# Relationship Between Phoma Black Stem Severity and Yield Losses in Hybrid Sunflower

M. L. CARSON, Former Associate Professor, Plant Science Department, South Dakota State University, Brookings 57007

**ABSTRACT**

Carson, M. L. 1991. Relationship between Phoma black stem severity and yield losses in hybrid sunflower. Plant Dis. 75:1150-1153.

Field trials were conducted over two growing seasons in South Dakota to determine the effect of artificial inoculation of hybrid sunflower (*Helianthus annuus*) with *Phoma macdonaldii* on seed yield and other agronomic characters. Treatments were a factorial arrangement of inocula (sterile water or $2 \times 10^5$ conidia per milliliter of *P. macdonaldii*) injected into the lower stem and time of inoculation (2 wk before anthesis, anthesis, and 2 wk after anthesis), as well as an uninoculated check. Inoculation with *P. macdonaldii* consistently caused greater internal decay, external symptoms, and premature ripening than did injection with sterile water. Seed yields were not significantly reduced by inoculation with *P. macdonaldii*, but 100-seed weights were significantly reduced when inoculation occurred at anthesis or 2 wk postanthesis. Percent oil content of seeds was slightly increased by inoculation with *P. macdonaldii* at anthesis. Regression analysis indicated that yield was reduced 0.5% and 100-seed weight reduced 0.3% for every 1% increase in premature ripening, and 100-seed weight was reduced 3.7% for each unit increase in severity of external symptoms (0–4 scale). *P. macdonaldii* is capable of causing causing extensive internal stalk decay and premature ripening of sunflower, but seed yield losses are slight. The small losses in seed weight indicate that premature ripening occurred late in the seed-filling stage.

Phoma black stem, caused by *Phoma macdonaldii* Boerema, has been reported as occasionally damaging to sunflower (*Helianthus annuus* L.) in North America (5), but no estimates of yield reductions attributable to *P. macdonaldii* under controlled experimental conditions have been reported. Recent evidence suggests that stem girdling cankers caused by *P. macdonaldii* are the primary cause of the early dying or premature ripening complex of sunflower observed in the northern Great Plains (3). Losses as great as 61% were documented when yields of adjacent healthy and prematurely ripened plants were compared in 13 fields in northeastern South Dakota in 1982 (7). Although there was some association in this study between stalk rot severity and premature ripening, in many instances, stalks of both healthy and prematurely ripened plants were severely rotted.

The purpose of this study was to confirm the role of *P. macdonaldii* in causing premature ripening of sunflower, determine its ability to cause stalk decay, and determine the relationship between disease severity and yield losses in hybrid sunflower. A preliminary report has been published (2).

Present address of author: Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Accepted for publication 29 April 1991 (submitted for electronic processing).



## MATERIALS AND METHODS

An experiment was conducted on the South Dakota State University Plant Science research farm at Brookings, SD, during the 1985 and 1989 growing seasons. Treatments consisted of a factorial arrangement of three times of inoculation (2 wk before anthesis, at anthesis, and 2 wk after anthesis; corresponding to the R1, R5.5, and R6 growth stages [6], respectively) and two inoculation treatments (injection with 2 ml of a $2 \times 10^5$ conidia per milliliter suspension of *P. macdonaldii* or 2 ml of sterile water) and an uninoculated check. Inoculum was prepared by chopping 3- to 4-wk-old cultures of *P. macdonaldii* (grown on potato-dextrose agar) in distilled water, filtering the resulting suspension through a double layer of cheesecloth, and adjusting the conidial concentration with a hemacytometer. Treatments were replicated four times in a randomized complete block design.

Experimental units were two rows of hybrid 894, 6 m long and 0.9 m apart. Thirty seeds were machine-planted in each row and plots were not thinned. Final average plant populations were 43,680 and 38,750 plants per hectare in 1985 and 1989, respectively. Plots were planted on 7 June 1985 and 2 June 1989. Plants were inoculated by injecting stalks 20–30 cm above soil level with a 50-ml Vaco pistol-grip syringe equipped with a custom-designed needle with exit holes on the side to prevent plugging. Needles were rinsed with 70% ethanol between inoculations, and wounds were covered with petrolatum to reduce the accidental introduction of stalk-decaying organisms with the sterile water treatments.

