# PATTERNS OF CELL DEATH IN STALKS OF NORMAL AND INJURED SORGHUM

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ABSTRACT. — The effects of root injury, removal of the distal two-thirds of all leaves, and removal of inflorescences on cell death were studied in four sorghum varieties (De Kalb C44B, C45, E56A, and F63). The removal of inflorescences decreased the rate of cell death in the stalk tissue while both removal of the distal two-thirds of all leaves and root injury increased the rate. This model system which provides methods of modifying cell death rates will be useful in the study of the role of living cells in resistance to spread of fungal pathogens in sorghum stalks, and in the study of the cell death process in normal and diseased plants.

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Katsanos and Pappelis (1965) studied the patterns of cell death in the lower stalk internodes of six varieties of grain sorghum and reported an increase in cell death after inflorescences emerged. The study was continued for two months. Internodal and nodal stalk tissue which macroscopically appeared white and fluffy when the stalks were cut longitudinally through the central axis, microscopically was found to be composed of dead, air-filled cells with no evidence of the protoplast. However, internodal and nodal stalk tissue that macroscopically appeared well hydrated and light cream in color, microscopically was found to be composed of living cells with protoplasts that could be plasmolyzed. Pappelis and Katsanos (1966) reported that root and leaf injury increased the rate of cell death in internodal and nodal tissue, whereas removal of the inflorescence decreased the rate of cell death. The study was limited to two varieties (De Kalb C44B and E56A). In the same year, Katsanos and Pappelis made two reports (1966a, 1966b) that *Colletotrichum graminicola* (Ces.) G. W. Wils in inoculated plants of three varieties (De Kalb C44B, E56A, and F63) and in stalks of root-injured plants of one variety (De Kalb F63) spread through areas of dead cells and was inhibited by living cells or factors associated with them. Since it is important to develop model systems to test the hypothesis that living cells are involved in disease resistance in stalk rot, the present investigation was conducted to verify and extend the information on the effect of injury on cell death.

## MATERIALS AND METHODS

(De Kalb C44B, C45, E56A, and F63) four varieties of grain sorghum, were planted in a three replicate experiment on May 16, 1965, on Southern Illinois

University Agronomy Farm, Carbondale. In each replicate, each variety was planted in plots of 24 rows spaced 100 cm apart, each row containing 40 plants spaced 15 cm apart.

The inflorescences emerged in more than 50% of the plants in each replicate on the following dates: E56A, July 18; C44B, July 20; C45, July 22; and F63, July 24. On the above dates, the plots were randomly divided into sub pots and the following treatments applied: normal; inflorescence removed as they emerged; distal two-thirds of all leaves removed; and roots cut by inserting a 15 cm wide spade, starting about 10 cm away from the stalk and pushing down at a 45 degree angle to intersect below the stalk. Roots were cut a second time three weeks later. Since weed control was by chemical means (Atrazine) and hand hoe, the spade patterns were easily observed in the soil, and the spade was inserted at the same place on the second date of root cutting.

Each variety was sampled seven times at biweekly intervals starting on the date the treatments were applied. On each day, the four elongated internodes above the uppermost adventitious roots of 10 plants in each sub-plot were rated by splitting the stalks longitudinally and rating the amount of dead (white, fluffy) internodal and nodal tissue using the following system: 0.0, no white and fluffy in the internode; 0.1, less than 1% white; 0.5, 2 to 12%; 1, 13 to 25%; 2, 26 to 50%; 3, 51 to 75%; 4, 76 to 100%; and 6, plant dead. When dead tissue in a node linked dead tissue in adjacent internodes, the letter T was added to the rating of the lower internode. For each sampling day, the pith condition ratings for each internode of all 10 plants of every treatment in each replicate of a variety were averaged. The mean for the three replicate averages for each internode was determined and will be referred to as pith condition field average.

## Results

The field averages for pith condition ratings are presented in Table 1. In all treatments of every variety, the pith condition ratings increased with time. During the greater part of the study, pith condition ratings of all treatments were progressively lower from first to fourth internodes for E56A, but at the end of the study the ratings became progressively higher. For variety C44B, ratings were progressively higher from first to fourth internodes for all treatments during the entire study. For varieties C45 and F63, pith ratings were progressively lower from first to fourth internodes for all treatments during the entire study. In general, C44B showed the greatest increase of pith ratings for all treatments, followed in order by C45, F63, and E56A.

