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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.

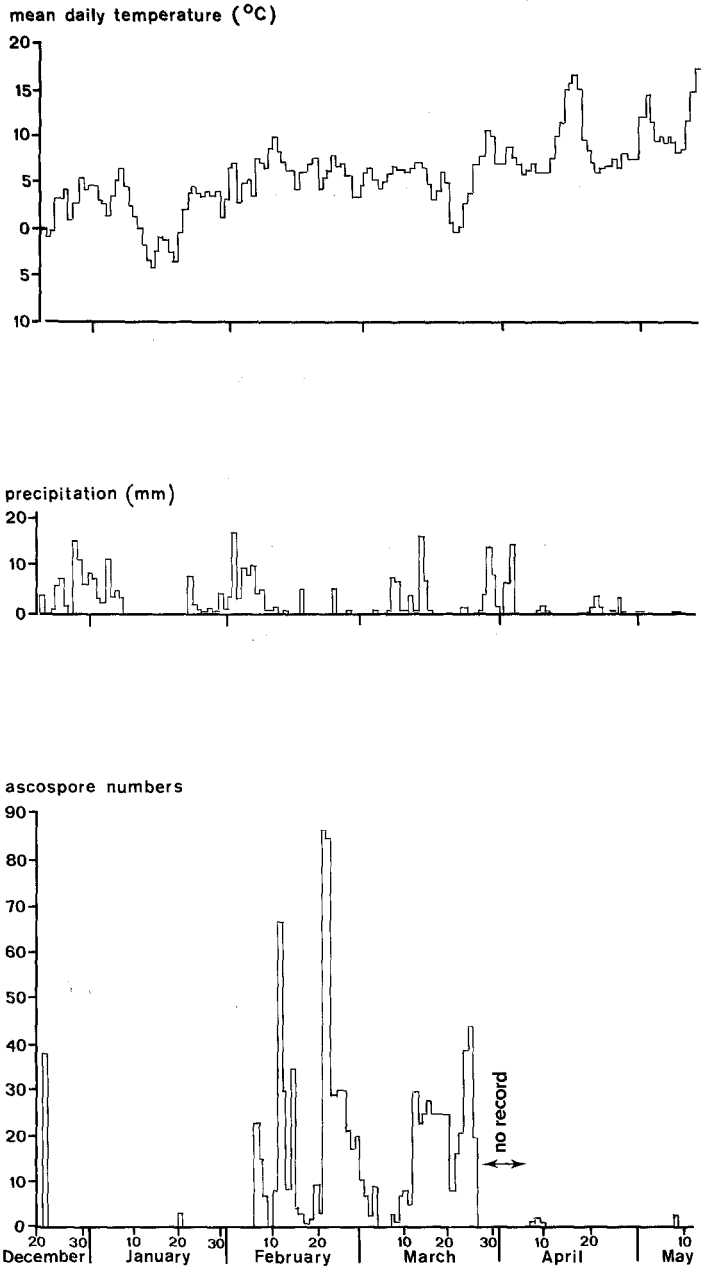

Fig. 1. Aantallen ascosporen van D.bryoniae die per dag van aangetaste stengeldelen van komkommer in de open lucht werden gevangen met een Burkard sporenvanger; de gemiddelde dagtemperatuur en neerslag vanaf 20 december 1979 tot 12 mei 1980.

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.

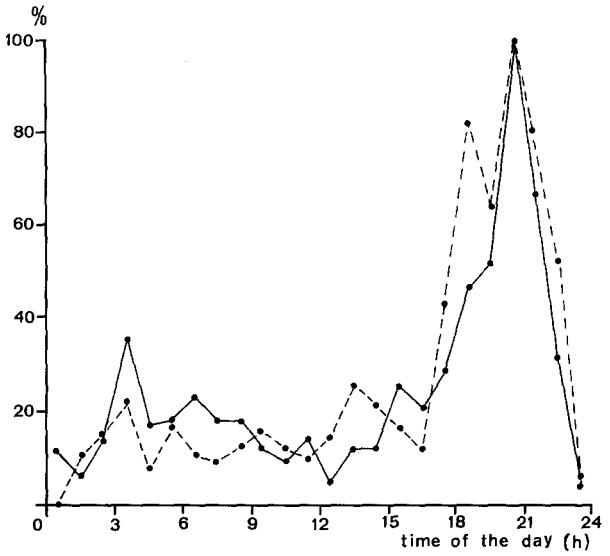

Fig. 2. Gemiddelde dagelijkse periodiciteitscurven van ascosporen van D.bryoniae, die van aangetaste stengeldelen van komkommer in de open lucht werden gevangen met een Burkard sporenvanger op dagen met (——) en zonder regen (- - -), uitgedrukt als percentage van de hoogste gemiddelde vangst van respectievelijk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

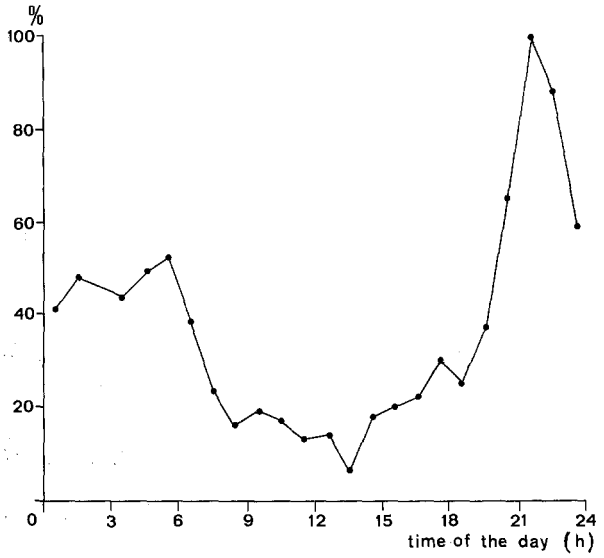

Fig. 3. Gemiddelde dagelijkse periodiciteitscurve van ascosporen van D.bryoniae, die in kaslucht werden gevangen met een Burkard sporenvanger op 15 dagen waarop de planten niet werden beregend in juni/juli 1980, uitgedrukt als percentage van de hoogste gemiddelde vangst.

