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# Root Plate Growth in Sunflower and Its Relevance to Sclerotinia Basal Stalk Rot

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## Abstract

Sclerotinia basal stalk rot (BSR) of sunflower (*Helianthus annuus* L.) is a fungal disease of the roots that causes symptoms of wilt and a basal stem lesion. Evaluating root plate growth could improve our understanding of BSR. Separate studies were conducted to determine the effect of sunflower growth stage or genotype on root plate diameter in North American environments. Root plate diameter of cultivated hybrids at reproductive growth stages was 3 to 4 times larger than vegetative stages. Cultivated hybrids had larger root plate diameter than interspecific lines. These results have implications for artificial inoculation methods that evaluate genotypes for BSR resistance in the field or greenhouse. Disease escapes can occur if field-grown plants are inoculated too far from the root plate and/or too early at vegetative growth stages. Side-dressing mycelium-infested cereal grain closer (*i.e.*, 10 cm) to plants at reproductive growth stages (*i.e.*, R1 - R4) can increase disease pressure and reduce disease escapes. These guidelines for the field can be used to validate results from artificial inoculations in the greenhouse.

## Keywords

Canopy Closure, Inoculation Method, Root Plate Diameter, Sclerotinia Wilt

## 1. Introduction

Sclerotinia basal stalk rot (BSR) or wilt is an economically important root rot disease of cultivated sunflower caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. This pathogen overwinters in soil as dormant sclerotia that can survive for many years [1]. In contrast to carpogenic germination, BSR originates from myceliogenic germination of sclerotia and subsequent infection of roots. The disease is observed around flowering when plants show symptoms of sudden wilt and a water-soaked lesion at the stem base [2]. Plants

with BSR are prone to lodging.

BSR is common in high sunflower production regions in the United States and Canada. Sclerotia-contaminated seed, dense seeding (e.g., solid seeding), poor crop rotation, and broadleaf weed hosts contribute to high disease incidence (60% - 95%) in surveyed fields [3]. An increase in inoculum density (*i.e.*, sclerotia population) will generally cause higher disease incidence [4]. Wilted plants infected from sclerotia are primary infection loci that can initiate secondary spread of disease to adjacent plants via root contact [5].

Since BSR infects the root system, studies evaluating the root plate may improve our understanding of the disease. In trees, the root plate is comprised of thickened lateral roots that provide structural support for aboveground biomass [6]. The root plate is commonly exposed on large trees that have been blown over by strong wind. Large sunflower plants have a similar root plate after root lodging. Soil remains intact on root plates because of high root density in this region, resulting in a sod-forming effect. In Argentina, root plate diameter of lodged sunflower plants was affected by environment, growth stage, genotype, and plant population [7]. One finding was that root plate diameter of two cultivated hybrids decreased as plant population increased from 3 to 16 plants/$m^2$.

Studies are needed to evaluate root plate diameter for sunflower growth stages and genotypes in North American environments, thus expanding on the work of Sposaro *et al.* [7]. However, my objective is to integrate findings on root plate growth with current knowledge on BSR (rather than root lodging). Guidelines will be presented for inoculating plants in the field with mycelium-infested cereal grain to validate BSR resistance of sunflower genotypes.

## 2. Materials and Methods

### 2.1. Environments

Two studies comprised of repeated experiments were conducted across multiple environments in the midwestern states of North Dakota and Minnesota, USA. Environments were assumed to represent a random sample from these states. Four dryland environments in eastern North Dakota had loam ($n$ = 3) or silty clay loam ($n$ = 1) textured soils. Two irrigated (center-pivot) environments with loamy sand and two dryland environments with silty clay loam were in northwestern Minnesota. All eight environments were farmed using conventional tillage practices.

