23.9 ± 0.7a	225.7 ± 8.6 a
Stargrass	14.1 ± 1.3a	22.8 ± 0.4a	140.7 ± 5.3 b
	$F = 0.2$; df = 1, 18; $P = 0.6607$	$F = 0.29$; df = 1, 14; $P = 0.5959$	$F = 16.5$; df = 1, 18; $P = 0.0007$

For each variable, means ± SE within colonies, strains, or hosts followed by the same letter are not significantly different.

Table 5. Description of fall armyworm colonies used in strain-specific feeding studies

Study	Colony name	Colony origin	Founder larvae host plant	No. generations in culture at time of exp	Molecular strain test
This study	CS-Lab	Tift Co., GA (unknown)	Corn	Unknown	COI
This study	CS-JS05	Miami-Dade Co., FL (2004)	Corn	4	COI
This study	RS-MS04	Washington Co., MS (2004)	Bermuda grass	8	COI
This study	RS-Ona03	Hardee Co., FL (2003)	Forage grasses	13	COI
This study	CS-DRU08	Alachua Co., FL (2008)	Corn	19	COI
This study	RS-Ona05	Hardee Co., FL (2003–5)	Forage grasses	69	COI
This study	RS-Chief09	Levy Co., FL (2009)	Bermuda grass	6	COI
Meagher et al. (2004)	Tifton	Tift Co., GA (unknown)	Corn	Unknown	COI
Meagher et al. (2004)	Ona	Hardee Co., FL (2002)	Forage grasses	11	COI
Whitford et al. (1992)	RS	E. Feliciana Parish, LA (1984)	Bermuda grass	Unknown	Electrophoretic
Whitford et al. (1992)	CS	MS (1985)	Corn	Unknown	Electrophoretic
Quisenberry and Whitford (1988)	LA (RS)	E. Feliciana Parish, LA (1984)	Bermuda grass	>25	Electrophoretic
Quisenberry and Whitford (1988)	GA-1, GA-2 (CS)	Tift Co., GA (1960)	Bermuda grass?	>300	Electrophoretic
Quisenberry and Whitford (1988)	MS (CS)	MS (1985)	Corn	Unknown	Electrophoretic
Whitford et al. (1988)	RS	E. Feliciana Parish, LA (1984)	Bermuda grass	Unknown	Electrophoretic
Whitford et al. (1988)	CS	MS (1985)	Corn	Unknown	Electrophoretic
Pashley et al. (1987)	LA	E. Feliciana Parish, LA (1984)	Bermuda grass	11	Electrophoretic
Pashley et al. (1987)	GA	Tift Co., GA (1960)	Bermuda grass?	>300	Electrophoretic
Pashley (1988)	RS	Puerto Rico (1985)	Rice	2	?
Pashley (1988)	CS	Puerto Rico (1985)	Corn	2	?
Pashley et al. (1995)	RS	E. Baton Rouge Parish, LA (1995)	Bermuda grass	1	Electrophoretic
Pashley et al. (1995)	CS	E. Baton Rouge Parish, LA (1995)	Corn	1	Electrophoretic
Veenstra et al. (1995)	RS	E. Baton Rouge Parish, LA (1992)	Forage grasses	2	Esterase, mitochondria
Veenstra et al. (1995)	CS	E. Baton Rouge Parish, LA (1992)	Corn	2	Esterase, mitochondria

lead to substantial changes in colony behavior, particularly because the relatively small number of specimens used to establish each generation and possible unintended selection pressures from the artificial rearing protocol, can rapidly alter allele frequency. For this reason, most laboratories use molecular tests, either strain-specific electrophoretic allozymes or mitochondrial markers to initially confirm the strain identity of a colony (Table 5). It is presumed that such molecular tests ensure that each colony is predominantly of the expected strain and thereby provide more consistent results when analyzing strain-specific behaviors. However, it has yet to be established that this is in fact the case. What is needed is for the same laboratory to repeat the feeding bioassays using different independently-isolated and molecularly defined colonies.