Leaves of root-cut plants of all varieties showed wilt symptoms within one hour after cutting. Within two days, the lower two or three leaves died, tips of some of the upper leaves were curled, and tips of some others had become yellow or brown. A few plants of each variety died. After this time, no other premature death of leaves or entire plants was observed, but all plants of this treatment generally appeared less healthy than the plants of the other treatments.

In all varieties, the root-cut treatment increased the pith condition ratings, that is, it increased the rate of cell death more than any other treatment. This increase was obvious early in the study, but it became most pronounced during the latter part. Root cutting also resulted in the greatest amount of death of cells in the nodes as indicated by the presence of the letter T in Table 1. It should be noted that normal nodal cell death in these varieties studied appears later in the season, occurring after many internodal cells have died.

In the leaf-cut treatment, plants of varieties F63 and C44B appeared less green and healthy than those of

TABLE 1.—Field averages[a] for pith condition (cell death) ratings[b] in normal stalk tissue of sorghum and those injured as follows: inflorescence removed (Head-cut), distal two-thirds of all leaves removed (Leaf-cut), and roots on both sides of the stalk cut on two dates using a spade 15 cm in width (Root cut).

| Treatment and Variety | Internodes | Pith Condition Ratings on Sample Dates[c] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| E56A | | | | | | | | |
| Normal | 4 | 0.0 | 0.3 | 0.6 | 1.7 | 2.0 | 2.8 | 3.0 |
| | 3 | 0.0 | 0.4 | 0.9 | 1.9 | 2.3 | 2.8 | 3.0 |
| | 2 | 0.2 | 0.5 | 1.1 | 2.2 | 2.5 | 2.8 | 2.5 |
| | 1 | 0.5 | 0.6 | 1.2 | 2.4 | 2.5T | 2.5 | 2.5 |
| Head-cut | 4 | ..... | 0.1 | 0.8 | 1.9 | 2.0 | 2.1 | 1.8 |
| | 3 | ..... | 0.3 | 1.2 | 2.0 | 2.0 | 2.1 | 1.9 |
| | 2 | ..... | 0.5 | 1.2 | 1.8 | 1.6 | 1.6 | 1.6 |
| | 1 | ..... | 0.8 | 1.0 | 1.4 | 1.3 | 1.2 | 1.4 |
| Leaf-cut | 4 | ..... | 0.2 | 0.7 | 1.8 | 2.2 | 2.6 | 3.2 |
| | 3 | ..... | 0.3 | 0.9 | 1.9 | 2.5 | 2.7 | 3.2 |
| | 2 | ..... | 0.5 | 1.0 | 2.1 | 2.6 | 2.7 | 3.1 |
| | 1 | ..... | 0.6 | 1.2 | 2.0 | 2.6 | 2.7 | 2.8 |
| Root-cut | 4 | ..... | 0.4 | 1.3 | 2.5 | 2.7 | 3.6T | 3.9T |
| | 3 | ..... | 0.6 | 1.5 | 2.6 | 2.9 | 3.7T | 3.9T |
| | 2 | ..... | 0.8 | 1.8 | 2.9T | 3.1T | 3.8T | 3.8T |
| | 1 | ..... | 0.9 | 2.0 | 3.0T | 3.1T | 3.8T | 3.8T |
| C44B | | | | | | | | |
| Normal | 4 | 2.0 | 2.8 | 3.4 | 3.7T | 3.5 | 3.7T | 4.0T |
| | 3 | 1.7 | 2.4 | 3.2 | 3.7T | 3.6T | 3.6T | 3.9T |
| | 2 | 1.2 | 1.6 | 2.6 | 3.7T | 3.6T | 3.6T | 3.8T |
| | 1 | 0.8 | 1.1 | 2.2 | 3.6T | 3.6T | 3.6T | 3.7T |
| Head-cut | 4 | ..... | 3.0 | 3.2 | 3.1 | 3.2 | 3.2 | 3.2 |
| | 3 | ..... | 2.6 | 2.5 | 2.6 | 2.9 | 2.7 | 2.9 |
| | 2 | ..... | 1.6 | 1.8 | 2.0 | 2.2 | 1.8 | 2.2 |
| | 1 | ..... | 1.0 | 1.2 | 1.5 | 1.6 | 1.5 | 1.5 |
| Leaf-cut | 4 | ..... | 3.1 | 3.4 | 3.7T | 3.7T | 3.9T | 4.1T |
| | 3 | ..... | 2.6 | 3.1 | 3.7T | 3.7T | 3.8T | 4.1T |
| | 2 | ..... | 1.8 | 2.7 | 3.6T | 3.6T | 3.8T | 4.0T |
| | 1 | ..... | 1.4 | 2.1 | 3.5T | 3.5T | 3.8T | 3.9T |
| Root-cut | 4 | ..... | 3.5 | 3.5T | 3.8T | 3.7T | 4.2T | 4.5T |
| | 3 | ..... | 3.1 | 3.3T | 3.8T | 3.7T | 4.2T | 4.5T |
| | 2 | ..... | 2.5 | 3.0T | 3.7T | 3.7T | 4.1Y | 4.5T |
| | 1 | ..... | 1.8 | 3.8T | 3.7T | 3.7T | 4.0T | 4.5T |
| C45 | | | | | | | | |
| Normal | 4 | 0.1 | 0.2 | 0.9 | 1.2 | 2.0 | 2.1 | 2.6 |
| | 3 | 0.1 | 1.4 | 1.0 | 1.6 | 2.2 | 2.3 | 2.8 |
| | 2 | 0.1 | 0.6 | 1.4 | 2.0T | 2.4T | 2.3 | 2.8 |
| | 1 | 0.2 | 0.7 | 1.4 | 2.2T | 2.5T | 2.4T | 2.8T |
| Head-cut | 4 | ..... | 0.2 | 0.9 | 1.0 | 1.0 | 1.4 | 1.5 |
| | 3 | ..... | 0.3 | 0.9 | 1.1 | 1.1 | 1.4 | 1.5 |
| | 2 | ..... | 0.4 | 1.0 | 1.3 | 1.2 | 1.4 | 1.6 |
| | 1 | ..... | 0.6 | 1.0 | 1.4 | 1.3 | 1.4 | 1.6 |