### 2.2. Establishment of Sunflower Plots

Single-row field plots (6 m long) were seeded in late May or early June using a four-row cone plot planter with row spacing of 76 cm. Sunflowers were thinned at the V2 to V4 growth stage [8] to a plant spacing of 25 to 30 cm. Weed control of plots was a combination of preplant incorporated herbicide and manual weeding. Traditional row-crop cultivators and flame weeders were used only if necessary.

### 2.3. Evaluation of Root Plate Diameter

Root plate diameter was evaluated in separate studies for sunflower growth stage or genotype. For each study, a single-factor experiment was repeated at multiple environments. Each experiment used a completely randomized design with sub-sampling. Four plants (sampling units) were vertically uprooted in each plot (experimental unit) and diameter of the root plate was measured. Soil was left intact on roots after uprooting. Diameter was measured perpendicular to the plot row where the plant was growing.

Pre-wetting of soil followed by artificially lodging plants is a suitable method to extract the root plate [7]. Vertical uprooting and lodging seem to be similar extraction methods for large plants. However, vertical uprooting is more suitable for vegetative plants and plants with low vigor. Soil moisture (wet versus dry) at the time of sampling may physically affect root plate diameter if plots are not pre-wetted. However, this specific effect of soil moisture during sampling was considered negligible in these studies.

### 2.4. Growth Stage Study

Root plate diameter was evaluated for three sunflower growth stages at four dryland environments in North Dakota and two irrigated environments in Minnesota. Growth stage levels (three replicates per environment) were V10 to V12, R1 to R2, and R4 to R5. Plots in each environment were planted on the same date with a seed mixture of various oilseed and confection hybrids. Data were collected when plants in assigned plots were at the respective growth stages.

Canopy closure was assessed across all plots by visually estimating percentage of interrow area covered by the sunflower canopy. One value was estimated at each growth stage level for all six environments in the study (18 observations total). Canopy closure of 100% in an environment would indicate a fully closed crop canopy with no ground showing between rows. Similar data in soybean [*Glycine max* (L.) Merr] were obtained by measuring width of the canopy and dividing that value by row width [9]. Direct measurement and visual estimation are both acceptable methods, though the latter will be slightly less accurate and precise.

### 2.5. Genotype Study

Root plate diameter was evaluated on eight sunflower genotypes grown at four environments in Minnesota. Genotype levels (two replicates per environment) included two cultivated hybrids, two interspecific lines, and four inbred lines. Data were collected when plants were at the R8 to R9 growth stage.

In one environment, a representative plant was selected and removed from one replicate plot of each genotype. Eight plants, one for each genotype, were then visually ranked by plant size (*i.e.*, vigor). Sunflower plants were similarly arranged by degree of competition to photograph top growth development in a study by Weaver [10]. The photograph (p. 119) depicts a visual ranking of plants

by size.

### 2.6. Statistical Analysis

For each study, a combined analysis of multiple experiments was conducted using PROC GLIMMIX in SAS [11]. Root plate diameter, assumed to have a Gaussian distribution, was the response variable. Data sets were analyzed using linear mixed models with treatment (growth stage or genotype) as a fixed effect. Random effects were environment, environment × treatment interaction, experimental error (*i.e.*, plot within environment after accounting for effects contributing to explained variation), and sampling error (*i.e.*, plant within plot). Assumptions regarding normality and homogeneity of variance were assessed by examining residuals. Least squares means and standard errors were calculated, and mean separation was conducted using the protected LSD multiple comparison procedure. Tests of treatment effect and differences between means were considered significant at $P < 0.01$.

Canopy closure data were summarized using PROC MEANS in SAS to obtain descriptive statistics for each growth stage treatment level. In addition, Kendall's tau coefficient was calculated using PROC CORR to measure the association between genotype mean root plate diameter and plant size ranking. Kendall's tau is more suitable than Spearman's rho when testing significance of rank correlations for small samples ($n < 10$) [12]. Degree of association was considered significant at $P < 0.01$.

## 3. Results

### 3.1. General Statistical Output

Tests of treatment effect were significant ($P < 0.0001$) in both studies (Table 1).

