Neth. J. Pl. Path. 98 (1992) 157-159 SHORT COMMUNICATION

Field inoculation of leek with Phytophthora porri

W.D. SMILDE[1] and M. VAN NES[2]

[1] Dept. of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, the Netherlands.
[2] DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, the Netherlands.

Accepted 21 January 1992

Abstract

Field inoculation of leek with zoospores of *Phytophthora porri* resulted in high infection within a short time. Inoculation with infected leaf tissue resulted in a more gradual increase of disease incidence. Inoculation with oospores was relatively unsuccessful. Zoospores were produced in Petri-dishes by treating fast-growing, young mycelium with a diluted soil extract for at least 2 days, followed by a cold treatment in sterile demineralized water. The successful methods can be used for evaluation of resistance or fungicide performance, and for epidemiological experiments.

Additional keywords: Allium porrum, inoculation methods.

Field experiments with *Phytophthora porri* Foister on leek (*Allium porrum* L.) depend on natural infection. The absence of the disease during some years has often hampered field evaluations of leek cultivars and breeding lines, and of fungicide performance (Vanparijs and Bockstaele, 1984; Van Bakel, 1964). Therefore, a reliable inoculation method was needed.

During the autumn of 1991 three different inoculation methods were tested in a field experiment, using three different kinds of inoculum: zoospores, infected leaf tissue of leek, and oospores.

Zoospore inoculum is produced as follows: 15 discs (diam. 5 mm) from a *Phytophthora porri* culture on leek agar (200 g leek leaf extract in 1 l of demineralized water and 17 g agar) are grown on 10-15 ml of 10% V8 broth in a Petri dish (diam. 9 cm) at 15 °C for 2-3 days. Then the V8 medium is decanted and a 10× diluted soil extract is added, which is produced by suspending 500 g of soil (Trios 17) in 1 l of demineralized water, leaving the suspension overnight, and autoclaving the filtrate. After at least 2 days incubation with soil extract at 15 °C numerous sporangia should be visible. Soil extract is then removed and 10 ml cold demineralized water is added. After 2-4 h at 3 °C this medium will contain about 1000 active zoospores per ml. Usually less than ten percent of the sporangia has germinated after the cold treatment. The described method is a modification of the method of Chen and Zentmyer (1970) and of Hamm and Koepsell (1984).

Zoospores will remain active for several hours when the suspension is kept cool. When sporangia are kept at 3 °C they will produce fresh zoospores for about 3 months.

Inoculation is achieved by dripping one ml of the zoospore suspension into a leaf sheath of a leek plant.

Infected leaf tissue is produced by placing an agar disc with mycelium or one drop of a zoospore suspension on a leaf piece of 5 cm length. After incubation in a moist chamber for about 4 days at 15-20 °C the leaf pieces are dried. Agar discs are removed. At the seventh or eighth day after inoculation greenish symptoms, typically lozenge-shaped, will develop. For inoculation, the infected tissue is placed into the leaf sheaths of leek plants, where a small quantity of water is often present for long periods.

Oospore inoculum is produced in leek agar. Colonies of *Phytophthora porri* are incubated for 2 months at ca 17 °C. To harvest the oospores, agar plates are homogenized in 50 ml sterile demineralized water per plate and incubated for 20 h at 18 °C with 0.5% (w/v) Novozym (Sigma L-2265) solution to digest the mycelium (Spielman et al., 1989). The surviving oospores (ca. 1000 per ml) are used as inoculum.

Each method was used to inoculate 600 leek plants. Five Wintertype cultivars were used. Uninoculated control plants were grown in neighbouring rows at 45 cm distance and in separate plots at 2.4 m.

Zoospore inoculation caused the highest infection (cf. Table 1): seven days after inoculation 50% of the inoculated plants showed disease symptoms. This percentage increased in the next few weeks till about 80%. Inoculation with infected leaves resulted in 30% infection at the eleventh day after inoculation. In the next two months the incidence increased gradually till 80%. Oospore inoculation was relatively unsuccessful. Thirteen days after inoculation only 7% of inoculated plants showed symptoms, and one month after inoculation 12% was infected.

Table 1. Disease incidence (n = 600) after inoculation with (1) zoospores, (2) infected leaf tissue, (3) oospores.

Days after inoculation	(1)	(2)	(3)
7-13 days	50%	30%	7%
30-60 days	80%	80%	12%

Uninoculated plants in neighbouring rows did not show symptoms until about 3 weeks after inoculation. Therefore, after 3 weeks, infection of inoculated plants may be due to secondary spread. During the reported period, no natural infection occurred in neighbouring control plots. This proves that soil-borne inoculum of *Phytophthora porri* was absent in the experimental plot initially.

Advantages of zoospore inoculation are the high infection percentage, the absence of contaminants from the inoculum, and the possibility to quantify the inoculum and to apply it homogeneously. A disadvantage of zoospore inoculation compared with infected leaf tissue inoculation may be the higher sensitivity to weather conditions. Zoospore inoculation was completely unsuccessful when temperatures were too high (>25 °C) during inoculation. Cool and wet conditions are supposed to favour the infection process.

Zoospores or infected leaf tissue may be applied to spreader plants in a field trial. Both methods may be useful for field evaluation of resistance and of fungicide performance.

Acknowledgement

This research is supported by the Technology Foundation (STW), the Netherlands.

References

Chen, D.W. & G.A. Zentmyer, 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. Mycologia 62: 397-402.

Hamm, P.B. & P.A. Koepsell, 1984. *Phytophthora* root rot of cabbage and cauliflower in Oregon. Plant Disease 68: 533-535.

Spielman, L.J., B.J. McMaster & W.E. Fry, 1989. Dominance and recessiveness at loci for virulence against potato and tomato in *Phytophthora infestans*. Theoretical and Applied Genetics 77: 832-838.

Van Bakel, J.M.M., 1964. Papiervlekkenziekte in prei (*Phytophthora porri* Foister). Mededelingen van de Directie van de Tuinbouw 27: 198-200.

Vanparijs, L & L. Bockstaele, 1984. Prei: Overzicht van het onderzoek in 1984. Beitem-Roeselare: Onderzoek- en Voorlichtingscentrum voor Land- en Tuinbouw, 87 pp.