DEATH OF CELLS IN PITH TISSUE OF SOYBEAN SEEDLINGS

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ABSTRACT.—Parenchyma cell death in the hypocotyl pith tissue of soybean seedlings was discovered in each of the 24 varieties studied representing seven maturity classes. Dead cells generally were observed on the fifth day after planting; and chlorenchyma tissue formed between the dead pith parenchyma cells and xylem in the above-ground stem. Dead parenchyma cells were observed in the below-ground cortex and in the pith of the elongating internode above the cotyledons. No dead cells were observed in the cotyledonary node during the 20-day study period.

The death of cells in pith parenchyma of the hypocotyl and the first and second internodes of seedlings of 18 soybean varieties was studied in relation to two or three planting depths (sub-surface, 2.5 cm, and 5.0 cm). Differences were noted in the rate of cell death in hypocotyls but not in epicotyl internodes as a result of planting depth differences. In the hypocotyls, most pith parenchyma cells died during the first week of growth, and the greater depth of planting resulted in the fastest rate of cell death in each variety. Cell death in pith parenchyma of internodes occurred as the internodes elongated.

Patterns of parenchyma cell death have been reported for normal and injured sorghum (Katsanos and Pappelis, 1969), sugarcane (Pappelis and Katsanos, 1965), and corn (Pappelis and Katsanos, 1969). It was considered desirable to seek similar parenchyma death in a plant more suited for study in growth chambers. This paper reports the discovery of parenchyma cell death in soybean seedlings and describes variations in cell death patterns associated with variations in planting depth.

MATERIALS AND METHODS

Twenty-four soybean varieties (Class 00, Acme, Flambeau, Portage; Class 0, Grant, Merit, Norcheif; Class I, A-100, Chippewa, Chippewa 64, Ontario; Class II, Hawkeye, Lindarin; Class III, Adams, Ford, Harosoy, Shelby, Wayne; Class IV, Clark, Kent, Midwest, P.I. 84.946-2, Clark 63; Class V, Dorman, Hill) were grown under greenhouse conditions at various times from December 29, 1964, to March 31, 1965. Seeds of each variety were planted in separate wood flats 2.5 cm apart at a depth of 5 cm in a soil mixture of 50% sand and 50% peat moss. Five plants from each variety were selected for study each day beginning at the second day after planting and continued until the first internodes had elongated. The maximum study period was 20 days.

Twelve soybean varieties were planted (April 29, 1965) in separate wood flats, 2.5 cm apart at depths of 2.5 or 5.0 cm. Watering was accomplished with a mist sprayer to reduce soil packing or washing. Five seedlings of each variety were selected from each planting depth on the seventh, tenth, and twentieth days after planting. Also, seedlings of six varieties were studied after planting (May 20, 1965) at three depths: 5.0 cm, 2.5 cm, and just below the soil surface. For the latter, seeds were placed on the soil surface and covered with a thin layer of soil to permit germination below the soil. Watering and sampling procedures were as those described

above but with two samples studied seven and fourteen days after planting.

Dead cells in hypocotyl and epicotyl pith tissue were discovered in cross section and longitudinal section using the plasmolyzing neutral red stain solution described by Tribe (1955).

The length of white hypocotyl tissue of the below-ground part was used as the final estimate of planting depth. The lengths of hypocotyls and internodes were measured before the stem was cut longitudinally. Lengths of pith composed of dead cells were measured and the per cent of total length with dead cells calculated.

Results

Dead and living pith cells were easily distinguished microscopically in cross and longitudinal sections of the hypocotyls using the plasmolyzing-neutral red stain. In stained sections, living cells contained dark-red, plasmolyzed protoplasts, while dead cells were light in color and contained no plasmolyzed protoplasts. In every case, dead cells contained a gas bubble. No remains of the protoplast or cell organelles were observed. When masses of dead cells occurred in the pith tissue, macroscopically the pith appeared white in color and was spongy. Tissue composed of living cells was well hydrated and appeared light green in color in the above-ground part, and cream in color in the below-ground part.

