The Association of Aspergillus flavus with Hemipterous and Other Insects Infesting Cotton Bracts and Foliage

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ABSTRACT

Aspergillus flavus was isolated from 61% of a surface-nonsterile and 33% of a surface-sterile sample of *Lygus hesperus* (lygus bug) and 79% of a surface-nonsterile and 37% of a surface-sterile sample of *Chlorochroa sayi* (stink bug). In addition, *A. flavus* was isolated from intestinal tissue of 20% and 23% of aseptically dissected surface-sterilized *L. hesperus* and *C. sayi*, respectively.

A. flavus was also isolated from *Collops vittatis, Zelus* spp., *Systena blanda, Chrysopa* spp., and *Diabrotica undecimpunctata*. However, these species, unlike *L. hesperus* and *C. sayi*, were not frequently encountered on cotton plants after regular applications of insecticide for control of pink bollworm commenced in early July.

A. flavus was isolated from 94% of the samples of floral bracts and 56% of the samples of foliar disks from leaves of cotton plants at Yuma. At Safford, however, *A. flavus* was isolated from only 15% of the samples of floral bracts and 8% of the samples of foliar disks. Examination of cultured intact and dissected floral bracts revealed that *A. flavus* was localized near the margins of the fimbriate projections.

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Additional key words: collops bettle, assassin bug, lace wing larvae.

Aspergillus flavus Link has been reported to incite boll rot (11, 12, 13), yellow stain of lint (10, 11), greenish yellow fluorescence of lint (1, 8, 13, 14, 15, 16), and aflatoxin accumulation (principally aflatoxins B_1 and B_2) in seeds of cotton (*Gossypium hirsutum* L.) (1, 2, 3, 4, 5, 13, 15, 17). Since the fungus has been isolated from cotton plants and soil in several cotton-growing regions of Arizona (Stephenson and Russell, *unpublished)* and Southern California (2), inoculum has been assumed to be soil-borne and wind-disseminated.

Brazzel (9) reported that boll-rotting fungi could invade bolls through pink bollworm (*Pectinophora gossypiella* Saunders) entrance tunnels. However, invasion by this mode was less frequent than through needle punctures made mechanically in the carpel wall or pink bollworm exit holes. In a subsequent investigation,

Ashworth et al. (6) further implicated pink bollworm exit holes as possible avenues of infection of fiber and seed of cotton. *A. flavus* was also reported to be associated with three species of beetles (Nitidulidae), but transmission of *A. flavus* to pink bollworm-damaged bolls by these beetles was discounted, since the amount of infection with beetles present did not exceed the amount obtained in the absence of beetles.

Since the source of inoculum, as well as the relative time and means by which squares were inoculated by *A. flavus*, could be major considerations in control schemes, sampling of foliar and floral parts of cotton for *A. flavus* was undertaken to establish the relative distribution of inoculum on the plant.

MATERIALS AND METHODS.—Random samples of cotton foliage and squares (bracts and floral bud) and insects were taken at 7- to 14-day intervals (June to September, 1973) from cotton plants at the University of Arizona experiment stations at Yuma and Safford. Samples of foliage and squares were placed in separate, sterile, polyethylene bags (Whirl-Pak 18 oz.), transported in ice chests and stored at 5 C in the laboratory. In most instances these materials were plated within 24 hours after collection.

Botran isolation medium (BIM) was prepared as described by Bell and Crawford (7) and dispensed (10 ml) in sterile plastic petri dishes (100 mm diameter).

Leaf disks (22 mm in diameter) were cut with a sterile cork borer and transferred to the surface of BIM. Equal numbers of disks with lower and upper leaf surfaces in contact with the medium were plated.

Squares were dissected to remove bracts from floral buds. Bracts and floral buds of squares were transferred to the surface of BIM both in groups and individually. In addition, the fimbriate projections and the remaining bract tissue were individually plated on the surface of BIM. After incubation at 32 C for 4-5 days, colonies of *A. flavus* were counted.

Insects were collected with insect nets from cotton plants and living insects were either placed in plastic petri dishes (100 mm in diameter) containing 10 ml BIM or aspirated into 250-ml Erlenmeyer flasks. Insects in flasks were surface-sterilized in 0.525% sodium hypochlorite (NaOCl) for 3 minutes and aseptically plated on BIM.

