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Colonization of wounded peanut seeds by soil fungi: selectivity for species from *Aspergillus* section *Flavi*

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Abstract: Soil is a source of primary inoculum for *Aspergillus flavus* and *A. parasiticus*, fungi that produce highly carcinogenic aflatoxins in peanuts. Aflatoxigenic fungi commonly invade peanut seeds during maturation, and the highest concentrations of aflatoxins are found in damaged seeds. A laboratory procedure was developed in which viable peanut seeds were wounded and inoculated with field soil containing natural populations of fungi, then incubated under different conditions of seed water activity and temperature. Densities of *Aspergillus* section *Flavi* in soil used for inoculating seeds were low relative to the total numbers of filamentous fungi (<1%). *Aspergillus* species from section *Flavi* present in soil included *A. flavus* morphotypes L and S strains, *A. parasiticus*, *A. caelatus*, *A. tamarii* and *A. alliaceus*. Wounding was required for high incidences of fungal colonization; viability of wounded seeds had little effect on colonization by *Aspergillus* species. Peanut seeds were colonized by section *Flavi* species as well as *A. niger* over broad ranges of water activity (0.82–0.98) and temperature (15–37 °C), and the highest incidences of seed colonization occurred at water activities of 0.92–0.96 at 22–37 °C. *A. parasiticus* colonized peanut seeds at lower temperatures than *A. flavus*, and cool soil temperatures relative to temperatures of aerial crop fruits might explain why *A. parasiticus* is found mostly in peanuts. Other fungi, dominated by the genera *Penicillium*, *Fusarium* and *Clonostachys*, colonized seeds primarily at water activities and temperatures suboptimal for section *Flavi* species and *A. niger*. *Eupenicillium ochrosalmoneum* frequently sporulated on the conidial heads of section *Flavi* species and showed specificity for these fungi. The inoculation of wounded viable peanut seeds with soil containing natural populations of fungi provides a model system for studying the infection process, the interactions among fungi and those factors important in aflatoxin formation.

Key words: aflatoxin, *Arachis hypogaea*, *Aspergillus alliaceus*, *Aspergillus caelatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus tamarii*, competitive saprophytic ability, *Eupenicillium ochrosalmoneum*, fungal competition, *Penicillium*, water activity

INTRODUCTION

Carcinogenic aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* are common contaminants of peanuts, corn, cottonseed and tree nuts (Diener et al 1987, Payne 1998). In Africa and southeastern Asia, aflatoxins have been shown to increase the incidence of human liver cancer by acting synergistically with hepatitis viruses (Wild and Turner 2002). In addition to the health concerns related to aflatoxins, the cost of managing aflatoxins is often very high, averaging \$26 million annually to the peanut industry in the southeastern United States alone (Lamb and Sternitzke 2001).

Aerial crops such as corn and cottonseed are infected predominantly with *A. flavus*, whereas peanuts (*Arachis hypogaea*) with their subterranean growth habit are invaded by both *A. flavus* and *A. parasiticus* (Horn 2003). *A. flavus* has been divided into two morphotypes: the typical L strain that produces large sclerotia >400 µm diam and the S strain, described as variety *parvisclerotigenus* (Saito and Tsuruta 1993), which produces numerous small sclerotia <400 µm (Cotty 1989). Other *Aspergillus* species belonging to section *Flavi* have been reported from peanuts, including *A. caelatus* and *A. tamarii* (Horn 1997, Horn and Greene 1995). Another species, *A. alliaceus* (teleomorph = *Petromyces alliaceus*), has been transferred to section *Flavi* based on molecular characters (Peterson 2000). None of these other species forms aflatoxins, although *A. tamarii* produces cyclopiazonic acid (Horn et al 1996) and *A. alliaceus* produces ochratoxin A (Bayman et al 2002a).

Soil serves as a reservoir for primary inoculum of *A. flavus* and *A. parasiticus* (Horn et al 1995, Horn and Dorner 1998), and peanut pods are in direct contact with soil populations of aflatoxigenic fungi. Infection of peanut seeds predominantly by primary inoculum differs from infection of corn and cottonseed in which secondary inoculum plays a dominant

role (Horn 2003). Two modes of infection are possible in peanuts. First, seeds from pods without visible damage may be invaded by aflatoxigenic fungi when plants are drought stressed (Sanders et al 1981), although undetected microscopic pod damage might account for some of this infection (Porter et al 1986). Second, pod and seed damage allows for direct invasion from soil. The highest levels of *A. flavus* and *A. parasiticus* infection and aflatoxin contamination are associated with seed damage (Blankenship et al 1984, Dowell et al 1990, Hill et al 1983, Sanders et al 1985b). Larvae of lesser cornstalk borer (*Elasmopalpus lignosellus*) scarify and penetrate peanut pods and largely are responsible for pod and seed damage in the United States (Lynch 1984, Lynch and Mack 1995, Lynch and Wilson 1991). In tropical regions where peanuts are cultivated, white grubs (scarab beetle larvae), termites and millipedes are important feeders on peanut pods (Lynch and Mack 1995). Growth cracks also may expose seeds to soil inoculum that results in an increase in fungal invasion and aflatoxins (Schroeder and Ashworth 1965).

Peanut seeds vary considerably in water activity (A_w) and exposure to soil temperatures. Water activities of individual seeds range from 0.75 to 1.00 (Dorner et al 1989, JW Dorner unpublished data), and soil temperatures in the pod zone range from 20 C in warm temperate regions to >38 C in semi-arid tropical areas (Craufurd et al 2003, Hill et al 1983). Peanuts are invaded by aflatoxigenic fungi primarily under conditions of late-season drought and elevated soil temperatures (Blankenship et al 1984, Hill et al 1983, Sanders et al 1981, Sanders et al 1985b).

For this study a laboratory technique was developed in which viable peanut seeds were wounded and the wound sites were inoculated with soil from the field. The effects of various factors on peanut seed colonization by *Aspergillus* section *Flavi* species from natural populations in soil, including water activity, temperature, wounding and seed viability, are reported.

MATERIALS AND METHODS

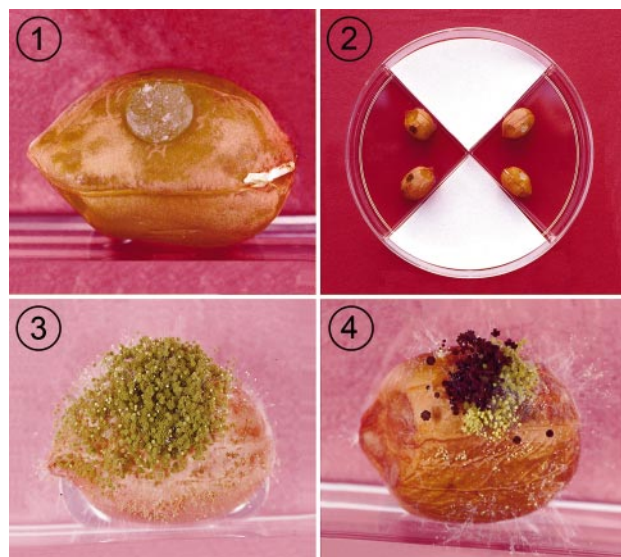
Soil collections.—Soil was collected 3 Jul, 30 Jul, 5 Sep, 13 Nov 2001; 24 Jan, 22 Feb, 3 Sep 2002; and 3 Feb, 2 Sep 2003 from cultivated Field A (1.6 km west of Graves, Terrell County, Georgia) and Field B (0.8 km south of Shellman, Randolph County). Soil consisted of Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults with 0–2% slope) and Greenville sandy loam (fine, kaolinitic, thermic Rhodic Kandiudults with 0–2% slope) for Fields A and B, respectively. Approximately 3 kg of soil was collected from the top 5 cm and thoroughly mixed. From a portion of this soil (600 g), three 50 g subsamples were removed and oven-dried (100 C, 48 h) to determine moisture con-

tent. The remaining soil was stored at 5 C in sealed plastic bags and used within 3 wk to determine fungal population densities and for inoculating seeds.

