**DIDYMELLA BRYONIAE**

**ON**

**GLASSHOUSE CUCUMBERS**

Promotor: Dr. Ir. J. Dekker, hoogleraar in de fytopathologie

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
Dr. C.C. Oosterlee,
in het openbaar te verdedigen
op woensdag 17 september 1986
des namiddags te vier uur in de aula
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**STELLINGEN**

1. Bij het optreden van inwendig vruchtrot bij komkommer is het bloempje van doorslaggevende betekenis.

   Dit proefschrift.

2. Gewasresistentie tegen Didymella bryoniae leidt ook tot minder vruchtrot.

   Dit proefschrift.

3. De benaming bladvlekkenziekte voor aantasting van komkommer door Didymella bryoniae is misleidend.

   Gewasbeschermingsgids 1985:310.

4. Vaak wordt ten onrechte aangenomen dat een ziekte of plaag afkomstig is uit het land waar het optreden ervan voor het eerst is beschreven.

5. Energieschermen in de glastuinbouw verhogen de luchtvochtigheid in de kas en verlagen de kans op schimmelinfecties.

6. Niet alleen chemische, maar ook biologische bestrijding van plagen kan de export van tuinbouwprodukten in gevaar brengen.

7. Wakimoto et al. hechten ten onrechte waarde aan de gevonden verschillen in concentratie bacteriën van Corynebacterium michiganense bij de door hen getoetste cultivars van tomaat.

   Wakimoto, S., Uematsu, T. and Mukoo, H., 1986.
   Bull. Nat. Inst. Agr. Sci. Ser. C 22: 269 - 281.

8. Bestrijding van nematoden met het middel methylbromide heeft de voorkeur boven het gebruik van aldicarb en oxamyl.

9. De teelten zonder aarde in kassen bieden bij uitstek mogelijkheden voor de biologische bestrijding van wortelpathogenen.

10. Gecomposteerd plantaardig tuinafval kan niet in de intensieve glastuinbouw worden hergebruikt.

11. Doorstroming van onderzoekresultaten naar de praktijk wordt belemmerd door het huidige overheidsbeleid ten aanzien van de voorlichting.

12. Het vak geestelijke stromingen dient behalve voor het basisonderwijs ook voor het voortgezet onderwijs verplicht te zijn.

13. Organisatiestructuren komen en gaan, maar onderzoek zal blijven bestaan.

N.A.M. van Steekelenburg.

Didymella bryoniae on glasshouse cucumbers.

Wageningen, 17 september 1986.

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**CONTENTS**

# INTRODUCTION

## The cucumber industry in the Netherlands

### Production systems

Commercial production of cucumber under glass in the Netherlands began about a century ago. Production of vegetables under frames started between 1880 and 1890 (Barendse, 1951). According to Hazeloop (1897) the frames in the Loosduinen area were mainly used for cucumber production. The first single span glasshouses, specially designed for cucumber cropping were built in the Netherlands, after English example, in the beginning of this century (Nederpel, 1954). In this type of glasshouse an inclined or arch training system was applied. Commercial cropping under frames and in single span cucumber houses has been abandoned nowadays. The largest development in cucumber production was in the fifties and sixties when the upright training system in multi-span glasshouses found acceptance.
This system requires less labour and gives higher yields. One of the benefits of multi-span glasshouses is that they allow a flexible change to another crop.

Early-planted crops were traditionally grown on horse manure, which was later replaced by straw bales. The decomposition of the manure and straw provided heating of the root zone. In the seventies the straw bales were more and more replaced by a soil heating system of buried pipes in the soil. Since 1975 there has been an increased use of rockwool as growing medium for cucumber. About 300 ha (60%) of the early-planted cucumbers were grown on rockwool in 1985. Rockwool is an inert, pathogen-free medium with good water retaining properties. Water, containing all the necessary nutrients, is applied to each plant by means of trickle irrigation. The nutrient solution is not recirculated.

Four production systems can be discerned:

1. Pipe-heated crops planted between December and end of February. Cropping extends to October. Part of the acreage of this early-planted crop is replaced by an autumn crop.
2. Air-heated crops planted in March/April. They are grown in rotation with other crops, mainly lettuce.
3. Unheated crops planted in May/June, also grown in rotation with other crops.
4. Autumn crops planted in July/August, usually following a main crop of tomato, with cropping to October/November, so they have to be heat assisted.

## Growing environment

The cucumber crop is the most thermophylic one of the glasshouse vegetables. Since the energy crisis of 1973 prices of fuel have risen enormously. The share of the heating costs in the total costs of an early-planted cucumber crop increased from about 20 to 30% in the past ten years. The glasshouse industry was forced to carry out energy saving measures. Leakages in the glasshouses were sealed, thermal screens and double glazed walls were installed, ventilation was restricted and heating temperatures were lowered. A more humid environment was the result of all these measures with its consequences on the incidence of diseases.

## Cultivars

The cultivars have changed throughout the years. They determined also the production system. The main objectives of breeding were earliness and high production. Nowadays more and more attention is given to fruits with a good quality and a long shelf life and to disease resistance.

Initially, mixed flowering cultivars were grown. Pollination had to be avoided as it resulted in low quality fruits. Since 1975 only female flowering hybrids are grown. They are very suitable for the upright training system, give no fruit pollination problem and are very productive.

## Economics

Cucumber is the second in importance , after tomato, of the glasshouse vegetable crops, in the Netherlands.

The acreage of cucumber was highest in 1977 (1330 ha). It decreased slowly to 1050 ha in 1983 with more than 500 ha early-planted crops, about 250 ha air-heated or unheated crops and 250 ha autumn crops (Rijksinstituut voor het Rassenonderzoek van cultuurgewassen, 1985).

Between 1970 and 1984 the auction supply increased from 242 million kg to 340 million kg (650 million fruits)per year and the turn-over from 125 to 350 million guilders (De Visser, 1981; Rijksinstituut voor het Rassenonderzoek van cultuurgewassen, 1985).

Production clearly increased in despite of a decreasing acreage since 1977. This implies a rise in yield per $m^2$, mainly caused by the continuous introduction of more productive cultivars and the steady development away from soil-based crops to rockwool crops.

About 80% of the production is exported, mainly to West-Germany.

## Fungal diseases

In the Annual Reports of the Glasshouse Crops Research and Experiment Station in Naaldwijk, scab (Cladosporium cucumerinum) has been reported for years and years as a serious disease on cucumber. Research on this disease started in 1927. After the second world war, good control was obtained with trichlorotrinitrobenzene (Bulbosan). Resistant cultivars were introduced in the mid fifties and at present all commercial cultivars are resistant.

Fusarium was considered a serious threat to the cucumber crop in 1936. After the second world war, Fusarium wilt and Fusarium foot and root rot were controlled by grafting onto Cucurbita ficifolia and by steaming the soil. On grafted cucumbers Fusarium solani f. sp. cucurbitae was observed in 1958, probably because the seed of the rootstock had been infected. Afterwards it occurred rarely.

With the scab resistant varieties no chemical control of scab was carried out and as a consequence the incidence of other leaf diseases, namely Corynespora cassiicola (= C. melonis) and Didymella bryoniae,

increased. Target leaf spot (C. cassiicola) could be solved by the development of resistant cultivars, but the occurrence of D. bryoniae increased gradually.

The development from frames to glasshouses in cucumber production and the intensification of the crop resulted in a considerable increase of powdery mildew (Sphaerotheca fuliginea) since 1955. The pathogen developed resistance to some of the fungicides, which had become available for control of the disease, particularly to systemic fungicides. Each time when this happened it was necessary to change to another chemical. The use of fungicides is still the most important control method. A few powdery mildew resistant cultivars have been introduced recently. However, they are hardly grown because they show leaf necrosis under poor light conditions and are less productive than the susceptible cultivars.

At present, the most important fungal diseases in cucumber are stem and fruit rot (Didymella bryoniae) and grey mould (Botrytis cinerea). Grey mould is controlled by avoiding humid conditions and by spraying chemicals. Black root rot (Phomopsis sclerotioides) can cause severe losses when no proper steam sterilisation is carried out. The rootstock Cucurbita ficifolia is less susceptible to this disease. Under humid conditions, in unheated and air-heated crops in particular, downy mildew (Pseudoperonospora cubensis) can have serious consequences. Verticillium wilt has increased in the last years, both in border soil crops and in rockwool crops. Occasionally severe attacks of white rot (Sclerotinia sclerotiorum) occur. Root rot in which Pythium spp. are included can be a problem in particular in rockwool systems.

Didymella bryoniae

The disease was first reported on cucumber in the same year in three countries, namely in France (Roumeguère, 1891), Italy (Saccardo, 1891) and Delaware U.S.A. (Chester, 1891). Leaves, stems and fruits of all kinds of cucurbits can be infected. The symptoms have been referred to as leaf-spot, stem canker, gummy stem blight, vine wilt and black fruit rot. The disease is geographically widespread and occurs both in outdoor and protected crops (Chupp and Sherf, 1960).

Both the perfect and the imperfect state of the fungus occur. The

occurrence on many plant species and plant parts is probably the main reason the fungus has been described under many different names. The correct name for the perithecial state is Didymella bryoniae (Auersw.) Rehm (Müller and Von Arx, 1962) and for the conidial state Phoma cucurbitacearum (Fr.) Sacc. (Boerema and Van Kesteren, 1972).

In protected crops in Europe it was not a major disease until the production of cucumber was intensified considerably. In the Netherlands, the disease was first observed on grafted cucumbers grown under frames in 1953. It caused brown and necrotic lesions on cotyledons, leaves and stems. With grafted cucumbers an infection at the graft union could kill the plant. Until 1967 the disease was of minor importance and symptoms were mainly restricted to lesions on the leaves. From then on an increase in stem lesions initiating from wounds made by trimming and picking was observed, in particular during summer and autumn. The lesions are initially brown and turn black by the formation of pycnidia and perithecia. Girdling stem lesions which remain wet result in dying of the plants. Some control of the disease was achieved with zineb. Infection of growing tips and external fruit rot are observed as well. The youngest leaves and growing tip are malformed, turn brown and become necrotic. With a severe infection the growing tip dies. With external rot, lesions all over the fruit occur, predominantly in the post harvest period during the warm summer months. Internal fruit rot was noticed in 1967 for the first time in the Netherlands in particular on all female flowering cultivars (Sweep and Govers, 1967). With internal fruit rot the tissue in the centre of the blossom end of the fruit is brown discoloured. Internal fruit rot is hard to observe externally. It was, like external fruit rot, mainly noticed during the summer months. Fruit rot, both internal and external, has adverse effects on the quality of the Dutch produce.

The disease has never been a problem in single span cucumber houses, in contrast to multi-span glasshouses. This fact may be explained by the differences in training system and micro climate between the two types of glasshouses. The increased incidence of the disease earlier in the season since the energy crisis of 1973 is probably a result of the more humid glasshouse climate, which in its turn is one of the consequences of the energy saving methods the glasshouse industry was forced to introduce. The change in cultivars throughout the years is probably also an important factor explaining

the increased incidence of stem and fruit rot. The breeding for resistance to scab and target leaf spot and the breeding for bitter-free fruits and all female flowering may have resulted in cultivars which are more susceptible to stem and fruit rot.

**Aim and outline of the present study**

In the previous part of this introductory chapter it has been outlined that in connection with production system, cultivar and environmental conditions fruit and stem rot, caused by D. bryoniae, has become the most harmful fungal disease of glasshouse cucumbers in the Netherlands. With no adequate method of control available a study was undertaken to develop a control strategy for the disease. For this, a better insight in the biology of the pathogen and in the epidemiology of the disease was required.

This thesis contains after the introductory chapter nine previously published papers. The survival of the fungus and the incidence of ascospores in glasshouses, outdoors and under controlled environmental conditions are described in the first paper. The occurrence of fruit rot is the most troublesome. Therefore the second and third paper deal with factors influencing external and internal fruit rot, respectively. As a result of energy saving measures cucumbers are produced under more and more humid conditions. The influence of the humidity on the incidence of the disease on leaves and growing tips under controlled environmental conditions is described in the fourth paper. The fifth, sixth and seventh paper deal with the influence of the glasshouse climate under commercial conditions on the incidence of the disease. In particular the influence of ventilation on disease development was studied.
In the eight paper inoculation methods are described and compared to support the breeding for resistance. The ninth paper, dealing with chemical control, is supplemented with recent developments. At the end a general discussion with conclusions and a summary is given.

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# Epidemiological aspects of Didymella bryoniae, the cause of stem and fruit rot of cucumber

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**Abstract**

The survival of *Didymella bryoniae* and the incidence of ascospores in glasshouses, outdoors and under controlled conditions were studied. The fungus was able to overwinter in the open as dormant mycelium. Dry and undecomposed crop residues remained a source of infection for more than one year. Moisture and a minimum temperature between 5 and 10 °C were needed for fructification. For ascospore release a high relative humidity was not sufficient, the substrate had to be moist during a short period. Ascospores could be trapped throughout day and night both outdoors and in glasshouses, but there was a marked peak during a period of 3 h in the evening. Both on days with and without rain about the same numbers of ascospores were trapped from crop residues in the open. Ascospore release was favoured by watering the plants in the glasshouse. Under controlled conditions the release of ascospores was determined by humidity and not by light or darkness.

In a cucumber crop in the glasshouse the first ascospores were trapped at about the same time the first symptoms on the plants appeared. In the glasshouse with introduced diseased plant debris, particularly when the debris became wet when the plants were watered, the disease was more severe and yield was less than in a glasshouse without introduced plant debris. Airborne ascospores may cause the primary infection of a cucumber crop. Therefore, hygienic measures must be taken to eliminate plant debris as source of infection, both in glasshouses and outdoors.

*Additional keywords: Cucumis sativus, Mycosphaerella citrullina, Mycosphaerella melonis,* spore trap.

## Introduction

Stem and fruit rot caused by *Didymella bryoniae* (Auersw.) Rehm, synonyms *Mycosphaerella citrullina* (C.O.Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu and Walker, is one of the most important diseases in cucumber crops in many countries. It occurs annually in every glasshouse with a cucumber crop in the Netherlands. Lesions with black fruiting bodies, pycnidia and perithecia, on stubs left after removal of the fruits are usually the first symptoms of the disease. This implies that the disease is not observed during the first months after planting. For a good control

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strategy the between-crop survival of the fungus must be known. Therefore, the overwintering and survival on crop residues in the open air and in glasshouses were studied during several years. In Madison (Wisconsin, USA), the fungus overwinters as dormant mycelium (Chiu and Walker, 1949).

Little is known about the role of ascospores in the epidemiology of the disease. The incidence of ascospores in a field crop of watermelon was studied by Schenck (1968a and 1968b) in Florida (USA). Fletcher and Preece (1966) collected data about the concentration of ascospores in glasshouse air. The incidence of ascospores in the open air during and after the winter in the Netherlands is reported here. Factors influencing release and concentration of ascospores in the air outdoors, in glasshouses and in a controlled environment were studied. The relation between ascospores in glasshouse air and disease development on plants and fruits was studied in an experiment on the importance of diseased plant debris as source of infection.

## Materials and methods

The experiments on the survival of the fungus and on the release of ascospores were conducted in a field, in glasshouses and in a growth chamber (Karl Weiss ZK 2200 E/+ 4 JU-P-S). In the field, temperature and humidity at 1.5 m above soil level and precipitation were recorded.

*Survival.* Stem pieces of cucumber plants, covered with pycnidia and perithecia of *D.bryoniae,* were placed in styropor boxes in the open and in a glasshouse in November 1973 and 1974. The boxes in the field were covered with wire netting to prevent the material from being blown away and the bottoms were perforated to drain rain water. The material in the glasshouse was kept dry at a temperature of about 20 °C. Diseased plant material was also buried into soil and placed both in the open and air-dry in a glasshouse in the autumn of 1974. Every month, of each treatment 10 stem pieces with a length of 5 cm were examined macro- and microscopically. The viability of the fungus was checked by plating out fruiting bodies on cherry decoction agar. The virulence of the isolates was checked on cucumber seedlings, cultivar Farbio, as described elsewhere (Van Steekelenburg, 1981). The survival of the fungus on plant debris in the open air was also studied with the aid of spore traps.

*Spore incidence.* Two types of spore traps were used, a self-made trap with glass slides and a Burkard volumetric spore trap.

The self-made traps consisted of wooden trays of 30 × 7 × 4 cm (length × width × height) with holes in the bottom and wire netting on top. They were filled with heavily diseased stem pieces of cucumber plants. Glass slides of 76 × 26 mm were placed a few mm above the diseased plant material in grooves of the standing sides of the trap just above the wire netting. At the end of November 1977 such traps were placed in the open air 30 cm above soil level and in a glasshouse at about 20 °C. In August 1978 the trap outdoors was provided with fresh material. In addition a trap was placed upside down with slides underneath the plant material. Each tray was provided with three slides which were changed at least monthly. After exposure, the slides were stained with cotton blue and examined under the microscope for presence of ascospores and conidia of *D.bryoniae.*

The Burkard volumetric spore trap is a modification of the trap designed by Hirst (1952). It is equipped with a seven-day recording clockwork-driven drum and provided with a special sampling tape. In December 1979, a wind-vane-mounted trap, with orifice at 0.5 m above soil level, was placed in the open air with diseased plant material in wire netting in a circle 0.5 m from the orifice, to determine the release of spores outdoors in more detail.

The incidence of ascospores indoors was determined using a trap without a wind-vane in the same glasshouses where the influence of debris of a previous crop on the outbreak of the disease was studied in 1980.

For a more detailed study of the effect of temperature, humidity and light on release of ascospores, a Burkard trap was placed in a growth chamber with air movement from the bottom to the top and equipped with an Elka Airfog atomizer. The atomizer was connected with a time-clock so that at any chosen time it could operate for 15 min to wet the material. The photoperiod was 12 h and light intensity was 30 000 lux (90% number 33 and 10% Philinea fluorescent tubes). For each experiment subsamples were taken from air-dry stored diseased stems and these were attached to the trap in wire netting at 10 cm from the orifice of the trap. Each experiment lasted 2 weeks after which the bundle of diseased stems had to be replaced because of development of saprophytic fungi.

In all experiments, sections of the exposed tape, representing periods of 24 h, were stained with cotton blue and the number of ascopores deposited on it were counted with the aid of a microscope.

*Crop residues as source of infection.* The influence of debris of diseased plants of a previous crop on disease development was studied in three glasshouse compartments of about 19 $m^2$ each in 1980 and 1981. Three rows each of ten plants of cultivar Farbio were planted in steam-sterilised soil in mid February in each compartment. Diseased stem pieces of plants of a previous crop were scattered on the soil in one compartment and suspended in wire netting above the plants in another. The plant debris on the soil was wetted when the plants were watered, but the plant debris suspended above the plants remained dry, as the spray irrigation system lay on the soil. In the third compartment no diseased plant debris was introduced.

During the 1980 season, a Burkard spore trap was used during 7 out of 14 days alternately in the two compartments in which diseased plant debris was introduced. When spores were trapped in either of these compartments, the trap was also run in the compartment without introduced plant debris. From then on the trap operated in each compartment during 7 out of 21 days. The number of *D.bryoniae* lesions on the main stem were counted every 2 weeks. The fruits were harvested twice a week and every fruit was cut in half lengthwise to check for internal rot (Van Steekelenburg, 1978a).

In 1981, the experiment was repeated in the same way, but without using a spore trap, in three other compartments of the same size. The heating and ventilation temperatures were 20 and 25 °C, respectively. The relative humidity was registered with a thermohygrograph.

If necessary powdery mildew was controlled with fenarimol (Rubigan). In order to avoid spreading of the disease from one compartment to another all operations with plants were carried out first in the compartment without introduced plant debris, then

in the compartment with plant debris suspended above the plants and subsequently in the compartment with plant debris on the soil. The compartments were separated by guard compartments with a sweet pepper crop. The first experiment was finished mid July and the second one at the end of August.

## Results

*Survival.* On stems stored above soil in the open during the winter of 1973/1974, only empty fruiting bodies were observed after one month. Perithecia filled with asci were found inside some stem pieces in February 1974. Pycnidia with some conidia were observed at the end of April 1974. Only empty fruiting bodies were found during and after the winter of 1974/1975 on material stored above soil in the open.

On stems stored dry in a glasshouse, only old perithecia with asci and old pycnidia with some conidia could be found throughout the storage periods, even after storage of stumps for 18 months. Young brown pycnidia, and occasionally a young perithecium, were found if stumps were kept wet in petri dishes for one week.

On stems in soil and stored in the open, or air-dry in the glasshouse, only empty fruiting bodies were found during the observation period. The plant material in the soil in the open was totally decomposed after nine months. *D.bryoniae* could still be isolated from plant debris in air-dry soil in the glasshouse stored for ten months.

Table 1. Numbers of ascospores and of conidia of *D.bryoniae* trapped with glass slides above or underneath diseased cucumber stem pieces in the open air during the 1977/78 and 1978/79 season, and the mean temperature per month.

| Month | 1977/78 | | | 1978/79 | | | | |
|---|---|---|---|---|---|---|---|---|
| | slides above | | mean temperature | slides above | | slides underneath | | mean temperature |
| | ascospores | conidia | (°C) | ascospores | conidia | ascospores | conidia | (°C) |
| August | | | | + | ++ | ++ | ++++ | 16.4 |
| September | | | | ++ | +++ | ++ | ++++ | 15.1 |
| October | | | | + | +++ | ++ | +++ | 12.5 |
| November | | | | − | ++ | + | ++ | 8.1 |
| December | ++[1] | +++ | 5.8 | + | ++ | + | ++ | 2.9 |
| January | − | − | 4.5 | − | − | − | + | −0.9 |
| February | − | − | 2.5 | − | − | − | − | 0.4 |
| March | − | + | 7.1 | − | + | − | + | 5.4 |
| April | − | + | 7.9 | − | − | + | + | 8.4 |
| May | + | + | 12.4 | − | + | − | + | 11.8 |
| June | ++ | − | 15.4 | − | + | + | + | 15.2 |

[1] − = no spores; + = 1-10 spores per glass slide; ++ = 11-50 spores per glass slide; +++ = 51-100 spores per glass slide; ++++ = > 100 spores per glass slide.

*Tabel 1. Aantallen ascosporen en conidiën van D.bryoniae die met behulp van objectglaasjes, aangebracht boven of onder aangetaste stengeldelen van komkommer, in de open lucht werden gevangen in de perioden 1977/78 en 1978/79, en de gemiddelde temperatuur per maand.*

Isolates of *D. bryoniae* obtained by plating empty fruiting bodies from plant debris stored during winter in the open or in a glasshouse were as virulent as isolates from a newly diseased crop. Even the winter of 1973/1974 with 14 days of frost and with a minimum temperature of −9 °C did not have any harmful effect on the viability of the fungus.

*Spore incidence.* No spores were caught on the slides in the self-made trap with air-dry plant material in the glasshouse, except one ascospore in February 1978. The numbers of spores caught on slides in the open air during each month of the experiment are given in Table 1. No or hardly any spores were trapped in January and February, both in 1978 and 1979. Spores were trapped only in months with a mean temperature higher than 5 °C, except in December 1978, when mean temperature was 2.9 °C. In this month temperatures fluctuated very much and there were two periods of seven days with a mean daily temperature higher than 7 °C.

The daily number of ascospores caught with the Burkard spore trap from diseased plant material stored in the open air, precipitation and mean daily temperature for the period 20 December 1979 to 12 May 1980 are given in Fig. 1. No spores were trapped when the mean daily temperature was below 5 °C, except in the first few days when the material was stored outdoors and apparently already mature ascopores were discharged. Spores were trapped both on days with and without rain. When spores were trapped in February and March, the mean daily number of spores was 21 on days without and 19 on days with rain, with a highest mean number of 3.2 and 3.1 spores per hour per 0.6 $m^3$ sucked air, respectively. The mean diurnal periodicity curves on these days with and without rain show the same evening peak between 19.00 and 22.00 h (Fig. 2).

In glasshouses with plant debris as a source of infection, no ascospores were trapped until the beginning of April. In all three compartments the first ascospores were recorded at about the time of first appearance of symptoms on the plants in the compartment concerned. The maximum number of trapped ascospores per day was 200 to 250 in April and May and 1000 to 2000 in the second half of June and the first half of July. Ascospores were caught nearly always throughout day and night with a marked peak in the evening. In June/July 1980 this peak was between 21.00 and 23.00 h (Fig. 3). The minimum and maximum mean numbers of ascospores trapped per hour per 0.6 $m^3$ sucked air, were 7 and 109, respectively. During the day the relative humidity was about 60%. Usually, in the evening at about 19.00 h it increased to more than 95% which was maintained during the night. At about 8.00 h it decreased again to about 60%. Obviously there is a correlation between humidity and ascospore release. The effect of watering the plants on ascospore release was checked on 12 days. On 9 days there was a peak in number of spores caught within 3 h after watering which did not coincide with the evening peak, and on 3 days there was no peak within 3 h after watering.

