Relationship of Nitrogen, Crude Fiber, Ether-Soluble Substances, and Mineral Nutrients to Cell Death in Corn Cob Parenchyma Tissue

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ABSTRACT

Various cell constituents were measured before and during the period of cell death in corn (*Zea mays*) cob parenchyma tissue. It was determined that, since cell elongation and cell death were occurring concurrently, the only valid basis of comparison of cell constituents was on a per cell basis, and all results were reported on that basis. The study period began on the day of silking, and lasted 3 weeks. K, Si, P, Fe, and Co concentrations increased during the 1st week (the period of greatest cell elongation), then decreased slowly as the cells died, to give an over-all increase. There was a continuous accumulation of Sr, Cu, crude fiber, and ether-soluble substances over the study period. Mo increased during the first week, then remained constant. Zn, Ba, and B increased until the 2nd week, when about 85% of the cells were dead; and then decreased. Mg and total N remained constant during the study period. Phytopathology 60:513-517.

Senescence of plant tissue has been investigated extensively. Most thoroughly studied have been the aging of seeds, cotyledons of germinating seeds, ripening fruit, and excised leaves. Much of this work has been reviewed (26, 28, 29).

Investigations of host-parasite relationships in stalk rot of corn (*Zea mays* L.) revealed that spread of stalk rot pathogens such as *Gibberella zeae* (Schw.) Petch. and *Diplodia zeae* (Schw.) Lev. is limited to areas of dead stalk parenchyma cells in the host (20, 21, 23); i.e., stalk parenchyma cells must die before the tissue can be invaded by the pathogen. Similar relationships were found with sorghum and sugarcane (7, 8, 22). Hence, resistance and susceptibility to infection by stalk rotting fungi in these three monocotyledons are functions of host cell senescence and death, and investigations of senescence of stalk parenchyma tissue were begun.

No causative relationship between amounts of stalk components (total N, 80% ethanol-soluble N, simple sugars, total sugars, inorganic elements) expressed as a percentage of dry wt or wt per unit volume of tissue and cell death was found (1, 3, 5, 17). Since these studies confirmed the observations of Pappelis (19) that fresh and dry wt of internodal tissue in elongated stalks are variables (determined by volume basis analysis), expression of amounts of tissue components as a percentage of fresh or dry wt is unsuitable for expressing physiological changes in maturing corn plants or for comparative purposes within or among cultivars, even on the same day.

Since corn stalk tissue sampled by the pith core method included both parenchyma cells and vascular tissue, the relationship between changes in the levels of components of the parenchyma cells and the death process was not clear because of the possibility of localized high concentrations of substances in vascular bundles; for, although parenchyma cells died, many cells in and around vascular tissue remained alive, and the vascular tissue continued to function, conducting nutrients through the area being studied. For this reason, BeMiller et al. (2) used corn cob parenchyma tissue free of vascular bundles as a model for a biochemical study of parenchyma cell senescence and death. Interpretation of their results was, however, limited because of an overlap of periods of cell elongation and of cell death.

The purpose of this investigation was to follow changes in amounts of crude fiber, ether-soluble substances, N, and certain other elements during the period of cell senescence, and to collect data so that contents could be expressed on a fresh wt, dry wt, volume, and per cell basis.

MATERIALS AND METHODS.—*Tissue.*—Corn plants were grown on the Southern Illinois University Agronomy Farm, Carbondale. On 3 August 1966, 120 ears of corn (*Zea mays* L. 'Funks G-72') which had just begun to silk were tagged. Thirty ears were collected that day, and the remaining 90 were collected at 1-week intervals, 30 each week. Pith tissue was removed from inside the ring of vascular bundles with a cork borer, and the density (g fresh wt/cc tissue) was calculated from tissue wt and volumes determined by water displacement. The tissue was dried at 70 C in a forced-air oven for 2 days.

