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Influence of humidity on incidence of Didymella bryoniae on cucumber leaves and growing tips under controlled environmental conditions

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Abstract

The influence of relative humidity, leaf wetting, mechanical injury and inoculum concentration on the incidence of *Didymella bryoniae* on growing tips and young and older leaves of cucumber was studied in growth chambers.

Infection was rare at 60% r.h. It increased at 95% r.h. and was most serious if the leaves were kept wet. A period of 1 hour of free water was sufficient for the initial stage of infection. For further expansion of the disease, leaf wetness was required.

A high relative humidity did not predispose leaves to infection by *D. bryoniae.*

Wounding was essential for infection of older leaves, but not for infection of young plant tissue.

A higher conidial concentration increased infection. Without keeping the leaves wet at 95% r.h. a tenfold conidial concentration was needed to get equal infection as with leaf wetting.

To control the disease by means of the climate, it is of major importance to prevent the presence of free water on plant parts.

Additional keywords: Cucumis sativus, Mycosphaerella citrullina, Mycosphaerella melonis.

Introduction

Fruits and foliage of a cucumber crop can be attacked by *Didymella bryoniae* (Auersw.) Rehm. (synonyms: *Mycosphaerella citrullina* (C.O. Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu and Walker). Symptoms and economic importance of the disease have been described elsewhere (Van Steekelenburg, 1978, 1982; Van Steekelenburg and Van de Vooren, 1981). The incidence of the disease is influenced by the glasshouse climate with humidity as a major factor (Van Steekelenburg, 1984, 1985; Van Steekelenburg and Van de Vooren, 1981). In the present study it has been investigated whether a high relative humidity as such or leaf wetness was required for infection and further expansion of the disease. As under glasshouse conditions humidity and temperature fluctuate and interact continuously, experiments were car-

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ried out under controlled environmental conditions. Svedilius and Unestam (1978), using low light intensities of 1000 to 10 000 lux, found continuously wetting of cucumber leaves necessary to obtain infection. In view of the fact that these intensities are much lower than those in practice, the experiments reported here were carried out at 30 000 lux, a light intensity present on an average spring day. In addition to infection of the leaves, also infection of growing tips was investigated, as it is known that the latter can cause yield reduction (Van Steekelenburg, 1985).

Materials and methods

Plants. Seedlings of cultivar Farbio were potted in 12 cm plastic pots three days after sowing and grown under glasshouse conditions until inoculation.

Pathogen. The virulent isolate M74-3 of *D. bryoniae* was used. This isolate had been taken from diseased cucumbers from a commercial glasshouse in 1974 and has been maintained on oatmeal agar.

Inoculation. Inoculum was prepared as described elsewhere (Van Steekelenburg, 1985).

To obtain infection on growing tips and young leaves, plants were inoculated in the first to fourth leaf stage. Per plant, about 4 ml inoculum suspension (10^6 conidia per ml) was sprayed with a Sprayon sprayset.

To obtain infection on older leaves, both the second and third leaf, each with a diameter of more than 10 cm, were inoculated with 0.02 ml droplets with suspended conidia. Per leaf 12 droplets were placed onto the upper surface of intact or mechanically injured leaves. Leaves were injured by pressing a 2 mm cork borer onto their surface.

Environmental conditions. Incubation took place in one of the two available growth chambers (Karl Weiss ZK 2200 E/+4JU-P-S). Each chamber was equipped with two Elka Airfog atomizers to spray the plants with demineralised water. To keep the leaves continuously wet atomizing took place with the aid of a time-clock for 2.5 min each half hour. The air temperature was 23 °C. The dew point temperature was 23 °C for a r.h. of 95% and 18 °C for a r.h. of 60%. The photoperiod was 12 h with a light intensity of 30 000 lux (90% number 33 and 10% Philinea fluorescent tubes).

Disease assessment. After one week incubation in a growth chamber the disease incidence on the growing tip and youngest leaves was rated. On the growing tip it was assessed according to a scale from 0 to 4 in which 0 = no symptoms, 1 = slight malformation, 2 = moderate malformation, 3 = severe malformation and 4 = dead growing tip. For the disease incidence on the young leaves only those which had a diameter of less than 6 cm at the time of inoculation were used for the assessment. Scale number 1 was given when 0.1 cm^2 necrotic leaf area or 10 small yellow lesions on a leaf were present. Scale number 2 was given when 0.2 cm^2 necrotic leaf area or 20 small yellow lesions were present, etc.

Inoculation sites of older leaves were inspected macroscopically for fungal infection one week after inoculation.

Statistical analysis. Effects of treatments were evaluated by analysis of variance.

Results

Disease incidence on growing tips and young leaves. Disease incidence on growing tips was rather variable and no effect of regular leaf wetting at 95% r.h. was observed. The growing tip was not diseased at 60% r.h. (Table 1).

With regular leaf wetting both small yellow lesions on the leaves and brown spots at leaf margins developed. Without leaf wetting only small yellow lesions on the leaves were observed. This resulted in a great difference in disease incidence between wetted and unwetted leaves at 95% r.h. Only occasionally a yellow lesion on the leaf developed at 60% r.h. (Table 1).

