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Studies of Didymella bryoniae: The influence of nutrition and cultural practices on the occurrence of stem lesions and internal and external fruit rot on different cultivars of cucumber

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Abstract

Cucumber plants of different cultivars grown under various levels of nutrient supply, climatic conditions and different pruning practices were inoculated with *Didymella bryoniae* (Auersw.) Rehm. Increased concentration of the nutrient solution in the rockwool slabs leading to a higher amount of dry matter content in the plant tissue resulted in a lower percentage of internal and external fruit rot in the cultivars Daleva and LD 290/82 (Dæhnfeldt), and in lower percentage of internal fruit rot in cv. Aminex, whereas the effect on cv. Dalibor was insignificant. Differences between cultivars were observed, cv. Dalibor showing the highest level of resistance against internal fruit rot and lowest incidence of nodal and internodal lesions.

Separate factors appear to affect the establishment of the pathogen in the nodal tissue and its invasion of the internodal tissue. By raising the temperature to daytime level 3 h before sunrise, the risk of extended periods with high humidity was limited and the incidence of internal fruit rot was reduced. An increased incidence of nodal and internodal lesions and external fruit rot was observed at reduced pruning, leaving wilted leaves and shoots on the plants.

Additional keywords: Gummy stem blight, glasshouse cucumber, flower infection, resistance, climatic conditions, pruning, nodal infection, internodal infection, dry matter content, nutrition, cultivar differences.

Introduction

Gummy stem blight of cucumbers (*Cucumis sativus* L.) caused by *Didymella bryoniae* (Auersw.)Rehm is regarded as one of the most serious diseases in glasshouse cucumber crops in Denmark since it causes severe losses of crop yield and quality. The symptoms appear on stems, leaves and fruits. Attack on the main stem – if not treated – usually kills the plant. Ascospores are airborne and thus responsible for the infection of the flowers. The pathogen invades the stigmatic tissue and follows the pollen transmitting tissue into the young fruit, where it later causes internal rot (de Neergaard, 1989a). Van Steekelenburg (1986) investigated the occurrence of internal fruit rot and concluded that a short period of flowering and a long style are factors which limit the incidence of internal rot. Internal rot often develops without any symptom visible on the fruit surface. The tissue of the central part is discolored, the symptom developing from the blossom end. In a severe epidemic, internal rot occurs in 5–10% of the harvested fruits.

Climatic conditions are generally the most important factors which influence the incidence of the disease. Although differences have been observed between cultivars, no highly resistant types are available (Van der Meer et al., 1978, Wyszogrodzka et al., 1986). Nutritional conditions leading to higher dry matter content of the plant tissues are generally believed to confer resistance. Hyphal perforation of cell walls is slower in tissue with a high degree of secondary growth in the cell walls (Gäumann, 1951). According to Kiraly (1976, 1980), infection by facultative parasites is suppressed by nitrogen fertilization, which keeps the plants in a more juvenile state. Trials with cucumbers grown at low levels of N_2-fertilization have shown earlier and heavier attack of gummy stem blight relative to cucumbers receiving normal nutrient level (Wendland, 1979). Studies of the infection of vegetative parts in a trial where EC (electric conductivity) levels varied from 2.5 to 5.0 have been performed by Van Steekelenburg and Welles (1988) who report a decrease in the frequency of diseased nodes with increasing EC at low Ca^{++} concentration, and the same effect of increasing Ca^{++} concentration at low EC level. Prokhorova (1976) investigated *D. bryoniae* infecting cucumber and found that increased supply of K^+ caused increased resistance.

The trials reported here were carried out in order to determine whether the disease development of the vegetative plant parts and the susceptibility of the fruits to gummy stem blight, especially the occurrence of internal fruit rot, was influenced by high concentrations of nutrients supplied to a culture of cucumbers leading to high dry-matter content of the tissues (a so-called "hard" plant).

Materials and methods

The host plants. Cucumber plants were sown at the end of January and transplanted to the research greenhouse 4 weeks later. They were grown on rockwool slabs in a greenhouse with 6 separated compartments, each measuring 7.25 × 9.25 m. Six plots with 14 plants each, 4 of which were guard plants, were arranged in each room (1.25 plant m^{-2} in 1985, 1.4 plant m^{-2} in 1986 and 1987). The cultivars Daleva (Bruinsma) and LD 290/82 (Dæhnfeldt) hereafter called D 290, were used in 1985 and 1986, and Aminex (Sluis en Groot) and Dalibor (Royal Sluis) in 1987.

