Mycoscience (2007) 48:267–273
DOI 10.1007/s10267-007-0363-7

FULL PAPER

Donald T. Wicklow · Bruce W. Horn

Association between vegetative compatibility and aflatoxin production by *Aspergillus* species during intraspecific competition

Received: February 16, 2007 / Accepted: April 19, 2007

Abstract Intraspecific competition is the basis for biological control of aflatoxins, but there is little understanding of the mechanism(s) by which competing strains inhibit toxin production. Evidence is presented that demonstrates a relationship between strength of the vegetative compatibility reaction and aflatoxin production in *Aspergillus flavus* and *A. parasiticus* using the suspended disk culture method. Combining wild-type aflatoxin-producing isolates belonging to different vegetative compatibility groups (VCGs) resulted in a substantial reduction in aflatoxin yield. Pairs of aflatoxin-producing isolates within the same VCG, but showing weak compatibility reactions using complementary nitrate-nonutilizing mutants, also were associated with reduced levels of aflatoxin B_1. In contrast, pairings of isolates displaying a strong compatibility reaction typically produced high levels of aflatoxins. These results suggest that interactions between vegetatively compatible wild-type isolates of *A. flavus* and *A. parasiticus* are cooperative and result in more aflatoxin B_1 than pairings between isolates that are incompatible. Successful hyphal fusions among spore germlings produce a common mycelial network with a larger resource base to support aflatoxin biosynthesis. By comparison, vegetative incompatibility reactions might result in the death of those heterokaryotic cells composed of incompatible nuclei and thereby disrupt the formation of mycelial networks at the expense of aflatoxin biosynthesis.

D.T. Wicklow (✉)
Mycotoxin Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Agricultural Research Service, Peoria, IL 61604, USA
Tel. +1-309-685-4011; Fax +1-309-681-6686
e-mail: wicklodt@ncaur.usda.gov

B.W. Horn
National Peanut Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Dawson, GA, USA

The content of this paper was presented at the 50th Anniversary Meeting of the Mycological Society of Japan, June 3–4, 2006, Chiba, Japan

Key words Aflatoxin · *Aspergillus flavus* · *Aspergillus parasiticus* · Heterokaryon · Intraspecific competition · Vegetative compatibility

Introduction

Intraspecific competition is the basis for biological control of aflatoxins using nontoxigenic *Aspergillus flavus* and *A. parasiticus*, but there is little understanding of the mechanism(s) by which competing strains inhibit toxin production (Cotty and Bayman 1993; Wicklow et al. 2003). Competition for substrate resources between the applied nontoxigenic strain and native aflatoxin-producing strains is a factor contributing to reduced levels of aflatoxin contamination (Cotty and Bayman 1993; Horn et al. 2000). Wicklow et al. (2003) examined the ability of nonaflatoxigenic strains of *A. flavus* to interfere with aflatoxin production by an aflatoxigenic *A. flavus* strain using a replacement series with the suspended disk culture method. Aflatoxin reductions were greater than expected based on the ratios for aflatoxigenic versus nonaflatoxigenic conidial inoculum within a replacement series. Furthermore, aflatoxin yields were also reduced when conidia from aflatoxin-producing strains were mixed in equal proportions. The authors suggested that the substantial inhibition of aflatoxin yield for inoculum mixtures might result from the failure of spore germlings to form heterokaryons and establish a cooperative mycelial network. Here we explore this hypothesis by pairing aflatoxin-producing wild-type isolates of *A. flavus* and *A. parasiticus* from both within and among vegetative compatibility groups (VCGs) as determined by Horn and Greene (1995) and Horn et al. (2000). Evidence is presented that demonstrates a relationship between strength of the vegetative compatibility reaction and aflatoxin production in *A. flavus* and *A. parasiticus*.