In all varieties dead cells were first observed in the central area of the below-ground hypocotyl pith five days after planting (Figure 1). Sev-

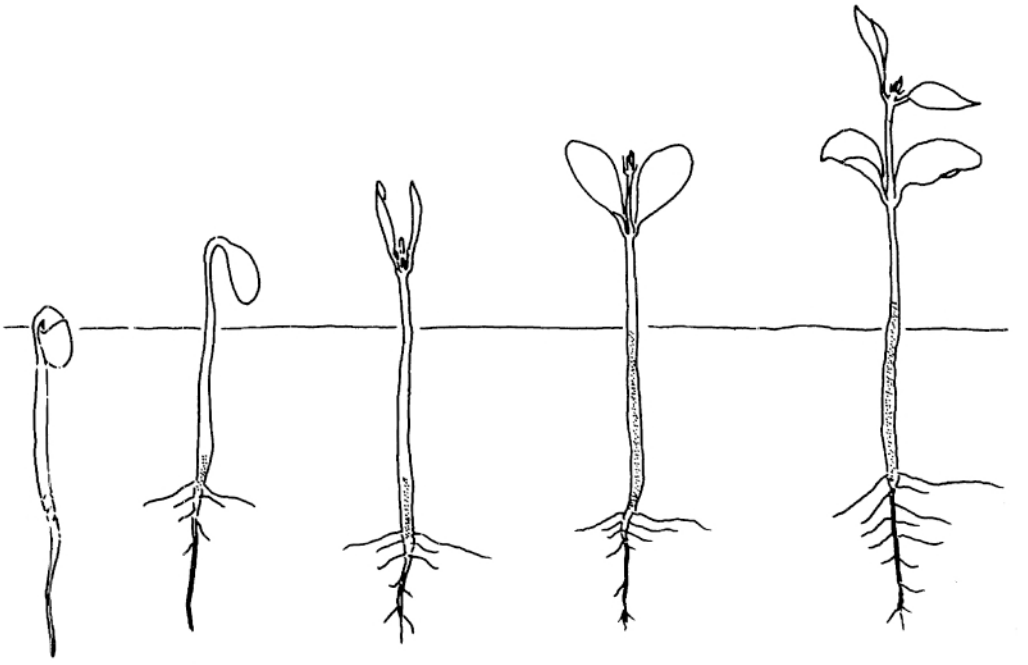

Figure 1. Dead pith parenchyma cells occur in hypocotyls five days after planting. From left to right, appearance of seedlings and areas of dead cells at 4, 5, 6, 8, and 10 days after planting; dotted areas representing areas of dead parenchyma cells in the pith.

en to eight days after planting, almost all of the cells in pith tissue of the below-ground hypocotyl were dead. Ten days after planting dead cells were observed in the central region of the pith of the above-ground hypocotyl and a hollow area formed in the below-ground hypocotyl pith. This was associated with an increase in below-ground diameter of the hypocotyl; a result of cortical expansion. At this stage, dead cells and hollow areas were also observed in the cortex. In the above-ground hypocotyl, pith cells adjacent to the xylem developed into a layer of chlorenchyma, four to five cells in thickness.

In the first internodes of all varieties studied, dead cells appeared in the pith as the internodes elongated. A chlorenchyma layer developed in the internode pith adjacent to the xylem tissue. No dead cells appeared in the cotyledonary node or in nodes above this point.

In the two experiments designed to determine the effects of planting depth on cell death in stem tissue, little differences were noted in the cell death patterns in the epicotyl internodes. For this reason, only the results from two varieties, one from each experimental group, will be presented (Table 1). In the hypocotyls, pith cells died in different patterns due to planting depth. The data for 12 varieties, planted at 2.5

TABLE 1.—Cell death percentages in the hypocotyl, first internode, and second internode pith tissue at different stages of seedling development from seeds of Harosoy planted at two depths and Kent planted at three depths. (% DC = per cent of the area of pith tissue with dead cells.)

Depth of Planting (cm)	Days After Planting	Seedling Part					
		Hypocotyl		First Internode		Second Internode	
		Length	% DC	Length	% DC	Length	% DC
Harosoy (Planted April 29)							
2.5	7	6.6	45	1.7	0		
	10	7.0	85	5.3	5		
	20	7.7	87	8.5	96	4.0	0
5.0	7	8.1	56	1.9	0		
	10	8.2	92	6.2	8		
	20	8.0	96	9.2	96	4.4	27
Kent (Planted May 20)							
Sub-surface	7	4.5	0	2.5	0		
	14	4.4	52	11.3	91	7.2	54
2.5	7	6.0	50	4.8	0		
	14	5.9	76	12.4	84	7.8	57
5.0	7	8.2	88	5.0	4		
	14	8.7	95	11.7	99	7.5	49

and 5.0 cm, and sampled 7, 10, and 20 days after planting are given in Table 2. The data for 6 varieties, planted at sub-surface, 2.5 cm, and 5.0 cm, and sampled 7 and 14 days after planting are given in Table 3.