Three different levels of mineral nutrition in the solution in the rockwool slabs were maintained: In 1985 and 1986 electrical conductivity (EC) levels of 1.5, 2.0, and 3.5 mS/cm and in 1987 2.0, 3.5, and 4.0 with the same ratios between N, P, K, Mg, S, and Ca and the same concentration of Fe, Mn, B, Cu and Mo at all levels. The nutrient solution in the rockwool slabs was analysed for EC-level and pH every second day, and for N, P, K, Mg, Ca, S, Fe, Mn, B, Cu, and Mo every second week. Adjustments of the applied solution were made if required during the growing season. Furthermore, the uptake of nutrients was determined by analysis of leaf samples for dry matter and composition of mineral contents. In 1987, during the flowering stage, 2/3 of the pistil including the style were removed from a number of guard plants once a month and the dry matter content and chemical composition were analysed.

From May, the temperature was 18 °C during night and 21 °C in the daytime. It was allowed to rise 4 °C before the ventilators were opened. When the outdoor temperature was above 10 °C, the ventilators were opened when the relative humidity was above 85%. In this way, the RH of the greenhouse was maintained at approximately 85% during the daytime. During the night it might occasionally be higher. CO_2 at 900 ppm was supplied when the ventilators were closed.

Pruning of the plants was performed as in a commercial greenhouse crop, i.e. lateral shoots were removed below the seventh leaf of the main stem. The following lateral shoots were cut over one leaf. When the main stem reached the suspending wire it was stopped by cutting, and three or four shoots were allowed to develop from the upper part of the plant.

In 1985, half of the plants were pruned according to the standard routine for commercial cultivation including removal of dead plant parts. In the other plots wilted leaves and shoots were left on the plants to study the effect of 'reduced pruning'. In 1986, half of the plants were subjected to the standard limitation of fruit setting on the main stem (i.e. development of seven fruits). No fruit-thinning took place on the other plants. This treatment was included in order to test the possible effect of stress-factors on the susceptibility of the plants. In 1987, the daytime climate programme was started 3 h before sunrise in half of the trial, whereas the rise to daytime temperature coincided with sunrise in the other half. The trial was terminated 14th September 1985, and 22nd August 1986 and 1987.

All trials were designed with three replicates of all cultivars and treatments.

The pathogen. The pathogen was originally isolated from stem lesions in a commercial greenhouse 20 km to the west of Copenhagen. The isolate is kept in the culture collection of The Plant Pathology Section at The Royal Veterinary and Agricultural University of Copenhagen (CP) as No. 1799. Inoculum was produced by growing *D. bryoniae* on potato cubes. After 7 days (12 h near-UV light alternating with 12 h dark periods, 20 °C), the cultures were flooded with distilled water for 10 min, and the resulting conidial suspension was adjusted to a concentration of c. 10^6 ml^{-1}.

Inoculation. The plants were inoculated at an age of 11–12 weeks (in the last half of April), at which time the first fruits of the lateral shoots were about to be harvested. Inoculation of the plants in the greenhouse took place under conditions with high humidity (in the evening with closed ventilators and after watering the floors of the greenhouse), using an electric sprayer (Bosch PSP 250). An average of 300 ml spore-suspension was used for each plant. After an incubation period of 5–7 days, symptoms appeared and the epiphytotic continued to develop without further inoculation for the rest of the season.

Recording. Harvest took place three times a week. All harvested fruits produced on the lateral shoots of the 360 plants in the trial were cut lenghtwise at the blossom end to ascertain whether internal fruit rot had developed. In addition to that, external fruit rot was recorded. Stem lesions were assessed by counting nodes and internodes of the main stem with sporulating lesions. For assessment, the internodes were divided (imaginarily) in three equal parts and lesions were recorded for each part, i.e. a totally affected internode was counted as three lesions. Six plants in the central part of each plot were examined 7 or 8 times during the entire growing season starting 1 week after the first symptoms appeared, (13–14 days after inoculation). Results were corrected for differences in total number of possible infection sites (nodes and internodes) and processed by analysis of variance.