In experiments with continuous and alternating periods at 95 and 60% r.h. development of the disease on a growing tip occurred even at continuously 60% r.h. The mean disease incidence on the growing tip became only substantial at continuously 95% r.h. The disease incidence on the leaves increased at a rate proportionate to the period the plants were first incubated at 95% r.h. and decreased at a rate proportionate to the period the plants were first incubated at 60% r.h. (Table 2).

Disease incidence on older leaves. Without wounding of the leaves hardly any infection was observed (Table 3). After wounding, the percentage of infected sites was significantly higher ($p < 0.01$) at 95% r.h. than at 60% r.h. (Table 4) and at 95% r.h. it was significantly higher ($p = 0.02$) with leaf wetting than without leaf wetting (Table 3). At infected sites only a yellow discoloration of leaf tissue around the circle made by the cork borer was observed at 60% r.h. A number of infected sites showed brown necrotic lesions at 95% r.h., especially at high inoculum concentrations. With leaf wetting at 95% r.h. the brown lesions were more common and larger than without leaf wetting. The formation of pycnidia was observed only in brown leaf tissue.

Table 1. Effect of humidity on the incidence of *D. bryoniae* on the growing tip and young leaves of cucumber plants (means of 4 experiments, each with 4 plants per treatment).

Humidity conditions	Disease incidence			
	first series of exp.		second series of exp.	
	growing tip[1]	leaf[2]	growing tip	leaf
95% r.h. + leaf wetting	1.2 a[3]	2.90 p	—	—
95% r.h., no leaf wetting	1.5 a	0.90 q	2.4 a	1.12 q
60% r.h., no leaf wetting	—	—	0 b	0.01 r

[1] Disease index from 0 = no symptoms to 4 = growing tip dead.

[2] Disease index in which 1 = 0.1 cm² necrotic leaf area or 10 small yellow lesions, 2 = 0.2 cm² necrotic leaf area or 20 small yellow lesions, etc.

[3] Values in one column followed by a different letter differ significantly at $p < 0.05$ (analysis of variance).

Table 2. Effect of varying periods of different humidities on the incidence of *D. bryoniae* on growing tip and leaves of cucumber plants (means of 5 experiments, each with 2 plants per treatment).

Length of periods (days)		95% r.h. followed by 60% r.h.		60% r.h. followed by 95% r.h.	
first period	second period	growing tip[1]	leaf[2]	growing tip	leaf
0	7	0.4 a[3]	0.02 a	2.0 a	1.36 a
1	6	0.4 a	0.12 a	0.6 b	0.58 b
2	5	0.8 a	0.20 a	0.6 b	0.30 b
4	3	0.6 a	0.54 a	0.4 b	0.20 b
7	0	2.0 b	1.36 b	0.4 b	0.02 b

[1] and [2] see Table 1.
[3] Values in one column followed by a different letter differ significantly at $p < 0.05$ (LSD test).

Table 3. Effect of wetting and wounding the leaf surface on the percentage of infected sites on cucumber leaves at a relative humidity of 95% after inoculation with droplets of different conidial concentrations (means of 4 experiments, each with 48 inoculation sites per treatment).

Number of conidia per site	Leaf wetting		No leaf wetting	
	no wounding	wounding	no wounding	wounding
20	1.6	42.2	1.0	7.8
200	2.6	68.2	1.0	38.0
2 000	4.7	85.9	3.1	65.1
20 000	5.2	95.3	4.2	82.3

A significant effect of wounding ($p < 0.01$), of inoculum concentration ($p < 0.01$) and of wetting wounded leaves ($p = 0.02$), but no effect of wetting unwounded leaves (analysis of variance).

Table 4. Effect of forced drying of the inoculum droplets at 95 and 60% r.h. on the percentage of infected sites on wounded cucumber leaves after inoculation with droplets of different conidial concentrations (means of 3 experiments, each with 48 inoculation sites per treatment).

Number of conidia per site	95% r.h.		60% r.h.	
	no drying	drying	no drying	drying
20	34.7	34.7	0	1.4
200	52.1	56.9	5.6	3.5
2 000	77.8	77.8	11.8	15.3
20 000	91.7	88.2	23.6	16.0

A significant effect of relative humidity ($p < 0.01$) and of inoculum concentration ($p < 0.01$), but no effect of forced drying (analysis of variance).

Table 5. Effect of relative humidity on plant characteristics, viz. dry matter content and diameter of the laminae of cucumber leaves, and on infectivity of wounded leaves with inoculum droplets of *D. bryoniae* (means of 2 experiments of 5 plants with 3 leaves each and 72 inoculation sites per treatment, respectively).

r.h. before inoculation	Plant characteristics		Percentage infected sites at r.h. after inoculation	
	dry matter (%)	diameter (cm)	60%	95%
60%	13.8 a[1]	12.7 a	8 a	85 a
95%	10.1 b	16.7 b	7 a	83 a

[1] Values in one column followed by a different letter differ significantly at $p < 0.05$ (analysis of variance).