Analyses.—Elemental analyses for K, P, Ca, Mg, Mn, Fe, B, Cu, Zn, Al, Sr, Mo, Co, Na, Si, and Ba were performed by emission spectrography at the Ohio Agricultural Research and Development Center with the cooperation of J. Benton Jones, Jr. Crude fiber, ether-soluble substances, and N (Kjeldahl) analyses were performed by the Rosner-Hixson Laboratories. All concentrations were calculated on a fresh wt, volume, and per cell basis.

Cell count.—Three fresh cobs from each sampling date were stored in 80% ethanol. Parenchyma cells in these cobs were counted to determine the extent of cell elongation as the cob grew. Sections for cell counts were taken along the entire length of the cob.

RESULTS.—Density of corn cob pith cores decreased

with time (Fig. 1). A density of 1.0 indicates that most of the cells are alive while, at densities below 0.3, most of the cells are dead (23). Since fresh wt/cc and dry wt/cc (Table 1) both varied with time, expression of amounts of substances per mg fresh wt or per mg dry wt were not considered to be a good basis for comparison, and a per cell basis was used for data expressions. For this purpose, the average number of cells per centimeter in longitudinal and cross sections and the calculated number of cells per cc were determined and found to decrease with time (Table 1).

Amounts of crude fiber and ether-soluble substances increased on a per cell basis over the study period (Fig. 2). Total N content remained constant on a per cell basis (Fig. 2), but plotted on a volume basis, the amount of N showed a steady decrease (Fig. 3) because of cell elongation. The same data calculated on a dry wt basis are presented in Fig. 4 to permit comparison with the standard method of reporting chemical contents of tissue.

As corn cob parenchyma cells age and die, there are changes in the per cell concentrations of common elements (Fig. 5-7). Concentrations of some of the elements studied (K, Si, P, Fe, Co) increased during the 1st week after silking, which is the period of greatest cell elongation, then decreased slowly, to give an over-all increase; the change was most pronounced in K, Fe, and Co. Concentrations of others (Zn, Ba, B) peaked on the 3rd week after silking, then decreased, to give an over-all increase; this pattern was most pronounced in Ba and B. Mo concentration increased during the 1st week, then remained constant, to give an over-all increase. Mg concentration showed very little change during the study period. Mn concentration remained almost constant during the 1st week, then decreased to give an over-all decrease. Perhaps the most surprising result is that there was a continuous accumulation of Sr and Cu, especially Cu, even when only a few living cells remained. Ca was present in measurable amounts on the first sampling date, but only traces were found thereafter. Only traces of Na and Al were found.

Discussion.—Improved interpretation of the concentration changes of tissue components during the period of cell elongation and death can be obtained using the per cell basis. Total N of corn cob parenchyma cells shows no change on a per cell basis over the sample period, but a decrease in amount per unit volume or per cent dry wt. This is explained by cell enlargement causing a reduction in cell number per unit volume and a continuous increase in dry wt per cell during the same period. Improved interpretation of the changes in amounts of crude fiber and ether-soluble substances is similarly obtained, using the per cell basis. On a per cell basis, crude fiber is shown to gradually and continuously increase. On a volume basis,

