# Effect of Plant Population and Inoculum Density on Incidence of Sclerotinia Wilt of Sunflower

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Journal Series Paper 1387 of the North Dakota Agricultural Experiment Station.

The authors are grateful to J. Hammond, D. Hertsgaard, and K. Kofoid for advice on statistical analysis. Also, the assistance of G. Fick, T. Gulya, H. Shands, J. Gardner, and K. Erickson is gratefully acknowledged.

Accepted for publication 5 August 1985 (submitted for electronic processing).

## ABSTRACT

Holley, R. C., and Nelson, B. D. 1986. Effect of plant population and inoculum density on incidence of Sclerotinia wilt of sunflower. Phytopathology 76:71-74

Eight field experiments were established at different sites with soils naturally infested with *Sclerotinia sclerotiorum* to study the effect of plant population and inoculum density (ID) on disease incidence (DI) of Sclerotinia wilt of sunflower. Plant populations between 37 to  $74.1 \times 10^3$  plants per hectare were established and replicated at each site and inoculum densities were determined for the upper 16.5-cm of soil. Analysis of covariance indicated no significant difference in DI between plant populations for DI recorded 3 wk after the initiation of anthesis or 17 wk after planting. Inoculum densities for the eight sites ranged from 0.09 to

1.55 germinable sclerotia per 800 cm³ of soil and disease incidence varied from 4.8 to 70.7% and 16.1 to 78.8% at the first and second evaluation dates, respectively. Quadratic regression analysis showed a positive correlation between DI and ID and indicated that as ID increased there was a decreasing effect on DI. Results indicated the importance of inoculum densities of Jess than one sclerotium per 800 cm³ of soil. The inoculum densities of S. sclerotiorum resulting in incidence of Sclerotinia wilt are the lowest reported for a soilborne pathogenic fungus in terms of propagules per unit of soil and a corresponding DI.

Sclerotinia wilt caused by Sclerotinia sclerotiorum (Lib.) de Bary is an important soilborne disease of sunflower in North Dakota. Disease is initiated by sclerotia which germinate myceliogenically and infect the sunflower roots (15,29,30). It is the only crop disease caused by S. sclerotiorum in which root infection consistently occurs; all other diseases are primarily initiated by ascospores or mycelium from sclerotia near the soil surface which infect above ground plant parts (1). First reported in North Dakota in 1948 (2), Sclerotinia wilt is currently widespread in the eastern part of the state (11). The greatest damage from this disease has been the removal of infested fields from sunflower production, thus disrupting rotation schedules and future economic gain from the crop.

At present, crop rotation is the only control for Sclerotinia wilt in North Dakota. There are no resistant hybrids or economical chemical means of control. The goal of crop rotation is to reduce populations of sclerotia to sufficiently low levels that sunflower can be replanted without major yield loss and rapid buildup of inoculum. However, the efficient use of crop rotation depends on information about the relationship between inoculum density (ID) and disease incidence (DI). Such information is lacking for Sclerotinia wilt. Also, plant population could be an important factor in a DI-ID relationship. Huang and Hoes (16) reported that efficiency of plant-to-plant spread by S. sclerotiorum was reduced by decreasing the sunflower population.

The objective of this research was to study the effect of natural inoculum and commercially used plant populations on the incidence of Sclerotinia wilt of sunflowers. A preliminary report on part of this study has been published (14).

## MATERIALS AND METHODS

Eight field experiments were established at four locations in eastern North Dakota and one in western Minnesota in 1981 and 1982. These locations were Carrington, Galesburg, Northwood, and Wyndmere in North Dakota and Moorhead in Minnesota. The sites chosen were in crop fields (naturally infested with S.

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sclerotiorum) that had been planted to susceptible crops within the previous 3 yr. The site designations and respective soil types were as follows: Carrington irrigated and Carrington dryland, Hiemdahl loam; Galesburg east and Galesburg west, Swenoda sandy loam; Northwood south and Northwood north, Gardena silt loam; Wyndmere, Overly silty clay loam; Fargo-Moorhead, Fargo silty clay. All sites were dryland except Carrington irrigated which was furrow irrigated with 23 cm of water.