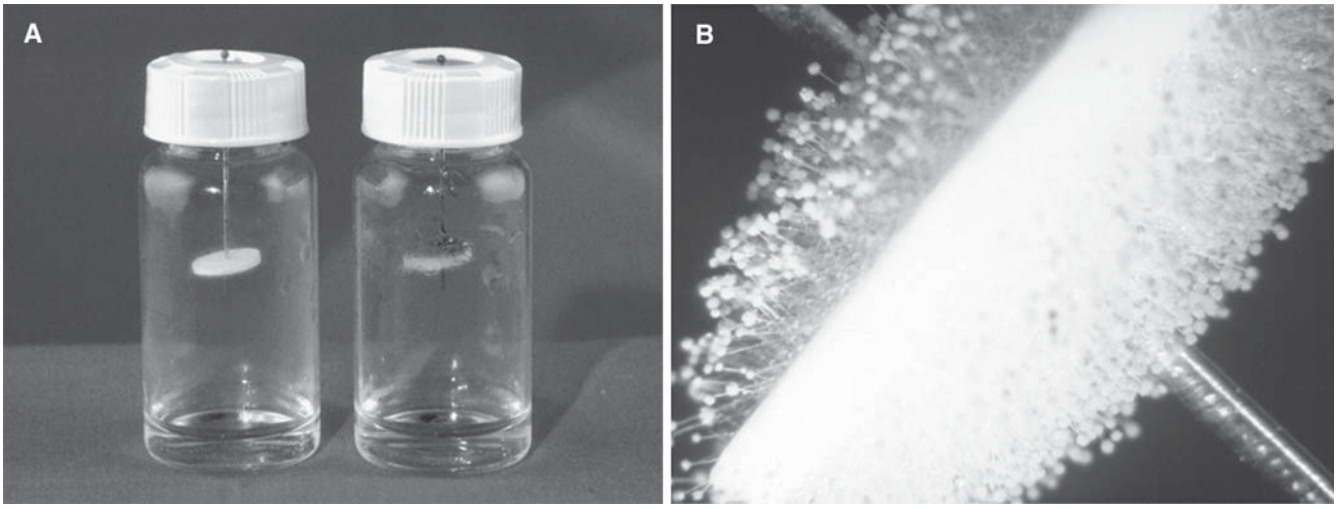

Fig. 1. Suspended disk culture method for determining aflatoxin by inoculum mixtures. **A** Scintillation vial with an open-type cap containing a thick Teflon-coated septum pierced by a pin on which a glass fiber disk is affixed. **B** *Aspergillus flavus* sporulation on glass fiber disk incubated for 5 days at 25°C

Materials and methods

Fungal strains

The *A. flavus* and *A. parasiticus* wild-type isolates used in this study were obtained from the Agricultural Research Culture Collection, Peoria, IL, USA, and included *A. parasiticus* isolates from peanut (*Arachis hypogaea*) field soil, Dawson, GA, USA (Horn and Greene 1995; Horn et al. 2000) NRRL 29555 (= NPL P5), NRRL 29565 (= NPL P43), NRRL 29568 (= NPL P19), NRRL 29575 (= NPL P52), NRRL 29592 (= NPL P12), NRRL 29593 (= NPL P48), NRRL 29604 (= NPL P23), NRRL 29605 (= NPL P53); *A. flavus* isolates from maize (*Zea mays*) grown in Georgia (Papa 1986) NRRL 20050 (= KEP 81-30b), NRRL 20032 (= KEP 81-37a), NRRL 20033 (= KEP 81-51), NRRL 20224 (= KEP 81-20-23); and from peanut field soil and peanut seeds, Dawson, GA (Horn and Greene 1995) NRRL 29460 (= NPL F40), NRRL 29459 (= NPL F35), NRRL 29462 (= NPL F53), NRRL 29461 (= NPL F42), NRRL 29463 (= NPL F76), NRRL 29465 (= NPL F13), NRRL 29467 (= NPL F48), NRRL 29466 (= NPL F14), NRRL 29473 (= NPL F86), NRRL 29478 (= NPL F44), NRRL 29474 (= NPL F8), NRRL 29479 (= NPL F45), NRRL 29476 (= NPL F37), NRRL 29499 (= NPL F3), NRRL 29500 (= NPL F17), NRRL 29501 (= NPL F9), NRRL 29502 (= NPL F79), NRRL 29464 (= NPL F1). The nitrate-nonutilizing (*nit*) mutants (*niaD*; *nirA*; *cnx*) produced from wild-type isolates are maintained at the National Peanut Research Laboratory (NPL), Dawson, GA, USA, by B.W. Horn.

