

The percentage of plants infected is highly variable — low for 9SC-69(4) and Stepniak 1-2 (11% maximum) and higher for 9SC-2(7) and PAC 1 (37% maximum). The levels of infection for the other cultivars were highly variable in each plot. Hence, from a breeding point of view, we consider it better to remove cultivars which have a high level of infection, even if this only occurs occasionally.

## DISCUSSION AND CONCLUSION

The data reported here does not claim to solve the problem of *S. sclerotiorum* but does provide data on inoculation techniques and variable reactions to the fungus. The following points can be made:

Undoubtedly inoculation techniques had some effect on disease expression as did the time of inoculation and the growth stage of the plant when inoculation occurred. Ascospore discharge occurred over something like a 10 day period, so cultivars with a wide flowering period may have avoided some infection. We might be able to overcome this by inoculating one head several times.

All things being equal, it seems that the best inoculation technique is to use an ascospore suspension with a concentration of  $40 \text{ mm}^{-3}$ . On some occasions, however, it may be useful to use the confetti method (although this wounds the head as we forced the plant microclimate interface) because the confetti can provide a nutritive medium which the fungus requires to develop its saprophytic phase (as reported by several authors).

High temperatures in the bugs may limit disease development and therefore, the success of a particular technique.

The low levels of infection obtained in the various cultivars do not indicate resistance but might be an expression of tolerance with the pathogen producing typical symptoms within a precise microclimate. High levels of infection

indicate that these conditions existed. The expression of tolerance in a given cultivar under field conditions, would then depend on favourable environmental conditions occurring at the same time as the correct growth stage of the plant. Hence, tolerance could only be acquired in appropriate controlled conditions when one could be sure that climatic conditions necessary for infection occurred at the growth stage most conducive to infection.

From these observations it can be seen that further study on cultivar response to infection by *S. sclerotiorum* should be first be carried out with 9SC-69(4) and Stepniak 1-2 and then with PAC 1 and 9SC-2(7).

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## FIELD INOCULATION OF SUNFLOWER FOR *SCLEROTINIA SCLEROTIORUM* BASAL STALK ROT AND VIRULENCE OF ISOLATES FROM VARIOUS HOSTS.

M.K. MANCL and S.E. SHEIN

Northrup King Co., 13410 Research Road, Eden Prairie, MN 55344, U.S.A.

## ABSTRACT

Effective field screening of sunflower (*Helianthus annuus* L.) for resistance to basal stalk rot, caused by *Sclerotinia sclerotiorum*, can be difficult in naturally infested soils because of uneven inoculum distribution. In 1979 and 1980 field trials, an inoculation method was used to achieve uniform field infection. For inoculum, *S. sclerotiorum* was grown on auto-claved oats. The inoculum was placed 2 — 3 cm below the base of 6 week-old plants (6 ml/plant), then covered with soil. In 1979, certain genotypes had a lower percent of infected plants compared with susceptible genotypes. The survivors were selfed, then selfed again in a winter nursery, and the S<sub>2</sub> generation screened in the 1980 field nursery. Progeny from surviving plants had a significantly lower percent of basal stalk rot than susceptible material, indicating that the parental genotypes had some resistance to *S. sclerotiorum*, and that resistance was transferable to the S<sub>2</sub> generation. One isolate each of *S. sclerotiorum* from soybean, carrot, and snapbean, and two from sunflower, were evaluated in the field for virulence on two susceptible and two resistant sunflower genotypes. There were significant differences in virulence between the isolates, based on a percent of plants infected. A sunflower isolate which had been repeatedly subcultured in the lab was significantly less virulent than an isolate recently obtained from sunflower.