Fungal soil populations.—*Aspergillus* species and total filamentous fungi were enumerated, and *Aspergillus* species were identified as described by Horn and Dorner (1998). Briefly, three 3.3 g subsamples of soil were vortexed in 10 mL of 0.2% water agar. Soil suspensions of subsamples each were dilution plated onto five plates (0.2 mL/plate) of modified dichloran-rose bengal medium and incubated 3–4 d at 37 C. Species from *Aspergillus* section *Flavi* were identified under a stereomicroscope directly from the dilution plates with the exception of *A. caelatus* and *A. tamarii*, which were subcultured to Czapek agar (Cz) slants for final identification. *Aspergillus* species from other sections not recognizable on dilution plates were subcultured and identified according to the techniques of Klich (2002) and Raper and Fennell (1965). The density of total filamentous fungi was determined on unmodified dichloran-rose bengal medium after 5 d at 25 C (Horn et al 1994), and those same plates were incubated an additional 2–3 d (30 C) to enumerate *Eupenicillium ochrosalmoneum* (anamorph = *Penicillium ochrosalmoneum*) and *Eup. cinnamopurpureum* (anamorph = *Pen. cinnamopurpureum*). *Eupenicillium* species were recognized on dilution plates by colonies dominated by ascostromata; isolates were subcultured to Cz slants and identified according to Pitt (1979). All fungal densities were corrected for soil moisture and were reported on a dry-weight basis.

Peanut seeds.—Irrigated peanut plants of the cultivar Georgia Green were grown using standard applications of fertilizer, herbicides, fungicides and insecticides (Guillebeau 2004). Peanut plants were dug from fields on 18 Oct 2000 (7.9 km northeast of Cuthbert, Randolph County, Georgia), 13 Sep 2001 (5.4 km southwest of Dawson, Terrell County), and 8 Sep 2002 and 11 Sep 2003 (2.1 km north of Sasser, Terrell County) with a digger-inverter, and pods immediately were hand picked. Fresh pods were abraded with a wet-impact blaster to expose the inner pericarp wall, then color sorted according to maturity (Williams and Drexler 1981, Williams and Monroe 1986). Mature pods (black and brown maturity classes) were removed and dried 14 d at ambient temperature on a forced-air drier. Dried pods were stored at 5 C and were used for experiments within 1 yr. At the end of 1 yr, the embryos and cotyledons of 100 seeds were tested for tissue viability after pod-surface sterilization using the tetrazolium method (Peters 2000).

Blasted pods without visible damage to the pericarp were surface sterilized 2 min with 2.0% Na hypochlorite followed by three sterile water rinses. Seeds were removed aseptically from the pods. Two seeds were placed loosely in each of two compartments of four-section plates (100 × 15 mm); the other two compartments contained 4 mL of NaCl solution (A_w = 0.82, 0.84, 0.85, 0.87, 0.90, 0.93, 0.96) or distilled water (A_w = 1.00) with a fitted piece of filter paper to keep the solution evenly spread (Fig. 2). To adjust seeds to a specific water activity, plates were incubated 7 d at 37 C in a covered desiccator jar over 900 mL of the same solution. Seed water activities were up to 0.02 units below



FIGS. 1–4. 1. Aseptically wounded peanut seed before inoculation with soil. 2. Four-sectioned plate containing wounded peanut seeds, two of which have been inoculated with soil. Two of the compartments contain NaCl solution with filter paper. 3, 4. Wounded peanut seeds inoculated with soil and incubated for 7 d at 37 C in a desiccator jar (salt solution $A_w = 0.93$); seeds are colonized by *Aspergillus* section *Flavi* species and *A. niger*.

those of the salt solutions and water after incubation. NaCl solutions for specific water activities were made up according to Chirife and Resnik (1984). Water activities of solutions and peanut seeds were determined with a Series 3TE water activity meter (Decagon Devices, Pullman, Washington); seed water activities were measured at the temperature of the experiment.

Seed inoculations.—After rehydration peanut seeds were examined under the stereomicroscope for fungal growth. One cotyledon from each fungus-free seed then was injured aseptically to a depth of approximately 1 mm with a borer (3 mm diam), and the peanut tissue was scraped from the inside of the borer cut with a dissecting needle (FIG. 1). Seeds were attached to the bottom of the plate adjacent to the partitions (wound facing outward) (FIG. 2) using multitemperature glue applied with a small glue gun.

For inoculating seeds, 33 g of soil was mixed with 3.0–6.5 mL of sterile water (depending on the initial moisture content of the soil) to form a near-saturated paste. Wound sites from 40 peanut seeds (10 plates) then were inoculated with 7.2 ± 0.002 mg of soil (\pm SD; $n = 20$; dry weight) using a small spatula. An additional 20 wounded seeds in each experiment were not inoculated and served as controls. Twenty seeds that were not wounded or inoculated also were included in each experiment; those seeds were macerated individually with a razor blade at the time of inoculation (7 d incubation) (10 seeds) and at the end of the experiment (10 seeds) for determining water activities.

Fungal colonization.—Peanut seeds were examined under the stereomicroscope at 4, 7 and 14 d after inoculation with

soil. Additional periods of incubation of up to 42 d were required for experiments involving low water activities and/or low temperatures that slowed the rate of fungal growth. At the end of the experiments, the extent of colonization by section *Flavi* was estimated as 0–100% of the visible seed surface. *A. niger*, *A. terreus* and *Emericella rugulosa* (anamorph = *A. rugulovalvus*) were identified directly from the peanut seed. *Aspergillus* species from section *Flavi*, with the exception of *A. alliaceus*, could not be distinguished based on conidial color when growing on peanut seeds and, therefore, were subcultured onto Cz slants for identification. One to 10 transfers were made from each seed, depending on the extent of colonization by section *Flavi*. *Eupenicillium* species, when present on the heads of species from section *Flavi*, were transferred simultaneously with their hosts to Cz slants where they typically continued to sporulate on the *Aspergillus* heads. Other fungal species that could not be identified directly on the seeds were subcultured and identified according to these references for each genus: *Aspergillus* and its teleomorphs *Petromyces*, *Emericella* and *Eurotium* (Klich 2002, Raper and Fennell 1965); *Penicillium* and its teleomorphs *Eupenicillium* and *Talaromyces* (Pitt 1979); *Paecilomyces* (Samson 1974); *Fusarium* (Nelson et al 1983); *Clonostachys* (Schroers 2001); *Alternaria* and *Cladosporium* (Ellis 1971); *Absidia* and *Syncephalastrum* (Domsch et al 1980); *Cunninghamella* (Zheng and Chen 2001); *Rhizopus* (Schipper and Stalpers 1984); and *Thermomucor* (Subrahmanyam et al 1977).

Other experiments.—Colonization of fresh seeds obtained directly from the field was compared with colonization of dried peanut seeds revived in water. Fresh pods from the field were abraded for color sorting, surface sterilized and shelled without drying. Dried seeds from surface-sterilized pods were revived to the water activity of fresh seeds by soaking in sterile water (16 h at 5 C). Forty seeds from each set were wounded and inoculated with soil from Field A. Inoculated seeds were incubated 14 d at 37 C over salt solution ($A_w = 0.89$) as previously described. The experiment was performed twice.

The effects of wounding and seed viability on colonization also were examined together in a combined experiment. Treatments included: (i) viable seeds, wounded; (ii) viable seeds, not wounded; (iii) nonviable seeds, wounded; and (iv) nonviable seeds, not wounded. Unwounded seeds (viable and nonviable) were inoculated at one of three locations on the seed surface: (i) cotyledon where normally wounded; (ii) raphe midway between the hilum and chalaza; and (iii) hilum. All treatments, including inoculations at three locations on the seed, were represented by 40 seeds. Dried seeds were rendered nonviable by autoclaving 1 min at 120 C. All seeds were rehydrated in desiccator jars over salt solution ($A_w = 0.96$) at 37 C for 7 d. Fungus-free seeds then were inoculated with soil from Field A and incubated 14 d under the same conditions. The experiment was performed twice.