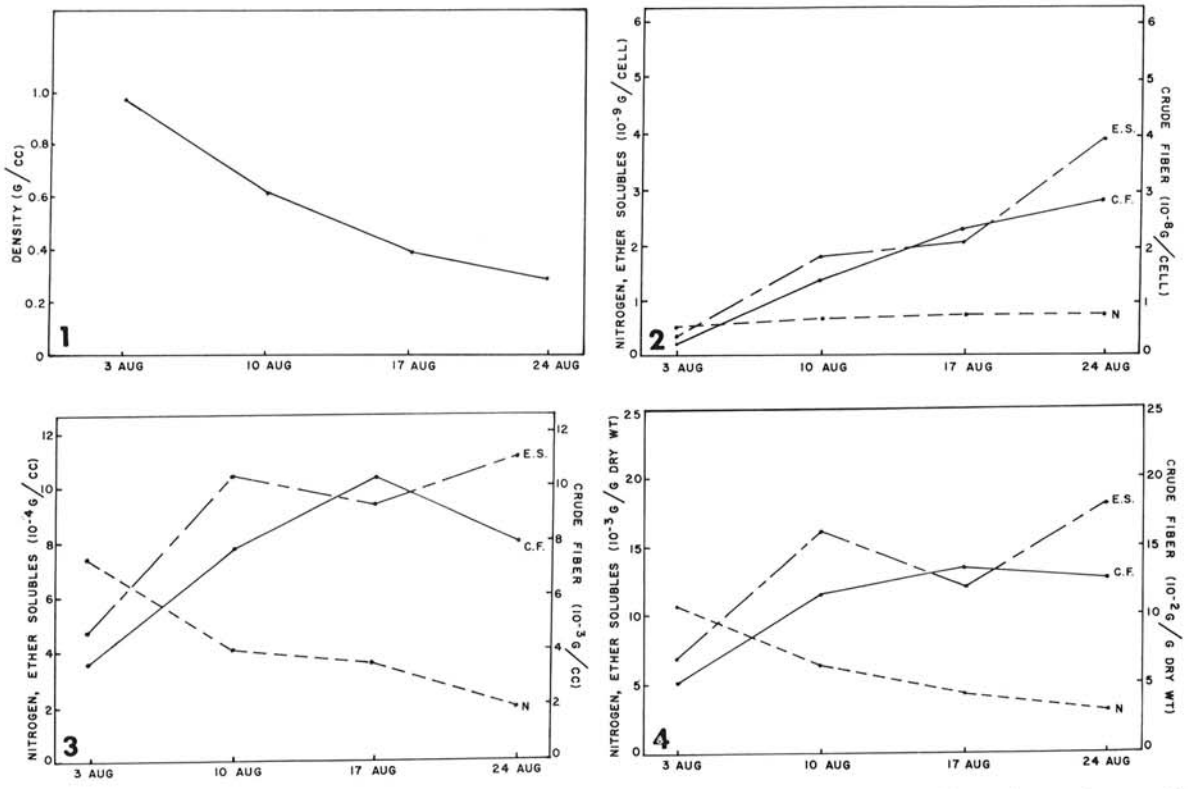

Fig. 1-4. **1)** Density changes with time in corn cob parenchyma tissue (Funks G-72). Silking date, 3 August. **2)** Amount per cell changes with time of crude fiber, N, and ether-soluble substances. **3)** Amount per cc changes with time of crude fiber, N, and ether-soluble substances. **4)** Amount per g dry wt changes with time of crude fiber, N, and ether-soluble substances.

TABLE 1. The effect of time after silking on the number and dry weight of cells per cc and on dry wt per cell

Sampling date (Aug. 1966)	Cells/cm[a]		10^{-3} Cells/cc	Dry wt/cc (10^{-2} g/cc)	Dry wt/cell (10^{-7} g/cc)
	Long sec	Cross sec			
3	173	91	1,400	7.0	0.5
10	116	70	568	6.6	1.2
17	84	72	452	7.7	1.7
24	72	62	282	6.1	2.3

[a] These values are an average of three cobs each, and reflect the entire cob length.

crude fiber appears to increase sharply during the 1st week of sampling, increase slightly during the 2nd week, and decrease slightly during the 3rd week. On a dry wt basis, crude fiber appears to increase sharply during both the 1st and 2nd weeks, and decrease in the 3rd week. The apparent decrease in crude fiber on the latter two bases would have required an interpretation of enzymic degradation during the latter stage of ear formation, an erroneous interpretation as shown by the per cell data.

The obvious improvement by the use of the per cell basis of data analysis for physiological and biochemical interpretation led us to abandon the percentage dry wt basis and to adopt the per cell basis for reporting concentration changes during cell elongation and senescence. The volume basis is unsuitable for comparative studies within cob parenchyma tissue over the period in which cell enlargement is occurring; however, the volume basis method used previously for comparison of amounts of substances in senescing stalk parenchyma tissue (1, 3, 6, 17, 27) should be valid, for samples were taken after stalk elongation was completed.

