colorin-A-accumulating mutant (9); however, no such biogenetic relationship was observed among the major aflatoxins when they were presented to this mutant. Therefore, we conclude that the major aflatoxins are produced simultaneously at the end of the aflatoxin pathway, and propose a branching pathway from a common precursor for the production of the aflatoxins B_1, B_2, G_1 and G_2.

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Effect of β-Ionone on *Aspergillus flavus* and *Aspergillus parasiticus* Growth, Sporulation, Morphology and Aflatoxin Production

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ABSTRACT

The ketone β-ionone is reported to be one of the naturally occurring volatile metabolites of developing corn ears. In testing the effects of volatile compounds on *Aspergillus flavus* and *A. parasiticus*, we found that β-ionone applied to the surface of PDA plates had a striking inhibition of growth and sporulation of these fungi. The colonies were restricted, remained buff-colored and had little or no sporulation. There were major effects on the morphology of the asexual reproductive structures. The conidiophore development was arrested and normal sporulation did not occur. Mycelial transfers from these atypical cultures to potato dextrose agar had normal growth and conidia. Incorporation of β-ionone at levels of 10-1000 μL/L, in liquid media seeded with spore suspensions of *A. parasiticus* (NRRL 2999) severely depressed aflatoxin accumulation in shake culture.

INTRODUCTION

A possible way to influence aflatoxin contamination of corn is to develop a corn variety that contains chemicals that do not allow growth of the *Aspergillus flavus* group or formation of aflatoxins. Aflatoxin contamination is possible following growth of *A. flavus* in corn, but these characters are not necessarily linked. In screening several volatile compounds reported (1,2) to be present in developing corn, we found that β-ionone had a striking and unexpected effect on growth and sporulation of *A. flavus*. The purpose of these experiments was to document the morphological response to β-ionone and to measure the effect of β-ionone on growth and aflatoxin accumulation in shake culture.

EXPERIMENTAL PROCEDURES

Aspergillus flavus Link (CP-22) isolated from Georgia corn and *Aspergillus parasiticus* Speare (NRRL 2999) were maintained on potato dextrose agar (pda) slants and used in these experiments. The *A. flavus* isolate (CP-22) does not produce aflatoxin and the *A. parasiticus* isolate (NRRL 2999) produces aflatoxins.

Direct contact tests with β-ionone were done using petri dishes (15 × 100 mm) containing 20 mL pda and maintained at 26 C. Varying amounts (1-20 μL) of β-ionone were pipetted directly onto the pda surface just before inoculation. Some of the cultures were placed in plastic bags during incubation. Gross observations, diameter measurements and microscopic observations were periodically taken.

Bioassays of volatile effects of β-ionone were also done using pda in divided plates. These bioassays were conducted using the following procedure: (a) all 4 quadrants were inoculated with stabs of a spore suspension; (b) different concentrations of β-ionone (1-50 μL) were placed in only 1 quadrant; (c) plates were stored in separate plastic bags and incubated at 26 C; (d) gross observations and diameter measurements were recorded after 3, 4 or 5 days of incu-

bation; (e) slides were made and microscopic observations recorded.

Shake cultures of *A. parasiticus* (NRRL 2999) were used to determine the effects of β-ionone on growth and aflatoxin production. The medium was prepared by dissolving 50 g Bacto mycological broth w/low pH (Difco), 15 g sucrose and 2 g yeast extract in 1,000 mL H_2O. One hundred mL of the medium were placed in 125-mL Erlenmeyer flasks and autoclaved. When the medium was cool, varying amounts of β-ionone (0-100 μL/flask) were pipetted into the flasks. All experiments were replicated at least 4 times. The cultures were inoculated with a spore suspension of *A. parasiticus*. The flasks were sealed with aluminum foil and rubber bands. The cultures were grown on a rotary shaker and dry weights taken at 1, 2, 3, 4, 5, 6, 7 and 10 days.

Aflatoxin was determined from 7-day cultures after extraction of the liquid medium with $CHCl_3$. Twenty-five mL of medium was removed from each flask and mixed with 25 mL of saturated sodium chloride solution. This mixture was extracted twice with 25 mL $CHCl_3$. The $CHCl_3$ layers were collected and taken to dryness using a rotary evaporator. The residue was suspended in 1 mL $CHCl_3$ then diluted with 1 mL hexane. The suspension was placed on a silica gel Sep-Pak (Waters Assoc.) and eluted with 5 mL hexane, 5 mL anhydrous ethyl ether, and 3 mL $CHCl_3/CH_3OH$ (90:10). The $CHCl_3/CH_3OH$ fraction was collected, taken to dryness under N_2 and reconstituted in HPLC mobile phase. The aflatoxins were determined by HPLC using the method of Thean et al. (3).