In the study of cell death in 12 varieties at two planting depths, seven days after planting, the lengths of hypocotyls of seedlings planted at 2.5 cm depths ranged from 6 to 9 cm. During the next 13 days, additional growth was generally less than 1 cm; Acme being most extreme with an additional 1.9 cm of growth. Hypocotyls of seedlings at 5.0 cm depth ranged in length from 7 to 9 cm 7 days after planting and increased about 1 cm in length during the next 13 days, Hawkeye being most extreme with an additional 2.5 cm of growth. The length of hypocotyl with dead pith cells on the seventh day after planting ranged from 41 to 65% for seeds planted at 2.5 cm depth and 53 to 88% for those planted at 5.0 cm depth. In all but one case, the seedlings from the deepest planting depth had the highest percentage of dead cells in each variety. By the twentieth day after planting, the amount of dead cells in the pith ranged from 88 to 100% in the plants from both planting depths

TABLE 2.—Per cent of the area of pith tissue of hypocotyls composed of dead cells in seedlings of 12 varieties of soybeans planted at two depths and sampled three times after planting (April 29).

Variety	Depth of Planting (cm)	Per cent of hypocotyl with dead cells; days after planting		
		7	10	20
A-100	2.5	53	96	96
	5.0	66	99	96
Acme	2.5	42	95	96
	5.0	53	96	98
Chippewa 64	2.5	64	61	97
	5.0	88	97	97
Chippewa	2.5	44	96	96
	5.0	68	98	98
Clark	2.5	46	84	95
	5.0	66	75	96
Dorman	2.5	57	94	96
	5.0	64	94	95
Flambeau	2.5	41	97	100
	5.0	66	97	96
Ford	2.5	57	96	97
	5.0	64	96	97
Grant	2.5	61	71	90
	5.0	57	95	95
Harosoy	2.5	45	85	87
	5.0	55	95	96
Hawkeye	2.5	50	40	97
	5.0	62	80	98
Hill	2.5	58	75	91
	5.0	66	68	88

with no apparent difference due to the depth of planting. Generally, most of the pith parenchyma cells had died before the tenth day after planting.

The above-ground growth in the 12 varieties showed some variations but within each variety, the growth of the first and second internodes and the per cent of pith tissue with dead cells were similar in the seedlings from both the 2.5 and 5.0 cm planting depths. The average growth of the first internodes seven days after planting ranged from none visible to 1 cm, 4 to 10 cm on the tenth day, and 6 to 14 cm on the twentieth day after planting. The second internodes began to elongate between the tenth and twentieth days after planting, ranging in size from about 3 to 7 cm on the twentieth day. Cell death in the first internodes occurred after the seventh day. On the tenth day, the amounts of dead cells in the pith ranged from 0 to 43% in seedlings from the 2.5 cm depth and 8 to 55% in those from the 5.0 cm depth. On the twentieth day after planting, the amounts of pith with dead cells in the first internodes ranged from 92 to 98% in seedlings from the 2.5 cm depth (except Grant, 45%) and 95 to 98% in those from the 5.0 cm depth. The amounts of dead cells in the pith tissue of the second internodes ranged from 0 to 82% in seedlings from the 2.5 cm depth and 25 to 82% in those from the 5.0 cm depth. Generally, death of pith cells began in the lower part of the pith tissue of the internodes after they had grown a few centimeters in length. The slight differences noted in lengths and percentages of pith length with dead cells for Harosoy grown at two planting depths (Table 1) are typical for the 12 varieties studied.

The effects of sub-surface, 2.5, and 5.0 cm planting depths on cell death in stem tissue were most noted for hypocotyl pith. The results with Kent (Table 1) were typical both for hypocotyl and epicotyl pith cell death observed in all six varieties.

