Neth. J. Pl. Path. 74 (1968) 85–93

Penicillium corymbiferum entering bulbous iris through wounds

G. J. Saaltink

Flower Bulb Research Centre, Lisse

Accepted 6 November, 1967

Abstract

Two types of wounding of iris bulbs occur: damage by handling (transport etc.) and ruptures in the bulb tissue made by the first growth of root tips.
Contamination of bulbs with spores of *Penicillium corymbiferum* demonstrated that the fungus is able to enter bulbs only through fresh wounds. At 17 °C and high humidity the wounds became unsuitable for infection within 3 days. However, at a lower temperature and/or lower humidity the wounds remained suitable for entry of the fungus over a longer period.
It is suggested that attack at the base of the bulb, which until now has not been appreciated, is the resultant of (1) the occurrence of ruptures in the tissue there, correlated with the speed of initial root growth, (2) susceptibility of the wound tissue, related to temperature and relative humidity, and (3) growth rate of the fungus.

Introduction

It has been assumed by different workers that *Penicillium corymbiferum* Westling enters bulbous iris only after damage due to handling, or after an attack by *Fusarium oxysporum*. The fungus grows easily in damaged tissue and bulbs often sustain damage during cleaning, grading or transportation (Fig.1).Attacks on damaged tissue may start either during storage under humid conditions or after planting.
However, infection experiments have proved that *P. corymbiferum* is also able to infect planted bulbs without such initial damage (Saaltink, 1965). In these experiments, if the bulbs were dipped in a suspension of spores they became heavily attacked after planting. It was noted that after inoculation the attack usually started at the base of the bulb, in the region of the roots, where no wounds due to mechanical injury were visible (Fig. 2). Any attack on the bulb scale at other spots was always accompanied by visible damage. Following these observations, decay at the basal plate was also found in greenhouse crops. The attacked plants were often noticeable by their stunted growth and a bending of their leaves.
Observations on the rooting of bulbs made it clear that the parenchymatic tissue is ruptured by the root tips (Saaltink, 1966) (Fig. 3). The present paper describes the results of some experiments to show that such ruptures in the tissue are used by the fungus to enter the bulb.

Fig. 1. Damaged bulbs of an imported stock

Fig. 1. Beschadigde bollen uit een importpartij

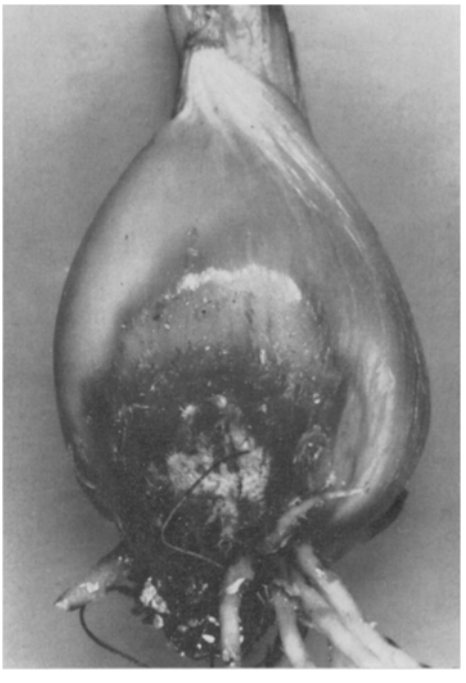
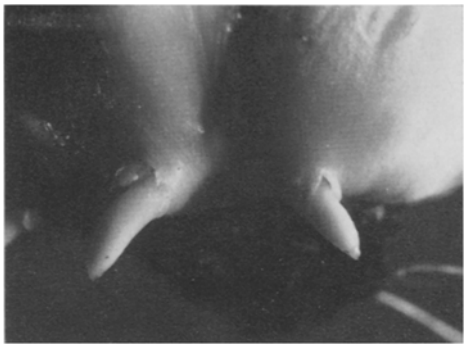


Fig. 2. A heavy attack of the base of the bulb due to the entrance of the fungus through fresh bursts

