Neth. J.PL. Path. 85 (1979) 143-150

A method to test wheat leaves for their reactions to inoculation with Septoria species

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Accepted 17 November 1978

Abstract

The construction and application is described of a polystyrol 'humidity box' in which wheat leaves, while continuing to function as parts of living plants, can be tested for their reactions to *Septoria* spp. in an atmosphere nearly saturated with water, as is required for successful infection. The method is simple, accurate ans inexpensive.

Additional keywords: Leptosphaeria nodorum, Septoria nodorum, S. tritici, humidity box (for attached leaves).

Introduction

Infection of wheat leaves by conidia of *Leptosphaeria nodorum* Müller (*Septoria nodorum* Berk.) and *Septoria tritici* Desm. only occurs under humid conditions. Not only the release of the conidia, but also their germination and the growth of the germ tube require a wet leaf surface or an atmosphere nearly saturated with water. For a successful infection, these conditions should be maintained for at least several hours and preferably several days (Shipton et al., 1971).

The same holds for an adequate indoor testing of wheat plants as to their reactions to infection by *Septoria* species (Holmes and Colhoun, 1974; Eyal et al., 1977). For this purpose several methods are applied. Most common is to spray the plants (mainly seedlings) with a conidial suspension of the fungus and to cover them subsequently with a polythene bag or to put them in a dew chamber for some time. However, this is likely to result in an uneven distribution of the inoculum and difficulties in the assessment of the leaf reactions. Moreover, quite apart from inoculation and air humidity, the whole plants suffer from the mere fact that they are covered during this incubation. Another method is to test detached leaves or leaf segments, which after inoculation are put in a closed dish, serving as a humid chamber and containing a benzimidazole solution to keep the leaf material in condition (Baker, 1970; Baker and Smith, 1978). This method has the disadvantage of the artificial condition of the material being tested and moreover the fungicidal properties of benzimidazole. This matters the more as higher concentrations of this chemical are needed in case of longer latency periods.

In this article a new method for obtaining the desired moisture conditions is described. This method is simple and effective and offers a number of advantages over existing methods.

Materials

Starting material is a rectangular box (inside measurements 115 × 75 × 30 mm) with an accurately fitting lid (118 × 78 × 7 mm), both made of clear polystyrol of 2 mm in thickness. With acrylate glue a small bar of polymetha-acrylate (115 × 12 × 12 mm) is fixed lengthwise in the middle of the bottom of the box. At a height of about 15 mm from the bottom, the box is then horizontally sawn in two. The upper edge of the ramaining part of the box and the lower edge of the sawn-off rectangle are made smooth and resilient by covering them with silicon-tubing (inner diameter 3 mm, outer diameter 4 mm), cut open lengthwise. Again acrylate glue is used for this purpose. Now the box consists of three fitting parts, as shown in cross-section in Fig. 1A.

Fig. 1. A humidity box in cross-section. A) The three parts of the box, from the top downwards: the lid, the detached piece of the wall with the tube-covered lower edge, the bottom part with tube-covered upper edge and a 'bridge'. B) A closed humidity box with a leaf of a wheat plant in it. Some water on either side of the bridge. A drop of a conidial suspension on the leaf.

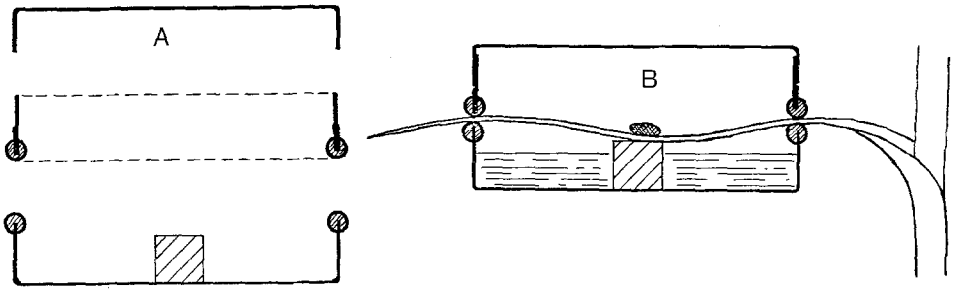


Fig. 1. Dwarsdoorsnede door een vochtdoos. A) De drie delen van de doos, van boven naar beneden: het deksel, het losse wandstuk met de beklede onderrand, het bodemgedeelte met beklede bovenrand en een 'brug'. B) Een gesloten vochtdoos met het blad van een tarweplant er doorheen. Aan weerszijden van de brug staat water. Op het blad een druppel conidiënsuspensie.