In the growth chamber experiments, no spores were caught when air and dew point temperature were both 23 °C (r.h. > 95%). No spores were caught either at an air temperature of 23 °C and an alternating dew point temperature of 19 °C (r.h. c. 70%) and 23 °C in a 12 h cycle. After wetting the plant material spores were caught mainly within the first 3 h both with 23/23 °C and 23/19 °C air/dew point temperature combinations (Fig. 4). Wetting at different points of time during the light and dark period

Fig. 1. Daily number of ascospores of *D.bryoniae* trapped from diseased cucumber stem pieces in the open air with a Burkard spore trap, mean daily temperature and precipitation from 20 December 1979 till 12 May 1980.

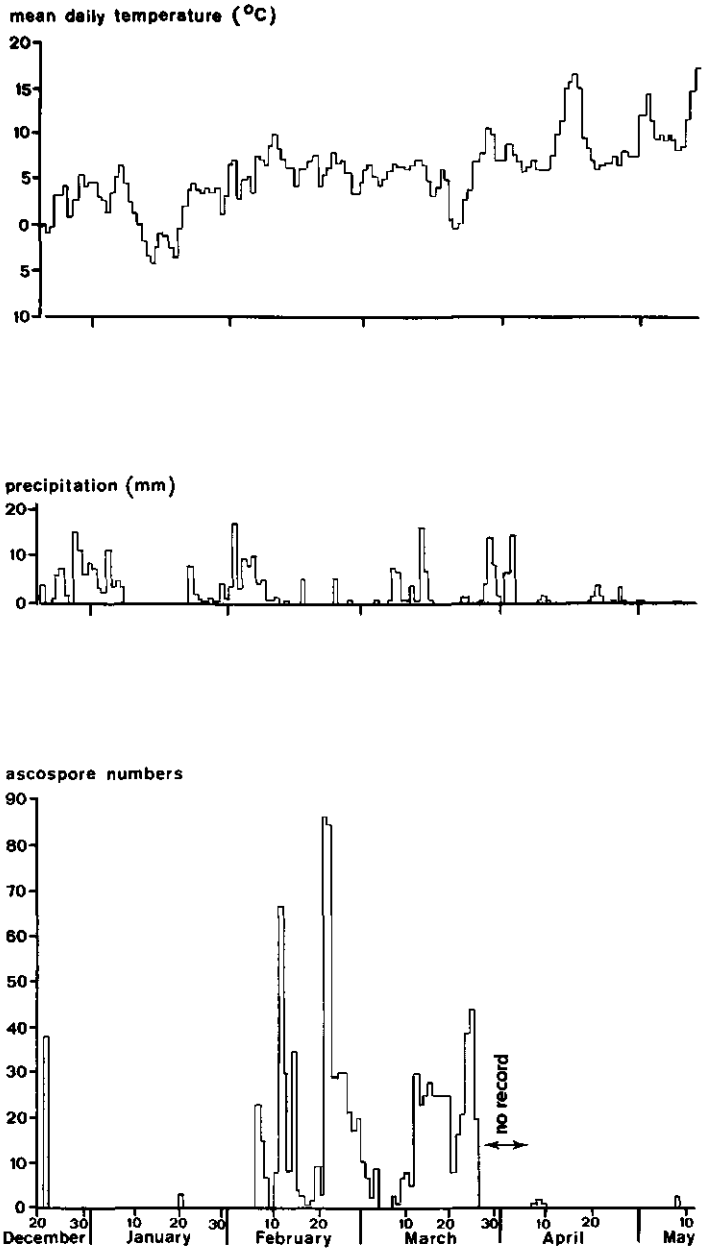


*Fig. 1. Aantallen ascosporen van D.bryoniae die per dag van aangetaste stengeldelen van komkommer in de open lucht werden gevangen met een Burkard sporenvanger; de gemiddelde dagtemperatuur en neerslag vanaf 20 december 1979 tot 12 mei 1980.*

Fig. 2. Mean diurnal periodicity curves of ascospores of *D.bryoniae* trapped from diseased cucumber stem pieces in the open air with a Burkard spore trap on days with (——) and without rain (- - -) expressed as percentage of the peak geometric mean concentration of 21 and 25 days, respectively, in het period 7 February to 26 March 1980.

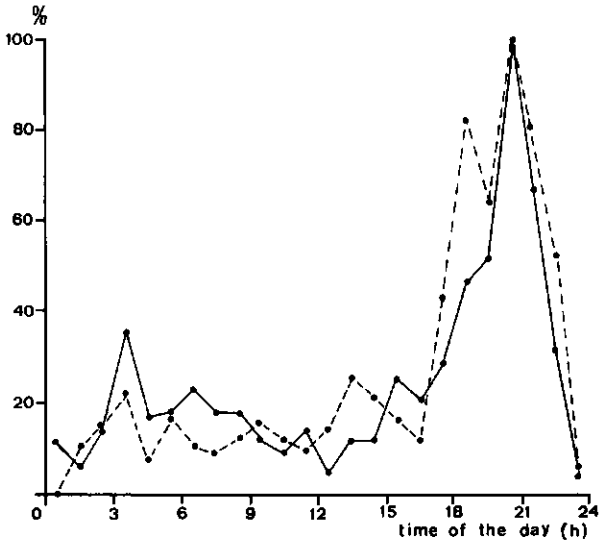


*Fig. 2. Gemiddelde dagelijkse periodiciteitscurven van ascosporen van D.bryoniae, die van aangetaste stengeldelen van komkommer in de open lucht werden gevangen met een Burkard sporenvanger op dagen met (——) en zonder regen (- - -), uitgedrukt als percentage van de hoogste gemiddelde vangst van respectievelijk 21 en 25 dagen in de periode van 7 februari tot 26 maart 1980.*

had no different effect on the spore catches. At air and dew point temperatures of both 5 °C some spores were caught after wetting the plant material but only during the first few days. Apparently these spores were mature already at the start of the experiment.

*Crop residues as source of infection.* In the 1980 experiment, the first lesions on the main stem were observed in the two compartments with introduced plant debris in April, about 2 months after planting. In the compartment without diseased plant debris, these symptoms were observed about 2 months later (Fig. 5). In the compartment with plant debris on the soil, the first fruit with internal rot was found about the same time the first symptoms on the plants appeared. In the two other compartments the first fruit with internal rot was observed about 2 to 4 weeks after the first stem lesions appeared (Fig. 5). The total numbers of harvested fruits from the compartments without debris, with debris above the plants and with debris on the soil were 1137, 915 and 876, respectively.

In the 1981 experiment, the first fruit with internal rot in the compartment without introduced plant debris was found in the beginning of May, without any lesions observed on the main stem. With plant debris on the soil, the first lesion and the first

Fig. 3. Mean diurnal periodicity curve of ascospores of *D.bryoniae* in glasshouse air trapped with a Burkard spore trap on 15 days on which the plants were not watered in June/July 1980, expressed as percentage of the geometric mean concentration.

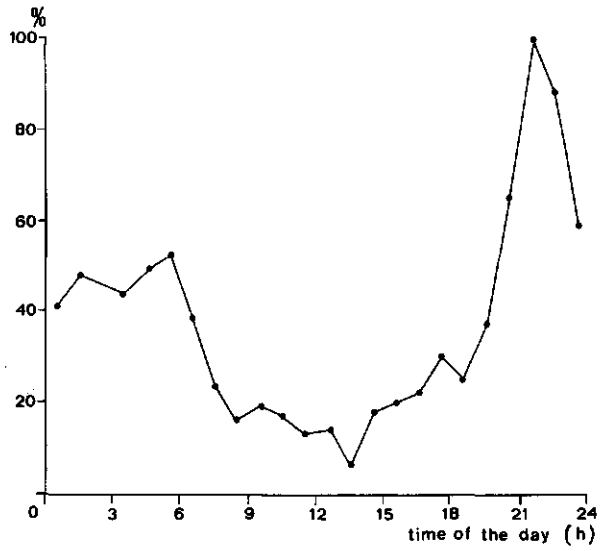


*Fig. 3. Gemiddelde dagelijkse periodiciteitscurve van ascosporen van D.bryoniae, die in kaslucht werden gevangen met een Burkard sporenvanger op 15 dagen waarop de planten niet werden beregend in juni/juli 1980, uitgedrukt als percentage van de hoogste gemiddelde vangst.*

fruit rot were observed in the beginning of June, about 4 months after planting. The development of the disease from then on was about similar as in 1980. Without plant debris and with debris above the plants, hardly any symptoms on the plants and fruits developed in 1981 (Fig. 5). The total numbers of harvested fruits from the compartments without, with debris above the plants and with debris on the soil were 1405, 1287 and 1240, respectively.

## Discussion

The fungus was able to survive periods with temperatures below 0 °C in diseased plant material, probably as dormant mycelium, as was also observed by Chiu and Walker (1949) in Wisconsin (USA). Data on spore catches outdoors (Table 1, Fig. 1) and in a growth chamber indicate that the minimum temperature for fructification of *D.bryoniae* is between 5 and 10 °C. This implies that ascospores can be present in glasshouse air throughout the year and are absent outdoors only during a few winter months. In vitro and in vivo, the minimum temperature for growth of the fungus was also between 5 and 10 °C (Van Steekelenburg, 1982; Wiant, 1945). Besides a temperature above this minimum, moisture is needed for fructification of the fungus on plant debris. After winter the conidial state was formed first and the perfect state appeared later on, usually less numerous on plant debris outdoors (Table 1). The same was found after inoculation of cucumber fruits (Van Steekelenburg,

Fig. 4. Periodicity curves of ascospores of *D.bryoniae* trapped from diseased cucumber stem pieces in a growth chamber with a 12 h dark/light cycle and 23/23 °C (——) and 23/19 °C (- - -) air/dew point temperature combinations after two times of wetting per 24 h, expressed as percentage of the peak geometric mean concentration per hour.

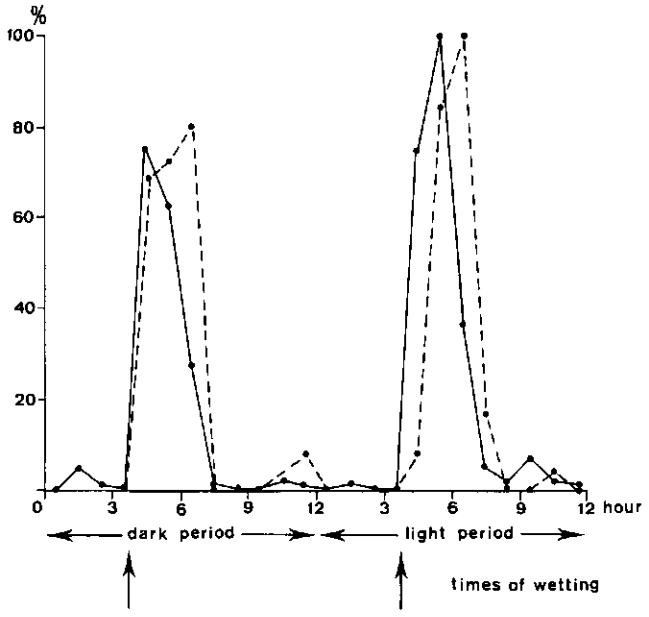


*Fig. 4. Periodiciteitscurven van ascosporen van D.bryoniae, die van aangetaste stengeldelen van komkommer werden gevangen in een klimaatkast met een 12-urige donker/licht cyclus en lucht-/ en dauwpunttemperatuur combinaties van 23/23 °C (——) en 23/19 °C (- - -), na tweemaal bevochtigen per etmaal, uitgedrukt als percentage van de hoogste gemiddelde vangst per uur.*

1982; Wiant, 1945) and after wetting air-dry stored debris of diseased plants. Although pycnidia were formed predominantly, ascospores are important in the epidemiology of the disease.

The fungus is very resistant to dryness and can survive in dry plant material present on glasshouse structures and in plant debris in and on the soil as long as the debris is not decomposed. The disease will occur earlier and more severely when plant debris from a previous crop is left in the glasshouse, particularly when this debris is wetted (Fig. 5). After finishing the crop, thorough cleaning of the glasshouse and washing down the structures, preferably with a disinfectant, is essential to eliminate sources of infection for the next crop. In order to control the disease a soil disinfestation is needed when a monoculture of cucumber is practised. Both sterilisation by steam and by methylbromide proved to be effective (unpublished data). Plant debris thrown outdoors can serve as an infection source too. Wind-borne ascospores originating from this debris may be responsible for the primary infection of the crop. As ascospores are already formed and released at rather low temperatures and moist conditions, the plant debris must be destroyed immediately after finishing the crop.

Fig. 5. Development of the total number of *D.bryoniae* lesions on the main stems (——) and of the total number of fruits with internal rot (- - -) in cucumber crops in glasshouse compartments (30 plants each) without introduced plant debris (I), with plant debris above the plants (II) and with plant debris on the soil (III) in 1980 and 1981.

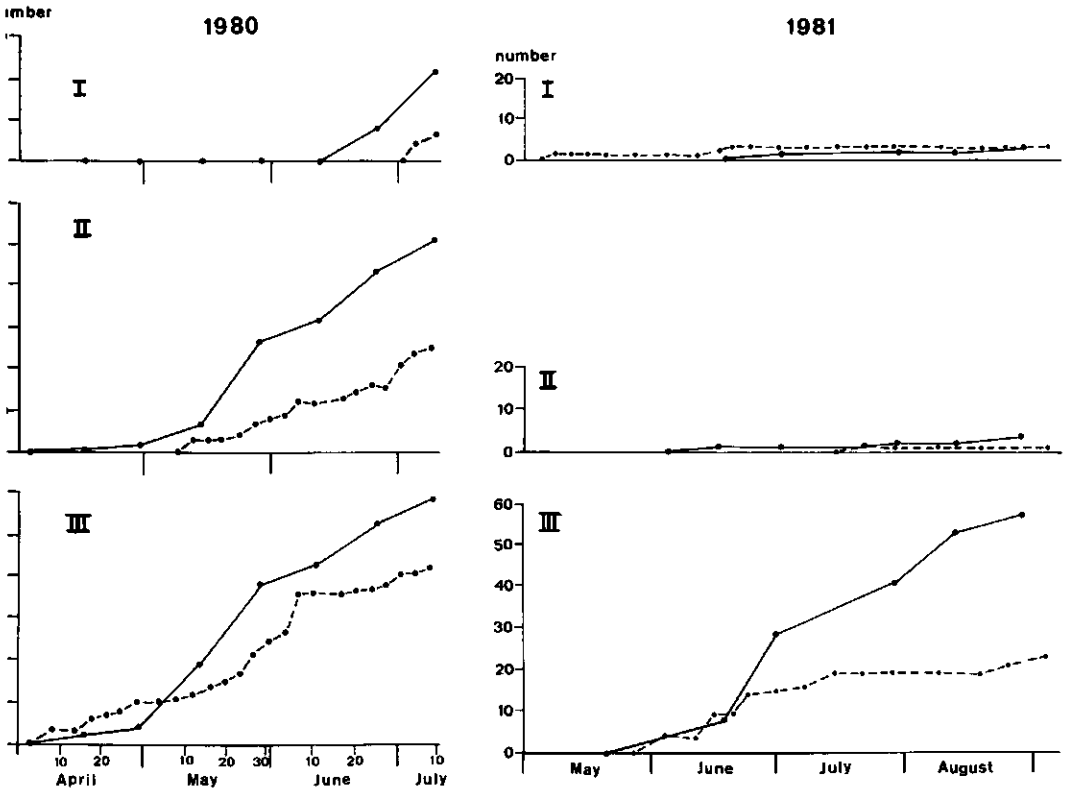


*Fig. 5. Het verloop van het totaal aantal stengellesies van D.bryoniae (——) en van het totaal aantal vruchten met inwendig rot (- - -) in een gewas komkommers in kasafdelingen (met elk 30 planten) zonder ingebrachte plantenresten (I), met plantenresten boven de planten (II) en met plantenresten op de grond (III) in 1980 en 1981.*

A daily peak concentration of ascopores in the air outdoors (Fig. 2) and in the glasshouse (Fig. 3) was observed during a period of about 3 h. Under controlled conditions the majority of the mature ascospores were also released within a period of 3 h after wetting the plant material (Fig. 4). Outdoors and under glass this peak occurred in the evening after sunset. The difference in time of sunset in spring and summer is reflected in the time of appearance of the peak in Fig. 2 and 3. No effect of light or darkness on release of ascospores of *D.bryoniae* was observed in the growth chamber experiments. Watering the plants in the glasshouse favoured nearly always ascospore release. With diseased plant material stored outdoors rain was not necessary for ascospore release (Fig. 1 and 2). Most probably rain has an effect on ascospore release but it could not be established under the experimental conditions

outdoors. Rain favoured ascospore release of *Mycosphaerella pinodes* on pea straw and on days without rain ascospores were released in a regular daily rhythm with an afternoon peak too (Carter, 1963). According to the results in the growth chamber experiments a high relative humidity is not sufficient, a certain supply of moisture to the perithecia is needed for the release of ascospores. After sunset, temperature decreases and humidity increases both outdoors and in glasshouses and apparently the substrate becomes sufficiently moist for ascospore release. The ultimate factor that determines the periodicity of ascospores release is humidity and not light. Ascospores can be present in the air at all times of the day and night outdoors (Fig. 2) and in glasshouses (Fig. 3). It is evident that at least a proportion of the perithecia on the plants or plant debris receive sufficient moisture at any moment of the day for ascospore release. Carter (1963) suggested the same for *Mycosphaerella pinodes* on pea. Fletcher and Preece (1966) and Schenck (1968a) reported a similar peak concentration of ascopores of *D.bryoniae* in the evening hours with cucumbers in glasshouses and with watermelons outdoors, respectively. Schenck (1968a) stated that periods of free moisture were needed to collect ascospores. Fletcher and Preece (1966) found highest ascospore counts in wet dull weather.

With an increase in disease level during the cropping period the number of ascospores in the glasshouse air increased and so did the number of fruits with internal rot (Fig. 5). This fruit rot, which can be caused both by ascospores and by conidia (unpublished data), is still under investigation. Due to an early incidence of *D.bryoniae,* a yield reduction of 10 to 20% is possible. These results were from unreplicated experiments in small glasshouses but were observed in two successive years and also in other experiments a higher disease incidence on the plants resulted in a reduction in number of harvestable fruits (Van Steekelenburg and Van de Vooren, 1981).

Trapping ascospores of *D.bryoniae* is of no use to forecast the disease. It is easier to look for the very first symptoms as these can be spotted about the same time the first ascospores can be caught. When the first symptoms of the disease appear control must be achieved by a combined action of preventing humid conditions (Van Steekelenburg and Van de Vooren, 1981) and frequent sprayings with fungicides (Van Steekelenburg, 1978a and 1978b).

**Acknowledgements**

Thanks are due to H. Barendse, B.C. van Dam and S.J. Paternotte for carrying out parts of the experiments and to C.A. Ammerlaan for providing the meteorological data.

**Samenvatting**

*Epidemiologische aspecten van Didymella bryoniae, de veroorzaker van stengellesies en vruchtrot bij komkommer*

De overleving van *Didymella bryoniae* en het voorkomen van ascosporen in kassen, buiten en onder geconditioneerde omstandigheden is onderzocht. De schimmel kon buiten overleven als rustend mycelium. Aangetaste plantenresten, die droog en niet

verrot waren, bleven gedurende meer dan een jaar een infectiebron. Voor fructificatie was vocht nodig en een minimum temperatuur tussen 5 en 10 °C. Voor het vrijkomen van ascosporen is een hoge relatieve luchtvochtigheid niet voldoende, maar moet het substraat gedurende een korte periode vochtig zijn. Zowel buiten als in de kas konden ascosporen gedurende de gehele dag en nacht worden gevangen, maar er was 's avonds een duidelijke piek gedurende een periode van 3 uur. Op dagen met en zonder regen waren de aantallen ascosporen die buiten in de nabijheid van aangetaste plantenresten werden gevangen ongeveer even hoog. Door het watergeven van de planten in de kas werd het vrijkomen van ascosporen bevorderd. Het vrijkomen van ascosporen werd onder geconditioneerde omstandigheden bepaald door de vochtigheid en niet door licht of donker.

De eerste ascosporen werden in een kas met een gewas komkommers gevangen op ongeveer hetzelfde moment als waarop aan de planten de eerste symptomen te zien waren. In vergelijking met de kas waar géén aangetaste plantenresten waren ingebracht was in de kas met ingebrachte plantenresten de ziekte ernstiger en de produktie minder, vooral als de plantenresten bij het watergeven van de planten nat werden.

Ascosporen in de lucht kunnen de eerste aantasting in een gewas komkommers veroorzaken en daarom moeten hygiënische maatregelen worden genomen om plantenresten, die zowel buiten als in de kas een bron van infectie vormen, te vernietigen.

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Neth. J. Pl. Path. 88 (1982) 47-56

# 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*.

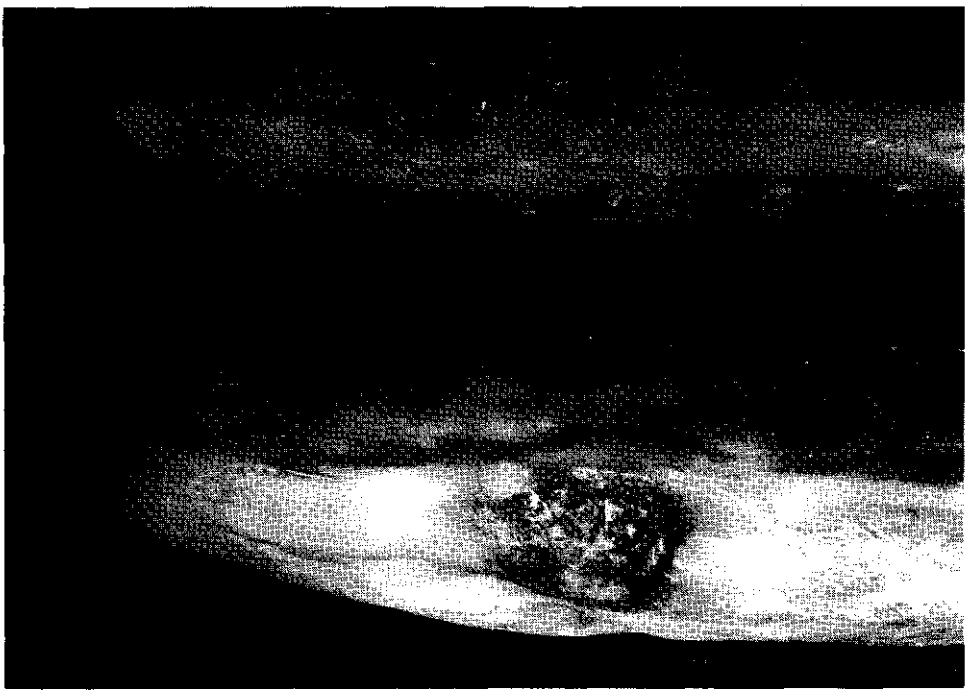

*Fig. 1. Uitwendig rot op komkommervruchten veroorzaakt door D. bryoniae.*

(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 com-

mercial 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).