## INTRODUCTION

Basal stalk rot of sunflower, caused by *Sclerotinia sclerotiorum*, is a significant problem in many of the sunflower growing regions of North America (Gulya, 1981; Sackston, 1981). Resistance or tolerance in *H. annuus* to basal stalk rot has recently been reported by various workers (Dueck and Campbell, 1978 and Zimmer and Hoes, 1978). In 1979, we evaluated 85 lines for resistance to *S. sclerotiorum* in the field. Surviving plants were self-pollinated, the S<sub>1</sub> progeny self-pollinated in a winter nursery, and the S<sub>2</sub> generation evaluated for reaction to *S. sclerotiorum* in 1980. In addition, we compared isolates of *S. sclerotiorum* from sunflower, soybean, snapbean and carrot for virulence to sunflower genotypes differing in reaction to basal stalk rot. Results of these studies are presented in this paper.

## MATERIALS AND METHODS

To obtain uniform field infection, a method similar to that described by Dueck and Campbell (1978) was used. All isolates of *S. sclerotiorum* were maintained on Difco potato-dextrose-agar (PDA) at 18 — 22°C under low light. The field inoculum was produced as follows. Oat seeds were steeped in hot water for six hours, soaked overnight under room conditions, then drained. The oat medium, in aliquots of 300 gms, was placed in 1 litre canning jars modified with a

35 mm foam stopper in the metal top, then auto-claved twice, 48 hours apart, at 18 psi at 121°C. The jars were seeded with agar plugs of *S. sclerotiorum* on PDA, then incubated in the dark at 18–22°C. After three to four weeks, the infested oats was removed, air-dried, and sealed in plastic boiling pouches for storage.

Field plots were established in 1979 and 1980 at the Northrup King Research Center, Eden Prairie, Minnesota. Trifluralin plus chloramben were preplant applied, and soil incorporated. Plots were single rows on 97 cm centers, with row lengths of 3 m, and 4.6 m, in 1979 and 1980, respectively. Plots were hand thinned to a single plant spacing of 30 cm.

Plots were inoculated 42 days after planting by placing 6 ml of inoculum near the root zone of each plant. Inoculum was placed 2–3 cm below the soil 2 cm away from the stalk, then covered with soil. In 1979, inoculations were done entirely by hand. In 1980, inoculations were done with a jab-type hand planter. Entries were replicated three times in 1979, and five times in 1980.

The effect of isolate source of *S. sclerotiorum* was evaluated in 1980 on two resistant and two susceptible sunflower entries which were chosen on the basis of 1979 results. One isolate each from soybean, snapbean and carrot, and two from sunflower were used. With the exception of one sunflower isolate, the isolates were subcultured no more than five times before inoculating the oat medium. The second sunflower isolate was subcultured seventeen times before being seeded to the oat medium. Each isolate was used to inoculate all four sunflower varieties. There were six replications per treatment. Data for all studies was recorded as the percent of surviving plants at 15, 51 and 71 days after inoculation.

## RESULTS

The inoculum, consisting of mycelium infested oats, and sclerotia, was highly effective in establishing the basal stalk rot phase of *S. sclerotiorum* in the 1979 and 1980 field nurseries. Symptoms appeared within four to six days after inoculation in susceptible genotypes.

In 1979, there were significant differences in survivability among genotypes (Table 1). Genotypes NSB16 and S59 had significantly high survivability than susceptible types such as Commander. The reactions of these lines did not significantly differ from HA61, which Dueck and Campbell (1978) reported to be partially resistant. The F<sub>1</sub> crosses of resistant x susceptible genotypes were not significantly better than the susceptible entries.

