Low aflatoxin levels and flowering delay in *Aspergillus flavus*-resistant maize lines are correlated with increased corn earworm damage and enhanced seed fumonisin content

Subbaiah Chalivendra[1,*], Fangneng Huang[2], Mark Busman[3], W. Paul Williams[4] Jong Hyun Ham[1] and Geromy G. Moore[5]

[1]Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, Baton Rouge, LA 70803, USA

[2]Department of Entomology, Louisiana State University AgCenter, Baton Rouge, LA 70803, USA

[3]Bacterial Foodborne Pathogens and Mycology Research Unit, USDA-ARS-NCAUR, Peoria, IL 61604, USA

[4]USDA-ARS, Corn Host Plant Resistance Research Unit, Mississippi State, MS 39762, USA

[5]USDA-ARS, Southern Regional Research Center, New Orleans, LA 70124, USA

*Author to whom correspondence should be addressed:

schalivendra@agcenter.lsu.edu

19 **Keywords & Research Areas:** Maize (*Zea mays*), *Aspergillus flavus*, *Fusarium*
20 *verticillioides*, ear rot, resistant and susceptible lines, corn earworm (*Helicoverpa*
21 *zea* Boddie), aflatoxin toxicity, fumonisin tolerance

ABSTRACT

Preharvest mycotoxin contamination of field-grown crops is influenced not only by the host genotype, but also inoculum load, insect pressure and their confounding interactions with seasonal weather. In two field trials, we observed a preferred natural infestation of specific maize (*Zea mays* L.) genotypes by corn earworm (*Helicoverpa zea* Boddie) and investigated this unexpected interaction. These studies involved four maize lines with contrasting levels of resistance to *Aspergillus flavus*. The resistant lines had 7 to 14-fold greater infested ears than the susceptible lines. However, seed aflatoxin B_1 levels, in mock- or *A. flavus*-inoculated ears were consistent with maize genotype resistance to *A. flavus*. Further, the corn earworm-infested ears had greater levels of fumonisin content in seeds than uninfested ears, indicating that the insect may have vectored native *Fusarium verticillioides* inoculum. The two maize lines with heavy infestation showed delayed flowering. The availability of young silk for egg-laying could have been a factor in the pervasive corn earworm damage of these lines. At the same time, *H. zea* larvae reared on AF-infused diet showed decreasing mass with increasing AF and >30% lethality at 250 ppb. In contrast, corn earworm was tolerant to fumonisin with no significant loss in mass even at 100 ppm, implicating the low seed aflatoxin content as a predominant factor for the prevalence of corn earworm infestation and the associated fumonisin contamination in *A. flavus* resistant lines. These results highlight the need for integrated strategies targeting mycotoxigenic fungi and their insect vectors to enhance the safety of crop commodities.

IMPORTANCE: *Aspergillus* and *Fusarium* spp. not only cause ear rots in maize leading to crop loss, they can also contaminate the grain with carcinogenic mycotoxins. Incorporation of genetic resistance into breeding lines is an ideal solution for mycotoxin mitigation. However, the goal is fraught by a major problem. Resistance for AF or FUM accumulation is quantitative and contributed by several loci with small effects. Our work reveals that host phenology (flowering time) and insect vector-mycotoxin interactions can further confound breeding efforts. A host genotype even with demonstrable resistance can become vulnerable due to seasonal variation in flowering time or an outbreak of chewing insects. Incorporation of resistance to a single mycotoxin accumulation and not pairing it with insect resistance may not adequately ensure food safety. Diverse strategies including host-induced silencing of genes essential for fungal and insect pest colonization and broad-spectrum biocontrol systems need to be considered for robust mycotoxin mitigation.

INTRODUCTION

Besides causing crop damage and economic loss to the grower, mycotoxigenic fungi pose a serious risk to human and livestock health due to the contamination of commodities with carcinogenic and neurotoxic secondary metabolites known as mycotoxins. Aflatoxin B_1 (**AF**) is the most dangerous among mycotoxins due to its very potent carcinogenicity. *Aspergillus flavus*, an opportunistic pathogen, is the predominant species that contaminates cereal and oil seed crops with AF. Although not as genotoxic as AF, fumonisins (**FUM**) are associated with esophageal cancer, particularly due to cytotoxicity of fumonisin B_1 (**FB_1**). They are also among the most common food- and feed-contaminating mycotoxins in many countries (BIOMIN Mycotoxin Survey 2015). FUM are produced by *Fusarium* species, *F. verticillioides* (formerly known as *F. moniliforme*) being the predominant contaminant of commodities (Munkvold 2003). *A. flavus* and *F. verticillioides* cause ear rots in maize (*Zea mays* L.), a globally important food, feed and fuel crop of high productivity. Co-contamination of commodities with AF and FUM has been reported, particularly, in high cancer-risk areas (Sun et al. 2011; Shirima et al. 2013; Guo et al. 2017). Studies in animal models indicate an additive or even synergistic effect on liver cancer due to an exposure to both mycotoxins (Lopez-Garcia 1998; World Health Organization 2018).

Aspergillus and Fusarium ear rots are more frequent in warmer and drier cropping seasons or a warmer and wetter weather combination at the time of harvest, and are often exacerbated by insect damage. Insect-vectored inoculum

can breach the natural plant defense. The invasive methods of inoculation by chewing and piercing insects would bypass resistance mechanisms, such as remote defense signals triggered in the husk, silk or seed surface in response to natural infection. Consequently, ear rot diseases are more common in the southern United States (US) and lowland tropics (Miller, 1994; reviewed in Cotty and Jaime-Garcia 2007; Santiago et al. 2015). Among insect pests infesting maize, European Corn Borer (**ECB**) causes the most serious damage (Boyd and Bailey, 2001; Hutchison et al. 2010). It not only injures plants, exposing them to infection, but also vectors ear rot and stalk rot fungi, particularly *F. verticillioides* and *F. graminearum* (Widstrom 1992). Extensive use of **Bt** (*Bacillus thurigiensis Cry*stal proteins-expressing) maize with its high efficacy against ECB, has reduced overall ECB populations in the US (Hutchison et al. 2010). Maize pests previously considered as secondary to ECB are now taking its position (Bowers et al. 2014). Corn earworm [**CEW**; *Helicoverpa zea* (Boddie); formerly in the genus *Heliothis*] has become the most economically important pest in the southern United States where non-freezing winters are conducive for CEW to multiply by 4-7 generations in a year. Resistance of this pest to a wide range of insecticides and to Bt maize has also been documented (Capinera 2004; Dively et al. 2016; Kaur et al. 2019). Although CEW has multiple crop and weed hosts, maize is its preferred host (Johnson et al. 1975). Annual yield loss due to CEW ranges from 2-17% for field corn and up to 50% in sweetcorn in the southern US. *A. flavus* and *F. verticillioides* invade the seed through silk and are also vectored by CEW and other ear-infesting insects (Munkvold and White 2016). *F. verticillioides* can grow

104 also as an endophyte through root or stem infection, and is vectored also by
105 insects such as ECB that feed on vegetative tissues (Blacutt et al. 2018). Unlike a
106 strong association observed in the case of FUM contamination (e.g., Smeltzer
107 1959; Dowd 2000; Mesterházy et al. 2012), seed AF levels were poorly correlated
108 with CEW damage caused by either natural invasion (Ni et al. 2011) or manual
109 infestation (Lillehoj et al. 1984). A meta-analysis of published work showed a 59%
110 reduction in the mean FB_1 concentration in Bt maize compared to the non-Bt
111 control (Cappelle 2018). A complete mitigation of AF or FUM, requires control of
112 multiple pests, including CEW (Abbas et al. 2013; Bowers et al. 2014; Porter and
113 Bynum 2018).

114 In addition to facilitating fungal colonization, insect infestation can also
115 enhance mycotoxin production in host tissues (Döll et al. 2013; Drakulic et al.
116 2015, 2016). In turn, mycotoxigenic fungi can affect insect vector infestation by
117 inducing volatile production in host tissues. This is particularly well documented
118 in the case of *Fusarium* species (Schulthess et al. 2002; Piesik et al. 2011; Drakulic
119 et al. 2016). For example, pre-inoculation of maize with *F. verticillioides* was
120 shown to enhance the fecundity and rate of development in Lepidopteran and
121 Coleopteran pests (Ako et al. 2003), while retarding larval development in
122 western corn rootworm (*Diabrotica virgifera virgifera*; Kurtz et al. 2010). We
123 observed a preferential CEW infestation and increased FUM contamination in *A.*
124 *flavus* resistant maize lines in our field trials. This previously unreported or
125 overlooked observation was pursued to unravel the factors underlying this novel
126 host-pathogen-insect interaction. Although late flowering might have facilitated

enhanced oviposition by *H. zea* in these maize lines, our analysis suggests that the toxicity of AF to CEW is a more compelling reason for the observed prevalence of ear damage in the low AF-accumulating genotypes.

RESULTS

Unusual weather pattern and corn earworm outbreak in 2018 summer

During the summer of 2018, daily profiles of rain fall and air temperature patterns were different from past years' average in Louisiana as well as many of the maize-growing states in US. The growing season was shorter (late April to early August) due to extended cold temperatures into the beginning of the planting season and relatively warmer and drier days during the early crop growth period (**Fig. S1**). April 2018 was the coldest April month since 1997 based on US average temperatures (and for Iowa and Wisconsin, it was the coldest April since records began in 1895). In contrast, May 2018 was the hottest May on record, breaking the record set in May 1934 during the Dust Bowl (National Oceanic and Atmospheric Administration: https://www.noaa.gov/). The unseasonal and steep warming after protracted cold seems to have favored an explosion of CEW population as indicated by a heavy infestation of ears in both of our experimental plots. CEW incidence was also reported from maize fields in other states in southern (Porter and Bynum 2018) as well as northern US (e.g., Handley 2018). In spite of two applications of a strong broad-spectrum insecticide before and after silking, the insecticide seems to have failed to reach silks covered by the husks. Further, all ears were bagged immediately after

149 inoculation/pollination, which concealed earworm damage until developing ears
150 were sampled for analysis.

151 **CEW infestation was significantly greater in *A. flavus* resistant maize lines**

152 During sampling of ears later in the season (July), we noticed that the two
153 resistant lines, the hybrid Mp313ExMp717 and the inbred CML322 showed
154 greater infestation by CEW than the susceptible lines GA209xT173 and B73 (**Fig.**
155 **1**, left panels). The infestation was <10% in susceptible lines and it ranged from
156 22% to 68% in the resistant lines. The maize lines used in the two field trials have
157 been extensively validated in the field and are often used as checks for evaluating
158 new genotypes and in mapping resistance loci (e.g., Mideros et al. 2012; Guo et
159 al. 2017). Despite our concerns that the distinctive patterns of CEW infestation
160 might potentially interfere with the genetic response of maize lines to *A. flavus*,
161 AF measurements showed that the genotype responses were robust in spite of
162 CEW infestation. As described in the **MATERIALS AND METHODS** section, we
163 harvested and utilized all ears in the plots to obtain robust AF data. The insect
164 infestation was 8-fold greater in CML322 than observed in B73 ears in the mock-
165 inoculated set. Inoculation with the highly toxigenic Tox4 strain resulted in a
166 significant ($p<0.01$) and nearly 4-fold decrease in the infestation of CML322, but
167 still 2-fold greater than infestation in B73. This is inversely correlated with >3-
168 fold increase in seed AF content in Tox4- inoculated CML322 ears. As expected
169 from its susceptibility to *A. flavus* colonization, B73 seeds accumulated >100 ppb
170 of AF even in mock-inoculated (Control) ears and >500 ppb in Tox4-inoculated

ears. These AF levels are >12-19 fold higher than those measured in CML322 seeds (**Fig. 1B**, right panel). CEW infestation was also greater in the resistant hybrid (Mp313E x Mp717) than in the susceptible hybrid by >30-fold in the control set and by 7-fold in the inoculated set (**Fig. 1A**, left panel). Infestation was inversely correlated with seed AF levels in hybrids as well. The susceptible hybrid (GA209×T173) had 100 ppb in control seeds and >400 ppb of AF in the inoculated set (i.e., 3 and 24-fold greater than in the resistant hybrid). Unlike the resistant inbred CML322, the resistant hybrid showed no difference in either AF content or CEW infestation between the control and CA14-inoculated ears. Analysis of variance (ANOVA) confirmed that only the host genotype (i.e., resistance to *A. flavus*) affected infestation highly significantly (>99.99% confidence level) and inoculation-induced differences were not statistically different (**Table S1 and S2**).

