A Systems Analysis Approach to Establishing Research Objectives for Control of Aflatoxin Production in Cotton Seed

H. H. Ramey, Jr.

Research Geneticist, Agricultural Research Service, U.S. Department of Agriculture, Knoxville, Tennessee 37916.
The many discussions with P. B. Marsh and C. D. Ranney and the helpful suggestions of G. T. Johnson and J. L. McMeans are gratefully acknowledged.

Accepted for publication 11 June 1974.

ABSTRACT

A system which defines parameters affecting aflatoxin production in cottonseed before harvest is set forth. Switching points in the system are identified. Factors contributing to aflatoxin production or no aflatoxin production are enumerated. This analysis can help investigators identify potential areas for fruitful research. In this system, major areas are shown to be (i) determination of the quantity and origin of *Aspergillus flavus* Link inoculum in the field; (ii) study of the environmental conditions leading to *A. flavus* infection of cotton bolls; (iii) analysis of the regulatory mechanisms governing the production of secondary metabolites; and (iv) development of additional information on genetic variability in *A. flavus* strains.

Phytopathology 64:1451-1454

Additional key words: Gossypium hirsutum L.

Aflatoxins are potent carcinogens, which are effective in minute amounts. First discovered in 1961 in peanuts colonized by *Aspergillus flavus*, they have been reported in other agricultural products (7). The presence of aflatoxins in cottonseed meal prevents use in livestock rations. These toxins can be elaborated in cottonseed (*Gossypium hirsutum* L.) in the field before harvest and during subsequent storage.

The purpose of this effort is to construct, in simplified terms, a system by which aflatoxin may be produced in cottonseed before harvest. Decision analysis as defined by Wagner (11) is a procedure for separating a large scale problem into its subparts, each of which is simpler to manipulate and diagram. After the separate elements are carefully examined, the results can be synthesized to give insights into the original problem. A decision-box network described by Eisner (6) provides for a series of alternate paths. The system with alternate paths to define critical switching points is used to delineate approaches for attack on the problem. These methods are adapted for the biological system.

The following assumptions are made at the outset: (i) aflatoxins are produced only by *Aspergillus flavus* Link and related species such as *A. parasiticus* Speare; (ii) *A. flavus* is a facultative parasite, which will not directly invade healthy tissue; (iii) any boll that has been thoroughly infested by *A. flavus* will be so structurally weakened that it will not be harvestable; (iv) aflatoxins are secondary metabolites and, as such, their production is subject to metabolic regulation; and (v) any detectable quantity of aflatoxin in a cottonseed is not tolerable for subsequent use in food or feed.

CONSTRUCTION OF THE SYSTEM.—The developing cotton boll will be the initial point of the analysis. Extending the system to earlier stages of boll development will complicate the system without serving a useful purpose.

As the cotton boll matures, senescence phenomena become operative, and the capsule dehisces longitudinally. In dehiscence the locule is exposed, and the exposed drying locule is a suitable growth medium for saprophytic fungi. After the bolls open, the seed cotton may be exposed on the plant for as long as 8 wk before the crop is harvested and become a target for infection by *A. flavus*.

The starting point for the construction of the flow chart (Fig. 1) is the opening boll. The system will be extended backward to the developing boll, but the opening boll is taken initially as the point from which to start. As the boll opens, the locules are exposed. Either path 7 or path 8 may be taken from this event. Path 7 will be followed if the boll opens and dries slowly, inoculum potential is sufficient, and environmental conditions are favorable for growth of *A. flavus*. If any one of these conditions is absent, path 8 will be followed, and lint infection will not occur. Path 11 will follow from path 8 because seed infection is rare if no lint infection occurs during the boll opening and drying process.

If lint infection by *A. flavus* occurs (via path 7), circumstances may prevent *A. flavus* from infecting the seed (path 10) (9). When the seed is infected after lint infection, the course is by path 9.

Seed that was not infected during the boll opening and drying process may remain on the plant for some time before harvest. During this weathering, which can include several cycles of wetting and drying from rain or heavy dew, the seed may (path 12) or may not (path 13) become infected by *A. flavus*. Lack of infection during weathering, path 13, will lead to the desired result, no aflatoxin contamination (path 19).