Fig. 1B demonstrates the use of this box. On either side of the 'bridge' some water has been poured in. The wheat leaf to be tested is laid across the bridge. It is inoculated with a drop of a conidial suspension. Then the detached piece of the wall and the lid are replaced. Thus the inoculated part of the leaf is enclosed in a practically completely closed humid chamber, while nevertheless the leaf is undamaged and still functioning as a part of a normal plant. The length of the box can accomodate a maximum of five leaves side by side.

Air humidity

Recordings of the humidity conditions in boxes which were in use, carried out by the Technical and Physical Engineering Research Service, Wageningen, taught us that a relative humidity of about 97% or even higher, was reached within an hour after closing the box. The presence of the water was found to be especially important in the dark to supplement the then decreased evaporation of the leaves. Changes in

environmental temperatures between 14 and 30°C., caused only slight and temporary changes of the relative humidity in the boxes. So the high humidity in boxes which are in use, is hardly influenced by common environmental conditions.

Position

There are several possible ways of arranging the humidity boxes near the plants to be tested. For this purpose a narrow shelf can be installed at the desired height along the plants. It should be possible to exert some vertical pressure on the closed boxes, in order to ensure proper sealing for the tubecovered edges. This can be achieved in a simple manner with the aid of elastic bands which are put round the shelf beforehand and each of which can afterwards be drawn over one of the boxes.

Fig. 2. Stand with five humidity boxes in which leaves of young wheat plants are being tested. In the open box on the left a drop of conidial suspension is visible on each of the leaves.

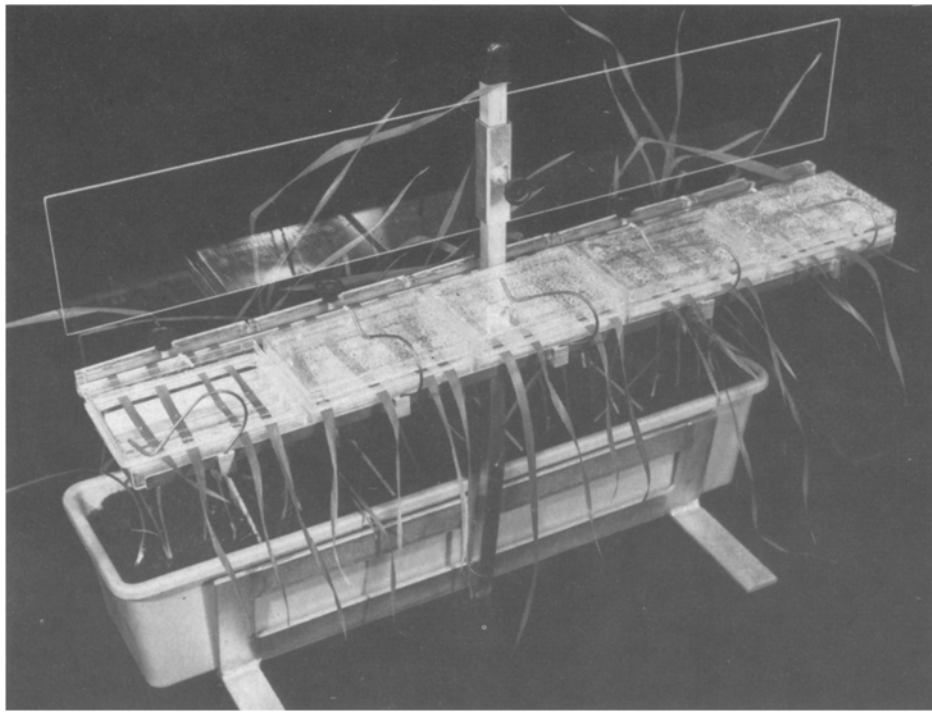


Fig. 2. Statief met vijf vochtdozen waarin bladeren van jonge tarweplanten worden getoetst. In de geopende doos (links) is op elk blad een druppel conidiënsuspensie te zien.

For the use of the humidity boxes the Technical and Physical Engineering Research Service designed and constructed the stand shown in Fig. 2. The frame is made of electrolytically galvanized steel. It can hold a plant-box of $600 \times 160 \times 120$ mm or four pots in which the material to be tested is grown. The central pole has a

height of 500 mm, but it can be lengthened up to 1000 mm with the aid of a separate extension piece. Attached to the pole there is a vertically adjustable aluminium shelf, which can accomodate five humidity boxes. The shelf can be adjusted at any height between 200 and 980 mm. On the side facing the plants the shelf is provided with five 'leaf conductors', thin plastic bars fastened with small springs, under which the leaves can be temporarily secured if necessary. On the other side the shelf is provided with five horizontally adjustable bowsprings, which, when put on the lids of the boxes, exert enough pressure to keep the boxes well closed. An acrylate plate ($620 \times 100 \times 3$ mm), adjustable on the pole above the shelf, restrains the plants to prevent them from hanging over the boxes. A detailed design of the stand is obtainable on request.

