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DETECTION OF CROTALARIA SEED IN MIXED FEEDS

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Several cases of poisoning by mixed feeds have occurred recently in which investigation has shown that the toxin came from seed of crotalaria species present in the feed. The detection and identification of such seed in mixed feeds has, therefore, become of considerable importance. This publication presents a method for such detection and identification.

While the genus crotalaria contains many species, only two, C. spectabilis and C. retusa, have been investigated extensively. These species have been used widely in the South for many years for soil conservation and improvement. Some work also has been done with C. juncea, C. striata and C. intermedia.

Seed of crotalaria species may remain dormant in the soil for several years, and then germinate and mature as volunteer plants in plantings of corn, soybeans, oats, grain sorghum or other grains which subsequently are harvested and used in the formulation of mixed feeds. Seed of crotalaria thus occur as a contaminant in the feed grains. Both the plant and the seed are toxic, but the seed are much more poisonous than the vegetative parts of the plant. The toxins in crotalaria seed are so powerful that only a few seed per pound of mixed feed can cause trouble ranging from loss of thrifty condition through severe liver damage to death of the animal.

Toxins

The toxicity of crotalaria seed is due to the presence of a number of alkaloids and closely related compounds. All crotalaria species investigated thus far are toxic, but the degree of toxicity varies with different species. Culvenor and Smith (7,8) isolated monocrotaline and a new alkaloid, spectabiline, from seed of C. spectabilis. Spectabiline, an O-acetylmonocrotaline, also was prepared by acetylation of monocrotaline, and has the empirical formula $C_{18}H_{25}O_7N$. Preliminary assays (8) showed an aklaloid content of 5.8 percent in the seed and 0.63 percent in the whole plant. In C. retusa, they found retronecine-N-oxide and three new aklaloids--retusine, retusamine and a noncharacterized base. Adams and coworkers (1, 2, 3, 4, 5) were the first to determine that C. retusa is a poisonous plant. They reported that its total alkaloid content ranged as high as 5 percent, of which a large part was monocrotaline. They also proved that this alkaloid had a cyclic diester structure.

Macroscopic Characteristics

Crotalaria seed in a mixed feed may occur either as whole seed or as fragments produced by grinding the grain in which the crotalaria seed are present. The identification of whole seed is rather simple, but fragments must be examined microscopically before they can be identified positively.

