Importance of Plant Spacing and Sclerotial Position to Development of Sclerotinia Wilt of Sunflower

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ABSTRACT

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Wilt of sunflower in Manitoba was due to infection of underground tissue by *Sclerotinia sclerotiorum*. The taproot-hypocotyl axis was the primary site of infection; lesion development on this tissue resulted in wilting of leaves and sudden death of the plant. The penetration site on the taproot lesion was within the zone of lateral roots. Studies in artificially infested field plots showed that sclerotia were the primary source of inoculum and that their spatial distribution affected the incidence of wilt. Wilt incidence was highest when sclerotia were buried next to seed and decreased with increasing distance between sclerotia and seed. The plant at the site of infestation developed wilt symptoms first and became a primary infection locus (PIL) from which the pathogen spread by root contact from plant to plant in sequential order. Plant spacing affected efficiency and time of spread to adjacent plants and the number of new infections developing from each PIL.

Additional key words: basal stem rot, *Helianthus annuus*, plant density, root rot, *Whetzelinia sclerotiorum*

Head rot and wilt or basal stem rot or root rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary [(=*S. libertiana* Fuckel (20);= *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont (13)] are important diseases of sunflower (*Helianthus annuus* L.) in Manitoba (8) and are limiting factors in production (10). Isolates from sunflower with Sclerotinia wilt in Manitoba (5), Montana (22), and Wisconsin (12) all belonged to the same species. Sunflower production in Manitoba dropped from about 77,000 hectares in 1972 to 8,500 hectares in 1974 (3). This decline was due in part to severe yield losses caused by Sclerotinia head rot and wilt.

Other diseases caused by *S. sclerotiorum* include white mold of bean (7), stalk rot of cruciferous seed crops (15), stalk rot of potato (17), and drop of lettuce (16). All these diseases are due to infection of aboveground parts by airborne ascospores produced from apothecia arising from sclerotia at or near the soil surface. Ascospore infection is conditioned by a long period of wetness and by senescent flower tissue that serves as a food base for the germinating ascospore (1). Ascospores likewise infect aboveground parts of sunflower and cause head rot. This disease is prominent when high moisture conditions exist during late July, August, and September, months when sunflowers are flowering, seed is being produced, and senescing florets are abundant.

In contrast to head rot, Sclerotinia wilt of sunflower may occur at the seedling stage. Most wilt symptoms develop during the flowering and seed development stages, but conditions are dry. Wilt symptoms are first observed in individual plants scattered throughout the field. As the disease progresses, plants are affected in series of two or more. Plants with incipient wilt are characterized by chlorosis and wilting of leaves, often on one side only. Water-soaked lesions occur on the taproot and on some fibrous roots. A severely diseased plant shows "drop" in which all leaves wilt suddenly; a lesion at the stem base is characteristic. The lesion is continuous and extends from the taproot along the hypocotyl to as much as 50 cm along the basal stem. Most fibrous roots are rotted and the cortex has sloughed off. White mycelium interspersed with sclerotia occur on the brown, wet lesion, underground as well as aboveground. Mycelium and sclerotia also occur on the outside of the taproot and the stem base and on the outside of the laterally extending fibrous roots (9). Wilting of leaves, the nature of the lesion, and the presence of large sclerotia and white mycelium are symptoms and signs characteristic of wilt disease (22).

Spread of the pathogen and development of new infections are aspects of wilt disease that are not fully understood. Bisby (4) believed that plants are "usually attacked at or near the soil surface" and noted that the disease occurred mainly in patches, a feature also observed by Jones (12). Transplanted sunflower plants artificially inoculated with mycelium (4) or already wilted and carrying mycelium (22) caused wilt of neighboring plants of sunflower (4) and of hemp (22). Young and Morris (22) concluded that the pathogen penetrated the sunflower mainly through roots and spread rapidly along the row unless the stalks were 30 cm apart. Direct evidence of spread of the pathogen by root contact was not presented, however, and the primary source of inoculum was not emphasized.

This paper describes the pattern of wilt distribution in the field, the site of primary infection, the mode of spread of the pathogen, and the effects of inoculum position and plant spacing on the epidemiology of Sclerotinia wilt of sunflower. The studies were conducted on naturally and artificially infested soils.