CEW infestation is negatively correlated with seed AF content

Not surprisingly, ANOVA of AF content revealed that the host genotype and inoculation with toxigenic *A. flavus* strains showed highly significant independent (or direct) as well as interaction effects on seed AF content. As indicated by the data presented in Fig. 1, infestation was also significantly related to AF content, although the interaction effect of genotype with infestation on AF was not significant (**Tables S3 and S4**). Both the resistant genotypes (CML322 and Mp313E×Mp717) manifested robust resistance to *A. flavus* and accumulated less than 30 ppb of AF in the seed either in the control (via colonization of native *A.*

193 *flavus* strains) or the inoculated set. Conversely, the susceptible inbred and
194 hybrid accumulated 100 and 500 ppb in control and inoculated sets, respectively.
195 AF content is inversely correlated with CEW infestation pattern in each of the
196 four maize genotypes. This relationship becomes clear when the data is
197 combined for control and inoculated sets in each genotype (**Fig. 2)** or when all
198 data is combined (**Fig. S2**). It is of interest to note that the uninfected controls
199 from both resistant lines showed a numerical but statistically insignificant
200 increase in AF in CEW-infested ears. AF was scarcely detectable levels in the
201 uninfested and uninoculated controls (a mean value of 6 ppb in Mp313E×Mp717
202 and <1 ppb in CML322) but increased by 5 and 14-fold in infested ears of
203 resistant hybrid and inbred respectively. This suggested that the resistance to *A.*
204 *flavus* colonization and AF contamination might have been compromised to
205 some extent in seeds heavily damaged by CEW.

206 **Kernel fumonisin content was enhanced in CEW-infested ears**

207 *Fusarium verticillioides* is among the most common mycotoxigenic fungi
208 colonizing field-grown maize. We observed symptoms of *F. verticillioides*
209 colonization (e.g., star-burst pattern on seeds) in our samples. We isolated the
210 fungus from seeds with visual symptoms using *Fusarium*-selective Malachite
211 Green Agar 2.5 medium (Alborch et al. 2009) and confirmed by genomic PCR
212 using *F. verticillioides*-specific primers (Baird et al. 2008). FUM content was
213 analyzed in the same seed samples used for AF determination (**Fig. 3 A**) and
214 compared between uninfested and CEW-infested samples (**Fig. 3B**).

Both maize hybrids used in this study have been previously shown to be resistant to FUM accumulation. In particular, Mp313ExMp717 (*A. flavus* resistant hybrid) was shown to be more robustly resistant than GA209xT173 across studies (Williams 2006; Henry et al. 2009; Williams and Windham 2009). In the current study, however, the Mp313ExMp717 accumulated >7-fold FUM in its seeds than GA209xT173 (**Fig. 3A**). Although CML322 accumulated a considerable amount of FUM, it was >4-fold less than that in B73, which is known to be among the most susceptible inbreds to Fusarium ear rot and FUM accumulation (Morales et al. 2019). However, when the data was parsed based on CEW infestation (only in sets where both clean and infested ears were available), infested ears showed >5-fold more FUM than uninfested ears (**Fig. 3B**). The differences were not significant probably due to the high variability in the colonization by native strains (the lowest p-value was 0.052 for CML322; also see **Fig. S2**). These data indicated that CEW may vector *Fusarium* spp. that produce FUM during its infestation.

Differential toxicity of AF versus FB1 to CEW

The preferential infestation of *A. flavus* resistant lines by CEW and a negative correlation between AF and infestation rate, taken together with greater FUM levels in infested ears, suggested that AF may be more toxic to *H. zea* than FUM. We tested this hypothesis by feeding experiments where CEW neonates were reared on artificial diet containing graded levels of AF or FB1. Results shown in **Fig. 4** and **5** clearly demonstrate that the pest is more susceptible to AF than to

238 FB1. As reported previously (Zeng et al. 2006), AF retarded CEW larval growth
239 even at the lowest concentration tested, although the effect was not significant
240 (**Fig. 5**) and was toxic above 200 ppb (**Fig. 4**). On the other hand, FB1 was non-
241 toxic to CEW even at the highest concentration tested. In fact, at lower
242 concentrations (below 30 ppm) the toxin seems to marginally enhance the growth
243 of the larvae (the effect was consistent although there was variability among the
244 bioassays). These results further support the proposal that the enhanced
245 infestation of *A. flavus* resistant maize lines by *H. zea* may be due to very low
246 levels of AF that are not inhibitory to larval growth.

247

248 **Delayed flowering in *A. flavus* resistant maize lines**

249 The tassel and ear development were delayed in CML322 by 3 weeks relative to
250 B73 and by 4-5 weeks in the resistant hybrid, Mp313E×Mp717 compared to
251 GA209×T173, although all four lines were planted together. CML322 is a tropical
252 inbred and shows delayed flowering under long days, i.e., ≥13 h photoperiod
253 (Hung et al. 2012). The parents of the resistant hybrid (Mp313Ex Mp717) are also
254 derived from the tropical maize race Tuxpeño (Scott and Zummo 1990; Williams
255 and Windham 2006) and known to show late-flowering phenotype. This is true
256 for most maize lines that are resistant to *A. flavus* and attempts to segregate the
257 two traits have been of limited success (Henry 2013). The availability of green
258 silks may be an important factor for the increased *H. zea* infestation of these late
259 flowering genotypes. However, in an adjacent plot where B73 was planted two

weeks later (unrelated to the current study), silk emergence coincided with that of CML322 plants used in the present study. Nonetheless, B73 ears had highly elevated levels of seed AF (400 ppb in controls and 800 ppb in inoculated plants) and low levels of CEW infestation in this plot as well, suggesting that high seed AF levels may act as a deterrent for CEW infestation because of its toxicity.

DISCUSSION

There are few studies where CEW infestation patterns have been compared in maize genotypes with varying resistance to *A. flavus* or AF accumulation. Nie et al (2011) compared spatial patterns of natural infestation of four ear-feeding insects (CEW, fall armyworm, maize weevil and brown stinkbug) with AF distribution due to colonization of a single commercial maize hybrid by native *A. flavus* strains. In the first year of the study, CEW infestation was very extensive (95% of ears) and in the second year, although less intense, it was as high as 41%. However, AF contamination was very low in both years (>80% of ears had ≤30 ppb and only ≤4% ears had ≤100 ppb). Although the predominantly low AF content makes it difficult to quantify the relationship, it is strongly indicative of a negative association between CEW damage and seed AF content. The maize genotypes in our study have proven resistance or susceptibility to *A. flavus*. Further, high AF contamination (≤100 ppb) in uninoculated as well as inoculated plots of only susceptible lines allowed to make robust comparisons.

The premise for this study is an unprecedented or unreported observation, in that two unrelated maize lines (Tuxpeño germplasm versus CML) with proven resistance to *A. flavus* were heavily infested by CEW. Conversely, the two *A. flavus* susceptible lines (stiff-stalk inbred B73 and non-stiff stalk hybrid GA209 x T173) were spared from heavy CEW damage. Although late flowering maize is known to be susceptible to CEW infestation by providing green silks, availability of silks alone could not fully explain our observations. Late flowering is more often a

problem in the northeastern US where it coincides with CEW migration from southern states. Furthermore, late planted B73 in an adjacent plot had delayed silk emergence but showed no CEW infestation. The other and more likely explanation is that the susceptible lines had very high levels of AF that were toxic to CEW. Even mock-inoculated controls had 100 ng of AF per gram of seed meal prepared from entire ears with both moldy and non-moldy seeds. This argument is supported by previous studies on AF toxicity to CEW in feeding experiments (Zeng et al. 2006) as well as our current work (**Fig. 4** and **5**). Zeng et al (2006) showed that AF at 200 ppb strongly inhibited the growth and development of first instar larvae, leading to >50% larval death after 9 d and 100% death after 15 d of feeding. Even lower concentrations (1-20 ppb; FDA-regulated levels) affected larval development, delayed pupation rate and led to >40% mortality when the exposure was longer than 7 d (Zeng et al. 2006). Although concentrations below 20 ppb were not tested in our study, we observed a steady decline in larval mass as AF concentration increased with ≥30% mortality at or above 250 ppb during 10-15 d exposure (**Fig. 5**). We did not continue our observations beyond the larval stage to assess the longer term developmental effects (e.g., pupation or emergence of adults). An apparent exception to the correlation between low AF and high CEW infestation was a significant decrease in CEW infestation observed in TOX4-inoculated ears compared to uninoculated ears in the *A. flavus* resistant inbred CML322, although average AF levels did not exceed 30 ppb. Given the highly variable distribution of AF in individual kernels of a maize ear (e.g., Lee et al. 1980), it is possible that AF content particularly in damaged kernels (close to

the silk canal, the site of inoculation as well as CEW infestation) was much greater than the average for the entire ear and high enough to be toxic to CEW survival. Furthermore, CEW may be sensitive also to other anti-insectan compounds made by *A. flavus* (Cary et al. 2018) that could act additively or synergistically with AF (e.g., Kojic acid; Dowd 1988). Future experiments would involve late-maturing lines with *A. flavus* susceptibility and early maturing lines with *A. flavus* resistance to clarify and quantify the effects of flowering time and AF content on CEW infestation.

It is not surprising that AF is toxic to insects, not merely to mammals. *A. flavus* is predominantly a soil-living saprophyte, feeding on decaying organic matter, including dead insects. It is also an opportunistic pathogen and can colonize a wide variety of insects, e.g., moths, silkworms, bees, grasshoppers, houseflies and mealy bugs among others (St. Leger et al. 2000; Gupta and Gopal 2002 and references therein). At the same time, *A. flavus* is known to survive ingestion by mycophagous insects. Among three *Aspergillus* species tested, *A. flavus* conidia phagocytized by insect hemocytes were still able to germinate (St. Leger et al. 2000). *A. flavus* may also proliferate in the hindgut of CEW (Abel et al., 2002). In spite of being a polyphagous pest with a remarkable capacity to metabolize a wide array of plant compounds, CEW has limited tolerance to AF and poor ability to metabolize the mycotoxin (Dowd 1988; Zeng et al. 2006). The fungus is known to make several anti-insectan compounds, beside AF (TePaske et al. 1992; Cary et al. 2018). Other insect pests that are more tolerant may vector *A. flavus* (Zeng et al. 2006; Opoku et al. 2019). Spatial correlation analysis of

natural infestation by different pests and seed AF content in field-grown maize plants indicated that AF content was correlated to the frequency of weevils and stink bug-affected kernels, but not with CEW damage (Ni et al 2011).

Our work also showed that FUM is not toxic to *H. zea* (**Fig. 4**). This may have allowed CEW to vector *F. verticillioides* and other FUM-contaminating fungi, as indicated by an increased seed FUM content in infested ears (**Fig. 3**). CEW damage is also frequently associated with the colonization by another mycotoxigenic fungus, *Stenocarpella maydis*, which causes diplodia ear rot (Munkvold and White 2016). In animal model systems, FB1 at 25-50 µM (i.e., 18-36 ppm) inhibits ceramide synthases and leads to the accumulation of toxigenic/carcinogenic sphinganine and related compounds (Riley et al., 2001; Riley and Merrill 2019). Conversely, FB1 was not toxic to yellow mealworm larvae even at 450 ppm when included in the diet or when injected into larva (Abado-Becognee et al. 1998). Recently, the brown marmorated stink bug (*Halyomorpha halys*) was shown to enhance *F. verticillioides* infection and FUM contamination in field corn (Opoku et al. 2019). Among other secondary metabolites produced by *F. verticillioides*, fusaric acid is only a weak antisectan compound (Dowd 1988). The lack of secondary metabolites with potent insecticidal properties in the biosynthetic repertoire of *F. verticillioides* could be one of the reasons for its frequently observed transmission via insect infestation (e.g., Smeltzer 1959; Dowd 2000; Mesterházy et al. 2012; Madege et al. 2018)

354 The association between CEW-infestation and high FUM content can also
355 be explained by host reaction to fungal infection potentially triggering enhanced
356 insect damage. Mycotoxin-producing *Fusarium* spp. trigger volatile production
357 by maize leaves that attract cereal leaf beetles (Piesik et al. 2011). Other examples
358 where insect species benefit from the presence of mycotoxigenic fungi are also
359 reported (Schulthess et al. 2002). Alternatively, insect-fungus interactions can
360 enhance production of secondary metabolites by plant host tissues (Döll et al.
361 2013; Drakulic et al. 2015, 2016).