Both living and dead (killed in ethyl acetate vapor) surface-sterilized insects were assayed for the presence of *A. flavus* propagules adhering to the exoskeleton by transfer of individual insects to culture plates containing BIM. Insects were removed from culture plates after 10-15 min and culture plates were incubated at 30 C for 4-5 days. Insects were also assayed for the presence of *A. flavus* carried internally by surface-sterilizing insects in a solution of 0.525% NaOCl for 3 minutes, aseptically dissecting insects to remove internal organs, transferring exoskeleton and internal organs to separate BIM plates and incubating at 30 C for 4-5 days.

RESULTS.—Cotton harvested in 1973 from Yuma and Safford, Arizona, revealed that frequencies of isolation of *A. flavus* were higher on bracts of squares than on foliage (Table 1). In addition, the levels of *A. flavus* on cotton bracts and foliage in the Yuma region increased progressively during the 1973 growing season. In contrast, frequencies of *A. flavus* isolated from bracts and squares of cotton in the Safford region decreased during the growing season (Fig. 1). However, the populations of *Alternaria* spp. encountered on Safford cotton early in the season remained high throughout the season.

Examination of intact and dissected bracts revealed that most of the *A. flavus* colonies arose from areas of insect damage or from the bases or the margins of the fimbriate regions of the bracts (Fig. 2). In addition, cultures from impressions made by aseptically pressing bracts against the surface of the agar medium indicated a similar pattern of distribution of *A. flavus* colonies.

A. flavus was frequently isolated from *Lygus hesperus* Knight, *Chlorochroa sayi* Stal, and *Collops vittatis* Say collected from cotton plants at the University of Arizona Experiment Stations in Yuma. In addition, *A. flavus* was occasionally isolated from *Zelus* spp., *Chrysopa* spp. and *Systena blanda* Melsheimer collected from cotton plants in Yuma but not Safford. *A. flavus* was more frequently associated with *Diabrotica undecimpunctata* Barber and *C. vittatis* in Safford (Table 2). However only *Lygus* and *Collops* in Safford and *Lygus* and *Chlorochroa* in Yuma were frequently encountered on cotton plants after regular applications of insecticide for control of pink bollworm commenced in early June. Although an insect-*A. flavus* association was frequently encountered with insects collected from cotton plants in Yuma, and *insect-Alternaria* spp. association was most frequently encountered from insects in Safford. The respective insect-*Alternaria* spp. association was most frequently were also similar to the results of isolations from bracts of squares collected in these localities.

When live *L. hesperus* (lygus bug) and *C. sayi* (stink bug) were aseptically plated on BIM, *A. flavus* grew from frass deposited on the BIM agar. After death of these

TABLE 1. Frequency of isolation[a] of *Aspergillus flavus* from floral bracts and foliar disks of naturally infested cotton in Yuma and Safford, Arizona

Plant part	Yuma			Safford		
	Samples (Total no.)	Samples infested with *A. flavus* (No.)	(%)	Samples (Total no.)	Samples infested with *A. flavus* (No.)	(%)
Bracts	385	360	94	60	9	15
Foliage	220	124	56	40	3	8

[a]Botran isolation medium.

insects, relatively pure cultures of *A. flavus* grew from spiracles, recta and mouth parts of both surface sterile and surface non-sterile insects (Table 3) (Fig. 3). When internal organs of lygus and stink bugs were aseptically removed and plated on BIM agar, colonies of *A. flavus* developed from intestinal tissues (Fig. 3) in four of 20 of *L. hesperus* and eight of 35 of *C. sayi*. These values are lower than values obtained for surface sterilized insects because propagules of *A. flavus* probably are carried between joints and beneath abdominal scales where they escape surface sterilization.

Nitidulid bettles (*Carpophilis* spp.) were encountered in ears of corn from plants adjoining cotton plants and from a few pink bollworm damaged bolls in Yuma. *A. flavus* was isolated from one of 10 and 11 of 100 surface nonsterile beetles from pink bollworm-damaged bolls and corn ears, respectively.

DISCUSSION. The localization of inoculum of *A. flavus* reported in this investigation suggests that factors other than wind could play a major role in dissemination of *A. flavus* propagules. Since *A. flavus* appeared to be more frequently isolated from bracts of squares than foliage, from fimbriate regions of bracts and from areas of insect damage, an association between insects which frequent and feed upon developing bolls and *A. flavus* was suspected. This hypothesis was supported by isolation of *A. flavus* from 61% and 79% of a sample of surface nonsterile *L. hesperus* and *C. sayi*, respectively. *A. flavus* was also isolated from a sample of 33% of surface-sterile *L. hesperus* and 37% of a sample of surface sterile *C. sayi*. In addition, *A. flavus* was isolated from frass and intestinal tissues of these insects. Interpretation of these data strongly suggest that insects play a role in dissemination of *A. flavus* and suggest that insects may be responsible for the localization of inoculum on bracts of squares. Since insects other than *L. hesperus* and *C. sayi* in Yuma and *L. hesperus* and *C. vittatis* in Safford were not frequently encountered after mid-July, when cotton boll development begins, other insects were not regarded to be of major importance in dissemination of *A. flavus*.