Paths 9a and 12a are "dummies" which lead to a common event, seed infected by *A. flavus*. During or after infection, aflatoxin may (path 14) or may not (path 15) be produced in the seed. Lack of aflatoxin elaboration during infection (path 15) or during weathering (path 18) will lead to the desired result (path 21), no aflatoxin contamination.

Although no aflatoxin is produced after infection, aflatoxin may be elaborated during weathering (field exposure), path 17. Hence, in either case, whether aflatoxin is produced following infection (path 14) or during weathering (path 17) the result is aflatoxin contamination of seed before harvest (paths 16 and 20).

An extension of the system from the opening boll to the developing boll accommodates any boll injury, particularly insect predation (8). A developing boll may

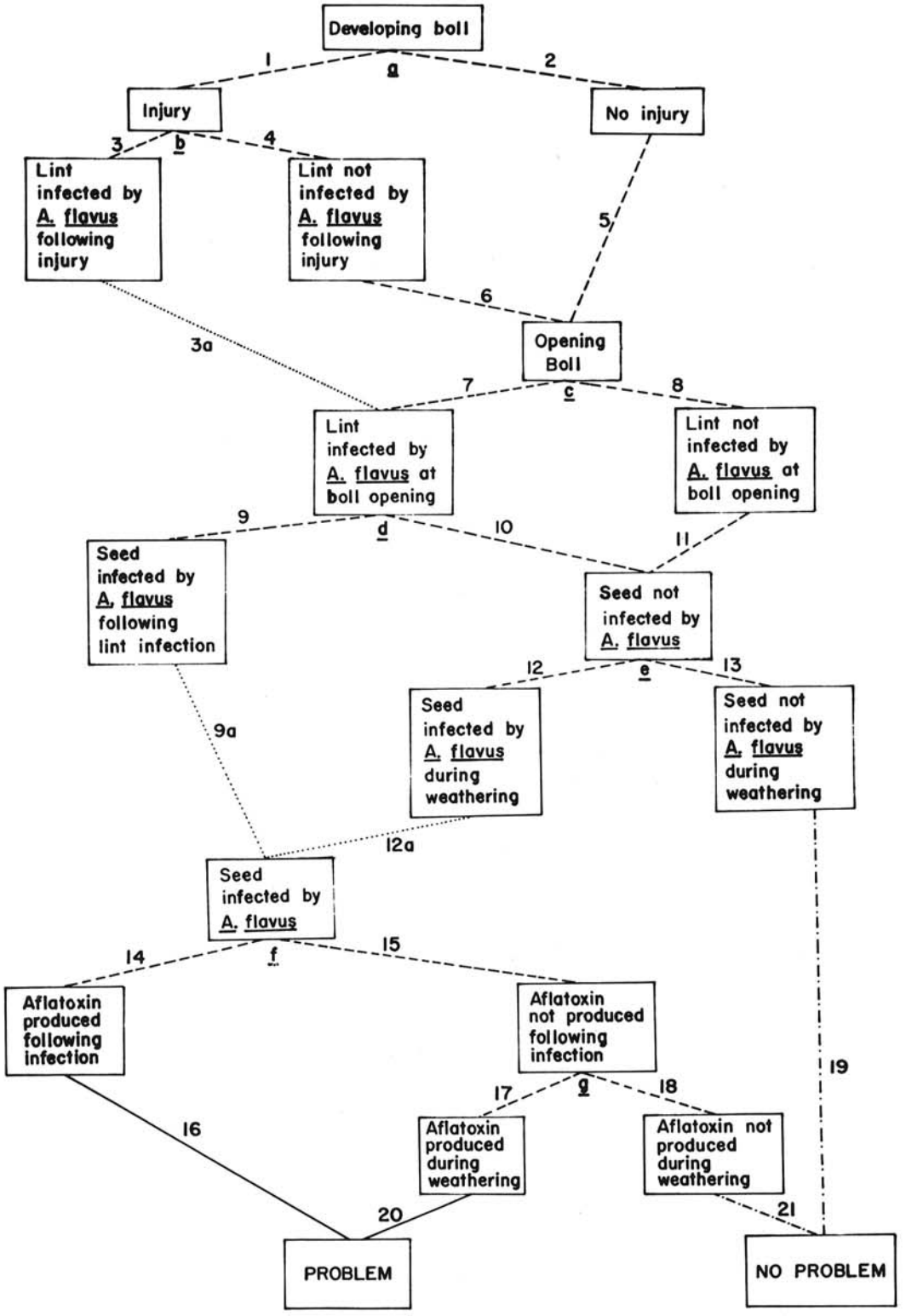

Fig. 1. Flow chart of system for systems analysis of preharvest aflatoxin contamination of cottonseed.

or may not be injured, paths 1 and 2. With no injury (path 2) the boll matures (path 5), and reaches the stage from which the flow chart construction was started, the opening boll.