Four weeks after the last inoculation treatment was applied, the percentage of plants prematurely ripened (no green tissue visible) in each plot was recorded. At this stage, healthy, uninoculated plants of hybrid 894 were at the R8 growth stage (6), where the back of the head is completely yellow with some green present on the bracts and physiological maturity is not complete. External and internal symptom severity on individual plants in one row of each plot was also recorded. External symptom severity was visually assessed on a 0–4 rating scale where 0 = no visible symptoms, 1 = small lesion immediately surrounding inoculation site covering <50% of the stalk circumference, 2 = lesion surrounding inoculation site covering >50% of stalk circumference, 3 = lesion completely girdling the stalk, and 4 = stalk exterior completely discolored. Internal stalk decay was assessed visually after cutting plants off 1 m above soil level and splitting stalks longitudinally. A 0–4 rating scale was used where 0 = no decay evident, 1 = some decay immediately surrounding the inoculation site, 2 = spread of decay >5 cm from inoculation site, 3 = extensive decay throughout stalk, some pith remaining intact, and 4 = pith completely disintegrated. The remaining row in each plot was harvested by hand, heads were dried and thrashed, and seed yields and 100-seed weights were determined. Seed oil contents were determined by nuclear magnetic resonance (NMR) analysis on a 10% seed moisture basis.

Analysis of variance was conducted on the combined data from the 1985 and 1989 experiments. Similiar experiments were planted in 1986, 1987, and 1988 but were abandoned because of poor stand establishment and/or downy mildew infection. Covariance analysis was used to adjust 100-seed weight, oil content, external symptoms, and percentage of plants prematurely ripened in 1985, and yield, 100-seed weight, external symptoms, and percentage of plants prematurely ripened in 1989 for variations in stand. In all cases, the adjustments were minor and did not change the outcome of statistical tests. Single degree of freedom contrasts were used to compare sterile water treat-

ments with the uninoculated check, treatments inoculated with *P. macdonaldii* with the uninoculated check, sterile water with *P. macdonaldii* treatments, and individual treatments inoculated with *P. macdonaldii* with sterile water treatments at the same growth stage.

The treatment × year interaction terms in the analyses of variance of external and internal symptom severities were significant and were used as the error term in tests of treatment means over years. Correlation analysis among variables was also conducted. Linear and quadratic regression analyses of yield and 100-seed weight on percent prematurely ripened plants and 100-seed weight on external symptom severity were conducted on individual year data. *F* tests of the models and polynomial terms were made using the error term from the analyses of variance. Estimates of the intercepts from the linear regressions of yield and 100-seed weight on percent plants prematurely ripened and 100-seed weight on external symptom severity in each year were used to estimate the maximum yield and maximum 100-seed weight (yield and 100-seed weight in the absence of disease). Percentage of maximum yield and 100-seed weights were then regressed on percent prematurely ripened plants and percent maximum 100-seed weight on external symptom severity to determine the relationship between disease severity and reduction in yield or seed weight.

## RESULTS

Injection of sunflower stalks with *P. macdonaldii* resulted in significant development of external symptoms (cankers), internal decay of the stalk, and increased the percentage of plants that ripened prematurely when these inoculation treatments were compared with either the uninoculated check or with the sterile water injections (Table 1). Inoculation of stalks with sterile water alone also resulted in an increase in internal stalk decay and premature ripening compared with the uninoculated check. When treatments injected with *P. macdonaldii* applied at a specific growth stage were compared with sterile water-injection treatments applied at the same time, injection with *P. macdonaldii* resulted in increased external symptoms, increased internal stalk decay, and, with the exception of treatments applied 2 wk postanthesis, increased the percentage of plants ripening prematurely.

Although all of the inoculation treatments resulted in lower yields compared with the inoculated check, none of the comparisons were statistically significant. One hundred-seed weights were significantly reduced when compared with either the uninoculated check or the sterile water treatments. When *P. macdonaldii* and sterile water treatments applied at the same time were compared, only inoculation with *P. macdonaldii* 2 wk before anthesis did not significantly reduce seed weight ($P > 0.10$). Oil content of seeds was significantly increased by inoculation with *P. macdonaldii* at anthesis.