We performed this experiment in three separate studies in 2004 (Meagher et al. 2004, 2005, 2010). The effectiveness of the COI markers used to confirm the strain identity of the different colonies had previously been demonstrated in field studies showing the host-specific distribution of the COI haplotypes, the primary characteristic that defines the two strains (Meagher and Gallo-Meagher 2003). In 2004, bioassays on a corn diet showed significantly faster development of rice strain larvae as indicated by higher larval weights and shorter larval duration. This could not be reproduced in the 2005 bioassays using a different set of colonies. In this case larval weight and larval duration were indistinguishable between the two strains, whereas corn strain pupal weight was heavier or equal to that of the rice strain. In 2010, we tested whether the strain differences observed in 2005

Table 6. Comparison of host strains (CS, corn strain; RS, rice strain) within diets and among different studies

Test host	Outcome	No. comparisons	Larval wt/growth rate	Frequency (%) larval duration	Pupal wt
Corn	CS = RS	9[a]	44.4	88.9	50.0
	CS > RS		44.4	11.1	50.0
	CS < RS		11.1	0	0
Pinto bean/meridic	CS = RS	12[b]	16.7	14.3	0
	CS > RS		75.0	14.3	100
	CS < RS		8.3	71.4	0
Grasses or rice	CS = RS	11[c]	40.0	20.0	72.7
	CS > RS		20.0	80.0	27.3
	CS < RS		40.0	0	0

Studies used are listed in Table 5.
[a] Pupal weight had 10 comparisons because one study looked at males and females.
[b] Larval duration had seven comparisons, pupal weight had 11.
[c] Larval weight and larval duration had 10 comparisons.

Table 7. Comparison of host strains (CS, corn strain; RS, rice strain) among diets (C, corn; D, meridic diet; and R, rice strain host, such as grass or rice) and among different studies

Study	Larval wt/growth rate	Larval duration	Pupal wt
Corn strain colonies			
2005 CS-Lab	C > D > R	R > D > C	D > C > R
2005 CS-JS05	C > R = D	R = D > C	D > C > R
2010 CS-DRU08	D = R	D = R	D > R
Whitford et al. (1988)	C = R	C = R	C = R
Pashley (1988)	R > C	C = R	C > R
Pashley et al. (1995)	R > C	C > R	C > R
Rice strain colonies			
2005 RS-MS04	C > R > D	D > R > C	D = C = R
2005 RS-Ona03	C > R > D	D > R > C	D = C = R (♀), D = C > R (♂)
2010 RS-Ona05	D = R	D = R	D > R
2010 RS-Chief09	R > D	D > R	D > R
Whitford et al. (1988)	R > C*	C > R	R > C
Pashley (1988)	R > C	C > R	C > R
Pashley et al. (1995)	R > C	C > R	C > R

* The comparison was not significantly different.

with respect to the pinto bean diet and stargrass could be reproduced. The results were mixed as similar results were obtained for larval and pupal weights on the pinto bean diet, but different observations found for larval duration and the developmental parameters when grown on stargrass. These results indicate that the observed variability in these feeding studies cannot be resolved by the use of molecular strain markers, at least those used in this study. This could indicate limitations in the effectiveness of the COI marker, such that its application to the characterization of the colonies was still not sufficient to identify pure lines. Indeed, there is some evidence that genetic polymorphisms at other loci display a higher correlation with host plants (Nagoshi 2010). Perhaps better characterization of the genes that determine strain differences will allow more accurate designation of colonies and facilitate the development of strain-diagnostic bioassays.

An alternative possibility is that host use is not (as yet) a strain-specifying characteristic. The observed asymmetric distribution of the two strains on different hosts may be driven by other influences (e.g., natural enemy pressure), with host differences in developmental performance a secondary consequence that is still in the process of divergence. If this is the case then the two strains could display considerable overlap in their feeding physiology such that even "pure" strain colonies would give inconclusive and variable results in feeding bioassays.

Potential for Unintended Selection of Corn Strain Traits When Using Pinto Bean Diet. We previously demonstrated that the average time of larval development of a colony could be substantially altered by a single generation of selection (Nagoshi 2011). This suggests a strong genetic component of multiple alleles influencing the rate of development. Therefore, the developmental performance of a given population will depend on its genotypic composition, which because of practical limitations in the number of specimens used to generate and maintain lines, will probably vary considerably between colonies. In addition, the observation seen in five of the seven studies (Table 6) that corn strain larvae tended to develop faster on the pinto bean meridic diet than the rice strain provides a potential selection advantage for corn strain genes that could significantly alter the genetic makeup and behavior of a colony after prolonged laboratory rearing. Even colonies that were initially primarily of the rice strain would be expected to display increasing corn strain-like behavior under that kind of selection pressure if corn strain alleles were present in the founding population. A possible example of this is the description of the corn strain colony (GA) by Quisenberry and Whitford (1988). This colony was initially founded with larvae isolated from Bermuda grass, a rice strain host, yet after several years in culture molecular tests indicated that it was primarily, if not entirely, of the corn strain (Table 5). Such selection pressure also could explain the anomalous results of the RS-Ona05 colony in our 2010 feeding study. This line displayed mitochondrial markers diagnostic of the rice strain but behaved more similarly to the corn strain with respect to developmental parameters. We believe that even with molecular screening using strain markers it is possible for corn strain alleles to be present in the founder population of a rice strain colony (or in subsequent supplementation with wild specimens) if these contain interstrain hybrids. Such hybrids have been generated in the laboratory where a cross between the two strains produce progeny that must contain genes from both strains yet still display the mitochondrial markers diagnostic of one strain (Nagoshi et al. 2006, Nagoshi 2010). In this situation, contamination by corn strain genes would go undetected in the rice strain colony, and selection for corn strain traits by the meridic diet could quickly shift the behavior of the population in the corn strain direction.