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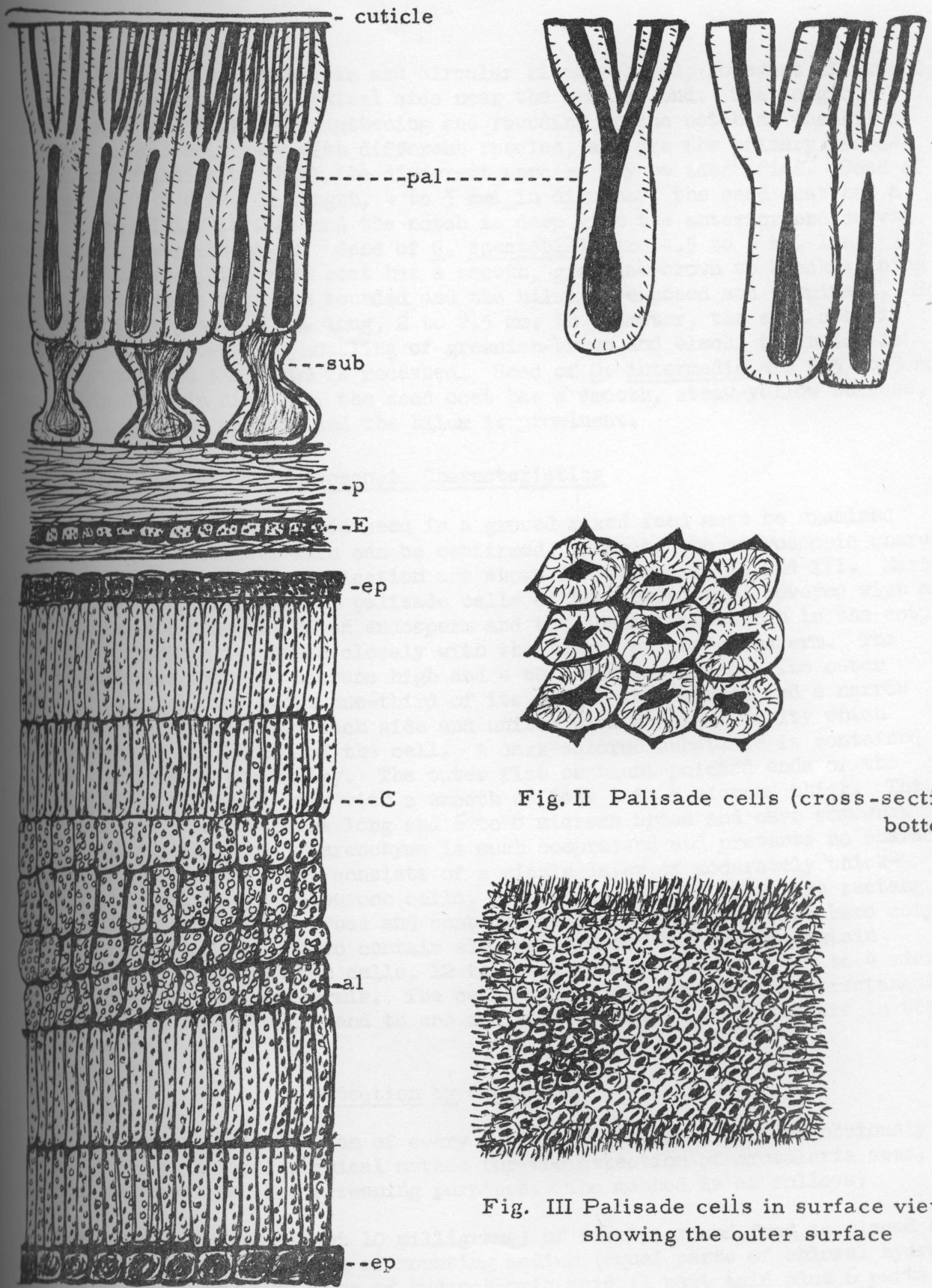

Fig. I Crotalaria juncea (cross-section of seed)
pal - palisade cells, sub-column cells (subepidermal layer), p-parenchyma, E-endosperm, C-cotyledon, with ep-epidermis and al-aleurone grains

Fig. II Palisade cells (cross-section-bottom)

Fig. III Palisade cells in surface view showing the outer surface

Whole seed of crotalaria are circular kidney-shaped, somewhat flattened, and have a deep notch on the ventral side near the smaller end. The length, diameter, color and degree of flattening and rounding of the notch at the hilum and of the hilum itself vary with different species, and are the primary macroscopic characteristics by which the different species may be identified. Seed of C. juncea are 7 to 8 mm. in length, 4 to 5 mm. in diameter, the seed coat has a smooth, black shining surface and the notch is deep with the anterior end curved and protruding over the hilum. Seed of C. spectabilis are 4.5 to 5 mm. long, 3.5 to 4 mm. in diameter, the seed coat has a smooth, greenish-brown to black shining surface, the notch is deep and rounded and the hilum is exposed and prominent. Seed of C. striata are 3 to 3.5 mm. long, 2 to 2.5 mm. in diameter, the seed coat is smooth and yellow-brown with mottling of greenish-brown and black, the notch is deep and rounded and the hilum is recessed. Seed of C. intermedia are 2.5 to 3 mm. long, 1.5 to 2 mm. in diameter, the seed coat has a smooth, straw-yellow surface, the notch is deep and rounded and the hilum is prominent.