Aflatoxin analyses

Tests measuring the effect of intraspecific competition on aflatoxin B_1 inhibition for strain pairings of wild-type isolates of *A. flavus* and *A. parasiticus* were performed using suspended disk cultures (Fig. 1) as previously described (Norton 1995; Wicklow et al. 2003). Aflatoxins were extracted twice by adding 2 ml $CHCl_3$ to the disk in the vial, vortexing for 15 s, and allowing to set overnight. Extracts were combined and evaporated to dryness with a stream of N_2 at room temperature, and the aflatoxins were analyzed and quantitated by high performance liquid chromatography using water:acetonitrile (67:33, v/v).

Strength of vegetative compatibility reactions

Complementary *cnx* and *niaD* nitrate-nonutilizing mutants of *A. flavus* and *A. parasiticus* strains with known VCG designations were obtained from the study of Horn and Greene (1995). When a *cnx* mutant was not available, a *cnx* mutant was created on plates of Czapek agar supplemented with 25 g/l potassium chlorate as described in Horn and Greene (1995). Five to 20 Czapek agar (Cz) plates (15 × 100 mm; sole nitrogen source = NO_3) were inoculated at their centers with conidial suspensions of *cnx* and *niaD* mutants (5 mm apart) using a transfer needle; plates were incubated for 10 days at 30°C (Horn and Greene 1995). Three plates showing a complementation zone from each pairing were randomly chosen for measuring the strength of the compatibility reaction.

Regions of complementation were initially outlined with a felt marker on the bottoms of plates. The bottoms of the three plates from each pairing were then simultaneously scanned with an Epson Perfection 3170 scanner; a metric ruler was included on the agar surface of one of the plates for calibration. Complementation zones were delimited with Photoshop CS (version 8), and total areas (cm^2) were measured in Fovea Pro 2 (Reindeer Graphics, Asheville, NC).

Nit mutant composition of *A. parasiticus* conidia

Complementary *cnx* and *niaD/nirA* mutants of *A. parasiticus* were inoculated onto Cz plates as previously described, then incubated 14 days at 30°C. Mass conidial transfers were made with a transfer needle from five different locations in the complementation zone. Conidia were suspended in 10 ml water with Tween 20 (100 µl/l), and 0.1 ml was spread on each of three hypoxanthine plates per zone location. *Cnx* mutants were identified directly on the plates (4 days; 30°C) by their sparse mycelial growth; wild-type colonies were transferred to Cz slants. All transfers of wild-type colonies to Cz resulted in *nit*-type growth. Subsequent transfers were made to NO_2 slants to identify *niaD* mutants by their wild-type growth and *nirA* mutants by their sparse growth on that nitrogen source (Horn and Greene 1995).

Statistical analyses

Analyses were performed using SigmaStat, version 3.1 (Jandel Scientific, San Rafael, CA, USA) and SAS statistical package, version 8 (SAS Institute, Cary, NC, USA). In the comparison of percent inhibition of aflatoxin B_1 production by *A. parasiticus* in pairings within VCGs and among VCGs, an analysis of variance (ANOVA) showed that percent inhibition values for experiments A and B were not significantly different ($P > 0.05$); therefore, mean values of experiments A and B were used in the comparison. Pearson product moment correlations were based on the means of experiments A and B for percent aflatoxin B_1 inhibition and on the means of the three replicate measurements of the area (cm^2) of complementation in *nit* pairings.

Results

The ability of aflatoxin-producing wild-type isolates of *A. flavus* and *A. parasiticus* to interfere with aflatoxin B_1 production in within- and among-VCG pairings was examined with the suspended disk culture method (Fig. 1; Table 1). Aflatoxin B_1 yields in *A. parasiticus* were substantially reduced (87.6%; range, 63.0%–97.6%) when conidia from isolates belonging to different VCGs were mixed in equal proportions. Combinations of isolates from within the same VCG overall showed less aflatoxin B_1 reduction (39.6%; range, –6.2% to 80.3%). Kruskal–Wallis ANOVA on ranks showed that percent aflatoxin B_1 inhibition in *A. parasiticus* for among-VCGs pairings was significantly greater ($H = 6.513$; $P < 0.01$) than percent inhibition for within-VCG pairings. An insufficient number of observations of strain pairings among isolates belonging to different VCGs did not permit a similar comparison in *A. flavus*.