Dry wt per cc is controlled by cell elongation and translocation. Some cell enlargement can, perhaps, take place primarily by the uptake of water, but then translocation into the cell may occur and cell walls may thicken. As a result of elongation, there are fewer cells per cc; as a result of wall thickening, there is more dry wt per cell. In this case, the total effect is of very little change. The over-all effect could be expected to vary with varieties, factors affecting "rate of growth", and seasons.

The similarities of the g per unit volume and percentage dry wt bases is a result of little change in total dry wt per unit volume during the changes in dry wt per cell because of cell elongation.

Comparison of the various values in Table 1 can, in itself, indicate something of the physiological changes occurring in the tissue. For example, comparison of the number of cells per unit volume of tissue (Table 1) with density changes (Fig. 1) shows that cell elongation and cell death occur simultaneously in corn cob parenchyma tissue. Another advantage of collecting all these data is that the amount of water per cc can be calculated and, hence, cellular components can be expressed on a molarity basis which may prove to be most important in host-parasite studies.

Previous work in this laboratory on the mineral nutrient content of corn stalk tissue had not revealed any relationship between concentrations of the macroelements (N, P, K, Ca, Mg) expressed on a volume or per cent of dry wt basis and density or resistance to stalk rot caused by *G. zeae* or *D. zeae* (1, 5). The trends for these nutrients reported on either basis during the period of senescence and death of cells in stalk parenchyma tissue are quite different from those found here for cob parenchyma tissue expressed on a per cell basis. This comparison can be made because a volume basis for stalk parenchyma tissue is essentially a per cell basis, as no elongation is occurring. Neither was any relationship found between tissue contents of P or K when expressed as percentage of dry wt or wt per unit volume and the rate of cell death in sugarcane stalk tissue (27). However, several workers have suggested that a high N:K ratio in fertilizers applied to corn increases stalk rot susceptibility, and that KCl applications delay death of corn plants. Martens & Arny (13, 14) reviewed the pertinent literature and reported that KCl applications increased pith density and organic N (on a dry wt basis) levels of stalk parenchyma tissue in two of three lines studied. Reducing sugars (on a dry wt basis) were sharply decreased by the KCl treatment in all three corn lines studied. In this work, it was found (on a per cell basis) that K increased in concentration while N remained constant. (The N level decreased on a dry wt basis with age.) Hence, the gross effect is an over-all decrease in the N:K ratio on a per cell basis as the tissue dies and becomes susceptible to stalk rot. Whether a N:K ratio change would occur due to changes in fertility levels remains to be tested.

Permeability changes have been proposed as a causative factor in aging (11, 24), but there is no evidence here that a general permeability change of the cell to inorganic constituents causes senescence of parenchyma tissue of corn cobs. It may be that some increases are due simply to cell and/or vacuole enlargement, or it may be that the per cell increases are the result of active transport and that the order in which active transport stops reflects the stage of senescence, i.e., that active transport of K, Fe, or Co stops as a result of aging before that of Ba or B. All mineral nutrients, with the exception of Mg, Mn, and Sr, increase in per cell amounts during the period of greatest cell enlargement, which is also the period of greatest water loss. Some apparently leave the cells after death, K, Fe, Co, and Mn leaving, as water is lost to other tissue, before Ba and B. The continuous increase in Cu might suggest that it is found mainly in the intercellular space from which water is not lost, and that it continues to accumulate by diffusion into this space as more is translocated into the tissue or as some is re-