Each experiment had 12-24 plots, hereafter referred to as experimental units, of various plant populations. An experimental unit consisted of four to six rows, each 6 m long with 76.2 or 91.4 cm (Galesburg east, Galesburg west, and Fargo-Moorhead sites only) row spacing. Plant populations of 37.0, 49.4, 61.7, and  $74.1 \times 10^3$ plants per hectare were replicated four to six times at each site. Sites were planted at the rate of  $98.8 \times 10^3$  plants per hectare and plant populations were established at the four-leaf stage by carefully thinning to the appropriate plant spacing. No disease symptoms occurred prior to thinning. The experimental design was a randomized complete block in 1981 and a completely random design in 1982. Conventional cultural practices for sunflower production were followed in each experiment. Sunflower hybrid 894A (Sigco Sun Research, Breckenridge, MN) was planted at Wyndmere and both Carrington sites and hybrid 894 (a USDA release) was planted at all other sites. These two hybrids do not differ in susceptibility to Sclerotinia wilt (B. D. Nelson, unpublished). Disease incidence in each experimental unit was monitored at 1- to 2-wk intervals from plant emergence to maturity and recorded as the number of wilted plants. Sclerotinia wilt was recognized by wilting of leaves and the presence of a basal stem canker (29). The 2-yr study involved 135 experimental units and  $17.5 \times 10^3$  plants.

The inoculum density of the upper 16.5-cm of soil in each experimental unit was determined from four soil samples taken in a zig-zag pattern with a soil bucket auger (Arts Machine Shop, American Falls, ID) (7.6 cm diam. × 16.5 cm deep, volume = 800 cm³) within 3 wk after planting. Samples were stored at 5 C after collection then air-dried and weighed prior to being processed. Each soil sample was processed separately. Sclerotia were extracted by wet-sieving soil through two nested sieves with openings of 1.4 mm and 2.0 mm, respectively. The debris on each sieve was examined, and sclerotia were removed and air-dried. This extraction method was determined to be highly accurate (98%

recovery) by pretesting with soil containing known numbers of sclerotia.

Sclerotia recovered from each site, up to a maximum of 100, were tested for germinability. Sclerotia were rinsed in cool running water for 30 min, surface sterilized in a 1:1 (v/v) solution of 95% ethanol and 5.25% sodium hypochlorite for 30 sec, and air dried in a laminar flow hood for 5 min. Sclerotia were then split with sterile forceps, placed on potato-dextrose agar blocks containing 0.15 mg

TABLE I. Plant populations of sunflower and incidence of Sclerotinia wilt caused by *Sclerotinia sclerotiorum* at eight different field sites in North Dakota and Minnesota

		Disease incidence (%) <sup>b</sup>		
Year/site	Population (× 10 <sup>3</sup> plants/ha) <sup>a</sup>	3 wk after anthesis <sup>c</sup>	17 wk after planting	
1981				
Galesburg east	37.0	51.2	65.3	
	49.4	40.6	53.7	
	61.7	48.0	61.8	
Galesburg west	37.0	28.1	38.9	
The state of the s	49.4	29.7	41.5	
Northwood south	37.0	35.5	64.8	
	49.4	38.2	64.2	
	61.7	34.2	61.9	
	74.1	38.8	66.9	
Wyndmere	37.0	4.8	14.4	
	49.4	2.5	12.0	
	61.7	7.3	22.0	
1982				
Carrington dryland	37.0	16.3	29.9	
	49.4	10.6	20.0	
	61.7	8.7	16.9	
Carrington irrigated	37.0	36.3	51.6	
	49.4	32.4	49.5	
	61.7	38.9	58.8	
Fargo-Moorhead	37.0	74.6	79.8	
	49.4	74.4	82.4	
	61.7	63.2	74.3	
Northwood/north	37.0	41.3	61.9	
	49.4	38.1	54.5	
	61.7	43.1	67.3	
	74.1	37.4	63.0	

<sup>&</sup>lt;sup>a</sup> Row spacing was 76.2 cm at all sites except the Galesburg east, Galesburg west, and Fargo-Moorhead sites where it was 91.4 cm.