362 Although this study was pursued to explain a serendipitous observation
363 made during two unrelated field studies, it has important implications in
364 mycotoxin control. AF and FUM are ubiquitous and unpredictable contaminants
365 of commodities, particularly maize. Our study clarifies a component of this
366 unpredictability. The late flowering trait of *A. flavus* resistant lines (owing to their
367 tropical origin) is known to delay harvest, potentially leading to frost damage
368 and/or high grain moisture. Our current work shows that delayed flowering
369 coupled with low AF accumulation can exacerbate CEW infestation, which in turn
370 can lead to contamination by other mycotoxins, such as fumonisins (Munkvold
371 and White 2016).

372 In contrast to a mutual antagonism reported previously between *A. flavus*
373 and *F. verticillioides* (Zummo and Scott 1992; also see **Fig. S3)**, we observed high
374 levels of AF and FUM co-contaminating our samples. B73, in particular with its
375 high susceptibility to both mycotoxigenic fungi, had very high levels of both AF

and FUM in many of its seed samples. Although CEW damage was very low in this inbred (**Fig. 1B and 2**), FUM levels were exacerbated in infested ears (**Fig. 3B**). There is some evidence for an additive or even synergistic effect on carcinogenicity from co-exposure to AF and FUM (World Health Organization 2018). Based on biomarker studies and food analyses, the co-occurrence of these two mycotoxins has been widely documented in developing countries (Shirima et al. 2013; Biomin Mycotoxin Survey 2019). It is important to examine the underlying factors as well as effects of mycotoxin co-contamination both by researchers and regulatory agencies to mitigate its impact on food safety (Lopez Garcia 1998).

388 ## MATERIALS AND METHODS

389 **Field planting of maize and application of *A. flavus* toxigenic strains**: The four
390 maize genotypes used in the study are non-transgenic and non-commercial lines.
391 The two hybrids, GA209×T173 (susceptible to AF accumulation) and
392 Mp313E×Mp717 (resistant to AF accumulation), were developed at the USDA-ARS
393 Corn Host Plant Resistance Research Unit, Mississippi (Williams and Windham
394 2009). The hybrids, along with two popular inbreds B73 (susceptible to AF
395 accumulation, (Campbell and White 1995) and CML322 (resistant to AF
396 accumulation, (Betrán et al 2002)) were planted in 4-row plots at the LSU
397 Agricultural Experimental Station in Baton Rouge (Louisiana) in the middle of
398 April. To keep the insect pressure low, Besiege (a broad-spectrum foliar
399 insecticide with fast knockdown and long-lasting residual effects; has
400 chlorantraniliprole and λ-cyhalothrin as active ingredients) was sprayed at ~V9
401 and R1 growth stages. Three days after the second insecticide application, plants
402 were inoculated with *A. flavus* strains by silk canal injections (Zummo and Scott
403 1992), with conidial suspensions as described before (Chalivendra et al. 2018).
404 The hybrids were inoculated with CA14, inbreds with Tox4. Plants were
405 maintained with standard agronomic practices of fertilizer and herbicide
406 applications and received irrigations during extended dry periods.

407 The inbred study was originally aimed at analyzing microbiome changes in
408 a susceptible and a resistant line in response to *A. flavus* colonization. We used
409 Tox4 in the study because it is an isolate from local maize fields (Chalivendra et

al. 2018), produces high AF levels and serves as a good model strain to study microbiome changes. The experiment with hybrids was an extension of recent studies on biofilm-like structure formation by *A. flavus* during maize seed colonization (Dolezal et al. 2013; Shu et al. 2014; Windham et al. 2018). The objective of our study was to localize the expression of *A. flavus* Medusa A gene by *in situ* hybridization in maize seeds in relation to the spatial distribution of the biofilm-like structure. *A. flavus* strain CA14 was used in the study, since it has whole genome sequence information and needed mutant resources (Chang et al. 2019). CA14 was obtained from the USDA Agricultural Research Service Culture Collection, Northern Regional Research Laboratory, Peoria, IL, USA.

HPLC analysis of aflatoxin B_1: One ear per plant from each genotype and treatment was harvested, resulting in 70-80 ears in inoculated plants and double the number from uninoculated plants. Ears in each lot were separated by the presence or absence of CEW infestation to monitor the effect of insect damage on mycotoxin levels. Only ears with visible internal damage (i.e., nibbled seed and cut silks, larval feeding tracks with frass; sometimes with dead or live CEW larvae) were considered as infested. No distinct spatial or other pattern of infestation was observed in our plots (as was also reported by Ni et al. 2011), except that a majority of resistant inbred or hybrid plants were infested, while only a few ears from susceptible lines showed damage by the earworm. At least three ears were used per replicate and each category had 3-5 replicates. Given the low frequency of CEW-damaged ears in B73 and GA209×T173, all ears in each category were used for AF analysis to have robust AF data. When the seed meal exceeded more

than 100 g (in uninoculated controls), we took more than one sample to minimize sampling error. AF from seed meal was extracted and measured as before (Chalivendra et al. 2018) with modified HPLC conditions. The equipment included Waters e2695 HPLC (Waters Corp., Milford, MA, United States) fitted with a Nova-Pak C18 column, a photochemical reactor (Aura Industries Inc., New York, United States) and a Waters 2475 FLR Detector (Waters Corp.). The signal was detected by excitation at 365 nm and emission at 440 nm. Aqueous methanol (37.5%) was used as the mobile phase.

LC-MS analysis of fumonisins: Maize kernel samples were analyzed for FB1, FB2 and FB3 by liquid chromatography–mass spectrometry (LC-MS) using an adaptation of a previously published method for mycotoxin analysis (Plattner 1999). Briefly, maize samples were ground with a laboratory mill. Portions (5 g) of the seed meal were extracted with 25 mL 1:1 acetonitrile/water for 2 h on a Model G2 Gyrotory Shaker (New Brunswick Scientific, Edison, NJ, USA). Extracts were filtered with a Whatman 125 mm 2V paper filter (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). A total of 10 µL of extract was applied to a Kinetex (Phenomenex, Torrance, CA, USA) C18 column (50 mm length, 2.1 mm diameter). Chromatography was conducted utilizing a Thermo Dionex Ultimate 3000 (Thermo Fisher, Waltham, MA, USA) ultrahigh-performance liquid chromatography (UPLC) system consisting of an autosampler coupled to a binary gradient pump. Elution of analyte was achieved with a 0.6 mL min−1 gradient flow of methanol and water (0.3% acetic acid was added to the mobile phase). The solvent program used a 35–95% gradient over 5 min. Flow was directed to a Q

Exactive (Thermo Fisher, Waltham, MA, USA) hybrid quadrupole-Orbitrap mass spectrometer equipped with an electrospray ionization source. The mass spectrometer was operated in full-scan mode over a range of 300 to 1200 m/z. Operation of the LC-MS and quantification of the eluting fumonisins were performed utilizing Thermo Xcalibur software. Quantification of fumonisins was based upon intensity of protonated ions for FB1 (m/z 722.3), FB2 (m/z 706.3) and FB3 (m/z 706.3) compared to calibration standards of the toxins. The limit of quantification for the analytical method was determined to be 0.1 µg per g for FB1, FB2 and FB3.

Toxicity bioassays

The toxicity of FUM to CEW larvae was tested in a pre-mixed meridic diet (WARD'S Stonefly Heliothis diet, Rochester, NY) containing 0. 3, 10, 30 60 or 100 µg/g FB1 (Cayman Chemical, MI) or 20, 50, 100, 250 or 500 ng/g of AFB_1 (Sigma Chemicals). The diet was prepared as per manufacturer's instructions. The FB1 stock, made in water, was diluted to the above rates before the dry diet was added and mixed thoroughly. AF was dissolved in methanol at a stock concentration of 2 mg/mL and diluted appropriately to provide the aforementioned concentrations. The highest concentration of methanol used (0.08% by w/w) was incorporated into the control diet. The assay was done in a 128 well bioassay plate (C-D International Inc., Pitman, NJ). A single CEW neonate from a laboratory CEW colony obtained from Benzon Research Inc. (Carlisle, PA) was added to each well

478 with 1 g diet using a camel hair brush (Kaur et al. 2019). At least 20 larvae were
479 tested per treatment and the assay was repeated four times.

480 **Statistical analysis of data**

481 Insect damage and aflatoxin levels were compared by ANOVA and post-hoc
482 analysis by Tukey's Honestly Significant Difference (HSD) test using R program
483 (version 3.6.2) in RStudio. Student's t-test was used for comparison of specific
484 pairs of data sets.

485

486

487 **Safety**

488 Aflatoxin B_1 and fumonisin B_1, being highly toxic mycotoxins, were handled with
489 care using a biohood, surgical gloves and nose as well as mouth masks. All
490 residues and containers were decontaminated using bleach and by autoclaving.

491 **SUPPLEMENTAL MATERIAL**

492 **SUPPLEMENTAL MATERIAL FOR THIS ARTICLE MAY BE FOUND AT:**

ACKNOWLEDGMENTS

SC thanks the National Corn Growers Association for the funding support through their AMCOE program. Excellent field and laboratory assistance by Mr. Anthony Nguyen is gratefully acknowledged. Dr. Z-Y Chen is thanked for his help in aflatoxin measurement.

REFERENCES

Abado-Becognee K, Fleurat-Lessard F, Creppy EE, Melcion D. 1998. Effects of fumonisin B1 on growth and metabolism of larvae of the yellow mealworm, Tenebrio molitor. Entomologia Experimentalis et Applicata 86:135-143. https://doi.org/10.1046/j.1570-7458.1998.00274.x

Abbas HK, Zablotowicz RM, Weaver MA, Shier WT, Bruns HA, Bellaloui N, Accinelli C, Abel CA. 2013. Implications of Bt Traits on Mycotoxin Contamination in Maize: Overview and Recent Experimental Results in Southern United States. Journal of Agricultural and Food Chemistry 61:11759-11770. https://doi.org/10.1021/jf400754g

Ako M, Schulthess F, Gumedzoe MYD, Cardwell KF. 2003. The effect of *Fusarium verticillioides* on oviposition behaviour and bionomics of lepidopteran and coleopteran pests attacking the stem and cobs of maize in West Africa. Entomologia Experimentalis et Applicata 106:201-210.

Alborch L, Bragulat MR, Cabañes FJ. 2010. Comparison of two selective culture media for the detection of *Fusarium* infection in conventional and transgenic maize kernels. Letters in Applied Microbiology 50:270-275.

Baird R., Abbas HK, Windham G, Williams P, Baird S, Ma P, Kelley R, Hawkins L, Scruggs M. 2008. Identification of select fumonisin forming *Fusarium* species using PCR applications of the polyketide synthase gene and its relationship to fumonisin production *in vitro*. International Journal of Molecular Sciences 9:554-570.

520 Betrán FJ, Isakeit T, Odvody G. 2002. Accumulation accumulation of white and
521 yellow maize inbreds in diallel crosses. Crop Science 42:1894-1901.

522 BIOMIN World Mycotoxin Survey. 2015

523 BIOMIN World Mycotoxin Survey. 2019 Quarter 1

524 Blacutt AA, Gold SE, Voss KA, Gao M, Glenn AE. 2018. *Fusarium verticillioides*:
525 advancements in understanding the toxicity, virulence, and niche adaptations of
526 a model mycotoxigenic pathogen of maize. Phytopathology 108:312-326.

527 Bowers E, Hellmich R, Munkvold G. 2014. Comparison of Fumonisin
528 Contamination Using HPLC and ELISA Methods in Bt and Near-Isogenic Maize
529 Hybrids Infested with European Corn Borer or Western Bean Cutworm. Journal
530 of Agricultural and Food Chemistry 62:6463-6472.

531 Boyd ML, Bailey WC. 2001. European Corn Borer: A Multiple-Crop Pest in Missouri.
532 Bulletin G7113. https://extension2.missouri.edu/g7113

533 Campbell KW, White DG. 1995. Evaluation of corn genotypes for resistance to
534 Aspergillus ear rot. kernel infection and aflatoxin production. Plant Disease
535 79:1039-1045.

536 Capinera JL. Encyclopedia of Entomology, 2004, Kluwer-Academic, New York.

537 Cappelle K. 2018. Fumonisin B1 in Bt and non-Bt maize: A meta-analysis. Iowa
538 State University Graduate Theses and Dissertations 16324.
539 https://doi.org/10.31274/etd-180810-5954

Cary JW, Gilbert MK, Lebar MD, Majumdar R, Calvo AM. 2018. *Aspergillus flavus* secondary metabolites: more than just aflatoxins. Food Safety 6:7-32.

