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Factors influencing external fruit rot of cucumber caused by Didymella bryoniae

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Abstract

Several factors influencing the occurrence and extent of external fruit rot caused by *Didymella bryoniae* on cucumbers in the post harvest period were studied.

The minimum, optimum and maximum temperatures for growth of the fungus on fruits were circa 10, 23 and 35 °C, respectively. The influence of the temperature on the growth of the fungus in vitro and in vivo was about similar. The fitness of the fungus diminished by storing inoculated fruits at about the maximum temperature for growth of the fungus for one day, but this temperature influenced fruit quality negatively. Storing at 10 to 12 °C is more advisable.

Isolates of *D. bryoniae* showed variation in virulence. There was a linear relationship between growth on fruits and growth in vitro of these isolates, but no correlation was found with disease incidence on plants.

The degree of fruit rot was increased by more severe wounding, by storing in the dark instead of in the light and by higher nitrogen fertilization of the crop. Relative humidity during storage had no effect on fruit decay. It is very likely that the amount and composition of available nutrients for fungus growth determine the degree of rotting of the fruits.

With the present cultivars, external fruit rot can be best controlled by reducing the changes of wounding in the pre- and post-harvest period.

Additional keywords: Cucumis sativus, Mycosphaerella citrullina, Mycosphaerella melonis, post harvest disease, stem and fruit rot.

Introduction

Didymella bryoniae (Auersw.) Rehm, synonyms: *Mycosphaerella citrullina* (C.O.Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu and Walker, causes a variety of symptoms in cucumber *(Cucumis sativus L.)* grown in glasshouses in the Netherlands and other countries. Foliage and fruits can be attacked. Fruit rot, both external and internal, is the most troublesome as often fruits decay during storage and handling after being harvested. Sometimes four out of the twelve fruits per box stored during one or a few warm days on a wholesale market show lesions. Rotting of stored cucumbers and melons can be caused by several pathogens like *Botrytis cinerea, Sclerotinia sclerotiorum, D. bryoniae* and bacteria as has been observed by myself and others

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Fig. 1. External rot on cucumber fruits, caused by *D. bryoniae.*

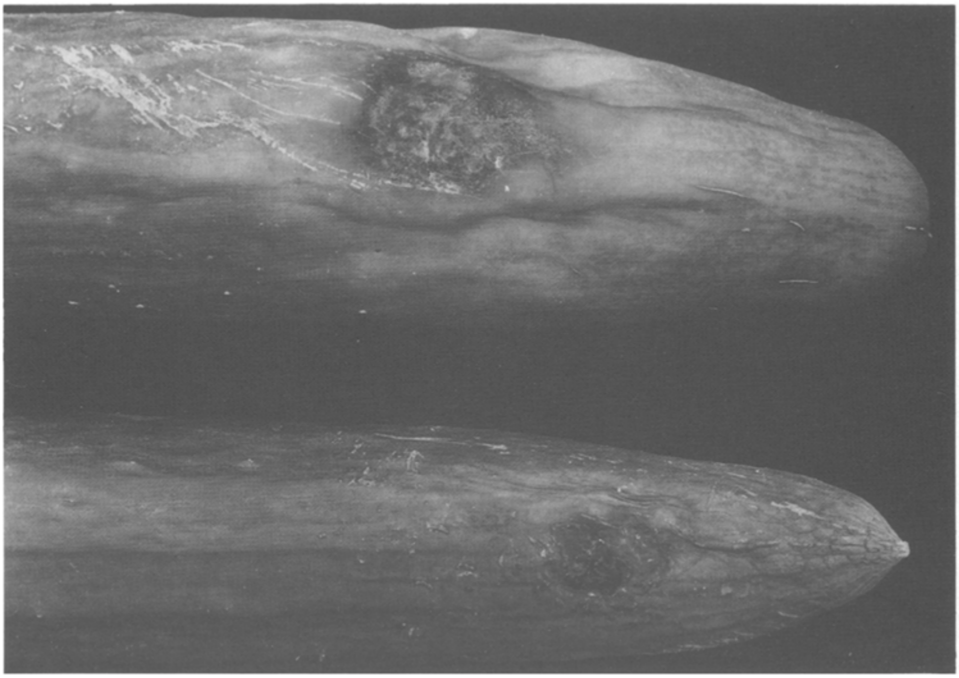

Fig. 1. Uitwendig rot op komkommervruchten veroorzaakt door D. bryoniae.