In another experiment, different seeds and grain ($n = 40$) were wounded and inoculated with soil from Field A to determine their susceptibility to invasion by *Aspergillus* species. Brazil nuts, unsplit pistachio nuts (Kermit cultivar),

TABLE I. Populations of *Aspergillus* species and their teleomorphs in soil used for inoculating peanut seeds

<i>Aspergillus</i> species ^b	Field A ^a		Field B ^a	
	CFU/g	Relative density ^c	CFU/g	Relative density ^c
Section <i>Flavi</i>				
<i>A. flavus</i> L strain	461 ± 141.4	33 ± 7.1	1301 ± 1717.8	41 ± 22.7
<i>A. flavus</i> S strain	136 ± 63.8	10 ± 3.4	7 ± 11.1	0.1 ± 0.20
<i>A. parasiticus</i>	178 ± 103.3	11 ± 4.6	532 ± 407.0	34 ± 15.7
<i>A. caelatus</i>	273 ± 163.1	18 ± 3.0	359 ± 259.8	20 ± 9.0
<i>A. tamarii</i>	81 ± 67.8	5 ± 1.2	86 ± 89.7	5 ± 3.1
<i>A. alliaceus</i>	371 ± 319.2	23 ± 6.1	0 ± 0.0	0 ± 0.0
Section <i>Nigri</i>				
<i>A. niger</i>	2919 ± 1025.0		3430 ± 2502.8	
<i>A. japonicus</i>	4 ± 5.4		2 ± 3.6	
Section <i>Terrei</i>				
<i>A. terreus</i>	7549 ± 2818.9		10512 ± 12694.4	
<i>A. carneus</i>	111 ± 76.2		3 ± 5.6	
Section <i>Nidulantes</i>				
<i>Emericella rugulosa</i>	115 ± 66.0		1093 ± 1880.8	
<i>A. ustus</i>	10 ± 16.2		55 ± 70.3	
Section <i>Fumigati</i>				
<i>A. fumigatus</i>	607 ± 440.0		9545 ± 4124.8	
Total filamentous fungi (× 10 ⁵)	1.27 ± 0.364		1.72 ± 1.065	

^a Mean ± SD based on five independent soil collections.

^b Deposition of species into sections according to Peterson (2000).

^c Percentage of section *Flavi*.

English walnuts (Chandler), shelled almonds (Nonpareil), cottonseed (Deltapine 458BR, acid delinted), corn (Pioneer 3394) and soybeans (Northrup King S73-Z5) were surface sterilized as described for peanuts. Brazil nuts, pistachio nuts and English walnuts were shelled aseptically after surface sterilization. These three nuts and almonds were wounded with a borer in the same manner as peanuts. A sterile scalpel was used to slice off a portion (<1 mm) of cottonseed, corn kernels and soybeans at the end opposite of the germ. Because of the high incidence of fungi in some of the seeds and grain that were not killed by surface sterilization (particularly in English walnuts, almonds and corn), seeds and grain were not rehydrated beforehand but instead were wounded in a dry state before inoculation with soil from Field A. Inoculated seeds and grain were incubated in desiccator jars over salt solution ($A_w = 0.96$) at 37 C for 14 d. The water activity was determined from 10 individual seeds and corn kernels (not wounded or inoculated) at the end of the experiment.

Statistics.—Data were analyzed using SigmaStat, version 1.0 (Jandel Scientific, San Rafael, California).

RESULTS

Soil populations.—Fresh soil was collected for each experiment to ensure the viability of all possible fungal colonizers of peanut seeds. As a consequence,

fungal densities in soil from a given field varied considerably because of different collection dates (TABLE I). Fields A and B differed in both composition and density of species belonging to *Aspergillus* section *Flavi*. Field A contained *A. alliaceus*, not detected in Field B, and Field B had higher densities of *A. flavus* L strain and *A. parasiticus* than Field A. *A. flavus* S strain was rare in Field B soil. *Aspergillus* species present at the highest densities included *A. niger* and *A. terreus* in Fields A and B and *A. fumigatus* in Field B. In both fields the relative density (percentage of total filamentous fungi) of combined species from section *Flavi* was <1%.

Seed viability and initial infection rate.—Cotyledon and embryo tissue in peanut seeds (100 seeds each for harvest years 2000, 2001 and 2002) were 100% viable based on the tetrazolium test. Of the wounded control seeds that were not inoculated with soil ($n = 752$), two seeds showed fungal colonization (*A. niger* and *Pen. pinophilum*) at the end of the experiments and none was infected with species from *Aspergillus* section *Flavi*.

Comparison of fresh and rehydrated peanut seeds.—The water activities of peanut seeds (± SD, $n = 10$) at

inoculation were similar for fresh seeds (0.99 ± 0.002) and rehydrated seeds (0.99 ± 0.002) in Experiment 1 and for fresh seeds (0.99 ± 0.001) and rehydrated seeds (0.98 ± 0.006) in Replicate Experiment 2. The incidence of section *Flavi* was 87.5% for both fresh and rehydrated seeds in Experiment 1 and 90.0% for both fresh and rehydrated seeds in Experiment 2. The respective incidences of *A. niger* in fresh and rehydrated seeds were 50.0 and 47.5% in Experiment 1 and 30.0 and 32.5% in Experiment 2.

Interactions of water activity and temperature.—Colonization of wounded viable peanut seeds (rehydrated) after inoculation with soil often was dominated by species from *Aspergillus* section *Flavi* and *A. niger* (FIGS. 3, 4). Application of soil from Fields A and B to wounds resulted in similar patterns of peanut seed colonization by *A. flavus* L strain (FIG. 5), *A. parasiticus* (FIG. 7), *A. tamarii* (FIG. 8) and *A. caelatus* (FIG. 9) over the range of water activities and temperatures tested. *A. flavus* S strain and *A. alliaceus* occurred at low densities or were not detected in soil from Field B (TABLE I); therefore seeds inoculated with Field B soil were not colonized by either of these species (FIGS. 6, 10). Within the 14 d incubation period after inoculation, all species from section *Flavi* colonized seeds within similar limits of seed water activity (0.82–0.98) and temperature (22–37 C) (FIGS. 5–10). The highest incidences of colonization by *A. flavus* L and S strains, *A. tamarii* and *A. alliaceus* generally occurred at seed water activities of 0.92 and 0.96 and temperatures of 30 and 37 C. *A. parasiticus* showed high incidences of colonization at 22 C (FIG. 7), and *A. caelatus* showed an increasing incidence of colonization with decreasing temperature (maximum of 17.9% at 37 C to 64.3% at 22 C) (FIG. 9). Increasing temperatures from 22 to 37 C also resulted in an increase in the ability of species to colonize peanuts at lower water activities (FIGS. 5–10).

The extent of seed colonization by *Aspergillus* section *Flavi*, measured as a percentage of the visible seed surface, increased with increasing water activity and temperature to optimal conditions of $A_w = 0.96$ at 37 C (73.4 and 81.4% of the seed surface for Fields A and B respectively). However colonization by section *Flavi* at a seed water activity of 0.98 accounted for only 8.0 and 10.1% of the seed surface at 37 C for respective Fields A and B, and growth of fungi in general similarly was inhibited at all temperatures at this water activity.

