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Topple Disease of Tulips

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With 3 figures

The toppling of tulips is a physiological disorder that occurs, almost without exception, only when the plants are forced in greenhouses. The disease is characterized by the appearance of glassy, water-soaked areas on the flower stem, usually just before or during the flowering period. This is generally followed by shrinkage and furrowing at the site of the disorder, after which the portion above the lesions topples over. An extensive investigation into the cause of this toppling and the conditions under which it occurs was carried out by PINKHOF (1929 a, b) whose work has been continued by UYLDERT (1934). On the basis of these studies, detailed observations were made concerning the occurrence of macroscopically and microscopically visible symptoms, and a number of external factors influencing this phenomenon were investigated in detail. The osmotic value and the permeability of the cells and the Ca content of both healthy and diseased tissue were also analysed.

Description of the symptoms

The first macroscopic symptom of toppling is an intercellular infiltration. This may occur in any of the stem tissues. If it begins in the chlorenchyma, the infiltration is externally observable as a glassy spot on the surface of the stem. When the initial localization lies deeper, it becomes superficially observable after one or two days.

At the onset of the infiltration the cells lying in the infiltrated areas, which initially consist of longitudinal streaks, have a normal appearance. However, microscopic examination shows that in many cases the flow of the protoplasm is slower locally than in the as yet unaffected areas. After being brought into a plasmolyzing solution (e.g. 1 M sucrose), the protoplasts of affected tissue

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become rounded more rapidly than cells showing no tendency toward infiltration; they are in all likelihood less viscous. The retardation of the protoplasmic streaming is, therefore, probably the result of a decreasing activity of the infiltrated cells.

The first microscopically visible changes take place in the nucleus which becomes hyaline, making it more difficult to observe its structure. The next change is seen in the cytoplasm which becomes lumpy and appears disorganized. It often shows 'caps', not only under plasmolysis in sucrose or $Ca(NO_3)_2$ but also in sections embedded in paraffin oil in which plasmolysis does not take place. This latter fact indicates that the 'caps' were already present in the diseased cells and are, therefore, not the result of the penetration of ions from the plasmolyzing solution. The cytoplasm may continue to swell until tonoplast plasmolysis develops. The cytoplasm also shows a marked Brownian movement. All these phenomena point to alteration of the plasma.

Lastly, the cells begin to shrink, which weakens the stalk locally and causes it to topple over (fig. 1, right).

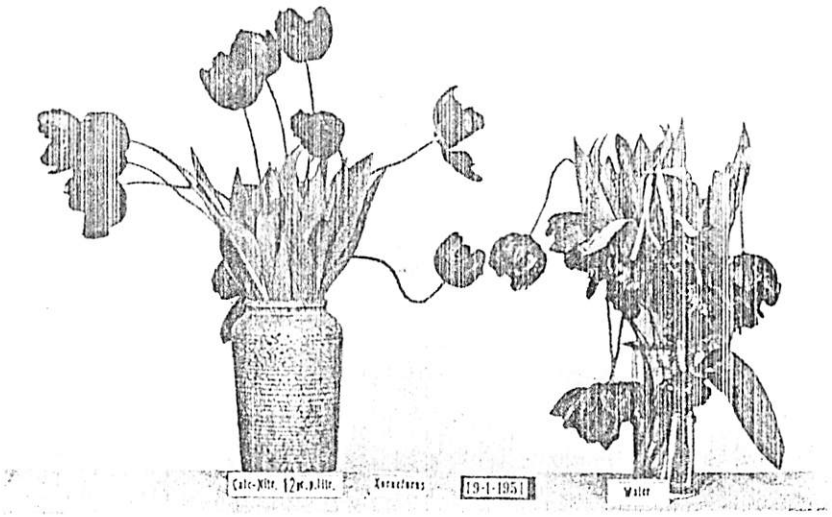

Fig. 1. Right: toppling of tulips (cultivar 'Korneforos'). Left: marked reduction of toppling after administration of calcium nitrate

Factors influencing the occurrence of infiltration

a) Relationship between the elongation zone in the stem and the site of infiltration

The site at which the disease appears is closely related to the elongation growth of the stem. During this growth, the elongation zone shifts from the base of the 1st (lowermost) internode via the 2nd and 3rd to the top of the upper-

most, or 4th internode of the stem. The closer to the base, the earlier the stem cells are completely elongated.