Results

The epiphytotic started rapidly, and the first conidia and ascospores were produced within 1 week after inoculation. During the growing season, the EC of the nutrition solution in the rockwool slabs became generally too high compared to the planned level, therefore the concentration of the solutions supplied was reduced accordingly. The level of dry matter content of the green leaves correlated with the nutrient treatment levels. In 1987 where the apical part of the pistil including the style were analysed, the dry matter content and chemical composition were in good agreement with the applied nutrient supply at each level.

Stem lesions. Level of nutrients had no effect on the frequency of nodal and internodal lesions, whereas differences between cultivars were observed, the weak growing cv. Aminex apparently being more susceptible than cv. Dalibor. In 1985 there was no significant difference between Daleva and D 290, whereas in 1986 D 290 was the most resistant of the two cultivars as for stem lesions (Table 1). The ratio between nodal and internodal symptoms (Table 2) is increasing during the growth season in all trials, indicating that the disease development starts from the nodes and moves gradually into the internodes. Comparison of cvs Aminex and Dalibor reveals differences in the IN/N ratio through the entire growing season. At the end of the season cv. Aminex has a ratio of 0.930, that is to say almost equal numbers of infected nodes and infected internodal sections, whereas cv. Dalibor has a ratio number of 0.366, i.e. three infected nodes for each infected internodal section.

An increased incidence of nodal and internodal lesions was observed where reduced pruning was applied, especially late in the season. At 14, 42 and 142 days after inoculation the nodes of the plants subjected to reduced pruning showed a lesion frequency of 18.9%, 46.4%, and 65.4% higher than in standard pruned plants (Table 3).

Table 1. Percentage of nodes and internodes showing lesions. Average of treatments.

Year	Stem part	Cultivar	Days after inoculation		
			14	42	142
1985	Nodes	Daleva	14.79 a[1]	38.09 a	67.23 a
		LD 290/82	10.01 a	35.63 a	63.19 a
	Internodes	Daleva	0.19 a	0.76 a	5.88 a
		LD 290/82	0.14 a	0.81 a	3.53 a
Year	Stem part	Cultivar	Days after inoculation		
			13	41	68
1986	Nodes	Daleva	32.80 a	48.57 a	52.44 a
		LD 290/82	29.95 b	43.83 b	47.75 b
	Internodes	Daleva	1.16 a	2.91 a	4.57 a
		LD 290/82	0.55 b	2.01 b	3.35 b
Year	Stem part	Cultivar	Days after inoculation		
			13	62	118
1987	Nodes	Aminex	19.88 a	46.71 a	54.75 a
		Dalibor	16.51 b	37.84 b	46.09 b
	Internodes	Aminex	0.37 a	9.07 a	16.98 a
		Dalibor	0.12 b	1.54 b	5.62 b

[1] For each pair, different letters indicate significance at the 95% level.

Table 2. Ratio between number of diseased internodes and nodes (IN/N ratio). Average of treatments.

Year	Cultivar	Days after inoculation		
		14	42	142
1989	Daleva	0.038	0.060	0.262
	LD 290/82	0.046	0.068	0.167
Year	Cultivar	Days after inoculation		
		13	41	68
1986	Daleva	0.056	0.138	0.211
	LD 290/82	0.107	0.180	0.262
Year	Cultivar	Days after inoculation		
		13	62	118
1987	Aminex	0.057	0.583	0.930
	Dalibor	0.022	0.123	0.366

Table 3. Effect of pruning on stem parts. Percentage of nodes and internodes showing lesions. Average of treatments and cultivars.

Stem part	Pruning[1]	Days after inoculation		
		14	42	142
Nodes	Normal	11.33 a[2]	29.92 a	49.18 a
	Reduced	13.47 b	43.81 b	81.24 b
Internodes	Normal	0.14 a	0.82 a	3.44 a
	Reduced	0.17 a	0.76 a	5.97 b

[1] Normal: Standard pruning. Reduced: No removal of wilted leaves.
[2] For each pair, different letters indicate significance at the 95% level.

Internal fruit rot. In 1985 a lower percentage internal fruit rot occurred in cv. Daleva compared to D 290 (6.1 vs. 8.9%) (Table 4a), and the incidence decreased with increasing level of nutrient supply (Table 4b). The type of pruning had no effect on internal fruit rot and there was no effect of fruit-thinning.

In 1986, the differences in internal fruit rot incidence between cultivars and levels of nutrition were similar to the results of 1985, but apparently lower (Table 4a and 4b).