(Ceponis and Butterfield, 1974; Luepschen, 1961; Saito 1976), but *D. bryoniae* is the main organism involved in the rotting of Dutch cucumbers. With internal rot, *D. bryoniae* produces a brown heart rot at the blossem end of the fruit with no external discolouration. With external rot, lesions can occur all over the fruit and somewhat irregularly circular spots, first yellow to light brown in colour but soon turning black, are produced by *D. bryoniae* (Fig. 1). Beneath this dark sunken lesions an extensive rot is found. The main diagnostic feature is the abundant development of black pycnidia followed by perithecia.

After storing apparently healthy fruits under warm and humid conditions up to 25% of the fruits, depending on the fungicides sprayed on the crop, showed lesions caused by *D. bryoniae* (Van Steekelenburg, 1978). The percentage of fruits with internal rot varies from harvesting date to harvesting date, with 14% as the highest recorded (Van Steekelenburg and Van de Vooren, 1981). Apparently external fruit rot is as important as, or even more important than, internal fruit rot. The influence of several factors on the occurrence and extent of external fruit rot are described in this paper. The internal fruit rot problem is still under investigation.

Materials and methods

Picked marketable fruits of the female flowering cultivar Farbio, grown under commercial conditions, were wounded with a cork borer of 5 mm ø to a depth of 5 mm, unless stated otherwise, and inoculated with a highly virulent isolate of *D. bryoniae.* The inoculum consisted of 5 mm discs of a 14-day-old culture of the fungus grown on cherry decoction agar and exposed to black light (Philips TL 20 W F20 T12 BLB) to induce sporulation. Each fruit was inoculated at three sites and incubated in darkness in cabinets of 0.6 m^3 in which temperature and relative humidity could be controlled very accurately or in a glass box of approximately 1 m^3 with an air humidity at saturation point. Usually the incubation temperature was 23 °C.

Two measurements, perpendicular to each other, of the diameter of each lesion on the fruit four to seven days after inoculation were taken. The mean diameter of the lesions minus the diameter of the wound is given in the tables and figures.

To study the effect of the soil nitrogen level, plants were grown separately in styropor boxes filled with 15 l of commercial potting soil and watered by drip irrigation. Per litre irrigation water 0.5 g K_2SO_4 and 0.2 g $MgSO_4.7H_2O$ was given and nitrogen was supplied at four levels, viz. 0, 0.15, 0.30 and 0.45 g NH_4NO_3 per litre water. Fruits of two harvesting dates, about 10 and 12 weeks after planting respectively, were inoculated. Subsequently the plants were sprayed with a conidial suspension of *D. bryoniae* to study the difference in plant susceptibility between the N levels. The number of *D. bryoniae* lesions on the main stem of the plants was counted and the total surface area of these lesions was measured four weeks afterwards.

Results

Wounding. Fruits were wounded with a 5 mm ø cork borer to a depth of 1, 2.5 and 5 mm and with a 1 mm ø needle to a depth of 1 and 5 mm to study the effect of wounds on the occurrence of rot. The inoculum was inserted into or applied to these wounds. No rot occurred on inoculated unwounded fruits or on fruits inoculated after needle puncture, to whatever depth. With wounds 5 mm in diameter the percentages of lesions and the diameter of these lesions increased with increased depth of wounding (Table 1).