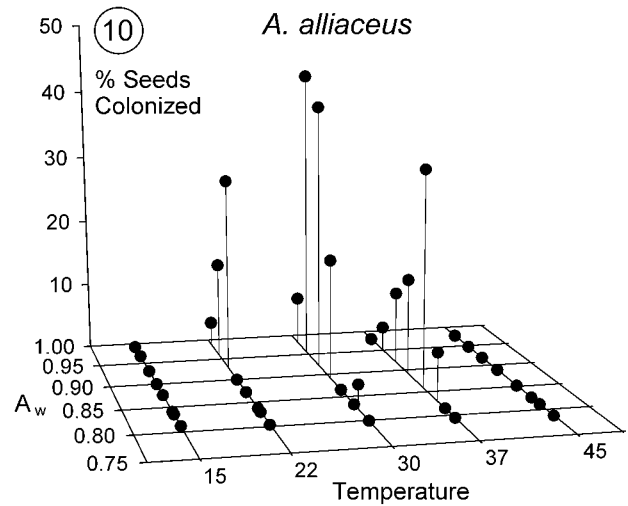
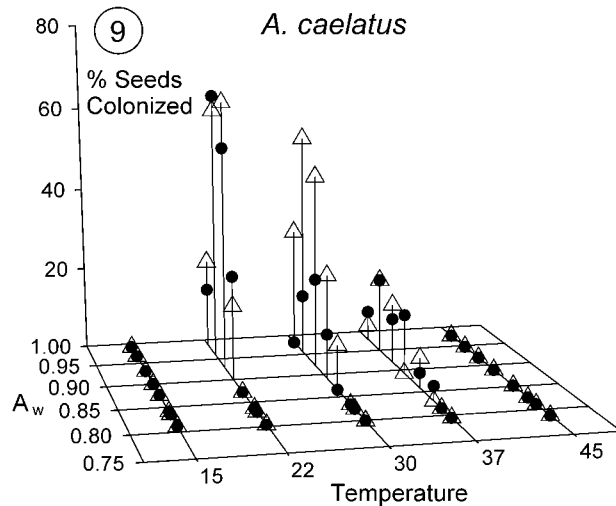
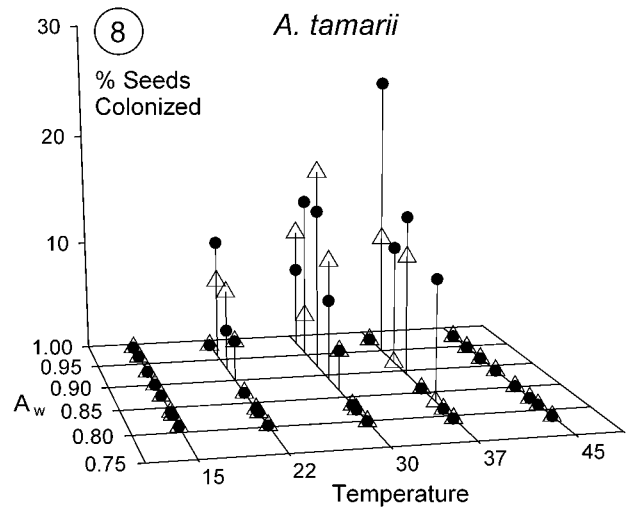
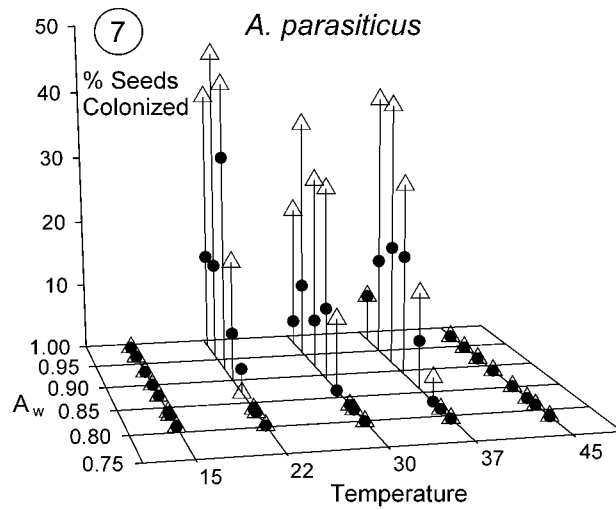
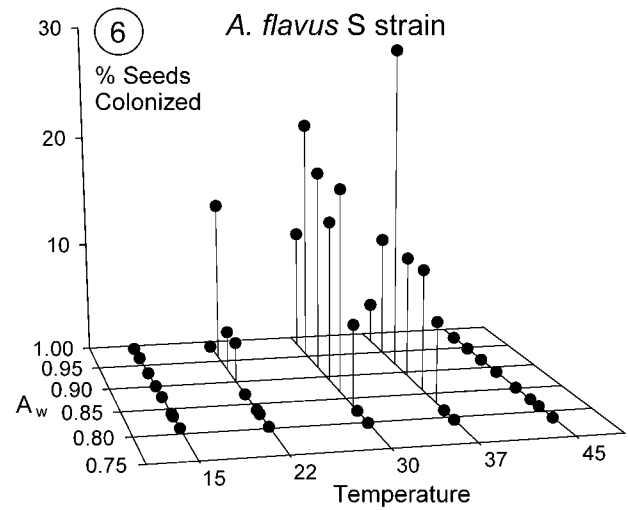
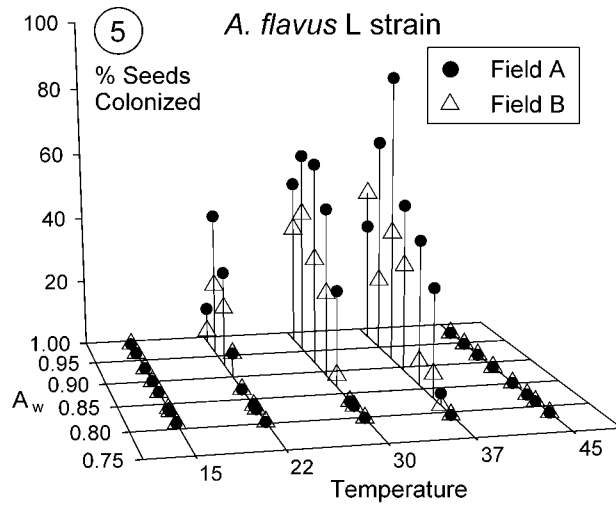
Incubation of seeds at lower water activities for periods of greater than 14 d showed slow increases in the incidence of seed colonization by section *Flavi* (FIG. 11). Highest incidences of colonization by sec-

tion *Flavi* generally were attained with 14 d incubation (seed $A_w = 0.96$) at 22, 30 and 37 C (FIG. 12). However at 15 C with seeds inoculated with Field B soil, the incidence of colonization by section *Flavi* increased from 0.0% at 14 d to 64.3% at 28 d. This increase was due solely to *A. parasiticus*. Similarly *A. parasiticus* was the only colonizer from section *Flavi* (14.3%) after 35 d incubation at 15 C (seed $A_w = 0.93$) when seeds were inoculated with soil from Field A.

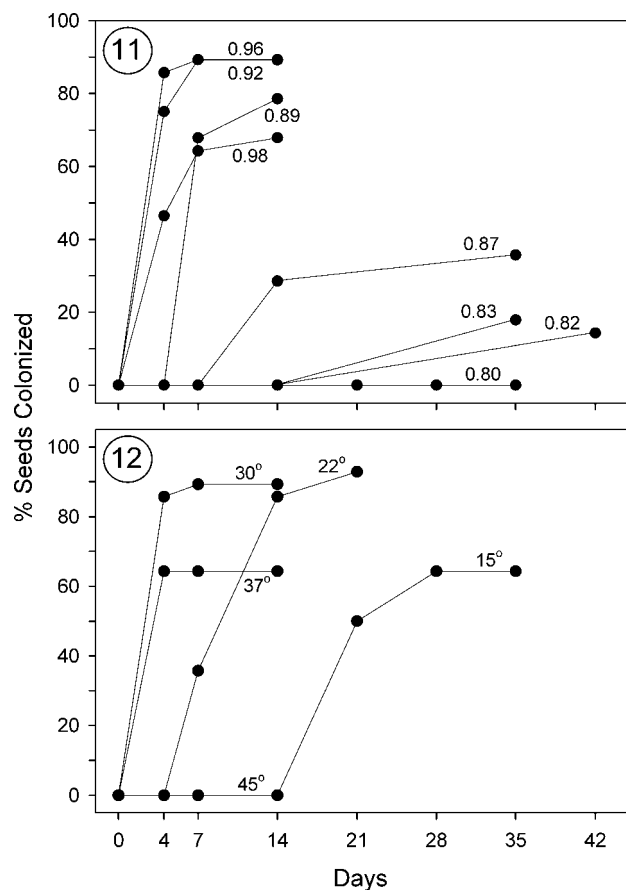
Colonization of seeds by *A. niger* (FIG. 13) occurred at water activities and temperatures similar to those for section *Flavi* species. In contrast *A. terreus* showed high incidences of colonization at 45 C (FIG. 14). Although *A. terreus* colonized a high proportion of seeds at 30 and 37 C, this slow-growing species generally was restricted to the wound site after 4 d of incubation and quickly was overrun thereafter by more aggressive section *Flavi* species and *A. niger*. At 45 C, the lack of competition with section *Flavi* species and *A. niger* allowed *A. terreus* to more fully colonize the seeds.

Other species of fungi, with the exception of *Eup. ochrosalmoneum*, were observed most frequently on peanut seeds under conditions of water activity and temperature that were suboptimal for colonization by section *Flavi* species and *A. niger* (TABLE II). *Penicillium* species were generally most common at lower seed water activities (≤ 0.89) and lower temperatures (15, 22 C) except for *Pen. pinophilum* and *Pen. funiculosum*, which occurred only at the highest seed water activity of 0.98. Species of *Fusarium*, *Clonostachys*, *Paecilomyces*, *Alternaria*, *Cladosporium*, *Cunninghamella* and *Syncephalastrum* occurred at higher seed water activities (≥ 0.92) and lower temperatures (15, 22 C). *A. fumigatus* and *Talaromyces* species colonized seeds primarily at 37 C with a seed water activity of 0.98. Growth at 45 C mostly was restricted to *A. terreus* (FIG. 14), *Em. rugulosa*, *Rhizopus microsporus* and *Thermomucor indica-seudaticae*.