**Table 1.** Statistical output for two studies evaluating effects of growth stage or genotype on sunflower root plate diameter in eastern North Dakota and northwestern Minnesota, USA.

| Statistical Output[a] | Study | |
|---|---|---|
| | Growth stage | Genotype |
| **Tests of treatment effect** | | |
| *F*-value | 44.0 | 15.0 |
| *P*-value | <0.0001 | <0.0001 |
| **Variance component estimates** | | |
| Treatment effect (F)[b] | 118.0 | 23.9 |
| Environment (R)[c] | 9.6 | 12.5 |
| Environment × treatment (R) | 15.7 | 3.3 |
| Experimental error (R) | −0.2[d] | 4.1 |
| Sampling error (R) | 10.1 | 12.2 |

[a]Estimation Technique: Restricted Maximum Likelihood. [b]F = Fixed effect. [c]R = Random effect. [d]Not set to zero.

Root plate diameter was also affected by environment. In the genotype study, environment accounted for 22% of the total variation in root plate diameter (Table 1). The negative estimate for experimental error in the growth stage study is preferable to setting the estimate to zero. Test statistics are unbiased if this estimate is allowed to be negative [13]. Diagnostic information regarding model assumptions and specific results (e.g., multiple comparisons) are reported for each study.

### 3.2. Growth Stage Study

A plot of residuals versus predicted values revealed unequal variances for the growth stage analysis. Variance of residuals increased as root plate diameter increased. Root plate diameter of vegetative plants varied less than larger plants at reproductive growth stages. However, all six experiments in the combined analysis were balanced. Balanced experiments minimize the effects of unequal variances on the analysis [14]. In addition, transforming the data was not warranted as it did not change conclusions.

Reproductive growth stages (R1 - R2, R4 - R5) had a larger root plate diameter than vegetative growth stages (V10 - V12) (Table 2). The root plate of vegetative plants was small and just starting to develop. As expected, vegetative plants had an open crop canopy (Table 2). Reproductive growth stages had greater canopy closure but also a wider range of values across environments (ranging from droughty to mesic). Results of this study are applicable to interspecific and inbred lines. However, root plate growth rates in most of these lines will probably differ from cultivated hybrids.

### 3.3. Genotype Study

Examination of residuals for the genotype study showed no departures from

Table 2. Mean root plate diameter for three sunflower growth stage levels. Descriptive statistics for canopy closure at each growth stage are provided. Data were from planted mixtures of various hybrids at six environments across eastern North Dakota and northwestern Minnesota, USA.

| Growth Stage | Root Plate Diameter (cm)[a] | Canopy Closure (%)[b,c] | |
|---|---|---|---|
| | | Median | Range |
| V10 - V12 | 6.5 c | 5 | 0 - 25 |
| R1 - R2 | 18.5 b | 50 | 25 - 85 |
| R4 - R5 | 28.4 a | 80 | 45 - 100 |
| **Measure of variability** | | | |
| SEM[d] | 2.08 | | |
| SED[e] | 2.34 | | |

[a]Means followed by the same letter are not significantly different according to protected LSD (0.01). [b]Percentage of interrow area covered by the sunflower canopy. [c]$n$ = 6 observations for each growth stage level. [d]SEM, standard error of the mean. [e]SED, standard error of the difference.

assumptions. Cultivated hybrids had larger root plate diameter than interspecific lines (Table 3). Root plate diameter for inbred lines ranged from large (HA-R3) to small (HA 482). Cultivated hybrids generally had more vigorous aboveground growth than most interspecific and inbred lines. Genotypes with larger plants generally had a larger root plate diameter ($\tau = 0.79$, $P = 0.003$, $n = 8$).