Microscopic Characteristics

Fragments of crotalaria seed in a ground mixed feed must be examined microscopically before detection can be confirmed. Some of the microscopic characteristics which serve as identification are shown in Figures I, II and III. Marked characteristics are the Y-shaped palisade cells of the spermoderm, covered with a thick cuticle, the presence of an endosperm and the absence of starch in the cotyledons. The spermoderm is united closely with the layers of the endosperm. The palisade cells are 15 to 20 microns high and 4 to 5 microns broad. The outer portion of each cell for about one-third of its length is Y-shaped and a narrow cavity extends centrally down each side and unites with a larger cavity which reaches nearly to the bottom of the cell. A dark-colored substance is contained in the inner portions of the cavity. The outer flat or blunt-pointed ends of the palisade cells are covered over with a smooth cuticle 3 to 4 microns thick. The column cells are 5 to 6 microns long and 6 to 8 microns broad and have somewhat thickened walls. The spongy parenchyma is much compressed and presents no characteristic features. The endosperm consists of a single layer of moderately thick-walled aleurone cells. The aleurone cells, as seen in surface view, are rectangular or polygonal, 6 to 8 microns broad and contain proteid substances. The hard cotyledons and radicle of the embryo contain aleurone grains, but do not contain starch. Two layers of palisade cells, 12 to 15 microns in length and 3 to 4 microns wide, underlie the inner epidermis. The cells toward the center become rectangular or polygonal, and are arranged end to end and side by side, since they are in the palisade layers.

Detection by Chemicals

Microscopic examination of every questionable sample of feed obviously is impracticable. A rapid chemical method for the detection of crotalaria seed, therefore, was developed for screening purposes. The method is as follows:

A small portion (about 10 milligrams) of finely ground feed is placed on a microscope slide. Three drops of mounting medium (equal parts of chloral hydrate, water and glycerol) and two drops of hydrochloric acid (1 part acid plus 6 parts water) are added. The feed and reagents are mixed thoroughly and spread evenly over about half of the slide and allowed to stand 3 to 4 minutes. Fragments of crotalaria seed, if present, will change from black or straw-yellow to a pink to cherry-red color. If the red color develops, an excess of sodium hydroxide (3 or 4

pellets in 30 cc water) is added to the mixture, which causes the fragments of crotalaria seed to turn blue. The changes in color of crotalaria fragments probably result from the presence of some anthocyanin within the cavities of the palisade cells.

Seed of several other plants, the bran coats of barley and rye and the seed coat of many kinds of weed seed contain anthocyanins which give their fragments a red, blue or black color under normal conditions, but the addition of acid or base causes no change in color. This color change from black or straw-yellow to red or blue with a change in acidity has not been found with fragments of any other material and appears to be specific for crotalaria seed. Consequently, fragments of these other materials will not be confused with fragments of crotalaria seed.

This detection method is of particular value in examining soybean meals suspected of containing ground crotalaria seed. The outer surfaces of the soya and crotalaria hulls are very similar in appearance when viewed under a compound microscope. In such cases, the two hulls may be differentiated quickly by adding one to two drops of the acid solution to the slide. The crotalaria hull fragment will turn slowly through pink to a cherry-red color; the color of the soya hull will remain unchanged.

The detection method is effective on seed of C. spectabilis, C. juncea, C. striata and C. intermedia, but is less distinctive on C. intermedia because it has a normal reddish straw-yellow color. Development of the blue color with sodium hydroxide is best in the case of this species.

The mounting medium has been checked to determine if any of the reagents used is essential to the development of the red color in crotalaria hull. Glycerol had no effect, but development of the red color in acid solution is hastened by the chloral hydrate.

This method also can be used for detecting the presence of sunflower meal. The anthocyanins in this plant are concentrated in the disk flowers of the modified calyx or pappus, seed coat (pericarp) and the epidermal tissues of the stalks and stems. All of these tissues turn to a blood-red color when a drop of the acid solution is added to them in the mounting medium. The color reaction depends on the use of both the acid and chloral hydrate. Glycerol appears to speed the reaction and causes the red color to spread out into the mounting medium. Sodium hydroxide alone or with the mounting medium does not produce a color change in any of the tissues of the mature plant. If any flowering heads are incorporated in the product, the yellow petals from the ray flowers will turn blue when a drop of sodium hydroxide is added to it in the mounting medium. The acid solution does not change the normal yellow color of the petal.

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