MATERIALS AND METHODS

Studies on naturally infested soils. *Pattern of wilt distribution in commercial fields in Manitoba.* In 1972, a field of the cultivar Peredovik at the seed development stage was severely diseased with Sclerotinia wilt. The rows were 90 cm apart, and variable distances separated the within-row sunflowers. A total of 3,231 plants were inspected for wilt. Whether diseased plants occurred singly or within clumps was noted. Single plants were taken as those separated from adjacent ones in the same row by more than 20 cm. A plant was considered belonging to a clump if it was separated from adjacent ones by less than 20 cm. The number of diseased and healthy plants in the same clump was counted, but the sequential order of diseased and healthy plants was not recorded.

In 1977, 2,791 plants of the cultivar Krasnodarets were inspected in a field in which wilt was severe. Row spacing was 90 cm and within-row spacing of plants varied. Series of one or more diseased plants bounded by one or more healthy plants were recorded, and the kind and size of series were noted. The data were analyzed by the "doublet" method of Van Der Plank (19), in which two adjacent diseased plants comprise a doublet, three diseased plants comprise two doublets, four diseased plants comprise three doublets, etc. The expected number N of doublets in a population of n plants of which m are diseased is $\frac{m}{n}$ (m-1), with a standard error of $\sqrt{N}$. Presence of

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significantly more than the theoretical number of doublets was taken to suggest transmission of the pathogen between adjacent plants.

Primary site of infection. The study was conducted on clay loam soil at the Research Station. Samples were taken at the beginning of flowering when only a few scattered sunflower plants showed advanced symptoms of Sclerotinia wilt. The plants were grown in rows 90 cm apart, and row-to-row spread of the pathogen was precluded (22). Twenty-one plants that had incipient wilt and were bordered by healthy plants were dug. Leaves on one side of the selected plants were wilting, and the lesions were underground and typical of those associated with Sclerotinia wilt. The length of the lesion and the distance between its center and the soil surface were measured. The center of the lesion was assumed to be the site of penetration by the pathogen. The depth of the zone along which lateral roots occurred was also recorded. For identification of the pathogen, the taproot along with the hypocotyl of each plant was washed under tap water and incubated in a plastic bag for 7 days at room temperature.

Transmission of the wilt pathogen. At a later date, 64 pairs of wilted plants were selected from the same Research Station field plot. One plant of each pair was severely wilted, while the other showed incipient wilt symptoms only. After the soil was carefully removed to a depth of 20 cm or more, the root systems of the pair were examined in situ for signs of transmission of the pathogen. The distance between the centers of the stems of the two plants was recorded. Five pairs of plants were dug, taken to the laboratory, and processed for identification of the pathogen.

Studies on artificially infested soil. Three experiments using the cultivar Krasnodarets were conducted in a field at the Research Station. The field was considered to be free of *S. sclerotiorum* because a cereal-fallow rotation had been followed for more than 12 yr. Plots were infested with sclerotia harvested from diseased sunflower heads the previous fall and stored in a dry condition at room temperature until use. Seed was planted in freshly dug furrows 5 cm deep and 600 cm long. Rows were 90 cm apart. After inoculation and planting, the furrows were filled and packed.

Effect of plant spacing on wilt development in neighboring plants. Ten sclerotia were placed at each of three sites in each furrow. The sites were centrally located and 150–160 cm apart, depending on the spacings between seeds subsequently planted. Seed was planted just above the inoculum, and planting of a row was completed by spacing seeds at 10, 20, 30, or 40 cm. Each treatment consisted of six rows. Wilt development was monitored weekly. Each wilted plant was marked with a stake and the date of wilt was recorded.

Effect of vertical and horizontal seed-inoculum distance on wilt development. For vertical seed-inoculum distance, seed was sown within the row at 30-cm spacing to preclude most of the plant-to-plant spread of the pathogen (22). Ten sclerotia were buried at each plant site at varying vertical distances from the seed. Treatment consisted of placing sclerotia 4 cm above the seed, at seed level, or 5, 15, or 25 cm below the seed. For horizontal seed-inoculum distance, seed was sown at 60-cm spacing to preclude infection from sclerotia belonging to different plants in the same row. Sclerotia were buried at seed level at varying horizontal distances from each seed site. Treatment consisted of placing one dose of inoculum (five sclerotia) adjacent to the seed or four doses crosswise in four directions 10, 20, or 30 cm from the seed. The study was conducted as a randomized block (single-row plots) with six replicates.