To determine the zone of optimal elongation, marks were made with India ink 10 mm apart on the 4th internode of the stems of 13 plants just before flowering. The distances between these marks were measured daily, the daily increase in the length of each zone being expressed as a percentage of the length of the same zone on the preceding day. Table 1 shows the increase in length on the day on which the internal infiltration could be considered to have started, i.e. the day before the infiltration became externally visible, this interval having been determined from prior observations. The zone in which infiltration began is indicated in table 1 by heavy type.

Table 1

Relationship between the site of initial infiltration and the zone of elongation growth in the 4th internode of tulip stems (cultivar 'Murillo').

Elongation growth is expressed as the percentual longitudinal increase of 10 mm zones in the preceding 24 hours. Values in heavy type indicate the zones in which the first signs of infiltration were observed

Zone No.		Plant No.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
top	11							33						
	10							29	25	20				
	9		40			26		43	31	33	22	36		
	8	35	42	27		8		38	33	44	40	38	28	36
	7	13	18	3	18	0	29	44	28	45	33	36	30	27
	6	0	5	4	20	0	28	36	6	31	50	38	40	22
	5	0	0	0	33	0	8	33	0	8	55	31	45	6
	4	0	0	0	38	0	0	15	0	0	30	0	31	0
	3	0	0	0	36	0	0	6	0	7	7	0	17	0
	2	0	0	0	21	0	0	0	0	0	0	6	0	0
basis	1	0	0	0	7	0	0	0	0	7	6	0	6	0

It can be seen that in most cases infiltration began in the zone in which elongation was most rapid on that day. In 4 of the 13 plants the infiltration appeared just below the zone of most rapid growth. From the occurrence of the infiltration in the zone showing the most rapid growth it follows that the infiltration occurs lower in the stem the earlier the developmental phase in which the disorder appears.

b) Influence of temperature

The stage in which the disorder develops is dependent on several factors. One of these is the rate of elongation of the stem. Any circumstance that contributes to the acceleration of the elongation is favourable to the occurrence of the disorder. Temperature plays an important part, not only during forcing but also during the storage period of the bulbs before planting. The temperatures to which the bulb is exposed during storage are determined by a pre-treatment

lasting until the complete flower has been formed and an after-treatment lasting until the bulbs are planted.

The results shown in table 2 indicate clearly that the percentage of toppling plants was lower the higher the treatment temperature and, therefore, the slower the rate of elongation. This holds for both pre- and after-treatment.

In addition to the effect of temperature during the storage period, elongation is also influenced by the temperature of the greenhouse. The higher the greenhouse temperature, the more rapid the elongation, and this is again accompanied by a higher percentage of toppling plants.

Generally speaking, the disorder occurs lower in the stalk the more the temperatures both before and after planting promote toppling.

Table 2

The influence of the temperature at which tulip bulbs are stored before planting on the occurrence of toppling in two susceptible cultivars (in % of the total number, 8 days after opening of the flowers).
One part of each series of plants was given tap water and the other a solution of $Ca(NO_3)_2$

treatment °C	cv. 'Mr. van der Hoef'		cv. 'Le Nôtre'	
	watering with tap water	watering with $Ca(NO_3)_2$ solution	watering with tap water	watering with $Ca(NO_3)_2$ solution
	arranged according to pre-treatment			
17 °C	55	34	53	9
20 °C	54	30	51	5
23 °C	25	18	35	1
2 weeks 25,5° + 23°	26	15	22	0
4 weeks 25,5° + 23°	12	4	26	0
	arranged according to after-treatment			
9 °C	59	42	54	10
13 °C	39	18	53	3
17 °C	28	14	27	0
20 °C	11	6	14	0

Physiological aspects of infiltration

The infiltration could be imagined as a loss of fluid from the vascular bundles, in other words as a kind of internal bleeding.

Anatomically, this assumption can be neither demonstrated nor refuted, because the distance between the individual vascular bundles is so small that even very restricted infiltration is seen in the neighbourhood of one or more bundles even when they are not a source of the infiltrate.

