Neth. J. Pl. Path. 92 (1986) 81-91

Factors influencing internal fruit rot of cucumber caused by Didymella bryoniae

N.A.M. VAN STEEKELENBURG

Research Institute for Plant Protection (IPO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands[1]

Accepted 27 January 1986

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

---

[1] Seconded to the Glasshouse Crops Research and Experiment Station, Zuidweg 38, 2671 MN Naaldwijk, the Netherlands.

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.

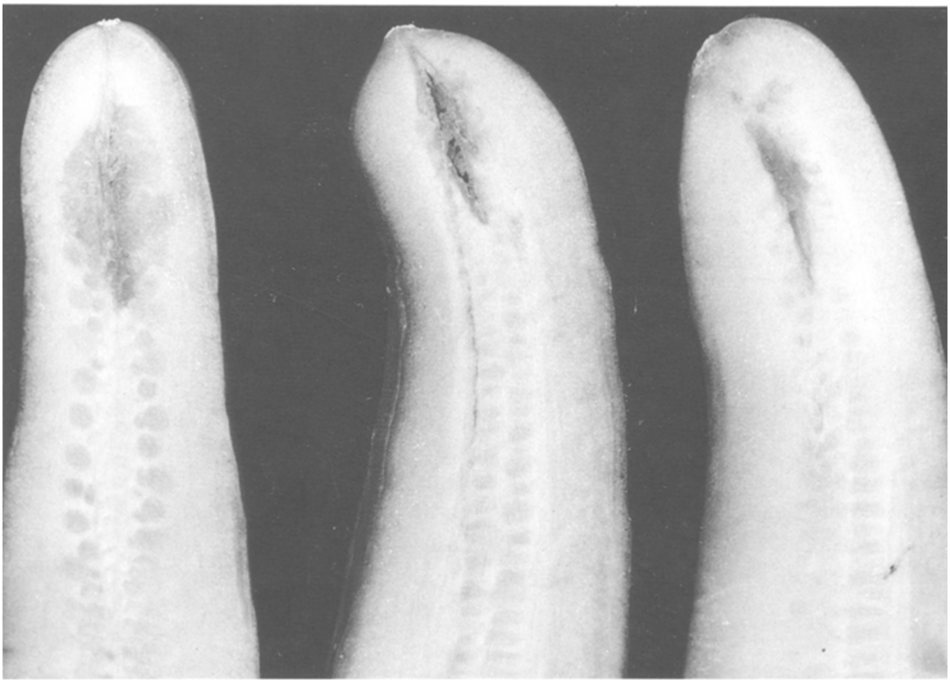

Fig. 1. Internal rot at the blossom end of cucumber fruits, caused by *D. bryoniae*.

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.

Acknowledgements

Thanks are due to B.C. van Dam, S.J. Paternotte and G.P. Verduyn for their help in carrying out the experiments, to J.C.M. Withagen for statistical analysis and to W.A. van Winden for correcting the English text.

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.

References

Bergstrom, G.C., Knavel, D.E. & Kuć, J., 1982. Role of insect injury and powdery mildew in the epidemiology of the gummy stem blight disease of cucurbits. Plant Disease 66: 682-686.

Callebaut, A., 1984. Induction of fungitoxic substances in plants, callus and suspension cultures of *Cucumis sativus.* Med. Fac. Landb.wet. Rijksuniv. Gent 49: 987-994.

Chiu, W.F., & Walker, J.C., 1949. Physiology and pathogenicity of the cucurbit black rot fungus. J. agric. Res. 78: 589-615.

Figueiredo, M.B., Cardozo, R.M.G. & Harruda, H.V., 1970. Blossom excision as a method of controlling fruit rot infection caused by *Mycosphaerella melonis* (Pass.) Chiu and J.C. Walker in Italian squash (*Cucurbita pepo* L.). Arq. Inst. biol., S. Paulo 37: 285-292.

Fokkema, N.J., Houter, J.G. den, Kosterman, Y.J.C. & Nelis, A.L., 1979. Manipulation of yeasts on field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum.* Trans. Br. mycol. Soc. 72: 19-29.

Heuvel, J. van den, 1981. Effect of inoculum composition on infection of French bean leaves by conidia of *Botrytis cinerea.* Neth. J. Pl. Path. 87: 55-64.

Kagiwata, T., 1970. Brown heart rot of the cucumber by *Mycosphaerella melonis* (Pass.) Chiu et Walker, and its control. Rev. Pl. Prot. Res. Tokyo 3: 94-97.

Leski, B., 1984. Black fruit and stem rot caused by *Didymella bryoniae* an important disease of glasshouse cucumber, new to Poland. Acta Horticulturae 156: 245-250.

Lint, P.J.A.L. de & Heij, G., 1982. Night temperature and flower abortion of glasshouse cucumber (*Cucumis sativus* L.). Neth. J. agric. Sci. 30: 331-339.

Sitterly, W.R., 1968. A new symptom of gummy stem blight (*Mycosphaerella melonis*) on cucumber fruit. Pl. Dis. Reptr 52: 49-51.

Steekelenburg, N.A.M. van, 1981. Comparison of inoculation methods with *Didymella bryoniae* on *Cucumis sativus*. Euphytica 30: 515-520.

Steekelenburg, N.A.M. van, 1982. Factors influencing external fruit rot of cucumber caused by *Didymella bryoniae*. Neth. J. Pl. Path. 88: 47-56.

Steekelenburg, N.A.M. van, 1984. Influence of ventilation temperature and low ventilation rates on incidence of *Didymella bryoniae* in glasshouse cucumbers. Acta Horticulturae 156: 187-197.

Steekelenburg, N.A.M. van, 1985. 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. Neth. J. Pl. Path. 91: 225-233.

Steekelenburg, N.A.M. van & Vooren, J. van de, 1981. 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*. Acta Horticulturae 118: 45-56.

Svedelius, G. & Unestam, T., 1978. Experimental factors favouring infection of attached cucumber leaves by *Didymella bryoniae*. Trans. Br. mycol. Soc. 71: 89-97.

Sweep, A.A.M. & Govers, J., 1967. Weer nieuwe ziekte in komkommers? Groenten en Fruit 23: 739.