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.

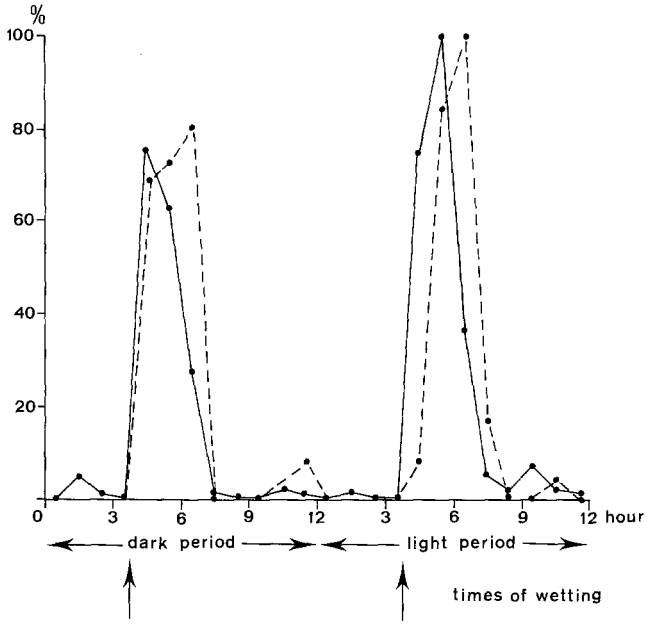

Fig. 4. Periodiciteitscurven van ascosporen van D.bryoniae, die van aangetaste stengeldelen van komkommer werden gevangen in een klimaatkast met een 12-urige donker/licht cyclus en lucht-/ en dauwpunttemperatuur combinaties van 23/23 °C (——) en 23/19 °C (- - -), na tweemaal bevochtigen per etmaal, uitgedrukt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

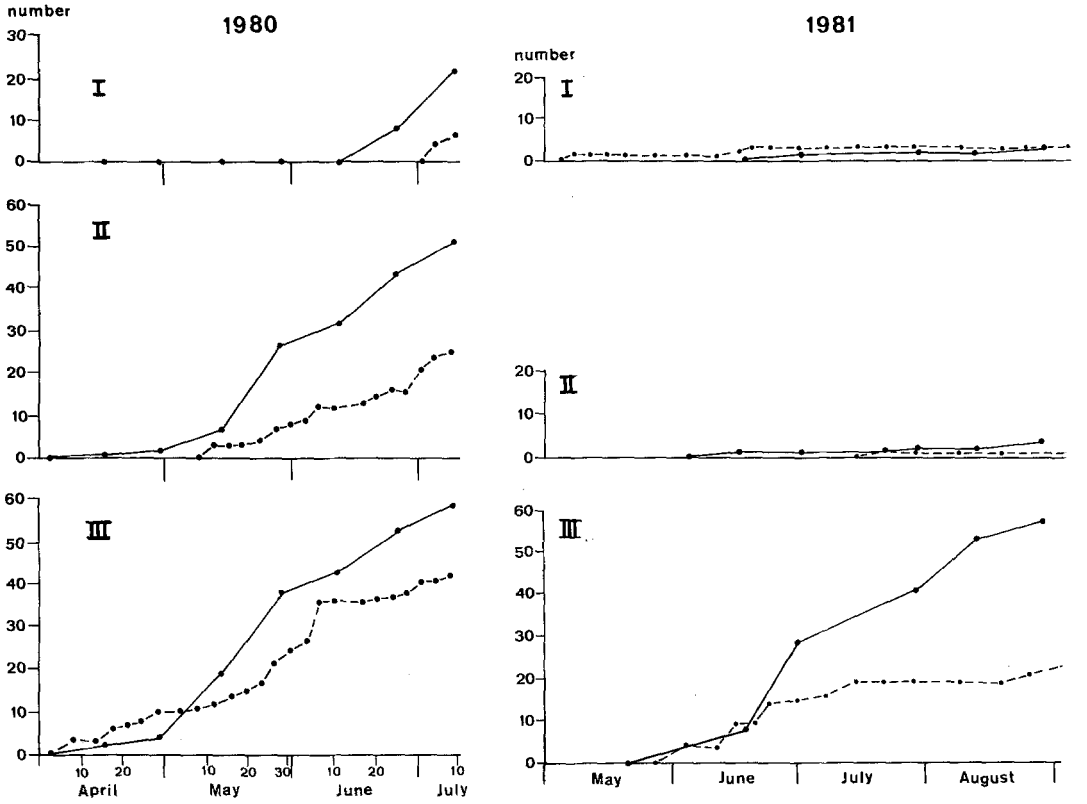

Fig. 5. Het verloop van het totaal aantal stengellesies van D.bryoniae (——) en van het totaal aantal vruchten met inwendig rot (- - -) in een gewas komkommers in kasafdelingen (met elk 30 planten) zonder ingebrachte plantenresten (I), met plantenresten boven de planten (II) en met plantenresten op de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

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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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