Chalivendra S, DeRobertis C, Reyes Pineda J, Ham JH, Damann K. 2018. Rice phyllosphere *Bacillus* species and their secreted metabolites suppress *Aspergillus flavus* growth and aflatoxin production *in vitro* and in maize seeds. Toxins 10: 159.

Chang P-K, Scharfenstein LL, Mack B, Hua SST. 2019. Genome Sequence of an *Aspergillus flavus* CA14 Strain That Is Widely Used in Gene Function Studies. Microbiology Resource Announcements 8:e00837-19.

Cotty PJ, Jaime-Garcia R. 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. International Journal of Food Microbiology 119:109-115.

Dively GP, Venugopal PD, Finkenbinder C. 2016. Field-evolved resistance in corn earworm to Cry proteins expressed by transgenic sweet corn. PLoS ONE 11:e0169115.

Dolezal AL, Obrian GR, Nielsen DM, Woloshuk CP, Boston RS, Payne GA. 2013. Localization, morphology and transcriptional profile of *Aspergillus flavus* during seed colonization. Mol Plant Pathol 14:898-909. doi:10.1111/mpp.12056

Döll K, Chatterjee S, Scheu S, Karlovsky P, Rohlfs M. 2013. Fungal metabolic plasticity and sexual development mediate induced resistance to arthropod fungivory. Proceedings of the Royal Society B: Biological Sciences 280:20131219.

561 http://doi.org/10.1098/rspb.2013.1219

562 Dowd PF. 1988. Toxicological and biochemical interactions of the fungal
563 metabolites fusaric acid and kojic acid with xenobiotics in *Heliothis zea* (F.) and
564 *Spodoptera frugiperda* (J.E. Smith). Pesticide Biochemistry and Physiology 32:123-
565 134.

566 Dowd PF. 2000. Indirect Reduction of Ear Molds and Associated Mycotoxins in
567 *Bacillus thuringiensis* Corn Under Controlled and Open Field Conditions: Utility
568 and Limitations. Journal of Economic Entomology 93:1669-1679.

569 Drakulic J, Ajigboye O, Swarup R, Bruce T, Ray RV. 2016. Aphid Infestation
570 Increases *Fusarium langsethiae* and T-2 and HT-2 Mycotoxins in Wheat. Appl
571 Environ Microbiol 82:6548-6556.

572 Drakulic J, Caulfield J, Woodcock C, Jones SPT, Linforth R, Bruce TJA, Ray RV.
573 2015. Sharing a host plant (wheat *Triticum aestivum*) increases the fitness of
574 *Fusarium graminearum* and the severity of Fusarium head blight but reduces the
575 fitness of grain aphids (*Sitobion avenae*). Appl Environ Microbiol 81:3492–3501.
576 doi:10.1128/AEM.00226-15.

577 Guo B, Ji X, Ni X, Fountain JC, Li H et al. 2017. Evaluation of maize inbred lines
578 for resistance to pre-harvest aflatoxin and fumonisin contamination in the field.
579 The Crop Journal, 5:259-264.

580 Gupta A, Gopal M. 2002. Aflatoxin production by *Aspergillus flavus* isolates
581 pathogenic to coconut insect pests. World Journal of Microbiology and
582 Biotechnology 18:329.

583 Handley DT. 2018. MAJOR CORN EARWORM FLIGHT THREATENS LATE CORN.
584 Sweet Corn IPM Newsletter– September 6, No. 11
585 (https://extension.umaine.edu/highmoor/blog/tag/pest-management/).

586 Henry WB. 2013. Maize aflatoxin accumulation segregates with early maturing
587 selections from an S2 breeding cross population. Toxins 5:162-172.

588 Henry WB, Williams WP, Windham GL, Hawkins LK. 2009. Evaluation of maize
589 inbred lines for resistance to Aspergillus and Fusarium ear rot and mycotoxin
590 accumulation. Agronomy Journal 101:1219-1226.

591 Hung H-Y, Shannon LM, Tian F, Bradbury PJ, Chen C, Flint-Garcia SA, McMullen
592 MD, Ware D, Buckler ES, Doebley JF, Holland JB. 2012. ZmCCT and the genetic
593 basis of day-length adaptation underlying the postdomestication spread of
594 maize. Proceedings of the National Academy of Sciences, 109:E1913-E1921.

595 Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie, TW, Fleischer SJ,
596 Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster LV, Hunt
597 TE, Wright RJ, Pecinovsky K, Rabaey TL, Flood BR, Raun ES. 2010. Areawide
598 suppression of European Corn Borer with Bt maize reaps savings to non-Bt maize
599 growers. Science 330:222-225.

600 Johnson MW, Stinner RE, Rabb RL. 1975. Ovipositional response of *Heliothis zea*
601 (Boddie) to its major hosts in North Carolina. Environmental Entomology 4:291-
602 297.

603 Kaur G, Guo J, Brown S, Head GP, Price PA., Paula-Moraes S, Ni X, Dimase M, Huang
604 F. 2019. Field-evolved resistance of *Helicoverpa zea* (Boddie) to transgenic maize
605 expressing pyramided Cry1A.105/Cry2Ab2 proteins in northeast Louisiana, the
606 United States. Journal of Invertebrate Pathology 163:11-20.

607 Kurtz B, Vidal S, Karlovsky P. 2010. Interaction Between Western Corn Rootworm
608 (Coleoptera: Chrysomelidae) Larvae and Root-Infecting *Fusarium verticillioides*.
609 Environmental Entomology 39:1532-1538.

610 Lee LS, Lillehoj EB, Kwolek WF. 1980. Aflatoxin distribution in individual com
611 kernels from intact ears. Cereal Chem 57:340-343

612 Lillehoj EB, McMillian WW, Widstrom NW, Guthrie WD, Jarvis JL, Barry D, Kwolek
613 WF. 1984. Aflatoxin contamination of maize kernels before harvest: Interaction
614 of *Aspergillus flavus* spores, corn earworm larvae and fungicide applications.
615 Mycopathologia 86:77-81.

616 Lopez-garcia R. 1998. Aflatoxin B(1) and Fumonisin B(1) co-contamination:
617 Interactive effects, possible mechanisms of toxicity, and decontamination
618 procedures. LSU Historical Dissertations and Theses, 6746.

619 Madege RR, Audenaert K, Kimanya M, Tiisekwa B, De Meulenaer B, Bekaert B,
620 Landschoot S, Haesaert G. 2018. Control of *Fusarium verticillioides* (Sacc.)

621 Nirenberg and Fumonisins by Using a Combination of Crop Protection Products
622 and Fertilization. Toxins 10:67.

623 Mesterházy Á, Lemmens M, Reid LM. 2012. Breeding for resistance to ear rots
624 caused by *Fusarium* spp. in maize – a review. Plant Breeding 131:1-19.

625 Mideros SX, Windham GL, Williams WP, Nelson RJ. 2012. Tissue-specific
626 components of resistance to *Aspergillus* ear rot of maize. Phytopathology
627 102:787–793; doi:10.1094/PHYTO-12-11-0355

628 Miller JD. 1994. Epidemiology of Fusarium ear diseases of cereals. In Mycotoxins
629 in Grain: Compounds Other Than Aflatoxin. 1st ed.; Miller JD, Trenholm HL Eds.,
630 Eagan Press, St. Paul, MN, USA., pp. 19-36.

631 Morales L, Zila CT, Moreta Mejía DE, Montoya Arbelaez M, Balint-Kurti PJ, Holland
632 JB, Nelson RJ. 2019. Diverse components of resistance to *Fusarium verticillioides*
633 infection and fumonisin contamination in four maize recombinant inbred
634 families. Toxins 11:86.

635 Munkvold, GP 2003. Epidemiology of Fusarium Diseases and their Mycotoxins in
636 Maize Ears. European Journal of Plant Pathology, 109:705-713.

637 Munkvold GP, White DG. 2016. Compendium of corn diseases. Fourth ed. St. Paul,
638 Minnesota, USA.

639 Ni X, Wilson JP, Buntin GD, Guo B, Krakowsky MD, Lee RD, Cottrell TE, Scully BT,
640 Huffaker A, Schmelz EA. 2011. Spatial Patterns of Aflatoxin Levels in Relation to
641 Ear-Feeding Insect Damage in Pre-Harvest Corn. Toxins 3:920.

642 Opoku J, Kleczewski NM, Hamby KA, Herbert DA, Malone S, Mehl HL. 2019.
643 Relationship between invasive Brown Marmorated Stink Bug (*Halyomorpha halys*)
644 and fumonisin contamination of field corn in the mid-Atlantic U.S. Plant Disease
645 103:1189-1195.
646 Piesik D, Lemńczyk G, Skoczek A, Lamparski R, Bocianowski J, Kotwica K, Delaney
647 KJ. 2011. Fusarium infection in maize: Volatile induction of infected and
648 neighboring uninfected plants has the potential to attract a pest cereal leaf
649 beetle, *Oulema melanopus*. Journal of Plant Physiology 168:1534-1542.

650 Plattner RD. 1999. HPLC/MS analysis of Fusarium mycotoxins, fumonisins and
651 deoxynivalenol. Natural toxins 76:365-70.

652 Porter P, Bynum Ed. 2018. Texas Panhandle: Bt Corn – What's Up With All the
653 Corn Earworms? AgFax July 25

654 (https://focusonagriculture.blogspot.com/2018/07/whats-up-with-all-corn-
655 earworms-in-bt.html).

656 Riley RT & Merrill AH. 2019. Ceramide synthase inhibition by fumonisins: a
657 perfect storm of perturbed sphingolipid metabolism, signaling and disease.
658 Journal of Lipid Research 60:1183-1189.
659 Riley RT, Enongene E, Voss KA, Norred WP, Meredith FI, Sharma RP, Spitsbergen
660 J, Williams DE, Carlson DB, Merrill AH Jr. 2001. Sphingolipid perturbations as
661 mechanisms for fumonisin carcinogenesis. Environ Health Perspect. 109 Suppl
662 2:301-8.

663 Santiago R, Cao A, Butrón, A. 2015. Genetic factors involved in fumonisin
664 accumulation in maize kernels and their implications in maize agronomic
665 management and breeding. Toxins, 7:3267-3296.

666 Schulthess F, Cardwell KF, Gounou S. 2002. The effect of endophytic *Fusarium*
667 *verticillioides* on infestation of two maize varieties by *Lepidopterous* stemborers
668 and Coleopteran grain feeders. Phytopathology 92:120-128.

669 Scott GE, Zummo N. 1990. Registration of Mp313E Parental Line of Maize. Crop
670 Science 30:1378-1378.

671 Shirima CP, Kimanya ME, Kinabo JL, Routledge MN, Srey C, Wild CP, et al. 2013.
672 Dietary exposure to aflatoxin and fumonisin among Tanzanian children as
673 determined using biomarkers of exposure. Mol Nutr Food Res 57:1874-81.

674 Shu X, Livingston DP, Franks RG, Boston RS, Woloshuk CP, Payne GA. 2014.
675 Tissue-specific gene expression in maize seeds during colonization by
676 *Aspergillus flavus* and *Fusarium verticillioides*. Mol Plant Pathol 16:662-74. doi:
677 10.1111/mpp.12224

678 Smeltzer, D. 1959. Relationship between Fusarium ear rot and corn earworm
679 infestation. Agronomy Journal 51:53-55.

680 St Leger R.J, Screen SE, Shams-Pirzadeh B. 2000. Lack of host specialization in
681 *Aspergillus flavus*. Appl Environ Microbiol 66:320-324.

682 Sun G, Wang S, Hu X, Su J, Zhang Y, Xie Y et al. 2011. Co-contamination of
683 aflatoxin B1 and fumonisin B1 in food and human dietary exposure in three areas
684 of China. Food Additives & Contaminants: Part A, 28:461-470.

685 TePaske MR, Gloer JB, Wicklow DT, Dowd PF. 1992. Aflavarin and β-aflatrem: new
686 anti-insectan metabolites from the sclerotia of *Aspergillus flavus*. Journal of
687 Natural Products 55:1080-1086.

688 Widstrom NW. 1992. Aflatoxin in developing maize: Interactions among involved
689 biota and pertinent econiche factors. in: Handbook of Applied Mycology Vol. 5,
690 Mycotoxins in Ecological Systems. Marcel Dekker, Basel, Pp. 59-86.