When cut and defoliated stems are placed in moisture-saturated air, most of them show extension of the already-present infiltration spots. Since no

water is transported by the vascular bundles of these stems, the infiltration must have originated and spread due to the exosmosis of fluid from the vacuoles. Such exosmosis could be caused by an increase in permeability. VAN SLOGTEREN (see PINKHOF 1929b) indeed succeeded in inducing infiltration by making the cells of the tulip stem abnormally permeable by treating them with narcotics. PINKHOF (1929a, b) also described experiments in which she was unable, using the plasmometric method of HÖFLER, to demonstrate accelerated endosmosis of glucose and sucrose in infiltrated cells. She found on the contrary an elevated exosmosis in sections of infiltrated tissue brought into water, and ascribed this elevation entirely to the abnormally high osmotic value of the diseased cells.

This problem seemed important enough to warrant further investigation, the more so because PINKHOF's measurements were carried out in a period in which the plants had already been diseased for some time. It was even more important to determine whether the permeability is already elevated at the first occurrence of the infiltration. For this investigation stem samples were taken for longitudinal sectioning from the site of the infiltration or, when infiltration was not yet present, from the site at which it could be expected to develop. The samples were then placed in a series of plasmolyzing solutions with increasing concentration, the solutions being made with glucose, urea, or a mixture of potassium and calcium nitrate. In both diseased and healthy cells, potassium nitrate caused 'cap' plasmolysis, and often even tonoplast plasmolysis. This could be prevented by the addition of calcium in a molar ratio of one part

Table 3

Osmotic value (in gmol/l) at incipient plasmolysis of the parenchyma cells before and after the onset of infiltration in tulip stems and the increase in this value after immersion in various plasmolytics.

Material was taken from the 4th internode unless otherwise indicated

Condition of the observed stem-part	osmotic value				increase in osmotic value by endosmosis in				
			glucose			urea		glucose	
	KNO_3 + $Ca(NO_3)_2$	urea	4th internode	2nd internode	KNO_3 + $Ca(NO_3)_2$ after 1 day	after 2 hrs.	after 4 hrs.	4th internode after 1 day	2nd internode after 1 day
before infiltration	0.36	0.64	0.51	0.51	0.025	0.08	0.19	0.000	−0.007
internal infiltration		0.68	0.58	0.49		0.15	0.31		
0—1 day external infiltration	0.36		0.55	0.49	0.045			−0.007	0.000
1—2 days external infiltration	0.38		0.63	0.50	0.060			−0.008	−0.020
2—3 days external infiltration			0.77	0.54				0.000	−0.010
3—4 days external infiltration	0.40				0.050				

calcium nitrate to 10.5 parts potassium nitrate. In these plasmolytics we determined the osmotic value at incipient plasmolysis and the increase in this value due to endosmosis after a period of time in the plasmolyzing solution.

The results shown in table 3 (columns 2—5) indicate that in the 4th internode there was little increase in the osmotic value at the onset of infiltration. The infiltration, therefore, cannot be ascribed to an increased osmotic value. In later stages of the disease the osmotic value shows a marked increase, which was clearly expressed when the non-permeating glucose was used as plasmolytic. In the 2nd internode of the same stems, which showed no symptoms of the disorder, the osmotic value was barely or not all higher.

The permeability (columns 6—10), to the contrary, showed an increase at the onset of infiltration. This was most marked when the rapidly permeating urea was used as plasmolytic. After a period of two or four hours in this solution, the osmotic value in tissue showing no infiltration increased by 0.08 and 0.19 mol/litre respectively. In newly infiltrated tissue this increase was 0.15 and 0.31 mol/litre respectively (columns 7 and 8). The rate of endosmosis in the nitrate mixture also indicates that the permeability of the already-infiltrated tissues was higher than that of the not yet infiltrated tissue (column 6). In the glucose solution there was no increase in the osmotic value, so no differences in permeability between the two kinds of tissue could be demonstrated (columns 9 and 10).

On the basis of these determinations, the infiltration can be ascribed to increased permeability. The cause of this increased permeability could be a disturbance of the balance of the various permeability-regulating ions.

UYLDERT (1934) investigated the influence of various mineral salts on the toppling process. She supplied these salts by applying solutions to the soil during forcing in the greenhouse and also by cutting the plants from the bulb before flowering and placing them in sucrose solutions with and without the addition of mineral salts. She found that the presence of the calcium ion in the medium reduced the occurrence of toppling. These experiments led us to make a further study of the influence of the mineral salts. In our experiments they were usually supplied in the form of potassium and calcium nitrate; in a few cases other cations and anions were used.