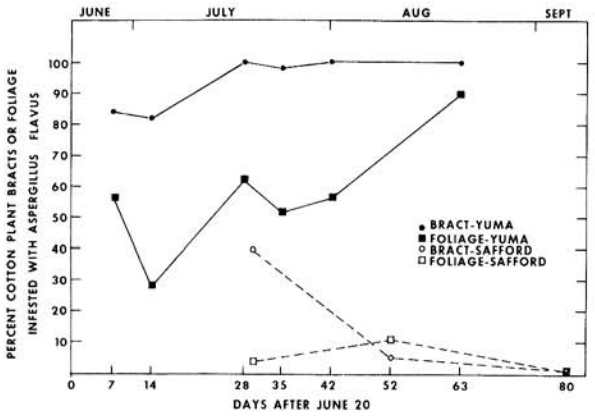

Fig. 1. Frequency of isolation of *Aspergillus flavus* from naturally infested bracts and foliage of cotton from Yuma and Safford, Arizona, during the 1973 season.

Although *A. flavus* was isolated from a nitidulid beetle removed from a pink bollworm damaged boll these beetles were rarely encountered on or in pink bollworm damaged bolls. We therefore did not consider nitidulids as a major carrier of inoculum. This conclusion agrees with the previous observations of Ashworth et al. (6) regarding these insects. It is postulated that squares and developing bolls are infested with *A. flavus* by *L. hesperus* and *C. sayi* in Yuma and *L. hesperus* and *C. vittatis* in Safford and that *A. flavus* subsequently gains entry into bolls via necrotic tissue surrounding pink bollworm exit holes and wounds.

Since Toumanoff (19) first reported *A. flavus* as a pathogen of the insect *Pyrausia nubilis* Hubner (European corn borer) more than 30 reports have appeared implicating this fungus as an insect pathogen. Several reports have also implicated *Aspergillus parasiticus* Speare as an insect pathogen (18). Since isolates of both *A. flavus* and *A. parasiticus* are known to produce aflatoxins on corn, wheat, oats, rice, sorghum, pea, soybean, peanut, pecan, and cotton seed substrates, their association with insects frequenting these plants is significant.

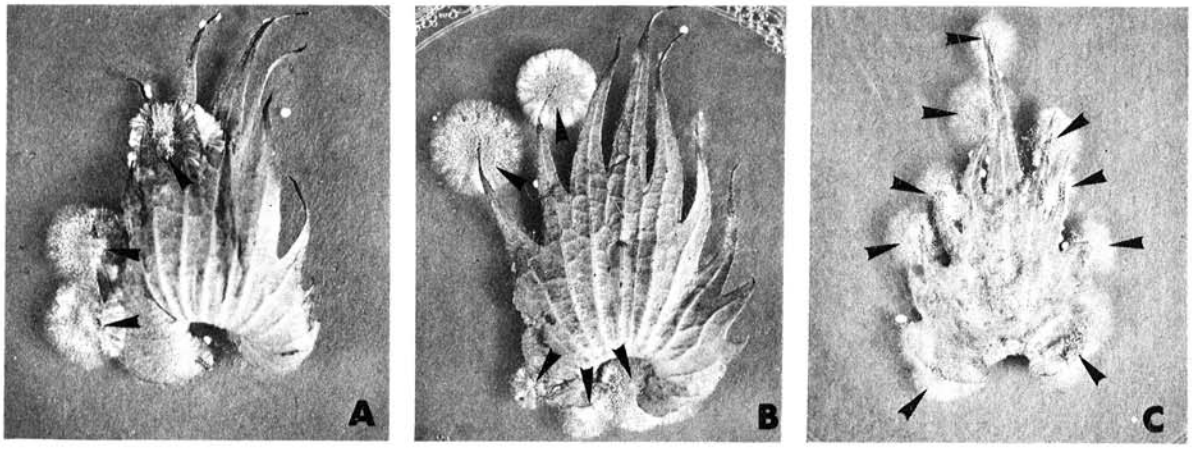

Fig. 2-(A to C). Colonies of *Aspergillus flavus* (see arrows) from naturally infested bracts after incubation for 2 days on Botran isolation medium. **A-C)** Localization of colonies of *A. flavus* about margins and fimbriate projections of bracts.