Fig. 2. Een heftige aantasting van de basis van de bol ten gevolge van het groeien van de schimmel door de nog verse barstjes bij de wortels

Fig. 3. Rupturing of the tissue during the growth of root tips

Fig. 3. Het barsten van bolweefsel door de eerste wortelgroei



Material and methods

In all experiments the iris cultivar 'Wedgwood' was used. The bulbs were stored at 30 °C and used all the year round. Before planting they were kept for 6 weeks at 17 °C, to get a normal development.
One isolate of *P. corymbiferum*, originating from bulbous iris, was used in the experiments. The identity of the fungus was determined at the "Centraalbureau voor Schimmelcultures" at Baarn. To prevent clustering of spores, suspensions were made in 0.02 % Tween 80 in water. The concentration of spores was 2×10^6 per ml.
Bulbs were damaged artificially by gently rubbing moistened carborundum powder over the bulb scale. They were planted in vermiculite in plastic pots. To obtain moist conditions, every pot, containing 75 g dry vermiculite and 5 bulbs, was weighed immediately after planting and then 200 ml water was added. To obtain dry conditions 80 ml water was added instead of 200 ml. During the experiment water loss by evaporation and transpiration was corrected by weighing the pots twice a week and adding water to bring them up to their original weight.
The bulbs were planted immediately after contamination with spores. The pots were kept at 17 °C unless otherwise stated. Planting in moist vermiculite gave the opportunity to make observations on the development of the infection during the experiment. The bulbs could be taken out of the vermiculite and replaced after the observations without damaging the roots. Observations were made 1 month after contamination.

Results

To study the importance of the bursts in the parenchymatic tissue around root tips as pathways for entrance by the fungus, the following experiments were carried out.
Uncontaminated bulbs were planted at 17 °C in moist vermiculite. After 1 day, drops of spore suspension were put into the first tissue bursts around the young root tips. The inoculated ruptures were marked. Of the treated wounds 50 % became infected. None of the roots were attacked.
This first experiment, which indicated that the fungus may indeed enter through these wounds, was followed by another in which the conditions favoured a different speed of root formation. If the initial growth of roots extends over a longer period, this means that rupturing of the parenchymatic tissue of the bulb scale also extends over a

longer period, with continued presentation of fresh wounds. One condition of influence was the moisture in the vermiculite, adjusted as explained above. With less water the development of roots was much slower. The initial root growth was also delayed by storing the pots with planted bulbs at 5° instead of 17 °C (Fig. 4). In these experiments the bulbs were contaminated with spores at 1, 5, 7, 9, 12 or 16 days after planting. Table 1 shows that at 17 °C combined with high moisture the bulbs became heavily infected only when they were contaminated with spores after 1 day. Contamination after 5 or 7 days gave less infection. A mild infection occurred when the bulbs were inoculated 9 or 12 days after planting. The results indicate that after a certain period wounds become unsuitable as infection paths for the fungus. When the roots develop more slowly, as at 5 °C, and new ruptures of the tissue occur over a longer period, heavy infections may occur even after 12 or 16 days (third column, Table 1). When the initial root growth is inhibited by drought (fourth column, Table 1) heavy infections resulted from contaminations 5 and 7 days after planting. The data in these two columns support the supposition that hyphae of *P. corymbiferum* are able to enter the bulbs only

Fig. 4. Root formation of iris bulbs at 17° and 5°C after 1, 5 and 9 days germination in humid vermiculite