Table 1. Effect of depth of 5 mm ø wounds on percentages of lesions produced and on lesion diameters four days after inoculation of cucumber fruits with *D. bryoniae.*

Depth of wounding (mm)	Percentage of lesions[1]			Lesion diameter (mm)		
	Exp. I	Exp. II	Exp. III	Exp. I	Exp. II	Exp. III
1	42	58	22	8	7	7
2.5	–	–	80	–	–	9
5	98	100	94	14	15	20

[1] Based on 60 sites per treatment.

Tabel 1. Invloed van de diepte van wonden, 5 mm in doorsnede, op het percentage gevormde lesies en op de lesie-diameter, vier dagen na inoculatie met D. bryoniae.

Fig. 2. Effect of temperature on lesion diameters of inoculated cucumber fruits (———) and on colony diameters of *D. bryoniae* on PDA (– – –) (means of 30 and 10 replicates respectively at each temperature tested in three experiments of 11-20°, 17-29° and 26-35°C respectively).

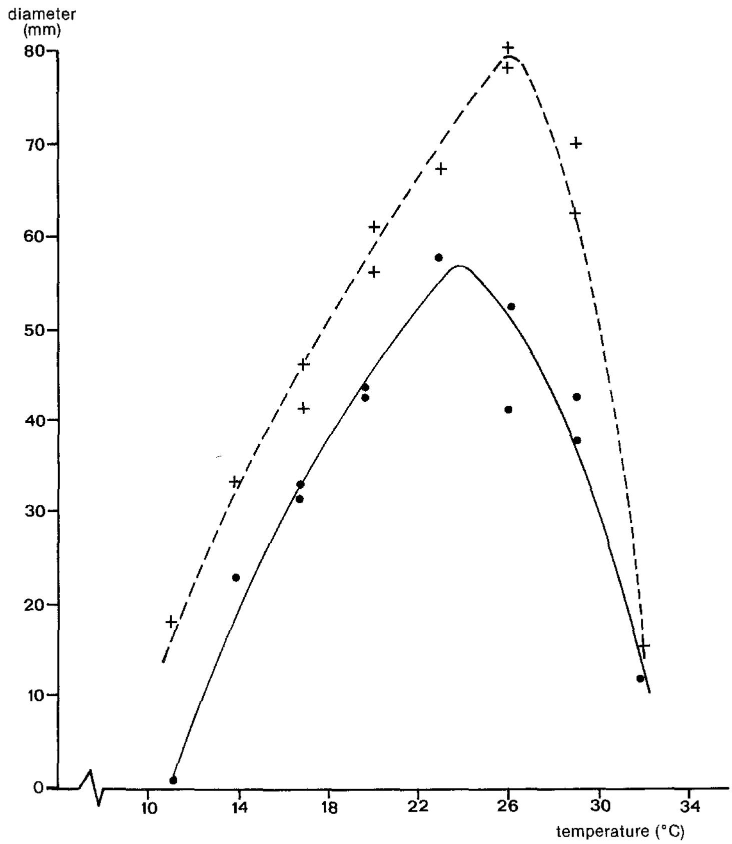

Fig. 2. Effect van de temperatuur op de diameter van de rotte plek van geïnoculeerde komkommervruchten (———) en op de koloniediameter van D. bryoniae op PDA (– – –) (gemiddelden van respectievelijk 30 en 10 herhalingen per getoetste temperatuur in drie experimenten van respectievelijk 11-20°, 17-29° en 26-35°C).

Temperature. The effect of temperature on lesion development on fruits and on colony diameter on potato dextrose agar (PDA) is given in Fig. 2. The minimum, optimum and maximum temperatures for growth in vitro and vivo were about 5, 26, 35 °C and 10, 23, 35 °C, respectively. Fruits stored during the first day after inoculation at about the minimum temperature for growth of the fungus rotted afterwards at 20 °C significantly more severely than fruits stored continuously at 20 °C, whereas fruits stored during the first day at about the maximum temperature for fungus growth rotted afterwards at 20 °C significantly less severely (Table 2). Inoculated fruits were incubated for 0, 4, 8, 16 and 24 h at 20 °C before storing at 11 °C, the normally recommended temperature for weekend storage. A 4 h incubation period

Table 2. Influence of storage temperature during the first day after inoculation with *D. bryoniae* on the lesion diameters of wounded cucumber fruits.