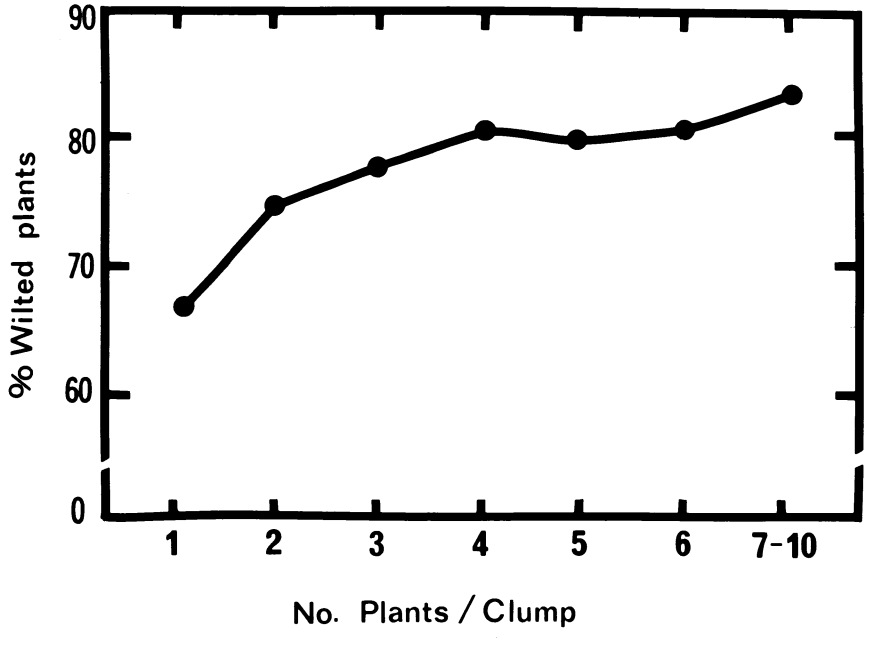

Fig. 1. Effect of plant density on incidence of sunflower wilt caused by *Sclerotinia sclerotiorum*. Data are based on 912 plants occurring singly; the number of plants belonging to clumps varied from 132 in clumps of six plants to 780 in clumps of two.

RESULTS

Wilt distribution in commercial fields in Manitoba. Of the 3,231 plants inspected in 1972, 912 occurred singly and 2,319 occurred in clumps of two to seven or more plants. The number of plants belonging to clumps of two or more and the number of clumps varied from 132 in 22 clumps of six plants to 780 in 390 clumps of two. The incidence of wilt increased from 66% in single plants to 83% in clumps of seven to 10 plants (Fig. 1).

Of the 2,791 plants inspected in 1977, 997 were wilted. Of the diseased plants, 129 occurred singly and 194, 174, 164, 130, 60, 14, 40, 18, and 10 occurred in series of two to 10 plants, respectively; 64 plants occurred in series of 12 to 21 plants. The estimated total number of doublets was 356, with a standard error of 19. However, 622 doublets were actually observed; the 266 more doublets than expected was 14 times the standard error of 19. Because the plant population was large, the difference was considered to be highly significant (19) and suggested that the wilt pathogen had been transmitted from plant to plant.

Primary site of infection. Lesions on taproots of the 21 plants with incipient wilt measured 2–7 cm in length and averaged 4.2 cm. All lesions yielded mycelium and sclerotia characteristic of *S. sclerotiorum* when incubated in the laboratory. The lesion centers or the sites of penetration occurred at soil depths of 1.5–8.5 cm, with the majority occurring at 4–6 cm. The depth of the soil profile containing lateral roots ranged from 5–12 cm and averaged 8 cm. The sites of penetration were thus always within the zone in which lateral roots occurred.