**Table 1. Field survival of various sunflower lines after inoculation with *Sclerotinia sclerotiorum* from sunflower, 1979.**

| Line                | Percent survival <sup>a</sup> |       |
|---------------------|-------------------------------|-------|
|                     | Mean                          | Range |
| Commander           | 13                            | 0–25  |
| Sunbred 254         | 33                            | 25–50 |
| NSB16-1 sib         | 53                            | 31–91 |
| S59-1               | 51                            | 33–83 |
| HA 61 3205-3        | 47                            | 25–63 |
| HA 61 437/438-2     | 50                            | 0–80  |
| RW647 x NSB16-1     | 20                            | 0–46  |
| RW637 x S59-1       | 16                            | 11–25 |
| RW637 x S59-1       | 9                             | 0–18  |
| 5H-651-10 x NSB16-1 | 6                             | 0–17  |
| LSD 5%              | 28                            |       |

a Final counts made 50 days after inoculation

**Table 2. Response of various sunflower genotypes after inoculation with *Sclerotinia sclerotiorum* from sunflower, 1980.**

| Line                                                 | Percent survival <sup>a</sup> |        |
|------------------------------------------------------|-------------------------------|--------|
|                                                      | Mean                          | Range  |
| Commander                                            | 16                            | 0–30   |
| RW647                                                | 24                            | 8–50   |
| RW637                                                | 28                            | 9–50   |
| Sunbred 254                                          | 25                            | 0–93   |
| NSB16                                                | 69                            | 31–88  |
| S59-1                                                | 84                            | 57–100 |
| HA 61 – 1 Sib                                        | 46                            | 20–100 |
| ((RW647 x NSB16-1) x NSB16-1 sib-4) – 3 <sup>b</sup> | 58                            | 31–83  |
| ((RW647 x NSB16-1) x NSB16-1 sib-4) – 2 <sup>b</sup> | 23                            | 8–50   |
| (RW647 x NSB16-1) – 1 – 8 <sup>c</sup>               | 75                            | 50–90  |
| (RW647 x NSB16-1) – 1 – 2 <sup>c</sup>               | 28                            | 13–50  |
| (RW637 x S59-1) – 1 – 1 <sup>c</sup>                 | 58                            | 10–90  |
| (RW637 x S59-1) – 1 – 4 <sup>c</sup>                 | 28                            | 20–43  |
| LSD 5%                                               | 26                            |        |

a Final counts made 50 days after inoculation

b S<sub>1</sub> material backcrossed to resistant parent in 1979 disease nursery and selfed in winter nursery

c S<sub>2</sub> material selfed in 1979 disease nursery and again in winter nursery

In 1980, genotypes identified as resistant in 1979, as well as some advanced generations of resistant and resistant x susceptible genotypes were significantly more resistant than the susceptible checks (Table 2).

Based on the 1980 test, there were significant differences in virulence between the isolates (Table 3). In Table 4 the mean survival of S59, NSB16, RW637 and Commander is compared against all isolates. Both of the genotypes considered resistant have significantly higher percent survival than the two susceptible genotypes. Sunflower isolate 002, and the isolate from snapbean, caused significantly less disease than did the isolates from soybean, carrot, and sunflower isolate 001 (Table 5).

**Table 3. Response of sunflower lines to inoculation with *Sclerotinia sclerotiorum* isolates from four different crops, 1980.**

| Line                   | Isolate origin             | Days after inoculation |    |    |
|------------------------|----------------------------|------------------------|----|----|
|                        |                            | 15                     | 51 | 71 |
|                        |                            | Mean percent survival  |    |    |
| Commander <sup>a</sup> | Sunflower 001 <sup>b</sup> | 58                     | 7  | 3  |
| Commander              | Sunflower 002 <sup>b</sup> | 88                     | 58 | 46 |
| Commander              | Snapbean                   | 97                     | 58 | 46 |
| Commander              | Soybean                    | 65                     | 17 | 11 |
| Commander              | Carrot                     | 73                     | 11 | 10 |
| S59                    | Sunflower 001              | 90                     | 50 | 44 |
| S59                    | Sunflower 002              | 99                     | 98 | 98 |
| S59                    | Snapbean                   | 100                    | 96 | 96 |
| S59                    | Soybean                    | 90                     | 61 | 57 |
| S59                    | Carrot                     | 93                     | 80 | 79 |
| NSB16                  | Sunflower 001              | 98                     | 65 | 56 |
| NSB16                  | Sunflower 002              | 100                    | 98 | 89 |
| NSB16                  | Snapbean                   | 99                     | 98 | 93 |
| NSB16                  | Soybean                    | 99                     | 93 | 73 |
| NSB16                  | Carrot                     | 100                    | 86 | 67 |
| RW637                  | Sunflower 001              | 91                     | 30 | 27 |
| RW637                  | Sunflower 002              | 95                     | 72 | 47 |
| RW637                  | Snapbean                   | 99                     | 84 | 69 |
| RW637                  | Soybean                    | 89                     | 47 | 30 |
| RW637                  | Carrot                     | 97                     | 52 | 44 |
| LSD 5%                 |                            | 11                     | 18 | 20 |