691 Williams WP. 2006. Breeding for resistance to aflatoxin accumulation in maize.
692 Mycotoxin Research, 22:27-32.

693 Williams WP, Windham GL. 2009. Diallel analysis of fumonisin accumulation in
694 maize. Field crops research, 114:324-326.

695 Williams WP, Windham GL. 2006. Registration of Maize Germplasm Line Mp717
696 Joint contribution of USDA-ARS and the Mississippi Agricultural and Forestry
697 Experiment Station. Miss. Agric. and Forestry Exp. Stn. Journal No. J-10802.
698 Registration by CSSA. Crop Science 46:1407-1408.

699 Windham GL, Williams WP, Mylroie JE, Reid CX, Womack ED. 2018. A Histological
700 study of *Aspergillus flavus* colonization of wound inoculated maize kernels of
701 resistant and susceptible maize Hybrids in the field. Frontiers in microbiology
702 9:799-799.

703 World Health Organization, Co-exposure of fumonisins with aflatoxins. Food
704 Safety Digest 2018, WHO/NHM/FOS/RAM/18.3.

705 Zeng RSL, Niu G, Wen Z, Schuler MA, Berenbaum MR. 2006. Toxicity of Aflatoxin
706 B1 to *Helicoverpa zea* and Bioactivation by Cytochrome P450 Monooxygenases.
707 Journal of Chemical Ecology, 32:1459-1471.

708 Zummo N, Scott GE. 1992. Interaction of *Fusarium moniliforme* and *Aspergillus*
709 *flavus* on Kernel Infection and Aflatoxin Contamination in Maize Ears. Plant
710 Disease 76:771-773.

FIGURE LEGENDS

Fig. 1. Rate of corn earworm infestation (left panels) and seed AF content (right panels) in maize lines. (**A**) Data is from hybrid plots. Infestation was significantly dependent on the host genotype with very little difference between control (mock-inoculated) and CA14-inoculated set. Seed AF content in CA14-inoculated set and the control were also similar in the resistant hybrid (Mp313E x Mp717). (**B**) Data shown is from inbreds. There was a similar negative relationship between CEW infestation rate and seed AF content as was observed in hybrids. Infestation was significantly dependent on the host genotype with very little difference between control (mock-inoculated) and Tox4-inoculated plots except in the case of CML322. The resistant inbred showed only 30% infestation in Tox4 inoculated set compared to the control. Seed AF levels were significantly higher in B73 both in control and inoculated ears than those of CML322. Values shown are average + SE. Significant differences (P value <0.05) between each data set were tested using an ANOVA (Supplemental Table 1) followed by Tukey's multiple-comparisons post hoc test (Supplemental Table 2) in R (version 3.6.2). Means are significantly different if marked by a different letter.

FIG. 2. CEW damage is negatively correlated with seed AF content in maize 20 lines. The infestation and AF data from control and infected ears is combined in each genotype. Significant differences (P value <0.05) between each data set were tested using an ANOVA (Supplemental Table 3) followed by Tukey's multiple-comparisons post hoc test (Supplemental Table 4) in R. Average (+SE) infestation

and AF values between *A. flavus* susceptible and resistant lines are highly significant ($p<0.01$).

Fig. 3. FUM contamination by native *Fusarium* strains. (**A**) Seed fumonisin content in the four maize lines. (**B**) Seed FUM content parsed by uninfested (clean) versus CEW infested ears in each genotype. The values are averages + SE in each genotype and were not significantly different at 95% confidence level.

Fig. 4. Effect of aflatoxin B_1 and fumonisin B_1 on the growth and mortality of *H. zea* larvae. Graded doses of AF or FB_1 was tested on CEW growth and mortality by incorporating them into an artificial insect diet. Larvae were grown in a 128 well bioassay plate for 10 d. Each well had 1 g of feed and a single neonate at the start of the assay. A representative assay from 4 replicates is shown. In an additional assay, 100 ppm of FB_1 and 300 ppb of AF were tested. Results were not different, except for a greater larval mortality at 300 ppb of AF (data not shown). Scale Bar = 1 cm.

Fig. 5. AF and FB_1 effects on CEW larval mass. At the end of the bioassay, larvae were removed from the well killed by chloroform vapors and weighed. Values are averages + SE of ≥16 larvae/treatment except at 250 ppb of AF, where mortality was 30% or greater (dead and dried were seen stuck to the bottom of the well). The values marked with the same letter are not statistically significant. FB_1 had no significant effect on larval growth at concentrations tested.

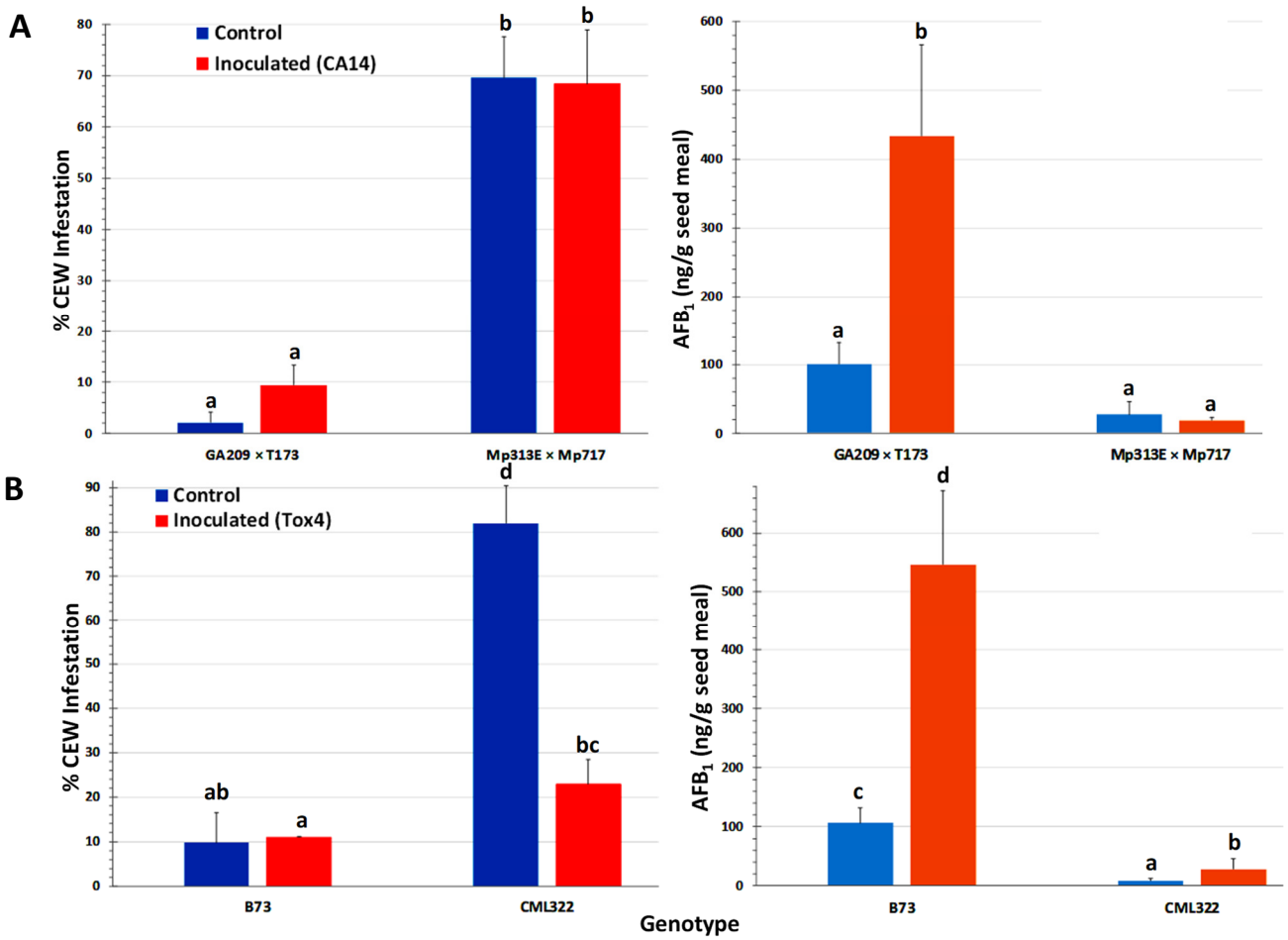

Fig. 1. Rate of corn earworm infestation (left panels) and seed AF content (right panels) in maize lines. (**A**) Data is from hybrid plots. Infestation was significantly dependent on the host genotype with very little difference between control (mock-inoculated) and CA14-inoculated set. Seed AF content in CA14-inoculated set and the control were also similar in the resistant hybrid (Mp313E x Mp717). (**B**) Data shown is from inbreds. There was a similar negative relationship between CEW infestation rate and seed AF content as was observed in hybrids. Infestation was significantly dependent on the host genotype with very little difference between control (mock-inoculated) and Tox4-inoculated plots except in the case of CML322. The resistant inbred showed only 30% infestation in Tox4

inoculated set compared to the control. Seed AF levels were significantly higher in B73 both in control and inoculated ears than those of CML322. Values shown are average + SE. Significant differences (P value <0.05) between each data set were tested using an ANOVA (Supplemental Table 1) followed by Tukey's multiple-comparisons post hoc test (Supplemental Table 2) in R (version 3.6.2). Means are significantly different if marked by a different letter.

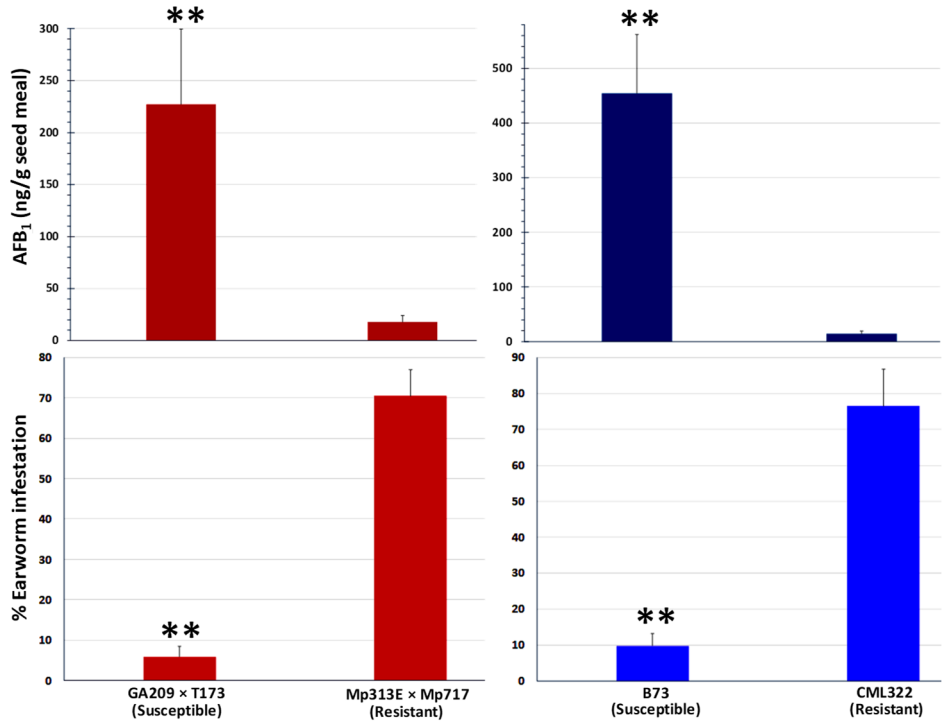

Fig. 2. CEW damage is negatively correlated with seed AF content in maize lines. The infestation and AF data from control and infected ears is combined in each genotype. Significant differences (P value <0.05) between each data set were tested using an ANOVA (Supplemental Table 1) followed by Tukey's multiple-comparisons post hoc test (Supplemental Table 2) in R (version 3.6.2). Average (+SE) infestation and AF values between *A. flavus* susceptible and resistant lines are highly significant ($p<0.01$).