The salts were supplied at various stages of the development of the plants. In the first experimental series they were given at the time of planting of cooled (9 °C) and uncooled bulbs, the bulbs being placed on a solution of the salt under study. The curves in figure 2 show the percentage of toppling before, during, and after the opening of the flower. For both cooled and uncooled series, toppling was much more rapid on a solution of the two potassium salts and distilled water than on the two calcium salts and (calcium-containing) tap-water. The effect of calcium nitrate was particularly favourable.

In a second experimental series the plants were watered after transfer to the greenhouse with a solution of potassium nitrate or calcium nitrate. Figure 3 shows that the administration of potassium nitrate accelerated the occurrence of toppling. Watering with calcium nitrate, to the contrary, gave a distinct retar-

dation. The favourable effect of watering with $Ca(NO_3)_2$ can also be seen in table 2 for the cultivar 'Le Nôtre' (see also fig. 1 for cultivar 'Korneforos').

In a third series, newly flowered plants were cut and placed in various salt solutions. Potassium, sodium, and ammonium nitrate had little influence on the number of toppled plants (table 4). They did promote infiltration, however, since the infiltrated areas of the stem were larger than those on plants placed in distilled water. The favourable effect of calcium nitrate is again very distinct. This also holds for manganese nitrate. On 0.075, 0.050, 0.025, and 0.0125 M solutions of these two salts, no toppling occurred in any of the plants (set 1), although the appearance of symptoms of intoxication makes it difficult to draw

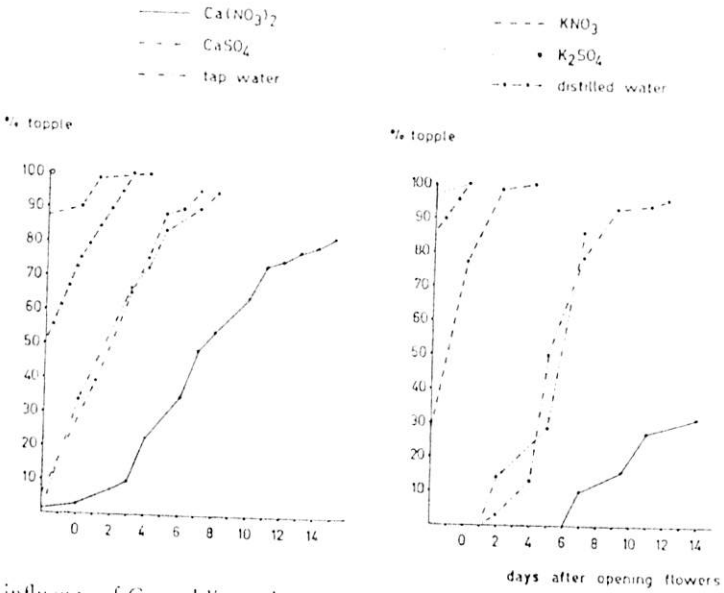

Fig. 2. The influence of Ca and K on the occurrence of toppling in tulips (cultivar 'Le Nôtre') cultured on water (0.013 gramion/l). Controls on distilled water and tap-water. Left: in bulbs cooled at 9 °C. Right: in uncooled bulbs. Greenhouse temperature: 21 °C

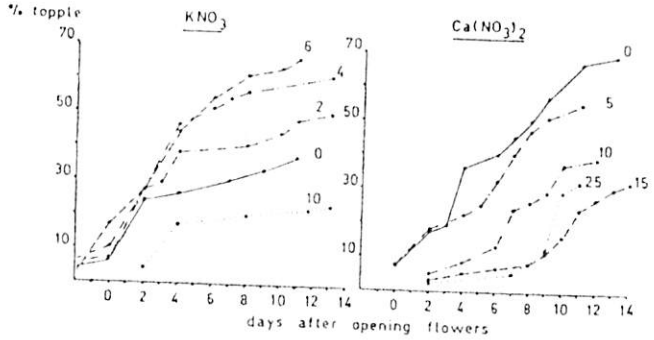

Fig. 3. The influence of Ca and K on the occurrence of toppling in tulips (cultivars 'Murillo' and 'Le Nôtre'). The plants in flats were watered regularly with solutions of KNO_3 (0, 2, 4, 6 and 10 g/l) and $Ca(NO_3)_2$ (0, 5, 10, 15 and 25 g/l). Greenhouse temperature: 21 °C