The droplets had visibly dried up after 12 h at 95% r.h., after 2 h at 60% r.h. and after 1 h with forced drying in an air stream. No effect of forced drying of the droplets on infection was observed (Table 4).

The inoculum concentration had a significant effect ($p < 0.01$) on the level of infection (Tables 3 and 4). The effective dose causing 50% infection on wounded leaves at 95% r.h. with leaf wetting was 40 and without leaf wetting 400 conidia per site.

No difference in disease incidence was observed after wounding and inoculating the leaves of plants grown constantly at 60 or 95% r.h. Plants grown at 95% r.h. were different from those grown at 60% r.h. At 95% r.h. the diameter of the leaves was 31% larger and the dry matter content of the lamina was 27% lower than at 60% r.h. (Table 5).

Discussion and conclusions

Wounding of young plant tissue such as the growing tip was not necessary for infection by conidia of *D. bryoniae*. However, wounding was essential for infection of older plant tissue such as leaves (Table 3) and fruits (Van Steekelenburg, 1982). The fact that wounding is not essential for infection of young plant tissue may be explained by the absence of a developed wax layer on young leaves. Penetration of host tissue is direct or through intercellular spaces around the basal cells of abraded trichomes (Chiu and Walker, 1949).

Infection of growing tips and young and older leaves was rare at 60% r.h. (Tables 1, 2, 4 and 5). The time needed for drying up of the water of the inoculum suspension at 60% r.h., viz. 1 h, was still occasionally sufficient for the initial phase of infection. A higher relative humidity favoured the disease incidence on growing tips and young and older leaves. The presence of free water increased the incidence (Tables 1 and 3) and the severity of the disease on young and wounded older leaves. Without leaf wetness usually small chlorotic lesions developed without any further expansion in necrotic tissue.

On young leaves the restricted infection, resulting in scattered small yellow lesions without further disease development, may be explained by the host defence

mechanisms. At higher humidities there may be more infection sites resulting in lesions close together by which the host defence mechanism may be broken down.

On wounded older leaves, the more severe disease development at higher humidities may be the result of an increase in the number of penetrating hyphae of the fungus. The infection rate on wounded older leaves increased at a higher conidial concentration (Tables 3 and 4; Svedelius and Unestam, 1978). Besides, there was an interaction between conidial concentration and humidity. The effect of leaf wetting at 95% r.h. on the number of infected sites was comparable with the effect of a tenfold conidial concentration.

According to Svedelius and Unestam (1978) the raised infectivity on wounded leaves is the result of the availability of nutrients for fungal growth. However, any mechanical damage to plant tissue may facilitate the entry of the fungus. *D. bryoniae* is a weak parasite requiring special conditions for infection and senescent tissue is readily invaded. The infectivity of the fungus is determined by the physiological age and health status of the plant tissue. *D. bryoniae* is in many ways comparable with *Botrytis cinerea,* another important fungal disease of glasshouse cucumbers and other crops.

Although plants grown at 95% r.h. were different from those grown at 60% r.h., no different susceptibility to infection by *D. bryoniae* was observed (Table 5).

For a serious disease development of *D. bryoniae* long periods of free water or a high relative humidity in combination with a high conidial concentration were needed. To decrease the incidence of the disease in commercial cucumber crops it is of utmost importance to prevent the presence of free water on plant parts. Long periods with high humidity and condensation on plant parts can be decreased by a proper ventilation practice (Van Steekelenburg, 1984, 1985; Van Steekelenburg and Van de Vooren, 1981).

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Samenvatting

Invloed van vocht op het optreden van Didymella bryoniae op komkommerbladeren en -groeipunten onder geconditioneerde klimaatsomstandigheden

De invloed van de relatieve luchtvochtigheid, het bevochtigen van het blad, mechanische beschadiging en inoculumconcentratie op het optreden van *Didymella bryoniae* op groeipunten en jonge en oudere bladeren van komkommer is in klimaatkasten onderzocht.

Aantasting kwam zelden voor bij 60% R.V., nam toe bij 95% R.V. en was het ernstigst als de bladeren nat werden gehouden. Voor de eerste fase van infectie was de aanwezigheid van vrij water gedurende 1 à 2 uur voldoende. Voor een verdere uitbreiding van de aantasting moest het blad nat zijn.

Een hoge relatieve luchtvochtigheid had geen predispositie-effect op de infectie van bladeren door *D. bryoniae.*

Voor de infectie van oudere bladeren was verwonding nodig, voor die van jong planteweefsel niet.

Een hogere concentratie van conidiën verhoogde de aantasting. Zonder het blad nat te houden, was een tienvoudige concentratie van conidiën nodig om een gelijke infectie te verkrijgen als met bladbevochtiging.

Voor de bestrijding van de ziekte via het klimaat is het tegengaan van de aanwezigheid van vrij water op plantedelen van het grootste belang.

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