Intraspecific pairings of *cnx* and *niaD* mutants produced from these same *A. parasiticus* and *A. flavus* wild-type isolates confirmed the compatibility based on previously established VCGs (Horn and Greene 1995; Horn et al. 2000) by the presence of an area of wild-type growth and sporulation

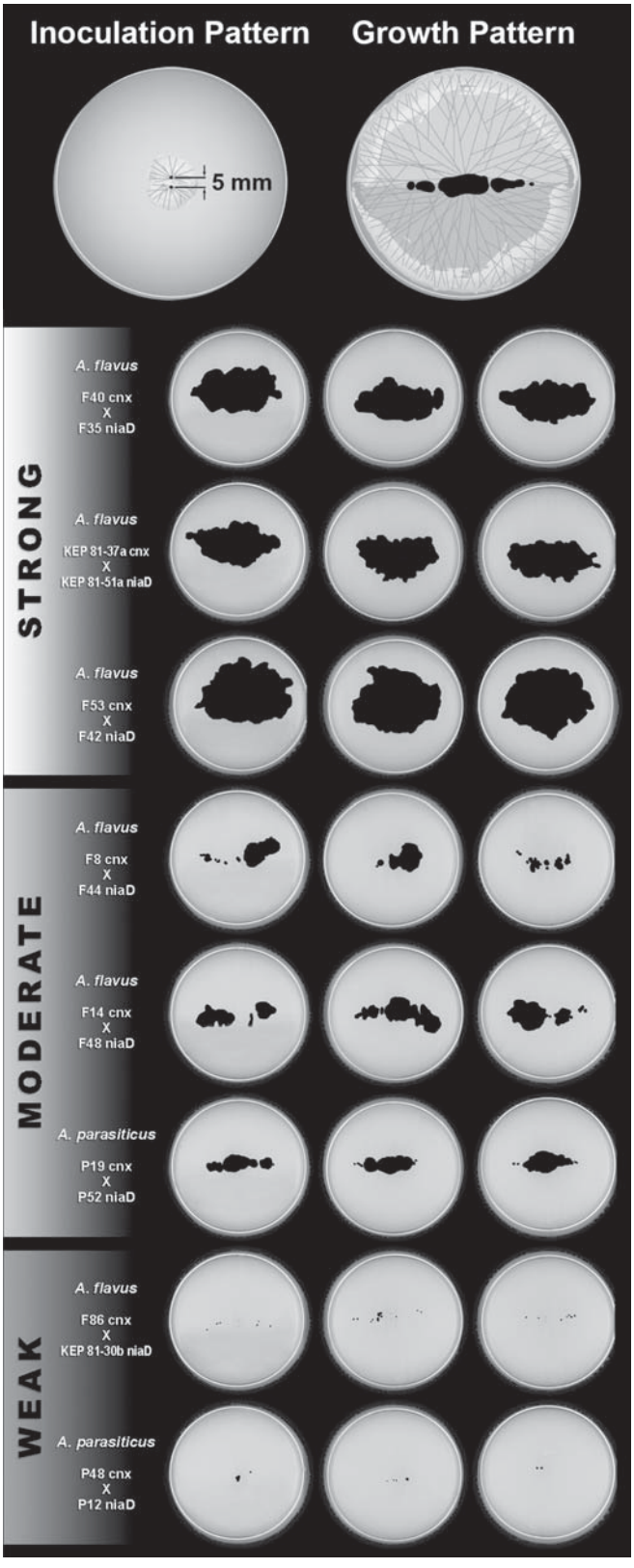

Fig. 2. Strength of vegetative compatibility reactions measured as an area of complementation (cm^2) using complementary *nit* mutants of *A. flavus* and *A. parasiticus*. Initial points of needle inoculation for strain pairings were separated by 5 mm; hyphal anastomoses extended outward following the line of contact between adjacent colonies (see diagram at *top*)

Table 1. *Aspergillus* strain pairings within and among vegetative compatibility groups (VCGs): reduction in aflatoxin and area of complementation zone