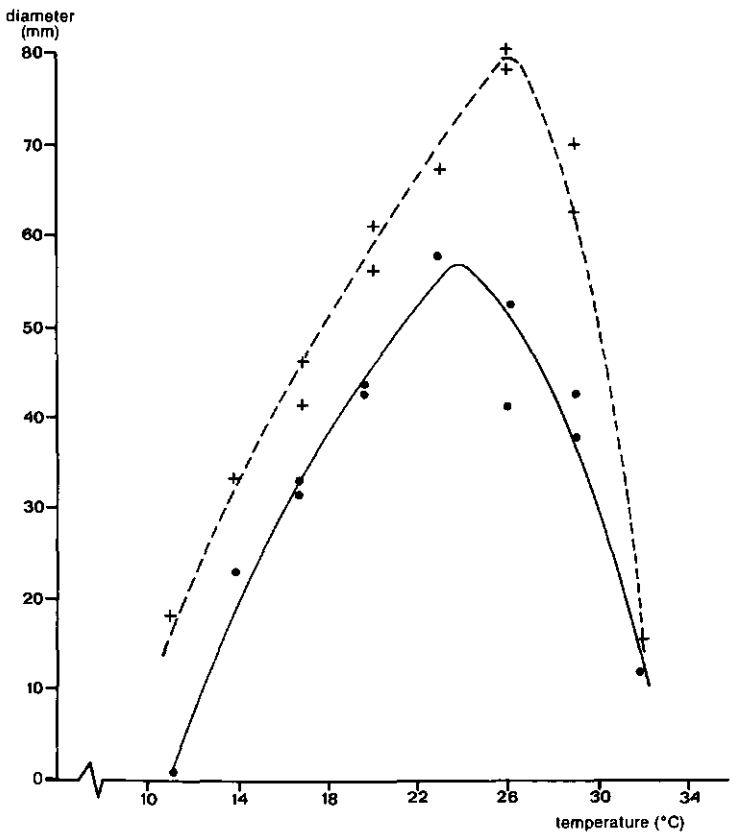


*Fig. 2. Effect van de temperatuur op de diameter van de rotte plek van geïnoculeerde komkommervruchten (———) en op de koloniediameter van D. bryoniae op PDA (– – –) (gemiddelden van respectievelijk 30 en 10 herhalingen per getoetste temperatuur in drie experimenten van respectievelijk 11-20°, 17-29°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

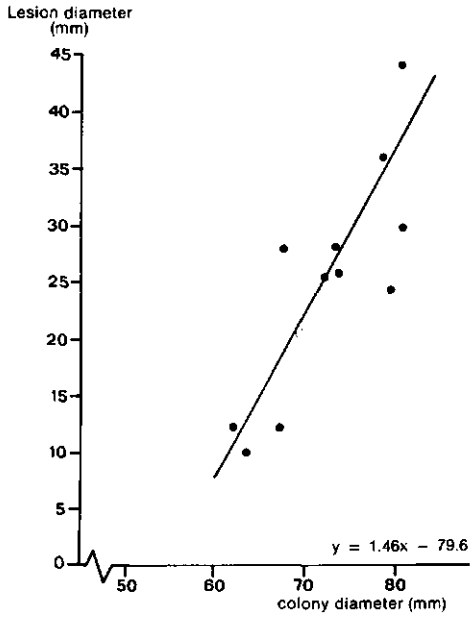


Fig. 3 Relationship between colony diameter on PDA (means of 3 replicates) and lesion diameter on cucumber fruits (means of 10 inoculation sites) of 11 isolates of *D. bryoniae*.

*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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# Factors influencing internal fruit rot of cucumber caused by Didymella bryoniae

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**Abstract**

Several factors influencing the incidence of internal fruit rot of cucumber caused by *Didymella bryoniae* were studied.

Internal infection of fruits is achieved via the flower. However, in most cases the majority of the fruits escaped infection after flower inoculation. It took more than two days for the fungus to reach the fruit after infection of the style. A mechanical barrier was not detected in the fruit tip within three days after inoculation of the open flower.

Inoculation of wilted flowers resulted in 60% less infection than inoculation of fresh flowers. Blossom excision reduced fruit infection with ca 75%. Growing plants under drought stress markedly increased the incidence of internal fruit rot.

Neither the method of inoculation, nor the composition of the inoculum, nor the relative humidity influenced the incidence of internal fruit rot. Fruit thinning, duration of fruit growth, flowering period and the removal of parts of the flower had no effect either on fruit infection.

Cultivars resistant to powdery mildew were also resistant to internal fruit infection. The resistance was associated with a long style and a short flowering period.

Growing cultivars in which the flowers quickly fall away from the fruitlets or in which the flowers have no style may solve the problem of internal fruit rot in cucumber.

*Additional keywords: Cucumis sativus,* disease resistance, Mycosphaerella citrullina, Mycosphaerella melonis.

## Introduction

Stem and fruit rot caused by *Didymella bryoniae* (Auersw.) Rehm (synonyms: *Mycosphaerella citrullina* (C.O. Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu and Walker), is an important fungal disease of cucumber *(Cucumis sativus* L.) and other cucurbits. The disease has adverse effects on both quality and quantity of the fruits (Van Steekelenburg, 1984, 1985). Fruit rot occurs in the pre- and postharvest periods. Fruits can be infected externally and internally. Aspects of external fruit rot are described earlier (Van Steekelenburg, 1982).

The internal rotting of fruits always starts at the blossom end. Initially, the tissue in the center of the infected fruit tip shows a brown discoloration over a length of 1

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to 2 cm and 2 mm in diameter. Subsequently the brown discoloration extends into the carpels (Fig. 1). In an advanced stage of infection the fruit rot spreads also externally from the blossom end. As the rot advances, the fruit gradually dries up and turns black. Fruiting bodies of the fungus appear on the diseased tissue. In the early stages of infection it is very difficult to judge externally if a fruit is infected internally. Sunken areas a few cm from the blossom end of a harvestable fruit indicate internal infection. Internal rot may result in a misshapen fruit with a tapering tip. Such a misshapen fruit, however, may be the result of a physiological disorder as well.

The occurrence of internal fruit rot in cucumber has been described by Kagiwata (1967) in Japan, Sweep and Govers (1967) in the Netherlands, Sitterly (1968) in British Honduras and Leski (1984) in Poland. Its incidence in Dutch glasshouses fluctuates and differs from nursery to nursery. It depends on the infection pressure and on the glasshouse climate (Van Steekelenburg, 1984, 1985; Van Steekelenburg and Van de Vooren, 1981). Occasionally, up to 46% of the fruits can be infected on a harvest date (Van Steekelenburg, 1984). Over a whole growing season up to 5% of the fruits can be infected.

In preliminary experiments it was not possible to achieve internal fruit rot by injecting a conidial suspension into the fruit stalk. Infection was 100% successful when young fruits were injected under the fruit skin. The first internal rot in harvested fruits of commercially grown plants was found 7 to 15 days after spraying the whole plants with a conidial suspension (Van Steekelenburg, 1985; Van Steekelenburg and Van de Vooren, 1981). This period is about equal to the time needed from flowering to harvest.

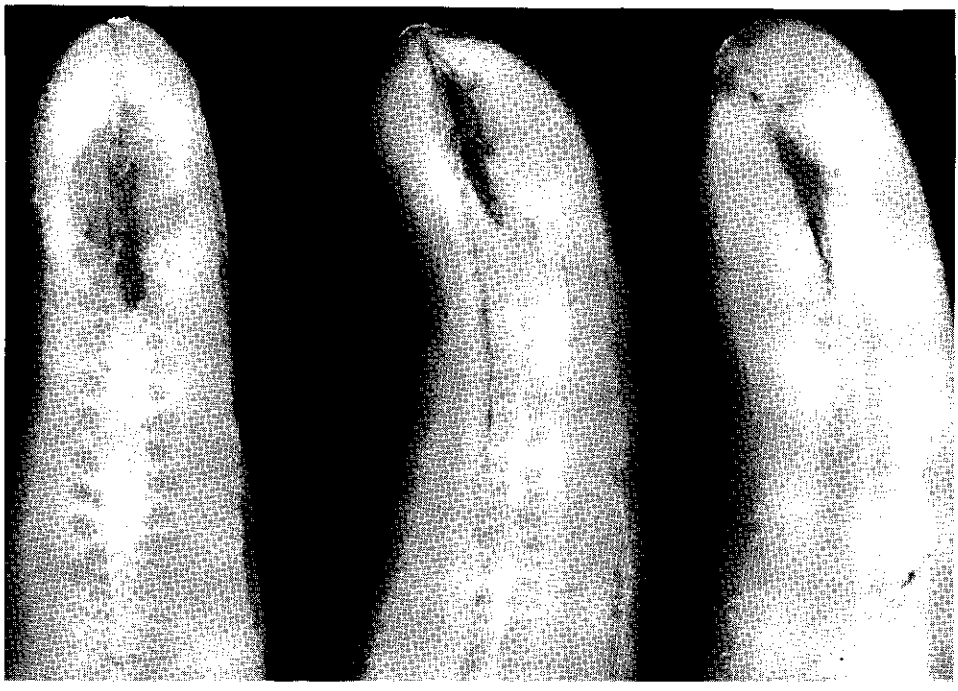

Fig. 1. Internal rot at the blossom end of cucumber fruits, caused by *D. bryoniae*.

Apparently natural infection of fruit takes place in the flowering period through flower parts. Methods for inoculation of the open flower and the effect of inoculum composition were studied. The role of the flower in fruit infection as well as plant conditions which might influence the incidence of internal fruit rot were investigated. The resistance of some cultivars to the disease was tested.

## Materials and methods

*Plants.* The all female flowering cultivar Farbio was planted in steam-sterilised border soil unless stated otherwise. Plants were grown vertically up to the suspending wire at about 2.1 m above soil level and then downwards. The side shoots along the whole stem were trimmed. The lower 0.8 m of the stem was also trimmed of fruits. Then two to three fruits were allowed to develop. The following three nodes were trimmed of fruits again to prevent abortion. Subsequently two to three fruits were allowed to develop, etc.

*Experimental design.* During several years experiments were carried out in a glasshouse compartment of 14.8 × 4.8 m with four rows of 22 plants each. Usually there were four to five replicates of three to four plants each per treatment in an incomplete latin square design.

*Glasshouse climate.* When plants were in production the heating temperatures were set a 17/19 °C (night/day) with a ventilation temperature of 24 °C.

*Inoculum and inoculation.* Isolate M 74-3 of *D. bryoniae,* obtained from a diseased cucumber plant of a commercial crop, was grown on cherry decoction agar or oat meal agar under black light. The conidia were washed off with water and filtered through cheese-cloth. The conidial suspension ($10^6$ conidia per ml) was brushed into the open flower unless stated otherwise. In each experiment inoculation was carried out during several days to get at least six inoculated fruits per plant.

*Disease assessment.* Fruits were harvested two to three times per week according to grower's procedures. Each fruit was cut longitudinally at the blossom end and classified internally infected if a brown discoloration over a length of at least 1 cm was observed.

*Statistical analysis.* Effects of treatments were evaluated by analysis of variance followed by Tukey's range test when appropriate.

## Results

### *Effect of inoculation methods and inoculum composition*

*Inoculation methods.* The following inoculation methods of open flowers were compared. (1) A 2-mm diameter disc of an agar culture of the fungus was placed on the stigma. (2) The conidial slime produced by pycnidia on agar cultures was brushed into the flower. (3) A conidial suspension ($10^6$ conidia per ml) was brushed or (4) dripped

Table 1. Comparison of inoculation methods of cucumber flowers with *D. bryoniae* on the incidence of internal fruit rot.

| Inoculation method | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | number of fruits harvested | percentage infected | number of fruits harvested | percentage infected |
| Agar disc | 137 | 12 | – | – |
| Brushing slime | 135 | 16 | 113 | 25 |
| Brushing suspension | 135 | 13 | 123 | 24 |
| Dripping suspension | 115 | 12 | – | – |
| Dipping flower | – | – | 118 | 21 |
| Control (water) | – | – | 116 | 3 |

(0.1 ml per flower) into the flower. (5) The whole flower was dipped into a conidial suspension.

No significant differences in the incidence of internal rot were found between the inoculation methods (Table 1). Only occasionally an uninoculated fruit was infected.

*Inoculum composition.* The inoculum suspension was amended with various nutrients (Table 2). The amendments neither stimulated nor decreased the incidence of the disease.

*Plant and fruit conditions*

*Fruit thinning.* Plants fully loaded with fruits may be in a stress situation leading to a higher incidence of internal fruit rot. Therefore the effect of fruit thinning was studied. Without fruit thinning all fruits on the stem above the lower 0.8 m were allow-

Table 2. Effect of adding nutrients to the inoculum suspension of *D. bryoniae* on the incidence of internal fruit rot after flower inoculation.

| Nutrients | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | number of fruits harvested | percentage infected | number of fruits harvested | percentage infected |
| None (conidial suspension) | 94 | 12 | 78 | 54 |
| 2% Cucumber flower extract | 96 | 10 | – | – |
| 2% Orange juice | 94 | 11 | – | – |
| 2% Sucrose + 0.5% yeast extract | 96 | 9 | 81 | 42 |
| 0.1 % Sucrose + 0.05% casein hydrolysate | – | – | 76 | 50 |
| 0.1% Glucose + 1% $KH_2PO_4$ | – | – | 76 | 42 |
| Control (water) | 92 | 0 | – | – |

ed to develop. With fruit thinning three nodes with a developing fruit above the lower 0.8 m of the stem were succeeded by three nodes with the fruits pruned in the flowering stage, etc. With more than 400 fruits per treatment, fruit infection of 21% with and 19% without fruit thinning did not indicate a significant effect.

*Fruit growth duration.* The time from flowering till reaching harvest size of a fruit varies (De Lint and Heij, 1982). The susceptibility to internal fruit infection of slow, medium and fast growing fruits, with a fruit growth duration of 11 to 14, 15 to 18 and 19 to 22 days, respectively, was compared. With about 20% infected fruits in each category, fruit growth duration had no effect on the incidence of internal fruit rot.

*Water supply.* Water stress of the plants may affect incidence of internal fruit infection. Seedlings were planted in 10-l plastic containers filled with a potting mixture of peat. Half the number of plants were grown with excess of water by keeping the lower 3 cm of the containers continuously in water. The other plants were watered only when wilting symptoms appeared. After flowering had started half the number of plants of each treatment kept the same watering regime or was transferred to the other regime.

Drought stress, both in the period before and after flowering, increased the incidence of internal fruit rot (Table 3). Drought stress in the period after flowering markedly reduced fruit production.

*Relative humidity.* The effect of relative humidity (r.h.) on the incidence of internal fruit rot after flower inoculation was tested in two growth chambers (Karl Weiss ZK 2200 E/+ 4 JU-P-S) at a temperature of 23 °C, a photoperiod of 12 h and a light intensity of 30 000 lux (90% number 33 and 10% Philinea fluorescent tubes). Plants in 10-l plastic containers filled with a potting mixture of peat were incubated either at 60% r.h. or at 95% r.h. after flowering had started. In each chamber twelve series of four plants each were run.

With more than 200 fruits per treatment and 21% fruit infection at 60% r.h. and 25% fruit infection at 95% r.h. no effect of r.h. was indicated.

Table 3. Effect of excess (+) or shortage (−) of water to cucumber plants in the period before or after flower inoculation with *D. bryoniae* on the incidence of internal fruit rot.

| Watering regime | | Number of fruits harvested | Percentage infected |
|---|---|---|---|
| before flowering | after flowering | | |
| + | + | 116 a[1] | 31 a |
| − | + | 119 a | 44 b |
| + | − | 74 b | 53 c |
| − | − | 79 b | 62 d |

[1] Values in one column followed by a different letter differ significantly at $p < 0.05$ (Tukey's range test).

Table 4. Effect of flowering stage on the incidence of internal fruit rot after inoculation with *D. bryoniae*.

| Flowering stage | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | number of fruits harvested | percentage infected | number of fruits harvested | percentage infected |
| Fresh open | 70 | 14 a[1] | 274 | 41 a |
| Starting to wilt | 92 | 15 a | 246 | 30 b |
| Completely wilted | 81 | 6 b | 272 | 17 c |
| Two days after wilting | 84 | 6 b | 249 | 12 c |
| Control (uninoculated) | 92 | 0 | – | – |

[1] Values in one column followed by a different letter differ significantly at $p < 0.05$ (Tukey's range test).

*Flower conditions*

*Flowering stage.* The flower opens early in the morning. The petals start wilting after 1 to 2 days and after another 1 to 2 days the flower is completely wilted. To investigate the most susceptible period for internal fruit infection, flowers were inoculated in different stages of development by dipping them into an inoculum suspension.

The more advanced stage of wilting, the less internal fruit rot occurred (Table 4).

*Duration of flowering.* The duration of flowering, the period from bud breaking to complete wilting, varied from 2 to 5 days. It did not have a significant effect on the incidence of internal fruit rot.

*Removal of flower parts.* Whole flowers or parts of these were removed prior to inoculation to investigate if certain parts were necessary for internal fruit infection and if wounds could increase the infection rate.

The incidence of internal fruit rot was equal without removing flower parts and with removing stigma, stigma and style, or petals. Removal of the whole flower before inoculation reduced the incidence of internal fruit rot by 70 to 85% (Table 5).

*Fungal growth through flower tissue.* To investigate the speed of fungal growth through the flower tissue into the fruit, flowers were removed at different intervals after inoculation.

Fruit rot occurred only when the flowers were left at the fruit for more than 2 days (Table 6). The incidence of rot in fruits with flower removed 3 days after inoculation was as high as in fruits with the flowers left.

Fungal growth through flower tissue and the possible formation of a mechanical barrier to fungal invasion of the fruitlet was examined with the aid of a light microscope. Flowers together with the tip of the fruitlet were cut 2 or 3 days after inoculation of the open flower. The petals were removed and the remainder was fixed and stored in a 5:5:90 mixture of 40% formaldehyde, 96% acetic acid and 70%

Table 5. Effect of removal of the whole flower prior to inoculation with *D. bryoniae* on the incidence of internal fruit rot.

| Flower removed | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | number of fruits harvested | percentage infected | number of fruits harvested | percentage infected |
| Yes | 64 | 3 | 94 | 7 |
| No | 65 | 17 | 96 | 25 |

ethanol. Longitudinal handsections were stained in 0.5% aniline blue in 50% ethanol for at least 30 seconds and subsequently rinsed in 90% lactic acid.

The stigma and the style of nearly all 100 examined flowers appeared to be infected. In most flowers, mycelium was observed in the nectaries as well. Neither the formation of some kind of cork layer, nor any change in the structure of the cells in the tip of the fruitlet was observed within three days after inoculation of the open flower.

*Disease resistance*

No different susceptibility to internal fruit rot was found between the cultivars Spotvrije and Farbio and no fruit resistance was established in breeding material showing plant resistance (Van Steekelenburg, 1981). Powdery mildew-resistant cultivars were released in the past few years. Some of them were tested for internal fruit rot resistance by flower inoculation.

The incidence of internal fruit rot in the powdery mildew-resistant cultivars appeared to be less ($p < 0.05$) than in the powdery mildew-susceptible cultivars (Table 7). The mean duration of the flowering period of 'K 0552' was shorter ($p < 0.05$) than of the other three cultivars (Table 7). Within one cultivar, however, no effect of a different flowering period on fruit infection could be established.

The length of 48 styles of each cultivar was measured with the aid of an ocular micrometer at 16 × magnification after picking the flowers and removing the petals.

Table 6. Effect of removal of the flower at different intervals after inoculation with *D. bryoniae* on the incidence of internal fruit rot.

| Period between inoculation and flower removal (days) | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | number of fruits harvested | percentage infected | number of fruits harvested | percentage infected |
| 1 | 89 | 0 | 85 | 0 |
| 2 | – | – | 72 | 0 |
| 3 | 66 | 45 | 68 | 19 |
| Control (not removed) | 85 | 39 | 65 | 17 |

Table 7. The incidence of internal fruit rot in and the duration of the flowering period of cucumber cultivars with or without powdery mildew resistance after flower inoculation with *D. bryoniae*.

| Cultivar | Powdery mildew resistant | Experiment 1 | | Experiment 2 | | |
|---|---|---|---|---|---|---|
| | | number of fruits harvested | percentage infected | number of fruits harvested | percentage infected | duration of flowering (days) |
| Saskia | – | – | – | 158 | 32 a | 3.4 a |
| Farbio | – | 311 | 47 a[1] | 154 | 42 a | 3.4 a |
| K 0552 | + | – | – | 121 | 8 b | 3.0 a |
| Millio | + | 323 | 28 b | 125 | 12 b | 3.4 a |

[1] Values in one column followed by a different letter differ significantly at $p < 0.05$ (Tukey's range test).

The mean style length of 'Saskia', 'Farbio', 'K 0552' and 'Millio' was 4.0, 4.1, 4.4 and 4.6, respectively. The powdery mildew-resistant cultivars had a longer style ($p < 0.05$) than the powdery mildew-susceptible cultivars.

**Discussion and conclusions**

Obviously internal fruit rot is achieved via the flower under natural conditions. Only a fraction of 0.1 to 0.6 of the fruits showed internal rot after flower inoculation. This fraction was neither influenced by the method of inoculation (Table 1), nor by the r.h. under controlled environmental conditions. For flower infection no free water seems to be needed, as brushing conidial slime and inoculating with an agar disc resulted in the same fraction of internally infected fruits as inoculation with a conidial suspension.

Orange extract stimulated spore germination of *D. bryoniae* (Chiu and Walker, 1949); sucrose and casein hydrolysate stimulated the disease on cucumber leaves (Bergstrom et al., 1982; Svedelius and Unestam, 1978). Inorganic phosphate and glucose stimulated *Botrytis cinerea*-infection of French bean leaves (Van den Heuvel, 1981) and sucrose and yeast extract increased the population density of phyllosphere yeasts on wheat (Fokkema et al., 1979). However, none of these amendments to an inoculum suspension of *D. bryoniae* influenced the incidence of internal fruit rot (Table 2). Lack of nutrients for spore germination or fungal growth is not likely to account for the escape to fruit infection. Besides, microscopic examination of flower tissue revealed that stigma and style were readily invaded by the fungus. The growth of the fungus through the flower tissue into the fruitlet took 2 to 3 days (Table 6). No evidence was found for the formation of a mechanical barrier in the fruit tip within these three days. A defence mechanism to fruit infection may be based on the formation of fungitoxic substances in the fruitlet. However, in preliminary experiments no evidence for the occurrence of substances inhibiting spore germination or fungal growth was found in the tip of the fruitlets. Evidence for the presence of fungitoxic substances in

cucumber seedlings grown in non-sterile conditions and their accumulation after inoculation of the seedlings with *D. bryoniae* was found by Callebaut (1984).

Fruit infection was influenced by the supply of water to the plant (Table 3). Under commercial conditions an ample water supply will increase the yield and decrease the incidence of fruit infection. Apart from the water supply other conditions, such as temperature, humidity, nutrition and presence of micro-organisms in and on the plant, will have varied in the glasshouse experiments and may be responsible for the variation of the fraction of fruits with internal rot between the experiments.

Inoculation of wilted flowers resulted in 60% less incidence of internal fruit rot compared to inoculation of open flowers (Table 4). The formation of some kind of mechanical barrier to fungal invasion of the growing fruit or the accumulation of fungitoxic substances in the fruitlet may explain this phenomenon.

Slight wounding by removing parts of the flower did not facilitate the entry of the fungus into the fruitlet, while severe wounding by blossom excision reduced the incidence of internal fruit rot with ca 75% (Table 5). The incidence of blossom end rot on Italian squash (*Cucurbita pepo* L.) in Brazil was reduced to the same extent by blossom excision within three days after flowering had started (Figueiredo et al., 1970). With blossom excision some kind of stress situation may be created in which fungal-growth-inhibiting substances are formed and released, although no evidence for it was found in preliminary experiments.

The powdery-mildew-resistant cultivars tested were resistant to internal fruit rot (Table 7). This may be attributed to a general defence mechanism, although it is not likely as the powdery mildew-resistant cultivars showed no plant resistance to *D. bryoniae* (unpublished data). The resistance to internal fruit rot is more likely to be a coincidence and may be better explained by the shorter flowering period and the longer style of the flowers of the powdery-mildew-resistant cultivars (Table 10). On the other hand, within a cultivar no effect of the duration of the flowering period on disease incidence was established. It cannot be excluded that resistance to fruit rot is based on other factors as well.

Breeding cultivars with rapidly wilting flowers may contribute to controlling the disease. The risk that an open flower is infected spontaneously is already decreased by a shorter flowering period. It would be even better if the flowers had no style. With the all female flowering cultivars with parthenocarpic fruit growth, fruit setting is not needed, is even not desired for high quality fruit production. As removal of flowers by hand is too laborious, breeding may give the solution to the internal fruit rot problem in cucumber by developing cultivars of which the flowers drop off quickly.

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**Samenvatting**

*Factoren die inwendig vruchtrot van komkommers, veroorzaakt door Didymella bryoniae, beïnvloeden*

Factoren die van invloed kunnen zijn op het ontstaan van inwendig vruchtrot van komkommer, veroorzaakt door *Didymella bryoniae,* werden onderzocht.

Inwendige vruchtinfectie vindt plaats via het bloempje. Bijna altijd ontsnapte echter het grootste deel van de vruchten aan een aantasting als de bloem werd geïnoculeerd. Het duurde meer dan twee dagen voordat de schimmel via de stijl de vrucht had geïnfecteerd. In de punt van de vrucht werd binnen drie dagen na inoculatie van de open bloem geen mechanische barrière gevonden.

Na inoculatie van verwelkte bloemen kwam 60% minder aantasting voor dan na inoculatie van bloemen die pas open waren. Het verwijderen van het bloempje reduceerde de aantasting met ca. 75%. Het optreden van inwendig vruchtrot nam aanzienlijk toe door de planten onder droge omstandigheden te telen.

Noch de methode van inoculatie, noch de samenstelling van het inoculum, noch de relatieve luchtvochtigheid beïnvloedden het optreden van inwendig vruchtrot. Vruchtdunning, duur van de vruchtgroei, bloeiduur en het verwijderen van delen van de bloem hadden ook geen effect op de aantasting.

