

A method for artificial inoculation of oats and barley for seed treatment trials on seedling-infecting smuts¹

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A method was developed to inoculate bulk amounts of seed of oats and barley for trials on the efficacy of seed-treatment fungicides in controlling the seedling-infecting smuts *Ustilago avenae*, *U. kollerl*, *U. nigra*, and *U. hordei*. The method is based on the partial vacuum principle, with the seed in a desiccator being evacuated before the spore suspension (100-200 mg spores/litre water) is introduced. It is suggested that this method be adopted as a standard for such trials in Canada. Since these four smuts have the same biology, it may be possible to use only *U. avenae* on oats as the test organism.

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On a élaboré une technique d'inoculation de semences d'avoine et d'orge en vrac en relation avec des essais sur l'efficacité de certains fongicides (traitement des semences) dans la lutte contre les charbons des plantules (*Ustilago avenae*, *U. kollerl*, *U. nigra*, et *U. hordei*). La méthode s'inspire du principe du vide partiel selon lequel la semence est mise dans un dessicateur et en est retirée avant l'inoculation avec la suspension de spores (100 à 200 mg de spores par litre d'eau). On recommande l'adoption de cette méthode comme étalon pour ce type d'essai au Canada. Puisque ces quatre espèces de charbon ont les mêmes propriétés biologiques, il serait possible de n'utiliser que *U. avenae* comme organisme-test sur l'avoine.

Field trials for testing the efficacy of fungicides to control the seedling-infecting smuts of oats and barley [*Ustilago avenae* (Pers.) Rostr., *U. kollerl* Wille, *U. nigra* Tapke, and *U. hordei* (Pers.) Lagerh.] should ideally be done on naturally infested seed. However, such seed is now very hard to obtain, and even if grain that is visibly contaminated by spores can be procured, infection of hulled cultivars of oats and barley is often very low. These two factors have forced most workers to use some form of artificial contamination or inoculation of the seed with spores. Such a method of artificial inoculation should fulfil the following requirements: 1) it should approach natural conditions as much as possible; 2) it should be reliable and reproducible, so that results of tests done in different areas or years are comparable; and 3) it should be easy to perform.

In nature, infection by the seedling-infecting smuts of oats and barley is caused by spores that lodge between hull and karyopsis (Kitunen 1937, Rusch 1957, Thiede 1963). Spores of loose smut of oats and false loose smut of barley are deposited between these structures at or shortly after flowering; spores of the covered smuts find their way under the hull at threshing or during other agitation of the grain. Any method of artificial inoculation should also attempt to deposit the spores under the hull, but it is immaterial when and how this is done. When dry spores are dusted on to the seed, some of them will in fact find their way under the hull. However, the number of spores deposited in this space, and consequently the level of infection, depends on how tightly the

hull encloses the karyopsis. Seed samples and cultivars differ in this characteristic, and this difference is largely responsible for the variability between tests, making this method of inoculation unreliable.

A dependable method to bring spores under the hull was developed by Zade (1928129). The spores were suspended in water, the seed immersed in this suspension, and the mixture subjected to a partial vacuum. The air that was removed from between hull and karyopsis was replaced by spore-suspension when the system was returned to atmospheric pressure. The seed was then dried. This method, in one modification or other, has been routinely used for testing of cultivars of oats and barley for their reaction to smut (Fischer and Holton 1957). Generally a spore-concentration of 1 g/litre water was used. The method has also been used to inoculate seed for seed-treatment trials, but the control by chemicals of smut on seed inoculated in this way appeared to be less effective than in tests with naturally infested seed (Leukel 1937, Purdy 1958). Purdy concluded "that artificial inoculation by the vacuum method produces unnatural and unnecessarily difficult problems of smut control by seed treatment", and "that testing of new materials should be done with naturally infested seed since it more closely represents the conditions encountered in commercial treating than does artificially inoculated seed". Purdy's data show that artificial inoculation with the vacuum method indeed produced an unnatural and unnecessarily difficult problem of smut control: his untreated check had up to 89% infection, a level that is never found under natural conditions. It is caused by an excess of spores on the seed. This, however, is not a flaw of the vacuum method, since any other method that introduced such a high spore load between hull and karyopsis would have

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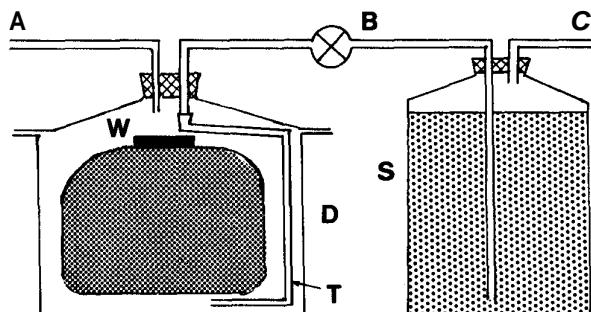


