Large scale field evaluations for Sclerotinia stalk rot resistance in cultivated sunflower

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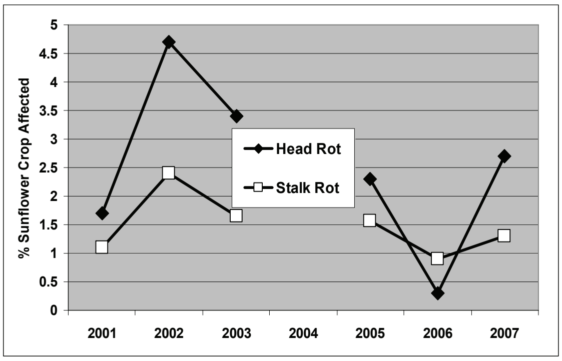
ABSTRACT

An artificial inoculation procedure to incite Sclerotinia basal stalk rot of sunflower was developed that is appropriate for large scale field evaluations. The procedure employs a dried, millet-based mycelial inoculum, without sclerotia. A measured amount of inoculum is deposited in a continuous furrow alongside each row, with ~ 60 g used per 7 m row. Thus, for each 1000 rows in a nursery we use ~ 60 to 70 kg of inoculum, allowing for spillage and inoculum deposited between rows. Preliminary results demonstrated that mycelium produced on oats and millet are equally infective, but the spherical shape of millet seeds facilitates the use of mechanized inoculation equipment. For large scale field inoculations, we modified a granular chemical applicator mounted on a tractor-driven cultivator to deposit uniform amounts of inoculum. The application needs to be made when the sunflower plants are at the V-6 stage or earlier, when the plants are shorter than the tool bar upon which the applicator is mounted. Since the inoculum is deposited in a furrow 20 to 25 cm away from the young plants, the initial symptoms of infection do not appear until 4 to 5 weeks after inoculation, at which time their root systems have contacted the inoculum. Based upon four years of field trials with commercial hybrids, this method has proven capable of producing sufficient and uniform levels of stalk rot, allowing statistical identification of the most resistant hybrids.

Key words: disease testing – *Helianthus* – inoculation methods – *Sclerotinia sclerotiorum* – sunflower.

INTRODUCTION

Basal stalk rot and wilt of sunflower caused by *Sclerotinia sclerotiorum* continues to be one of the major diseases affecting sunflower in North America, along with Sclerotinia head rot (Berglund, 2007; Gulya, 2003, 2004a; Lamey et al., 2002). During the period from 2001 to 2007, the incidence of stalk rot affected fields has ranged from 16 to 35% while head rot in the same period has ranged from 9% to 51% fields affected (Fig. 1). The severity, or percentage of the crop affected, during the same period has ranged from 0.9 to 2.4% for stalk rot and from 0.3 to 4.7% for head rot (Fig. 2). In an effort to develop resistant germplasm and to assess hybrid resistance to both Sclerotinia diseases, we have made an effort to develop artificial inoculation procedures to generate consistent and statistically sound data. While many papers have been published on inoculation procedures for head rot, there is a dearth of information on stalk rot inoculation techniques. We began a study in 2002 to develop a field inoculation method which would produce a reliable level of stalk rot with which to identify sunflower germplasm with improved levels of resistance. This initial study demonstrated the superiority of mycelial inoculum compared to sclerotia (Gulya, 2004b). The next objectives were (1) to adapt this to large scale evaluations encompassing thousands of rows at multiple locations, and (2) verify the method with commercial hybrids tested over several years.



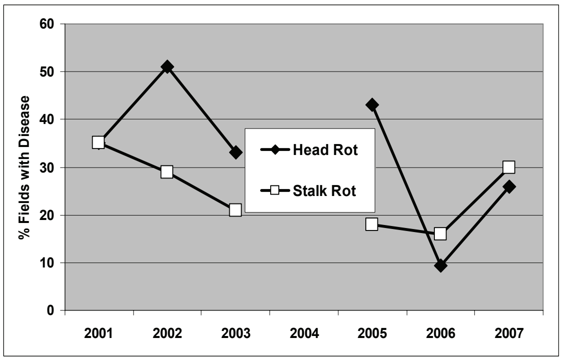


Fig. 1 and 2. Changes in disease severity (% of crop affected, left graph) and disease incidence (% of fields with disease, right graph) for Sclerotinia stalk rot and head rot in North Dakota fields surveyed between 2001 and 2007, using data obtained from the National Sunflower Association annual survey. The survey was not conducted in 2004.