Table 4

The influence of several monovalent and divalent cations on the occurrence of toppling in tulips (cultivar 'Peach Blossom').
Cut flowers were placed for 24 hours in a series of solutions with decreasing molarity (10 flowers per treatment)

	set 1. Number of toppled flowers (n = 10) 4 days after cutting					
	0.075 M.	0.050 M.	0.025 M.	0.0125 M.	0.0063 M.	dest. water
$NaNO_3$	1*	2*	5	6	5	6
KNO_3	2*	1*	3	7	4	2
NH_4NO_3	2*	3*	4	6	4	4
$Ca(NO_3)_2$	0*	0*	0*	0	3	7
$Mn(NO_3)_2$	0*	0*	0*	0*	1	5
	set 2. Number of toppled flowers (n = 10) 5 days after cutting					
	0.0125 M.	0.0063 M.	0.0031 M.	0.0016 M.	dest. water	
$NaNO_3$	4	4	4	7	3	
KNO_3	3	3	4	6	5	
NH_4NO_3	4	3	5	3	6	
$Ca(NO_3)_2$	1	2	5	4	5	
$Mn(NO_3)_2$	0	1	3	5	6	

* Symptoms of intoxication on leaf tops.

reliable conclusions with respect to this series of concentrations. At still lower concentrations (set 2) the favourable effect is lower, the lower the concentration.

It may be concluded from the results of this study that of all the ions used, only the calcium and manganese ions can prevent toppling. Since the quantity of manganese present in the plant is negligible, the question arises of whether the toppling is caused by too low a calcium content in the plant, particularly in that part of the plant in which the disorder occurs. If this is the case, the calcium content would be lower the more favourable the conditions under which the plant develops are for the occurrence of the disorder. One of these conditions is temperature. By means of the oxalate precipitation method (LOOMIS and SHULL 1937) the calcium content of the stem was determined in plants which had come into flower slowly at relatively low field temperatures and in plants forced in the greenhouse at temperatures of 15 ° and 27 °C. The 1st, 2nd, 3rd, and 4th internodes were investigated separately just after the plants had flowered.

Table 5 gives the calcium content expressed as a percentage of the dry weight and of the total ash weight. Both conversions give essentially the same result. The calcium content of the lowermost internode is the same for all temperatures. This is probably due to the fact that at the time at which the plants were transferred to the greenhouse this internode was completely or almost completely elongated. In the higher internodes the calcium content of the field plants shows little or no decrease; the plants cultivated in the greenhouse show a mark-

Table 5

The calcium content of stem internodes of tulips grown at various temperatures (cultivar 'Mr. van der Hoef'), expressed as percentages of the dry weight and the ash weight

part of the stem	as % of dry weight			as % ash weight		
	80 °F greenhouse	60 °F greenhouse	field	80 °F greenhouse	60 °F greenhouse	field
internodium 1 (lowest one)	0.27	0.32	0.29	4.3	4.0	3.7
internodium 2 and 3	0.12	0.32	0.45	1.9	4.4	5.2
internodium 4 (upper one)	0.06	0.13	0.23	1.1	2.8	4.0

ed decrease; especially at 27 °C. At this temperature the calcium content of the 4th internode is only a quarter of that of the field plants. It is just this 4th internode in which more than half of the plants showed symptoms of toppling at 27 °C at a later stage of development.

Discussion

The favourable effect of divalent cations such as Ca^{++} and the low content of this ion in the part of the stem in which infiltrations may occur justify the assumption that the occurrence of toppling is caused by or is partially due to a local calcium deficiency. To explain this deficiency we have evidence (see table 5) that the Ca ions are more slowly taken up, mobilized, or transported than the other mineral components. When the stem elongates rapidly, the calcium is not supplied with sufficient rapidity in the zone of the most rapid growth. The relative deficiency will increase upwards in the stem until it becomes so great in this particular zone that the cells can no longer develop normally and become diseased. This hypothesis provides a simple explanation of the following observations:

1. The disorder occurs higher in the stem the later the plants are transferred to the greenhouse. The longer the plants remain in the field at low temperatures, the greater the part of the slowly elongating stem with an adequate calcium content and the higher the region in which a later rapid elongation will lead to a calcium deficiency.

2. The disorder appears lower in the stem the higher the temperature in the greenhouse. At higher temperatures the elongation of the stem is accelerated and the calcium content shows more lag during growth than at lower temperatures.