Yield was significantly negatively correlated to the percentage of plants prematurely ripened, as was 100-seed weight (Table 2). One hundred-seed weight was also significantly negatively correlated to external symptoms of Phoma black stem. Percent prematurely ripened plants was also positively correlated to both external symptoms and internal stalk decay, which were also highly correlated with each other. The linear regression of percent maximum yield on percentage of plants prematurely ripened was significant in 1985 ($r^2 = 0.104$, $P < 0.10$,), was not significant in 1989 ($r^2 = 0.016$), and was highly significant ($r^2 = 0.173$, $P < 0.01$) when data from both years were combined (Fig. 1A–C). The linear

**Table 1.** Effect of inoculation of sunflower stalks (Hybrid 894) with *Phoma macdonaldii* or sterile water at three different growth stages[a]

| Treatment | Yield (kg/ha) | 100-seed weight (g) | Oil content (%) | External symptoms[b] | Internal symptoms[c] | Prematurely ripened plants (%) |
|---|---|---|---|---|---|---|
| Uninoculated check | 1,707 | 4.81 | 41.5 | 1.5 | 0.8 | 17 |
| Water—preanthesis | 1,686 | 4.71 | 41.6 | 1.0 | 1.5 | 21 |
| Water—anthesis | 1,600 | 5.06 | 40.3 | 1.1 | 1.8 | 16 |
| Water—postanthesis | 1,613 | 4.72 | 41.1 | 2.0 | 2.3 | 29 |
| *Phoma*—preanthesis | 1,638 | 4.58 | 42.2 | 2.8 | 3.1 | 34 |
| *Phoma*—anthesis | 1,580 | 4.67 | 41.6 | 2.7 | 3.0 | 25 |
| *Phoma*—postanthesis | 1,568 | 4.40 | 41.4 | 2.7 | 3.0 | 32 |
| Contrast[d] | | | | | | |
| Water treatment vs. check | NS | NS | NS | NS | ** | + |
| *Phoma* treatment vs. check | NS | + | NS | ** | ** | ** |
| *Phoma* vs. water treatment | NS | ** | + | ** | ** | ** |
| *Phoma* vs. water—preanthesis | NS | NS | NS | ** | ** | ** |
| *Phoma* vs. water—anthesis | NS | * | + | ** | ** | * |
| *Phoma* vs. water—postanthesis | NS | + | NS | * | * | NS |

[a]Data are the means of four replications each of 2 yr at Brookings, SD.
[b]External symptoms rated on a scale of 0–4 where 0 = no visible symptoms and 4 = stalk exterior completely discolored.
[c]Internal symptoms rated on a scale of 0–4 where 0 = no decay evident and 4 = pith completely disintegrated.
[d]NS = Contrast is nonsignificant; + = significant at $P = 0.10$; * = significant at $P = 0.05$; and ** = significant at $P = 0.01$.

**Table 2.** Correlation coefficients[a] among symptoms of Phoma black stem, premature ripening, and agronomic traits of hybrid sunflower

| Trait | Yield | 100-seed wt | Oil content (%) | External symptoms | Internal symptoms | Prematurely ripened (%) |
|---|---|---|---|---|---|---|
| Yield | ··· | 0.56**[b] | 0.30* | −0.18 | −0.17 | −0.37** |
| 100-seed wt | ··· | ··· | 0.20 | −0.31* | −0.17 | −0.58** |
| Oil content (%) | ··· | ··· | ··· | 0.17 | 0.17 | −0.15 |
| External symptoms | ··· | ··· | ··· | ··· | 0.78** | 0.61** |
| Internal symptoms | ··· | ··· | ··· | ··· | ··· | 0.48** |

[a]Data are from individual experimental units over 2 yr at Brookings, SD.
[b]* and ** = Correlation coefficient is significant at the $P = 0.05$ and 0.01 levels, respectively.