of tetracycline and 0.15 mg of streptomycin per milliliter, incubated at 19 C, and evaluated weekly for 1 mo. Sclerotia that germinated myceliogenically and produced sclerotia were considered germinable. Germinability of sclerotia from each site was expressed as percent germinable sclerotia. When 25 or fewer sclerotia were recovered from a site, we considered the sample size insufficient to accurately test germinability. Therefore, for those sites, germinability was estimated by calculating the mean germinability of all sclerotia recovered in the study. The ID of each experimental unit was calculated as the number of sclerotia recovered × proportion of germinable sclerotia for the site per 800 cm³ soil. Also, an ID for each site was calculated based on the total number of sclerotia recovered from the site × the proportion of germinable sclerotia. Furthermore, ID not adjusted for germinability of sclerotia (i.e., sclerotia recovered per 800 cm³ soil) was calculated for each site.

Disease incidence was analyzed 3 wk following the initiation of anthesis and 17 wk after planting. The two dates were about 5 wk apart. Initiation of anthesis was defined as 50% of the stand in anthesis. These evaluation dates were chosen to assess DI within 4 wk following anthesis when wilt has the greatest effect on seed yield (6) and at the end of the season when DI is at a maximum. Plants that wilt later in the season can also contribute to yield reduction through seed loss from plants lodging due to basal stem and root decay (unpublished). Moreover, all infected plants potentially contribute sclerotia to the soil. When studying the biology of Sclerotinia wilt it is important to consider that DI in the current season may contribute to increased ID in following seasons.

Statistical analysis was performed by using the General Linear Models procedures of the Statistical Analysis System (SAS) (23). Analysis of covariance was performed on data from each experiment to determine if plant population affected DI. The concomitant variable was the ID of experimental units. The relationship between DI and ID was examined with regression analysis. Inoculum density adjusted for germinable sclerotia and ID nonadjusted were regressed against DI. Two regression models with regression lines forced through the origin were fitted to the data: a standard linear equation (y = bx) and a quadratic equation  $(y = ax^2 + bx)$  in which y = DI and x = ID. Standard methods for evaluating model goodness of fit were followed (7).

## RESULTS

Analysis of covariance indicated no significant difference (P = 0.05) in DI at either evaluation date between plant populations in the eight field experiments (Table 1). Disease incidence never varied more than 13% among any two populations at a site for either date; the mean difference was 6.8 and 8.1% for 3 wk after anthesis and 17 wk after planting, respectively.

Since plant populations did not significantly affect DI, the data from experimental units were combined and averaged to obtain the DI for each site. The DI and ID of sites were used in regression analysis. The ID for the eight sites ranged from 0.09 to 1.55

TABLE 2. Soil sampling results, inoculum density, and incidence of Sclerotinia wilt for eight sunflower fields naturally infested with sclerotia of Sclerotinia sclerotiorum

Site	Soil samples <sup>a</sup> (no.)	Sclerotia recovered	Inoculum density per 800 cm <sup>3</sup> soil		Disease incidence (%)	
			Germinable sclerotia	Sclerotia	3 wk after anthesis <sup>b</sup>	17 wk after planting
Carrington dryland	72	8	0.09	0.11	11.9	22.3
Wyndmere	48	6	0.10	0.13	4.8	16.1
Galesburg west	48	12	0.20	0.25	28.9	40.2
Galesburg east	48	25	0.42	0.52	46.6	60.3
Fargo-Moorhead	60	47	0.59	0.78	70.7	78.8
Carrington irrigated	72	86	0.97	1.19	35.8	53.3
Northwood north	96	140	1.15	1.46	40.0	64.2
Northwood south	96	160	1.55	1.67	36.7	64.4

<sup>&</sup>lt;sup>a</sup>One sample = 800 cm<sup>3</sup> of soil.

<sup>&</sup>lt;sup>b</sup> Based on analysis of covariance, no significant differences (P = 0.05) were found between disease incidence of plant populations at any site on either date.

<sup>&</sup>lt;sup>c</sup> Anthesis was defined as 50% of the stand in anthesis.