Aspergillus parasiticus					Aflatoxin B_1 Inhibition	Complementation Zone
Within VCGs						
Strain	VCG		Strain	VCG	(% ± SD)[a]	(cm^2 ± SD)[b]
NPL P19	3	×	NPL P52	3	–6.2 ± 12.49	2.92 ± 0.264
NPL P5	2	×	NPL P43	2	36.3 ± 7.99	3.19 ± 1.727
NPL P23	9	×	NPL P53	9	47.9 ± 42.78	1.71 ± 0.973
NPL P12	5	×	NPL P48	5	80.3 ± 10.68	0.04 ± 0.033
Among VCGs						
NPL P5	2	×	NPL P12	5	63.0 ± 4.17	—[c]
NPL P52	3	×	NPL P48	5	82.1 ± 0.07	—
NPL P43	2	×	NPL P48	5	84.5 ± 1.63	—
NPL P43	2	×	NPL P52	3	91.3 ± 0.42	—
NPL P5	2	×	NPL P19	3	93.4 ± 0.07	—
NPL P19	3	×	NPL P12	5	92.9 ± 8.7	—
NPL P12	5	×	NPL P23	9	95.7 ± 1.63	—
NPL P19	3	×	NPL P23	9	97.6 ± 0.35	—
Aspergillus flavus						
Within VCGs						
KEP 81-30b	17	×	NPL F86	17	22.2 ± 19.21	0.10 ± 0.068
NPL F 9	29	×	NPL F79	29	24.9 ± 5.66	10.72 ± 1.868
NPL F40	6	×	NPL F35	6	30.7 ± 20.29	11.86 ± 0.937
NPL F53	6	×	NPL F42	6	34.2 ± 34.86	16.34 ± 1.970
NPL F76	6	×	NPL F53	6	34.4 ± 37.12	8.44 ± 9.187
KEP 81-37a	6	×	KEP 81-51	6	35.2 ± 20.29	10.80 ± 0.266
NPL F14	14	×	NPL F48	14	53.8 ± 20.58	6.22 ± 2.579
NPL F42	6	×	NPL F40	6	57.0 ± 4.88	11.32 ± 2.963
NPL F44	23	×	NPL F8	23	58.0 ± 15.20	3.95 ± 2.029
NPL F13	14	×	NPL F1	14	80.0 ± 3.18	0.08 ± 0.070
KEP 81-20-23	14	×	NPL F1	14	86.5 ± 4.60	0.01 ± 0.014
NPL F 3	28	×	NPL F17	28	89.3 ± 2.19	0.00 ± 0.000
NPL F35	6	×	KEP 81-51	6	93.8 ± 7.42	0.85 ± 0.440
NPL F45	23	×	NPL F37	23	93.8 ± 5.94	0.33 ± 0.496
Among VCGs						
NPL F1	14	×	NPL F76	6	49.6 ± 29.56	—
KEP 81-37a	6	×	KEP 81-20-23	14	83.6 ± 9.83	—

[a] Wild-type strains grown in suspended disk culture (Wicklow et al. 2003); means based on two independent experiments
[b] Nitrate-nonutilizing (*nit*) mutants (*cnx*; *niaD*) paired on Czapek agar containing $NaNO_3$ as a nitrogen source (Horn and Greene 1995); means based on three replicate plates
[c] Incompatible pairings not tested
Culture collection designations: KEP, K.E. Papa Laboratory Collection, Dept. Plant Pathology, Univ. Georgia, Athens, GA [maintained at ARS Culture Collection (NRRL), Peoria, IL]; NPL, National Peanut Research Laboratory, Dawson, GA

extending along the line of interaction between the growing colonies (Fig. 2). However, pairings among individual isolates belonging to the same VCG exhibited substantial differences in the area of complementation (Table 1). Examples of strong (>8 cm^2), moderate (1–8 cm^2) and weak (<1 cm^2) compatibility reactions in *A. parasiticus* and *A. flavus* are illustrated in Fig. 2. *Aspergillus flavus* NPL F40 produced a strong reaction when paired with NPL F35, but the reaction was weak when NPL F35 was paired with KEP 81-51 (see Table 1). At the same time, KEP 81-51 reacted strongly when paired with KEP 81-37a.

Conidium composition in the complementation zone of vegetatively compatible pairings of *A. parasiticus* on Cz plates consisted entirely of parental genotypes (*cnx*, *niaD*, and *nirA*), and no heterokaryotic conidia were detected (Table 2). *Cnx* mutant conidia dominated in all pairings. The proportionality of genotypes differed according to location within the complementation zone. Some locations consisted entirely of *cnx* or *niaD/nirA* conidia whereas other locations contained a mixture of the two. There was no observable pattern according to the location of the genotypes, and various genotypes were detected in the middle of the complementation zone, at the edges, and at the extreme ends.