Procedure

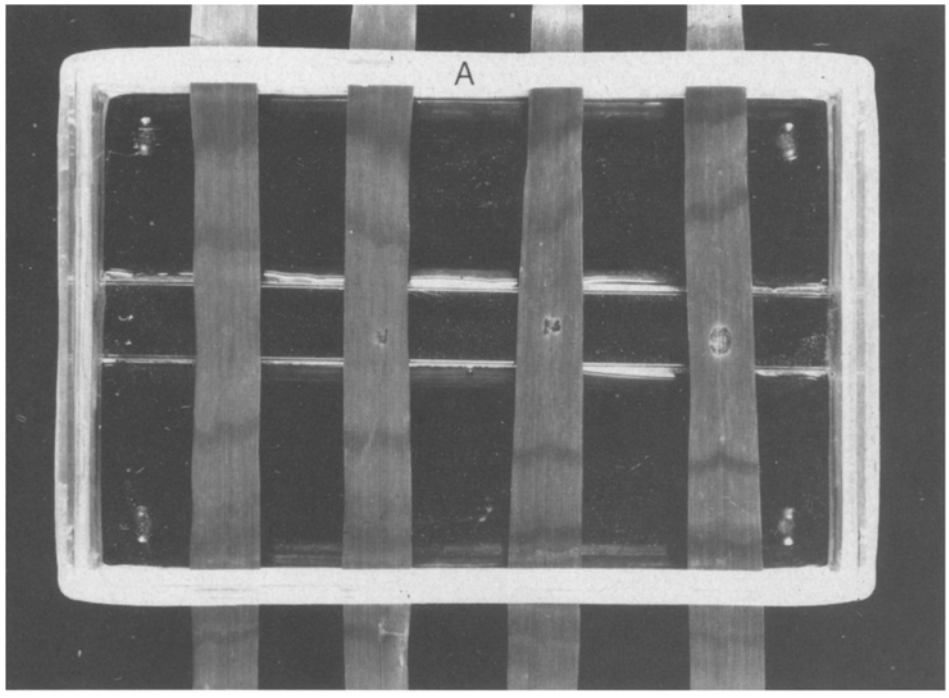
The leaves to be tested are put in position in the way described above and water is added up to some millimeters below the upper surface of the bridge, so that the leaves themselves don't touch the water. Each leaf is inoculated on the upper side with a drop of a conidial suspension of the fungus, with the aid of a micro pipette. A drop size of 10^{-2} ml is suitable. In order to distinguish between the differences in leaf reactions occurring in the wheat cultivars currently grown in the Netherlands, the following variation in conidium concentrations proved the most suitable: of *L. nodorum* 10^5 and 10^6 conidia/ml and of *S. tritici* 10^6 and 2.10^6 conidia/ml. A control can be achieved with a drop of sterile water. Immediately after inoculation the boxes are closed. After inoculation of wheat with *L. nodorum,* the boxes should remain closed for 48 hours. At light/dark conditions of 12/12 hours and 20/15°C the symptoms then develop within a week (Fig. 3A). With highly susceptible cultivars the first signs of reaction are already visible on opening the boxes. After inoculation with *S. tritici* the boxes should remain closed for four days. Under the above-mentioned light and temperature conditions the symptoms of *S. tritici* will then emerge after a latency period of about three weeks (Fig. 3B).