## 4. Discussion

### 4.1. Root Plate Diameter in Sunflower

Root plate diameter is an indirect measurement of root biomass and growth. Diameter and biomass (taproot excluded) of the root plate are positively associated to a degree, based on results reported in Manzur *et al.* [15]. However, biomass can be expected to vary somewhat among root plates of comparable diameter. Morphological differences in roots (e.g., lateral root thickness) exist among sunflower genotypes [16] and may account for this variability.

### 4.2. Environmental Effects on Root Plate Diameter

Under exceptional drought, poor root plate development in dry topsoil has been observed from R1 to R5 growth stage. Root plate diameter is reduced in size and fine roots are sparse under these conditions. Plant survival depends on roots and moisture deep in the soil profile, far beyond the root plate. However, environmental effects on root plate diameter are often influenced by management. Water stress is exacerbated by negative effects of soil compaction on sunflower root

Table 3. Mean root plate diameter for eight sunflower genotypes grown at four environments in northwestern Minnesota, USA. A ranking of these genotypes by plant size (*i.e.*, vigor) at one environment is provided. Data were collected at R8 to R9 growth stage.

| Genotype | Type | Root Plate Diameter (cm)[a] | Plant Size Ranking[b] |
|---|---|---|---|
| HA-R3 | Inbred | 25.3 a | 6 |
| Northrup King 277 | Cultivated hybrid | 21.7 ab | 7 |
| Croplan 343 | Cultivated hybrid | 20.4 abc | 8 |
| RHA 483 | Inbred | 18.1 bcd | 5 |
| HA 288 | Inbred | 15.7 cde | 4 |
| AP ANO | Interspecific | 14.6 de | 3 |
| HA 482 | Inbred | 11.4 e | 2 |
| AP MAX | Interspecific | 10.9 e | 1 |
| **Measure of variability** | | | |
| SEM[c] | | 2.21 | |
| SED[d] | | 1.85 | |

[a]Means followed by the same letter are not significantly different according to protected LSD (0.01). [b]1 = Smallest, 8 = Largest. [c]SEM, standard error of the mean. [d]SED, standard error of the difference.

development [17]. In more mesic environments, early season weed competition during vegetative growth of sunflower can severely reduce plant vigor.

Root plate growth is enhanced by good growing conditions that result in vigorous plants. Interspecific lines AP ANO and AP MAX had vigorous growth at one environment with silty clay loam (4.5% organic matter) that had been fertilized. Despite moderate drought, sunflower growth was not negatively affected because of timely rains during vegetative growth stages. High fertility and abundant soil moisture are favorable for vegetative growth but also BSR [2].

### 4.3. Artificial BSR Inoculations in the Field

BSR inoculation methods developed for the field inoculate either the stem base region (former hypocotyl) [18] [19] [20] or the roots [21] [22] (**Table 4**). Most natural infection, however, occurs through the roots rather than the stem base [2]. These methods differ in timing of inoculation and placement (distance from stem base × depth). Reliability of any method depends on many factors, especially the environment. In field inoculations, BSR is sensitive to inoculum placement and soil moisture. Rate of inoculum is less critical [20].

BSR development under natural conditions has implications for artificial inoculation methods. First, BSR disease incidence is typically low (0 - 8%) during vegetative growth stages [23] [24] [25] when there is minimal contact between roots and sclerotia [26]. Second, most primary sites of sclerotial infection on roots are at the 4- to 6-cm soil depth [5]. Third, plant-to-plant spread of BSR during reproductive growth stages is favored by plants being close (*i.e.*, 10 cm apart) within the row [3] [5] [27]. On the basis of this evidence, most natural infection probably occurs at the root plate.