Transmission of the wilt pathogen. Transmission of *S. sclerotiorum* by root contact was evident from the diseased-pair study. In 54 pairs of plants separated by 30 cm or less, wilting of the "receptor" plant with incipient wilt always occurred on the leaves facing the severely infected "donor" plant. The taproots of the 54 receptor plants were characterized, without exception, by a lesion on the same side as the wilting leaves. Lateral roots originating from lesion areas on donor and receptor plants intermingled and showed signs of the wilt pathogen. When plant distance ranged from 31 to 40 cm, however, transmission by root contact of the pathogen was evident in only five of the eight pairs examined. It was not evident in the two pairs with

plant distance of more than 40 cm, indicating that infection arose independently in these pairs. Mycelium and sclerotia characteristic of *S. sclerotiorum* were produced from the lesions on taproots of all 10 plants that had been removed from the field and incubated in plastic bags at room temperature.

Effect of plant spacing on wilt development in neighboring plants. Wilt developed at 37 of the 72 sites where inoculum had been deposited. In each of the 37 cases, the plant at the inoculum site developed wilt first and became a primary infection locus (PIL). In turn, plants adjacent to the PIL developed wilt as a result of the spread of the pathogen. Continued plant-to-plant spread of *S. sclerotiorum* ultimately produced series of wilted plants. The longest series consisted of nine plants and occurred in a row with 10-cm plant spacing. The series was represented as (-8-7-5-4-3-1-2-4-5-), each figure indicating the number of the week during which a plant wilted. Week 1 began on 22 June 1976. The difference between two successive figures in the series indicated the time required by the pathogen to spread from plant to plant and to cause new infections. The example shows that the pathogen spread in two directions and caused wilt of plants in sequential order. The symptom severity during development of a series was always greater in the PIL than in the extreme plants of the series.

Plant spacing affected efficiency and time of spread of the pathogen and thus the number of new infections. The pathogen spread from nine of 12 PIL at the 10-cm spacing but from only one of nine PIL at the 40-cm spacing. The time needed for pathogen spread averaged 1.5 wk at the 10-cm spacing, 2.6 wk at the 20-cm spacing, and 4.3 wk at the 30-cm spacing. Spread at the 40-cm spacing occurred in only one instance and to one plant only. As a result of pathogen spread, about four to five times as many plants were killed at the 10-cm spacing than at the 30-cm spacing. Pathogen spread was negligible at the 40-cm spacing (Table 1).

Effect of vertical and horizontal seed-inoculum distance on wilt development. Proximity of seed and sclerotia markedly affected the incidence of wilt. More than 50% of the plants developed wilt symptoms when sclerotia were buried at seed level near the seed (Tables 2 and 3). Wilt incidence decreased significantly when sclerotia were buried 4 cm above or 5 or 15 cm below the seed (Table 2). Similarly, wilt incidence decreased significantly when seed and sclerotia were separated horizontally by 10 cm or more (Table 3).

DISCUSSION

Data on the distribution of Sclerotinia wilt of sunflower in commercial fields indicated that when more plants were grouped together, more wilt developed. Once a plant was infected, the possibility of other plants in the same clump developing wilt increased, and the number of doublets that developed exceeded expectation significantly. These data suggested that wilt developed as a result of spread of the pathogen and that plant spacing was an important factor in wilt development. The hypothesis was confirmed in studies in artificially infested plots. Wilt developed fastest when the inoculum was closest to the seed. The pathogen then spread from these primary infection loci to adjacent and other neighboring plants in sequential order. The closer the plant spacing, the more efficient the spread of the pathogen, the less time needed for infection, and the more plants that developed wilt. One primary infection locus caused the death of as many as eight neighboring plants spaced at 10 cm in the row. Transmission of the pathogen was minimal at spacings of 30 and 40 cm, confirming the observation of Young and Morris (22). Wide spacing promoted disease escape because of fewer potential infection loci and because of decreased incidence of transmission. Burdon and Chilvers (6) found that varying the plant density of *Lepidium sativum* had an effect on the frequency of primary infection loci of *Pythium irregulare* similar to that produced by varying the density of the inoculum. An analogous situation likely exists in the *Helianthus-Sclerotinia* system.