Temperature (°C) during		Lesion diameter (mm)[1]
first day	next six days	
6	20	60[a2]
20	20	48[b]
36	20	24[c]

[1] Based on 30 inoculation sites per treatment.
[2] Entries marked with different letters differ significantly at $P < 0.05$ (Studentized range in connection with an analysis of variance).

Tabel 2. Invloed van de temperatuur gedurende de eerste dag na inoculatie met D. bryoniae op de diameter van de rotte plek op verwonde komkommervruchten.

at 20 °C did not, but a 8 h period did stimulate the rotting of fruits subsequently held at 11 °C, compared with fruits continuously incubated at 11 °C.

Relative humidity. The results of the experiments on the influence of relative humidity are given in Table 3. No significant differences in rotting were observed between the humidities tested except that in one experiment the fruits at 50% r.h. rotted significantly less than at higher humidities.

Light and darkness. The experiments on the influence of light on fruit decay were conducted in a serial thermostat. The boxes, 50 cm long, 50 cm wide and 25 cm high were closed with double glass either covered with a black plastic sheet or illuminated

Table 3. Influence of relative humidity on lesion diameters on wounded cucumber fruits inoculated with *D. bryoniae*.

Relative humidity (%)	Lesion diameter (mm)[1]		
	Exp. I	Exp. II	Exp. III
50	38[a2]	29[a]	46[a]
65	45[b]	32[a]	47[a]
80	45[b]	34[a]	43[a]
95	45[b]	34[a]	47[a]

[1] Based on 30 sites per treatment.
[2] Entries of one experiment marked with different letters differ significantly at $P < 0.05$ (Studentized range in connection with an analysis of variance).

Tabel 3. Invloed van de relatieve luchtvochtigheid op de diameter van de rotte plek op verwonde komkommervruchten die met D. bryoniae zijn geïnoculeerd.

Table 4. Lesion diameters of *D. bryoniae*-inoculated wounded cucumber fruits stored in the light or dark.

Ligt or darkness	Lesion diameter (mm)[1]		
	Exp. I	Exp. II	Exp. III
Light	10[a2]	30[a]	52[a]
Dark	20[b]	48[b]	62[b]

[1] Based on 30 sites per treatment.
[2] Entries of one experiment marked with different letters differ significantly at $P < 0.05$ (analysis of variance).

Tabel 4. Diameter van de rotte plek op verwonde, met D. bryoniae geïnoculeerde komkommervruchten die in het licht of in het donker werden bewaard.

with two fluorescent tubes of 20 W each. The results are given in Table 4. Fruits rotted significantly more in the dark than in the light. There was no difference in fungal growth on PDA in the light and in the dark.

Isolate. Various isolates collected during several years were tested for their virulence on cucumber fruits. The diameter of the lesion on the fruit was compared with the radial growth of the fungus on PDA. The isolates showed differences in virulence, and the growth in vitro and in vivo of these isolates was linearly related with a correlation coefficient of 0.83 (Fig. 3). Variation in virulence between isolates was not

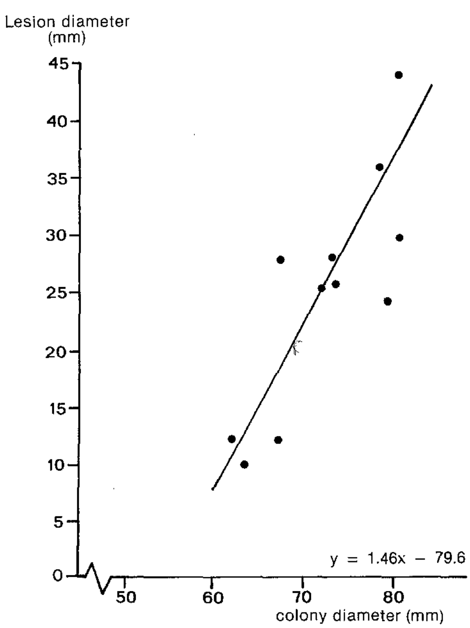

Fig. 3 Relationship between colony diameter on PDA (means of 3 replicates) and lesion diameter on cucumber fruits (means of 10 inoculation sites) of 11 isolates of *D. bryoniae*.