Effects of wounding and seed viability.—Water activities of viable and nonviable (autoclaved) seeds ($n = 10$) at the time of inoculation were not significantly different (t -test; $P > 0.05$) within each of the two replicated experiments (seed $A_w = 0.95$). The incidences of *Aspergillus* species on viable wounded seeds were considerably greater than the incidences on viable unwounded seeds (TABLE III). The location of inoculation (cotyledon, raphe or hilum) on viable unwounded seeds had no apparent effect on colonization. Although the incidence of *A. terreus* was high on viable unwounded seeds, colonization was confined to the area of soil application and consisted of sparse mycelium with few conidial heads. Only 2.9%



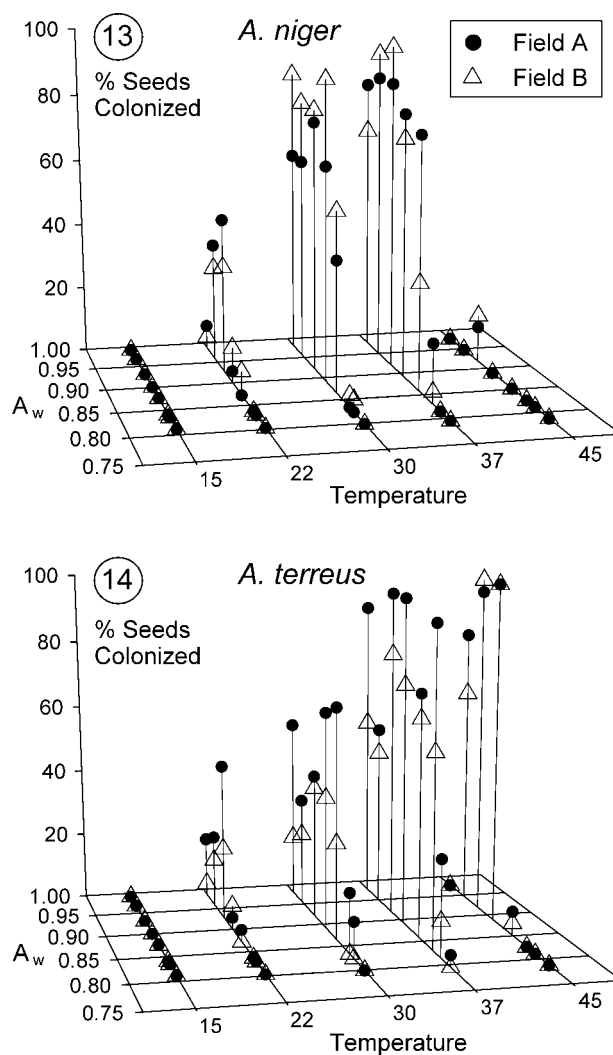
FIGS. 5–10. Percentages of wounded viable peanut seeds ($n = 40$) colonized by species from *Aspergillus* section *Flavi* at different combinations of seed water activity and temperature. Wounds were inoculated with soil from Fields A and B, and seeds were subsequently incubated for 14 d. 5. *A. flavus* L strain. 6. *A. flavus* S strain; colonization was not observed with soil from Field B. 7. *A. parasiticus*. 8. *A. tamarii*. 9. *A. caelatus*. 10. *A. alliaceus*; colonization was not observed with soil from Field B.



FIGS. 11, 12. Effect of incubation time on colonization by combined species within *Aspergillus* section *Flavi* of wounded peanut seeds ($n = 40$) inoculated with soil from Field B. 11. Percentages of seeds colonized at different seed water activities (30°C). 12. Percentages of seeds colonized at different temperatures (seed $A_w = 0.96$). The increase in colonization at 15°C was entirely due to *A. parasiticus*.

of viable unwounded seeds from both experiments ($n = 240$) showed extensive colonization (>50% visible seed surface) by section *Flavi* species and *A. niger*.

Loss of seed viability through autoclaving coupled with wounding resulted in little overall change in the incidences of colonization shown in viable wounded seeds (TABLE III). The respective incidences of section *Flavi* in nonviable wounded seeds and viable wounded seeds were 82.5 and 95.0% in Experiment 1 and 65.9 and 60.0% in Experiment 2. In contrast, inoculation of unwounded seeds with soil showed a higher overall incidence of *Aspergillus* species in nonviable seeds compared to viable seeds, particularly for *A. niger* and *Em. rugulosa*. In Experiment 1, for example, *A. niger* occurred on 50.0–67.5% of nonviable unwounded seeds compared to 0.0–5.0% of viable unwounded seeds and *Em. rugulosa* occurred on 55.0–72.5% of nonviable unwounded seeds com-



FIGS. 13, 14. Percentages of wounded viable peanut seeds ($n = 40$) colonized at different combinations of seed water activity and temperature. Wounds were inoculated with soil from Fields A and B, and seeds were subsequently incubated for 14 d. 13. *A. niger*. 14. *A. terreus*.

pared to 0.0% of viable unwounded seeds. Colonization was extensive when nonviable unwounded seeds were invaded by *Aspergillus* species, including *A. terreus*. The location of inoculation (cotyledon, raphe or hilum) had no apparent effect on colonization of nonviable unwounded seeds.

Other seeds and grain.—Wounded uninoculated seeds and grain (controls, $n = 20$) showed varying degrees of fungal colonization despite surface sterilization: almonds (95.0%), corn (80.0%), English walnuts (45.0%), pistachio nuts (15.0%), Brazil nuts (10.0%), cottonseed (5.0%), soybeans (5.0%) and peanuts (0.0%). However only one seed each of almond and walnut and two corn kernels showed colonization by species from section *Flavi* (all *A. flavus* L strain).

TABLE II. Other fungal species colonizing peanut seeds that were wounded and inoculated with soil^a

	Temperature	Seed water activity	Maximum % seeds colonized ^b
ASCOMYCOTA			
<i>Alternaria alternata</i>	15, 22, 30	0.89, 0.92, 0.96 , 0.98	17.9 (B)
<i>Aspergillus carneus</i>	30, 37	0.82, 0.86 , 0.89	7.1 (A)
<i>A. flavipes</i>	15	0.89	3.6 (B)
<i>A. fumigatus</i>	22, 30, 37 , 45	0.96, 0.98	89.3 (B)
<i>A. melleus</i>	15, 22	0.86 , 0.89 , 0.92	3.6 (A, B)
<i>A. penicillioides</i>	22	0.82	3.6 (A)
<i>A. restrictus</i>	15, 22	0.83 , 0.92	3.6 (A)
<i>A. ustus</i>	22, 30 , 37	0.92, 0.96, 0.98	7.1 (B)
<i>A. wentii</i>	15, 22 , 30	0.86 , 0.89 , 0.92 , 0.96	3.6 (A, B)
<i>Cladosporium cladosporioides</i>	15, 22	0.89, 0.92 , 0.98	25.0 (B)
<i>C. sphaerospermum</i>	15	0.89, 0.92, 0.96 , 0.98	7.1 (A)
<i>Clonostachys rosea</i> f. <i>catenulata</i>	22 , 30	0.98	50.0 (B)
<i>C. rosea</i> f. <i>rosea</i>	15, 22 , 30	0.98	57.1 (B)
<i>Emericella rugulosa</i>	30, 37, 45	0.82, 0.86, 0.89, 0.92, 0.96	64.3 (B)
<i>Eupenicillium cinnamopurpureum</i>	22 , 30	0.83, 0.89 , 0.96	7.1 (A)
<i>Eup. ochrosalmoneum</i>	22, 30 , 37	0.83, 0.86, 0.89, 0.92 , 0.96 , 0.98	96.4 (A)
<i>Eurotium amstelodami</i>	22	0.89 , 0.96	3.6 (A, B)
<i>E. chevalieri</i>	15, 22 , 30 , 37	0.80 , 0.82, 0.83, 0.86, 0.89, 0.92	14.3 (A, B)
<i>E. repens</i>	15, 22	0.83, 0.86 , 0.92, 0.96	7.1 (A, B)
<i>E. rubrum</i>	22	0.80, 0.89	7.1 (A)
<i>Fusarium acuminatum</i>	15, 22, 30	0.92, 0.96, 0.98	78.6 (A)
<i>F. equiseti</i>	15, 22, 30	0.89, 0.92, 0.96 , 0.98	28.6 (B)
<i>F. oxysporum</i>	15, 22 , 30	0.92, 0.96, 0.98	17.9 (A)
<i>F. proliferatum</i>	15, 22 , 30	0.96 , 0.98	3.6 (A, B)
<i>F. solani</i>	15, 22 , 30	0.96, 0.98	25.0 (A, B)
<i>Paecilomyces</i> sp.	15, 22 , 30	0.98	28.6 (A)
<i>Penicillium brevicompactum</i>	15, 22	0.82, 0.83, 0.86, 0.89 , 0.92, 0.96	42.9 (B)
<i>Pen. citrinum</i>	15, 22 , 30, 37	0.80, 0.82, 0.83, 0.86 , 0.89, 0.92, 0.96, 0.98	39.3 (A)
<i>Pen. corylophilum</i>	15, 22 , 30	0.82, 0.83, 0.86, 0.89 , 0.92, 0.96, 0.98	35.7 (A)
<i>Pen. funiculosus</i>	22 , 30, 37	0.98	35.7 (B)
<i>Pen. glabrum</i>	15, 22	0.86, 0.89 , 0.92 , 0.96, 0.98	10.7 (B)
<i>Pen. pinophilum</i>	22, 30 , 37	0.98	82.1 (A)
<i>Pen. cf. roseopurpureum</i>	15, 22	0.82, 0.83, 0.86 , 0.89, 0.92	10.7 (B)
<i>Pen. restrictum</i>	15, 22, 30	0.80, 0.82, 0.83, 0.86, 0.89	25.0 (A)
<i>Pen. sclerotiorum</i>	15, 22	0.89 , 0.92, 0.96, 0.98	7.1 (B)
<i>Pen. cf. vinaceum</i>	15, 22 , 30, 37	0.80, 0.82, 0.83 , 0.86, 0.89, 0.92	50.0 (B)
<i>Talaromyces flavus</i>	22, 30, 37	0.96, 0.98	92.9 (A)
<i>T. trachyspermus</i>	37	0.98	3.6 (B)
ZYGOMYCOTA			
<i>Cunninghamella echinulata</i>	15, 30	0.96, 0.98	17.9 (B)
<i>C. elegans</i>	22 , 37	0.98	7.1 (A)
<i>Rhizopus microsporus</i>	15, 22, 30, 37, 45	0.96, 0.98	100.0 (B)
<i>Syncephalastrum racemosum</i>	15, 22	0.92 , 0.96, 0.98	14.3 (B)
<i>Thermomucor indica-seudaticae</i>	45	0.92 , 0.96	14.3 (A)