RESULTS AND DISCUSSION

Direct contact of *A. flavus* or *A. parasiticus* with 1, 2.5, 5, 10, 20 and 100 μL of β-ionone placed on the surface of pda resulted in severely restricted growth and arrested sporulation. The colonies remained light brown-white and the growth habit was compact. No sporulation occurred at levels of 5 μL or above even after 4 weeks' incubation. Sporulation occurred after 1-2 weeks with 1 and 2.5 μL of applied β-ionone. These colonies were restricted and many conidial heads were atypical. Frequently, vesicles of reduced size were formed, sometimes with irregular sterigmata (Fig. 1). When mycelial fragments from any treatments (1-100 μL) were transferred to fresh pda, normal growth and sporulation occurred.

The volatile effects of β-ionone on opposite or adjacent quadrants were somewhat different than the effects of direct contact. One μL of β-ionone produced effects only in the quadrant containing β-ionone; the growth and sporulation of *A. flavus* or *A. parasiticus* were not affected in the other quadrants. In plates with 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μL β-ionone in 1 quadrant, an increasing effect on growth and sporulation in other quadrants was noted in plates containing 5-20 μL of β-ionone. The effects of increasing from 5 to 20 μL included increasing restriction of colony diameters and decreasing levels of sporulation after 7 days. Radial growth in plates receiving 20-50 μL β-ionone was about half that of the controls. Little sporulation occurred after 7 days in any quadrant of these plates as long as they remained in unopened bags.

Direct contact with β-ionone at levels of 1-20 μL resulted in very restricted growth, little or no sporulation, and arrested asexual reproductive development. Few, if any, mature conidia were produced. The primary thallus consisted of vegetative hyphae and conidiophore initials that were atypical or of reduced size. The volatile effects of β-ionone were evidenced by morphological changes, growth inhibition, and sporulation reduction in adjacent and opposite quadrants. Microscopic observations included: reduced size of vesicle and conidiophore diameter; arrested asexual reproduction with many immature conidiophores; increased vegetative growth when compared to direct contact; atypical distribution of sterigmata, similar to direct contact; elongated, irregular sterigmata; atypical branching of conidiophores (Fig. 2); and abnormal conidiophore appearance. These effects are concentration-dependent at levels of 1-5 μL/plate for direct contact and at levels of 5-20 μL/plate for volatile effects in divided plates.

The effects of β-ionone on growth (dry wt) and aflatoxin synthesis of *A. parasiticus* are given in Table I. The effects

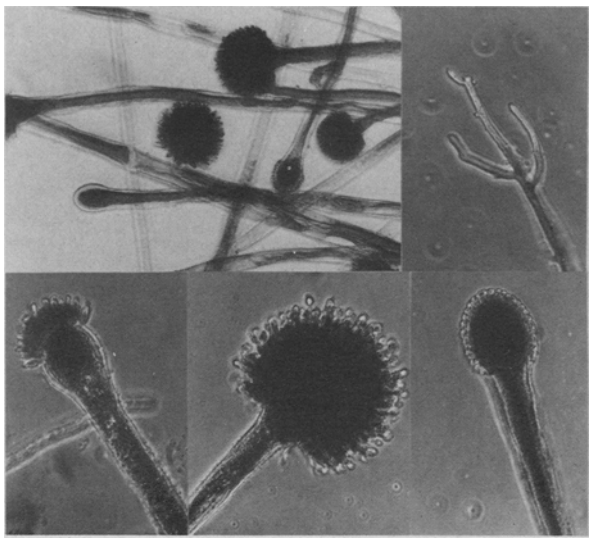

FIG. 1. Effect of direct contact of β-ionone on the conidial apparatus of *Aspergillus flavus*. Note the lack of mature conidiophores.

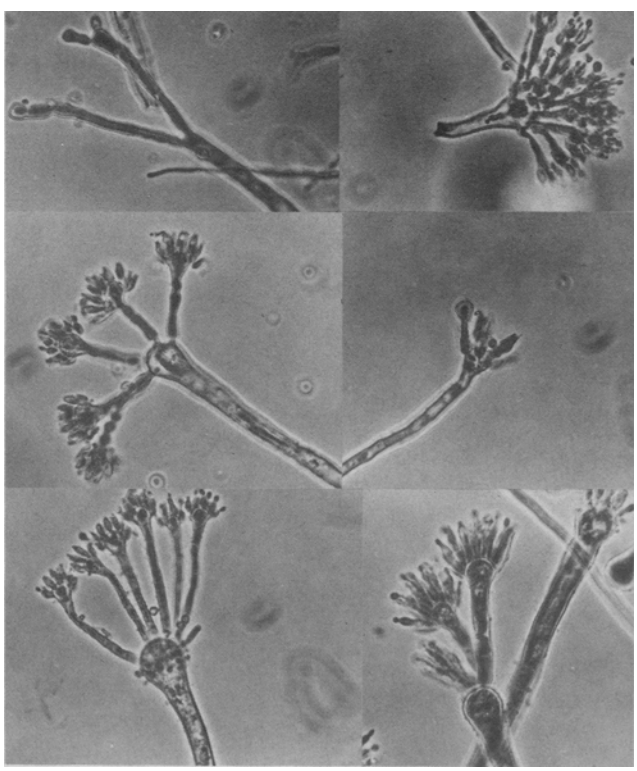

FIG. 2. Effect of β-ionone vapor on morphology and development of the conidial apparatus of *Aspergillus flavus*.