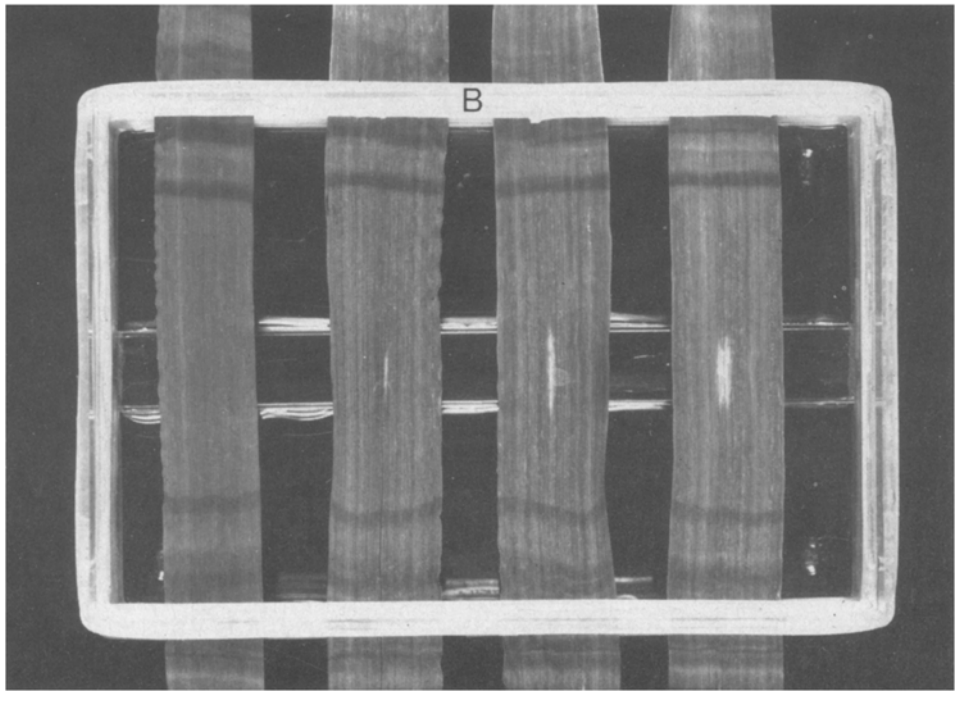
Fig. 3A. Symptoms of *L.nodorum* on leaves of a moderately susceptible wheat cultivar 7 days after inoculation in a humidity box. Conidium concentrations applied (in a drop of 10^{-2} ml per leaf), from left to right: 0, 10^4, 10^5, 10^6 conidia/ml.

Fig. 3B. Symptoms of *S. tritici* on leaves of a moderately susceptible wheat cultivar 25 days after inoculation in a humidity box. Conidium concentrations applied, from left to right: 0, 10^5, 10^6 and 2×10^6 conidia/ml.

Fig. 3A. Symptomen van L. nodorum op bladeren van een matig vatbaar tarweras, 7 dagen na inoculatie in een vochtdoos. Toegepaste conidiumconcentraties (in een druppel van 10^{-2} ml per blad), van links naar rechts: 0, 10^4, 10^5, 10^6 conidiën/ml.

Fig. 3B. Symptomen van S. tritici op bladeren van een matig vatbaar tarweras 25 dagen na inoculatie in een vochtdoos. Toegepaste conidiumconcentraties van links naar rechts: 0, 10^5, 10^6 en 2×10^6 conidiën/ml.


A


B

In the open boxes regular observations are possible about:
- length of incubation and latency periods;
- size and growth of the spot (*S. tritici*) or of the yellow halo round the spot (*L. nodorum*);
- degree and speed of chlorotisation and/or necrotisation of the spot;
- density of the pycnidia and their further increase (Fig. 4).

Fig. 4. Leaf spots and pycnidia formed by *S. tritici* in a very susceptible wheat cultivar, 28 days after inoculation of the leaves in a humidity box. Conidium concentrations applied (in a drop of 10^{-2} ml per leaf), 2×10^6 conidia/ml. The leaf on the left is a control.