In summary, the performance of fall armyworms in laboratory feeding studies is highly influenced by the specific characteristics of each individual colony. Even colonies genetically characterized as being of the same strain can differ substantially. We have identified several factors that could contribute to this inconsistency and believe these should be taken into account when designing feeding studies. Fall armyworm is a major agricultural pest in the Western

Hemisphere whose threat is compounded by evidence of developing field resistance to *Bacillus thuringiensis* insecticidal proteins (Storer et al. 2010) and that it may become a pest of grass species being considered for use as biofuels (Prasifka et al. 2009, but see Nabity et al. 2011). The generation of plant cultivars resistant to fall armyworm would be an important contributor to future control efforts, but these will require development of feeding bioassays that accurately and consistently reflect the diversity and behavior of wild populations. Our studies indicate that current protocols for such bioassays are potentially inadequate, requiring improvements in the identification of strain identity, increased characterization of strain-specific behaviors and physiology, and the development of rearing methods that reduce the artificial and unintended selection of strain-specific traits.

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References Cited

Groot, A. T., M. Marr, D. G. Heckel, and G. Schöfl. 2010. The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ecol. Entomol. 35: 105–118.

Guy, R. N., N. C. Leppla, J. R. Rye, C. W. Green, S. L. Barette, and K. A. Hollien. 1985. *Trichoplusia ni*, pp. 487–494. *In* P. Sing and R. F. Moore (eds.), Handbook of insect rearing, vol. 2. Elsevier, Amsterdam, The Netherlands.

Ke, L. D., and D. P. Pashley. 1992. Characterization of fall armyworm mitochondrial DNA (Lepidoptera: Noctuidae). Arch. Insect Biochem. Physiol. 21: 263–269.

Levy, H. C., A. Garcia-Maruniak, and J. E. Maruniak. 2002. Strain identification of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) insects and cell line: PCR-RFLP of cytochrome oxidase C subunit I gene. Fla. Entomol. 85: 186–190.

Lima, E. R., and J. N. McNeil. 2009. Female sex pheromones in the host races and hybrids of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Chemoecology 19: 29–36.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, NC.

Lu, Y.-J., M. J. Adang, D. J. Isenhour, and G. D. Kochert. 1992. RFLP analysis of genetic variation in North American populations of the fall armyworm moth *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Mol. Ecol. 1: 199–208.

Lu, Y.-J., G. D. Kochert, D. J. Isenhour, and M. J. Adang. 1994. Molecular characterization of a strain-specific repeated DNA sequence in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insect Mol. Biol. 3: 123–130.

Luginbill, P. 1928. The fall armyworm. U.S. Dep. Agric. Tech. Bull. 34.

Meagher, R. L., Jr., and M. Gallo-Meagher. 2003. Identifying host-strains of fall armyworm (Lepidoptera: Noctuidae) in Florida using mitochondrial markers. Fla. Entomol. 86: 450–455.

Meagher, R. L., and R. N. Nagoshi. 2004. Population dynamics and occurrence of *Spodoptera frugiperda* host strains in southern Florida. Ecol. Entomol. 29: 614–620.

Meagher, R. L., R. N. Nagoshi, C. Stuhl, and E. R. Mitchell. 2004. Larval development of fall armyworm (Lepidoptera: Noctuidae) on different cover crop plants. Fla. Entomol. 87: 454–46.

Meagher, R. L., P. Mislevy, and R. N. Nagoshi. 2007. Caterpillar (Lepidoptera: Noctuidae) feeding on pasture grasses in central Florida. Fla. Entomol. 90: 295–303.

Mislevy, P., W. F. Brown, L. S. Dunavin, D. W. Hall, R. S. Kalmbacher, A. J. Overman, O. C. Ruelke, R. M. Sonoda, R. L. Stanley, Jr., and M. J. Williams. 1989. 'Florona' stargrass. Fla. Agric. Exp. Stn. Circ. S-362.