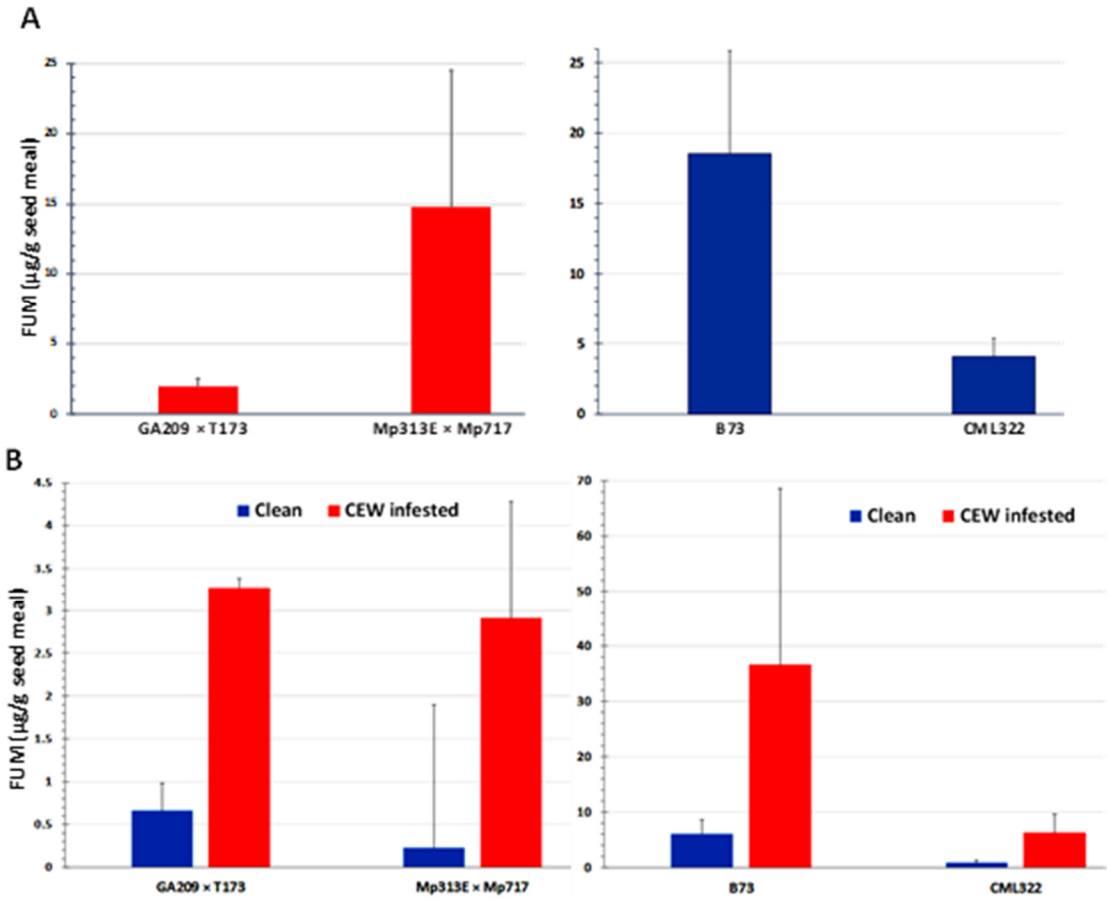

Fig. 3. FUM contamination by native *Fusarium* strains. (**A**) Seed fumonisin content in the four maize lines. (**B**) Seed FUM content parsed by uninfested (clean) versus CEW infested ears in each genotype. The values are averages + SE in each genotype and were not significantly different at 95% confidence level.

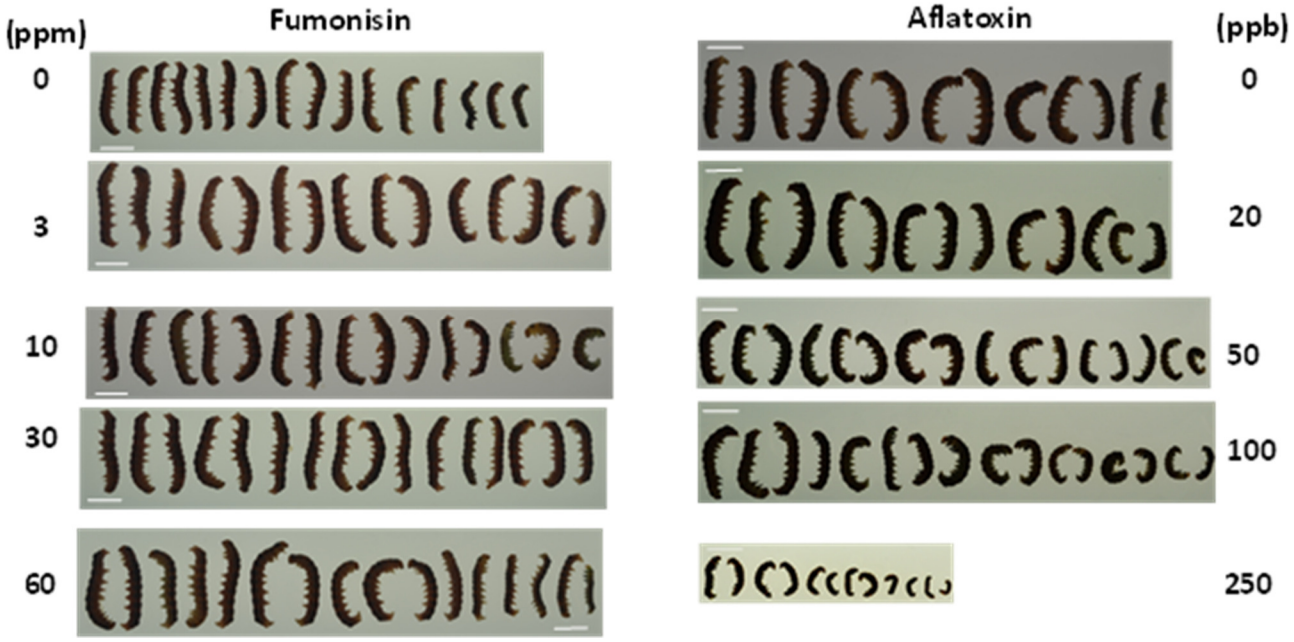

Fig. 4. Effect of aflatoxin B_1 and fumonisin B_1 on the growth and mortality of *H. zea* larvae. Graded doses of AF or FB_1 was tested on CEW growth and mortality by incorporating them into an artificial insect diet. Larvae were grown in a 128 well bioassay plate for 10 d. Each well had 1 g of feed and a single neonate at the start of the assay. A representative assay from 4 replicates is shown. In an additional assay, 100 ppm of FB_1 and 300 ppb of AF were tested. Results were not different, except for a greater larval mortality at 300 ppb of AF (data not shown). Scale Bar = 1 cm.

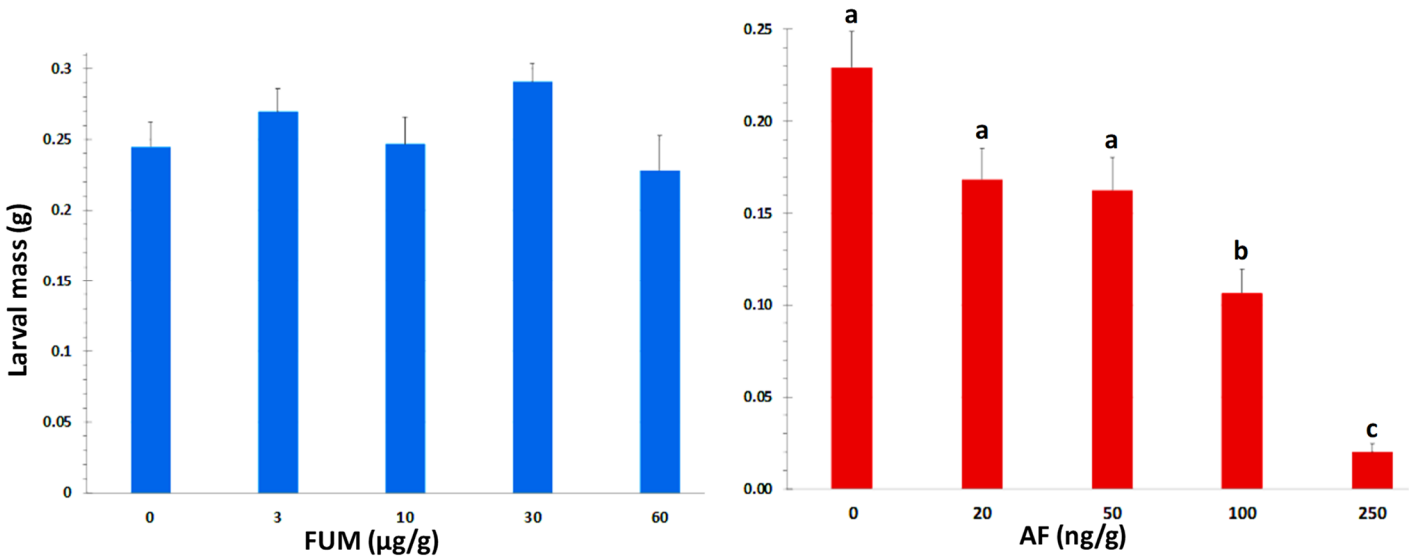

Fig. 5. AF and FB_1 effects on CEW larval mass. At the end of the bioassay, larvae were removed from the well killed by chloroform vapors and weighed. Values are averages + SE of ≥16 larvae/treatment except at 250 ppb of AF, where mortality was 30% or greater (dead and dried were seen stuck to the bottom of the well). The values marked with the same letter are not statistically significant. FB_1 had no significant effect on larval growth at concentrations tested.

Supplemental data

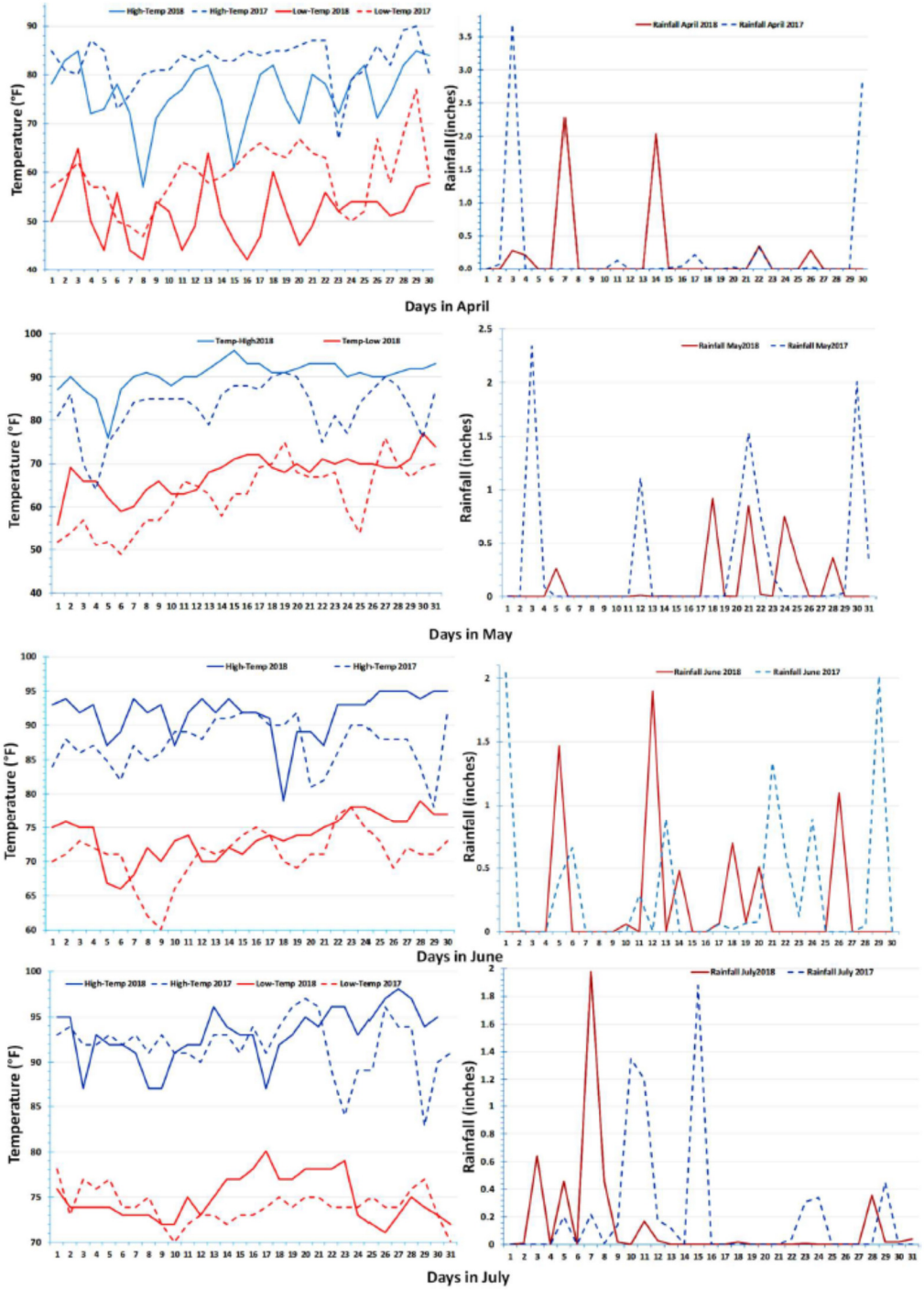

Figure S1. Weather data for the months of April-July in 2018 (solid lines) and 2017 (dashed lines). Daily high (blue lines) and low (red lines) temperatures are shown in the left panel. Rain fall is shown in the right panel.