**Table 4.** Methods for field evaluation of sunflower genotypes for Sclerotinia basal stalk rot resistance. These methods use mycelium-infested cereal grain for inoculum. Guidelines based on temporal and spatial aspects of natural infection are provided in the last row.

| Organ Inoculated | Within-row Plant Spacing (cm) | Inoculation Timing | Placement | | Citation |
|---|---|---|---|---|---|
| | | | Distance[a] (cm) | Depth (cm) | |
| Stem base | 12 - 15 | 40 cm PH[b] | <8 | Shallow | [18] |
| Stem base | 30 | 42 DAP[c] | 2 | 2 - 3 | [19] |
| Stem base | - | 26 DAP | <8 | 2 | [20] |
| Roots | 25 | Early bud GS[d] | 10 | 10 | [21] |
| Roots | - | ≤45 cm PH or V6 GS | 20 - 25 | 8 - 10 | [22] |
| Roots | 25 - 30 | R1 GS | 20 | 8 - 9 | Unpublished |
| Roots | 25 - 30 | R1 - R4 GS | 10 | 5 - 7 | - |

[a]Measured from the stem base. [b]PH, plant height. [c]DAP, days after planting. [d]GS, growth stage.

Disease escapes resulting from insufficient disease pressure are a common issue in many testing environments. Susceptible genotypes cannot be statistically separated from more resistant genotypes when overall disease incidence is low. Inoculating roots by side-dressing 20 cm away from vegetative plants allows the use of mechanized equipment [22]. However, use of this method for inoculating less vigorous genotypes on coarse-textured (*i.e.*, sandy) soils often results in little to no BSR. In addition, high replicate-to-replicate variability of disease incidence within genotypes has been reported [18]. Possible reasons are soil moisture variability (shallow inoculation depth) and/or plant-to-plant spread (close within-row plant spacing).

Artificial BSR inoculations in the field are risky and require strict management to obtain useable data. Increasing the number of replicates per entry may improve statistical separation of genotypes but requires more field space, labor, and inoculum. Larger evaluation nurseries often have more spatial variability (e.g., topography). Runoff from heavy rains in a large nursery increases variability in both soil moisture and BSR disease incidence.

### 4.4. Artificial BSR Inoculations in the Greenhouse

BSR inoculations in the greenhouse eliminate most of the risk discussed previously for field inoculations. Differences in root plate diameter, root morphology, and/or days to maturity among sunflower genotypes are also eliminated. However, the disease is sensitive to temperature [28] [29] and the total plant biomass to pot volume ratio (BVR) [30] in the greenhouse. Maintaining relatively uniform soil moisture among experimental units (pots or trays) after inoculation is important. Excessive watering can be detrimental to mycelia [31] if growing media does not drain adequately.

Effective methods for evaluating sunflower genotypes in the greenhouse have been developed [29] [31] and can be described as high BVR methods. These methods inoculate root-bound plants grown in small pots (e.g., 0.19 L) at the V4 to V6 growth stage (BVR ≥ 2.5 g/L). When root growth is impeded, the outer edge of a pot has a high percentage of the total root biomass [30]. Inoculum placement is adjacent to a region of high root density (*i.e.*, an artificial root plate), leaving little opportunity for plants to escape BSR infection. Susceptible genotypes will usually have 90% to 100% plant mortality at 14 days post-inoculation (DPI) [29] [31].

Sunflower plants grown in larger pots (e.g., 2.45 L) are not root-bound at the V4 to V6 growth stage (BVR < 1 g/L). A low BVR method has been used to inoculate these plants by vertically side-dressing 2 g inoculum along the pot edge (5 cm deep) (unpublished). Under intense disease pressure (21˚C day, 15˚C night), susceptible genotypes have chlorotic leaves and stunted growth (based on plant height). Some plants wilt and die, whereas other plants are still alive at 28 DPI. This method has lower plant mortality and longer time to plant death (16 - 28 days) than high BVR methods. A low BVR method lacks efficiency and is unreliable for evaluation in the greenhouse because plants of susceptible genotypes

can survive the test. However, it may have use in studying plant physiological response to BSR.

Vegetative sunflower plants seem to avoid or at least tolerate BSR when root density is low and lateral root growth is not restricted (in both field and greenhouse). BVR values of about 1 g/L have been estimated for vegetative plants growing in the field [30]. A simple validation test shows that inoculating field plants before R1 growth stage can result in low disease incidence of a susceptible genotype (Table 5).