TABLE 1.—Concluded.

| Treatment and Variety | Internodes | Pith Condition Ratings on Sample Dates[c] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Leaf-cut | 4 | ..... | 0.3 | 0.8 | 1.1 | 1.6 | 2.3 | 2.2 |
| | 3 | ..... | 0.4 | 0.9 | 1.4 | 1.8 | 2.3 | 2.3 |
| | 2 | ..... | 0.6 | 1.2 | 1.7 | 2.0 | 2.3 | 2.4 |
| | 1 | ..... | 0.6 | 1.2 | 2.0 | 2.0 | 2.3 | 2.4 |
| Root-cut | 4 | ..... | 0.8 | 1.5 | 1.9 | 2.4 | 2.6 | 4.0T |
| | 3 | ..... | 1.0 | 1.7 | 2.2 | 2.5 | 2.7 | 4.1T |
| | 2 | ..... | 1.3 | 2.0 | 2.5T | 2.8T | 2.8T | 4.1T |
| | 1 | ..... | 1.3 | 2.0 | 2.5T | 2.8T | 2.8T | 4.1T |
| F63 | | | | | | | | |
| Normal | 4 | 0.1 | 0.5 | 0.5 | 0.9 | 1.3 | 1.7 | 2.6 |
| | 3 | 0.1 | 0.6 | 0.6 | 1.2 | 1.4 | 1.8 | 2.7 |
| | 2 | 0.2 | 0.7 | 0.8 | 1.4 | 1.7 | 1.8 | 2.8 |
| | 1 | 0.3 | 0.8 | 0.8 | 1.5 | 1.9 | 1.8 | 2.8 |
| Head-cut | 4 | ..... | 0.5 | 0.7 | 0.9 | 0.9 | 1.2 | 1.4 |
| | 3 | ..... | 0.7 | 0.9 | 0.9 | 1.0 | 1.2 | 1.5 |
| | 2 | ..... | 0.7 | 0.9 | 1.0 | 1.0 | 1.1 | 1.5 |
| | 1 | ..... | 0.8 | 0.9 | 1.1 | 1.0 | 1.1 | 1.5 |
| Leaf-cut | 4 | ..... | 0.4 | 0.9 | 1.3 | 1.7 | 2.2 | 2.6 |
| | 3 | ..... | 0.6 | 1.0 | 1.7 | 1.9 | 2.5 | 2.6 |
| | 2 | ..... | 0.7 | 1.3 | 2.0 | 1.9 | 2.6T | 2.9T |
| | 1 | ..... | 0.7 | 1.4 | 2.2 | 2.0 | 2.7T | 2.9T |
| Root-cut | 4 | ..... | 0.9 | 1.2 | 1.7 | 2.5 | 3.0T | 4.1T |
| | 3 | ..... | 1.0 | 1.3 | 2.2 | 2.6 | 3.0T | 4.1T |
| | 2 | ..... | 1.3 | 1.5 | 2.4 | 2.7 | 3.2T | 4.1T |
| | 1 | ..... | 1.4 | 1.5 | 2.5 | 2.8T | 3.2T | 4.1T |