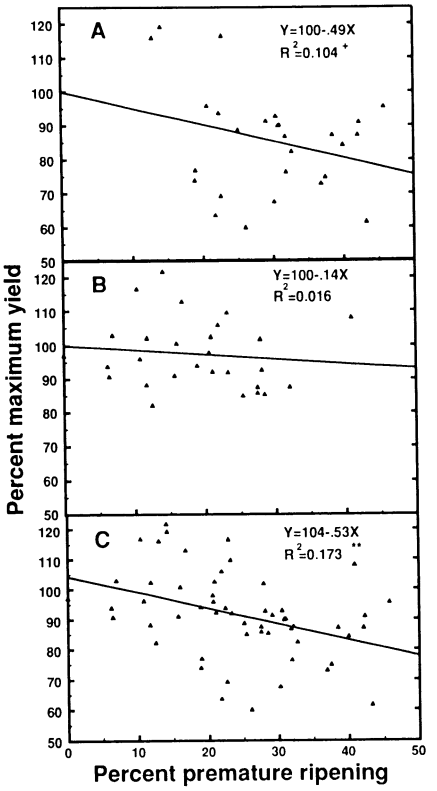


**Fig. 1.** Relationship between percent maximum yield and percent premature ripening of hybrid sunflower in **(A)** 1985, **(B)** 1989, and **(C)** both years combined. +, *, And ** indicate the coefficient of determination ($r^2$) is significant at the 0.10, 0.05, and 0.01 levels of probability, respectively.

regression of percent maximum 100-seed weight on percentage of plants prematurely ripened was not significant in 1985 ($r^2 = 0.096$) but was significant in 1989 and in the combined analysis ($r^2 = 0.121$, $P < 0.10$ and $r^2 = 0.133$, $P < 0.01$, respectively) (Fig. 2A–C). The linear regression of percent maximum 100-seed weight on external symptom severity was not significant in 1985 ($r^2 = 0.054$) but was significant in 1989 ($r^2 = 0.144$, $P < 0.05$) and in the combined data ($r^2 = 0.100$, $P < 0.05$) (Fig. 3A–C).

## DISCUSSION

These experiments demonstrated the ability of *P. macdonaldii* to cause stalk decay and premature ripening of sunflower under field conditions. Injection of stalks with sterile water also resulted in some stalk decay, but it was always less severe than that produced by injection with *P. macdonaldii*. This indicates that efforts to prevent the accidental introduction of stalk-decaying organisms into the stalks during injection with sterile water may not have been completely successful. This was particularly apparent when postanthesis inoculation treatments were compared, as the difference in internal stalk decay was significant only at the $P = 0.10$ level, and the difference in percentage of plants prematurely ripened was not significant. At this growth stage, naturally occurring lesions caused by *P. macdonaldii*, *Alternaria helianthi* (Hansf.) Tubaki & Nishihara, and *A. zinniae* M. B. Ellis were common on the stalk surface and these pathogens could easily have been introduced into the stalk during injection.

The effect of injection of sunflower stalks with *P. macdonaldii* on yield and other agronomic traits was not pronounced. Although yields resulting from inoculation treatments were always lower than that from the uninoculated check, none of the comparisons were statistically significant. One hundred-seed weight, however, was significantly reduced by injection with *P. macdonaldii* at anthesis or 2 wk postanthesis, indicating that seed weight may be a more sensitive indicator of disease damage than yield per se, as was found in another sunflower yield loss study (1). Seed weight is the yield component most likely to be affected by Phoma black stem, because the disease usually does not become evident until after anthesis, by which time head size and seeds per head has been determined. The greater oil content of seeds from plots inoculated with *P. macdonaldii* may have resulted from their reduced seed weight. Generally, there is a negative correlation between sunflower seed weight and oil content (4), although no significant association between these traits was found in this study.

Premature ripening, external symptom severity, and internal stalk decay were all positively correlated with each other, indicating that they are all measures of disease severity. However, internal stalk decay was not significantly correlated with either yield or seed weight and is probably not an important factor in the damage caused by Phoma black stem. A previous study found that outwardly healthy appearing plants often had as much internal stalk rot as adjacent prematurely ripened plants, even though the prematurely ripened plants yielded significantly less (7). Premature ripening and, to a lesser extent, external stem lesions appeared to be the most important factors in the damage caused by Phoma black stem.

None of the regressions of percent maximum yield or percent maximum seed weight on percent prematurely ripened plants or external symptom severity resulted in a particularly good fit, indicating that much of the variability in yield and seed weight could not be attributed to Phoma black stem. The slopes of the regression equations from the combined data indicate a 0.5 and 0.3% reduction in yield and seed weight, respectively, for each percent increase in percent prematurely ripened plants. By extrapolation, these would translate into 50 and 30% losses in yield and seed weight, respectively, if 100% of plants were prematurely ripened. These estimates are in general agreement with those obtained by comparison of yields of adjacent healthy and prematurely ripened plants in production fields (7). The slope of the regression of percent maximum 100-seed weight on external symptom severity indicated a 3.66% loss in seed weight for each unit increase in external symptom severity, or a loss of 15% if the stalk exterior was completely discolored.