### 4.5. Validation of Greenhouse Results in the Field

Sunflower genotypes that have high BSR resistance in the greenhouse should be inoculated in the field to validate results [28]. Little information exists for field validation methodology that complements greenhouse evaluations and reduces risk. High disease pressure is needed to statistically confirm the resistance of genotypes (Table 5). Ideally, susceptible checks included in the small-scale field test should attain 70% to 100% mean disease incidence. Analyzing disease incidence data (assumed distribution: binomial) using a generalized linear mixed model that accounts for unit-level variation is recommended [13]. Overdispersion is common with BSR field data if the unit-level (*i.e.*, plot-level) effect is not accounted for in the analysis.

Inoculation methods for BSR in the field need to be based on temporal and

Table 5. Mean disease incidence of Sclerotinia basal stalk rot for two sunflower genotypes inoculated in the field at vegetative or reproductive growth stages. Mycelium-infested millet was side-dressed 10 cm from the plant row (10 g/m).

| Genotype | Disease Incidence (%)[a,b] | |
|---|---|---|
| | Vegetative stage inoculation V8 - V12[c] | Reproductive stage inoculation R1 - R5[d] |
| Hybrid 894 (moderately susceptible) | 10 | 70 |
| Croplan 305 (partially resistant) | 4 | 18 |
| *P*-value | 0.0848 | 0.0260 |
| | 95% CL[e] (lower - upper) | |
| Hybrid 894 | 3 - 27 | 42 - 88 |
| Croplan 305 | 1 - 15 | 6 - 43 |

[a]Percentage of plants within a row showing symptoms of basal stalk rot at R8 to R9 growth stage. [b]Means are from a combined analysis (for each growth stage) of experiments repeated at three random environments (one in North Dakota, two in Minnesota). Experiment design was a completely randomized design. [c]Three replicates of each genotype per environment. [d]Two replicates of each genotype per environment. [e]CL, confidence limits.

spatial aspects of natural infection (Table 4, last row). Recall that disease incidence under natural conditions is low during vegetative growth stages when the root plate is small or absent. Inoculating plants at R1 to R4 growth stage allows more time for root system development, resulting in a larger root plate (*i.e.*, denser roots). In addition, this range of growth stages provides flexibility to schedule timing of inoculation with ideal soil moisture conditions. Precise placement of inoculum into moist soil is preferable to dry soil. High disease pressure can develop in moist, medium- to fine-textured soils when prolonged dry weather occurs after inoculation.

Inoculating closer to plants is effective for reducing disease escapes and is necessary for validating resistance of interspecific and inbred lines. Moving the inoculation distance from 20 to 10 cm has increased ($P < 0.0001$) mean disease incidence by threefold from 13% to 44% (unpublished). This effect of inoculation distance was the same (*i.e.*, no interaction) across four genotypes of varying susceptibility inoculated at reproductive growth stages. For cultivated hybrids at R1 to R2 growth stage, side-dressing 10 cm from plants will place inoculum adjacent to the root plate. Excessive disease pressure has occurred using a 10 cm inoculation distance [21], although this is rare (in my experience) for root inoculations.

## 5. Conclusions

Farming practices that benefit plant vigor and root plate growth are essential to field validation of BSR resistance in sunflower. High soil fertility, control of weeds, and adequate within-row plant spacing (25 - 30 cm) all contribute to a larger root plate. Late cultivation can dry out soil and prune roots [32] and should be avoided if possible. Limiting compaction (controlled traffic) after planting and side-dressing in interrow areas without tracks is important. Nevertheless, good growing conditions are not always guaranteed in dryland environments. Irrigated environments, however, are more reliable for BSR evaluation because root development and subsequent inoculation are not limited by inadequate soil moisture.

## Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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