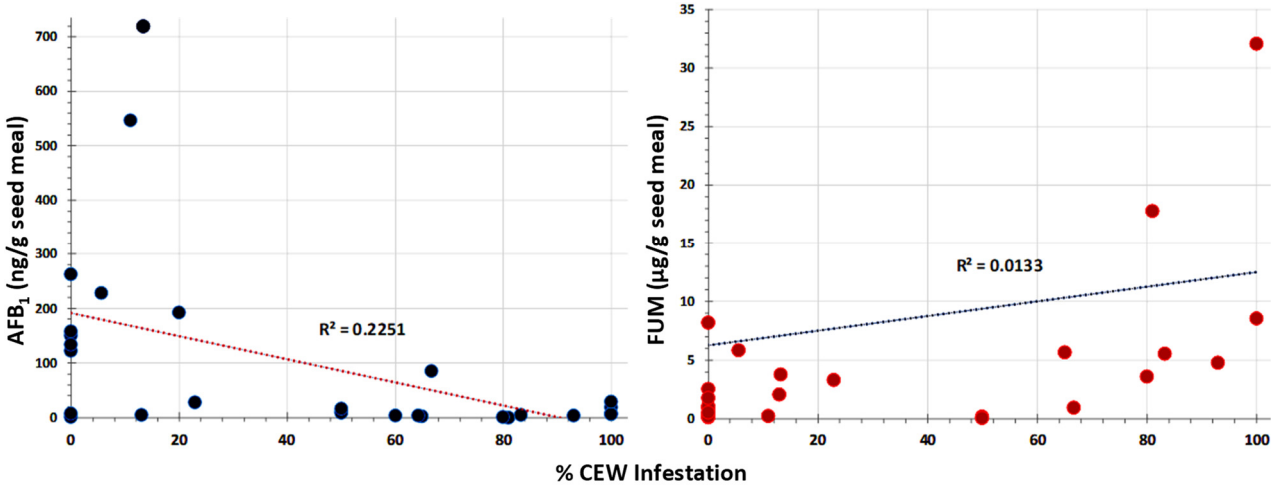

Fig. S2. Correlation between CEW infestation of ears and seed AF or FUM levels in maize. Combined data from inbred and hybrid maize lines is plotted. CEW showed a negative relationship with AF and a positive trend with FUM. The greater correlation observed with AF (Pearson correlation coefficient, R = -0.47) was likely because of manual inoculation with specific strains of *A. flavus* (dominant to native strains), whereas more random infestation by native *Fusarium* strains may have led to poor correlation (R = 0.115).

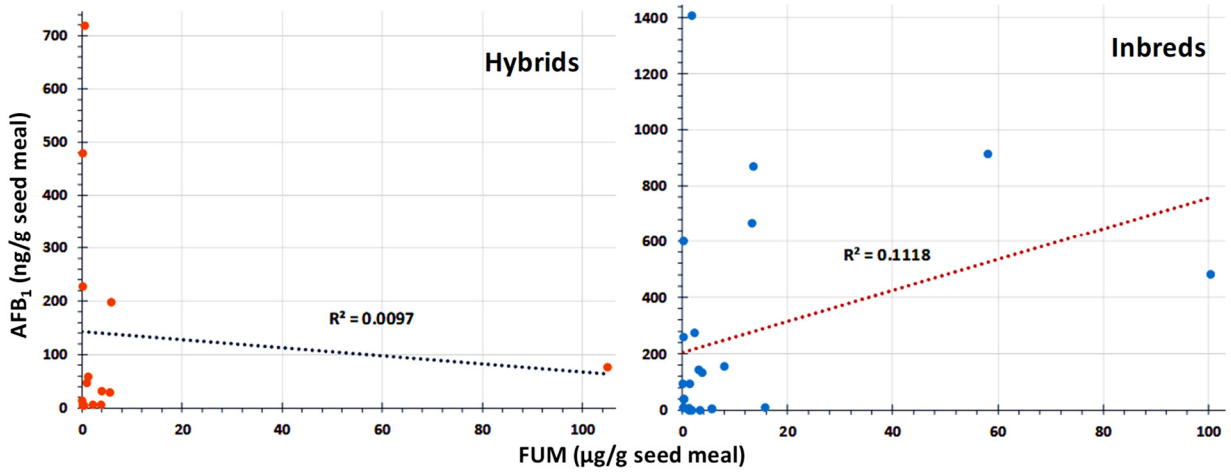

Fig. S3. Correlation of Seed FUM and AF contents in hybrids and inbreds. Contents of the two mycotoxins from the same seed sample are poorly correlated in both sets as indicated by Pearson correlation coefficient values (r = -0.0983 for hybrids and 0.3344 for inbreds). This lack of correlation indicated that there was no mutual effect in the production of the two mycotoxins by the fungi infecting seeds from same ears.

Table S1. Analysis of variance for CEW infestation in maize inbreds and hybrids with differential resistance to aspergillus ear rot.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Infection	1	693	693	1.595	0.225	
Genotype	3	20828	6943	15.970	4.54e-05	***
Inoculation :Genotype	3	321	107	0.246	0.863	
Residuals	16	6956	435			

Signif. codes: 0 ‘***’ 0.001 ‘**’

Table S2. Tukey HSD for Infestation data

> TukeyHSD(fm)

Tukey multiple comparisons of means

95% family-wise confidence level

Fit: aov(formula = Infestation ~ Infectio n + Genotype + Infecti on:Genoty + Genotype:Infection, d

data = ANOVA_infestation

$Infection

	diff	lwr	upr	p adj
uninfected-Infected	-10.75	-28.79485	7.29 4852	0.224718

$Genotype

	diff	lwr	upr	p adj
SusHybrid-ResHybrid	-68.266667	-102.70743	-33.82590	0.0001845
SusInbred-ResInbred	-46.500000	-80.94076	-12.05924	0.0067744

$`Infection:Genotype`

	diff	lwr	upr	p adj
uninfected:ResHybrid-Infected:ResHybrid		-82.14037	47.34037	0.9964093
Infected:SusHybrid-Infected:ResHybrid		-198.673707	-10.9263	0.0146146
uninfected:SusHybrid-Infected:ResHybrid		-215.473707	-19.3263	0.0055849
Infected:SusHybrid-uninfected:ResHybrid		-175.473707	0.673702	0.0538366
uninfected:SusHybrid-uninfected:ResHybrid		-192.273707	-7.7263	0.0210435
uninfected:ResInbred-Infected:ResInbred		-102.273707	37.2737	0.8962415
Infected:SusInbred-Infected:ResInbred		-172.273707	2.273702	0.0640846
uninfected:SusInbred-Infected:ResInbred		-174.94037	0.940369	0.0554305
Infected:SusInbred-uninfected:ResInbred		-128.94037	23.94037	0.4787105
uninfected:SusInbred-uninfected:ResInbred		-131.607033	22.60704	0.4352576
uninfected:SusHybrid-Infected:SusHybrid		-75.74037	50.54037	0.9995344
uninfected:SusInbred-Infected:SusInbred		-61.607033	57.60704	1
Infected:SusInbred-Infected:ResInbred		-177.819737	9 -38.6221	0.0000303
uninfected:SusInbred-Infected:ResInbred		-180.48641	3 -39.9554	0.0000241
Infected:SusInbred-uninfected:ResInbred		-204.858497	9 -52.1414	0.0000033
uninfected:SusInbred-uninfected:ResInbred		-207.52517	3 -53.4748	0.0000027
uninfected:SusHybrid-Infected:SusHybrid		-50.32517	3 25.1251	0.9851455
uninfected:SusInbred-Infected:SusInbred		-36.191833	6 32.1918	0.9999999

Table S3. Analysis of variance for seed aflatoxin content in maize inbreds and hybrids with differential resistance to aspergillus ear rot.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Genotype	3	3623512	1207837	34.373	3.99e-10	***
Infection	1	726043	726043	20.662	7.41e-05	***
Infestation	1	308549	308549	8.781	0.005705	**
Genotype:Inoculation	3	765751	255250	7.264	0.000753	***
Genotype:Infestation	3	423363	141121	4.016	0.015605	*
Inoculation:Infestation	1	1979	1979	0.056	0.813916	
Genotype:Inoculation :Infestation	3	125570	41857	1.191	0.328698	
Residuals	32	1124456	35139			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Table S4. Tukey's HSD for AF data

$Genotype

	diff	lwr	upr	p adj
ResInbred-ResHybrid	-3.009286	-210.3515	204.3329	0.9999777
SusHybrid-ResHybrid	313.154609	105.8124	520.4968	0.0014703
SusInbred-ResHybrid	664.271320	456.9291	871.6135	0.0000000
SusHybrid-ResInbred	316.163895	108.8217	523.5061	0.0013187
SusInbred-ResInbred	667.280606	459.9384	874.6228	0.0000000
SusInbred-SusHybrid	351.116711	143.7745	558.4589	0.0003654

$Infection

	diff	lwr	upr	p adj
Infected-Control	245.9747	135.7491	356.2003	7.41e-05

$Infestation

	diff	lwr	upr	p adj
Uninfested-Infested	160.3508	50.12523	270.5765	0.0057046

$`Genotype:Infection`

	diff	lwr	upr	p adj
SusHybrid:Control-ResHybrid:Control	108.9676382	-241.61198	459.5473	0.970012
ResHybrid:Infected-ResHybrid:Control	-13.3826243	-363.96224	337.197	1
SusHybrid:Infected-ResHybrid:Control	503.9589558	153.37934	854.5386	0.001249
SusInbred:Control-ResInbred:Control	384.7115015	34.13189	735.2911	0.023423
ResHybrid:Infected-ResInbred:Control	5.6059339	-344.97368	356.1856	1
ResInbred:Infected-ResInbred:Control	18.5759208	-332.00369	369.1555	1
SusInbred:Infected-ResInbred:Control	968.4256307	617.84602	1319.005	0
ResHybrid:Infected-SusHybrid:Control	-122.3502624	-472.92988	228.2294	0.944971
SusHybrid:Infected-SusHybrid:Control	394.9913176	44.41170	745.5709	0.018472
ResInbred:Infected-SusInbred:Control	-366.1355806	-716.71519	-15.556	0.035609
SusInbred:Infected-SusInbred:Control	583.7141292	233.13452	934.2937	0.000156
SusHybrid:Infected-ResHybrid:Infected	517.3415800	166.76197	867.9212	0.000884
SusInbred:Infected-ResInbred:Infected	949.8497099	599.27010	1300.429	0

$`Genotype:Infestation`

	diff	lwr	upr	p adj
SusHybrid:Infested-ResHybrid:Infested	211.6737740	-138.9	0584	562.2 5339	0.5252719
ResHybrid:Uninfested-ResHybrid:Infested	-11.1046741	-361.6	8429	339.4 7494	1.0000000
SusHybrid:Uninfested-ResHybrid:Infested	403.5307702	52.9	5116	754.1 1038	0.0151219
SusInbred:Infested-ResInbred:Infested	442.6270845	92.0	4747	793.2 0670	0.0058831
ResInbred:Uninfested-ResInbred:Infested	5.6720109	-344.9	0760	356.2 5162	1.0000000
SusInbred:Uninfested-ResInbred:Infested	897.6061377	547.0	2652	1248. 8575	0.0000000
ResHybrid:Uninfested-SusHybrid:Infested	-222.7784480	-573.3	5806	127.8 0117	0.4613626
SusHybrid:Uninfested-SusHybrid:Infested	191.8569962	-158.7	2262	542.4 3661	0.6418312
ResInbred:Uninfested-SusInbred:Infested	-436.9550736	-787.5	3469	-86.3 7546	0.0067638
SusInbred:Uninfested-SusInbred:Infested	454.9790532	104.3	9944	805.5 5867	0.0043306