In 1987, internal fruit rot occurred at a significant lower frequency in cv. Dalibor than in cv. Aminex (7.8 vs. 13.7%, $LSD_{.05} = 2.0$). The differences between the nutrient levels were not significant in Dalibor, whereas Aminex responded significantly: 15.6% internal fruit rot at EC = 2.0 and 12.4% at EC = 4.0 (Table 4a). Where daytime temperature regime was applied 3 h before sunrise, the percentage internal fruit rot was significantly lower

Table 4a. Percentage fruits with internal fruit rot in different cultivars at all or different (1987) levels of nutrient supply EC-data are average of pruning treatments (1985), resp. fruit thinning treatments (1986), resp. climatic regimes (1987).

Year	Cultivar	Internal fruit rot (%)				$LSD_{.50}$
1985	Daleva	6.1				2.4
	D 290	8.9				
1986	Daleva	5.3				0.8
	D 290	7.5				
Year	Cultivar	EC level				
		2.0	3.5	4.0	Average	LSD.05
1987	Aminex	15.6	13.0	12.4	13.7	2.0
	Dalibor	7.7	7.7	8.0	7.8	n.s.*

* n.s. = not significant.

Table 4b. Percentage fruits with internal fruit rot for all cultivars at different levels of nutrient supply. EC-data are average of pruning treatments (1985), resp. fruit thinning treatments (1988), resp. climatic regimes (1987)

Year	EC level	Internal fruit rot (%)	$LSD_{.05}$
1985	1.5	8.8	0.9
	2.0	7.7	
	3.5	6.0	
1986	1.5	7.5	1.5
	2.0	6.9	
	3.5	4.7	
1987	2.0	11.7	n.s.*
	3.5	10.4	
	4.0	10.2	

* n.s. = not significant.

compared to light dependent transition to daytime temperature (9.7 vs. 11.8%, data not shown in the tables).

External fruit rot. The percentage of external fruit rot was similar for the two cultivars Daleva and D 290 in 1985 and 1986, and decreased with increasing nutrient level (Table 5). Plants subjected to reduced pruning showed considerably higher frequency of external fruit rot than standard pruned plants: 9.7 vs. 5.6% ($LSD_{.05}$ = 0.8) (data not shown in the tables). Fruit-thinning had no effect on the ocurrance of external fruit rot. In 1987, percentage external fruit rot was lowest in cv. Dalibor (3.9 vs. 4.9%, data not shown in the tables), but independent of climate program and level of applied nutrients.

Table 5. Percentage fruits with external fruit rot at different levels of nutrient supply. Average of cultivars and treatments.

Year	EC level	Internal fruit rot (%)	$LSD_{.05}$
1985	1.5	8.3	0.7
	2.0	7.6	
	3.5	6.9	
1986	1.5	2.7	0.4
	2.0	2.6	
	3.5	2.4	

Discussion

Although direct comparison of all four cultivars cannot be made as the cultivars were grown in different years, it seemed as if cv. Aminex was generally the most susceptible to both types of fruit rot. Cv. Daleva seemed to be the most resistant of the four cultivars against internal rot. Both cvs Aminex and Daleva are weak growing cultivars. Hence, these results do not support the current assumption that weak growing cultivars are relatively more resistant due to their relatively 'harder' tissue.

High levels of supplied nutrients resulted in high dry matter content and a lower incidence of fruit rot in the cultivars Daleva, D 290 and Aminex, whereas no effect was recorded for cv. Dalibor. However, cv. Dalibor is generally more resistant to fruit rot than Aminex even at high levels of nutrient supply. The concentration of Ca^{++} in the dry matter increased at high nutrient level. The role of Ca^{++} in stabilizing the host tissue may be a key factor and should be further studied (cf. the work of Van Steekelenburg and Welles (1988)).

A certain level of internal fruit rot also appeared in the experimental plot showing the lowest frequency of disease. It must be noted that even a frequency of 5% internal fruit rot may cause big problems because most of the diseased fruits are externally symptomless, and hence can not be diagnosed and discarded before being forwarded to the consumer.