Cultivars die resistent waren tegen echte meeldauw vertoonden ook resistentie tegen inwendige vruchtaantasting. De resistentie was gecorreleerd met een lange stijl en met een korte bloeiduur.

De teelt van cultivars waarvan de bloemdelen snel van de vruchtbeginsels afvallen, of waarvan de bloempjes geen stijl hebben, zou het probleem van inwendig vruchtrot bij komkommer kunnen oplossen.

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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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# INFLUENCE OF THE GLASSHOUSE CLIMATE ON DEVELOPMENT OF DISEASES IN A CUCUMBER CROP WITH SPECIAL REFERENCE TO STEM AND FRUIT ROT CAUSED BY DIDYMELLA BRYONIAE

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## Abstract

In an autumn crop of cucumber no significant differences in disease development of D. bryoniae were observed between three temperature regimes and time of transition from night to day conditions. Plants grown under drier conditions were less affected than plants under more humid conditions. Powdery mildew was more severe under drier conditions.
In a spring crop the disease incidence was higher on plants grown at lower night temperatures in the pre-inoculation period. Once D. bryoniae had been established in the crop the development of the disease passed on parallel. The incidence of D. bryoniae was lower and of Botrytis cinerea higher under drier than under more humid conditions.

## Introduction

Didymella bryoniae (Auersw.) Rehm, synonyms: Mycosphaerella citrullina (C.O. Sm.) Gross. and Mycosphaerella melonis (Pass.) Chiu and Walker, does occur every year in every glasshouse with a cucumber crop in the Netherlands. The disease is known as fruit and stem rot, black fruit rot and gummy stem blight. Losses vary from nursery to nursery and from time to time. Most damage is done by fruit infection from May and later on in the season. The lesions with black fruiting bodies, just visible to the naked eye as black dots, on the stubs left after removal of the fruits are the most characteristic symptoms of the disease. These lesions can progress on the main stem.
Control of the disease by spraying fungicides is difficult (Van Steekelenburg, 1978). Some resistance in certain cucumber lines has been found (Van de Meer et al., 1978), but resistant cultivars suitable for Dutch conditions are not yet available. In practice and in inoculation experiments with young plants it was shown that humid conditions favour the disease. This was one of the reasons to study the influence of the climate on disease development in cucumber crops grown in a commercial way. The increase of costs of energy in the recent years forces the glasshouse industry to bring down these costs and to minimize the waste of energy. Therefore is was necessary to study the effect of ventilating less than usual and of lower temperature regimes on the development of diseases.

## Materials and methods

The influence of the glasshouse climate on disease development has been studied in two cucumber crops in the 24 compartments of a glasshouse with a computer controlled climate (Van de Vooren and Koppe, 1975).

The first experiment was with an autumn crop in 1976 and the second one with a spring crop in 1979, both with cultivar Farbio. Plants were grown, while the side-shoots were taken away, until they reached the wire at about 2.1 m above soil level. Then the growing tip was removed and two side-shoots were allowed to grow down from the top. Powdery mildew was controlled by regu larly spraying of pyrazophos ('Curamil') or ditalimfos ('Plondrel'). In some treatments the closing of the ventilators was prevented by a minimum ventilator opening of 0 to 10% depending on outdoor conditions. The inoculum of D. bryoniae was prepared by growing the fungus for two weeks on cherry decoction agar under black light (Philips TL 20W F20 T12 BLB) to induce sporulation. The conidia were washed off with water and each plant was sprayed under high pressure with about 0.3 l suspension with a concentration of $10^5$ conidia per millilitre. The numer of lesions on the main stem were counted several times during the experiments to characterize the disease development. The fruits were harvested twice a week and any suspect fruit was cut in half lengthwise to check for internal rot.

Autumn crop

Cucumbers were planted in six rows each of eleven plants in each glasshouse compartment in mid June. The setting of the climate was divided into two periods:

- In the first period, the first five weeks after planting, two different regimes each in twelve replicates were set in order to obtain "sturdy" and "weak" plants. Sturdy plants were obtained by irrigating three times a week as little as possible, a minimum ventilator setting and a minimum water temperature of the heating system of 30°C. Weak plants were obtained by daily irrigating and no limitations on ventilation and water temperature. The temperature regime was 21/23°C (night /day).
- In the second period, at the start of production, different climate regimes were set in order to obtain variations in humidity. The temperature regimes were 18/23, 21/23 and 21/21°C. Transition from night to day was set one hour before or one hour after sunrise. So there were two replicates of both sturdy and weak plants.

At the beginning of the second period half of each compartment (three rows) was inoculated once. The crop was finished at the end of September, ten weeks after inoculation.

Spring crop

This experiment was set up to study the effect of day/night temperature regimes on growth of cucumbers, earliness, production rate (see Van de Vooren, 1980) and on disease development. Observations were made on 40 plants per compartment with a planting date end of December. The setting of the climate was divided into three periods:

- In the first period, the first eight weeks after planting, there were eight temperature regimes (see table 5) in three replicates.
- In the second period, the following six weeks, the temperature regime was 16/23°C.
- In the third period, the three replicates of the first period got a different climate regime in order to obtain different humidity conditions. The transition from night to day temperature and the ventilator setting were as follows:
  1. slow transition over a period of four hours starting two hours before sunrise and no minimum ventilation.

2. quick transition in the shortest period possible starting half an hour before sunrise and no minimum ventilation.
3. normal transition in half an hour starting at sunrise and a minimum ventilation.

In the treatments with no minimum ventilation the ventilators were closed when the air temperature was raised till three hours after sunrise. In the third period, the foliage was inoculated three times with intervals of two weeks. The crop was finished at the end of June, ten weeks after the first inoculation.

## Results

The first symptoms of the disease, small yellow and necrotic lesions in young leaves, could be observed about one week after inoculation. Diseased stubs in leaf axils could be observed about two weeks after inoculation. The first fruits with internal rot were found seven to ten days after inoculation.

### Autumn crop

The percentages diseased leaf axils of the main stem at the end of the crop are given in table 1. The disease development during the experiment on inoculated and non-inoculated sturdy and weak plants is given in fig. 1. The spread of the disease from inoculated to uninoculated sturdy and weak plants is given in table 2. The percentage infected fruits varied from harvesting date to harvesting date. The highest percentage infected fruits on a harvesting date was nearly 7% with inoculated weak plants. The average percentages fruits with internal rot in the period in which this symptom occurred are given in table 3. The differences in yield between non-inoculated and inoculated plants and between weak and sturdy plants are given in table 4.

Healthy looking fruits of two cropping dates, about 1000 fruits on each date, were stored at 20°C under humid conditions under a plastic sheet for two weeks. External fruit rot was found on 1.0% and 3.2% of the fruits of these two dates respectively. About 70% of the infected fruits were from weak plants and 30% from sturdy plants. There was no correlation between external fruit rot and temperature regime or time of the night-day temperature change. Of the fruits judged healthy of these two storages 0.2% and 1.0% showed internal rot.

The number of lesions on the main stem due to a natural infection of Botrytis cinerea (grey mould) were counted at the end of the crop. There was only an average of 0.3 lesions per plant. No significant differences between treatments did occur.

No mildewicide was sprayed in the last few weeks of the experiment. More than 50% of the surface of many leaves of sturdy plants was colonised by powdery mildew at the end of the crop. Weak plants had only some small colonies on a few leaves. No significant differences were observed between the other treatments.

### Spring crop

Since the number of leaves on the main stem differed between the treatments, percentages diseased leaf axils are given in tables and figures. The disease incidence at the end of the crop is given in table 5. The disease development during this experiment is given in fig. 2 and 3. The first two fruits with internal rot were found nine days after the first inoculation. The percentage infected fruits increased to 14% twenty days after the first inoculation, then decreased to 5% and in-

creased subsequently to 13% thirty days after the second inoculation. No pattern was observed later on. The average percentages of internally rotten fruits in the period in which this symptom occurred are given in table 6. No significant differences in yield did occur between the three treatments with different humidity conditions.
A natural infection of leaf axils caused by Botrytis cinerea was observed in the last two months of the experiment. The Botrytis lesions on the main stem were counted four times with intervals of two weeks. The percentage infected leaf axils doubled in these two months (fig. 4). The incidence of Botrytis at the end of the crop is given in table 7.

## Conclusions and discussion

In the autumn crop no significant differences in disease incidence between the temperature regimes and between the time of the night-day temperature change in the post-inoculation period was observed. Sturdy plants which were grown with minimum ventilator and minimum heating settings were significantly less affected than weak plants which were grown without minimum ventilator and heating settings (table 1). The spread of the disease to non-inoculated plants was quicker on weak than on sturdy plants (table 2). Fruits from sturdy plants were also significantly less affected internally (table 3) and externally than fruits from weak plants. However, sturdy plants produced obviously fewer healthy fruits than weak plants, especially on the side shoots (table 4). A high incidence of D. bryoniae reduced the number of healthy fruits. The incidence of D. bryoniae was not high in the period in which the fruits of the main stem were picked but still the number of healthy fruits was higher from non-inoculated than from inoculated plants. This difference was doubled, however, in the period in which the fruits from the side shoots were picked and a severe incidence of the disease occurred (table 4).
In the spring crop there was a postponed effect of the night temperature maintained during the pre-inoculation period on the disease incidence on the main stem later on in the season. The disease was more severe on plants grown at lower night temperatures in the pre-inoculation period (table 5). The day temperature in the pre-inoculation period had no effect on the incidence of D. bryoniae lesions later on in the season. Differences in percentage internal fruit rot were too small to observe significant differences between climate regimes (table 6).
The differences in disease incidence on the main stem in the spring crop were not as substantial as in the autumn crop. This explains probably that in the spring crop no effect of climate regimes on internal fruit rot could be found. A possible reduction in yield due to a D. bryoniae infection could not be established either in the spring crop. The percentage of fruits with internal rot was much higher in the spring crop than in the autumn crop. This may partly be a direct effect of the spraying of the conidial suspension. The autumn crop was inoculated once and the spring crop three times. However, the curve of the percentages of infected fruits during the experiment did not have peaks with the same time interval of two weeks as the three inoculations. The first peak, about 20 days after the first inoculation, indicates that fruit infection takes place in the flowering period.
The outbreak of D. bryoniae was favoured by humid conditions in the autumn crop. The daily maximum relative humidity was about 10% lower in the treatments with a minimum ventilator opening than in the treatments without it. The same tendency was found in the spring crop although

differences in disease incidence were not great. Once D. bryoniae being established in the spring crop the development of the disease passed on parallel (fig. 3). So to control the disease the main emphasis must be on preventing its establishment in the crop.
The outbreak of powdery mildew was more severe in the treatments with minimum ventilator and heating settings, so under drier conditions. This confirms the results obtained by Abiko and Kishi (1979).
The incidence of grey mould, Botrytis cinerea, was very low. Contrary to D. bryoniae it was most severe on plants grown at a temperature regime of 16/17°C in the first period after planting. The development of the plants in this treatment was disturbed amongst others by fasciation and the development of many fruits per leaf axil. Many fruits aborted and as Botrytis is known to infect plants via dead parts this may explain the high incidence of Botrytis in this treatment. Less grey mould was observed in the treatments with high night temperatures during the first period after planting as was found with D. bryoniae. Contrary to D. bryoniae the incidence of B. cinerea was most severe in the treatment with a minimum opening of the ventilators. The conclusions regardering B. cinerea have to be chequed in crops inoculated with this pathogen.
It is a matter of economics to calculate whether it is profitable to grow plants at lower temperatures and at higher humidities. In this economical model the risk of outbreaks of pathogens must be included. The results obtained so far indicate that it is possible to grow glasshouse cucumbers at lower temperature regimes and at higher humidities than in the past without getting great problems with fruit and stem rot, grey mould and powdery mildew.

Acknowledgements

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Table 1 - Percentages leaf axils of the main stem with D. bryoniae lesions at the end of an autumn crop of cucumbers with different plant types, grown at different climate regimes (33 plants per treatment in 2 replicates).

| Plant type | Night-day temperature in °C | Transition before (B) or after (A) sunrise | Non-inoculated plants | Inoculated plants |
|---|---|---|---|---|
| Sturdy | 18-23 | B | 16 | 48 |
| Sturdy | 18-23 | A | 14 | 41 |
| Sturdy | 21-23 | B | 11 | 44 |
| Sturdy | 21-23 | A | 13 | 37 |
| Sturdy | 21-21 | B | 13 | 45 |
| Sturdy | 21-21 | A | 17 | 53 |
| Weak | 18-23 | B | 44 | 65 |
| Weak | 18-23 | A | 41 | 70 |
| Weak | 21-23 | B | 44 | 61 |
| Weak | 21-23 | A | 43 | 62 |
| Weak | 21-21 | B | 35 | 53 |
| Weak | 21-21 | A | 33 | 63 |

Table 2 - Spread of D. bryoniae from inoculated (A, B and C) to uninoculated rows of plants (D, E and F) in an autumn crop of cucumbers. Mean percentages leaf axils of the main stem with D. bryoniae lesions (11 plants per row; 12 replicates).

| Plant type | Weeks after inoculation | Row | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| Sturdy | 2 | 7 | 7 | 4 | 0 | 0 | 0 |
| Sturdy | 5 | 27 | 29 | 27 | 7 | 0 | 0 |
| Sturdy | 10 | 47 | 51 | 46 | 14 | 11 | 11 |
| Weak | 2 | 11 | 11 | 11 | 0 | 0 | 0 |
| Weak | 5 | 40 | 46 | 43 | 18 | 7 | 7 |
| Weak | 10 | 61 | 68 | 64 | 47 | 37 | 36 |

Table 3 - Number and percentages of internally rotten fruits by D. bryoniae on non-inoculated and inoculated plants of an autumn crop of cucumbers grown at different climate regimes (396 or 264 plants per treatment).

| Treatment | | non-inoculated plants | | inoculated plants | |
|---|---|---|---|---|---|
| | | number | % | number | % |
| Sturdy plants | | 62 | 0.9 | 182 | 2.6 |
| Weak plants | | 172 | 2.2 | 325 | 4.3 |
| Night-day temperature change | 1 h before sunrise | 130 | 1.7 | 281 | 3.8 |
| | 1 h after sunrise | 104 | 1.4 | 226 | 3.2 |
| Night-day temperature | 18-23 | 91 | 1.8 | 167 | 3.4 |
| | 21-23 | 71 | 1.5 | 166 | 3.6 |
| | 21-21 | 72 | 1.5 | 174 | 3.5 |

Table 4 - Differences in number and in percentage of not by D. bryoniae infected fruits between non-inoculated (N) and D. bryoniae inoculated (I) plants and between weak (W) and sturdy (S) plants (792 plants per treatment).

| Harvesting period | N - I | | W - S | |
|---|---|---|---|---|
| | number | % | number | % |
| Stem fruits | 325 | 4.4 | -203 | -2.9 |
| Side shoot fruits | 856 | 9.1 | +1666 | +16.8 |
| All fruits | 1181 | 7.0 | +1463 | + 8.6 |

Table 5 - Percentages leaf axils of the main stem with D. bryoniae lesions at the end of a spring crop of cucumbers with different temperature regimes in the pre-inoculation period and a slow (S), quick (Q) and normal (N) transition from night to day temperature, S and Q without and N with minimum ventilation, in the post-inoculation period (means of 40 plants).

| Pre-inoculation climate night-day temperature in °C | Post-inoculation climate | | | |
|---|---|---|---|---|
| | S | Q | N | mean |
| 12-23 | 65 | 65 | 51 | 60 |
| 16-23 | 54 | 52 | 53 | 53 |
| 20/12-23 | 58 | 53 | 53 | 55 |
| 20-23 | 50 | 47 | 42 | 46 |
| 24-23 | 49 | 42 | 33 | 41 |
| 16-17 | 51 | 43 | 51 | 48 |
| 16-20 | 60 | 57 | 53 | 57 |
| 16-23 | 54 | 52 | 53 | 53 |
| 16-26 | 56 | 51 | 44 | 50 |
| mean | 55 | 51 | 47 | 51 |

Table 6 - Percentages of internally rotten fruits by D. bryoniae in a spring crop of cucumbers with different pre- and post-inoculation climates (40 plants per treatment).

| Pre-inoculation climate night-day temperature in °C | Post-inoculation climate | | | |
|---|---|---|---|---|
| | S | Q | N | mean |
| 12-23 | 9.9 | 9.3 | 7.2 | 8.8 |
| 16-23 | 7.4 | 8.0 | 6.9 | 7.4 |
| 20/12-23 | 9.5 | 6.7 | 6.8 | 7.6 |
| 20-23 | 8.2 | 9.5 | 7.5 | 8.4 |
| 24-23 | 6.8 | 6.9 | 7.9 | 7.2 |
| 16-17 | 9.2 | 7.3 | 7.9 | 8.1 |
| 16-20 | 10.4 | 7.7 | 10.0 | 9.4 |
| 16-23 | 7.4 | 8.0 | 6.9 | 7.4 |
| 16-26 | 9.3 | 9.5 | 8.6 | 9.0 |
| mean | 8.9 | 8.1 | 7.8 | 8.2 |

Table 7 - Influence of climate regimes during the first two and the last three months on percentages leaf axils of the main stem diseased by Botrytis cinerea at the end of a spring crop of cucumbers (means of 40 plants).

| Night-day temperature in °C in the first two months | Climate regime in the last three months | | | |
|---|---|---|---|---|
| | S | Q | N | mean |
| 12-23 | 0.2 | 1.7 | 3.2 | 1.7 |
| 16-23 | 3.4 | 2.5 | 4.8 | 3.6 |
| 20/12-23 | 2.7 | 5.7 | 4.3 | 4.2 |
| 20-23 | 0.1 | 0.1 | 2.3 | 0.8 |
| 24-23 | 0.1 | 0.9 | 0.7 | 0.6 |
| 16-17 | 4.8 | 4.2 | 5.5 | 4.8 |
| 16-20 | 1.1 | 3.0 | 5.3 | 3.1 |
| 16-23 | 3.4 | 2.5 | 4.8 | 3.6 |
| 16-26 | 0.8 | 1.3 | 2.8 | 1.6 |
| mean | 1.7 | 2.4 | 3.6 | 2.6 |

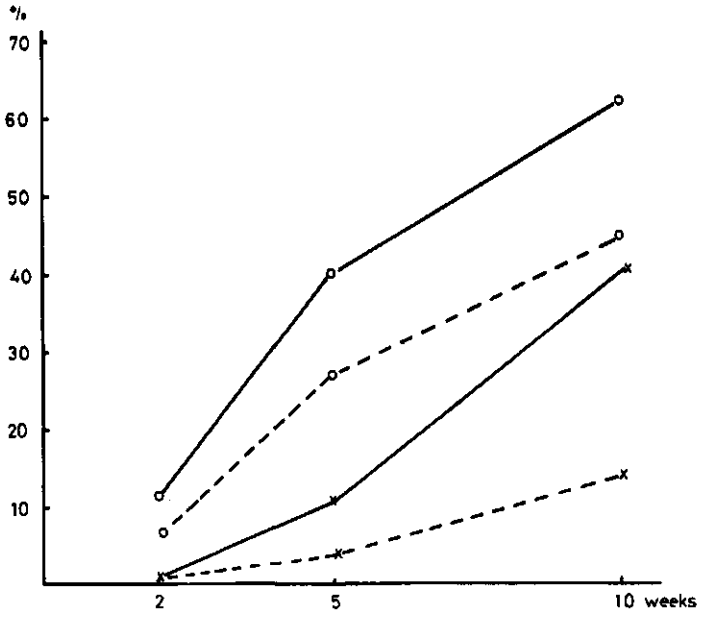


Figure 1

Effect of climate regimes on percentages of D. bryoniae diseased leaf axils of the main stem of uninoculated (x) and inoculated (o) plants of an autumn crop of cucumbers during 10 weeks after inoculation. Weak plants (——) were grown without and sturdy plants (---) with minimum ventilator and minimum heating settings.

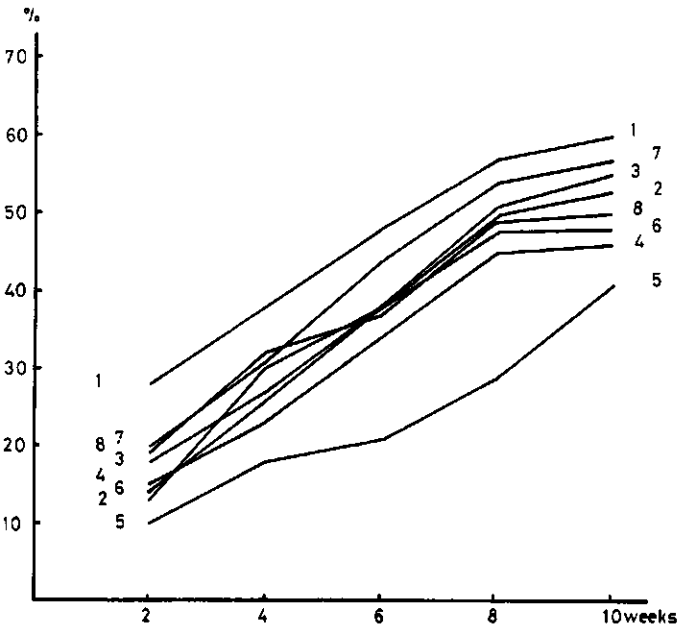


Figure 2

Influence of 8 temperature regimes in the pre-inoculation period on percentages leaf axils of the main stem with D. bryoniae lesions during 10 weeks after the first inoculation of a spring crop of cucumbers.
1 = 12/23, 2 = 16/23, 3 = 20-12/23, 4 = 20/23, 5 = 24/23, 6 = 16/17, 7 = 16/20, 8 = 16/26°C.

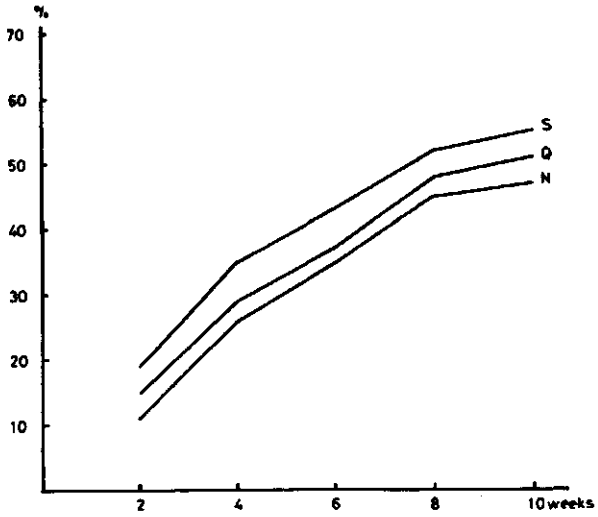


Figure 3

Influence of the post-inoculation climate on percentages leaf axils of the main stem with D. bryoniae lesions during 10 weeks after the first inoculation of a spring crop of cucumber.
S slow, Q quick and N normal transition form night to day temperature; S and Q without and N with minimum ventilation.

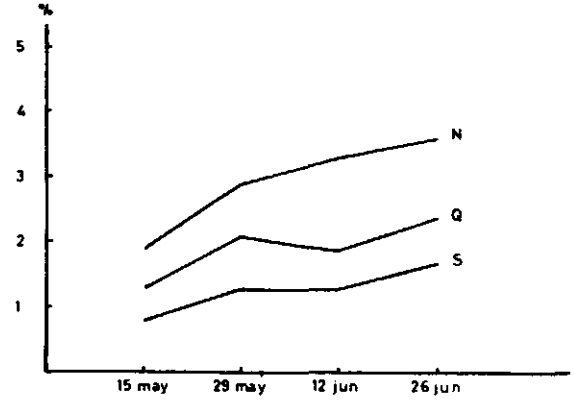


Figure 4

Influence of climate regimes during the last three months of a spring crop of cucumbers on percentages leaf axils of the main stem diseased by Botrytis cinerea.
S slow, Q quick and N normal transition from night to day temperature; S and Q without and N with minimum ventilation.

# INFLUENCE OF VENTILATION TEMPERATURE AND LOW VENTILATION RATES ON INCIDENCE OF DIDYMELLA BRYONIAE IN GLASSHOUSE CUCUMBERS

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## Abstract

The influence of ventilation temperature and of little ventilation with an electric fan, with an air exchange of four compartment volumes per hour, on the development of D i d y m e l l a b r y o n i a e in glasshouse-grown autumn cucumber crops was studied on both inoculated and uninoculated plants.

Depending on whether the plants were inoculated or not and on the period after inoculation the number of lesions on the main stem was 0.5 to 3 times higher at a ventilation temperature of 26°C than at one of 23°C when the heating temperature was set at 19/21°C (night/day). Internal fruit rot occurred two to three times more frequently at a ventilation temperature of 26°C than at one of 23°C. In another year when heating temperature was 19°C (night and day) disease incidence on the main stem did not differ at a ventilation temperature of 23°C and one of 26°C, but internal fruit rot occurred three to four times more frequently at a ventilation temperature of 26°C.