Fig. 3. Relatie tussen de diameter van de kolonie op PDA (gemiddelden van 3 herhalingen) en de diameter van de rotte plek op komkommervruchten (gemiddelden van 10 inoculatieplaatsen) bij 11 isolaten van D. bryoniae.

Table 5. Lesion diameter of *D. bryoniae*-inoculated wounded cucumber fruits of plants fertilized with four levels of NH_4NO_3.

Level of NH_4NO_3 ($g.l^{-1}$)	Lesion diameter (mm)[1]	
	Exp. I	Exp. II
0.0	5^{a2}	4^{a}
0.15	8^{ab}	9^{b}
0.30	10^{b}	10^{bc}
0.45	18^{c}	13^{c}

[1] Based on 60 (Exp. I) or 40 (Exp. II) sites per treatment.
[2] Entries of one experiment marked with different letters differ significantly at $P < 0.05$ (Studentized range in connection with an analysis of variance).

Tabel 5. Diameter van de rotte plek op verwonde, met D. bryoniae geïnoculeerde komkommervruchten van planten die met verschillende hoeveelheden NH_4NO_3 zijn bemest.

correlated with the duration of their cultivation on an artificial medium. Virulent isolates did not lose their virulence even after subculturing in vitro for more than five years.

Nitrogen fertilization. Seven weeks after planting, leaf colour differed between the treatments with different N levels. Where no nitrogen was added some leaves had turned yellow. The colour of the leaves varied from light green to dark green in the treatments with 0.15, 0.30 and 0.45 g NH_4NO_3 per litre irrigation water. Soil analysis at that time proved that the amount of nitrogen in the soil of the four treatments was very low, low, moderate and normal, respectively. The results of inoculating picked fruits from the different treatments in two consecutive experiments are given in Table 5. Fruits of plants grown at a higher nitrogen level rotted more severely. In the treatments with low nitrogen levels the yield of healthy fruits was lower than in the treatments with higher nitrogen levels. At the end of the cultivation period, the number and the total surface area of *D. bryoniae* lesions on the main stem of the plants at the two highest nitrogen levels was four- to fivefold that at the two lowest nitrogen levels.

Discussion and conclusions

D. bryoniae is a wound parasite as inoculated non-wounded and slightly wounded fruits did not rot at all. Apparently the peel of the fruit forms a mechanical and/or physiological barrier to fungal infection. Svedelius and Unestam (1978) demonstrated that mechanical injury facilitates fungal invasion of cucumber leaves because of release of nutrients from damaged cells rather than as a result of the rupture of the protective cutin layer.

The rotting of cucumber fruits increased progressively from 12 to 23 °C; thereafter it fell and was very limited at 32 °C. Similar results were obtained by Luepschen

(1961) with the same pathogen on watermelon. Mycelial growth of the fungus in vitro paralled decay development on cucumber fruits (Fig. 2). The in vitro growth curve is in close accordance with in vitro results of Wiant (1945) and Luepschen (1961), although Chiu and Walker (1949) reported a somewhat lower optimal temperature for fungal growth (20-24 °C). The fitness of the fungus was diminished by a short storage period of inoculated fruits at the maximum temperature but not by a short storage period at the minimum temperature for growth of the fungus (Table 2). Fruits stored at 10 to 12 °C did not rot, but an 8 h period at 20 °C, followed by storing at 11 °C, was enough to stimulate the rotting process. If cucumbers have to be stored during warm days, for instance for a weekend, it is still advisable to do so at about 12 °C to reduce the chance and/or the extent of rotting. Storing at higher temperatures will diminish also the shelf life and quality of the fruits.