Internal fruit rot occurred at different frequencies dependant on the cultivar in this study. This indicates some form of chemical resistance e.g. the accumulation of polyphenolic substances as demonstrated by de Neergaard (1989a) or certain types of pseudo resistance such as short or closed flowering. Consequently, further work concerning resistance breeding is needed, aiming at cultivars with shorter period of flowering or with no style as suggested by Van Steekelenburg (1986), preferably also without nectarium. Plants with increased or earlier accumulation of polyphenolics in the pollen transmitting tissue of stigma, style or the apical region of the fruitlet might be obtained by selection or breeding as proposed by de Neergaard (1989a).

The decrease in the incidence of internal rot when the greenhouse temperature was raised 3 h before sunrise may be caused by the lower humidity which limits the release of ascospores. Van Steekelenburg (1983) found that ascospore release was dependent of humid conditions for a certain period.

The percentages of internal fruit rot recorded in the trial are believed to be slightly lower than in a typical serious attack, occurring spontaneously in a commercial greenhouse. It is likely that spore concentrations obtained by artificial inoculation in the trial were unnaturally high, presumably leading to increased frequency of aborted fruits (Van

Steekelenburg 1985), not recorded as diseased fruits. Maximum flower infection takes place at somewhat lower inoculum pressure according to Van Steekelenburg (1976) and has been confirmed by own observations.

External fruit rot occurs mainly after wounding, but also starts from sites with certain amounts of available organic nutrients, for example decaying tissue such as petals adherent to the fruit surface. A high level of nutrition resulted in lower incidence of external rot in the cultivars Daleva and D 290, but without showing any difference between the cultivars. The effect of high nutrition level might be strengthening the superficial layers of the fruit or keeping the fruit in an optimal physiological state, and thereby reducing the occurrence of tiny cracks in the outermost layers of the fruit. Van Steekelenburg (1981) has demonstrated that the thickness of the fruit peel is a determining factor in external fruit rot frequency.

Cultivar differences were demonstrated between cvs Dalibor and Aminex, the former being more resistant to external rot, whilst increased levels of nutrition played no role in the resistance of these cultivars. External fruit rot occurred at a much higher frequency under conditions with reduced pruning. With wilted leaves left on the plants, there were high amounts of organic nutrients available for the fungus, leading to increased spore production and better organic nutrient conditions for the establishment of new infections. It was characteristic that the amount of external fruit rot increased under such conditions, similar to the increased incidence of lesions on the vegetative parts. This is in accordance with the histological similarities in pathogen development in the host tissue in vegetative parts and in fruits after superficial penetration (= external fruit rot), whereas internal fruit rot, originating from the flower, has a quite different way of development, the pathogen causing far less necrosis in the initial stages after invasion of the host tissue (De Neergaard 1989a, 1989b).

The level of mineral concentration of the applied nutrient solutions had no significant effect on disease development on the stem parts in the trial, but a tendency to fewer lesions was noticed at EC level 3.5, compared to lower levels, whereas an EC-level at 4.0 seemed to confer a slightly increased susceptibility (data not shown). Cv. Aminex was more susceptible than cv. Dalibor, both in terms of fruit rot, and disease development on vegetative plant parts when assessed in the same trial. Lesions on stem nodes of cv. Aminex occurred approximately at a 20% higher frequency, and on internodes they were 3 times as frequent than on cv. Dalibor. However, when observing cvs Daleva and D 290, comparison of disease incidence on generative and vegetative organs leads to the opposite result. Daleva had a lower incidence of internal fruit rot but a 15–25% higher frequency of stem lesions compared to cv. D 290. Apparently disease susceptibility of fruits is not always correlated to that of the vegetative parts.

Besides the factors limiting the occurrance of the disease on the nodes, it seems as if another mechanism is active when the pathogen invades the internode. This is demonstrated by the fact that the IN/N ratios are different in different cultivars, especially late in the season. Cv. Dalibor has a very low internodal disease frequency, much lower than could be explained merely by the lower frequency on the nodes. Whether this resistance works in the nodal tissue, externally or internally in the transition zone to the internode, or inside the internodal region, or whether it is active against superficial invasion of the internodes, is still to be elucidated. Nevertheless it can be concluded that more than one resistance mechanism is active in the stem parts of the studied cultivars.

In addition to the selection of the optimal cultivar and nutrient level, other precautions to avoid gummy stem blight must still be taken into consideration, mainly climatic and sanitary precautions. A uniform temperature 24 h should be considered. Besides reducing the risk of condensation, caused by high humidity, it generally improves fruit quality.

Efficient sanitary measures, such as total removal and immediate destruction of diseased plant parts should be taken.

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