The use of a fan during 2 h in the early morning at a ventilation temperature of 26°C did not or hardly decrease the disease incidence on plants and fruits. The use of a fan during daylight at a ventilation temperature of 26°C sometimes reduced plant infection but fruit infection occurred even more frequently in one experiment.

Inoculation of the plants reduced fruit production by 0 to 20%, depending on growing and climatic conditions.

The influence of the ventilation regimes on the development of a natural infection of B o t r y t i s c i n e r e a was about the same as it was on the development of D . b r y o n i a e on plants not inoculated with D . b r y o n i a e.

## 1. Introduction

Stem and fruit rot caused by D i d y m e l l a b r y o n i a e (Auersw.) Rehm (synonyms: M y c o s p h a e r e l l a c i t r u l l i n a (C.O.Sm.)Gross. and M y c o s p h a e r e l l a m e l o n i s (Pass.) Chiu and Walker) is an important disease in glasshouse-grown cucumber crops. Symptoms and economic importance of the disease have been described elsewhere (Van Steekelenburg, 1978 a, 1982; Van Steekelenburg and Van de Vooren, 1981). Frequent sprayings with fungicides control the disease to a certain level (Van Steekelenburg, 1978 a, b). Resistance to D . b r y o n i a e in cucumber has been found in Russia (Belova, 1970; Boos and Belova, 1974; Prokhorova, 1975) and in the Netherlands (Van der Meer et al., 1978), but suitable resistant cultivars are not yet available (Van Steekelenburg, 1981).

In glasshouse crops, the incidence of the disease can be influenced by controlling the climate. Humid conditions favoured the development of the disease on young plants in inoculation experiments (unpublished data; Svedelius and Unestam, 1978) and on commercially grown plants (Van Steekelenburg and Van de Vooren, 1981). The majority of the glasshouses in the Netherlands is heated by circulating warm water in a system of steel pipes when the air temperature is below the setpoint for heating. The ventilation windows are opened when, as a result of solar radiation, the glasshouse air temperature rises above the setpoint for ventilation, which is usually 1 to 2 degrees above the setpoint for heating. Fuel costs can be reduced by minimizing ventilation and allowing solar radiation to raise the temperature without opening the ventilation windows. Adverse effects of the resulting higher humidity might be avoided by using an electric fan. The effect of differences in ventilation rates with the aid of an electric fan on plant development and production were studied in co-operation with the glasshouse climate department of the Research Station. The phytopathological consequences of it are reported here.

## 2. Materials and methods

The influence of ventilation temperature and ventilation rate on development of stem and fruit rot was studied in three autumn crops of cucumber during 1979 till 1981. The experiments were conducted in four glasshouse compartments of 192 $m^2$ each. Cultivar Farbio was planted out at a spacing of 1.6 x 0.5 m (one plant per 0.8 $m^2$) in mid-August. The growing tip of the main stem was removed just above the suspending wire at about 2.3 m above border soil level. Side shoots were removed along the main stem, except for the top two or three. Temperature and ventilation were controlled automatically by analogue electronic equipment. The first 5 to 7 weeks equal climate regimes were set in all four compartments with heating at 21°C (night and day) and ventilation at 22/24°C (night/day). From the beginning of October the heating temperature was 19/21°C (night/day) and differences in ventilation were set.

In 1979, one compartment was normally ventilated at 23°C and had a minimum water temperature of the heating system of 40°C. The other three compartments were ventilated at 26°C and had no minimum water temperature of the heating system. In two of them an exhaust-fan, with an air exchange of four compartment volumes per h, which is equivalent to a ventilation rate of about 12 $m^3$ per $m^2$.h, was installed in a gable. This fan ran in these two compartments in the mornings for 2 h starting half an hour behore sunrise. The fan in one compartment ran also during the other daylight hours at a rotation speed proportionate to the relative humidity within the range of 50 to 95%.

In 1980, two treatments were applied in duplicate. In all compartments the ventilators were opened at 26°C; two compartments were without a fan and in two others a fan ran for 2 h in the early morning.

In 1981, the experiment of 1979 was repeated, except that the heating temperature was not raised during the day and in the compartment ventilated at 23°C no minimum temperature of the heating system was set.

Within the first week after differences in ventilation were set, six plots of six plants or four plots of eight plants in each compartment were inoculated by spraying each plant with 0.3 l suspension ($10^6$ conidia per ml). The inoculum was prepared by washing off the conidia of two-week-old cultures of the fungus grown on cherry decoction agar under black light (Philips TL 20W F20 T12 BLB). Observations on disease development and fruit production were made on inoculated and uninoculated plants, which were separated by guard plants. Every 2 weeks the numbers of lesions on the main stems were counted. The fruits were harvested twice a week and any suspect fruit was cut longitudinally at the blossom end to check for internal rot. Fruits with external rot due to D . b r y o n i a e or B o t r y t i s c i n e r e a at the time of harvest were noted separately (see also Van Steekelenburg, 1982). The fungicides sprayed were pyrazophos (Curamil), ditalimfos (Plondrel) and fenarimol (Rubigan) to control powdery mildew.

## 3. Results

### Experiment in 1979

The plants had on average 29 internodes on the main stem. Differences in percentage of D . b r y o n i a e -diseased leaf axils of the main stem were already noticed 2 weeks after inoculation, with the highest disease incidence in the compartments ventilated at 26°C without or with a fan during 2 morning hours (fig. 1). On inoculated plants, the increase of the disease in the period after these 2 weeks was about equal in all four compartments. On uninoculated plants, both the disease incidence 2 weeks after inoculation and the increase of the disease afterwards was highest on plants grown at a ventilation temperature of 26°C without using a fan (fig. 1).

The production in number of fruits is given in table 1. The mean fruit weight was about 450 g. At a ventilation temperature of 26°C there was a tendency that the less the fan was used the more fruits were produced. Inoculation resulted in an average reduction of 18% of the total number of harvested fruits in all compartments, of 12% in the compartment with a ventilation temperature of 23°C and of 20% in the three compartments with a ventilation temperature of 26°C. Fruit production of inoculated plants was reduced even more, a mean of 24%, if only healthy fruits are taken into account.

The percentages of infected fruits depended on harvesting date and varied between 0 and 46 for internal rot and between 0 and 24 for external rot. The average percentages internally rotten fruits during the 8 weeks of the experiment were two to three times higher with a ventilation temperature of 26°C than with one of 23°C (table 1). With external fruit rot the difference between the two treatments was even greater. At a ventilation temperature of 26°C, fruit infection decreased when in addition a fan was used during 2 morning hours, but increased when the fan was used during daylight.

A spontaneous infection of B o t r y t i s c i n e r e a was observed. On D . b r y o n i a e - inoculated plants, 2 to 3% of the leaf axils of the main stem were diseased by B . c i n e r e a at the end of the experiment. On D . b r y o n i a e - uninoculated plants, B . c i n e r e a infection was more severe.

It decreased when the fan was used during a longer period (fig. 2). The percentages of fruits infected by B . c i n e r e a were very low; less than 1% of the fruits from D . b r y o n i a e - inoculated plants and about 2% of the fruits from D . b r y o n i a e - uninoculated plants.

Experiment in 1980

The mean number of internodes on the main stem was 25. At the time of inoculation, 5 to 8% of the leaf axils of the main stem were already diseased by D . b r y o n i a e and 6 to 8% by B . c i n e r e a. The crop was terminated early, 4 weeks after inoculation, due to the rather poor condition of the plants. At that time, 18% of the leaf axils of the main stem was diseased by D . b r y o n i a e and 23% by B . c i n e r e a with hardly any differences between the treatments and between inoculated and uninoculated plants.

The production of fruits in the post-inoculation period was highest in the glasshouses where an electric fan was used (table 2), but over the whole cropping period no differences in fruit production between the treatments were observed. Inoculation did not reduce the number of fruits harvested. The percentages internally and externally infected fruits were low and no significant differences between the treatments occurred (table 2).

Experiment in 1981

The mean number of internodes on the main stem was 28. On inoculated plants, no differences in percentage diseased leaf axils of the main stem were observed during the experiment (fig. 3). On uninoculated plants, the disease incidence on the main stem differed only slightly between the treatments during the first 4 weeks, but the increase of the disease in the last 2 weeks was lowest in the two compartments in which a fan was used (fig. 3).

Production of fruits in the compartment ventilated at 23$^{o}$C cannot be compared with the production in the other compartments. Due to technical troubles with the irrigation system this compartment had to be fertilized differently. Inoculation did not influence the number of fruits harvested (table 3). The percentages internally infected fruits were rather low but were highest when the fan was not used at all or only during 2 early morning hours (table 3).

The development of a natural infection of B . c i n e r e a on the main stem of D . b r y o n i a e - uninoculated plants is given in fig. 4. The effect of ventilation on the incidence of B . c i n e r e a lesions was about the same as on the incidence of D . b r y o n i a e lesions on uninoculated plants. On D . b r y o n i a e - inoculated plants, the initial infection of B . c i n e r e a on the main stem after 2 weeks was about as high as on uninoculted plants; it did not increase much. After 6 weeks, it was in all four compartments three times less than on uninoculated plants. Only occasionally a fruit was infected by B . c i n e r e a.

## 4. Discussion and conclusion

In spite of attempts to control the glasshouse climate it will differ from day to day and from year to year as the climate indoors depends on weather conditions outdoors. The difference in growing conditions from one year to another, is reflected in the number of internodes of the main stem. In 1980, the plants grew vigorously with more than 10% fewer internodes on the main stem than in the two other years. This resulted in weak plants with thick stems and large leaves, which are very susceptible to diseases (Van Steekelenburg and Van de Vooren, 1981) by which the high spontaneous infection of both D . b r y o n i a e and B . c i n e r e a in 1980 can be explained.

The main stem of the plants of the various treatments was grown each year under the same conditions during the pre-inoculation period. Differences in disease development on the main stem between the treatments must be ascribed to the direct or indirect influence of the ventilation regimes during the post-inoculation period. The main factor causing an increase in disease incidence is humidity. At a higher ventilation temperature, the relative humidity is maintained at the high night level during a longer period, as long as the ventilation windows have not been opened.
Moreover, as a result of solar radiation in the morning, the plant temperature rises slower than the glasshouse air temperature and comes below the dew point temperature, resulting in condensation when there is no ventilation (Mihara and Hayashi, 1978).

In the treatments with a ventilation temperature of 23°C, the interval between heating and ventilation temperature during the day was 2°C in 1979 and 4°C in 1981. Therefore the conditions in 1981 will have been more humid than in 1979. This may explain the difference in disease incidence on the main stem 2 weeks after inoculation in the treatments with a ventilation temperature of 23°C and 26°C in 1979 (fig. 1), whereas hardly any difference in disease incidence between these two treatments was observed in 1981 (fig. 3).

Apart from causing fruit rot D . b r y o n i a e can reduce the production of fruits as was observed in the 1979 experiment and previously (Van Steekelenburg, 1983; Van Steekelenburg and Van de Vooren, 1981).

A higher infection pressure on inoculated plants resulted in more internally infected fruits but not always in more externally infected fruits (tables 1 and 3). The highest incidence of internally rotten fruits in the compartment with a fan running during daylight in the 1979 experiment (table 1) might be a result of the dispersal of ascospores of D . b r y o n i a e with the air currents caused by the fan. Air-borne ascospores can cause internal fruit rot by flower infection (unpublished data) and in the glasshouse air they can be present in high numbers (Fletcher and Preece, 1966; Van Steekelenburg 1983). However, results of 1979 are in contradiction with those of 1981 when internal fruit rot was not observed at all in the compartment with a fan running during daylight (table 3).

B . cinerea and D . b r y o n i a e are competitive in their infection of leaf axils of the main stem. The highest incidence of B . c i n e r e a will therefore occur on D . b r y o n i a e - uninoculated plants. As far as the effect of ventilation on the incidence of B . c i n e r e a could be checked, it was the same as on that of D . b r y o n i a e.

A greater interval between heating and ventilation temperature will increase substantially the risk of the incidence of both D. bryoniae and B. cinerea in cucumber crops. The effect of little ventilation by using a fan under the conditions of a great interval between heating and ventilation temperature is doubtful and insufficient and will increase costs of energy. Whether or not a glasshouse should be ventilated by opening the ventilator windows is a matter of costs and returns. However, earning money by energy-saving climate regimes can be at the cost of both the quality and quantity of the cucumber fruits produced.

## 5. Acknowledgements

Thanks are due to D. Bokhorst, Institute for Agricultural Engineering, for the cooperation and the care for the climate regimes, to B.C. van Dam and S.J. Paternotte for their help in carrying out the experiments and to W.A. van Winden for his help with the English text.

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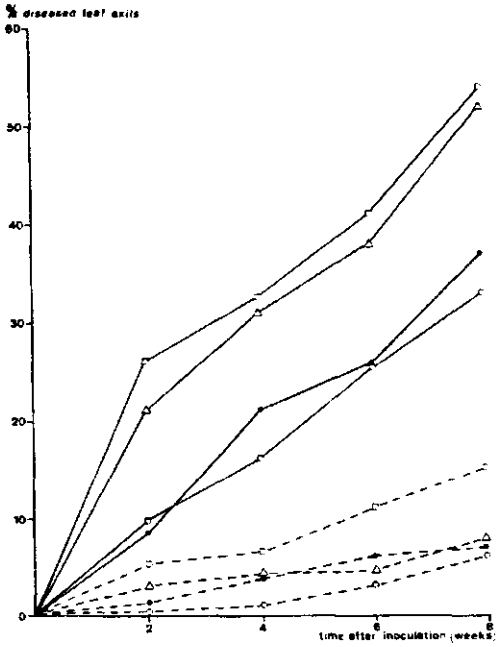



● window ventilation at 23°C and no fan

o window ventilation at 26°C and a fan during daylight

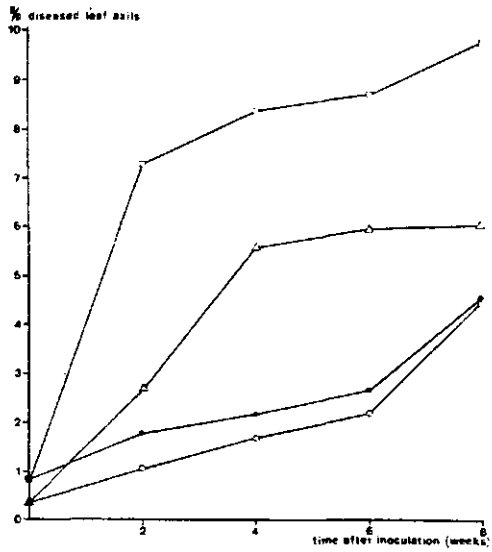
△ window ventilation at 26°C and a fan during 2 h in the early morning

□ window ventilation at 26°C and no fan

Figure 1 - Effect of ventilation regime on percentage of D . b r y o n i a e - diseased leaf axils of the main stem of inoculated (——) and uninoculated (---) cucumber plants during 8 weeks in an autumn crop in 1979 with night/day heating temperatures of 19/21°C (means of 36 plants).

Table 1 - Effect of ventilation regime and inoculation with D . b r y o n i a e on total number of fruits harvested and on percentage of infected fruits during 8 weeks after inoculation of an autumn crop of cucumbers in 1979 with night/day heating temperatures of 19/21°C (36 plants per treatment).

| Treatment | | Inoculated plants | | | Uninoculated plants | | |
|---|---|---|---|---|---|---|---|
| Ventilation temperature (°C) | Use of fan | Fruits per m2 | Internal rot (%) | External rot (%) | Fruits per m2 | Internal rot (%) | External rot (%) |
| 23 | no | 10.1 | 6.2 | 0.3 | 11.5 | 2.1 | 0.3 |
| 26 | daylight | 8.8 | 17.4 | 2.8 | 10.9 | 8.3 | 5.7 |
| 26 | 2 hours | 9.0 | 9.6 | 1.2 | 11.3 | 3.1 | 2.2 |
| 26 | no | 9.9 | 11.3 | 4.6 | 12.5 | 6.6 | 6.9 |




● window ventilation at 23°C and no fan

o window ventilation at 26°C and a fan during daylight

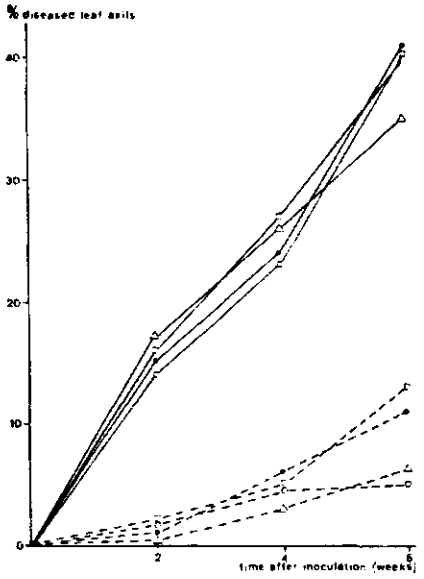
△ window ventilation at 26°C and a fan during 2 h in the early morning

□ window ventilation at 26°C and no fan

Figure 2 - Effect of ventilation regime on percentage of B o t r y t i s c i n e r e a-diseased leaf axils of the main stem of D . b r y o n i a e-uninoculated cucumber plants during 8 weeks in an autumn crop in 1979 with night/day heating temperatures of 19/21°C (means of 36 plants).

Table 2 - Effect of ventilation at 26°C with and without a fan during 2 h in the early morning on total number of fruits harvested and on percentage of infected fruits during 4 weeks after inoculation with D . b r y o n i a e of an autumn crop of cucumbers in 1980 (36 plants per treatments; two replicates).

| Ventilation | Inoculated plants | | | Uninoculated plants | | |
|---|---|---|---|---|---|---|
| | Fruits per m2 | Internal rot (%) | External rot (%) | Fruits per m2 | Internal rot (%) | External rot (%) |
| With fan | 12.1 | 1.1 | 0.7 | 12.1 | 2.3 | 0.6 |
| Without fan | 10.5 | 0.8 | 0.5 | 10.2 | 1.4 | 1.2 |




● window ventilation at 23°C and no fan

o window ventilation at 26°C and a fan during daylight

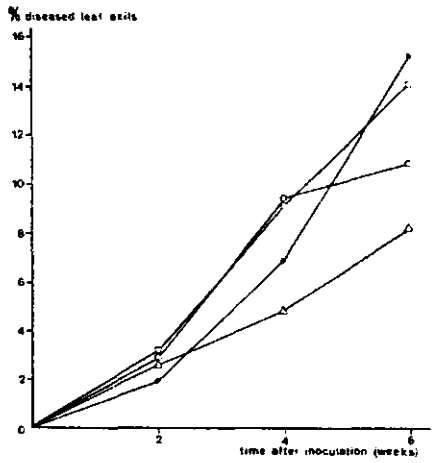
△ window ventilation at 26°C and a fan during 2 h in the early morning

□ window ventilation at 26°C and no fan

Figure 3 - Effect of ventilation regime on percentage of D . b r y o n i a e-diseased leaf axils of the main stem of inoculated (——) and uninoculated (---) cucumber plants during 6 weeks in an autumn crop in 1981 with a night and day heating temperature of 19°C (means of 32 plants).

Table 3 - Effect of ventilation regime and inoculation with D . b r y o n i a e on total number of fruits harvested and on percentage of infected fruits during 6 weeks after inoculation of an autumn crop of cucumbers in 1981 with a night and day heating temperature of 19°C (32 plants per treatment).

| Treatment | | Inoculated plants | | | Uninoculated plants | | |
|---|---|---|---|---|---|---|---|
| Ventilation temperature (°C) | Use of fan | Fruits per m2 | Internal rot (%) | External rot (%) | Fruits per m2 | Internal rot (%) | External rot (%) |
| 23 | no | 7.4 | 0.9 | 1.0 | 7.5 | 0.2 | 0.7 |
| 26 | daylight | 4.2 | 0.0 | 0.6 | 4.1 | 0.0 | 0.3 |
| 26 | 2 hours | 5.5 | 2.8 | 1.2 | 5.7 | 0.6 | 0.0 |
| 26 | no | 4.9 | 2.6 | 1.7 | 4.7 | 0.9 | 0.0 |




● window ventilation at 23°C and no fan

○ window ventilation at 26°C and a fan during daylight

△ window ventilation at 26°C and a fan during 2 h in the early morning

□ window ventilation at 26°C and no fan

Figure 4 - Effect of ventilation regime on percentage of *Botrytis cinerea*-diseased leaf axils of the main stem of *D. bryonia*e-uninoculated cucumber plants during 6 weeks in an autumn crop in 1981 with a night and day heating temperature of 19°C (means of 32 plants).

Neth. J. Pl. Path. 91 (1985) 225-233

# Influence of time of transition from night to day temperature regimes on incidence of Didymella bryoniae and influence of the disease on growth and yield of glasshouse cucumbers

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**Abstract**

The influence of transition from night to day temperature 3 h before, 1 h before, 1 h after and 3 h after sunrise on the incidence of *Didymella bryoniae* was studied both on inoculated and on uninoculated glasshouse-grown cucumber plants. The effect of inoculation on plant growth and fruit production was studied as well.

The later the transition to day temperature took place, the longer were the periods with a high relative air humidity and of condensation of water on fruits.

The time of transition had no effect on plant growth, yield, disease incidence on growing tips, number of lesions on the main stems of uninoculated plants and external fruit rot. The later the transition to day temperature took place, the more lesions on the main stem of inoculated plants appeared and the higher was the incidence of internal fruit rot.

Inoculation of plants increased the number of lesions on the main stem, the disease incidence on growing tips, the production of misshapen fruits and the internal and external fruit rot. The number of secondary side shoots was increased but the total number of their internodes was reduced by inoculation.

Inoculation caused an 18% reduction in number of internodes over a period of four weeks and a 10% reduction in number of fruits in the corresponding harvest period.
The consequences of a more humid glasshouse climate and of a high infection pressure of *D. bryoniae* for the grower are briefly discussed.

*Additional keywords: Cucumis sativus,* glasshouse climate, *Mycosphaerella citrullina, Mycosphaerella melonis,* stem and fruit rot.

## Introduction

*Didymella bryoniae* (Auersw.) Rehm (synonyms: *Mycosphaerella citrullina* (C.O. Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu and Walker) causes stem and fruit rot in cucumber crops. Symptoms and economic importance of the disease have been described earlier (Van Steekelenburg, 1978, 1982; Van Steekelenburg and Van de Vooren, 1981). Under controlled environmental conditions, infection of cucumber leaf tissue occurred only if the surface was kept wetted and for further expansion of the

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disease high humidity was required (Svedelius and Unestam, 1978).

In glasshouse crops, the incidence of the disease is directly and indirectly influenced by the glasshouse climate (Van Steekelenburg, 1984; Van Steekelenburg and Van de Vooren, 1981). The glasshouse climate is determined by a number of factors, of which outdoor climate, heating, ventilation and transpiration of the plants are the most important ones. After sunrise plant transpiration and, consequently, humidity increase. Moreover, plant and fruit temperatures rise slower than the air temperature. This results in water condensation on plant parts, especially fruits, with a temperature below dew point (Mihara and Hayashi, 1978). In glasshouse crops a low night temperature is maintained to save energy. However, in combination with a great interval between night and day temperature, it will increase humidity and condensation on fruits. In heated glasshouse crops the air temperature and, consequently, the plant temperature can be raised before the air temperature is heated up through sun heat. When the day setpoint is reached ventilation will decrease the air humidity. Therefore, the effect of time of transition from night to day conditions on the glasshouse climate and, consequently, on the incidence of stem and fruit rot was studied.

In previous experiments it was observed that *D. bryoniae* can reduce the production of fruits (Van Steekelenburg, 1983, 1984; Van Steekelenburg and Van de Vooren, 1981). The influence of the incidence of *D. bryoniae* on fruit production and plant growth is reported in this paper as well.

## Materials and methods

*Climate regimes.* The experiment was conducted under computer-controlled climate conditions in eight compartments of the glasshouse described by Van de Vooren and Koppe (1975). Equal climate regimes were set in all compartments during the first five weeks until the growing tip of the main stem was removed. Subsequently the time of transition from night to day temperature was set in two replicates 3 h before, 1 h before, 1 h after and 3 h after sunrise. The period of transition was 2 h. The heating temperatures were 16/24 °C (night/day) and ventilation temperatures were 17/26 °C (night/day).

Transition from day to night conditions was equal for all treatments: over a period of 2 h, starting 2 h before sunset. Temperatures were measured with copper constantan thermo-couples. Humidity was calculated from dry and wet bulb measurements.

