

Protocol for nematode resistance screening

The TystofteFoundation has since the seventies been testing new varieties of spring barley, spring wheat, and oat for resistance to cyst nematodes (*Heterodera avenae* race 1 and race 2). In the present test, resistance is determined from the prevalence of nematode cysts on the roots of the tested varieties.

The test is a semi-field test run over one season with sowing in April and harvest in early August.

The nematode ditches

The ditches consist of a concrete frame approx. 1.5 m x 1.5 m with room for 20 x 20 rows of PVC tubes (7 cm in diameter and 30 cm long) standing upright, with the top in soil level. The growth medium consist of a base of clean soil with an inoculum of infected soil on top.

For each nematode race, there are two ditches of which one is used for screening and one is used for multiplication.

Growth medium

The growth medium is a mixture of 75 % sandy loam and 25 % coarse sand free from nematodes.

The soil is strained and mixed three times with the sand. The uncontaminated soil is filled loosely into clean PVC tubes in the ditch. Subsequently, each tube is compressed to 8-10 cm under the edge of the tube.

Inoculum

Inoculum is produced over a three-year cycle. Every year, 120 tubes of inoculum are produced for each nematode race (photo 4).

1st year: Infected roots from the present year's screening trial are placed in tubes with clean soil, and clean soil is spread on top. Tubes with infected roots are left in the ditch until next growing season.

2nd year: Susceptible spring barley is sown in the tubes, in order to differentiate between nematode races. For race 1: Emir, (susceptible to race 1 and 2). For race 2: Ortolan (resistant to race 1, and susceptible to race 2).

3rd year: Oat variety Sun II (very susceptible to races 1 and 2) is sown in order to multiply the nematodes.

4th year: The soil from the tubes are taken out and mixed well on a plastic sheet. The infected soil is then distributed on top of the clean soil in the tubes for screening new varieties (Photo 1).

Reference varieties

The following varieties are used as references with regard to susceptibility. Resistant (r) and susceptible (s) to (Race 1/Race 2):

Spring barley: Emir, (s/s), Ortolan (r/s), Meltan (r/r).

Oat: Sun II (s/s), Fix (r/r) and 640318-40-2-1 (r/r).

Spring wheat: Capa (s/s), Aus (r/r), 63/11/17/15/12 (r/r).

Sowing

Each new variety is tested in 4 tubes. In each tube, three seeds are sown in the inoculum, and covered with approx. 3 cm of clean soil (Photo 2). The reference varieties are grown in 10 tubes each.

A labelled stick is placed in each tube. (Photo 3). The plants grow outdoors from April to August, until they reach stage BBCH 75-79 (medium to late milk).

The plants are fertilized with NPK and irrigated regularly. It is important to keep the soil moist but not wet.

Screening the roots for cyst nematodes

In the beginning of august, the tubes with plants and soil are taken up, and placed vertically in a container with water for 1 hour (Photo 5). Tubes should not be submerged completely. When the soil has softened, plants are gently released from the tube, and the roots are rinsed in water. Rinsing must be done carefully in order to retain the cysts on the roots. Then roots are placed in a small bowl with fresh water, and examined under a magnifying lamp (Photo 6). The number of cysts are counted. For the reference varieties, all cysts are counted. Normally, we have 50 to 150 cysts in each tube of a susceptible variety. (Photo 7; an early stage, and Photo 8; a later stage).

A variety of spring barley is considered resistant if the number of cysts are less than 5 % of the average for the susceptible reference variety.

A variety of spring wheat or oat is considered resistant if the number of cysts are less than 10% of the average for the susceptible reference variety.



Photo 1:



Photo 2



Photo 3

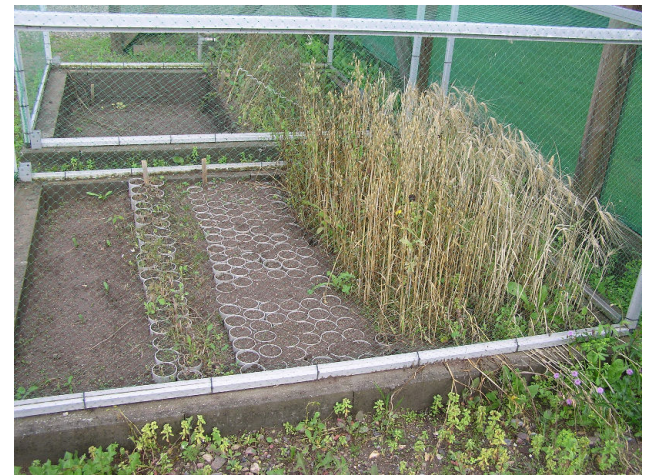


Photo 4



Photo 5



Photo 6



Photo 7

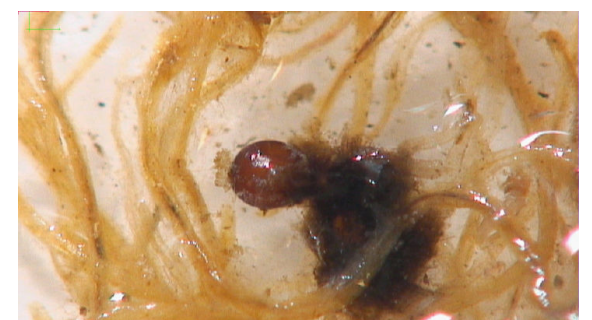


Photo 8