MATERIALS AND METHODS

The inoculation procedure we developed is a version of that initially developed by Mancl and Shein (1982). *Sclerotinia sclerotiorum* inoculum was produced by growing the fungus on autoclaved white proso millet for 7 to 9 days (before any sclerotia developed), drying the inoculum to 10% moisture, and storing it at 4ºC until needed. Using a granular chemical applicator (Gulya et. al, 2005), the inoculum was placed in a furrow ~ 25 cm from each row, about 8 to 10 cm deep. Each 7 m row received ~ 50 to 60 g of inoculum. For each 1000 rows, we used approximately 60 kg. or 135 pounds of millet-based inoculum. Each location was inoculated 5 to 6 wk after planting when the plants were approximately at the V-6 stage, or no more than 45 cm tall. This permitted the use of a tractor-drawn inoculator with minimal damage to the plants. Plots were evaluated for disease incidence at least twice, with the first evaluation in late August (12 to 14 wk after planting and 7 to 9 wk after inoculation), and the second evaluation two weeks later. A plant showing wilt and/or a basal stalk rot lesion was recorded as diseased, and the percent of diseased plants was calculated. Statistical analysis was done using SAS software.

Each year, starting in 2004, U.S. seed companies were asked to submit experimental or commercial hybrids for inclusion in both a stalk rot trial and a head rot trial, the latter which was conducted by personnel from North Dakota State University. Since both diseases are of major concern to U.S. producers, it was felt that information on a hybrid's performance against both diseases was essential. The stalk rot trials have been planted at five locations in eastern North Dakota and northwestern Minnesota each year. Four replications of single row plots, each 7 m long and on 75 cm centers, were planted, starting in late May, with the last location usually planted within three weeks of the earliest planting. A widely grown oilseed hybrid, Cargill 270, was chosen as the long-term susceptible check variety, while the resistant check was a hybrid produced using two USDA inbreds specifically developed for stalk rot resistance (HA 412 x RHA 409). There were six to eight rows of both the resistant and susceptible varieties per replication.

RESULTS

The use of a granular chemical applicator, driven by an electric motor, and mounted on a tractor driven cultivator, allowed us to uniformly deposit *Sclerotinia* mycelial inoculum (grown on millet) beside rows of young sunflowers. Initial symptoms of Sclerotinia wilt do not appear until 5 to 6 wk following inoculation, by which time the roots of the plants had grown and reached the inoculum. By having clear plastic tubes attached to the granular chemical applicator, a person could observe if the millet inoculum was flowing freely and thus minimize the possibility of rows not receiving a uniform dosage.

Each year from 2004 to 2007 some field trials had unforeseen problems, such as flooding, hail storms, extended drought, and downy mildew infestation, that either ruined the plot for Sclerotinia evaluations or made the results statistically insignificant. Thus, the number of stalk rot trials yielding usable information varied from two to four in any given year. In consultation with seed company researchers, a minimum of three statistically sound data sets were considered necessary for the data to be published. In addition to presenting the information annually at the Sunflower Research Workshop (Gulya and Henson, 2006), the stalk rot and head rot ratings are submitted to North Dakota State University which annually publishes a bulletin on sunflower hybrid performance (containing agronomic data as well as disease ratings), available in hard copies and on-line (Berglund and Grady, 2006).

Starting in 2004, when 75 hybrids were tested, we observed that the gradation in disease incidence from the most resistant to the most susceptible hybrids was continuous, as would be expected from a polygenically controlled quantitative trait, which precludes categorizing the entries into discrete groupings such as "resistant" or "susceptible" (Fig. 3). In 2004, there were four hybrids with less infection than the resistant USDA check (21% infection), but there was no statistical difference between them and the check. While confection hybrids are generally considered to have less resistance to most diseases than oilseed hybrids, there were some confection hybrids with reasonably high levels of resistance, as shown by the banded bars in Fig. 3. For complete information on the performance of the hybrids tested, please consult NDSU publication A-652 (Berglund and Grady, 2006) which is revised annually with new data posted in January.