3. The promotion of toppling by the administration of glucose to cut stems (PINKHOF 1929b, our observations). The taking up of glucose leads to a more rapid elongation of the stem.
4. The infiltration begins in the zone where elongation is the most rapid. The sharp increase in volume would cause the calcium content to decrease in this zone, in which the chance would then be greatest that the calcium content would drop below the required minimum.
5. In rare cases toppling is observed in field plants grown on calcium-poor soil. In this situation a relative calcium deficiency also develops in the stem, not, however, because elongation is too rapid but because too little is taken up.

It is difficult to visualize how the calcium deficiency would cause the symptoms of toppling. The first observable symptom is infiltration. This points to an increase in permeability, which has indeed been demonstrated; such an increase might be caused by a deficiency of calcium, since calcium has the effect of decreasing permeability. The reduced viscosity already present at the onset of infiltration and the signs of disintegration visible in the cytoplasm and nucleus at a later stage both point to radical structural changes in the contents of the cell. Of interest in this connection are the experiments of MARINOS (1962) who studied the apical stem meristem of barley plants with a calcium deficiency and demonstrated electron-microscopically that in the cells of the meristem not only the cytoplasm showed disintegration but also the plasmalemma and the membranes of the nucleus and plastids. It is conceivable that a similar degeneration also occurs in the stem of the tulip, which develops a calcium deficiency during the period of maximum elongation. It is highly probable that this disintegration greatly increases permeability, which causes the contents of the vacuoles to flow outward and at a later stage leads to the death of the cell.

This loss of the cellular fluid causes a loss of turgor, leading to shrinking and local weakening of the stem, which then topples.

Evaporation can also cause an increase in the concentration of the infiltrate, which would explain the high concentrations of sugar found in this fluid (see also PINKHOF 1929b). Other experiments, not reported here, have indicated that the nitrogen compounds and the ash components (with the exception of calcium) tend to accumulate in the diseased portion of the stem. Since the dry weight per centimeter of stem is higher in diseased than in healthy stems, this accumulation must be caused not only by evaporation of the escaped cellular fluid but also by the concentration of substances transported from another source (accumulation from the leaves and taking-up from the bulb and soil).

Summary

The toppling of tulips is a physiological disease which manifests itself as a local infiltration of the intercellular spaces, soon followed by a degeneration of the cell contents and shrinkage of the stem. Which part of the stem becomes

infiltrated depends upon the storage conditions and temperature during development. These factors also influence the susceptibility of the plants. The more these conditions favour a rapid development of the plant the higher the percentage of diseased plants. This is in agreement with the fact that the infiltration site is closely related to the zone of optimal elongation.

At the onset of the infiltration there is little increase in osmotic value but a marked increase in permeability. The infiltration can be ascribed to increased permeability and the latter could be caused by a disturbance of the balance of the various permeability-regulating ions.

Application of various salt solutions at various stages of development of the plants shows, that potassium, sodium, and ammonium promote infiltration and calcium and manganese inhibit this process.

The calcium content in the toppling internodes shows a marked decrease in comparison with healthy ones.

The assumption, that the occurrence of toppling is caused by or is partially due to a local calcium deficiency is discussed.

Zusammenfassung

Die Umfallkrankheit der Tulpen ist eine physiologische Erkrankung; sie beginnt als lokale Infiltration von Interzellularräumen im Stengel, gefolgt von Zelldegeneration und Schrumpfung des Stengels. Krankheitsanfälligkeit und Ort des Krankheitsausbruchs im Stengel hängen von den Lagerungsbedingungen und von der Temperatur während des Wachstums ab. Unter Umweltbedingungen, welche die rasche Entwicklung der Pflanzen begünstigen, ist die Zahl kranker Pflanzen am höchsten; die Infiltrationen treten in der Zone stärksten Längenwachstums auf.

Zu Beginn der Infiltration ist eine geringe Erhöhung des osmotischen Wertes und eine starke Erhöhung der Permeabilität festzustellen. Die Infiltration ist die Folge der erhöhten Permeabilität, die ihrerseits auf eine Störung im Ionengleichgewicht zurückgeführt werden kann. Kalium, Natrium und Ammonium begünstigen die Infiltration, während Calcium und Mangan hemmend wirken. In den geschädigten Stengelteilen ist der Calciumgehalt vermindert. Die Hypothese eines lokalen Calciummangels als Krankheitsursache wird diskutiert.

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