Soil Water Matric Potential Requirements for Root-Hair and Cortical Infection of Chinese Cabbage by *Plasmodiophora brassicae*

R. Dobson, R. L. Gabrielson, and A. S. Baker

Research assistant, plant pathologist and soil scientist, Western Washington Research and Extension Center, Puyallup 98371.
Scientific Paper 6136, Project 0267, Washington State University, College of Agriculture Research Center, Pullman 99164.
Supported in part by grants from the Northwest Agricultural Research Foundation, Inc.
Accepted for publication 7 July 1982.

ABSTRACT

Dobson, R., Gabrielson, R. L., and Baker, A. S. 1982. Soil water matric potential requirements for root-hair and cortical infection of Chinese cabbage by *Plasmodiophora brassicae*. Phytopathology 72:1598-1600.

This research was undertaken to define the soil moisture necessary for infection of Chinese cabbage by *Plasmodiophora brassicae*. Matric potentials required for infection were determined by using both tension plates and a semipermeable membrane/polyethylene glycol system. Both root-hair and cortical infections decreased with decreasing availability of water. Water potential necessary for cortical infection was measured in three soil types: no infection was observed at <-150 mbars in silt and sandy loam soils, but was found at -200 mbars in a muck soil. In a silt loam soil, cortical infection required more moisture (>-150 mbars) than root-hair infection (<-200 mbars). The requirement for more soil moisture (and thus, larger water-filled soil pores) is consistent with the postulated need for fused and, therefore, larger secondary zoospores necessary to initiate cortical infection.

The biology of *Plasmodiophora brassicae* Wor. is not fully understood, although there has been much research in this area since Woronin (13) first described this crucifer pathogen in 1878 (6). Basically there are two infection stages in the life cycle. In the first, primary (I°) zoospores arising from resting spores infect the root hairs and form sporangia. In the second, secondary (II°) zoospores released from the root-hair sporangia either reinfect root hairs or infect root cortical cells, initiating development of clubroot symptoms (5,6). Soil moisture requirements for the development of clubroot symptoms have been reported (1,4,6), but this is only a portion of the life cycle.

Previously, investigators reported that cortical infection requires a soil wetted to above 40% of its maximum water-holding capacity (1,4,6). However, maximum water-holding capacity is dependent upon soil type and its use should be avoided and replaced by a more comparable measure such as soil water matric potential (ψ_m) (2). This term has advantages over percent maximum water-holding capacity in that it represents an energy term independent of soil type that is directly related to the idealized diameter of water-filled soil pores (2,8). Duniway (3) and Cook and Papendick (2) showed that ψ_m as it relates to the size of water-filled soil pores can explain the effects of moisture on the movement of zoospores within soil.

The present study was initiated to define the ψ_m requirements for both the root hair and cortical infection by *P. brassicae*.

MATERIALS AND METHODS

Regulation of soil matric potential with tension plates. The method of Duniway (3), who used Büchner funnels with fritted glass disks as tension plates, was followed. The height of the water column, from funnel tension plate to reservoir, created a suction or matric potential on the soil according to the proportion, 1 mbar = 1.022 cm. Water column heights were maintained at 10, 50, 100, 150, and 200 cm, and each was replicated three times. Resting spores of *P. brassicae* were prepared according to William's (12) method. The soil, infested with 10^7–10^8 spores per gram dry wt, was placed in the funnels to a depth of 2.0–2.5 cm and wetted by raising the water reservoir to the same height as the tension plate. After the soil was wetted, water reservoirs were lowered to the desired column heights and allowed to equilibrate for at least 24 hr before six seedlings of a susceptible Chinese cabbage (*Brassica campestris* L. spp. *pekinensis* (Louv.) Olsson.), previously germinated for 24–48 hr, were planted in each funnel. Funnels were capped with a loose-fitting plastic bag to reduce water loss and kept at 25–30 C under cool-white fluorescent lighting (~4,000 lux). Root-hair infection was assessed by removing plants 4 days after planting, washing the roots, staining with 0.05% cotton blue in 50% acetic acid for more than 1 hr, and counting the number of sporangia present in the first 2-cm of the root from the soil surface (9). Cortical infection (club-root symptoms) were rated after 5 wk.

Matric potentials were monitored in a few funnels with a tensiometer (Cat. No. 2100; Soil Moisture Equipment Corp., Santa Barbara, CA 93105). Matric potentials in some funnels set at <-100 mbars began to slowly dry out after 4–5 days, presumably due to slow water flux across the tension plates. However, the problem was not as serious as first expected because cortical infection was found to occur between the third and fourth day.

