Neth. J. Pl. Path. 72 (1966): 319–321

A MODIFIED BLOTTER TEST FOR SEED HEALTH[1,2]

Een gewijzigde filtreerpapierproef voor gezondheidsonderzoek van zaaizaad

T. LIMONARD
Government Seed Testing Station, Wageningen

In seed health testing for seed-borne fungal pathogens the blotter test is no doubt one of the most important methods available. In this method, seeds are incubated for a certain period of time on or between blotters. The seeds may then be allowed to germinate and fungal seed-borne infections may manifest themselves by any pertinent signs or symptoms. The manifestations of the pathogen are influenced by the environmental conditions during incubation.

Looking into the background of the blotter test, it was found (LIMONARD, in press) that environmental conditions may also influence the pathogen's expression in an indirect way, e.i. by their effect on the whole microflora of the seed. One might speak of "blotter test ecology" in this connection. However, besides these environmental factors (physical and biotic), another one which may have an important bearing on test results has been observed. This is the factor of the "resistance" offered by the tissues of seed or seedling to the manifestation of the pathogen.

In blotter testing for certain infections (e.g. *Phoma lingam* in cabbage seed), it has been customary to add the selective herbicide 2,4-D (2,4 dichlorophenoxyacetic acid) as an inhibitor of germination in order to facilitate the inspection of the seeds after incubation. It was found by the author that the use of this chemical increased, often to quite a considerable degree, the percentage of infection found for a number of seed-borne pathogens. This observation was most striking for the infection of corn salad *(Valerianella locusta)* by a pathogenic *Phoma* species.

It was noted that the normal blotter test showed 5% infection, but with a 2,4-D blotter test for the same sample applied simultaneously 40% was found. This led to the realization of the importance of the factor "resistance", because of the fact that this effect could also be obtained by maltreating the seedlings in various ways.

One of the methods used for this purpose was the freezing of seeds or seedlings in blotter testing, which proved to be the most convenient and efficient way of doing so. In case of the sample mentioned above it gave 60% infection, which corresponded with the same percentage found when seeds dissected out of the fruits were plated on potato glucose agar. Harming or killing of seeds or seedlings in the blotter test thus proved to be an excellent way of stimulating the development of pathogens.

In the modified blotter test that is described below the preceding information is applied. However, various details of the procedure are still under study. A

[1] Accepted for publication 9 September, 1966.
[2] This research has been financed in part by a grant made by the United States Department of Agriculture under P.L. 480.

general description of the modified test based on the work done so far is as follows. The seeds are incubated for a certain period of time on moist blotters (or they may be soaked in water to which antibacterial antibiotics like terramycin have been added) long enough to give them a moisture content sufficient for germination. The moist seeds on the blotters are then put into a deepfreezer at a temperature of –20 °C for at least four hours. After freezing, the seeds are returned to incubators provided with near ultraviolet light (General Electric BL fluorescent tubes). This light slows down the mycelial development of most fungi and stimulates their fructification. Low substrate moisture and high relative air humidity are likewise advocated. In some cases it may be advantageous to freeze the seedlings instead of the seed at some time after germination. This often facilitates the inspection which may be exemplified by the *Phoma* infection of corn salad as mentioned above. Extra care should be taken to avoid contamination by laboratory contaminants such as *Mucor* spp.

This method was tried on many kinds of seeds for which a normal blotter test may also be used (e.g. cornsalad, cabbage, wheat, oats, barley, rice, grasses, lettuce, spinach, carrot, pea, etc.). It did not only facilitate inspection greatly but also in many cases gave a higher percentage of infection than the normal blotter test.

The modified blotter test seems to be of special value for wheat, as it does allow the use of a single test for infections caused by *Helminthosporium* spp., *Fusarium* spp. and *Septoria nodorum*. The existing standard blotter tests for *Fusarium* spp. and *S. nodorum* have serious drawbacks which are not inherent in the freezing method. These drawbacks mainly concern the doubtful recognition of the produced symptoms.

In the modified blotter test for wheat, the seeds are pre-germinated by keeping them between moist blotters for three days at 10 °C followed by one or two days at 20° and are then frozen. Postfreezing incubation takes place at 20 °C in near ultraviolet light and in a relative air humidity of 95% or higher for one week without the top blotters. The *S. nodorum* present will then have formed pycnidia producing reddish spore exudate on the embryo end of the seed and on the coleoptile. Pycnidia also occur frequently on the roots, but usually these are without spore exudate. The presence of *S. nodorum* may very often be suspected by the appearance of the typical finely tufted white mycelium on the seed. *Fusarium* spp. may be recognized by their mycelial colours and orange spore masses.

For a large number of wheat samples tested in this manner the percentages of infection found for *S. nodorum* corresponded very closely with those found by means of the agar method.

Further investigations about the details of this new method are being carried out at the moment, and are also directed at the underlying causes of the observed facts.

SAMENVATTING

Er werd gevonden dat één van de factoren welke de „expressie" van pathogenen in de filtreerpapierproef voor gezondheidsonderzoek van zaden beïnvloedt, de weerstand is die het pathogeen ondervindt van de levende weefsels van zaad of kiemplant. Door deze te breken door middel van bevriezing van kiemend of gekiemd zaad wordt het verschijnen van pathogene schimmels vergemakkelijkt.

Incubatie onder nabij-ultraviolet licht (General Electric BL fluorescentiebuizen) en bij hoge luchtvochtigheid geeft na bevriezing sterke fructificatie en sporulatie. Deze methode bleek uitstekend geschikt voor beoordeling van vele zaadinfecties, in het bijzonder van *Septoria nodorum* en *Fusarium*-soorten in tarwe.

REFERENCE

LIMONARD, T., – 1966. Bacterial antagonism in seed health tests. Neth. J. Pl. Path. (in press).