Effect of Root and Leaf Injury on Cell Death and Stalk Rot Susceptibility in Corn

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ABSTRACT

Cell death, stalk rot response following inoculation with *Diplodia zeae* or *Gibberella zeae*, and the incidence of natural basal stalk rot are related. Injuries to roots and leaves increased susceptibility to stalk rot and the rate of cell death, the greatest increases to both being caused by root injury. Inoculation of the stalks did not increase the incidence of natural basal stalk rot. Death of cells in nodal tissue occurs concomitantly with the change from resistance to susceptibility to natural stalk rots and to spread of stalk rot pathogens from the roots to the stalk and from internode to internode within the stalk. Phytopathology 60:355-357.

The literature on stalk rot of corn reviewed by Christensen & Wilcoxson (1) and Koehler (2) indicates that in artificially inoculated stalks of corn, the spread of *Diplodia zeae* (Schw.) Lev. and *Gibberella zeae* (Schw.) Petch is correlated with cell death in the host (5). Hail damage, leaf blight, removal of parts of leaves, insect damage to root or stalk tissue, prevention of pollination, removal of ears, and various diseases have been studied in relation to change in stalk rot response.

This paper tested the hypothesis that changes in stalk rot response and cell death can be induced by injury, using the double inoculation system (3) with the same pathogen inoculated in one plant. Two stalk rot pathogens were used to determine the similarity of their spread in the stalk relative to cell death. The improved pith condition and stalk rot rating systems suggested from earlier work (5) were used to determine the role of cell death in nodal tissue in the spread of stalk rot pathogens in stalk tissue.

Materials and Methods.—In 1961, four corn (*Zea mays* L.) inbreds (B2, C103, 38-11, and Os420) were planted in a split plot design with three replicates on the Southern Illinois University Agronomy Farm. Each plot contained plants 30-cm apart, 13 plants/row, with rows 1-m apart. The planting date was 2 June 1961. The mid-silking dates were: Os420, 2 August; B2, 4 August; C103, 8 August; and 38-11, 9 August. On 21 August, roots were cut with a spade 15 cm in width at a 45-degree angle starting about 8 cm on both sides of the plant to intersect directly below the stalk. The removal of the distal half of leaves and the lower four leaves and sheaths were completed on the same day roots were cut.

On 23 August, plants of control and treated groups were inoculated in the first and fourth elongated internodes above the uppermost brace roots by inserting sterilized oats infested with *D. zeae* or *G. zeae* into holes made with a hand drill. The pathogens were never mixed within the same plant, and the location for each treatment was randomly selected in each plot. Four weeks after inoculation, 10 plants from each treatment were rated for pith condition in noninoculated plants, discoloration following *D. zeae* and *G. zeae* inoculation, and natural basal stalk rot.

The stalk rot rating system used was as follows: 0.5, 0-12% of the inoculated internode discolored; 1, 13-25%; 2, 26-50%; 3, 51-75%; 4, 76-100%; 4.5, like 4, and with the upper node and less than 50% of the adjacent internode discolored; 5, like 4.5, and with more than 50% of the adjacent internode discolored; 5.3, discoloration of three internodes including the inoculated internode; 5.4, discoloration of four internodes; 5.5, discoloration of five or more internodes; and 6, plant dead.

The pith condition rating in noninoculated plants was based on areas of spongy, dry tissue composed of dead cells that appeared white and fluffy in stalks cut through the central axis. The rating system used was as follows: 0.0, no white tissue in the internode; 0.1, less than 1% white; 0.5, 2-12%; 1, 13-25%; 2, 26-50%; 3, 51-75%; 4, 76-100%; 5, white areas in internodes linked by white tissue in the node; and 6, like 5 but no green color in leaves or rind of the stalk.

Natural stalk rot ratings were based on red-brown discoloration of tissue in the crown or basal stalk, the presence of sclerotia of *Macrophomina phaseoli* (Maubl.) Ashby, or both. Separate groups of 10- and 50-plant samples in the control plots were examined in each replicate to determine the reliability of a 10-plant sample.

Results.—Field averages for pith condition ratings based on 10 plants/replicate for the first through fourth internodes on 24 August (about 2-3 weeks after silking) were as follows: C103, 0.0, 0.4, 2.1, 3.1; B2, 0.2, 1.1, 2.5, 3.9; 38-11, 2.3, 3.2, 3.9, 4.0; and Os420, 4.0, 4.0, 4.0, 4.0. The lower internodes of the four inbreds would be rated as follows on that date: C103 and B2, resistant; 38-11, intermediate; and Os420, susceptible. The upper internodes of each of the inbreds would be rated susceptible. The data for pith condition ratings, stalk rot ratings, and natural rot in the plots 4 weeks after inoculation are given in Table 1. At that time, the percentage of natural rot was recorded in 50 additional plants in the control plot of each replicate to determine the reliability of a 10-plant sample/plot. The field averages for these observations are as follows: C103, 15% natural rot, no sclerotia of *M. phaseoli* present; B2, 7%, none with sclerotia; 38-11, 21%, 2% with sclerotia; and Os420, 65% natural