Artificial inoculation with *P. macdonaldii* did not always result in premature ripening, because no plots with more than 50% prematurely ripened plants were observed. This supports the concept that premature ripening of sunflower may also be related to environmental stress. There was a weak but significant positive association between percent premature ripening and plant population in both years of the study, indicating that population stress may be a factor in the disease as well. The higher plant population and greater moisture stress in 1985 (total precipitation from June through August was 10.6 cm below normal in 1985, compared with 3 cm above normal in 1989) may partially explain the greater premature ripening seen that season, again suggesting environmental factors could play an important role in disease development.

There is a clear connection between

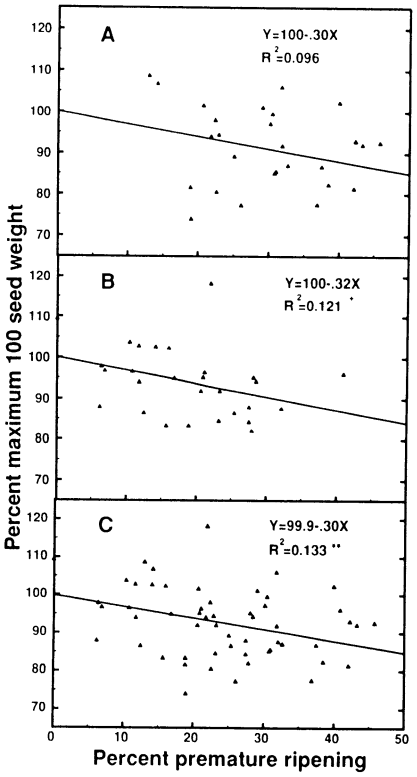


**Fig. 2.** Relationship between percent maximum 100-seed weight and percent premature ripening of hybrid sunflower in **(A)** 1985, **(B)** 1989, and **(C)** both years combined. +, *, And ** indicate the coefficient of determination ($r^2$) is significant at the 0.10, 0.05, and 0.01 levels of probability, respectively.

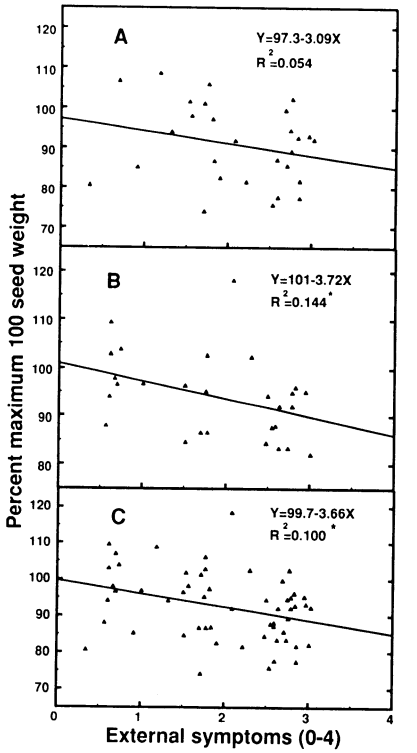


**Fig. 3.** Relationship between percent maximum seed weight and external symptom severity of Phoma black stem in **(A)** 1985, **(B)** 1989, and **(C)** both years combined. +, *, And ** indicate the coefficient of determination ($r^2$) is significant at the 0.10, 0.05, and 0.01 levels of probability, respectively.

Phoma black stem and the premature ripening complex of sunflower in the northern Great Plains of the United States (3). It remains to be established, however, whether infection with *P. macdonaldii* alone is sufficient to cause the disease or if other predisposing environmental factors are also necessary. Stem-boring insects, plant population stress, moisture stress, and defoliation attributable to foliar pathogens may be important factors that need to be examined further. The losses caused by artificial inoculation with *P. macdonaldii* were relatively small, but greater losses could have resulted if 100% premature ripening had occurred earlier during the seed-filling stages. The small reduction in seed weight in this study indicated that losses occurred late in the seed-filling process.

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