*Plants.* Five-week-old cucumber plants of cv. Profito, resistant to powdery mildew, were planted in six rows of ten plants in each compartment on 21 April 1982. The plants had been grafted on *Cucurbita ficifolia.* The side shoots were removed along the main stem until the plants reached the suspending wire at about 2.1 m above soil level. Then the growing tip of the main stem was removed and two side shoots were allowed to grow down from the top. At about 0.8 m above soil level the growing tips of the side shoots were removed and secondary side shoots were allowed to develop.

*Pathogen and inoculation.* Virulent isolates of *D. bryoniae* were grown for two weeks on oat meal agar under black light (Philips TL 20 W F20 T12 BLB). The conidia were washed off with water. All plants of two neighbouring rows were sprayed with 0.3 l suspension each ($10^6$ conidia per ml) in the beginning of June, when three to four

leaves on the side shoots had developed. Inoculation was repeated one month afterwards.

*Plant growth measurements.* The number of internodes on side shoots and secondary side shoots and the number of secondary side shoots were counted at weekly or fortnightly intervals on two rows of plants in each compartment. Plant growth was recorded during a period of seven weeks after the first inoculation.

*Fruit production measurements.* Twice a week, fruits were harvested, counted and weighed, after grading in full-grown and misshapen fruits.

*Disease assesment.* The number of lesions on the main stem was counted several times. The disease incidence on the growing tip and youngest leaves was rated according to an arbitrary scale from 0 to 4, in which 0 = no symptoms, 1 = small yellow lesions in the youngest leaves, 2 = slight malformation of leaves, 3 = severe malformation of leaves and 4 = growing tip (nearly) dead. At harvest, suspect fruits were cut longitudinally at the blossom end to check for internal rot. The fruits were also checked for external rot caused by *D. bryoniae.*

## Results

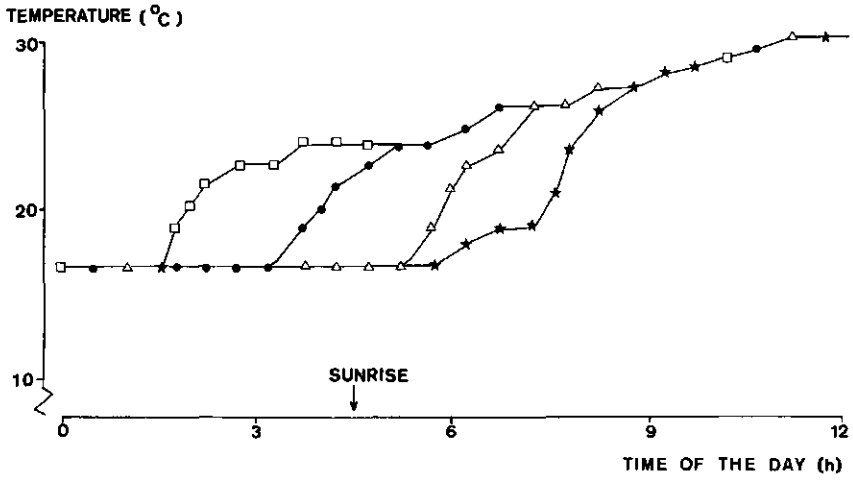
### *Glasshouse climate*

The temperature and humidity were only different for the four regimes during the morning hours between 3 h before sunrise and about 4 h after sunrise. The later the transition to day conditions took place, the longer the period a high relative humidity was maintained (Fig. 1). It was observed that in the early morning hours fruits were often wet from condensation in all treatments. The earlier the transition to day conditions, the earlier the fruits dried up. In the treatments with transition after sunrise the period the fruits remained wet was about two hours longer than with transition before sunrise.

### *Influence of climate on disease*

*Disease incidence on the main stem.* No spontaneous infection was observed at the time of the first inoculation. Data on the development of the disease on the main stem are presented in Table 1. Disease incidence on inoculated plants was always more severe than that on uninoculated plants ($p < 0.01$). The climate regimes had no significant effect on the incidence of the disease on uninoculated plants. Transition to day conditions after sunrise increased the number of *D. bryoniae* lesions on inoculated plants after four weeks. Later in the season the differences in disease incidence decreased.

*Disease incidence on the growing tip.* Three weeks after inoculation the disease incidence on growing tips of side shoots was only slight with no significant differences for the different climate regimes. Uninoculated plants did not show symptoms at all. The climate regimes had no effect either on the disease incidence on growing tips of secondary side shoots. The mean disease index of the secondary side shoots increased from 0.04 to 0.45 on uninoculated plants and from 0.41 to 1.03 on inoculated plants during the period between 4 and 7 weeks after the first inoculation.



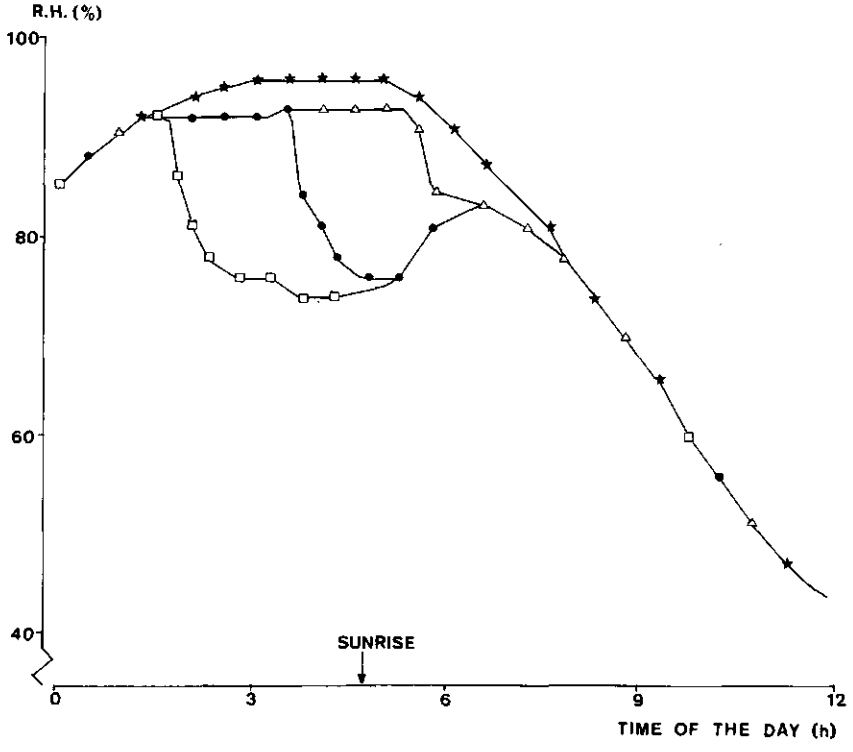




Fig. 1. Temperature and relative humidity of the glasshouse air in a cucumber crop with transition to day conditions 3 h before (□), 1 h before (●), 1 h after (▵) and 3 h after (★) sunrise in the morning of 10 June.

Table 1. Number of lesions caused by *D. bryoniae* on the main stem of uninoculated (U) and inoculated (I) cucumber plants grown at different times of transition to day conditions. Treatments included 20 plants in each of two replicates.

| Transition time to sunrise | 30 June | | 5 August | | 22 September | |
|---|---|---|---|---|---|---|
| | U | I | U | I | U | I |
| −3 h | 0.3[a1] | 1.8[a] | 2.6[a] | 5.5[a] | 6.5[a] | 9.2[a] |
| −1 h | 0.3[a] | 3.4[a] | 3.5[a] | 6.2[a] | 5.6[a] | 9.7[a] |
| +1 h | 0.9[a] | 6.3[b] | 3.4[a] | 7.9[a] | 5.9[a] | 9.3[a] |
| +3 h | 0.5[a] | 7.3[b] | 3.9[a] | 8.4[a] | 5.9[a] | 9.0[a] |

[1] Entries of one column marked with different letters differ significantly at $p < 0.05$ (Student test).

*Internal fruit rot.* The first fruits with internal rot were found on 17 June, 15 days after the first inoculation. The percentage of fruits with internal rot was higher on inoculated plants than on uninoculated ones in the harvest period until 12 July (Table 2). No effect of inoculation was observed later in the season, however, over the whole period until 20 September there was still some effect. The climate regimes had a significant effect ($p < 0.05$) on the incidence of internal rot (Fig. 2). The later the transition to day conditions took place, the higher was the number of fruits with internal rot. The average percentage of fruits with internal rot of inoculated and uninoculated plants for the regimes with transitions to day conditions 3 h before, 1 h before, 1 h after, 3 h after sunrise were 2.1, 4.1, 6.4 and 6.8, respectively.

Table 2. Effect of inoculation of cucumber plants with *D. bryoniae* on total fruit production and on percentage of fruits with internal and external rot in two harvest periods. Treatments included 20 plants in each of eight replicates.

| Harvest period | Inoculation | Fruit production | | Internal fruit rot (%) | External fruit rot (%) |
|---|---|---|---|---|---|
| | | kg per plant | number per plant | | |
| 14 June - 12 July | − | 4.2[a1] | 7.5[a] | 2.6[c] | 0.9[a] |
| | + | 3.6[b] | 6.7[b] | 4.5[d] | 5.2[b] |
| 14 June - 20 Sept. | − | 15.1[a] | 29.8[c] | 4.4[e] | 1.3[a] |
| | + | 13.9[b] | 28.0[d] | 5.6[f] | 4.0[b] |

[1] Entries of one harvest period in one column marked with a and b differ significantly at $p < 0.01$; marked with c and d at $p < 0.05$; marked with e and f at $p = 0.06$ (analysis of variance).

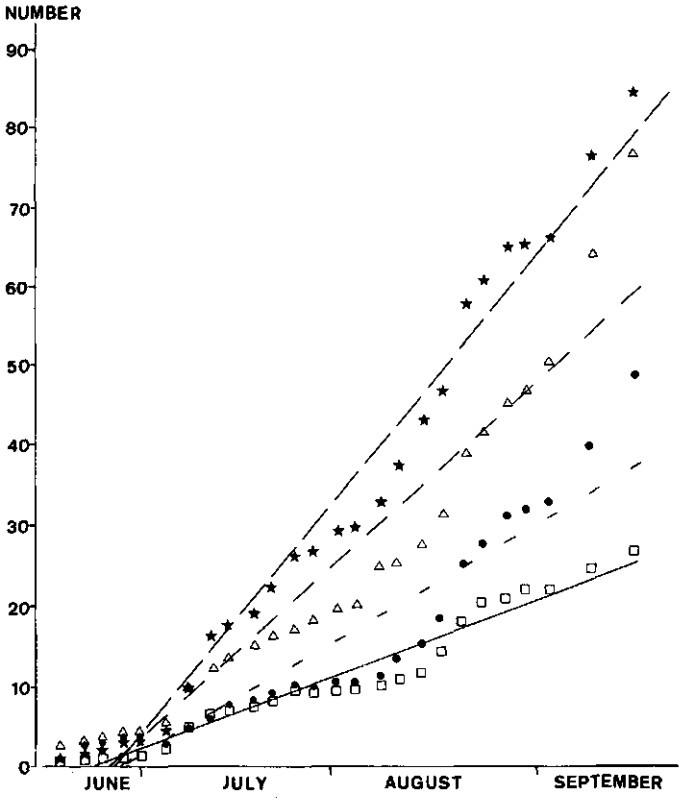



Fig. 2. Development of the total number of fruits with internal rot per 40 plants, 20 plants being uninoculated and 20 plants inoculated with D. bryoniae, in a glasshouse cucumber crop with transition to day conditions 3 h before (□), 1 h before (●), 1 h after (▵) and 3 h after (★) sunrise (means of two replicates).

*External fruit rot.* Only inoculation had a significant effect on the occurrence of external fruit rot. The percentage of fruits with external rot from inoculated plants was three times higher than that from uninoculated plants (Table 2).

*Influence of the disease on growth and yield*

*Plant growth.* The difference in climate regime had no significant effect on the total number of internodes, neither on side shoots, nor on secondary side shoots. Inoculation had no effect either on the number of internodes on the side shoots. On the secondary side shoots, inoculation reduced the total number of internodes per plant with 18 to 15%, depending on the growing period, whilst the number of secondary side shoots had increased compared with uninoculated plants (Table 3). The data of 30 June are the result of the first inoculation and the data of 23 July of both the first and the second inoculation.

Table 3. Effect of inoculation of cucumber plants with *D. bryoniae* on the total number of internodes and on the number of secondary side shoots per plant. Treatments included ten plants in each of eight replicates.

| Inoculation | Number of internodes | | Number of secondary side shoots | |
|---|---|---|---|---|
| | 30 June | 23 July | 30 June | 23 July |
| – | 26.4[a1] | 79.1[a] | 7.4[a] | 11.6[a] |
| + | 21.7[b] | 67.3[b] | 9.5[b] | 12.8[a] |

[1] Entries of one column marked with different letters differ significantly at $p < 0.05$ (analysis of variance).

*Fruit production.* Fruit production on the main stem was not influenced by climate regimes or inoculation. Therefore, only fruits from the side shoots and secondary side shoots were taken into account. The differences in climate regime had no significant effect on the total number and weight of the harvested fruits. Inoculation reduced the weight of the fruits with 13.5 to 7.9 %, depending on the duration of the harvest period (Table 2). As it takes 10 tot 14 days from flowering to harvest, the production until 12 July was influenced only by the first inoculation. The misshapen fruits are included in the total fruit production. Inoculation increased the production of misshapen fruits significantly ($p < 0.05$) from 0.94 to 1.11 kg per plant (18%).

**Discussion and conclusions**

With an early transition to day temperature the air humidity decreased as a result of the higher temperature (Fig. 1) and of the earlier opening of the ventilator windows. With a decreased air humidity the water on plant parts evaporated earlier. As a consequence environmental conditions for infection and expansion of the disease were less favourable. The incidence of stem and fruit rot was reduced substantially by heating up the glasshouse to the day setpoint before sunrise. The greater the interval between night and day temperature, the more important the early heating of the glasshouse will be as with lower temperatures a higher humidity will prevail.

An earlier transition to day temperature resulted in fewer lesions of *D. bryoniae* on the main stem only under circumstances of a high infection pressure after inoculation (Table 1). No difference in disease incidence was observed on growing tips. Apparently the microclimate on the growing tips and to a lesser extent on the main stem differed not very much for the four climate regimes. The most pronounced effect of the climate regime was on internal fruit rot (Fig. 2). It is most likely an indirect effect. Under more humid conditions and after condensation of water on diseased plant parts the release of ascospores of *D. bryoniae* will be enhanced (Fletcher and Preece, 1966; Schenck, 1968; Van Steekelenburg, 1983). This will result in an increased chance of flower infection, and flower infection is necessary to get internal fruit rot (unpublished data).

The climate regimes had no effect on the occurrence of external fruit rot. Wounding but not humidity is the most important factor in inciting external fruit rot (Van Steekelenburg, 1982).

Inoculation of plants increased infection pressure and, consequently, disease incidence on the main stem (Table 1), the growing tips and fruits (Table 2). Moreover, a higher infection pressure caused a reduction in fruit production (Table 2) and an increase in the incidence of misshapen fruits.

Inoculation caused a reduction of 4.7 internodes per plant (18 %) over a period of four weeks (Table 3) and a reduction of 0.8 fruits per plant (10 %) in the corresponding harvest period (Table 2). Every internode has a leaf axil with the initials of at least one fruit. Part of the young fruits abort spontaneously (De Lint and Heij, 1982). Although no effect of *D. bryoniae* on fruit abortion was observed in preliminary experiments it cannot be totally excluded that the disease increases abortion. With a high infection pressure of *D. bryoniae* there is not only the qualitative aspect of the rotten fruits, but also the invisible quantitative aspect of yield reduction may be important.

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**Samenvatting**

*Invloed van het tijdstip van overgang van de nacht- naar de dagtemperatuur op het optreden van Didymella bryoniae en de invloed van de ziekte op groei en produktie van kaskomkommers*

De invloed van het 3 uur vóór, 1 uur vóór, 1 uur na en 3 uur na zonsopgang overgaan van de nacht- naar de dagtemperatuur op het optreden van *Didymella bryoniae* werd zowel op geïnoculeerde als op niet-geïnoculeerde planten van kaskomkommers onderzocht. De invloed van inoculatie op de groei van de planten en de produktie van vruchten werd eveneens nagegaan.

Hoe later naar de dagtemperatuur werd overgegaan, hoe langer de perioden met een hoge relatieve luchtvochtigheid waren en hoe langer de perioden waarin condensatie van water op vruchten optrad.

Het tijdstip van overgang had geen effect op de groei van de planten, de opbrengst, de aantasting van groeipunten, het aantal lesies op de hoofdstengel van niet geïnoculeerde planten en uitwendig vruchtrot. Hoe later naar de dagtemperatuur werd overgegaan, hoe meer lesies na vier weken op de hoofdstengel van geïnoculeerde planten en hoe meer vruchten met inwendig rot voorkwamen.

Door inoculatie van de planten nam het aantal lesies op de hoofdstengel, de aantasting van groeipunten, de produktie van stekvruchten en het aantal vruchten met in- en uitwendig rot toe. Het aantal zijscheuten van de tweede orde nam toe, maar het totaal aantal internodiën ervan nam door inoculatie af. Inoculatie reduceerde het aantal internodiën met 18% over een periode van vier weken en die van het aantal vruchten met 10% in de overeenkomstige oogstperiode. De praktische consequenties van een

vochtig kasklimaat en van een hoge infectiedruk van *D. bryoniae* worden kort aangegeven.

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# COMPARISON OF INOCULATION METHODS WITH *DIDYMELLA BRYONIAE* ON *CUCUMIS SATIVUS*

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INDEX WORDS

*Cucumis sativus*, cucumber, *Didymella bryoniae*, *Mycosphaerella melonis*, gummy stem blight, stem and fruit rot, disease resistance.

SUMMARY

A good correlation between foliage attack of young and mature plants was observed. No evidence could be found that plants showing some resistance to plant infection produce fruits less susceptible to internal fruit rot. The extent of external fruit rot depended on the extent of wounding and on the thickness of the peel.

## INTRODUCTION

Stem and fruit rot caused by *Didymella bryoniae* (AUERSW.) REHM(synonyms: *Mycosphaerella citrullina* (C.O.SM.) GROSS. and *M. melonis* (PASS.) CHIU and WALKER occurs annually in every glasshouse with a cucumber crop in the Netherlands. Control of the disease by spraying fungicides is difficult to achieve (VAN STEEKELENBURG, 1978). The incidence of the disease is influenced by climatic conditions (VAN STEEKELENBURG & VAN DE VOOREN, 1980). Since good control is not yet possible, growing resistant cultivars could solve the problem. Lines, of which young plants showed some resistance were found by VAN DER MEER et al. (1978). However, most damage is done by fruit infection. For this reason research was done to discover if the damage on young plants correlated with the damage on mature plants and whether or not it correlated with internal and external fruit rot.

## MATERIALS AND METHODS

A highly virulent isolate of *D. bryoniae* served as inoculum. This isolate was grown on cherry decoction agar and exposed to black light (Philips TL 20W F20 T12 BLB) to stimulate sporulation. The conidia of 14-days-old cultures were washed off with sterilized water and the inoculum was adjusted to a concentration of $10^6$ conidia per ml unless stated otherwise. In the first experiment the male and female flowering cultivar Spotvrije was compared with the female flowering hybrid cultivar Farbio. Two series of breeding lines, which had shown some resistance when screened as young plants, were obtained from the Institute for Horticultural Plant Breeding (IVT) at Wageningen. These lines were compared with the cucumber cultivar Farbio and the gherkin cultivar Hokus.

The temperature in the glasshouse experiments was set thermostatically at 20°C. During the day, the temperature gradually increased during periods with sunshine.

EXPERIMENTS WITH YOUNG PLANTS

Plants were potted in 12 cm plastic pots four days after sowing and inoculated when the first true leaf had a diameter of about 5 cm. Per plant 3 to 4 ml suspension of $10^6$ conidia per ml was sprayed with a Sprayon spray-set. Subsequently the plants were incubated under a transparent plastic tent, resulting in a relative humidity of more than 95%. During the first 36 hours the plants were kept in the dark by covering the tent with a black plastic sheet. The transparent plastic was removed seven days after the inoculation. The temperature was maintained at about 25°C.

The first symptoms of the disease could be observed three to four days after inoculation. On the leaves small yellow lesions appeared and the edges of the growing tip turned brown. Disease assessments were made one and two weeks after inoculation. Symptoms on each leaf or growing tip were recorded on an arbitrary scale from 0 = no symptoms to 4 = severely attacked or dead. The disease slightly increased in the second week after inoculation. The results of two weeks after inoculation are given in the tables.

EXPERIMENTS WITH MATURE PLANTS

Usually the same crop was used for internal fruit infection and for observations on the disease development on mature plants. They were inoculated by spraying each plant

Table 1. Comparison of the different disease symptoms caused by *D. bryoniae* on two cucumber cultivars.

| Cultivar | Young plants disease index (n = 30) | Mature plants number of lesions per plant (n = 15) | Internal fruit rot % infected fruits (n = 467 and 299 resp.) | External fruit rot diameter of lesions in mm (n = 50) |
|---|---|---|---|---|
| Spotvrije | 3.0 | 15.0 | 16.6 | 46 |
| Farbio | 3.0 | 18.4 | 18.4 | 38 |

Table 2. *D. bryoniae* disease incidence on young and mature cucumber plants of four breeding lines and the cultivar Farbio.

| Line/cultivar | Young plants (n = 10) disease index | Mature plants (n = 20) | |
|---|---|---|---|
| | | number of lesions per plant | surface affected per plant in $mm^2$ |
| 108 a ⊗ | 1.2 | 13.6 | 43 |
| 75178 × 23 a | 1.2 | 7.8 | 46 |
| 73404 | 1.8 | 10.3 | 70 |
| 20 a ⊗ | 1.3 | 10.2 | 64 |
| Farbio | 2.9 | 10.4 | 93 |

Table 3. *D. bryoniae* disease indices of young and two-months-old cucumber plants of breeding lines compared with the cultivars Farbio and Hokus.

| Line/cultivar | Young plants (n = 15) | Two-months-old plants (n = 5) | |
|---|---|---|---|
| | | growing tip | older leaves |
| M2-91 × 53 | 1.2 | 0 | 0.8 |
| M3-34 × 563 | 1.5 | 0 | 0.7 |
| M3-33 × 213 | 2.0 | 0.5 | 1.2 |
| 108 a ⊗ | 1.7 | 0.6 | 2.4 |
| Hokus | 2.5 | – | – |
| Farbio | 2.9 | 0.8 | 3.4 |

with about 0.3 l of a suspension at a concentration of $10^6$ conidia per ml. In the experiment with 'Spotvrije' and 'Farbio' plants were inoculated three months after planting and the number of *D. bryoniae* lesions on the main stem were counted five months afterwards (Table 1). The first series of breeding lines was inoculated four months after planting. Five weeks afterwards the surfaces of the lesions with black fruiting bodies on the main stem were measured (Table 2). The plants of the second series of breeding lines were not suitable for investigating the disease incidence on mature plants after having been used for internal fruit infection. Of this series two-months-old plants in 10 l plastic containers were inoculated. The plants were incubated in a transparent plastic tent for one week. Two weeks after inoculation the growing tips and the older leaves were rated separately as with young plants (Table 3).

INTERNAL FRUIT ROT

In all experiments the full-grown fruits were cut in half lengthwise to check for internal rot. In the experiment with 'Spotvrije' and 'Farbio' the fruits harvested in the five months after spraying the foliage with the conidial suspension, were checked (Table 1). In the experiments with the breeding lines the female flowers were pollinated by hand in the morning and inoculated the same day at about 16.00 h. In the first series of breeding lines about 0.1 ml of a conidial suspension (1000 con/ml) was dripped into each flower. The mean percentages of infected fruits of five subsequent tests are given in Table 4. The second series of breeding lines was planted both in spring and autumn. One half of

Table 4. Internal and external rot of fruits, caused by *D. bryoniae*, of four cucumber breeding lines compared with the cultivar Farbio.

| Line/cultivar | Internal rot | | External rot (n = 20) |
|---|---|---|---|
| | number harvested | % fruits infected | diameter of lesions in mm |
| 108 a ⊗ | 42 | 2 | 50 |
| 75178 × 23 a | 85 | 9 | 42 |
| 73404 | 44 | 21 | 67 |
| 20 a ⊗ | 71 | 24 | 46 |
| Farbio | 81 | 17 | 38 |

Table 5. *D. bryoniae* internal rot of inoculated and uninoculated fruits of six cucumber genotypes in a spring crop (inoculation by brushing conidial slime).

| Line/cultivar | Inoculated | | Uninoculated | |
|---|---|---|---|---|
| | number harvested | % infected | number harvested | % infected |
| M2-91 × 53 | 43 | 42 | 48 | 8 |
| M3-34 × 563 | 38 | 42 | 33 | 18 |
| M3-33 × 213 | 29 | 59 | 31 | 7 |
| 108 a ⊗ | 34 | 59 | 39 | 5 |
| Hokus | 40 | 20 | 29 | 0 |
| Farbio | 21 | 43 | 20 | 0 |

Table 6. Comparison of two inoculation methods for internal fruit rot, caused by *D. bryoniae*, in six cucumber genotypes, autumn crop.

| Line/cultivar | Brushing conidial slime | | Dripping conidial suspension | |
|---|---|---|---|---|
| | number harvested | % infected | number harvested | % infected |
| M2-91 × 53 | 46 | 70 | 43 | 33 |
| M3-34 × 563 | 33 | 61 | 58 | 33 |
| M3-33 × 213 | 25 | 60 | 43 | 30 |
| 108 a ⊗ | 37 | 73 | 73 | 37 |
| Hokus | 21 | 33 | 64 | 14 |
| Farbio | 36 | 75 | 29 | 14 |

the number of plants of the spring crop was left uninoculated, the other was inoculated by brushing conidial slime produced by pycnidia on agar cultures into the open flowers. The mean percentages of infected fruits of nine subsequent tests are given in Table 5. In the autumn crop the flowers of one half of the number of plants were inoculated by dripping the conidial suspension into the flowers and those of the other half by brushing the conidial slime into them. The mean percentages of infected fruits of six subsequent tests are given in Table 6.