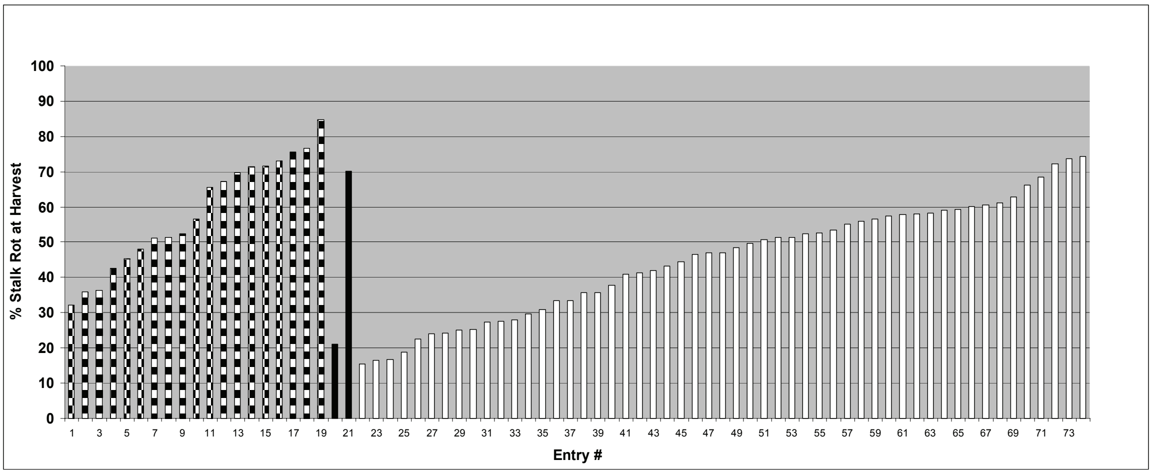


Fig. 3. Histogram of the stalk rot ratings (% diseased plants at physiological maturity) of 75 hybrid entries averaged over two locations in the 2004 tests. The banded bars on the left represent the confection hybrids while the remainder of the entries were oilseed hybrids. The black bars are the resistant check, with 21% infected plants, and the susceptible check, with 70% infected plants.

The stalk rot ratings of hybrids tested in subsequent years followed the pattern observed in the first year, with a continuous range of reaction and no discernible categories. While the range of infection varied from location to location and between years (Table 1), we were able to separate the most susceptible and the most resistant hybrids in each year. In 2007, for example, there were 18 hybrids which had stalk rot levels less than the resistant USDA check, but none of them were statistically different from each other (Fig. 4). Hybrids performing better than the resistant check were tested a second year, at up to five locations. Thus, under ideal conditions a hybrid may have been evaluated at up to 10 locations over two years.

Table 1. Summary statistics for stalk rot evaluations (% diseased plants at maturity) of commercial hybrids in inoculated field trials during 2004 to 2007.

	2004	2005	2006	2007
Number of Hybrids	75	89	97	97
Average % Stalk Rot	48	37	14	27
Minimum	15	10	2	3
Maximum	85	71	42	58
Number of Locations	2	3	4	3
Susceptible Check	70	54	23	35