Experiments with tension plates were repeated using three soil types from Puyallup, WA: a Sultan silt loam, a Puyallup sandy loam, and a muck soil. Soil water content at each ψ_m value was determined gravimetrically. This was then expressed as percent of maximum water-holding capacity (w/w) and as percent water retained per unit volume (w/v) of soil. Soil volume was measured with dried soil.

Matric potentials regulated with a membrane and polyethylene glycol (PEG). To obtain ψ_m values below -200 mbars, a technique similar to that of Tingey and Stockwell (10) was followed. Infested soil (10^7–10^8 spores/gm dry soil) was carefully pressed into the open end of a 50-mm-diameter cylinder of a semipermeable membrane (spectrapore 1, Spectrum Medical Industries, Inc., Los Angeles, CA 90054) tied tightly at one end. The soil-membrane unit was about 5 cm long and was placed in a 100-ml beaker filled with solutions of PEG (20,000 mol wt) at 0, 1, 3, 6, 8, and 10% w/v. After equilibration for at least 12 hr, four seedlings (pregerminated for 24 hr) were planted in each cylinder of soil and maintained in a Percival growth chamber (model MB-60B) at 25 C, 80% RH, and 12 hr per day of cool-white fluorescent light (~8,000 lux). Root-hair infection was assessed after 4 days as described above. Matric potential was determined by inserting the porous cup of a tensiometer into the cylinder of soil surrounded by the membrane and osmotic solution of PEG. At the higher concentrations of PEG (8 and 10%), the ψ_m fell below the range of the tensiometer (-700 mbars) and ψ_m values were estimated from data of Lagerwerff et al

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0031-949X/82/12159803/$03.00/0

(7).

The membranes were short-lived and began to disintegrate after 6–7 days of contact with the soil. Therefore, plants used to rate cortical infection and requiring 6 wk of growth had to be removed from the membranes after 5 days and transplanted into plastic cylinders in the greenhouse. These cylinders, 10 cm in diameter and 20 cm high, were placed in shallow watering pans and then filled up to about 10 cm from the top with a packed, well-moistened, but not saturated soil. A 2-cm layer of moist sand was then added. On top of the sand more soil premoistened to the ψ_m being tested was added. The soil/seedling units were transplanted into this top layer of soil. Water was added only to the bottom of the cylinders in quantities that did not allow the bottom soil to become saturated. Under these conditions, the top layer of soil slowly dried. Therefore, the constant, selected soil ψ_m was only maintained for the first 5–6 days while the soil was in the membranes, then the ψ_m slowly decreased as monitored by a tensiometer in representative cylinders. If the bottom soil became saturated, moisture crossed the sand barrier and saturated the top soil, invalidating the experiment.

RESULTS

The effect of matric potential in a silt loam soil on root hair and cortical infection by *P. brassicae* using tension plates and a membrane/PEG system to maintain desired ψ_m are shown in Table 1. With both methods, cortical infection did not occur below −150 mbars, while root-hair infection, although decreasing as the ψ_m decreased, occurred down to −500 and −800 mbars, respectively.

The effect of ψ_m values obtained with tension plates on cortical infection in a silt loam, sandy loam, and muck soil is given in Table 2. In both the silt and sandy loam soils, there was no cortical infection below −150 mbars, whereas in the muck soil cortical infection occurred down to −200 mbars.

The relationship of matric potential to percent maximum water-holding capacity is given in Fig. 1, while Fig. 2 shows the relationship of ψ_m to the percent water retained per unit volume of soil.

TABLE 1. Root-hair and cortical infection of Chinese cabbage by *Plasmodiophora brassicae* in a silt loam soil at different water matric potentials maintained with tension plates (TP) and with a membrane-polyethylene glycol (PEG) system

Matric potential[x] (mbar)	Method used to maintain matric potential	Root-hair infections[y] (no. per cm root)	Plants with cortical infection[z] (%)
0	PEG	167 ± 44	83 a
−10	TP	160 ± 50	96 a
−85	TP	125 ± 15	55 b
−90	PEG	129 ± 35	40 b
−100	TP	100 ± 20	28 c
−150	TP	70 ± 20	0 d
−200	TP	36 ± 8	0 d
−200	PEG	83 ± 25	0 d
−500	PEG	12 ± 5	0 d
−800	PEG	2 ± 1	0 d
−1,000	PEG	0	0 d

[x] Matric potentials determined by height of water column when using tension plates (TP) and by a tensiometer down to −700 mbars when using a membrane-polyethylene glycol (PEG) system. Matric potentials below −700 mbars in the PEG system were estimated from J. V. Lagerwerff et al (7).