Mislevy, P., W. F. Brown, L. S. Dunavin, D. W. Hall, R. S. Kalmbacher, A. J. Overman, O. C. Ruelke, R. M. Sonoda, R. L. Stanley, Jr., and M. J. Williams. 1993. Registration of 'Florona' stargrass. Crop Sci. 33: 359–360.

Nabity, P. D., A. R. Zangerl, M. R. Berenbaum, and E. H. DeLucia. 2011. Bioenergy crops *Miscanthus* × *giganteus* and *Panicum virgatum* reduce growth and survivorship of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Econ. Entomol. 104: 459–464.

Nagoshi, R. N. 2010. The fall armyworm triose phosphate isomerase (*Tpi*) gene as a marker of strain identity and interstrain mating. Ann. Entomol. Soc. Am. 103: 283–292.

Nagoshi, R. N. 2011. Artificial selection for developmental rates in fall armyworm (Lepidoptera: Noctuidae) and its implications on the design of feeding studies. Ann. Entomol. Soc. Am. 104: 88–94.

Nagoshi, R. N., and R. L. Meagher. 2003. *FR* tandem-repeat sequence in fall armyworm (Lepidoptera: Noctuidae) host strains. Ann. Entomol. Soc. Am. 96: 329–335.

Nagoshi, R. N., and R. L. Meagher. 2004. Seasonal distribution of fall armyworm (Lepidoptera: Noctuidae) host strains in agricultural and turf grass habitats. Environ. Entomol. 33: 881–889.

Nagoshi, R. N., R. L. Meagher, J. J. Adamczyk, Jr., S. K. Braman, R. L. Brandenburg, and G. Nuessly. 2006. New restriction fragment length polymorphisms in the cytochrome oxidase I gene facilitate host strain identification of fall armyworm (Lepidoptera: Noctuidae) populations in the southeastern United States. J. Econ. Entomol. 99: 671–677.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? Ann. Entomol. Soc. Am. 79: 898–904.

Pashley, D. P. 1988. Quantitative genetics, development, and physiological adaptation in host strains of fall armyworm. Evolution 42: 93–102.

Pashley, D. P., S. J. Johnson, and A. N. Sparks. 1985. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 78: 756–762.

Pashley, D. P., S. S. Quisenberry, and T. Jamjanya. 1987. Impact of fall armyworm (Lepidoptera: Noctuidae) host strains on the evaluation of Bermuda grass resistance. J. Econ. Entomol. 80: 1127–1130.

Pashley, D. P., T. N. Hardy, and A. M. Hammond. 1995. Host effects on developmental and reproductive traits in fall armyworm strains (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 88: 748–755.

Prasifka, J. R., J. D. Bradshaw, R. L. Meagher, R. N. Nagoshi, K. L. Steffey, and M. E. Gray. 2009. Development and feeding of fall armyworm on *Miscanthus* X *giganteus* and switchgrass. J. Econ. Entomol. 102: 2154–2159.

Quisenberry, S. S., and F. Whitford. 1988. Evaluation of bermudagrass resistance to fall armyworm (Lepidoptera:

Noctuidae): influence of host strain and dietary conditioning. J. Econ. Entomol. 81: 1463–1468.

SAS Institute. 2008. SAS 9.2 for Windows. SAS Institute, Cary, NC.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62: 82–87.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. D. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J. Econ. Entomol. 103: 1031–1038.

Stuhl, C. J., R. L. Meagher, and R. N. Nagoshi. 2008. Genetic variation in neonate behavior of fall armyworm (Lepidoptera: Noctuidae). Fla. Entomol. 91: 151–158.

Veenstra, K. H., D. P. Pashley, and J. A. Ottea. 1995. Host-plant adaptation in fall armyworm host-strains: comparison of food consumption, utilization, and detoxication enzyme activities. Ann. Entomol. Soc. Am. 88: 80–91.

Whitford, F., S. S. Quisenberry, T. J. Riley, and J. W. Lee. 1988. Oviposition preference, mating compatibility, and development of two fall armyworm strains. Fla. Entomol. 71: 234–243.

Whitford, F., S. S. Quisenberry, and D. J. Moellenbeck. 1992. Nutritional response by rice and corn fall armyworm (Lepidoptera: Noctuidae) strains to dietary component substitution in artificial diets. J. Econ. Entomol. 85: 1491–1496.

Yu, S. J. 1992. Detection and biochemical characterization of insecticide resistance in fall armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 85: 675–682.

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