When the developing boll is injured (path 1) the lint subsequently may (path 3) or may not (path 4) be infected by *A. flavus*. If infection and abscission do not occur after the injury (path 4), the boll will reach the stage of opening (path 6), or the point from which the flow chart construction was started. If lint infection by *A. flavus* does occur, but abscission does not (path 3) this event is reached before the opening of the boll. Even so, the sequence can be advanced by a dummy pathway, path 3a, to the stage of lint infection. The dummy pathway is used here to advance the sequence and simplify the flow chart, although it is recognized that seed infection may occur after the injury and before boll opening.

ANALYSIS OF SWITCH POINTS IN THE FLOW CHART.—a) *Boll injury.*—Any injury to a boll is caused by an agent outside the system. The exit holes of the pink bollworm [*Pectinophora gossypiella* (Saunders)], for example, may make the interior of the boll accessible to *A. flavus* (3). The prevalence of the pink bollworm injury is such that control of this injurious agent must be a requirement for the alleviation of the aflatoxin problem in cotton.

b) *Lint infection in the injured boll.*—An injury that exposes the locule provides a ready means of entry for *A. flavus*. The *quantity of inoculum* at the time of injury must play a major role in the infection of lint in damaged locules. Without an adequate level of inoculum present, an exposed locule will not become infected. *Accessory agents* such as *Nitidulidae* beetles (3) in feeding on the injured boll, may carry *A. flavus* spores into the exposed locule and thereby aid in establishing an infeciton. *Microclimatic conditions* determine whether the locule remains a favorable growth medium for a sufficient span of time for the fungus to grow and establish an infection.

c) *Lint infection during boll opening.*—As the boll opens, the locules are exposed and are a favorable medium for growth of *A. flavus*. The *quantity of inoculum* must play a major role in lint infection at this stage of boll development. A lack of inoculum will allow locules to escape infection. *Microclimatic conditions* affect this process in at least two ways. External conditions affect the time span that a locule is a favorable growth medium, particularly in water content. Drying conditions reduce the length of time a locule is vulnerable to infection (1). External conditions also affect the growth rate of *A. flavus*. High temp (2, 9) favors establishment of the fungus in the drying locule. Slow drying and high temp favor the growth of the fungus in the lint and can lead to the problem.

d) *Seed infection after lint infection.*—Once the lint is infected by *A. flavus,* the subsequent infection of the seed is affected by *microclimatic conditions.* Drying conditions will prevent seed penetration by the fungus, even though mycelia are growing in the lint. Temperatures can affect mycelial growth or provide a competitive advantage to other fungi (2).

e) *Seed infection during weathering.*—The moisture content of the seed determines whether the fungus can penetrate and become established. It appears that the fungus can penetrate a dried and subsequently remoistened seed (1). *Microclimatic factors* such as moisture and temp play a major role. The *quantity of inoculum* level of *A. flavus* should reduce the probability of infection, even though other conditions were favorable. However, if the lint had been infected previously, enough inoculum would probably be present.

f) *Aflatoxin elaboration following seed infection.*—*Genetic capabilities* of the *A. flavus* strain will be a factor in aflatoxin production. *Growth conditions* can affect aflatoxin production. Since aflatoxins are secondary (shunt) products (12), conditions that prevent the formation of secondary metabolites will result in the absence of aflatoxin contamination even in infected seed.

g) *Aflatoxin elaboration during weathering.*—Aflatoxins will not be produced by *A. flavus* strains that lack the *genetic capabilities* to do so. *Growth conditions* will determine whether mycelia having genetic capability will produce aflatoxins. Favorable moisture conditions are a prime requirement. The nutritive value of the seed for the fungus will affect the amounts of aflatoxins elaborated, within the genetic capabilities of the mycelia.