a. Field averages are the means of three replicate averages based on 10-plant samples in each treatment sub-plot.
b. Pith condition ratings: 0.0, no white tissue composed of dead cells; 0.1, less than 1% white; 0.5, 2 to 12%; 1, 13 to 25%; 2, 26 to 50%; 3, 51 to 75%; 4, 75 to 100%; and 6, plant dead. When dead tissue in a node linked dead tissue in adjacent internodes, the letter T was added to the rating of the lower internode.
c. Sample dates: E56A, July 18, August 2, 16, 30, September 13, 18, and October 13; C44B, July 20, August 4, 16, 30, September 13, 28, and October 13; C45, July 22, August 5, 19, September 2, 16, 30, and October 15; and F63, July 24, August 6, 19, September 2, 16, 29, and October 15.

the normal and head removal treatments. For these two varieties, leaf cutting resulted in pith condition ratings exceeded only by those of the root-cut treatment. For C45 and E56A, pith ratings in leaf-cut plants were higher than those of normal plants during most of the sampling days, but the differences were not as marked as that observed between plants of the root cutting and normal treatments. For variety E56A, leaf cutting resulted in a slight increase in pith condition ratings during the last three sampling days, whereas during the first three sampling days, rating decreased slightly as compared to those of the normal plants rated on the same days. In variety C45, pith condition ratings in leaf-cut plants were lower than those of the normal plants during the entire study.

Plants with inflorescences removed, appeared greener and healthier dur-

ing the entire period than the plants of the other treatments, including those of the normal one. Inflorescence removal resulted in many auxilliary buds forming vigorously growing new shoots and developing new inflorescences (which were removed as they appeared). Except for a slight increase in some internodes during the first, second, or both sampling days for varieties E56A, C44B, and F63, pith condition ratings were lower than the ratings in the plants of the other treatments. Inflorescence removal prevented nodal cell death during the period of study.

## Discussion

The treatment of root and leaf cutting had, in general, the same effect since both increased cell death in the stalks. The reasons for this are probably not the same. Root cutting results in decrease of water uptake, whereas leaf removal would reduce transpiration. Synthesis of carbohydrates and other substances would greatly be reduced by both injuries. The removal of the inflorescence would be expected to increase the level of carbohydrates in the stalk, whereas continued production of new shoots would require carbohydrates. The hormonal balance would be expected to be greatly changed by these injuries. Whatever may be the reasons for modification of the cell death rate resulting from plant injury, the change in rate will provide new model systems to test the effect of the living cells on resistance to spread of fungal stalk rot pathogens in sorghum stalk tissue. Since consistent differences appear between the cell death patterns in each variety, sorghum may prove to be useful in the study of genetics of cell death. Since the pattern of cell death appear to follow predictable patterns within the stalks, the use of this tissue may prove useful in the study of cell death at the cytochemical and ultrastructural level.

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