For all six varieties, hypocotyl lengths from the sub-soil plantings ranged from 4 to 6 cm on the seventh day and had little additional growth. The percentages of dead cells ranged from 0 to 84% on the seventh day and from 5 to 92% on the fourteenth day (Table 3). When these six varieties were planted at a depth of 2.5 cm, hypocotyls at 7 days after planting ranged in length from 5 to 7 cm with little or no additional growth during the next week. The amount of dead pith parenchyma ranged from 38 to 92% 7 days after planting and 14 to 93% 14 days after planting. The hypocotyls of the seedlings of the six varieties planted at 5.0 cm depths ranged in length from 7 to 12 cm 7 days after planting with little additional growth occurring during the next week. The ranges of cell death percentages for the hypocotyl pith tissue were 88 to 93% on the seventh day and 95 to 97% on the fourteenth day after planting. For all six varieties planted at sub-soil depth, the first internode lengths ranged from 3 to 11 cm 7 days after planting with 0 to 43% of the pith parenchyma cells dead, and 9 to 13 cm 14 days after planting with 80 to 100% of the cells dead. The second internodes began to elongate during the

TABLE 3.—Per cent of the area of pith tissue of hypocotyls composed of dead cells in seedlings of six varieties of soybeans planted at three depths and sampled two times after planting (May 20).

Variety	Depth of Planting (cm)	Per cent of hypocotyl with dead cells; days after planting	
		7	14
Kent	sub-surface	0	52
	2.5	50	76
	5.0	88	95
Lindarin	sub-surface	37	45
	2.5	66	82
	5.0	93	96
Merit	sub-surface	17	5
	2.5	38	14
	5.0	88	96
Norcheif	sub-surface	32	92
	2.5	62	93
	5.0	96	95
Portage	sub-surface	58	58
	2.5	66	46
	5.0	97	97
Wayne	sub-surface	84	58
	2.5	92	90
	5.0	89	97

second week and ranged from 5 to 8 cm in length 14 days after planting with 39 to 60% of the pith parenchyma cells dead. For the six varieties planted at a depth of 2.5 cm, first internodes lengths ranged from 5 to 13 cm 7 days after planting and 7 to 11 cm one week later. The dead cell percentages ranged from 0 to 38% and 84 to 99% on the seventh and fourteenth days, respectively. The second internodes began to elongate after the first sampling and ranged in length from 6 to 9 cm on the fourteenth day with cell death percentages ranging from 39 to 79%. For the six varieties planted at a depth of 5.0 cm, the first internode lengths for the six varieties ranged from 4 to 8 cm on the seventh day with 3 to 29% of the length containing dead cells and 8 to 17 cm on the fourteenth day with 68 to 99% of the lengths containing dead pith cells. The second internodes began to elongate during the second week after planting and, on the fourteenth day, the lengths ranged from 7 to 9 cm with cell death averages ranging from 49 to 74%.

No death of pith cells was observed in nodal areas in any of the experimental groups.

DISCUSSION

The death of parenchyma cells in the pith tissue of soybean occurred in all varieties studied, regardless of maturity class. Dead cells first

appeared in the central rows of pith cells in the below-ground part of the hypocotyl, usually within 5 days after planting. In all three planting groups, dead cells were observed in the pith of the above-ground and below-ground parts of the hypocotyl within two weeks. Hollow areas sometimes formed in the pith of the hypocotyl, especially in areas where increased cortical diameter had occurred. Whether expansion caused cortical cells to die is not known.

Death of pith parenchyma in the first and second internodes appeared after internode elongation began. Death of cells was first observed in the lower part of the internode.

In both planting depth experiments, the patterns for cell death were like those observed for all varieties at one planting depth, death of cells first occurring in the lower part of the internode or hypocotyl (Fig. 2). The death patterns were similar in all varieties studied but the rate of cell death differed between varieties.

In the study of the six varieties at 3 planting depths, it was very clear that the differences in growth and cell death in the first and second internode of any variety was very small. When compared as a group, some differences were noted in growth or cell death trends between varieties, but these too were small. The obvious differences in each variety were the percentages of cell death in pith cells of the hypocotyls of seedlings planted at the three depths, the greatest amount of cell death occurring at the deepest planting depth and the least amount occurring when the seeds were planted just below the soil surface. Varietal differences were noted (Table 3), with cell death percentages in Norcheif showing little difference due to planting depth while great differences occurred in Merit. Since cell death patterns in parenchyma tissue in stalks of corn, sorghum, and sugarcane is correlated with the areas susceptible to stalk rot in those crop plants (Katsanos and Pappelis, 1969; Pappelis and Katsanos, 1965 and 1969), it may be that the patterns of cell death discovered in the stem of soybeans will provide a new approach to the study of the nature of resistance to spread of *Cephalosporium gregatum* Allington and Chamberlain, the causal organism for the brown stem rot disease in soybean.

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Literature Cited

Katsanos, R. A. and A. J. Pappelis. 1969. Relationship of living and dead cells to spread of *Colletotricum graminicola* in sorghum stalk tissue. Phytopathology 59:132-134.