Pairings of isolates displaying strong compatibility reactions typically showed low percentages of aflatoxin B_1 inhibition (Fig. 3). In contrast, pairings of aflatoxin-producing isolates showing weak compatibility reactions were associated with high percentages of aflatoxin B_1 inhibition. Pearson product moment correlations showed that the area of growth (cm^2) in the zone of complementation was negatively correlated with percent aflatoxin B_1 inhibition in strain pairings of *A. parasiticus* ($R = -0.891$; $n = 12$; $P = 0.0001$) and *A. flavus* ($R = -0.674$; $n = 16$; $P < 0.01$). Pairings of isolates belonging to different VCGs did not show compatibility reactions (Horn and Greene 1995), and areas of

Table 2. *Nit* mutant composition of *A. parasiticus* conidia from the wild-type complementation zone arising from vegetatively compatible pairings[a]

Pairing	VCG	No. locations sampled in complementation zone	Total no. colonies	% *cnx*	% *niaD/nirA*
P19 *cnx* × P22 *niaD*	3	5	179	90.5	9.5
P19 *cnx* × P22 *nirA*	3	5	224	79.5	20.5
P19 *cnx* × P52 *niaD*	3	5	264	66.3	33.7
P19 *cnx* × P52 *nirA*	3	5	239	83.7	16.3
P37 *cnx* × P39 *niaD*	2	5	272	62.9	37.1

[a] Conidia generally contain 3–4 nuclei (Yuill 1950); heterokaryotic conidia were not detected

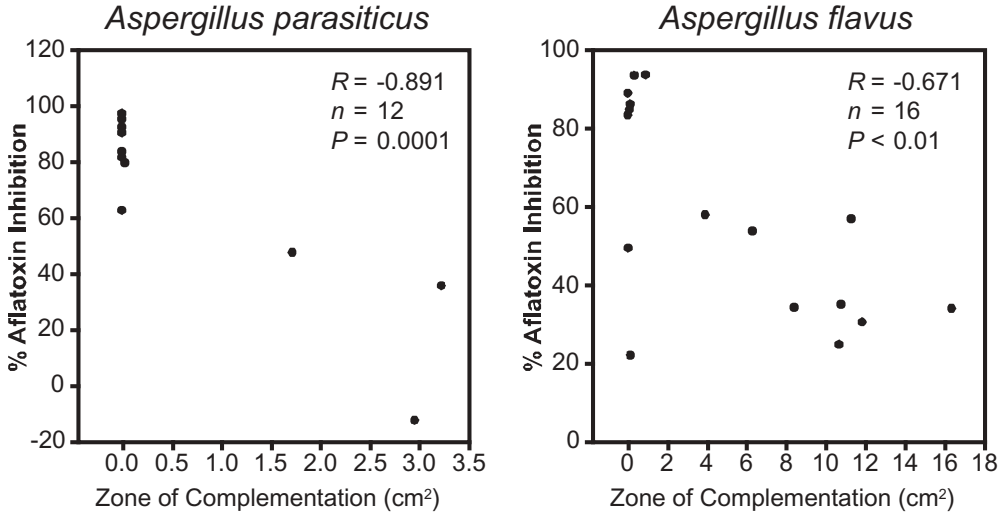

Fig. 3. Scatter diagrams showing negative correlations between area of growth (cm^2) in the zone of complementation and percent aflatoxin B_1 inhibition for within- and among-vegetative compatibility group (VCG) strain pairings of *A. parasiticus* and *A. flavus*

complementation were entered as zero in statistical calculations.

Discussion

Aspergillus flavus and *A. parasiticus* populations within restricted geographic areas comprise large numbers of VCGs that can be examined as subpopulations of vegetatively compatible individuals (Papa 1986; Bayman and Cotty 1991; Horn and Greene 1995; McAlpin et al. 1998; Wicklow et al. 1998). The current study suggests that interactions between strongly compatible wild-type isolates of *A. flavus* and *A. parasiticus* are cooperative and result in more aflatoxin B_1 than pairings between isolates which are weakly compatible or incompatible. Successful hyphal fusions would produce a common mycelial network with a larger resource base to support aflatoxin biosynthesis. By comparison, if two individuals differ at any heterokaryon in compatibility (*het*) loci, vegetative incompatibility reactions result in the death of those heterokaryotic cells composed of incompatible nuclei (Leslie 1993). Therefore, incompatibility reactions might disrupt the formation of mycelial networks at the expense of aflatoxin biosynthesis (Wicklow et al. 2003).