^a Based on maximum incubation periods (14–42 d).^b Maximum percentage of seeds colonized based on temperatures and water activities in bold. Soil from Field A and/or Field B shown in parentheses.

Therefore the source of infection by these fungi in inoculated seeds and grain was primarily soil. The incidence of colonization in inoculated seeds and grain (n = 40) by *Aspergillus* section *Flavi*, which in-

cluded all species present in soil from Field A (*A. flavus* L and S strains, *A. parasiticus*, *A. tamaraii*, *A. caelatus* and *A. alliaceus*), was: almonds (97.5%), peanuts (90.0%), pistachio nuts (77.5%), soybeans

TABLE III. Effect of peanut seed viability and wounding on incidence of fungal colonization^{ab}

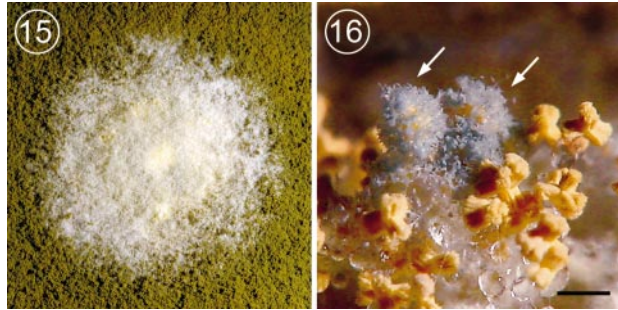
Peanut seed			Section <i>Flavi</i>						Other fungi			<i>Eup. ochrosalmoneum</i>
Viable	Wounded	Location ^c	<i>A. flavus</i> L strain	<i>A. flavus</i> S strain	<i>A. parasiticus</i>	<i>A. caelestis</i>	<i>A. tamarii</i>	<i>A. alliaceus</i>	<i>A. niger</i>	<i>A. terreus</i>	<i>Em. rugulosa</i>	
Experiment 1												
+	+	C	50.0	10.0	25.0	25.0	2.5	27.5	100.0	70.0	32.5	52.5
+	—	C	0.0	2.5	2.5	0.0	0.0	0.0	0.0	100.0	0.0	0.0
		R	2.5	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
		H	0.0	0.0	0.0	2.5	2.5	0.0	5.0	100.0	0.0	0.0
—	+	C	30.0	17.5	15.0	27.5	12.5	20.0	87.5	77.5	67.5	10.0
—	—	C	12.5	0.0	5.0	2.5	0.0	5.0	67.5	100.0	60.0	0.0
		R	7.5	10.0	7.5	5.0	7.5	2.5	50.0	95.0	55.0	0.0
		H	10.0	2.5	15.0	12.5	5.0	2.5	52.5	100.0	72.5	0.0
Experiment 2												
+	+	C	22.5	12.5	7.5	15.0	7.5	20.0	95.0	85.0	22.5	30.0
+	—	C	2.5	0.0	0.0	0.0	0.0	0.0	2.5	67.5	0.0	2.5
		R	2.5	2.5	0.0	0.0	0.0	0.0	5.0	75.0	0.0	2.5
		H	2.5	0.0	5.0	0.0	2.5	2.5	2.5	82.5	0.0	2.5
—	+	C	15.0	10.0	7.5	20.0	10.0	10.0	100.0	95.0	7.5	12.5
—	—	C	5.0	2.5	0.0	5.0	0.0	2.5	55.0	100.0	7.5	2.5
		R	2.5	0.0	5.0	2.5	2.5	10.0	60.0	90.0	25.0	12.5
		H	5.0	2.5	2.5	10.0	0.0	7.5	50.0	92.5	25.0	0.0

^a Two replicated experiments are shown. Percentages based on 40 seeds.
^b Seeds inoculated with soil from Field A; inoculated seeds incubated for 14 d at 37 C (salt solution A_w = 0.96).
^c Location on seed where inoculated: C = center of cotyledon; R = raphe midway between hilum and chalaza; H = hilum.

(72.5%), corn (65.0%), cottonseed (55.0%), English walnuts (47.5%), and Brazil nuts (42.5%). *A. niger* and *A. terreus* also were prevalent on all commodities, whereas other *Aspergillus* species were uncommon. *Eup. ochrosalmoneum* was observed frequently on peanuts (60.0%), soybeans (32.5%), cottonseed (27.5%), Brazil nuts (20.0%) and corn (12.5%), where the species was associated with section *Flavi* species. In contrast *Eup. ochrosalmoneum* was rare on English walnuts (2.5%) and absent on pistachio nuts and almonds despite a high incidence of species from section *Flavi* on those seeds. Inoculated corn kernels also had a high incidence of *Penicillium* species (42.5%) that initially were not present on kernels; otherwise colonization by other fungi on seeds was uncommon. Water activities of unwounded, uninoculated seeds and grain (\pm SD, n = 10) at the end of the experiment (14 d) ranged from 0.94 \pm 0.009 (cottonseed) to 0.96 \pm 0.003 (peanuts).

Observations on Eup. ochrosalmoneum.—The incidence of *Eup. ochrosalmoneum* was positively correlated ($P < 0.0001$, Pearson product-moment correlation) with both the incidence of seed colonization by *Aspergillus* section *Flavi* ($r = 82.0$) and the extent of

seed colonization (percentage of the seed surface) by section *Flavi* ($r = 85.7$) based on an examination of peanut seeds from 48 experiments (maximum incubation periods) involving soils from Fields A and B, three temperatures (22, 30, 37), and eight water activities (0.80–0.98). Small colonies of *Eup. ochrosalmoneum* were observed sporulating at the inoculated wound site on the peanut seed. As conidiophores of section *Flavi* species developed on the seed surface, hyphae of *Eup. ochrosalmoneum* grew upward along the *Aspergillus* stipes to the exposed surfaces of the *Aspergillus* heads. *Eup. ochrosalmoneum* then produced conidium-bearing penicillia on the heads of section *Flavi* species and formed an expanding colony over the mass of *Aspergillus* heads (FIGS. 15, 16). In most instances, however, *Eup. ochrosalmoneum* initially was not observed on the seed but was detected only after section *Flavi* species had established themselves on the seed. In addition, *Eup. ochrosalmoneum* often was detected only after conidia from section *Flavi* species were transferred to Cz slants for identification and *Eup. ochrosalmoneum* subsequently sporulated on *Aspergillus* heads within the slant. Of the 394 seeds with *Eup. ochrosalmoneum* from the 48



FIGS. 15, 16. *Eup. ochrosalmoneum* sporulating on the heads of species from *Aspergillus* section *Flavi*. Plates of malt extract agar were coinoculated with conidia and incubated at 30 C. 15. *A. caelatus* colony with sporulating *Eup. ochrosalmoneum* spreading across the mass of *Aspergillus* heads. 16. *Eup. ochrosalmoneum* sporulating on the heads of *A. alliaceus*, indicated by the arrows. Scale bar = 400 μ m.

experiments indicated above, 91.9% involved sporulation on the heads of section *Flavi* species and only 8.1% sporulated directly on the seed in the absence of section *Flavi*. *Eup. ochrosalmoneum* occurred at high incidences on *A. flavus* L and S strains, *A. parasiticus*, *A. caelatus* and to a lesser extent, *A. tamarii*. The fungus also sporulated commonly on conidial heads and immature ascostromata of *A. alliaceus*. In no instance was *Eup. ochrosalmoneum* observed sporulating on *A. niger* or other *Aspergillus* species outside section *Flavi* or on other genera of fungi. *Eup. cinnamomipurpureum* was observed on only four seeds that were inoculated with soil from Field A, and with those seeds the species sporulated on the heads of *A. flavus* L strain, *A. caelatus*, *A. tamarii* and *A. alliaceus* in a manner similar to *Eup. ochrosalmoneum*.