<sup>&</sup>lt;sup>b</sup>Anthesis was defined as 50% of the stand in anthesis.

germinable sclerotia per 800 cm<sup>3</sup> of soil (Table 2). The number of sclerotia recovered at some sites was extremely low. For example, from the Carrington dryland site only eight sclerotia were recovered from 72 soil samples. Germinability of sclerotia from sites varied from 76 to 93% with a mean of 80.4% for all sclerotia tested. Disease incidence in the eight sites ranged from 4.8 to 70.7% for the first evaluation date and from 16.1 to 78.8% for the later date (Table 2).

A positive correlation between DI and ID was shown at both dates with regression analysis. Quadratic regression models ( $R^2 = 0.90$  to 0.96) fit the data better than linear models ( $R^2 = 0.65$  to 0.81) (Table 3). Coefficients for terms in both equations were significant (P = 0.01). Quadratic regression indicated a curvilinear relationship between DI and ID. Regression results were similar using either ID nonadjusted or adjusted for germinable sclerotia; predictions of DI with these two quadratic regression equations differed by only about 1%.

## DISCUSSION

Huang and Hoes (16) studied the effect of plant spacing on Sclerotinia wilt and concluded that manipulation of plant spacing via plant population could be important in minimizing yield loss. Our study, however, showed no significant effect of plant population on DI in eight field experiments with different inoculum densities and at two row spacings and two evaluation dates. Also, the fact there were no differences in DI between plant populations at any site at both evaluation dates indicated that disease progress was not affected by plant population. The plant spacings tested were within the range studied by Huang and Hoes (16) and the plant populations and row widths were within the commercial range used by growers. We, therefore, believe that manipulation of plant population within the commercial range may not be important in minimizing yield loss from Sclerotinia wilt in North Dakota, because there is no effect on DI. Whether populations lower than  $37 \times 10^3$  plants per hectare (15 × 10<sup>3</sup> plants per acre) could decrease DI is unknown, but if so, they would most likely have no practical use in managing Sclerotinia wilt because of the potential for low yield (24).

The results of this study challenge current concepts regarding the effect of plant population on incidence of disease caused by soilborne plant pathogens. Burdon and Chilvers (4), in a review article, state that host density directly affects DI by changing the number of host plants available to intercept inoculum in a given area and by changing plant spacing which would affect the distance a pathogen must traverse successfully to spread between plants. Their statement is supported by results of a number of studies of soilborne diseases that show increased DI with increasing plant population (3,8,21,26). However, those factors may be less important to the development of Sclerotinia wilt in the plant populations studied because of characteristics of the sunflower root system and the method of pathogen spread. Sunflowers fill the upper 18 cm of soil with dense masses of highly branched lateral roots that form a complete network (28). A dense root system increases the chances of root-sclerotia contact which most likely would favor infection (15). Individual sunflower root systems differ in volume depending on the plant density, but within a unit of soil, the total root volume for different plant populations such as those in this study may not be markedly different (28). Therefore, the lower plant populations may not have reduced the total volume of roots enough to decrease the chances of root-sclerotia contact. Also, the extensive lateral root system spreads parallel to the soil surface and spans the distance between adjacent plants at the populations studied (28, and unpublished). These lateral roots of adjacent plants intermingle (28,29). This is especially important in disease development since S. sclerotiorum is spread within rows via root contact between diseased and adjacent healthy plants (16,29). Successful plant-to-plant spread at all populations studied could be another major reason why plant population did not affect DI.

The eight sites had relatively low ID compared to fields in Canada (13), Nebraska (25), and others in North Dakota (18,22). A large amount of soil was collected and processed from each site to

determine ID. For example  $57.6 \times 10^{5}$  cm<sup>3</sup> of soil (56 kg of air-dried soil) was collected from the Carrington dryland site, but only eight sclerotia were recovered. In some areas of North Dakota where soils infested with *S. sclerotiorum* are common, sunflower growers have expressed an interest in the determination of ID through soil sampling as a basis for disease control decisions. Private crop consultants could provide the service. However, this research indicated that a large number of soil samples might be required to detect low ID, especially in large fields. Perhaps that would limit the usefulness of soil sampling on a commercial scale.