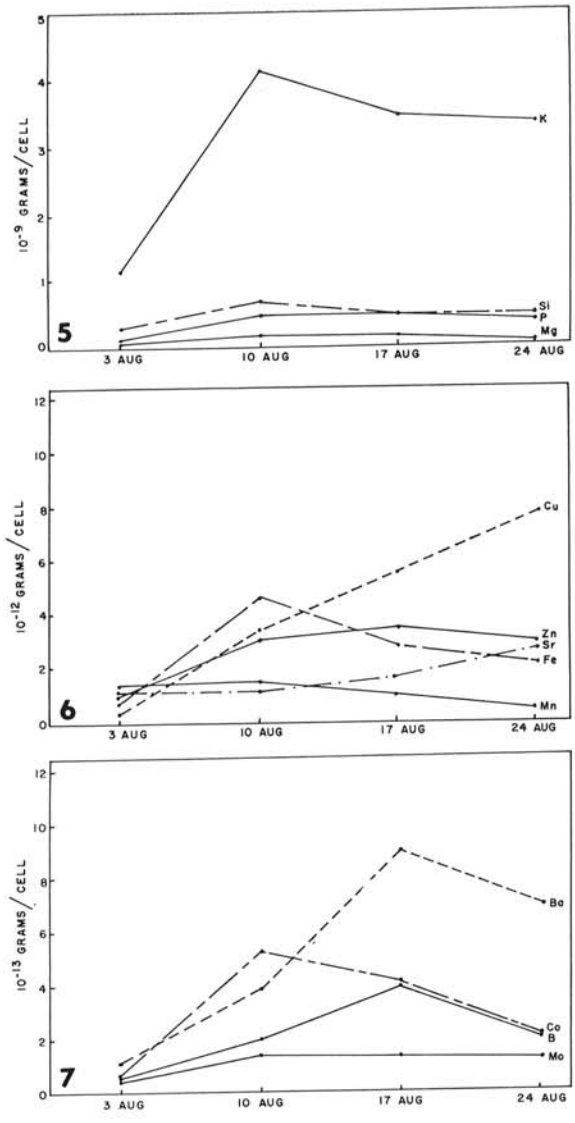

Fig. 5-7. 5) Amount per cell changes with time of K, Si, P, and Mg in corn cob parenchyma tissue (Funks G-72). Silking date, 3 August. **6)** Amount per cell changes with time of Fe, Cu, Zn, Mn, and Sr. **7)** Amount per cell changes with time of Ba, Co, B, and Mo.

moved as insoluble Cu, shifting an equilibrium. All those explanations are highly speculative.

No decrease in ether-soluble substances (which might have indicated a deterioration of membranes) or a decrease in crude fiber (which might have indicated a deterioration of cell walls) was found. In fact, both crude fiber and ether-soluble substances increased on a per cell basis, indicating that there is elongation and thickening of cell walls, and that changes in total lipid content in senescing cells are not related to membrane intactness; the total dry wt per cell also increases (Table 1). Neither is there evidence that an accumulation of Ca is responsible for the aging and death of cells as reported for other tissues and organisms (10, 11, 12, 15), since there appears to be an early decrease in Ca.

Zuber et al. (30) associated Diplodia and Gibberella corn stalk rot resistance with per cent N (dry wt basis). However, others found no relationship between total N or soluble N content of corn stalk parenchyma tissue on a volume basis and tissue density (1, 17). The results reported in Fig. 2 indicate that a corn cob parenchyma cell gets a certain amount of N when formed, and retains this amount throughout its life. The same is indicated for Mg (Fig. 5). This observation could be related to senescence and death of a cell if there is a shift from a steady state turnover of this N in nucleic acids and proteins to an increased anabolic activity with respect to these substances (2, 4, 9, 16, 18, 25).

Ca and Na, found in relatively high concentrations in stalk tissue (5), were found only in trace amounts in cob tissue. All stalk analyses were done with inbreds, and variations in these were shown. This work was done with ears from a commercial hybrid. It may be that some of the observed differences are due to germ plasm variations and heterosis. So at this time it is not known whether these differences reflect different mechanisms for senescence and death or whether they are effects rather than causes. Although the latter is suspected, further work is needed to determine the mechanism of senescence in both tissues. We suggest that studies of stalk and ear parenchyma cell death be continued and, where possible, simultaneously within the same plant.

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