EXTERNAL FRUIT ROT

Picked fruits of one or more harvesting dates were wounded 5 mm deep with a cork borer of 5 mm ⌀ and inoculated with a 5 mm disc of an agar culture of the fungus. Each fruit was inoculated on 1 to 3 places, depending on the size of the fruit. In the second series of breeding lines one part of the fruits was wounded 1 mm and another 2.5 mm deep, in both cases 5 mm wide. The fruits were incubated at 23 °C in the dark and at a relative humidity of over 95% for seven days.

Two measurements, perpendicular to each other, of the diameter of each lesion of the inoculated fruits were taken. The mean diameter of the lesions is given in the tables. No rot occurred if inoculated fruits were left unwounded.

## DISCUSSION AND CONCLUSIONS

The female flowering hybrid Farbio, introduced in 1973, was as susceptible to *D. bryoniae* as the male and female flowering cultivar Spotvrije, which was grown on a large area 10 to 15 years ago (Table 1). The idea that 'Farbio' was more susceptible to stem and fruit rot was not borne out. The number of lesions on the main stem was about similar on plants of breeding lines and on the standard cultivar Farbio, but the surfaces of these lesions differed (Table 2). This gives a reduced infection pressure on the breeding lines. The foliage of the breeding material was less affected than that of the standard cultivars and there was a good correlation between damage on young and mature plants (Tables 2 and 3).

The percentage fruits of 'Farbio' with internal rot was about the same after a more or less natural infection (Table 1) as after dripping a conidial suspension into the open flower (Tables 4 and 6). Some breeding lines of the first series showed a promising result with regard to internal fruit rot (Table 4). However, this was not confirmed in the second series (Tables 5 and 6) and even a natural fruit infection occurred in the breeding material (Table 5). The gherkin 'Hokus' showed less internal fruit rot than the other cultivars tested. In practice gherkins are also less affected than cucumbers. About twice as many fruits showed internal rot after brushing conidial slime than after dripping a conidial suspension. An explanation may be that the stigma is wounded slightly by brushing. However, in preliminary tests no differences in percentages of internal fruit infection were found between the two methods of inoculation. The internal fruit rot problem is still under investigation.

Wounding is essential for external fruit rot as inoculated unwounded fruits did not rot at all. Severely wounded fruits of the breeding lines rotted as severe as or even more than fruits of 'Farbio' (Table 4). Slightly wounded fruits of breeding lines were less affected than fruits of the standard cultivars and 'Hokus' was less affected than 'Farbio' (Table 7). The peel of the fruits of the breeding material and of 'Hokus' was thicker than of the fruits of 'Farbio'. The extent of fruit rot is probably determined by the thickness of the peel. Part of the fruit rot problem may be solved by growing cultivars with a thicker fruit peel. Such fruits are not so easily wounded and even no fruit rot will occur with wounds restricted to the peel.

Table 7. External rot of cucumber fruits wounded 1 or 2.5 mm deep and 5 mm wide, and inoculated with *D. bryoniae*.

| Line/cultivar | 1–5 mm | | 2.5–5 mm | |
|---|---|---|---|---|
| | number of inoculation sites | mean lesion diameter in mm | number of inoculation sites | mean lesion diameter in mm |
| M2-91 × 53 | 43 | 2 | 44 | 5 |
| M3-34 × 563 | 76 | 7 | 58 | 20 |
| M3-33 × 213 | 90 | 5 | 51 | 3 |
| 108 a ⊗ | 79 | 7 | 53 | 22 |
| Hokus | 118 | 19 | 61 | 40 |
| Farbio | 177 | 28 | 90 | 54 |

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# Chemical control of Didymella bryoniae in cucumbers

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**Abstract**

The effect of a number of fungicides on mycelial growth of *Didymella bryoniae* on agar was tested and compared with the effect on inoculated plants and in commercial crops. Activities in vitro and vivo are not always correlated. Spraying of inoculated young plants is a reliable and quick method for testing fungicides against *D. bryoniae*.

In commercial crops the treatments in decreasing order of effectiveness were weekly sprays of benomyl 0.025 – 0.05% active ingredient (a.i.), chlorothalonil 0.15% and triforine 0.02% a.i. These chemicals should be sprayed alternately to prevent the development of strains tolerant to benzimidazole derivates.

## Introduction

*Didymella bryoniae* (Auersw.) Rehm causes a variety of symptoms in cucumber (*Cucumis sativus* L.) grown under glass in the Netherlands. Leaves, stems, growing tips and fruits can all be attacked. Especially infection of the fruits causes economic losses. Often fruits decay during storage and handling after harvest (Veenman, 1972). Sometimes the fungus attacks melon (*Cucumis melo* L.) and gherkin (*Cucumis sativus* L.) in the Netherlands. Many other species of the cucurbit family can be infected (Chupp and Sherf, 1960).

The disease is known as black fruit rot or gummy stem blight. Many synonyms of the pathogen are used in the phytopathological literature, most frequently *Mycosphaerella citrullina* (C.O. Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu & Walker. The correct names are *Didymella bryoniae* (Auersw.) Rehm for the perfect state and *Phoma cucurbitacearum* (Fr.) Sacc. for the imperfect state (Boerema and Van Kesteren, 1972).

Dithiocarbamates are usually recommended for control (Anonymus, 1975; Fletcher and Preece, 1966), but results are often unsatisfactory. For that reason Naaldwijk Station has tested several chemicals under different circumstances. So it was possible to check the correlation between results in vitro and in vivo. We also investigated whether fungicides used for the control of powdery mildew, *Sphaerotheca fuliginea* (Schlecht. ex Link) Pollacci, had any side-effects on *D. bryoniae*.

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## Materials and methods

*Mycelial growth in vitro.* Aqueous suspensions of commercial products of the fungicides (Table 1) were added to molten cherry decoction agar after cooling to about 45 °C. Concentrations of 0, 1, 10 and 100 μg active ingredient (a.i.) per millilitre were tested. The agar plates were inoculated with 5 mm discs of agar with *D. bryoniae*. Colony diameters were measured after incubation for five days at 20 °C.

*Trials with young plants.* Cucumber plants of cultivar Uniflora D were potted in 12 cm plastic pots three days after sowing and were inoculated when the second leaf had a diameter of 5 cm. Per plant, about 4 ml suspension of $10^6$ conidia/ml was sprayed with a Sprayon spray-set. The fungicides (Tables 2 and 3) were sprayed at a concentration of 250 μg a.i. per ml and at the concentration normally recommended, either 24 h before or 24 h after inoculation. There were six plants per treatment in each of four replicates. The plants were incubated in the greenhouse under a transparent plastic tent at a temperature fluctuating between 20 and 30 °C and a relative humidity almost always of 100 % for the first seven days. For the next seven days the plastic was removed and the relative humidity fluctuated between 40 and 90 %.

*Trials in commercial crops: Weekly sprayings.* Cultivar Uniflora D was planted at the beginning of July 1974. Three and a half months later, this autumn crop was cleared. The crop was sprayed weekly with the fungicides mentioned in Table 4, for the first time 10 days after planting. The first two times, 0.2 liter/$m^2$ was sprayed with a mist-sprayer at a pressure of 4–5 atm. Afterwards 0.4 liter/$m^2$ was used, with fourteen sprays in all. An exception was dimethirimol, for which the soil was drenched six weeks after planting when the first spots of powdery mildew appeared. The number of plants per treatment was 24 in each of two replicates. The temperature was set thermostatically at 18 °C. During the day, higher temperatures were reached with sunshine.

*Weekly and fortnightly sprayings.* The most promising fungicides were tested with different frequencies in 1975. Cultivar Uniflora D was planted at the end of July. Two months afterwards, when the first attack of *D. bryoniae* was seen, the crop was first sprayed as described earlier. The dimethirimol treatment was applied once at that time. In the weekly scheme, crops were sprayed six times and in the fortnightly scheme three times. The number of plants per treatment was 16 in each of four replicates, each in a separate but identical greenhouse. The temperature was set initially at 20 °C and the setting was lowered after two months to 17 °C. During the day, the temperature rose higher with sunshine.

## Results

*Mycelial growth in vitro.* Results are summarized in Table 1. The growth of the mycelium is inhibited to a certain extent by all the fungicides except dimethirimol. Best results were with benomyl.

*Trials with young plants.* One week after inoculation, the infection of the second and third leaf of each plant was rated from 0 = healthy to 2 = severely attacked. The

Table 1. Effect of some fungicides on mycelial growth of *D. bryoniae* on cherry decoction agar (means of the sum of two perpendicular diameters of three isolates in three replicates, in mm).

| Active ingredient | Proprietary name | Content a.i. and formulation | Concentration in μg a.i./ml | | |
|---|---|---|---|---|---|
| | | | 1 | 10 | 100 |
| benomyl | Benlate | 50% w.p. | 58 | +[1] | 0 |
| triforine | Funginex | 20% e.c. | 77 | 23 | + |
| dinocap | Karathane | 43% e.c. | 53 | 32 | 10 |
| chloraniformethan | Imugan | 25% e.c. | 95 | 68 | 25 |
| pyrazophos | Curamil-Hoe 2873 | 30% e.c. | 94 | 80 | 25 |
| chlorothalonil | Daconil 2787 | 73% w.p. | 63 | 56 | 41 |
| zineb | AAphytora | 70% w.p. | 87 | 77 | 58 |
| dinobuton | Acrex-S | 48.5% e.c. | 78 | 62 | 61 |
| quinomethionate | Morestan | 25% w.p. | 93 | 75 | 60 |
| dimethirimol | Milcurb | 12.5% e.c. | 86 | 93 | 93 |
| control (water) | | | 92 | 92 | 92 |

[1] Growth very restricted, not measurable.

*Tabel 1. Invloed van verschillende fungiciden op de groei van het mycelium van D. bryoniae op kersagar (gemiddelden van twee loodrecht op elkaar staande diameters van drie isolaten in drie herhalingen, in mm).*

Table 2. Effect of some fungicides in a concentration of 250 μg a.i./ml on attack of young cucumber plants one week after inoculation with a conidium suspension of *D. bryoniae* (six plants per treatment; four replicates).

| Active ingredient | Sprayed 24 hours before inoculation | | Sprayed 24 hours after inoculation | |
|---|---|---|---|---|
| | % healthy plants | disease index[1,2] | % healthy plants | disease index[1,2] |
| benomyl | 0 | $2.1^{a}$ | 71 | $0.3^{p}$ |
| triforine | 0 | $2.5^{ab}$ | 25 | $1.1^{pq}$ |
| chlorothalonil | 0 | $2.5^{ab}$ | 0 | $1.5^{qr}$ |
| dinocap | 0 | $2.6^{ab}$ | 0 | $2.0^{qr}$ |
| zineb | 0 | $3.0^{ab}$ | 4 | $2.0^{qr}$ |
| chloraniformethan | 0 | $3.0^{ab}$ | 0 | $2.0^{qr}$ |
| pyrazophos | 0 | $3.1^{ab}$ | 0 | $2.5^{rs}$ |
| quinomethionate | 0 | $3.1^{ab}$ | 0 | $2.5^{rs}$ |
| dinobuton | 0 | $3.0^{ab}$ | 0 | $3.2^{s}$ |
| control | 0 | $3.3^{b}$ | 0 | $3.3^{s}$ |

[1] Mean of the sum of the infection rates of the second and third leaf per plant; each leaf rated from 0 = healthy to 2 = severely attacked.
[2] Means marked with different letters differ significantly at $P < 0.05$.

*Tabel 2. Invloed van verschillende fungiciden in een dosering van 250 μg a.i./ml op de aantasting van jonge komkommerplanten een week na inoculatie met een sporensuspensie van D. bryoniae (zes planten per behandeling; vier herhalingen).*

Table 3. Effects of some fungicides in the normally recommended dosage on attack of young cucumber plants one week after inoculation with a conidium suspension of *D. bryoniae* (six plants per treatment; four replicates).

| Active ingredient | µg a.i. per ml | Sprayed 24 hours before inoculation | | Sprayed 24 hours after inoculation | |
|---|---|---|---|---|---|
| | | % healthy plants | disease index[1,2] | % healthy plants | disease index[1,2] |
| benomyl | 500 | 17 | 1.7[a] | 75 | 0.3[p] |
| chlorothalonil | 1460 | 4 | 2.4[ab] | 29 | 0.9[p] |
| triforine | 200 | 0 | 2.5[ab] | 0 | 2.1[qr] |
| zineb | 1400 | 0 | 3.0[b] | 17 | 1.2[pq] |
| dinocap | 135 | 0 | 3.2[b] | 0 | 2.2[qr] |
| chloraniformethan | 82.5 | 0 | 2.9[b] | 0 | 2.4[rs] |
| pyrazophos | 120 | 0 | 3.2[b] | 0 | 2.7[rs] |
| quinomethionate | 75 | 0 | 3.2[b] | 0 | 2.8[rs] |
| dinobuton | 485 | 0 | 3.4[b] | 0 | 2.9[rs] |
| control | | 0 | 3.3[b] | 0 | 3.3[s] |

[1] See Table 2.
[2] Means marked with different letters differ significantly at $P < 0.05$.

*Tabel 3. Invloed van verschillende fungiciden in de normaal aanbevolen dosering op de aantasting van jonge komkommerplanten een week na inoculatie met een sporensuspensie van D. bryoniae (zes planten per behandeling; vier herhalingen).*

disease index was calculated as the mean of the sum of the leaf infection rates per plant (Tables 2 and 3). Two weeks after inoculation, the infection of the second, third and fourth leaf and of the growing tip was rated. These data are not given, as the effects of the treatments were nearly the same as one week after inoculation, although the disease increased and the number of healthy plants decreased in the second week after inoculation.

When sprayed after inoculation, benomyl, chlorothalonil and zineb in both concentrations and triforine 250 µg/ml were significantly better than before inoculation ($P < 0.05$). Triforine and zineb in the highest concentration gave better results than the lower ones ($P < 0.05$). There was no significant difference with concentration of the other fungicides. Dinocap at 250 µg/ml, nearly twice the recommended concentration, was phytotoxic; it caused growth reduction and necrotic leaves.

*Trials in commercial crops: Weekly sprayings.* The first symptoms of the disease, lesions of black fruiting bodies on the stubs left after removal of fruits or leaves, were seen six weeks after planting out. The main stem can be infected through these stubs. The lesions with black fruiting bodies (pycnidia and perithecia) on the main stem were counted and measured at the end of the crop. As there was a correlation between the number and surface, only the number of lesions per plant is given in Table 4.

The fruits were harvested twice a week and any suspect fruit was cut in half lengthwise to check for internal rot (Fig. 1), which is sometimes hard to see on the outside of the fruit. Samples showed that less than 1 % of the fruits judged healthy were infected. A week after symptoms were seen on foliage, the first fruit with internal rot was found.

Table 4. Effect of weekly sprays with fungicides on *D. bryoniae* in commercial crops (24 plants per treatment; two replicates). A = mean number of lesions per plant on the main stem; B1 = percentage internally rotted fruits; B2 = percentage externally rotted fruits.

| Active ingredient | μg a.i./ml | A[2] | B1 | B2 |
|---|---|---|---|---|
| benomyl | 500 | 0.3[a] | 0.5 | 7.5 |
| triforine | 200 | 1.2[a] | 3.5 | 16.5 |
| zineb | 1400 | 3.1[b] | 5.6 | 11.1 |
| dinocap | 135 | 4.0[b] | 5.8 | 20.8 |
| quinomethionate | 75 | 4.3[b] | 3.7 | 25.0 |
| dimethirimol | 0.25[1] | 3.3[b] | 3.4 | 20.5 |
| control | | 3.4[b] | 4.9 | 5.1 |

[1] ml active ingredient per plant.
[2] Means marked with different letters differ significantly at $P < 0.05$.

*Tabel 4. De invloed van wekelijkse bespuitingen met fungiciden onder praktijkomstandigheden op de aantasting van D. bryoniae (24 planten per behandeling; twee herhalingen). A = gemiddeld aantal lesies op de hoofdstengel; B1 = percentage vruchten met inwendig rot; B2 = percentage vruchten met uitwendig rot.*

This rot occurred mostly in the last three weeks. The percentage of fruits with internal rot over the period starting from the first attacked fruit in this trial is given in Table 4.

The picked fruits from the last harvest were kept in boxes covered with plastic at a temperature of about 25 °C. The number of fruits per treatment varied from 63 to 80. After 7 and 14 days, the fruits were checked for external rot (Fig. 2, Table 4).

*Weekly and fortnightly sprayings.* The data on infection of the stem and fruit were collected in the same way as described above and the results are given in Table 5.

Fig. 1. Internal rot at the blossom end of a cucumber fruit, caused by *D. bryoniae*.

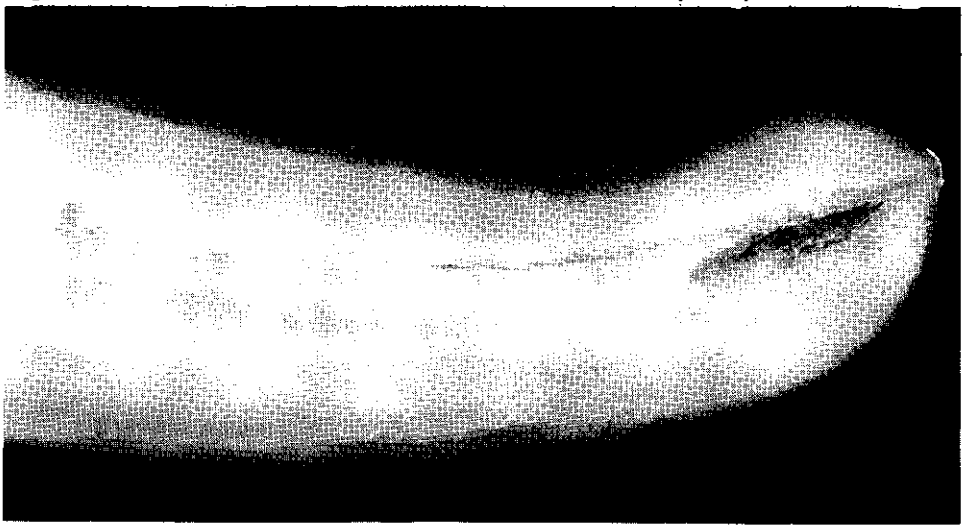

*Fig. 1. Inwendig rot bij het bloemeinde van een komkommervrucht veroorzaakt door D. bryoniae.*

Three times the picked fruits were kept under humid circumstances to check for external rot. The number of fruits per treatment varied between 64 and 116. Infection of the stem varied in extent. Still the effects of all treatments differ significantly from that with dimethirimol ($P < 0.01$). Weekly sprays of the fungicides differed significantly from fortnightly ones ($P < 0.05$), except for zineb. Internal and external fruit infection is best controlled with benomyl sprayed in a weekly scheme.

Fig. 2. External rot of a cucumber fruit, caused by *D. bryoniae*.

*Fig. 2. Uitwendig rot bij een komkommervrucht veroorzaakt door D. bryoniae.*

Table 5. Effect of weekly and fortnightly sprays with fungicides on *D. bryoniae* in commercial crops (16 plants per treatment; four replicates). A = mean number of lesions per plant on the main stem; B1 = percentage internally rotted fruits; B2 = percentage externally rotted fruits.

| Active ingredient | µg a.i./ml | Frequency | A[2] | B1 | B2 |
|---|---|---|---|---|---|
| benomyl | 250 | weekly | 1.0[a] | 2.1 | 2.2 |
| benomyl | 250 | fortnightly | 1.8[b] | 4.0 | 17.0 |
| chlorothalonil | 1460 | weekly | 0.8[a] | 6.1 | 12.9 |
| chlorothalonil | 1460 | fortnightly | 1.5[b] | 5.3 | 10.9 |
| triforine | 200 | weekly | 1.1[a] | 3.5 | 19.8 |
| triforine | 200 | fortnightly | 1.9[b] | 4.2 | 26.7 |
| zineb | 1400 | weekly | 1.6[b] | 8.8 | 26.5 |
| zineb | 1400 | fortnightly | 1.8[b] | 5.4 | 9.9 |
| dimethirimol | 0.25[1] | | 3.6[c] | 4.1 | 26.9 |

[1] ml active ingredient per plant.
[2] Means marked with different letters differ significantly at least at $P < 0.05$.

*Tabel 5. De invloed van wekelijkse en tweewekelijkse bespuitingen met fungiciden onder praktijkomstandigheden op de aantasting van D. bryoniae (16 planten per behandeling; vier herhalingen). A = gemiddeld aantal lesies op de hoofdstengel; B1 = percentage vruchten met inwendig rot; B2 = percentage vruchten met uitwendig rot.*

## Discussion

Results with benomyl and triforine are good both in vitro and in vivo. Those with chlorothalonil are better in vivo than in vitro, but with dinocap the opposite was found. Dinocap can be toxic to plants under certain conditions. Results with young plants are closely correlated with those in commercial crops. With inoculation of young plants, fungicides can be tested reliably for their effect on *D. bryoniae* in a simple way, on a small surface and in a short time.

It is difficult to explain why spraying after inoculation gives better results than spraying before inoculation. For control of *Didymella chrysanthemi* (Tassi) Garibaldi & Gullino with chlorothalonil, the opposite was found (Van Steekelenburg, 1976).

An effect on *D. bryoniae* of dinocap, chloraniformethan, pyrazophos, quinomethionate, dinobuton and dimethirimol in the normally recommended dosages for control of powdery mildew, could not be demonstrated (Tables 3 and 4).

There was an interaction between *D. bryoniae* and powdery mildew in the trials in commercial crops. A heavy attack of powdery mildew kills many leaves. The circumstances of a dry microclimate favouring powdery mildew are unfavourable for infection with *D. bryoniae*. A heavy attack of powdery mildew did occur in the control, less in the fortnightly treatments with chlorothalonil and zineb and in the last weeks of the experiments also with dimethirimol. So the strange results of *D. bryoniae* control with these treatments can be explained (Tables 4 and 5). Best results were obtained with benomyl followed by chlorothalonil and triforine. The good results with benomyl may be connected with the mechanism of action and high stability of methyl benzimidazole carbamate (MBC), the fungicidal principle. Though benomyl and triforine are both systemic fungicides, they differ in their mode of action; benomyl has an antimitotic activity (Davidse, 1973) and triforine interferes with sterol synthesis (Sherald and Sisler, 1975). Chlorothalonil is known as a surface protectant and its fungicidal action is attributed to thiol inactivation (Vincent and Sisler, 1968).

With most fungicides, the control of fruit infection is rather disappointing. The proportion of internally rotten fruits was lower than 6% in nearly all treatments. In some cases about 25% of the picked fruits rotted externally. An equal percentage was sometimes found in certain lots on the market (unpublished data). So external rot seems to cause more economic losses than internal rot.

Protection with a good fungicide is difficult to achieve because of the continuous production of wounds and the dense growth of a cucumber crop. The crop must be sprayed nearly every week to have reasonable effect. The life cycle of *D. bryoniae* can be very short. Four days after inoculation, sporulating pycnidia were present under ideal conditions of tests with young plants. Frequent sprays with benomyl or other fungicides producing MBC increase the incidence of strains tolerant to MBC. *Didymella chrysanthemi*, a fungus closely related to *D. bryoniae*, illustrates this phenomenon (Van Steekelenburg, 1973; Grouet, 1974). So benomyl, triforine and chlorothalonil have to be sprayed alternately for control of *D. bryoniae*. In this way, other diseases like powdery mildew, *Botrytis* and *Sclerotinia* will be controlled too.