The density of *Eup. ochrosalmoneum* in soil (\pm SD; $n = 4$) was 4077 ± 2930.0 CFU/g in Field A and 27315 ± 6782.4 CFU/g in Field B; the relative density (percentage of total filamentous fungi) in Fields A and B was 3.1 ± 1.76 and 16.7 ± 6.62 , respectively.

DISCUSSION

Effect of wounding on fungal colonization.—This research was designed to assess the capacity of fungi to invade living tissues of wounded peanut seeds directly from soil. Rehydrated seeds were used throughout the study because it was determined that they showed similar incidences of colonization by section *Flavi* and *A. niger* as seeds from freshly dug pods. Additional experiments showed that wounding was required for extensive colonization of peanut seeds by soil fungi. The low incidence of colonization in viable unwounded seeds supports earlier research showing the importance of injury for invasion by micro-organ-

isms and the role of the seed coat as a barrier for invasion (Carter 1973). Tannins, waxes, amino compounds and structural features in the peanut seed coat have been implicated in resistance to invasion by *A. flavus* and *A. parasiticus* (Amaya-F et al 1977, LaPrade et al 1973, Sanders and Mixon 1978, Zambettakis and Bockelee-Morvan 1976). Inoculation of different regions of the unwounded peanut seed, including the hilum, which has been postulated as route for *A. flavus* invasion (Taber et al 1973), had no effect on seed colonization. The few seeds that were colonized extensively might have had cracks or imperfections in the seed coat (Taber et al 1973). These results are in contrast to those of Xu et al (2000), who reported that peanut seeds are invaded readily by *A. parasiticus* through the seed coat. In the present study inoculation of autoclaved seeds with intact seed coats resulted in increased colonization, suggesting that the inhibitory properties of the seed coat had been partially lost.

Selectivity of peanut seeds for Aspergillus section Flavi.—Despite the low soil densities of *Aspergillus* section *Flavi* relative to the total numbers of filamentous fungi ($<1\%$), peanuts were colonized preferentially by species within the section over much of the water activity and temperature ranges of the study. Incidences of section *Flavi* species and *A. niger* were similar in viable and nonviable wounded peanut seeds under conditions (seed $A_w = 0.95$ at 37 C) that are optimal for colonization but in viable seeds are not conducive to resistance in the form of phytoalexin production (Dorner et al 1989). This suggests that despite the invasion of living tissue, colonization of viable peanut seeds under such conditions has features more characteristic of saprotrophy than a specialized form of parasitism. If the fungus-host relationship is viewed as more saprotrophic than parasitic, then the selectivity of peanut seeds for section *Flavi* species and *A. niger* indicates that these species have a high “competitive saprophytic ability” as defined by Garrett (1970) to measure the capacity of a species to achieve dominance in the colonization of dead organic matter. *A. terreus* appeared at the wound site soon after inoculation and was quickly overrun by section *Flavi* species and *A. niger*. Therefore competition with this slow-growing species was minimal. Competition among species within section *Flavi* and between section *Flavi* species and *A. niger* was likely of greater importance, but the extent of this competition and its effect on species composition on seeds are not known. After establishment of section *Flavi* species and *A. niger* on peanut seeds, there was little evidence of further successional changes in species composition, with the exception

of the appearance of *Eup. ochrosalmoneum*, which might not have been competing directly with the dominant colonists for the peanut substrate.

Competitive saprophytic ability in fungi is complex and depends on environmental variables such as substrate composition, water activity, temperature and inoculum density (Griffin 1972a, Rayner and Webber 1984). *Aspergillus* species from section *Flavi* have been reported from diverse substrates but are found most frequently in oil-rich seeds and grains (Diener et al 1987, Payne 1998) where they often have a competitive advantage over other fungi at water activities of <0.96 and a temperature of approximately 30 °C (Dorner et al 1989, Marín et al 1998a). Although these fungi invaded peanut seeds over wide ranges of water activities and temperatures in the present study, the lengthy incubation required for colonization at suboptimal water activity/temperature combinations typically would not be encountered in the field. Therefore the ranges of water activities and temperatures for colonization of peanuts grown under standard cultivation practices would be much narrower than the ranges shown in this study. Finally, inoculum density determines the probability of a propagule being present in soil at the peanut wound site. Griffin and Garren (1974) estimated that pod infection is possible with as few as 2.0 propagules of *A. flavus* in the 0.5 mm layer of the geocarposphere. The precise relationship between soil population density and the incidence of colonization in wounded seeds currently is being examined.

In addition to environmental parameters, physiological characters such as rate of spore germination, rate of mycelial growth and enzymatic capability greatly influence competitive saprophytic ability in fungi (Garrett 1970). Conidia of *A. flavus* do not germinate in the peanut geocarposphere due to soil fungistasis (Griffin 1972b). However injury to the pod releases sugars and amino-N compounds that let conidia overcome fungistasis and to germinate at high percentages (Hale and Griffin 1976). The production of diverse enzymes for degrading plant components such as cellulose, pectin, lignin and lipids (Betts and Dart 1989, Cotty et al 1990, Long et al 1998, Olutiola 1976) also likely contributes to the competitive advantage of section *Flavi* species in invading peanut seeds.

In this study seeds and grain other than peanuts also were colonized readily by section *Flavi* species when wounded and inoculated with soil. Instances of exposure of these aerial crops to airborne dust (Doster and Michailides 1994, Lee et al 1986) and to soil when plants are downed by wind or harvesting equipment (Doster and Michailides 1994, Zummo and Scott 1990) have led to colonization by *A. flavus*; oth-

erwise aerial crops are typically not in contact with soil. Infection of aerial crops instead occurs primarily through wind and insect dispersal of conidia, and wounding greatly enhances colonization by *A. flavus* and aflatoxin contamination (Diener et al 1987, Payne 1998). A mixture of species at the wound site, whether aurally dispersed by wind/insects or applied as soil, likely would result in selection for section *Flavi* species under moisture and temperature conditions favorable for these fungi.

Interactions of water activity and temperature.—Conditions for peanut seed colonization by section *Flavi* species in the current study (ranges: $A_w = 0.82$ – 0.98 and 15–37 °C; optima: $A_w = 0.92$ – 0.96 at 22–37 °C) were similar to conditions reported for spore germination and growth in pure culture (Holmquist et al 1983, Pitt and Miscamble 1995, Mislivec et al 1975, Sautour et al 2002). The observation of greater tolerance for low water activities at temperatures optimal for growth also has been reported from controlled culture conditions (Ayerst 1969, Holmquist et al 1983, Mislivec et al 1975, Pitt and Miscamble 1995). Water activities ≥ 0.98 are suboptimal for growth of section *Flavi* species (Pitt and Miscamble 1995, Sautour et al 2002), which was evident in the current experiments at the highest seed water activity of 0.98. However phytoalexin production by injured peanut seeds also is optimal at high water activities (Dorner et al 1989, Wotton and Strange 1987) and this might account for the inhibition of all fungal species observed at wound sites at the highest water activity. Peanut seeds under field conditions are most susceptible to invasion by *A. flavus* and aflatoxin contamination at water activities of 0.90–0.95 because above this range phytoalexins are inhibitory to *A. flavus* and below this range the reduced water activity restricts fungal growth (Dorner et al 1989).