Sclerotia of *S. sclerotiorum* are the primary source of inoculum, and infection of underground parts results in development of wilt symptoms in sunflower. This was clearly evident from a study in an artificially infested soil. Hyperparasitism (11) by a soilborne microorganism may have been the cause of failure at the 35 sites where wilt did not develop. However, infections were established in plants at the remaining 37 sites where sclerotial inoculum was introduced. The infections were due to mycelium originating from the sclerotia, because conditions were not conducive to carpogenic germination and apothecia were not observed. Sclerotial germination by production of mycelium was apparent in studies in which *S. sclerotiorum* produced secondary sclerotia in soil (7,21) and also on tap water agar (21). Parent sclerotia on agar produced a mass of mycelium that developed into a secondary sclerotium. There was little doubt that the secondary sclerotia produced in soil and on agar had developed by the same process (21). In

Table 1. Effect of plant spacing on time and efficiency of *Sclerotinia sclerotiorum* to spread from primary infection locus[a] (PIL) and cause wilt in sunflower

Variable	Within-row plant spacing (cm)			
	10	20	30	40
Efficiency of spread[b]	9/12	3/7	5/9	1/9
Time (wk) for plant-to-plant spread of pathogen				
Range	1–5	1–5	3–7	...
Mean	1.5	2.6	4.3	4.0
No. wilted plants neighboring PILs				
Range[c]	0–8	0–5	0–2	0–1
Mean[d]	3.3	1.7	0.7	0.1

[a] Primary infection locus = first of usually a series of plants to develop wilt as a result of infection from sclerotia.
[b] No. PIL/total, from which pathogen spread to adjacent plants.
[c] Based on all PIL of each plant spacing.
[d] Based on totals of 40, 12, 6, and 1 plant and on all PIL of respective spacings.

Table 2. Effect of vertical distance between seed and sclerotia of *Sclerotinia sclerotiorum* on incidence of wilt in sunflower[x]

Distance of sclerotia above or below seed[y] (cm)	Wilted plants[z] (%)
4 (above)	18 bc
0 (seed level)	52 a
5 (below)	16 bc
15 (below)	21 b
25 (below)	8 c

[x] Seed, 5 cm deep; rows, 90 cm apart; within-row spacing, 30 cm.
[y] Ten sclerotia per site of infestation.
[z] Data based on 126 plants per treatment; six replicates. Figures followed by same letter do not differ significantly (Duncan's multiple range test, $P = 0.05$).

Table 3. Effect of horizontal distance between seed and sclerotia of *Sclerotinia sclerotiorum* on incidence of wilt in sunflower[x]

Distance between seed and sclerotia[y] (cm)	Wilted plants[z] (%)
0	57 a
10	18 b
20	0 c
30	3 bc

[x] Rows, 90 cm apart; within-row spacing, 60 cm.
[y] Five sclerotia per site, deposited at seed level (5 cm). At 0 cm, one site; at 10, 20, and 30 cm, four sites in crosswise pattern.
[z] Data based on 56–60 plants per treatment; six replicates. Figures followed by same letter do not differ significantly (Duncan's multiple range test, $P = 0.05$).

contrast, Adams and Tate (2) stated that sclerotia of *S. sclerotiorum* germinated occasionally by production of stipes but never by production of mycelium. Marcum et al (14) stated that in California, infection of lettuce by *S. sclerotiorum* almost always occurred at ground level and probably originated from ascospore infection of senescent lower leaves. *S. sclerotiorum* in Manitoba causes head rot due to ascospore infection and wilt due to mycelial infection of underground tissues, including roots. Isolates of *S. sclerotiorum* from different geographic areas may differ physiologically and biologically.

The conclusions from our studies are of practical significance to sunflower production in areas such as Manitoba where wilt caused by *S. sclerotiorum* is a problem. Sunflowers may be sown in rows or solid-seeded. Plant spacing may be close or wide, depending on the rate of seeding, and may be uniform or highly variable, depending on the type and precision of the seeding equipment. Wilt incidence was 95% in a field sown with seed contaminated with 1% sclerotia and in which disease development was highly favored by within-row plant spacings of 10 cm (10). Solid-seeded fields were also severely wilted (10). Potentially high yields could be obtained from a population as low as 37,000 plants/ha, and row spacings ranging from 55 to 95 cm were relatively unimportant to yield (18). Manipulation of population size and plant spacing may be important in minimizing yield losses due to Sclerotinia wilt.

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