The factors involved in the switching points of the flow chart are: (i) inoculum level of *A. flavus,* (ii) the environmental or microclimatic conditions affecting growth of this fungus, (iii) metabolic pathways directed to the production of secondary metabolites, and (iv) genetic capabilities of the fungus. These factors are, then, the focal points for research directed toward alleviation of the problem of preharvest aflatoxin contamination of cottonseed.

METHODS OF ATTACK ON THE PROBLEM.—*Inoculum level.*—*A. flavus* appears to be ubiquitous. The inoculum level at one time during the boll development period was assessed in each of two areas of California (1). A lower inoculum level occurred in the area where aflatoxin contamination of cottonseed was found. Also the level differed from one year to the next, while aflatoxin levels were equivalent. A more detailed assessment of inoculum level during the time span from fruit initiation to maturity appears to be needed to clear up this seemingly conflicting information. Major sources of inoculum apparently are not defined, although all plant debris particles in soil were infested (1). Increasing inoculum on the soil surface resulted in greater infection and aflatoxin contamination (1). Reduction of inoculum level on the soil surface by chemical or cultural means could aid the control.

Growth conditions.—Conditions for the growth of *A. flavus* in pure culture are well known. However, temp considerably above the optimum for the growth of the fungus in pure culture are necessary for the development of *A. flavus* boll rot of cotton (2, 9). There may be an interaction between the fungus and the cotton boll such that optimum temp for growth in the field are not the same as for growth in pure culture in the laboratory. An investigation of the influence of environmental conditions on the fungus, the cotton boll, and the interaction of the two could provide leads to ways of reducing *A. flavus* boll rot.

Regulation of the Production of Secondary Metabolites.—Aflatoxin production by *A. flavus* has been the subject of many investigations (7). However, carbohydrates have been used as the carbon and energy

sources in most of these. Since cottonseed is primarily lipo-protein, it may be questioned whether the investigations with carbohydrate carbon sources are relevant to the problem of preharvest aflatoxin contamination of cottonseed. Verification of the results by using lipids and proteins as carbon and energy sources is in order. As secondary metabolites, production of aflatoxins should be subject to metabolic regulation. For example, zinc is known to be required for aflatoxin production (12). Increased levels of zinc result in lowered aflatoxin production (4). Cottonseed accumulates large quantities of zinc in neutral or acid soils (10), but preharvest aflatoxin contamination of cottonseed occurs in areas where the soil pH is high and zinc accumulation may be low (9). Provided that the low zinc-accumulation level is confirmed, increasing zinc accumulation in cottonseed in areas of high soil pH may be a fruitful approach to alleviation of the problem. Other targets for regulating secondary metabolite production may be found by determining the metabolic pathways leading to aflatoxin formation. The branching points in the metabolic pathways would be potential targets for control procedures.

Genetic capabilities of the fungus.—Some strains of the fungus lose aflatoxin-production capability during serial transfer on laboratory media (5). This phenomenon should be investigated. Finding the cause of the loss of aflatoxin-production capability could aid investigations into regulation of aflatoxin production. Furthermore, the inoculum source in the field apparently does not cause any appreciable loss of aflatoxin-production capability, as evidenced by the number of aflatoxin-producing isolates reported. However, based on the serial-transfer experience, there is a possibility that field inoculum sources could be altered so that inoculum produced lacks the capability to produce aflatoxin.

DISCUSSION.—A careful definition of a problem is a necessary first step to its solution. This flow-chart technique provides a mechanism by which a problem may be defined. As applied herein to preharvest aflatoxin contamination of cottonseed, it allows the identification of several switching points. These switching points are dichotomous, in that one direction from the point leads to the problem, while the other leads away from the problem.

Analysis of this series of switching points, to identify the factors most likely to be involved in a "yes" or "no" direction from each point, reveals only a few. These factors are considered the prime areas for research on the problem.

While this may be considered as belaboring the obvious, the technique focuses on the pertinent factors involved in the problem. Whereas a direct approach to the problem may center on peripheral factors, this technique tends to avoid trivia and highlight the essential. The technique has not been tried with other problems in cotton production, but it appears to have broad application in establishing research objectives.

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