Comparison of Techniques for Inoculating Sunflower Heads with Three Species of *Rhizopus*

S. M. Yang and C. A. Thomas

Conservation and Production Research Laboratory, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture, Bushland, TX 79012; and Plant Protection Institute, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture, Beltsville, MD 20705, respectively.
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ABSTRACT

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Seven methods of inoculating sunflower (*Helianthus annuus*) heads with *Rhizopus arrhizus, R. oryzae,* and *R. stolonifer* were compared. The insertion of a wheat-grain culture of *Rhizopus* into the sunflower receptacle with forceps consistently produced severe head rot in susceptible hybrids grown either in the field or in the greenhouse. Six alternate methods of inoculation either did not produce rot on all susceptible hybrids, or when rot developed following inoculation by these methods it was not always severe. The results of this study indicate that the insertion inoculation technique is suitable for mass screening of sunflowers for resistance to *Rhizopus* head rot.

Rhizopus head rot has become an important disease of sunflower (*Helianthus annuus* L.) in the USA (5,7). The disease has been reported from sunflower in India (1,6), Israel (2), and the Soviet Union (3). The species of *Rhizopus* that cause head rot of cultivated sunflower are *R. arrhizus* Fischer (1–3,10,11), *R. oryzae* Went & Prinsen-Geerligs (5,7), and *R. stolonifer* (Ehrenb. ex Fr.) Vuill (3,10,11). Various inoculation techniques have been used (2,5,6,10) to verify the pathogenicity of *Rhizopus* species in cultivated sunflower.

The development of resistant sunflower cultivars is a desirable way to reduce losses caused by *Rhizopus* species. Therefore, efforts are being made to identify sunflower germplasm that is resistant to *Rhizopus* head rot. Yang and Thomas (8) developed a dependable insertion-inoculation technique for testing the susceptibility of greenhouse-grown sunflower to *Rhizopus* spp. This paper reports the results of 2 yr of additional testing of the insertion-inoculation technique in both the greenhouse and the field, and compares the efficacy of this technique with six alternate methods of inoculating sunflower.

MATERIALS AND METHODS

Sunflower hybrids 894 and 896, both susceptible to *Rhizopus* spp. were grown in 30-cm pots (two plants/pot) in the greenhouse. Supplementary incandescent (1978/1979) and fluorescent lighting (1979/1980) was provided from 0600 to 2000 hours. Minimum night and maximum day temperatures during greenhouse inoculations were 16 and 30 C, respectively (the mean temperature for the first 3 days of incubation was about 24 C). Field inoculations were made on 10 July and 29 August 1979, with a minimum night and maximum day temperature of 13 and 36 C, respectively (the mean temperature for the first 3 days of incubation was about 25 C).

Three species of *Rhizopus* were used for the study. *Rhizopus arrhizus* and *R. stolonifer* were isolated from sunflower heads collected in Texas, and *R. oryzae* was supplied by J. M. Klisiewicz, USDA, Davis, CA.

Pathogens for the insertion inoculation were grown on wheat-grain medium (8). Twenty g of wheat grains were soaked overnight in 300 ml of tap water. The water was then decanted, and the grains were washed twice with tap water. After 60 ml of tap water had been added, the flask was plugged with cotton and autoclaved for 2 hr at 121 C on 2 consecutive days. In the second year, the initial weight of wheat grains was reduced to 5 g and the amount of tap water added after washing was reduced to 15 ml. The sterile wheat-grain medium was seeded with one or two blocks of agar containing mycelium (from 18- to 24-hr cultures on potato-dextrose agar [PDA]) and incubated at 20 to 25 C from 2 to 10 days.

Spore suspensions (10^5 to 10^7 spores/ml) were prepared from a 10- to 14-day-old culture on PDA in sterile distilled water. Cylinders of agar with mycelium were cut from an 18- to 24-hr-old culture on PDA with a cork borer (5 mm in diameter). Sunflower moth larvae were provided by C. E. Rogers, USDA, Bushland, TX.

The inoculation methods studied in the greenhouse (Table 1) included: insertion inoculation—wheat-grain cultures were removed from the flask, cut into small pieces (about 0.2 g) and inserted with forceps to a depth of approximately 5 mm into the receptacle; atomizer spraying—atomization of sunflower heads with *Rhizopus* spore suspensions (5 ml/head); cork-borer-plunge-mycelium and cork-borer-plunge-spore—removal of a plug of tissue (approximately 5 mm in depth) from a sunflower receptacle by a cork borer (5 mm in diameter) followed by introduction of an agar block with mycelium or of spore suspensions (0.5 ml/head) into the cavity, and then replacement of the tissue plug; larvae-atomizer spraying—atomization with spore suspension (5 ml/head) onto sunflower heads that had been infested for 1 wk with larvae (first to third instar, 10 larvae/head) of the sunflower moth (*Homoeosoma electellum* Hulst.) (the heads were covered with plastic bags immediately after the larvae were released on the flower, and the bags were left until the end of test); scalpel injury—injury of the receptacle with a scalpel that had been dipped in spore suspensions between cuttings; and syringe injection—injection of spore suspension (1 ml/head) into subepidermal tissue of receptacle (3 to 5 mm deep) by syringe. The efficacy of insertion inoculation and scalpel injury inoculation techniques was also compared in field tests.