TABLE 1. Field averages[a] pith condition (PC), *Diplodia zeae* (*Dz*) and *Gibberella zeae* (*Gz*) stalk rot ratings, and percentages of natural[b] and charcoal[c] rots of the basal internodes in normal and injured plants on 20 September 1961. Stalk rot ratings are from plants inoculated in both first and fourth internodes. Pith condition ratings are from noninoculated plants

		Stalk Rot %						PC, *Dz*, *Gz* Ratings					
		Natural			Charcoal			First Internode			Fourth Internode		
Var.	Treatment	PC	*Dz*	*Gz*	PC	*Dz*	*Gz*	PC	*Dz*	*Gz*	PC	*Dz*	*Gz*
C103	Control[d]	3	20	10	0	0	3	3.6	3.3	3.1	4.1	3.9	3.9
	Leaf-cut[e]	30	23	23	0	0	0	4.4	3.7	4.6	4.6	3.8	4.5
	Leaf-strip[f]	10	0	7	0	0	0	4.0	3.2	3.5	4.2	4.2	4.1
	Roots cut[g]	90	97	97	27	10	20	5.5	5.9	5.7	5.5	5.8	5.3
B2	Control	3	17	10	0	0	0	3.7	3.4	3.6	4.0	3.8	3.9
	Leaf-cut	47	17	53	7	13	3	4.4	4.1	4.0	4.5	3.9	4.0
	Leaf-strip	23	17	10	3	3	0	3.7	3.3	3.2	4.0	3.8	3.8
	Roots cut	77	83	47	33	13	3	5.3	5.3	5.2	5.3	5.1	5.1
38-11	Control	3	7	17	0	3	0	4.1	4.2	4.0	4.3	4.6	4.8
	Leaf-cut	50	40	43	3	7	3	4.8	4.4	4.1	4.9	4.7	4.8
	Leaf-strip	13	23	13	0	0	3	4.0	4.0	3.9	4.3	4.7	4.8
	Roots cut	70	80	73	17	30	17	5.4	5.4	5.3	5.4	5.4	5.3
Os420	Control	77	60	77	20	10	13	5.4	5.4	5.7	5.4	5.3	5.5
	Leaf-cut	83	80	83	20	6	13	5.4	5.7	5.6	5.5	5.5	5.3
	Leaf-strip	60	83	80	10	7	7	5.2	5.7	5.5	5.2	5.4	5.0
	Roots cut	93	97	97	23	17	17	5.9	6.0	5.9	5.9	5.7	5.9

[a] Average of the three replicate means, 10 plants sampled/replicate for each treatment.
[b] Natural rot means basal stalk rot with symptoms similar to those incited by *G. zeae*, with or without the presence of sclerotia of *M. phaseoli*.
[c] Charcoal rot refers to basal stalk rot in which the sclerotia of *M. phaseoli* were observed. Sclerotia were present only in plants showing symptoms like those incited by *G. zeae* and were never observed alone.
[d] Uninjured plants.
[e] Distal half cut from all leaves.
[f] Lower four leaves and leaf sheaths removed.
[g] Roots were cut with a spade on two sides of the plant.

rot, 6% with sclerotia. These are in good agreement with the 10-plant sample data presented for control plants in Table 1.

In B2, removal of the distal half of each leaf or cutting the roots increased the *D. zeae* and *G. zeae* stalk rot and pith condition ratings in both internodes studied. Spread of the stalk rot pathogens through the nodal tissue occurred most frequently following root cutting. Natural stalk rot increased from 10 to 50% when the distal half of each leaf was removed, and to 70% in the root-cutting treatment. Similar increases in susceptibility due to these injuries were observed in C103, 38-11, and Os420. The increase in natural rot in C103 root-cutting treatment from 10 to 90% was most striking. In Os420, the high amount of natural rot in controls was increased to about 100% by root cutting. In all inbreds, removal of the lower four leaves had little effect on the stalk rot or pith condition ratings.

The pith condition ratings for the first and fourth internodes of plants in control plots for each inbred had increased from 24 August to 20 September. The greatest difference in inbreds was noted when the death of cells in nodal tissue was studied. In B2 and C103, dead cells in nodal tissue were observed in about half of the plants with the distal halves of leaves removed, and in almost all plants in the root-cut plots. For B2, only about 3% of the plants in the control plants and 13% of the plants with lower leaves removed had dead cells in nodal tissue. For C103, about 6% of the control plants and 20% of the plants with lower leaves removed had dead cells in nodal tissue. In 38-11, the percentages of plants per treatment with dead cells in nodes were: control, 30%; distal halves of leaves removed, 60%; lower leaves removed, 20%; and root-cut, 90%. For Os420, the percentages were: control, 90%; distal halves of leaves removed, 100%; lower leaves removed, 90%; and root-cut, 100%.