A. niger was the dominant colonist of wounded peanut seeds outside *Aspergillus* section *Flavi*. This species has moisture and temperature requirements that are slightly broader than those of *A. flavus* and grows faster (Ayerst 1969, Marín et al 1998b). *A. niger* and *A. flavus* commonly co-occur on agricultural commodities such as peanuts, corn, cottonseed and tree nuts (Abdulla 1974, Ashworth et al 1971, Austwick and Ayerst 1963, Bayman et al 2002b, Hesseltine et al 1981, Hill et al 1983, Joffe 1969). Other *Aspergillus* species were less prominent on peanut seeds. *A. terreus* and *Em. rugulosa*, present at the early successional stages of peanut seed colonization, were limited in growth by the more aggressive section *Flavi* species and *A. niger*. However, because of their higher temperature ranges for growth (Ayerst 1969), *A. terreus* and *Em. rugulosa* were capable of extensively col-

onizing peanut seeds at 45 C in the absence of other *Aspergillus* competitors.

Other fungal species colonized seeds primarily at water activities and temperatures suboptimal for section *Flavi* species and *A. niger*. The majority of these species has been reported previously from freshly harvested peanut seeds, with dominant genera comprising *Aspergillus*, *Penicillium* and *Fusarium* (Hanlin 1973, Joffe 1969, Pettit and Taber 1968). Fungal species were detected and identified in this study by their aerial growth and sporulation on the surfaces of peanut seeds. The extent to which species were confined to the internal tissues of seeds and remained undetected is not known.

Conditions resulting in the highest incidences of *Penicillium* species and their teleomorphs on peanut seeds in the present study fell into four categories: (i) low water activities and cool to moderate temperatures (majority of species); (ii) high water activities and moderate temperatures (*Pen. pinophilum* and *Pen. funiculosum*); (iii) high water activities and high temperatures (*Talaromyces* species); and (iv) moderate water activities and moderate temperatures (*Eup. ochrosalmoneum*). These generalized conditions were consistent with reported optima for spore germination and growth of *Penicillium* species under culture conditions (Hocking and Pitt 1979, Mislivec and Tuite 1970, Pitt 1979), although data for many of the species are absent or incomplete. *Fusarium* and *Clonostachys* species colonized peanut seeds primarily at high water activities and cool temperatures, and these conditions also were consistent with reports of maximum growth of *Fusarium* species at water activities >0.98 and temperatures near 25 C (Magan and Lacey 1984, Marín et al 1995).

Notably absent on peanut seeds in the present study were peanut pathogens prevalent in southern Georgia such as *Rhizoctonia solani*, *Sclerotium rolfsii* (white mold) and *Macrophomina phaseolina* (charcoal rot), species that infect peanut seeds through hyphal growth from infested plant debris and through myceliogenic germination of sclerotia (Kokalis-Burelle et al 1997). The absence of these pathogens might reflect the low probability of propagules being present in the small amount of soil applied to the seed wound. On the other hand, the experimental conditions might not have been conducive for colonization by these fungi.

Crop specificity in A. parasiticus.—Past research does not adequately explain why peanuts are more frequently infected by *A. parasiticus* than aerial crops such as corn and cottonseed in which *A. flavus* is the predominant aflatoxigenic species (Diener et al 1987, Hill et al 1985, Horn 2003). Angle et al (1982)

and Horn et al (1995) reported approximately equal proportions of *A. flavus* and *A. parasiticus* in soil from cornfields in Missouri and Georgia respectively, yet corn from these regions is infected primarily by *A. flavus*. Soil insects harbor both species and are capable of vectoring them to aerial crops (Lillehoj et al 1980), suggesting that selection for *A. flavus* likely occurs in the crop. *A. parasiticus* readily colonizes corn ears that are artificially wounded and inoculated with *A. parasiticus* alone (Wilson et al 1986, Zummo and Scott 1990), but competition greatly favors *A. flavus* when ears are co-inoculated with the two species (Calvert et al 1978).

This study showed that the incidence of peanut seed colonization by *A. parasiticus* was optimal at 22 C compared to 30–37 C for *A. flavus*. Furthermore, when peanut seeds were incubated for extended periods at 15 C, *A. parasiticus* was the only species from section *Flavi* to colonize seeds. *A. parasiticus* grows more rapidly at 22–25 C than *A. flavus* on culture media (Pitt and Miscamble 1995) and irradiated pecans (Vázquez-Barrios et al 2001), and aflatoxin production is greatest in *A. parasiticus* at 25 C as opposed to 30 C for *A. flavus* (Faraj et al 1991). Overall temperature differences between subterranean peanut pods and aerial corn and cotton fruits in part might account for the preferential infection of peanuts by *A. parasiticus*. In water-stressed peanut plants grown in southern Georgia, soil and pod temperatures were 23–28 C and 19–31 C respectively, whereas aerial stem temperatures reached a maximum of 40–45 C (6–10 C greater than air temperature) (Sanders et al 1985a). Temperatures within corn ears and cotton bolls also often exceed daytime air temperatures by at least several degrees (Anderson 1940, Chu and Bariola 1987, Khabba et al 2001). Therefore elevated temperatures at potential infection sites on corn and cotton might give *A. flavus* a competitive advantage over *A. parasiticus*.

Association of Eupenicillium species with Aspergillus section Flavi.—There have been sporadic reports of *Penicillium* species within subgenus *Biverticillium* (*Pen. purpurogenum* and *Pen. rugulosum*) parasitizing and sporulating on the heads of *A. niger* and occasionally section *Flavi* species (*A. tamarii*, *A. flavus* and *A. oryzae*) (Raper and Thom 1949, Tatarenko 1959), but this paper is the first report of *Eupenicillium* species showing a similar growth habit. Furthermore the present research suggests some degree of specificity of *Eup. ochrosalmoneum* and *Eup. cinnamomopurpureum* for *Aspergillus* species belonging to section *Flavi*. The precise nature of this association and whether parasitism is involved remain to be determined. Because *Eup. ochrosalmoneum* was capable of

colonizing peanut seeds in the absence of section *Flavi*, this association, regardless of its nutritional basis, is clearly facultative. Colonization of the substrate by *Eup. ochrosalmoneum* might be required for subsequent sporulation on *Aspergillus* heads, which is one possible explanation for the failure of *Eup. ochrosalmoneum* to establish itself on section *Flavi* species sporulating on pistachio nuts and almonds.

Eupenicillium species are isolated primarily from soil (Stolk and Samson 1983). However both *Eup. ochrosalmoneum* and *Eup. cinnamopurpureum* have been reported frequently from seeds and grains (Pitt 1979, Stolk and Samson 1983). *Eup. ochrosalmoneum* invades preharvest corn (Wicklow and Cole 1984, Wicklow et al 1984, Wicklow et al 1988) and peanuts (B.W. Horn unpublished data), where it produces the neurotoxic mycotoxin citreoviridin, and *Eup. cinnamopurpureum* has been reported from corn in storage (Wicklow et al 1998). *Aspergillus* species from section *Flavi* are common on these substrates and likely would compete with *Eup. ochrosalmoneum* and *Eup. cinnamopurpureum*. In this study the relatively slow-growing *Eupenicillium* species sporulated close to the substrate and were overrun quickly by more aggressive section *Flavi* species that produced much taller conidiophores. A long history of interactions between the two groups of fungi might have resulted in an adaptation in which *Eupenicillium* species sporulate on the heads of section *Flavi* species for efficient aerial dispersal of their conidia.

The high frequency of *Eup. ochrosalmoneum* associated with section *Flavi* on peanut seeds might be due to the abundance of the species in soil, with densities in the current study of up to 3.6×10^4 CFU/g. *Eup. ochrosalmoneum* produces immature ascostromata on preharvest corn (Wicklow et al 1984) and peanuts (B.W. Horn unpublished data). Immature ascostromata in corn are dispersed to the soil surface by combine harvesters (Wicklow et al 1984) and subsequently mature to form ascospores (Horn and Wicklow 1986). An accumulation of resistant ascospores over many growing seasons could account for the high soil populations.

Conclusions.—Wounding of peanut seeds and inoculation of the wounds with soil in the laboratory have parallels with activities in nature; for example, the sudden exposure of a resource base when an insect creates a peanut wound that is contact with the surrounding soil. Furthermore, there are similarities between species composition on seeds in this study and reported species from peanuts in the field. A comparison between the incidence of seed colonization in the laboratory and in nature is difficult, but a higher incidence under laboratory conditions would be

expected due to the highly controlled conditions. Despite the dissimilarities with infection of peanuts under field cultivation, inoculation of wounded viable peanut seeds with soil containing natural populations of fungi provides a model system for studying the infection process, the interactions among fungi and those factors important in aflatoxin formation.

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