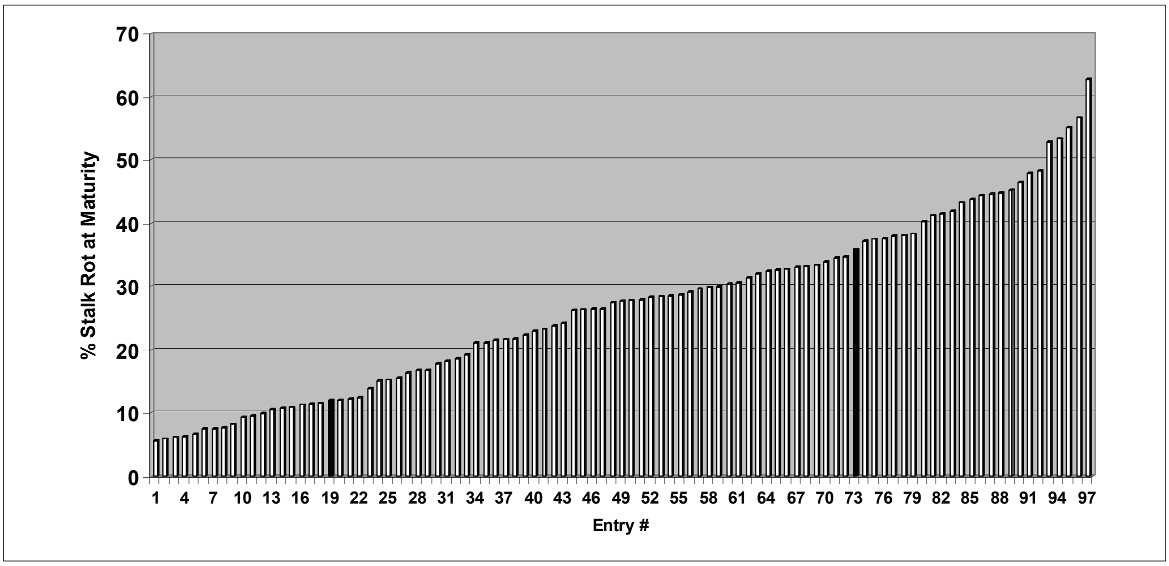


Fig. 4. Histogram of the stalk rot ratings of 97 commercial hybrids entered in the 2007 field trials, averaged over three locations. The black bar at position 19 is the resistant check, with 12% infected plants, and the black bar at position #73 is the susceptible check with 36% infected plants.

DISCUSSION

During the period 1970 to 2000, our USDA Sunflower Unit relied on fields naturally infested with *S. sclerotiorum* to screen sunflower for resistance to stalk rot. The uniformity of disease was often spotty and after repeated crops we often observed declines in disease incidence due to naturally occurring biological control. The field plots were also often located at long distances from our laboratory, all of which contributed to our decision to look for an artificial inoculation technique. Our initial method of placing a measured amount of inoculum beside each plant with a "corn jab planter" was satisfactory, but not practical with large numbers of rows requiring large numbers of people. Thus, the mechanization of the inoculation process not only produced a uniform amount of disease, but allowed us to greatly expand our efforts and test at multiple locations.

The current method of field testing for Sclerotinia stalk rot resistance is also used to evaluate USDA breeding material and other germplasm of interest. For example, the USDA Plant Introduction Collection of cultivated sunflower currently has ~ 800 accessions recently added which do not have stalk rot data listed in the USDA's Germplasm Resource Information Network (GRIN) database (http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?7), so these are currently being evaluated, initially for stalk rot and subsequently for head rot resistance. While this method could be used for evaluating wild *Helianthus*, with the variable phenotype of each species, it may be more appropriate to either inoculate wild species by hand in the field, or in the greenhouse with a technique modeled after Grezes-Besset et al. (1994) and modified by Block et al. (2007, 2008).

We have made minor modifications to our stalk rot evaluation methods over the past four years. For example, to minimize the loss of plants due to downy mildew infection, we treat all seeds with a fungicide mix, regardless if they already have a commercial coating. We use fenamidone and zoximide (Gulya, 2002), at rates of 125 g and 250 g, respectively, which effectively protects against downy mildew with no effect on *Sclerotinia*. We have noted that field plots which do not receive any precipitation for several weeks following inoculation often develop little or no stalk rot. On small plots, this problem could be partially prevented by applying some water at the time of inoculation, or alternately, if drip or furrow irrigation were available, this also would minimize the negative impact of dry soils. We have yet to observe any decline in disease incidence at locations which are reused annually, but we do follow a three-year rotation at these locations. In an attempt to make statistical separation of entries more precise, we are continuing to study ways to improve our evaluations, including increasing the number of replications, modifying the experimental design, and increasing the amount of inoculum.

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