Pappelis, A. J. and R. A. Katsanos. 1965. Spread of *Physalospora tucumanensis* in stalk tissue of sugarcane. Phytopathology 55:807-808.

———. 1969. Ear removal and cell death rate in corn stalk tissue. Phytopathology 59:129-131.

Tribe, H. T. 1955. Studies on the physiology of parasitism. XIX. On the killing of plant cells by enzymes from *Botrytis cinerea* and *Bacterium aroideae*. Ann. Bot. 19:351-369.

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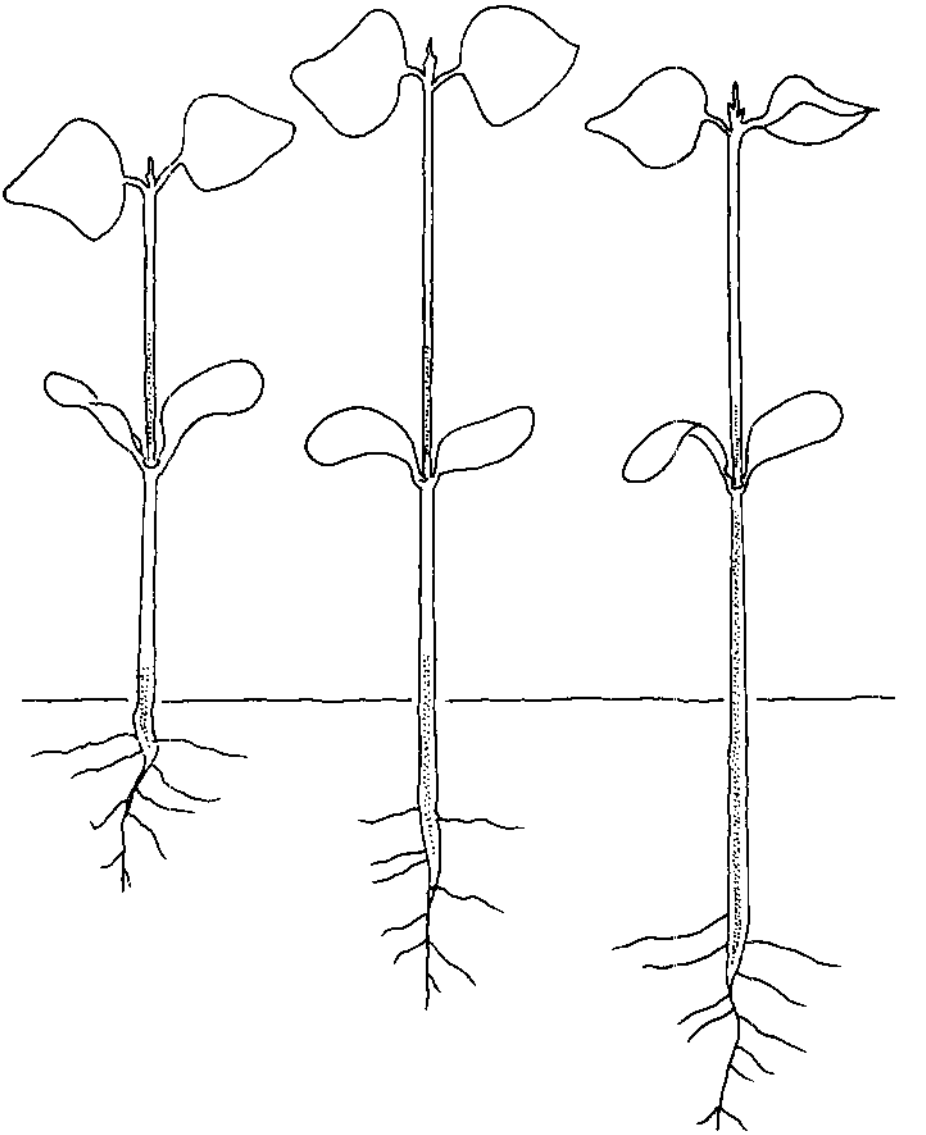

FIGURE 2. Effect of planting depth on the death of cells in pith tissue of soybean seedlings. From left to right, seedlings from sub-surface, 2.5 cm, and 5.0 cm depths, respectively. Dotted areas in the stem represent the areas (averages of six varieties) where dead cells occurred one week after planting (May 20; greenhouse conditions; equal parts sand and peat).