Quadratic regression showed a positive correlation between DI and ID, however, as ID increased, there was a decreasing effect on DI. Other soilborne diseases have similar DI-ID relationships (10,12,19,20). Aggregated horizontal inoculum patterns or multiple infections could be responsible for this phenomena (10). Although the quadratic equations indicated a decrease in DI at ID above one sclerotium per 800 cm<sup>3</sup> of soil, we believe that has no biological significance. There is no evidence DI would decrease under high ID. Further studies are necessary to quantify the relationship between DI and high ID.

The Fargo-Moorhead site had the greatest DI but ranked only fourth highest in ID. The site had been disked twice in the fall and field cultivated twice in the spring prior to planting. That likely resulted in more uniform horizontal distribution of inoculum compared to the other sites with higher ID which had fewer cultivations. A more uniform distribution of inoculum could result in higher DI since more plants would potentially contact sclerotia.

Regression lines were forced through the origin so models would conform to Vanderplank's "law of the origin" (27). Regression also was performed without forcing lines, but they intersected the y-axis above the origin which is biologically impossible. Interpretations, however, from nonforced models were the same as from forced models. Based on the data from 1981, we estimated in 1982 that the Carrington dryland, Carrington irrigated, Fargo-Moorhead, and Northwood north sites would have DIs of 16, 62, 61, and 64%, respectively, at 17 wk after planting. The actual results were 22.3, 53.3, 78.8, and 64.2%, respectively, for those sites: this indicates that a regression model could be useful for predicting the incidence of Sclerotinia wilt.

The results clearly indicated that ID of less than one sclerotium per 800 cm<sup>3</sup> of soil (about 850 g of dried soil) can result in a high incidence of Sclerotinia wilt. The IDs found in this study are very low compared to those reported for other sclerotia-producing

TABLE 3. Linear and quadratic regression analysis of incidence of Sclerotinia wilt of sunflower caused by *Sclerotinia sclerotiorum* against inoculum density (sclerotia per 800 cm<sup>3</sup> of soil)

	Coefficient <sup>b</sup>			
Inoculum density	Linear (x)		$R^2$	
Germinable sclerotia per 800 cm³ of soil Linear				
3 wk after anthesis <sup>c</sup>	39.26	***	0.65	
17 wk after planting	58.79		0.78	
Quadratic				
3 wk after anthesis	124.43	-69.30	0.90	
17 wk after planting	153.68	-77.21	0.94	
Sclerotia per 800 cm <sup>3</sup> of soil Linear				
3 wk after anthesis	34.37	***	0.70	
17 wk after planting	50.99	***	0.81	
Quadratic				
3 wk after anthesis	117.29	-60.13	0.94	
17 wk after planting	140.75	-65.09	0.96	

Linear regression: Y = bx and quadratic regression:  $Y = ax^2 + bx$  in which Y = disease incidence and x = inoculum density. Regression lines were forced through the origin, so intercept terms = 0. Disease incidence was percent wilted plants.

<sup>&</sup>lt;sup>h</sup>Regression coefficients were all significant at P = 0.01.

<sup>&</sup>lt;sup>e</sup>Anthesis was defined as 50% of the stand in anthesis.

soilborne pathogens (5,9,17), Indeed, the IDs of *S. sclerotiorum* important for predicting the DI of Sclerotinia wilt are the lowest reported for a soilborne pathogenic fungus, in terms of propagules per unit of soil and a corresponding DI. For example, the ID of the Carrington dryland site was 0.09 germinable sclerotia per 800 cm<sup>3</sup> of soil, but 22.3% of the plants were diseased at the end of the season. Low ID may result in high DI because the dense lateral root system of sunflower (28) facilitates contact with inoculum and the root contact between plants within a row (28,29) promotes spread of the fungus. Even if few plants are infected by sclerotia, plant-to-plant spread could markedly increase DI (16,29).

The germinability of inoculum is generally assessed in research on ID of soilborne fungal pathogens. However, the low ID of some sites in this study made it difficult to recover sufficient sclerotia to test germinability accurately. Therefore, regression analysis was performed with ID expressed as sclerotia recovered and ID adjusted for germinability. Results of both analyses were similar, indicating that it may be possible to eliminate germinability tests in future studies on ID of Sclerotinia wilt, especially when working with low ID.

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