The pith condition ratings and *D. zeae* and *G. zeae* stalk rot ratings in both internodes of plants in all treatments were highly correlated (*D. zeae*, $r = 0.85$; *G. zeae*, $r = 0.90$). The interrelationship between pith condition ratings, stalk rot ratings, and natural stalk rot percentages was well demonstrated with the first internode data in which the pith condition ratings and per cent natural rot in noninoculated plants in all treatments were highly correlated ($r = 0.96$). The percentage of natural stalk rot occurring in the noninoculated and inoculated plants in all treatments were highly correlated ($r = 0.94$), and demonstrates that inoculation had little or no effect on the susceptibility of the host to natural stalk rot. Both *D. zeae* and *G. zeae* stalk rot ratings were highly correlated with percentage of natural stalk rot ($r = 0.93$). Thus, both the pith condition and stalk rot ratings following inoculation are good indicators of basal stalk rot susceptibility.

This study supports the concept that the same mechanism is involved in the nature of resistance to *D. zeae* and *G. zeae* (5), since ratings for the two diseases are highly correlated ($r = 0.96$). The development of charcoal rot always followed other pathogens, and it is concluded that resistance to charcoal rot also is related to the presence of living cells in host tissue.

DISCUSSION.—Since there was a striking relationship between cell death and stalk rot in this study, it is concluded that the natural increase in susceptibility to *D. zeae, G. zeae,* and possibly other fungal stalk rot pathogens is related to rates of cell death in the corn plant following stalk elongation. Injuries to the plant changed the rate of cell death, and this was accompanied by a similar change in stalk rot response. Root damage resulted in the greatest increase in cell death and stalk rot susceptibility. Removal of the lower four leaves and sheaths resulted in little or no change in cell death rates in stalk tissue, indicating that the increase in the rate of cell death in stalks following root cutting was a result of root damage, and not of the accompanying death of the lower leaves.

New root growth following root injury was greatest in resistant inbreds and least in susceptible inbreds. This agrees with similar observations in later studies (4) where root cutting induced symptoms of stalk rot in the absence of root or stalk rot. In that study, the extent of symptom development in root-cut plants varied, but plants least affected had new root growth past the point of root cutting. This indicated that severe root rot may cause symptoms of stalk rot, and may increase the rate of cell death in the stalk. Basal stalk rot would occur as an extension of the root rot. This is in agreement with the concept presented by Whitney & Mortimore (7, 8), that stalk rot is the natural extension of root rot into the lower part of the stalk after some physiological activity associated with resistance has passed. I conclude from the data of the present study that cell death in nodal tissue is that event. The work of Pappelis & Boone (6) also confirms this conclusion, and extends it to include susceptibility to nodal stalk rot occurring separately from basal stalk rot. Stalk rot pathogens could spread from the rotted roots into the stalk, or from one internode to another when death of cells in the nodal tissue would link areas of dead cells in the root and stalk or in adjacent internodes. The explanation for cell death remains unknown.

Since pith condition ratings can be accomplished without inoculation and without the usual 4-week period to permit disease development, this system would require less time in breeding projects which include stalk rot resistance studies than the inoculation methods commonly used to determine stalk rot resistance. The pith condition rating system also should prove useful as (i) a diagnostic tool in plots with no plants available for inoculation but where stalk rot resistance estimates are desirable after the primary study has been completed; (ii) an estimate of change in stalk rot resistance with time in large experimental plots; and (iii) a tool to estimate stalk rot susceptibility in farm studies conducted by extension agents or commercial representatives where no inoculated plants are available.

LITERATURE CITED

1. CHRISTENSEN, J. J., & R. D. WILCOXSON. 1966. Stalk Rot of Corn. Monograph No. 3, Amer. Phytopathol. Soc. 59 p.
2. KOEHLER, B. 1960. Cornstalk rots in Illinois. Ill. Agr. Exp. Sta. Bull. 658. 90 p.
3. PAPPELIS, A. J. 1962. Double inoculation for corn stalk rot studies. Phytopathology 52:24 (Abstr.).
4. PAPPELIS, A. J. 1963. Corn stalk rot symptoms induced by root injury. Phytopathology 53:624-625 (Abstr.).
5. PAPPELIS, A. J. 1965. Relationship of seasonal changes in pith condition and density to *Gibberella* stalk rot of corn. Phytopathology 55:623-626.
6. PAPPELIS, A. J., & L. V. BOONE. 1966. Effects of planting date on stalk rot susceptibility and cell death in corn. Phytopathology 56:829-831.
7. WHITNEY, N. J., & C. G. MORTIMORE. 1957. Root and stalk rot of field corn in Southwestern Ontario. I. Sequence of infection and incidence of the disease in relation to maturation of inbred lines. Can. J. Plant Sci. 37:342-346.
8. WHITNEY, N. J., & C. G. MORTIMORE. 1961. Root and stalk rot of field corn in southwestern Ontario. II. Development of the disease and isolation of organisms. Can. J. Plant Sci. 41:854-861.