<sup>a</sup> Commander and S59 are single-headed types, NSB16 and W637 are branched lines  
<sup>b</sup> Sunflower isolate 001 was recently isolated culture and sunflower isolate 002 was repeatedly subcultured in the lab.

**Table 4. Response of sunflower lines to *Sclerotinia sclerotiorum*,<sup>a</sup> 1980.**

| Line                  | Days after inoculation |    |    |
|-----------------------|------------------------|----|----|
|                       | 15                     | 51 | 71 |
| Mean percent survival |                        |    |    |
| NSB16                 | 99                     | 88 | 76 |
| S59                   | 94                     | 77 | 75 |
| RW637                 | 94                     | 57 | 43 |
| Commander             | 76                     | 30 | 23 |
| LSD 5%                | 5                      | 8  | 9  |

<sup>a</sup> One isolate each from snapbean, soybean and carrot, and two from sunflower.

**Table 5. Virulence of five *Sclerotinia sclerotiorum* isolates on sunflower.<sup>a</sup>**

| Isolate Origin             | Days after inoculation |    |    |
|----------------------------|------------------------|----|----|
|                            | 15                     | 51 | 71 |
| Mean percent survival      |                        |    |    |
| Sunflower 001 <sup>b</sup> | 84                     | 38 | 32 |
| Soybean                    | 86                     | 55 | 43 |
| Carrot                     | 91                     | 57 | 50 |
| Sunflower 002 <sup>b</sup> | 95                     | 81 | 68 |
| Snapbean                   | 99                     | 84 | 76 |
| LSD 5%                     | 5                      | 9  | 10 |

<sup>a</sup> The four sunflower lines used were S59, Commander, NSB16 and W637

<sup>b</sup> Sunflower isolate 002 was subcultured repeatedly on PDA in the lab, whereas sunflower isolate 001 was recently obtained from field sclerotia and subcultured a minimal number of times before using to produce oat inoculum.

## DISCUSSION

Effective field screening of sunflower for resistance to basal stalk rot, caused by *S. sclerotiorum*, may be difficult because it is not always possible or convenient to locate a uniformly infested screening site. Our results and those of Dueck and Campbell (1978), show that field inoculation using laboratory produced *S. sclerotiorum* inoculum is an effective and efficient method for establishing basal stalk rot in the field.

Field tests in 1979 and 1980 showed that lines NSB16 and S59 had some resistance to basal stalk rot, but that this

resistance was not expressed in F<sub>1</sub> crosses with susceptible genotypes. However, it was expressed in certain advanced generations of the parental lines and crosses made with them. Inbred HA61 also showed some resistance, confirming the results of Dueck and Campbell (1978). Although it would appear that resistant inbred lines can be developed, this resistance has not yet been incorporated into a hybrid. Our preliminary results indicate resistance in the lines we crossed is not dominant.

Both NSB16 and S59 are slow to mature, even though their flowering dates are similar to earlier maturing types. Gulya (1980) also reported that resistance was often greater in longer season varieties, and this may be related to resistance, since *S. sclerotiorum* attacks senescing tissue more readily.