[y] Combined means with standard error based on three TP experiments and four PEG experiments.

[z] Combined means of three TP experiments with three replications and four PEG experiments with four replications. Numbers followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Cortical infection of Chinese cabbage by *Plasmodiophora brassicae* in three soil types at different matric potentials maintained with tension plates

Matric potential[y] (mbar)	Percentage of plants infected[z]		
	Silt loam	Sandy loam	Muck soil
−10	96 a	98 a	100 a
−50	...	88 a	100 a
−100	28 b	47 b	83 a
−150	0 c	0 c	87 a
−200	0 c	0 c	58 b

[y] Matric potentials determined by height of water column from reservoir to tension plate.

[z] Based on cortical infection after 6 wk of plant growth. Combine percentages of at least two experiments. Numbers in each column followed by the same letter are not significantly different (Duncan's multiple range test, $P = 0.05$).

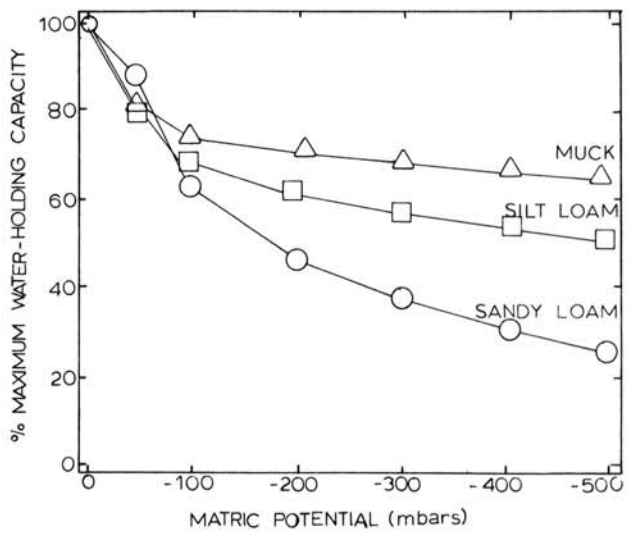

Fig. 1. Relationship between matric potential and soil moisture as percent maximum water-holding capacity (actual weight H_2O/weight H_2O at saturation per unit weight of soil × 100) in three soil types. At saturation, the ratio of soil wt: wt H_2O was 1:1 for muck, 1:0.46 for silt loam, and 1:0.37 for sandy loam soils.

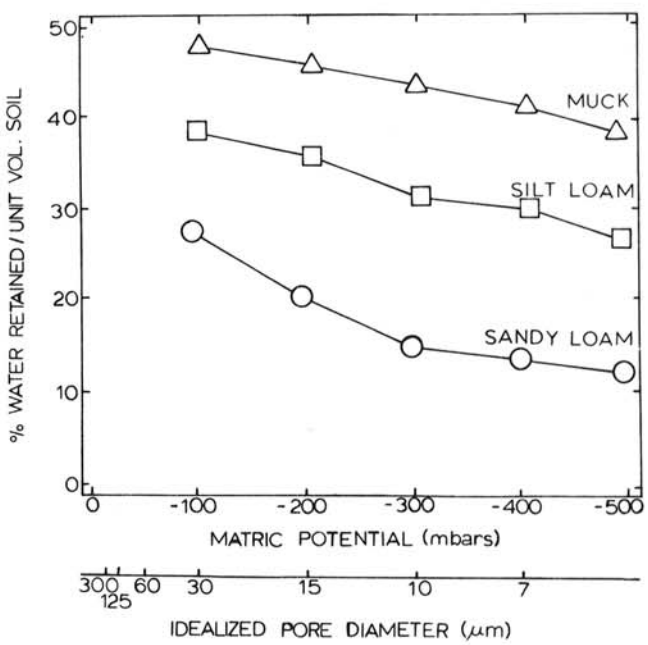

Fig. 2. Water retention (cm^3 of H_2O/cm^3 of soil × 100) in three soil types as a function of soil matric potential (mbars) and diameter of idealized water-filled soil pores (μm). Relationship between idealized pore diameter and matric potentials are taken from Cook and Papendick (2).