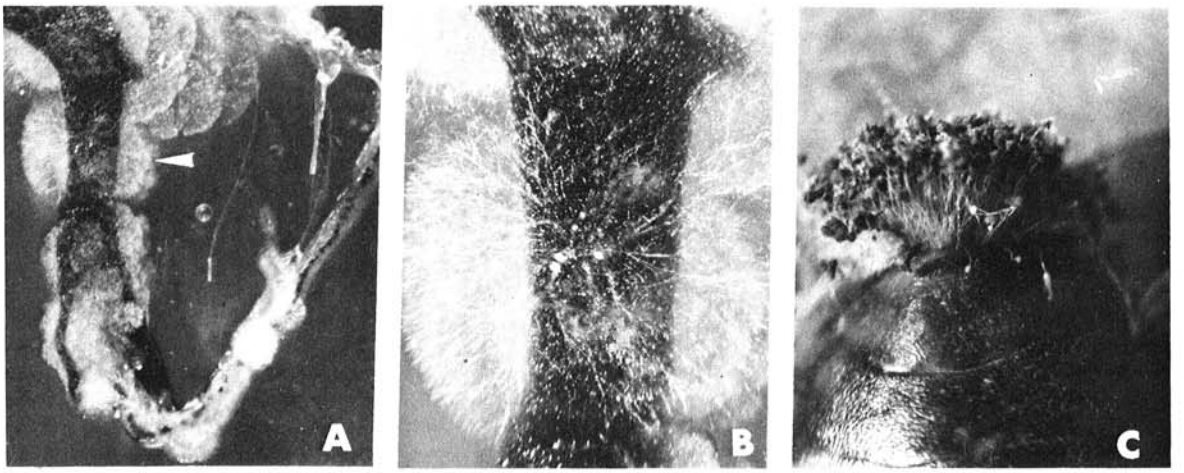

Fig. 3-(A to C). Mycelium (see arrow) of *Aspergillus flavus* growing from intestinal tissue of *Chlorochroa sayi* (stink bug). **A)** *A. flavus* associated with the intestine of a stink bug. **B)** Enlargement of a portion of A. **C)** *A. flavus* emerging from the anus of a stink bug.

TABLE 2. Frequency of isolation[a] of *Aspergillus flavus* from surface-nonsterile insects collected from cotton plants in Yuma and Safford, Arizona

Insect species	Yuma			Safford		
	Samples (Total	Samples associated with *A. flavus*		Samples (Total	Samples associated with *A. flavus*	
	(no.)	(No.)	(%)	(no.)	(No.)	(%)
Lygus hesperus (Lygus bug)	112	68	61	43	8	19
Chlorochroa sayi (Stink bug)	14	11	79	14	0	0
Collops vittatis (Collops beetle)	8	7	88	17	5	29
Zelus spp. (Assassin bug)	16	9	56	0	0	0
Chrysopa spp. (Lace wing larvae)	3	1	33	0	0	0
Systena blanda (Pale striped flea beetle)	35	16	46	0	0	0
Diabrotica undecimpunctata (Spotted cucumber beetle)	0	0	0	38	11	29

[a]Botran isolation medium.

TABLE 3. Frequency and percentage of isolation[a] of *Aspergillus flavus* from surface-nonsterile and surface-sterile *Lygus hesperus* and *Chlorochroa sayi* collected from cotton plants at Yuma, Arizona

Insect Species	Surface-nonsterile insects			Surface-sterile insects		
	Total no. sampled	*A. flavus* isolations		Total no. sampled	*A. flavus* isolations	
		(No.)	(%)		(No.)	(%)
Lygus hesperus	112	68	61	24	8	33
Chlorochroa sayi	14	11	79	30	11	37

[a]Botran isolation medium.

Whether all of the isolates of *A. flavus* from insects are aflatoxin-producing strains remains to be determined. Preliminary investigation of a few isolates from insects has indicated that some do produce aflatoxin.

Although *A. flavus* is a well-established pathogen of insects, the exact relationship (e.g., pathogen, symbiont, or contaminant) between the fungus and insect species reported in this investigation, has not been determined. The isolation of *A. flavus* from predators such as collops beetle, lace wing larvae and assassin bugs (Table 3) could be related to acquisition from the insect species upon which the predators feed. Since *A. flavus* was isolated from both predatory and nonpredatory insects, reduction of inoculum density would appear to be best achieved through early chemical control of insects frequenting cotton.

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