Horn et al. (2000) demonstrated that, in pairings among *A. parasiticus* strains from the same and different VCGs combined on plates of modified yeast extract sucrose agar, the mycelium derived from hyphal anastomoses has a larger substrate base for producing sclerotia than that of more restricted colonies resulting from competition between different VCGs, as postulated by Wicklow (1990). At the same time, Horn et al (2000) reported that mixtures of compatible *A. parasiticus* strains were not associated with higher aflatoxin B_1 production compared to mixtures from different VCGs and that the effect might have been localized at the point of anastomosis and was not detectable by the analyses used in their study. The authors concluded that there was no indication that inhibition of aflatoxin B_1 production was caused by factors other than competition for nutrients.

The cumulative negative effects on aflatoxin production associated with the death of heterokaryotic cells from the failure of incompatible aflatoxin-producing strains to fuse, as proposed by Wicklow et al. (2003), should be much greater at conidial densities using the suspended disk culture method (see Table 1) than at conidial densities used in experiments by Horn et al. (2000). In studies of intraspecific competition using the suspended disk culture, including the work reported herein, mixtures of *A. parasiticus* and *A. flavus* conidia suspended in the 90 µl SL

medium added to individual glass fiber disks totaled 100000 conidia/ml (Wicklow et al. 2003). In the experiments reported by Horn et al. (2000), mixtures of *A. parasiticus* conidia representing the same or different vegetative compatibility groups suspended in molten agar and distributed in Petri dishes totaled 1250 conidia /ml of agar. Ishitani and Sakaguchi (1956) showed that the frequency of hyphal fusions in *A. oryzae* and *A. sojae*, considered domesticated forms of respective *A. flavus and A. parasiticus* used in food fermentations (Kurtzman et al. 1986), is proportionate to the density of conidia per unit volume of medium; the higher frequencies of hyphal fusions were obtained when conidia were less than 10 µm apart. Interference with aflatoxin production in liquid shaken culture from a mixed conidial inoculum of *A. flavus* representing different VCGs (Cotty and Bayman 1993) might also involve a failure of hyphal anastomosis within the closely intertwined mycelial balls formed as agglomerations of numerous conidial germlings.

In the current study, the strength of vegetative compatibility reactions was measured from pairings of *nit* mutant strains on a nitrate medium, the same approach used in characterizing VCGs within populations of *Aspergillus* section *Flavi* species (Papa 1986; Horn and Greene 1995). In addition, by quantifying the zone of complementation, we were able to characterize compatibility reactions as "weak," "moderate," or "strong" (see Fig. 2). Likewise, in *F. oxysporum* f. sp. *radicis-lycopsersici*, the assignment of isolates to specific subgroups within VCG 094 from Florida and Europe was based on their weak, moderate, and strong compatibility reactions (Rosewich et al. 1999). The authors further described relationships between VCG 094 isolates from Florida as "a continuum of varying degrees of compatibility across the population." The inability of a relatively few isolates from other fungal taxa [e.g., *Fusarium oxysporum*, *Gibberella fujikuroi* (*F. moniliforme*), *Verticillium alboatrum*, and *Rhizoctonia solani*] to form robust macroscopic heterokaryons when complementary mutants from the same strain are paired have been designated heterokaryon self-incompatible (HIS) phenotypes (Correll et al. 1989). The same authors suggest that self-incompatible *nit* mutants of *A. flavus* reported by Papa (1986) are HIS phenotypes. Isolates with this phenotype participate in fewer hyphal fusions, resulting in an apparent vegetative incompatibility reaction resulting from the lack of a robust heterokaryon (Klein and Correll 2001).

Conidia of *A. flavus* and *A. parasiticus* are predominantly multinucleate (Yuill 1950) and can potentially be heterokaryotic. Heterokaryotic conidia were not detected in the complementation zone of compatible *A. parasiticus* pairings on Czapek agar plates when using the same culture technique for measuring the strength of complementation. Therefore, nuclear migration from the point of hyphal anastomosis appeared to be limited on Czapek agar plates. The wild-type growth and sporulation in the complementation zone was likely the result, to some extent, of nutritional cross-feeding, either through cytoplasmic movement in hyphae or from nutritional factors released into the medium (Ishitani and Sakaguchi 1956). The extent of heterokaryotic proliferation on the culture disks using wild-type strains is not known. Heterokaryosis is dependent on the types of mutations used for complementation (e.g., conidium color and nutritional markers) as well as on the culture medium (Ishitani and Sakaguchi 1956). Heterokaryotic conidia are unstable and generally segregate into component parental types following germination; heterokaryons are maintained in culture by subsequent anastomoses when mixtures of both parents are transferred (Ishitani and Sakaguchi 1956; Papa 1978; Benkhemmar et al. 1985).