Inoculations were made during the full-bloom stage, when head diameter ranged from 5 to 13 cm in the greenhouse and from 5 to 15 cm in the field. The inoculation site on the receptacle was near the bottom of the bracts. Heads in the greenhouse were covered with plastic bags for 3 days after inoculation except those heads infested with sunflower moth and inoculation with *Rhizopus*. Heads inoculated in the field were covered with paper bags for the duration of the tests to prevent injury by insects and birds. In the

TABLE 2. Development of head rot in sunflower hybrid 894 in the field 14 days after inoculation with three *Rhizopus* species by insertion-inoculation and scalpel-injury inoculation techniques[a]

Rhizopus species	Inoculation method	Percentage of plants in rot rating class[b]: 0	1	2	3	4	5
R. arrhizus	Insertion	0	0	0	0	0	100
	Scalpel injury	57	27	0	0	0	16
R. oryzae	Insertion	0	0	0	0	0	100
	Scalpel injury	66	9	0	0	0	25
R. stolonifer	Insertion	0	66	17	0	5	12
	Scalpel injury	87	10	0	0	0	3
Control	Insertion	100	0	0	0	0	0
	Scalpel injury	100	0	0	0	0	0

[a]Average percentage of head rot for two tests (30 heads per treatment in each test).

[b]Rot rating classes: 0 = no rot; 1 = rot limited to the site of inoculation injuries; 2 = rot greater than the site of inoculation but less than one fourth of the receptacle; 3 = rot greater than one fourth but less than one half of the receptacle; 4 = rot greater than one half but less than three fourths of the receptacle; and 5 = rot encircling the peduncle or greater than three fourths of the receptacle.

of the smooth and elastic tissue of the receptacle; therefore, cork-borer inoculation methods are not suitable for large-scale inoculation.

Infestation of sunflower heads in the field by larvae of the sunflower moth has been reported to cause increased incidence of Rhizopus head rot (4,7). In the greenhouse, exposure of sunflower heads to first-to-third instar larvae of the sunflower moth for 1 wk before inoculation did not increase the incidence of rot (Table 1). This inoculation method was time-consuming and did not produce 100% infection.

Atomizing spore suspensions onto heads caused 20–30% infection of hybrid 896 in the first-year tests, but no infection of hybrid 894 in the second-year tests. The scalpel-injury and syringe-injection inoculations produced neither 100% infection nor severe rot on all infected heads. Injection of inoculum was difficult, because the needle often became plugged by tissue as it was forced into the receptacle.

In the field, insertion inoculation produced 100% infection by *Rhizopus arrhizus, R. oryzae,* and *R. stolonifer* but the scalpel-injury method resulted in only 13–34% infection (Table 2). The relative severity of head rot for sunflower grown in the field and inoculated by the insertion-inoculation technique was similar to that of greenhouse-grown sunflowers inoculated with the three species of *Rhizopus*. Thus, the field inoculation results also indicated that the insertion technique was better than the scalpel-injury technique for inoculating sunflower.

The insertion-inoculation technique has been used successfully to evaluate 32 *Helianthus* spp. and to reevaluate 27 interspecific hybrids selected from *H. annuus* X *H. petiolaris* for resistance to *Rhizopus* head rot in the field (9). Four *Helianthus* spp. were slightly infected by *R. arrhizus* and *R. oryzae* (9). The 27 interspecific hybrids were resistant to *R. arrhizus* in the field when they were inoculated by the scalpel-injury inoculation technique (10), but all hybrids were severely infected by *R. arrhizus* and *R. oryzae* following inoculation of the heads by using the insertion-inoculation technique (9). Their reaction to *R. stolonifer* was not tested (9).

Although the efficacy of each of the other methods of inoculation was not compared with that of the insertion-inoculation technique in the field, our results suggest that the insertion-inoculation technique is the most effective and suitable for screening sunflower for resistance to *Rhizopus* in either the field or the greenhouse.

Each of the three *Rhizopus* spp. was reisolated from all sampled heads into which it had been inoculated regardless of the inoculation method. No *Rhizopus* species was isolated from the control plants.

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