DISCUSSION

Cortical infection required a soil ψ_m greater than −150 mbars in the mineral soils and down to −200 mbars in muck soil. These ψ_m represent water contents of 50 to above 70% of the maximum water-holding capacity depending on the soil type. This is in close agreement with moisture levels (45% maximum water-holding capacity) reported (6) necessary for infection by *P. brassicae*.

The advantage of expressing soil water in terms of ψ_m is that it measures the free energy of water and is directly related to the idealized diameter of water-filled soil pores as calculated by Cook and Papendick (2). A ψ_m of −150 mbars corresponds to idealized pores up to about 20 μm in diameter being filled with water (2). Below this ψ_m (smaller water-filled pores) we observed no cortical infection. The diameter of zoospores of *P. brassicae* is about 3 μm. Other zoospores have also been reported to require water-filled pores many times larger than their own diameter for movement (2,11), which probably reflects the less-than-ideal conditions in field soils.

In a muck soil, however, cortical infection occurred at −200 mbars. This may be explained by ease of zoospore movement or by more rapid movement of water in muck soils.

Zoospore movement would be less restricted in soils with a greater frequency and continuity of water-filled pores of appropriate size. Muck soil retained more moisture per unit volume than the mineral loam soils at ψ_m of −100 mbars or greater (Fig. 2). Thus, muck soils must have greater attractive forces to retain water and/or a larger number of pores in which water is retained, thereby increasing the frequency and continuity of pores. This is supported in that more infection (Table 2) occurred at −100 mbars in sandy loam soil (47%) with a greater frequency of larger pores than in the silt loam soil (28%) with a lower frequency of larger pores.

As roots remove water from soils, the ψ_m in the rhizosphere may be significantly lower than that measured for the entire soil mass. If there are more and/or larger water-filled pores in muck soil than in mineral soils and, therefore, increased hydraulic conductivity, water could move more rapidly into the depleted rhizosphere. Thus, the ψ_m measured in muck soil may more closely approximate the ψ_m in the rhizosphere in mineral soils. However, the relatively high level of conductivity in both muck and mineral soils above field capacity (~−300 mbars) should minimize this effect.

There was no reason to suspect that I° and II° zoospores would show different requirements for soil moisture considering that they are indistinguishable in size and motion (6). Data collected from both the tension plates and the membrane/PEG system (Table 1), however, showed that while cortical infection in the silt loam soil was confined to ψ_m values greater than −150 mbar, root-hair infection occurred at ψ_m values between −150 and −500 mbars and even at about −800 mbars in the membrane/PEG system. Although care was taken in the membrane/PEG system to eliminate all membranes showing signs of disintegration, we have some concern for the validity of the root-hair infection data at −500 and −800 mbars.

There are several possible explanations why root-hair infection can occur at lower soil ψ_m than cortical infection. (i) Resting spores of *P. brassicae* found immediately adjacent to root hairs may be able to germinate and infect the root hair with little need to move through water-filled pores, thus allowing such infection to occur at low ψ_m. In contrast, if II° zoospores must emerge from root hairs and swim from exit pores to cortical cells, this would require significant movement through water-filled pores and, thus, a higher ψ_m. (ii) Amoeboid behavior of I° zoospores has been reported (6) and this may allow movement in soils at lower ψ_m than II° zoospores. (iii) Cortical infection, leading to clubroot formation, may require the fusion of I° or II° zoospores prior to penetration. These fused zoospores, being larger, might require larger water-filled soil pores for movement. *Polymyxa betae* (Keskin), which is closely related to *P. brassicae* and has a similar life cycle, requires zoospore fusion prior to infection (6). (iv) If one assumes that cortical infection must follow root-hair infection and the release of II° zoospores, then removal of water from the rhizosphere by the plants for 3–4 days before II° zoospores are present may result in a rhizosphere ψ_m considerably lower than that measured for the soil mass (8). However, relatively high water conductivity rates in soils above field capacity (~−300 mbars) argues against this possibility. (v) Finally, the high ψ_m required for cortical infection may reflect its influence on some other factor (eg, the release of zoospores from root-hair sporangia, host predisposition, or other factors).

The data presented herein do not directly identify the infection process (such as sporangial and/or spore germination, zoospore movement in soils, root penetration, or root colonization) affected by soil moisture. However, the effects appear to be explainable by the relation of soil ψ_m and its influence on zoospore movement.

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