TABLE I

Effect of β-Ionone on Growth and Aflatoxin B_1 Accumulation in Shake Liquid Cultures of *Aspergillus parasiticus*

β-Ionone added (μL/L)	Dry wt (g)[b]	Aflatoxin B_1 (ng/mL)[b]
0	1.92	9528
10	1.93	10200
50	1.50	11240
100	1.23	2496
200	1.29	1568
250	1.02	1368
300	0.79	176
400	0.84	280
500	0.71	16
1000	0.74	2

[a]Culture flasks contained 100 mL medium.
[b]Numbers are averages from 4 flasks/treatment.

of growth were noticeable beginning at 50 μL/L of medium. Concentrations above 250 μL/L had little further effect on growth. The primary effect of β-ionone on growth in shake culture seemed to be on the rate of growth; however, sporulation of *A. parasiticus* in shake or submerged culture is inhibited and was not measured. Concentrations of 100 μL and above of β-ionone/L inhibited aflatoxin accumulations whereas 10 and 50 μL/L slightly stimulated aflatoxin production. This shows that the ability of the toxigenic strain of *A. parasiticus* to produce aflatoxin is not necessarily linked to growth; but aflatoxin synthesis may be positively correlated with the asexual reproductive process.

Other investigations on the effects of β-ionone on fungi have not been concerned with asexual morphogenesis of the fungi imperfecti or aflatoxin production. Carotenogenesis is stimulated by β-ionone in *Phycomyces blakesleeanus* (4) and *Blakeslea trispora* (5) Carotenogenesis is inhibited by β-ionone in *Verticillium agaricinum* (6) *Rhodotorula rubra* (7) and *Actinomyces chrysomallus* var. *carotenoides* (8).

The effect of β-ionone on carotene synthesis in the *A. flavus* growth should be investigated. The cause of the effects of β-ionone on growth and aflatoxin production in the *A. flavus* group is unknown and certainly should be investigated.

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Reducing Aflatoxin Contamination in Peanut Genotypes by Selection and Breeding

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ABSTRACT

The potential for developing agronomically suitable cultivars using peanut genotypes that exhibit resistance to seed colonization by aflatoxin-producing strains of *Aspergillus* species is explored. Some factors found to be associated with the nature of resistance to seed colonization by the toxin-producing fungi are cell structure, cell arrangement, permeability, waxy surface, tannin content and amino acid components of the seed testae. The practical implications of developing resistant cultivars are presented in data for yield, value and seed quality for 6 advanced peanut lines that were developed by breeding and selection from crosses.

INTRODUCTION

Aflatoxin contamination of peanuts is a vital concern to the peanut industry. Prevention, removal and inactivation are 3 approaches to coping with the aflatoxin problem in peanuts. However, the best approach to controlling the aflatoxin contamination is to develop cultivars that are resistant to toxin-producing strains of *Aspergillus*. Several workers have reported that certain peanut genotypes are resistant to seed colonization by aflatoxin-producing strains of *Aspergillus* spp (1-4) or to the production of aflatoxin in the seed following contamination by the fungus (5-7). In a study to determine the percentage of susceptible samples from 28 F_3 generation families from crosses between resistant and susceptible genotypes when 30-100% seed was infected after laboratory inoculation, it was concluded that the genetic and environmental influences were interacting to produce variation in seed colonization by the *Aspergillus* fungus (8). This variation allows the breeder to make progress in selecting for resistance in the segregating population of crosses. Further studies in the F_2 generation of seed from plants of crosses between peanut genotypes varying in *A. flavus* seed susceptibility levels gave evidence that crosses between certain genotypes could produce genetic variation greater than that attributed to additive genetic effect. Therefore, selection for resistance from peanut crosses is possible.

Many environmental and biological conditions in and around the peanut fruit may influence the *A. flavus* invasion of the peanut fruit. The incidence of *A. flavus* invasion is influenced by the amount and population of *A. flavus* in the soil (9), the type of plant residue in the soil (10), and crop rotation practices (11,12). Several workers have noted that drought stress before digging peanuts is associated with aflatoxin contamination (13-17). Diener and Davis (18) presented evidence that peanut pods are most vulnerable to infection by *A. flavus* when seed moisture is between 12 and 30%. Such seed moisture in combination with other