Figure 1. Diagram of the apparatus used to inoculate bulk amounts of seed of oats or barley with spores of *Ustilago* spp.

the same effect. The spore-concentrations of 1 or 2 g/litre water as used by Leukel (1937) and Purdy (1958) were the same as those used in tests of resistance or susceptibility of oat or barley cultivars to smut. In such experiments, maximum infection is desired. For seed-treatment trials, the concentration should obviously be much lower. To determine the concentration, and with it the spore-load that approaches natural conditions one should, in my opinion, be guided by the highest level of infection likely to be found in farmers' fields, because this is the level of infection that seed treatment with a fungicide should be able to prevent. Thus, an infection level of between 10% and 15% appears to be desirable. Since a certain number of spores between hull and karyopsis will cause a certain level of infection, regardless of how the spores get there, the vacuum method should provide the desired level of infection if the appropriate spore concentration is used. Thiede (1963) showed that in oats there is a linear correlation between spore-load and level of infection and established that with the partial vacuum method an infection of 10% to 15% was obtained with a concentration of 100 mg spores/litre water. Thiede's study also showed that when the spore-load was adjusted to give a natural level of infection, there was no difficulty in controlling smut by fungicides that were known to be effective. No similar study has been done with barley, but we found in our own trials that it is necessary to increase the spore-concentration to at least 200 mg/litre to obtain a similar level of infection. This difference between oats and barley is most likely due to the fact that in barley the hull adheres much tighter to the karyopsis than it does in oats.

Using partial vacuum and a low spore concentration, natural conditions are approached as closely as possible, and the tests become reliable and reproducible, fulfilling two of the three requirements mentioned above. The following description will show that the technique is also easy to use.

Seed, 3.5 kg of oats or 4 kg of barley, in a bag of loosely woven fabric was placed in a 25 cm diam desiccator (Fig. 1D) fitted with a cover with a rubber stopper. A

weight (W) of about 2 kg was placed on the bag to prevent it from floating. The inoculum was prepared by suspending spores in part of the required amount of water, using a Waring Blender running at approximately 2000 rpm for 1 min. The final concentration of the inoculum was 100 mg (oats) or 200-300 mg (barley) spores/litre water, and enough was prepared each time to fill the sturdy polyethylene storage bottle (S), which had a capacity of about 8 to 10 litres.

Valve B was closed when seed and spore-suspension were in place, and suction was applied at A until the vacuum reached about 600 to 650 mm. While evacuation continued, valve B was opened slightly to draw in spore suspension through rubber tube T until the inoculum covered the seed to a depth of about 5 cm. Evacuation continued for a further 10 min, after which the vacuum was released. The inoculum was transferred back into the storage vessel by applying gentle suction at C. The recovered inoculum could be used for successive inoculations done the same day. Excess inoculum was removed from the seed by draining the bag for 30 min. The seed was then spread in a layer about 1 cm thick and dried quickly and thoroughly with a fan before it was treated with fungicide.

For maximum infection, the tests should be seeded after the soil has warmed up. Spores for the following year's trials should be obtained by harvesting infected heads in paper bags just after heading and preferably before they have been subjected to rain. After drying the infected heads at room temperature for 2 weeks, the spores are separated by passage through a 40 sieve, sometimes after cutting up the dry heads in a blender. The spores are stored in a glass jar at about 5°C, where they remain viable for at least 2 years. Generally, however, it is advisable to use the freshest spores available.

To compare results of seed-treatment trials conducted at different times and at different geographical locations across Canada, it is desirable to standardize the methods used in such trials. The method of inoculation described here should be part of this standardization. Since loose and covered smuts of oats have the same biology, and false loose and covered smut of barley have the same biology, trials on only one of the smuts on each crop are needed. It is convenient to use loose smut of oats and false loose smut of barley, because they are easy to recognize in the field, and because spores of these smuts disperse more readily when the inoculum is prepared. A further aspect should also be considered, particularly in preliminary tests of a large number of prospective fungicides. Decades of past trials have shown that whenever a fungicide at a certain dosage was effective against the smuts of oats, it was also effective at this dosage against the seedling-infecting smuts of barley. Since there is no difference in biology of these smuts, and since they appear to react similarly to fungicides, it is possible that the required information can be obtained by testing prospective fungicides with only one species of smut, and with only one of the two crops. Oats would

be preferable, because it is less prone to attack by other diseases, and it is less susceptible to adverse weather and soil conditions.

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