**Samenvatting**

*Chemische bestrijding van Didymella bryoniae in komkommers*

Een aantal chemische middelen zijn in vitro en op verschillende manieren in vivo getoetst op hun werking tegen *Didymella bryoniae*. De resultaten in vitro zijn niet altijd positief gecorreleerd met die in vivo (Tabel 1 en 2). De resultaten na inoculatie van jonge plantjes komen wel overeen met die onder praktijkomstandigheden (Tabel 3, 4 en 5). Op jonge plantjes kunnen dus middelen op een snelle wijze en in een beperkte ruimte betrouwbaar worden getoetst.

Onder praktijkomstandigheden werd het beste resultaat verkregen met benomyl 0,025–0,05 % actieve stof, zowel wat betreft de gewas- als de vruchtaantasting. Hierop volgde chloorthalonil 0,15 % a.s. en triforine 0,02 % a.s. (Tabel 4 en 5). Indien *D. bryoniae* met chemische middelen moet worden bestreden, is het noodzakelijk dat er bijna elke week wordt gespoten (Tabel 5). Teneinde het ontstaan van resistentie van schimmels tegen benomyl en andere MBC-producerende fungiciden te voorkomen, wordt aanbevolen om benomyl, chloorthalonil en triforine afwisselend toe te passen. Met dit schema worden vele andere schimmelziekten in komkommer eveneens bestreden.

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## RECENT DEVELOPMENTS IN CHEMICAL CONTROL OF DIDYMELLA BRYONIAE

In the first part of the chapter on chemical control it is described that best results were obtained with benomyl, followed by chlorothalonil and triforine. Apart from triforine, more ergosterol biosynthesis inhibitors (bitertanol, fenarimol, imazalil) became available for control of powdery mildew and dicarboximides (iprodione, procymidone, vinclozolin) for control of grey mould. Several fungicides from these groups were tested for the control of Didymella bryoniae on young cucumber plants as described in the first part of this chapter. Results are summarized in Table 1. Bupirimate was phytotoxic under the test circumstances; it caused necrotic stems and leaves. Apart from triforine and chlorothalonil, spraying of the fungicides before or after inoculation made no difference. Triforine gave better results when sprayed after inoculation, and chlorothalonil when applied before inoculation ($p < 0.05$). With chlorothalonil the opposite was found in previous experiments. Best results were obtained with benomyl and tolylfluanid.

Strains of D. bryoniae resistant to benzimidazole fungicides were observed in Greece (Malathrakis and Vakalounakis, 1983). After ten successive weekly sprayings with benomyl no decreased sensitivity of fungal isolates was observed in own experiments (unpublished data). Isolates of the fungus from commercial crops showed no decreased sensitivity to benomyl either. Benzimidazole fungicides are not frequently used in the Netherlands, as they are harmful to the predator Phytoseiulus persimilis of the red spider mite. In the period powdery mildew was controlled by benzimidazole fungicides stem and fruit rot was probably controlled as well. However, powdery mildew rapidly became resistant to this group of fungicides which is hardly used since.

To control stem and fruit rot benzimidazole fungicides, tolylfluanid and chlorothalonil are recommended. However, benzimidazole fungicides can only be used if red spider mite is not controlled biologically. When powdery mildew has to be controlled as well, ergosterol biosynthesis inhibitors can be used. To control both grey mould and stem and fruit rot dicarboximides can be applied as well as tolylfluanid and chlorothalonil. A decreased sensitivity of

powdery mildew to ergosterol biosynthesis inhibitors (Schepers, 1983) and of grey mould to dicarboximides (Fletcher, 1985) has been reported. It is advised to apply fungicides with a different mechanism of action alternately to prevent the development of fungal strains resistant to fungicides.

Table 1. Effects of some fungicides sprayed in the normally recommended dosage 24 h before (B) or 24 h after (A) inoculation with D. bryoniae on disease incidence on young cucumber plants (four plants per treatment; four replicates).

| Active ingredient | µg a.i. per ml | Disease index[1] B | | Disease index[1] A | |
|---|---|---|---|---|---|
| benomyl | 250 | 0.8 | a2 | 0.9 | p2 |
| tolylfluanid | 1250 | 0.9 | a | 0.9 | p |
| mancozeb | 2400 | 1.4 | a | 1.4 | p |
| iprodione | 500 | 2.4 | a | 1.6 | p |
| procymidone | 250 | 2.6 | a | 2.4 | pq |
| vinclozolin | 250 | 3.6 | abc | 3.4 | pqr |
| imazalil | 50 | 3.1 | ab | 3.3 | pq |
| triforine | 200 | 5.8 | bc | 2.5 | pq |
| chlorothalonil | 1460 | 2.6 | a | 6.2 | r |
| fenarimol | 24 | 6.2 | c | 5.1 | qr |
| bupirimate | 625 | 5.8 | bc | 6.1 | r |
| control (water) | | 10.7 | d | 10.7 | s |

[1] Sum of the infection rate of the second and third leaf and the growing tip; each leaf or growing tip rated from 0 = healthy to 5 = dead.

[2] Numbers marked with different letters differ significantly at $p < 0.05$ (Tukey's range test).

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## GENERAL DISCUSSION AND CONCLUSIONS

Stem and fruit rot caused by Didymella bryoniae has become the most harmful fungal disease of glasshouse cucumbers. A number of factors may have contributed, among which changes in production system, cultivars, fungicide usage and environmental conditions. Especially the occurrence of internal and external fruit rot causes economical damage. Rotten fruits are lower graded or unsalable. The occurrence of rot in the marketing phase, which jeopardizes the quality of the whole produce, is of special importance.

The disease can be controlled in several ways. By hygienic measures infection sources must be eliminated or at least restricted. The fungus survives on diseased plant debris in glasshouses and outdoors as long as the debris is not decomposed. Therefore, the glasshouse must be thoroughly cleaned, the structures washed down with a disinfectant and the plant debris must be burned or eliminated in another way at the end of each crop. Ascospores originating from diseased plant debris are most likely responsible for the primary infection of the crop. Trapping ascospores is of no use in forecasting the disease, as the spores cannot be detected before the first symptoms of the disease appear. Usually, the first symptoms of the disease are observed on the stubs of the fruit stalk left after the picking of the fruits.

When the first symptoms appear, precautions have to be made to prevent a quick spread and increase of the disease. From then on plants have to be sprayed with fungicides. However, adequate chemical control is hard to achieve as cucumber is a fast and dense growing crop with a continuous production of wounds through picking and trimming. Spraying has to be carried out frequently, which increases the risk of resistant fungal strains. This risk has to be avoided by alternating fungicides with a different mechanism of action.

Control of the disease by influencing the glasshouse climate is the most important way of control. The glasshouse grower has the ability to manipulate, within certain limits, the environmental conditions in which the plants are growing. The glasshouse climate has a direct and an indirect effect on the incidence of the disease. There

is an indirect effect via the plant. The conditions in which plants are grown determine the degree of the plant's susceptibility to the disease. A higher night temperature in the period before picking resulted in plants less susceptible to the disease. For spore release, infection and further expansion of the disease free water is needed. By a proper ventilation practice with simultaneous heating, the occurrence of free water on plant parts must be restricted. This control measure requires heating. It is therefore not cheap and counteracts the effects of the energy saving methods the glasshouse grower is forced to. On top of that, too dry conditions may decrease the yield. On the other hand, there is the loss of rotten fruits and the additional yield reduction in severely infected crops, which is often not noticed by the grower. What also remains are the incalculable losses due to the bad quality when rot occurs in the post harvest period. Problems with chemical residues and phytotoxicity are avoided when control is based on climate regulation instead of fungicides. With a combination of environmental control measures and chemical control, the negative aspects of chemical control can be minimized.

With all the measures of hygiene, chemical control and control by means of the glasshouse climate it is still not possible to eliminate the disease under commercial conditions. Resistance breeding may contribute to solve the problem of stem and fruit rot in cucumbers. With plant resistance the chances of flower and fruit infection can still be reduced even when the fruit itself is not resistant. In cultivars with a shorter flowering period the chances of flower infection and consequently the occurrence of internal fruit rot are decreased. Because fruit infection is achieved via the flower it is likely that no internal fruit rot will occur if the flower drops off quickly or if the flower has no style. These characteristics of the flower can possibly be found and bred into the present cultivars. The occurrence of external fruit rot is decreased by diminishing the chances of wounding during handling. Breeding cultivars with a thicker fruit peel than the present cultivars may also help to solve this problem.

It should be investigated whether host defence mechanisms, e.g. the formation of phytoalexins, might play a role in the occurrence of internal fruit infection.

**SUMMARY**

The increasing incidence of fruit rot, caused by Didymella bryoniae, in glasshouse cucumbers in the Netherlands gave rise to a study of the biology of the pathogen, the epidemiology of the disease, and control methods. Stems, leaves and growing tips may be attacked, and fruits may be infected internally as well as externally. Internal infection of fruits starts at the blossom end and is initially hard to observe externally. With external fruit rot, lesions occur on any place of the fruit, predominantly in the post harvest period.

The survival of the fungus outdoors and in glasshouses was studied. Outdoors, the fungus was able to survive winters with 14 days of frost. A temperature above 5 °C was needed for growth and fructification of the fungus. Diseased plant residues remained an infection source as long as they were not decomposed. Deliberate introduction of diseased plant debris in the glasshouse caused an earlier appearance of symptoms, and increased the severity of the disease. Hygienic measures are advised to eliminate infection sources.

The number of daily trapped ascospores showed a peak after sunset, both outdoors near diseased plant debris and in glasshouses with diseased plants. Watering the plants in the glasshouse favoured ascospore release. Under controlled environmental conditions, ascospores were not released at a high relative humidity or after a change in relative humidity. They could only be trapped after wetting of the substrate. The first ascospores in glasshouses were trapped at about the same time the first symptoms on the plants appeared.

Wounding was essential to achieve external fruit rot. The degree of rot was increased by more severe wounding. The rotting of the fruits increased progressively from 12 to 23 °C; it decreased at higher temperatures and no rot at all occurred at 35 °C. To reduce rotting without diminishing the shelf life, fruits have to be stored at 12 °C in the post harvest period. Relative humidity during storage had no effect on fruit decay. A low nitrogen fertilization of the crop decreased the degree of fruit rot but reduced also the production. The difference in fruit rot in the light and in the dark had no practical significance.

Internal fruit rot was achieved by inoculation of the open flower; however, the majority of the fruits escaped infection. It took

more than two days for the fungus to reach the fruit after infection of the style. Neither the method of inoculation, nor amending nutrients to the inoculum suspension influenced the incidence of internal fruit rot. Slight wounding by removing parts of the flower had no effect on the incidence of internal fruit rot, whereas severe wounding by blossom excision reduced it with 75%. Inoculation of wilted flowers resulted in 60% less infection than inoculation of fresh flowers. Some powdery mildew resistant cultivars showed resistance to internal fruit infection. This resistance was associated with a long style and a short flowering period. However, no obvious effect of the flowering period within a cultivar on the incidence of internal fruit rot was observed. Growing plants under drought stress markedly increased internal fruit infection. Duration of fruit growth and fruit thinning had no effect on fruit infection. Relative humidity had no effect either. For flower infection no free water seems to be needed. It is not yet clear whether some kind of defence mechanism to fruit infection existst from which the fungus may escape under certain circumstances.

Under controlled environmental conditions infection of growing tips and leaves was rare at 60% r.h., it increased at 95% r.h. and was most serious if the plants 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. Wounding was essential for infection of older leaves, but not for infection of young plant tissue. A higher conidial concentration increased infection. The effect of leaf wetting on the number of infected sites was comparable with the effect of a tenfold conidial concentration.

In glasshouses with cucumbers grown in a commercial way, a lower night temperature in the pre-inoculation period, between 12 and 24 $^{o}$C, increased the disease incidence later on in the season, whereas no postponed effect of the day temperature, between 17 and 26 $^{o}$C, was observed. The daily maximum relative humidity was about 10% lower in glasshouse compartments with a minimum ventilator opening than in compartments without a minimum ventilator opening. This decreased the disease incidence on uninoculated plants to 35% and on fruits to 40%. The spread of the disease from inoculated plants to uninoculated ones was more rapid under humid than under drier conditions. However,

production was higher under more humid conditions.

Disease incidence was higher at an interval of 5 °C between heating and ventilation than at an interval of 2 °C. Little ventilation with an electric fan at an interval of 5 °C between heating and ventilation hardly influenced the incidence of the disease.

The influence of transition to day temperature 3 h before, 1 h before, 1 h after and 3 h after sunrise on the incidence of the disease was studied. The later the transition to day temperature took place, the longer the periods with high relative humidity were maintained, the longer the periods the fruits stayed wet by condensation of water and the higher the incidence of the disease on inoculated plants and fruits was.

Inoculation of plants resulted in a high infection pressure and in an increase of the disease incidence on plants and fruits as well as in an increase of the number of misshapen fruits. Inoculation reduced the number of internodes by 18% after four weeks and the number of fruits by 10%.

The male and female flowering cultivar Spotvrije was as susceptible to stem and fruit rot as the female flowering hybrid Farbio. The tested breeding material showed plant resistance, both in the young and mature stage, and resistance to external fruit rot if the fruits were slightly wounded. Resistance to internal fruit rot was not observed.

Not always a correlation was found between the activities of fungicides in vitro and in vivo. Testing fungicides on young inoculated plants under humid conditions was a reliable method. Best control was obtained with benzimidazole fungicides, tolylfluanid and chlorothalonil. Ergosterol biosynthesis inhibitors, used to control powdery mildew, and dicarboximides, used to control Botrytis, gave also some control of D. bryoniae. Frequent, preferably weekly sprays were necessary to obtain a reasonable effect.

The disease has to be controlled by a combination of hygienic measures, chemical control and manipulation of the glasshouse environment, especially by preventing humid conditions. A contribution of resistance breeding is expected in the future.

**SAMENVATTING**

**Didymella bryoniae bij kaskomkommers**

Het toenemend optreden van vruchtrot, veroorzaakt door Didymella bryoniae, in de intensieve glasteelt van komkommer in Nederland was aanleiding tot nader onderzoek over de biologie, de epidemiologie en de bestrijdingsmogelijkheden van de schimmel. Behalve stengels, bladeren en groeipunten worden ook vruchten, zowel inwendig als uitwendig, aangetast. Inwendig rot, dat bij het bloemeinde begint, is in het beginstadium moeilijk aan de buitenkant van de vrucht waar te nemen. Uitwendig rot komt voor op willekeurige plaatsen op de vrucht, voornamelijk als bewaarrot na de oogst.

De overleving van de schimmel in de open lucht en in kassen werd nagegaan. Op aangetaste planteresten in de open lucht overleefde de schimmel winters met 14 dagen vorst. Voor de groei en fructificatie van de schimmel was een temperatuur boven 5 °C nodig. Aangetaste restanten van planten bleven een infectiebron zolang ze niet verteerd waren. Het bleek dat de ziekte eerder en daardoor ook ernstiger optrad in kassen waarin opzettelijk aangetaste planteresten waren ingebracht dan in kassen waarin dit niet was gedaan. Geadviseerd wordt om de primaire infectie tegen te gaan door hygiënische maatregelen, zoals het vernietigen van het aangetaste afgedragen gewas, het uitspuiten van de kas met formaline en het stomen van de grond.

Zowel buiten bij aangetaste planteresten, als in kassen met aangetaste planten, trad 's avonds na zonsondergang een piek op in de aantallen gevangen ascosporen. Door het watergeven van de planten in de kas werd het vrijkomen van ascosporen bevorderd. Onder geconditioneerde omstandigheden bleek een hoge relatieve luchtvochtigheid, of een wisseling in r.v., niet voldoende voor het vrijkomen van ascosporen. Slechts nadat de aangetaste plantedelen waren bevochtigd, werden ascosporen gevangen. In de kas werden de eerste ascosporen gevangen op ongeveer hetzelfde moment als de eerste symptomen van de ziekte werden waargenomen. Vangen van ascosporen heeft daarom geen voorspellende waarde met het oog op de te treffen bestrijdingsmaatregelen.
Gewoonlijk is de eerste aantasting waar te nemen op de restanten van de vruchtsteeltjes die aan de stengel blijven zitten na het oogsten

van de vruchten.

Voor het ontstaan van rotte plekken op de vrucht, het zogenaamde uitwendig vruchtrot of bewaarrot, was verwonding nodig. De mate van aantasting nam toe naarmate de verwonding ernstiger was. Het rotten van de vruchten nam toe in het traject van 12 tot 23 °C; bij hogere temperaturen nam het af tot bij 35 °C helemaal geen rotting meer optrad. Teneinde rotting tegen te gaan en de bewaarduur niet te verkorten moeten de vruchten na de oogst tot kort voor de consumptie bij 12 °C worden bewaard.

De relatieve luchtvochtigheid tijdens de bewaarperiode had geen effect op de vruchtaantasting. Door een te lage stikstofbemesting tijdens de teelt nam de mate van vruchtrot af. Dit ging echter ten koste van de produktie. Minder rot trad ook op door de vruchten in het licht te bewaren in plaats van in het donker; van praktische betekenis was dit verschil echter niet. Uitwendig vruchtrot kan worden voorkomen door vruchten met een dikkere schil te telen. Bij de huidige cultivars dienen de mogelijkheden van verwonding, tijdens alle handelingen die de vruchten ondergaan, te worden verkleind.

Door open bloempjes te inoculeren werd inwendig vruchtrot verkregen; de meeste vruchten werden echter niet aangetast. De stempel en de stijl van een open bloempje werden gemakkelijk geïnfecteerd. Voordat de schimmel via het bloemweefsel de vrucht bereikte waren er meer dan twee dagen verstreken. Noch de wijze van bloeminoculatie, noch het toevoegen van voedingsstoffen aan de inoculumsuspensie, beïnvloedde het optreden van inwendig vruchtrot. Het licht verwonden, door delen van de bloem te verwijderen, had geen effect op de fractie vruchten met inwendig rot. Een ernstige verwonding, door het gehele bloempje te verwijderen, reduceerde de aantasting met 75%. Na inoculatie van verwelkte bloempjes trad 60% minder vruchrot op dan na inoculatie van open bloempjes. Enkele cultivars met resistentie tegen echte meeldauw waren minder vatbaar voor inwendig vruchtrot. Deze resistentie ging samen met een lange stijl en een korte bloeiduur. Een duidelijk effect van een verschil in bloeiduur op het optreden van inwendig vruchtrot kon echter binnen één cultivar niet worden aangetoond. Vruchten van planten die onder watergebrek waren geteeld, waren vatbaarder voor inwendig rot dan vruchten van planten die geen watergebrek hadden. Een verschil in vatbaarheid tussen langzaam en snel groeiende vruchten werd niet gevonden. Ook vruchtdunning had geen

effect op de aantasting. Evenmin kon worden aangetoond dat de luchtvochtigheid van invloed was. Voor bloeminfectie was geen vrij water nodig. Mogelijk is er een bepaald afweermechanisme tegen vruchtaantasting, waaraan de schimmel onder bepaalde omstandigheden ontsnapt. De kans op bloeminfectie kan worden verkleind door de bloeiduur te verkorten. Het optreden van inwendig vruchtrot kan worden tegengegaan door het telen van cultivars waarvan de bloemdelen snel van de jonge vruchten afvallen, of waarvan de bloempjes geen stijl hebben.

Onder geconditioneerde omstandigheden kwam aantasting van bladeren en groeipunt zelden voor bij 60% r.v., ze nam toe bij 95% r.v. en was het ernstigst als de planten nat werden gehouden. De aanwezigheid van vrij water gedurende een uur was voldoende voor de eerste fase van infectie. Het blad moest nat zijn voor een verdere uitbreiding van de aantasting. Bij 60 en 95% r.v. ontstond een verschil in planttype. De r.v. had echter geen predispositie-effect op de infectie van verwonde bladeren. Voor de infectie van oudere bladeren was, evenals bij vruchten, verwonding nodig, voor die van jong plantenweefsel niet. De schimmel is voornamelijk een wondparasiet. De aantasting nam toe als de concentratie van conidiën hoger was. Het effect van bladbevochtiging op de aantasting was gelijk aan die van een tienvoudige concentratie van conidiën.

Onder kasomstandigheden bevorderde een lagere nachttemperatuur in de periode voor de inoculatie, in het traject van 12 tot 24 °C, later in het seizoen de aantasting van komkommerplanten, terwijl van de dagtemperatuur in het traject van 17 tot 26 °C geen uitgesteld effect werd waargenomen. In kasafdelingen met een ingestelde minimum raamopening was de dagelijkse maximum relatieve luchtvochtigheid 10% lager dan in kasafdelingen zonder een minimum raamopening. Dit verminderde, bij niet geïnoculeerde planten, de gewasaantasting tot 35% en de inwendige vruchtaantasting tot 40%. De verspreiding van de ziekte van geïnoculeerde naar niet geïnoculeerde planten was onder vochtigere omstandigheden sneller dan onder drogere. De produktie in aantallen gezonde vruchten was echter onder de vochtigere omstandigheden hoger dan onder de drogere.

Een interval tussen stook- en ventilatietemperatuur van 5 °C in plaats van 2 °C bevorderde de aantasting. Een geringe ventilatie met behulp van een ventilator, bij een interval van 5 °C tussen stook- en ventilatietemperatuur, had nauwelijks of geen effect op de aantasting.

De invloed van het 3 uur voor, 1 uur voor, 1 uur na en 3 uur na zonsopgang overgaan van de nacht- (16 °C) naar de dagtemperatuur (24 °C) op het optreden van de ziekte werd onderzocht. Hoe later naar de dagtemperatuur werd overgegaan, hoe langer de perioden met hoge relatieve luchtvochtigheid waren, hoe langer de vruchten nat waren van condensatie en hoe meer aantasting in het gewas en de vruchten voorkwam bij geïnoculeerde planten.

Bij een hoge infectiedruk, na inoculatie van planten, nam de gewas- en vruchtaantasting toe en was de produktie van stekvruchten hoger dan bij niet geïnoculeerde planten. Inoculatie van planten reduceerde na vier weken het aantal internodiën met 18% en die van het aantal vruchten met 10%.

De gemengd bloeiende cultivar Spotvrije was even vatbaar voor gewas- en vruchtaantasting als de vrouwelijk bloeiende hybride Farbio. Het veredelingsmateriaal dat werd getoetst was zowel in het jonge als volwassen plantstadium minder vatbaar en vertoonde tevens minder uitwendig vruchtrot, indien de vruchten licht werden verwond, dan de standaardcultivar. Resistentie tegen inwendig vruchtrot werd in dit materiaal niet gevonden.

De werking van fungiciden in vitro en in vivo kwam niet altijd overeen. Het toetsen van chemische middelen op jonge, geïnoculeerde planten onder vochtige omstandigheden bleek een betrouwbare methode te zijn. De beste bestrijding werd verkregen met benzimidazoolfungiciden, tolylfluanide en chloorthalonil. Ergosterolbiosyntheseremmers, die tegen echte meeldauw worden gebruikt, en dicarboximiden, die tegen Botrytis worden toegepast, vertoonden ook een werking tegen D. bryoniae. Voor een goed resultaat moet frequent, zo mogelijk wekelijks, worden gespoten. Middelen met een verschillend werkingsmechanisme moeten zo veel mogelijk worden afgewisseld om de ontwikkeling van resistente schimmelstammen tegen te gaan.

Door de bestrijdingsmogelijkheden van hygiëne, kasklimaat en chemische bestrijding te integreren is het mogelijk de ziekte tot aanvaardbare proporties terug te dringen. Van deze mogelijkheden is bestrijding via het klimaat het belangrijkst, waarbij vooral door een juiste ventilatie de aanwezigheid van vrij water op plantedelen moet worden tegengegaan. De maatregelen die hiervoor moeten worden genomen zijn echter strijdig met de noodzaak tot energiebesparing. Mede daarom is te hopen dat ook de resistentieveredeling een bijdrage zal leveren door minder vatbare cultivars te kweken.

**CURRICULUM VITAE**

Nicolaus Aloisius Maria van Steekelenburg werd op 23 november 1941 geboren te Wateringen. Na het behalen van het diploma MULO-A in 1957 en het diploma HBS-B in 1961, volgde de studie aan de Landbouwhogeschool te Wageningen. In maart 1969 werd het ingenieursdiploma in de richting Planteziektenkunde behaald. Daarna was hij werkzaam bij Shell Nederland Chemie B.V. Op 1 januari 1971 trad hij in dienst van het Instituut voor Plantenziektenkundig Onderzoek dat hem aanvankelijk bij het Proefstation voor de Boomkwekerij te Boskoop stationeerde. Op 1 april 1973 volgde een overplaatsing naar het Proefstation voor Tuinbouw onder Glas te Naaldwijk, waar naast het onderwerp van dit proefschrift ook andere schimmel- en bacterieziekten van glastuinbouwgewassen onderwerp van studie waren.