In summary, we have shown that strength of vegetative compatibility reactions in *nit* mutants of *A. parasiticus* and *A. flavus* is negatively correlated with amount of aflatoxin inhibition in pairings of wild-type strains. To our knowledge, this is the first time that the strength of a vegetative compatibility reaction in intraspecific competition has been associated with the production of aflatoxins or any other fungal metabolite.

Acknowledgments We thank Jennifer Thompson and Travis Walk for their excellent technical assistance. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

References

Bayman P, Cotty PJ (1991) Vegetative compatibility and genetic diversity in the *Aspergillus flavus* population of a single field. Can J Bot 69:1707–1711

Benkhemmar O, Gaudemer F, Bouvier-Fourcade I (1985) Heterokaryosis between *Aspergillus oryzae* cyclopiazonic acid-defective strains: method for estimating the risk of inducing toxin production among cyclopiazonic acid-defective industrial strains. Appl Environ Microbiol 50:1087–1093

Correll JC, Klittich CJR, Leslie JF (1989) Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycol Res 93:21–27

Cotty PJ, Bayman P (1993) Competitive exclusion of a toxigenic strain of *Aspergillus flavus* by an atoxigenic strain. Phytopathology 83: 1283–1287

Horn BW, Greene RL (1995) Vegetative compatibility within populations of *Aspergillus flavus*, *A. parasiticus*, and *A. tamari* from a peanut field. Mycologia 87:324–332

Horn BW, Greene RL, Dorner JW (2000) Inhibition of aflatoxin B_1 production by *Aspergillus parasiticus* using nonaflatoxigenic strains: role of vegetative compatibility. Biol Control 17:147–154

Ishitani C, Sakaguchi K-I (1956) Hereditary variation and recombination in koji-molds (*Aspergillus oryzae* and *Aspergillus sojae*) V. Heterocaryosis. J Gen Appl Microbiol 2:345–400

Klein KK, Correll JC (2001) Vegetative compatibility group diversity in *Fusarium*. In: Summerell BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW (eds) *Fusarium*: Paul E. Nelson Memorial Symposium. APS Press, St. Paul, MN, pp 83–96

Kurtzman CP, Smiley MJ, Robnett CJ, Wicklow DT (1986) DNA relatedness among wild and domesticated species in the *Aspergillus flavus* group. Mycologia 78:955–959

Leslie JF (1993) Fungal vegetative compatibility. Annu Rev Phytopathol 31:127–150

McAlpin CE, Wicklow DT, Platis CE (1998) Genotypic diversity of *Aspergillus parasiticus* in an Illinois corn field. Plant Dis 82: 1132–1136

Norton RA (1995) A novel glass fiber disc culture system for testing of small amounts of compounds on growth and aflatoxin production by *Aspergillus flavus*. Mycopathologia 129:103–109

Papa KE (1978) The parasexual cycle in *Aspergillus parasiticus*. Mycologia 70:766–773

Papa KE (1986) Heterokaryon incompatibility in *Aspergillus flavus*. Mycologia 78:98–101

Rosewich UL, Pettway RE, Katan T, Kistler HC (1999) Population genetic analysis corroborates dispersal of *Fusarium oxysporum* f. sp. *radicis-lycopersici* from Florida to Europe. Phytopathology 89: 623–630

Wicklow DT (1990) Adaptation in *Aspergillus flavus*. Trans Mycol Soc Jpn 31:511–523

Wicklow DT, McAlpin CE, Platis CE (1998) Characterization of the *Aspergillus flavus* population within an Illinois maize field. Mycol Res 102:263–268

Wicklow DT, Bobell JR, Palmquist DE (2003) Effect of intraspecific competition by *Aspergillus flavus* on aflatoxin formation in suspended disc culture. Mycol Res 107:617–623

Yuill E (1950) The numbers of nuclei in conidia of aspergilli. Trans Br Mycol Soc 33:324–331