SusHybrid:Uninfested-ResHybrid:Uninfested	414.6354442	64.0	5583	765.2	1506	0.0116165
SusInbred:Uninfested-ResInbred:Uninfested	891.9341268	541.3	5451	1242.	1374	0.0000001

$`Infection:Infestation`

	diff	lwr	upr	p adj
Infected:Infested-Control:Infested	258.81725	51.47506	4 66.1594	0.(98206
Control:Uninfested-Control:Infested	173.19341	-34.14878	3 80.5356	0.1 282735
Infected:Uninfested-Control:Infested	406.32553	198.98334	6 13.6677	0.(461
Control:Uninfested-Infected:Infested	-85.62384	-292.96603	1 21.7184	0.(807700
Infected:Uninfested-Infected:Infested	147.50828	-59.83391	3 54.8505	0.2 370108
Infected:Uninfested-Control:Uninfested	233.13212	25.78993	4 40.4743	0.(227130

$`Genotype:Infection:Infestation`

		diff	lwr	upr
SusHybrid:Control:Infested-ResHybrid:Control:Infested		34.26629	-533.27882	601.8114(
ResHybrid:Infected:Infested-ResHybrid:Control:Infested		-43.6601	-611.20516	523.8850(
SusHybrid:Infected:Infested-ResHybrid:Control:Infested		345.4212	-222.12391	912.96631
ResHybrid:Control:Uninfested-ResHybrid:Control:Infested		-41.3821	-608.92721	526.16301
SusHybrid:Control:Uninfested-ResHybrid:Control:Infested		142.2869	-425.25823	709.8319(
ResHybrid:Infected:Uninfested-ResHybrid:Control:Infested		-24.4873	-592.03241	543.05781
SusHybrid:Infected:Uninfested-ResHybrid:Control:Infested		621.1146	53.56949	1188.65972
SusInbred:Control:Infested-ResInbred:Control:Infested		57.88487	-509.66025	625.4299(
ResInbred:Infected:Infested-ResInbred:Control:Infested		-0.85515	-568.40026	566.6899(
SusInbred:Infected:Infested-ResInbred:Control:Infested		826.5142	258.96905	1394.05927
ResInbred:Control:Uninfested-ResInbred:Control:Infested		-13.7591	-581.30417	553.7860(
SusInbred:Control:Uninfested-ResInbred:Control:Infested		697.7791	130.23397	1265.32419
ResInbred:Infected:Uninfested-ResInbred:Control:Infested		24.24793	-543.29718	591.7930(
SusInbred:Infected:Uninfested-ResInbred:Control:Infested	1	96.57805	529.03294	1664.12316
ResHybrid:Infected:Infested-SusHybrid:Control:Infested		-77.9263	-645.47146	489.6187(
SusHybrid:Infected:Infested-SusHybrid:Control:Infested		311.1549	-256.39020	878.70002
ResHybrid:Control:Uninfested-SusHybrid:Control:Infested		-75.6484	-643.19351	491.89672
SusHybrid:Control:Uninfested-SusHybrid:Control:Infested		108.0206	-459.52452	675.5657(
ResHybrid:Infected:Uninfested-SusHybrid:Control:Infested		-58.7536	-626.29870	508.79152
SusHybrid:Infected:Uninfested-SusHybrid:Control:Infested		586.8483	19.30320	1154.39343
ResInbred:Infected:Infested-SusInbred:Control:Infested		-58.74	-626.28512	508.8051(
SusInbred:Infected:Infested-SusInbred:Control:Infested		768.6293	201.08418	1336.1744(
ResInbred:Control:Uninfested-SusInbred:Control:Infested		-71.6439	-639.18903	495.9011(
SusInbred:Control:Uninfested-SusInbred:Control:Infested		639.8942	72.34910	1207.43933
ResInbred:Infected:Uninfested-SusInbred:Control:Infested		-33.6369	-601.18205	533.90818
SusInbred:Infected:Uninfested-SusInbred:Control:Infested	1	38.69318	471.14807	1606.23829
SusHybrid:Infected:Infested-ResHybrid:Infected:Infested		389.0813	-178.46386	956.62637
ResHybrid:Control:Uninfested-ResHybrid:Infected:Infested		2.27795	-565.26716	569.8230(
SusHybrid:Control:Uninfested-ResHybrid:Infected:Infested		185.9469	-381.59818	753.4920(
ResHybrid:Infected:Uninfested-ResHybrid:Infected:Infested		19.17276	-548.37236	586.71787
SusHybrid:Infected:Uninfested-ResHybrid:Infected:Infested		664.7747	97.22955	1232.31977
SusInbred:Infected:Infested-ResInbred:Infected:Infested		827.3693	259.82419	1394.91441
ResInbred:Control:Uninfested-ResInbred:Infected:Infested		-12.9039	-580.44902	554.6412(

SusInbred:Control:Uninfested-ResInbred:Infected:Infested		698.6342	131.08912	1266.17934
ResInbred:Infected:Uninfested-ResInbred:Infected:Infested		25.10308	-542.44203	592.64819
SusInbred:Infected:Uninfested-ResInbred:Infected:Infested	1	97.43319	529.88808	1664.97831
ResHybrid:Control:Uninfested-SusHybrid:Infected:Infested	-	386.8033	-954.34842	180.74181
SusHybrid:Control:Uninfested-SusHybrid:Infected:Infested	-	203.1343	-770.67943	364.41079
ResHybrid:Infected:Uninfested-SusHybrid:Infected:Infested	-	369.9085	-937.45361	197.63661
SusHybrid:Infected:Uninfested-SusHybrid:Infected:Infested		275.6934	-291.85171	843.23852
ResInbred:Control:Uninfested-SusInbred:Infected:Infested	-	840.2732	-1680.55	
SusInbred:Control:Uninfested-SusInbred:Infected:Infested	-	128.7351	-696.28019	438.81004
ResInbred:Infected:Uninfested-SusInbred:Infected:Infested	-	802.2662	-1604.53	
SusInbred:Infected:Uninfested-SusInbred:Infected:Infested		270.0639	-297.48122	837.60900
SusHybrid:Control:Uninfested-ResHybrid:Control:Uninfested		183.669	-383.87613	751.21410
ResHybrid:Infected:Uninfested-ResHybrid:Control:Uninfested		16.8948	-550.65031	584.43992
SusHybrid:Infected:Uninfested-ResHybrid:Control:Uninfested		662.4967	94.95160	1230.04182
SusInbred:Control:Uninfested-ResInbred:Control:Uninfested		711.5381	143.99303	1279.08325
ResInbred:Infected:Uninfested-ResInbred:Control:Uninfested		38.00699	-529.53812	605.55210
SusInbred:Infected:Uninfested-ResInbred:Control:Uninfested	1	110.3371	542.79199	1677.88222
ResHybrid:Infected:Uninfested-SusHybrid:Control:Uninfested	-	166.7742	-734.31929	400.77093
SusHybrid:Infected:Uninfested-SusHybrid:Control:Uninfested		478.8277	-88.71739	1046.37284
ResInbred:Infected:Uninfested-SusInbred:Control:Uninfested	-	673.5311	-1347.06	
SusInbred:Infected:Uninfested-SusInbred:Control:Uninfested		398.799	-168.74614	966.34408
SusHybrid:Infected:Uninfested-ResHybrid:Infected:Uninfested		645.6019	78.05679	1213.14702
SusInbred:Infected:Uninfested-ResInbred:Infected:Uninfested	1	72.33012	504.78500	1639.87523

		p adj
SusHybrid:Control:Infested-ResHybrid:Control:Infested	1	0
ResHybrid:Infected:Infested-ResHybrid:Control:Infested	1	0
SusHybrid:Infected:Infested-ResHybrid:Control:Infested	0	0.65447
ResHybrid:Control:Uninfested-ResHybrid:Control:Infested	1	0
SusHybrid:Control:Uninfested-ResHybrid:Control:Infested	0	0.999818
ResHybrid:Infected:Uninfested-ResHybrid:Control:Infested	1	0
SusHybrid:Infected:Uninfested-ResHybrid:Control:Infested	0	0.021302
SusInbred:Control:Infested-ResInbred:Control:Infested	1	0
ResInbred:Infected:Infested-ResInbred:Control:Infested	1	0
SusInbred:Infected:Infested-ResInbred:Control:Infested	0	0.000575
ResInbred:Control:Uninfested-ResInbred:Control:Infested	1	0
SusInbred:Control:Uninfested-ResInbred:Control:Infested	0	0.005792
ResInbred:Infected:Uninfested-ResInbred:Control:Infested	1	0
SusInbred:Infected:Uninfested-ResInbred:Control:Infested	0	4.1E-06
ResHybrid:Infected:Infested-SusHybrid:Control:Infested	0	1
SusHybrid:Infected:Infested-SusHybrid:Control:Infested	0	0.790392
ResHybrid:Control:Uninfested-SusHybrid:Control:Infested	1	0
SusHybrid:Control:Uninfested-SusHybrid:Control:Infested	0	0.999994
ResHybrid:Infected:Uninfested-SusHybrid:Control:Infested	1	0
SusHybrid:Infected:Uninfested-SusHybrid:Control:Infested	0	0.037011
ResInbred:Infected:Infested-SusInbred:Control:Infested	1	0
SusInbred:Infected:Infested-SusInbred:Control:Infested	0	0.001645
ResInbred:Control:Uninfested-SusInbred:Control:Infested	1	0

SusInbred:Control:Uninfested-SusInbred:Control:Infested	0	0.015601
ResInbred:Infected:Uninfested-SusInbred:Control:Infested	1	0
SusInbred:Infected:Uninfested-SusInbred:Control:Infested	0	1.17E-05
SusHybrid:Infected:Infested-ResHybrid:Infected:Infested	0	0.468305
ResHybrid:Control:Uninfested-ResHybrid:Infected:Infested	1	0
SusHybrid:Control:Uninfested-ResHybrid:Infected:Infested	0	0.996433
ResHybrid:Infected:Uninfested-ResHybrid:Infected:Infested	1	0
SusHybrid:Infected:Uninfested-ResHybrid:Infected:Infested	0	0.010244
SusHybrid:Infected:Infested-ResInbred:Infected:Infested	0	0.510234
SusInbred:Infected:Infested-ResInbred:Infected:Infested	0	0.000566
ResInbred:Control:Uninfested-ResInbred:Infected:Infested	1	0
SusInbred:Control:Uninfested-ResInbred:Infected:Infested	0	0.005706
ResInbred:Infected:Uninfested-ResInbred:Infected:Infested	1	0
SusInbred:Infected:Uninfested-ResInbred:Infected:Infested	0	0.000004
ResHybrid:Control:Uninfested-SusHybrid:Infected:Infested	0	0.477757
SusHybrid:Control:Uninfested-SusHybrid:Infected:Infested	0	0.991473
ResHybrid:Infected:Uninfested-SusHybrid:Infected:Infested	0	0.549387
SusHybrid:Infected:Uninfested-SusHybrid:Infected:Infested	0	0.898356
ResInbred:Control:Uninfested-SusInbred:Infected:Infested	0	0.000447
SusInbred:Control:Uninfested-SusInbred:Infected:Infested	0	0.999946
ResInbred:Infected:Uninfested-SusInbred:Infected:Infested	0	0.000895
SusInbred:Infected:Uninfested-SusInbred:Infected:Infested	0	0.911522
SusHybrid:Control:Uninfested-ResHybrid:Control:Uninfested	0	0.996857
ResHybrid:Infected:Uninfested-ResHybrid:Control:Uninfested	1	0
SusHybrid:Infected:Uninfested-ResHybrid:Control:Uninfested	0	0.01065
SusInbred:Control:Uninfested-ResInbred:Control:Uninfested	0	0.004551
ResInbred:Infected:Uninfested-ResInbred:Control:Uninfested	1	0
SusInbred:Infected:Uninfested-ResInbred:Control:Uninfested	0	3.2E-06
ResHybrid:Infected:Uninfested-SusHybrid:Control:Uninfested	0	0.998874
SusHybrid:Infected:Uninfested-SusHybrid:Control:Uninfested	0	0.175766
ResHybrid:Infected:Uninfested-SusInbred:Control:Uninfested	0	0.006694
ResInbred:Infected:Uninfested-SusInbred:Control:Uninfested	0	0.008817
SusInbred:Infected:Uninfested-SusInbred:Control:Uninfested	0	0.42876
SusHybrid:Infected:Uninfested-ResHybrid:Infected:Uninfested	0	0.014177
SusInbred:Infected:Uninfested-ResInbred:Infected:Uninfested	0	6.4E-06