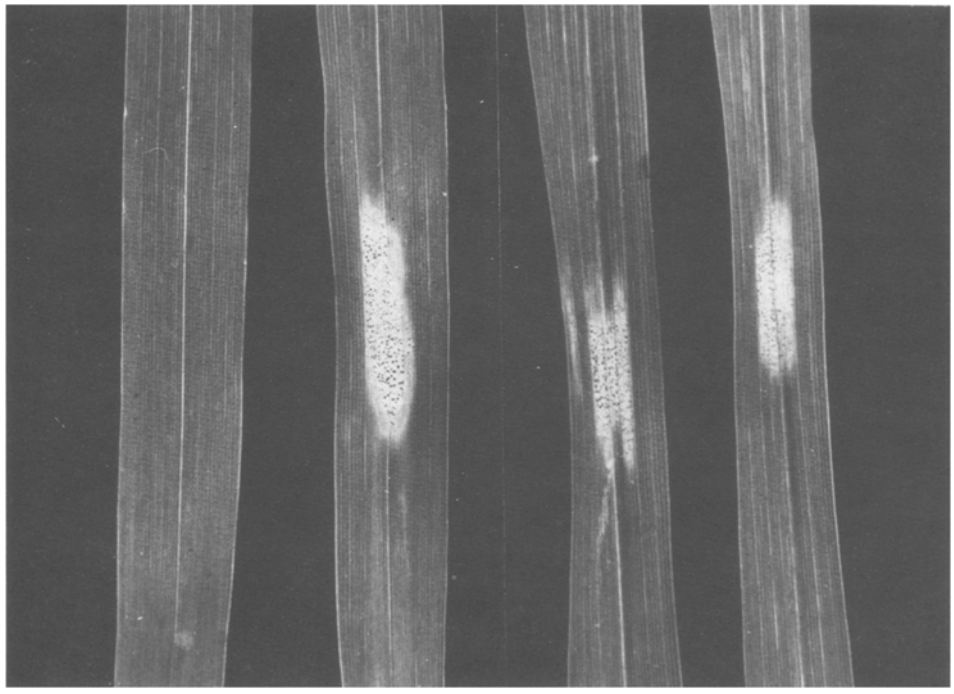


Fig. 4. Bladvlekken en pycniden gevormd door S. tritici in een zeer vatbaar tarweras, 28 dagen na inoculatie van de bladeren in een vochtdoos. Toegepaste conidiumconcentratie (in een druppel van 10^{-2} ml per blad), 2×10^6 conidiën/ml. Het linker blad is een blanco.

In one box it is possible to test a number of comparable leaves of different plants of one cultivar. In this way not only young plants can be tested, from about the fourth leaf, but all later leaves of mature plants as well, including the flag leaf. During testing the plants can be tended quite normally and if necessary they can be given different treatments. Of course it is also possible to use the method to compare different isolations or strains of the fungi.

Important features

The following features of this testing method are especially important.

- The method is reliable, simple and inexpensive. The required high degree of air humidity can be achieved whenever and for as long as desired without complicated or costly equipment and without causing the plants to suffer from this treatment. This possibility is especially important in connection with the phenomenon that varietal differences in reaction to inoculation with *Septoria* spp., can depend also on the length of the postinoculation wet period (Holmes and Colhoun, 1974: Eyal et al., 1977).
- The method requires very little infection material. One petri dish with a well sporulating *Septoria* culture on an agar medium is sufficient to test several thousands of leaves in this way.
- Accuracy. This method involves precisely localized infections caused by different precisely known quantities of inoculum. Both the components and the speed of the leaf reactions can be observed closely. The value of these possibilities is supported by recent data of Shearer (1978), concerning the effects of the inoculum density of *S. tritici* on the latency period and the symptom expression in wheat cultivars. One of his conclusions is that screening wheat cultivars for resistance to *S. tritici*, should be done with several inoculum densities and not without determination of the latency periods.
- The plant material to be tested is hardly or not at all influenced by the method itself. This results in quite natural reactions and compares favourably with, for instance, the testing of detached leaves or leaf fragments artificially kept in a good condition with the aid of benzimidazole.
- The suitability of the method for research into te effects of various factors on the plant/pathogen reaction, such as variety, growth stage or treatment of the plant and type or concentration of the inoculum. This is due to the fact that infection, incubation and reaction do indeed occur on the plants, but localized and isolated in the boxes. This as opposed to the methods in which whole plants are infected and incubated. This latter procedure requires much more infection material and also impedes a proper incubation, an effective isolation of the objects and an accurate evaluation of the reactions.
- Because the tested plant is damaged hardly at all, it remains available for further tests, other observations and harvesting seed.

Samenvatting

Een methode voor het toetsen van tarwebladeren op hun reactie op inoculatie met Septoriasoorten

Voor dit doel is een z.g. vochtdoos geconstrueerd (Fig. 1A). Het te toetsen blad wordt daar doorheen geleid en in de doos geïnoculeerd met een druppel conidiënsuspensie van de schimmel. Onder het blad staat wat water in de doos (Fig. 1B). Na afsluiting ontstaat in de doos de hoge luchtvochtigheid van bijna 100% die nodig is voor de infectie. Dit wordt op deze wijze eenvoudig en goedkoop gerealiseerd. De bladeren die zo worden getoetst blijven onbeschadigd functioneren aan de plant. Na

enkele dagen kan de doos worden geopend en kan de symptoom-ontwikkeling worden afgewacht en gevolgd (Fig. 3A, 3B en 4). De methode leent zich voor nauwkeurig werk en vereist zeer weinig infectiemateriaal. De Technische en Fysische Dienst voor de Landbouw (TFDL), Wageningen, ontwierp en construeerde een statief voor het gebruik van de vochtdozen in serie (Fig. 2).

Acknowledgments

We are indebted to the Technical and Physical Engineering Research Service, Wageningen, for their valuable help, in particular to Mr G.J.W. Visscher for the measurements of the air humidity in the boxes, to Mr J.H. Oosterveld for the design and construction of the stand and to Mr A.N. Brandsen for drawing the figures.

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