The differences in virulence between the isolates on the sunflower genotypes studied, may be due to differences in virulence of *S. sclerotiorum* isolates on different crops, or to the isolates themselves, regardless of source. Environmental factors may also influence virulence. No differential interaction between sunflower genotypes was observed, and ranking of host genotypes remained the same regardless of isolate source. Nevertheless, the use of several isolate sources would be appropriate to minimize the chance of using a single isolate with low virulence. More research on the effect of isolate handling and isolate source would be worthwhile. All *S. sclerotiorum* isolates we studied were virulent on sunflower.

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## CONIOTHYRIUM MINITANS AS A TREATMENT FOR SCLEROTINIA WILT OF SUNFLOWERS.

P. TRUTMANN<sup>1</sup>, P. KEANE<sup>1</sup> and P. MERRIMAN<sup>2</sup>

<sup>1</sup> Botany Dept., La Trobe University.

<sup>2</sup> Dept. of Agriculture, Victoria.

### ABSTRACT

Application of the hyperparasitic fungus *Coniothyrium minitans* for control of wilt in sunflowers caused by *Sclerotinia minor* is under investigation. Laboratory tests have shown that spores of *C. minitans* will germinate, infect and parasitize sclerotia of *S. minor* and *S. sclerotiorum* between 4° and 25°C at high relative humidities. Results also indicated that the levels of *C. minitans*, artificially introduced into field soil, remained high over 2 years at depths of at least 20 cm. Preliminary field tests consistently showed that sclerotia were killed at the soil surface within 1 — 2 months of an autumn application of *C. minitans*. Further field tests in 1981 — 82 are being conducted to determine whether post harvest applications of *C. minitans* may be used to reduce sclerotial populations in soil to levels where disease is insignificant. Spore suspensions in water have been applied to slashed sunflower stubble infected with *S. minor*. The parasitic activity of *C. minitans* in soil and its effect on numbers of sclerotia are being monitored at intervals, and ultimately effects on disease in the subsequent crop of sunflowers will be assessed.

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## THE USE OF HIGH FREQUENCY MICROWAVES ON SCLEROTIA TO CONTROL INOCULUM OF *SCLEROTINIA SCLEROTIORUM*

### UTILISATION DES MICRO-ONDES DE HAUTE FREQUENCE DANS LE CONTROLE DU RESERVOIR D'INOCULUM (SCLEROTES) DE *SCLEROTINIA SCLEROTIORUM*.

Claudine LAMARQUE<sup>1</sup>, R.A. PERNY<sup>2</sup> et M. BERTOUMIE<sup>3</sup>

<sup>1</sup> INRA, Pathologie, Route de Saint-Cyr, 78000 Versailles.

<sup>2</sup> CETIOM, 174, avenue Victor Hugo, 75116 Paris.

<sup>3</sup> ONERA, 2, avenue E. Belin, 31000 Toulouse, France.

### ABSTRACT

In order to germinate and produce apothecia, sclerotes must have remained in soil, having normal biological activity, for 4 months, but they are able to germinate and produce mycelium whenever they are in the vicinity of plant roots. The effect of internal heating by microwave irradiation on these sclerotes was tested under various laboratory conditions. The following changes were observed: (1) treated sclerotia were unable to germinate and form mycelium; (2) treated sclerotia germinated to form mycelium but this mycelium was unable to form new sclerotia; (3) treated sclerotia were unable to produce apothecia after 4 months in soil. The effectiveness of

irradiation depended on whether the sclerotes were soaked.

### RÉSUMÉ

Pour germer sous forme apothécies, les sclérotés doivent rester pendant 4 mois dans un sol en activité biologique normale tandis que tous les sclérotés, initiés ou non à la carpogénèse sont capables de germer sous forme mycélienne dès qu'ils sont en contact avec une racine par exemple. L'effet des micro-ondes qui produit un échauffement interne des sclérotés a été testé au laboratoire sur les