External fruit rot was not, or hardly, influenced by relative humidity (Table 3). High humidity is necessary for disease development of uninjured mature leaves (unpublished data; Svedelius and Unestam, 1978). It seems probable that the release of nutrients from a damaged fruit is more important than the relative humidity.

No difference was observed between fungal extension on an agar medium in the light and the dark, although fruits rotted more extensively in the darkness than in the light (Table 4). The only possible explanation seems to be a light-induced change in the biochemistry of resistance or susceptibility of the fruit. Disease development on leaves is also increased by darkness (unpublished data; Svedelius and Unestam, 1978).

Isolates of the fungus showed variation in virulence on cucumber fruits and a good correlation could be established between extent of fruit rot and growth of the fungus in vitro (Fig. 3). Chiu and Walker (1949) reported some variation in virulence among isolates on squash plants. In unpublished tests the isolates differed in virulence on cucumber seedlings (inoculation method described by Van Steekelenburg, 1981) but no correlation could be established between the disease incidence on seedlings and the growth of the fungus in vitro.

Both stem and fruit rot were more severe in the treatments with high nitrogen levels. For stem rot this may partly be due to the more unfavourable drier microclimate for disease development in the treatments with low nitrogen levels where the plants had fewer leaves than in the treatments with higher nitrogen levels. It may also be that the sturdier plants of the lower nitrogen levels form a mechanical barrier to fungal penetration. But it is more likely that there are some differences in nutrients available for fungus growth between plants and fruits of the different treatments as, on severely wounded fruits, rot increased with higher fertilization (Table 5). For economic reasons, a lower nitrogen fertilization is not the solution to the stem and fruit rot problem in cucumber as yield will diminish too much and some rot is still possible.

The only way to control external fruit rot is to avoid wounding during picking, grading, packing and shipping by careful handling. A thicker and smoother peel may prevent wounding in the pre- and post-harvest period and subsequent rotting, but it is claimed that consumers do not want a thick peel. Still, a thicker peel or fully resistant cultivars seem to be the final solution if a zero tolerance for external fruit rot is wanted.

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Samenvatting

Factoren die uitwendig vruchtrot van komkommers, veroorzaakt door Didymella bryoniae, beïnvloeden

Verschillende factoren die van invloed kunnen zijn op het ontstaan en de mate van uitwendig vruchtrot op komkommers in de periode na de oogst, veroorzaakt door *Didymella bryoniae,* zijn onderzocht.

De minimum, optimum en maximum temperatuur voor de groei van de schimmel op vruchten waren respectievelijk circa 10, 23 en 35 °C. De invloed van de temperatuur op de groei van de schimmel in vitro en in vivo was nagenoeg gelijk. Door geïnoculeerde vruchten een dag bij de maximum temperatuur voor de groei van de schimmel te bewaren, werd de groeikracht van de schimmel verminderd, maar de vruchtkwaliteit werd door deze temperatuur negatief beïnvloed. Het is raadzamer de vruchten bij 10-12 °C te bewaren.

Isolaten van *D. bryoniae* vertoonden een variatie in virulentie. Tussen de groei van deze isolaten op vruchten en de groei in vitro bleek een lineair verband te bestaan, maar er bestond geen verband met de aantasting van planten.

De mate van vruchtrot nam toe door de vruchten ernstiger te verwonden, ze in het donker in plaats van in het licht te bewaren en door een hogere stikstofbemesting tijdens de teelt. De relatieve luchtvochtigheid tijdens de bewaarperiode had geen effect op de vruchtaantasting. De hoeveelheden en de samenstelling van de voor de groei van de schimmel beschikbare voedingsstoffen bepalen zeer waarschijnlijk de mate van vruchtrot.

Uitwendig vruchtrot kan bij de huidige cultivars nog het best worden tegengegaan door de mogelijkheden van verwonding, zowel in de periode voor als na de oogst, te verkleinen.

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