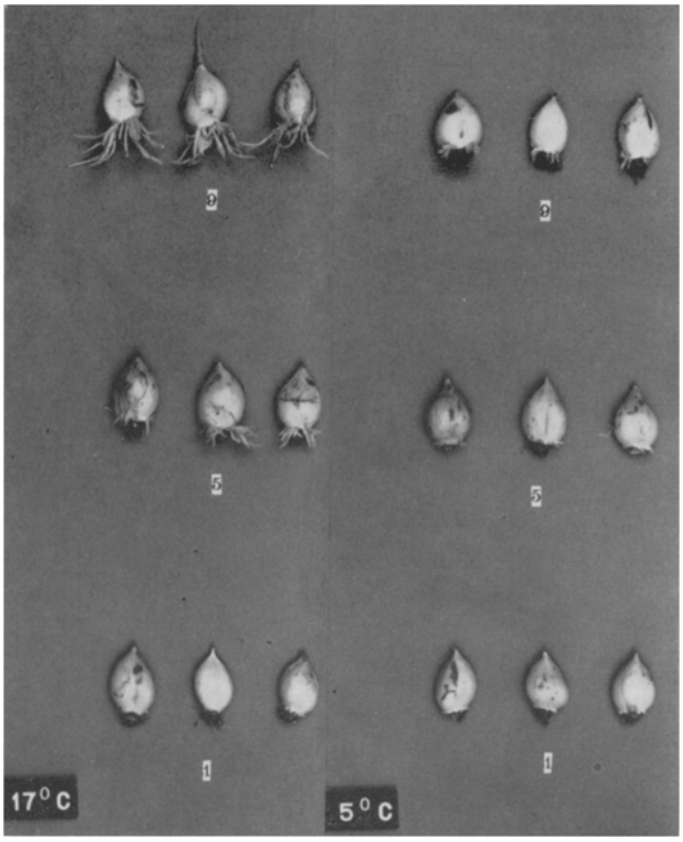


Fig. 4. De wortelvorming van irisbollen bij 17° en 5° C na 1, 5 en 9 dagen kieming in vochtige vermiculiet

Table 1. Influence of the speed of root formation (as influenced by temperature or humidity) on infection of iris bulbs by *P. corymbiferum*, when spores were placed at the basal area of the bulb at different periods after planting. The figures give the average disease intensity (0 = not attacked, 5 = completely decayed).

Number of days between planting and contamination	*Bulbs grown, before and after contamination at 17 °C, wet*	*Bulbs grown, before contamination at 5 °C; thereafter at 17 °C, wet*	*Bulbs grown, before and after contamination at 17 °C, dry*
1	4.8	4.9	5.0
5	2.4	4.9	3.9
7	2.2	–.–	4.0
9	1.2	5.0	1.4
12	1.5	3.6	1.1
16	–.–	3.3	–.–
not contaminated	0.5		

Tabel 1. De invloed van de snelheid van wortelvorming (beïnvloed door temperatuur of vochtigheid) op de mogelijkheid van binnendringen door P. corymbiferum als de sporen een bepaald aantal dagen na het planten op de basis van de bol worden gebracht. De gemiddelde ziektegraad is uitgedrukt in cijfers (0 = niet aangetast, 5 = totaal verrot).

through fresh wounds, which under the circumstances described may occur during a prolonged period.

In three further experiments under the conditions shown in column 2 of Table 1 similar results were obtained.

To gain more information concerning the unsuitability of older wounds for the entrance of the fungus, bulbs were damaged artificially by gently rubbing of the bulb scale with carborundum powder after partial removal of the brown tunics. It was ob-

Table 2. Influence on the extent of infection of iris bulbs of the period between artificial damage to the bulb scale and subsequent contamination with spores of *P. corymbiferum*. Two storage conditions. Temperature 17 °C.

Conditions after damaging and before contamination (in all cases vermiculite after contamination)	*Percentage of diseased bulbs*		
	exp. I	*exp. II*	*exp. III*
moist vermiculite			
1 day	30	84	100
2 days	0	21	4
3 days	–	0	0
4 days	–	0	–
storage room (70 % R.H.)			
6 days	–	100	96
12 days	–	100	96
contaminated immediately after damaging	100	84	100

Tabel 2. De invloed van het tijdsverloop tussen kunstmatige beschadiging en besmetting van de bolrok op de mate van aantasting. Twee bewaaromstandigheden. Temperatuur 17 °C.

served in three experiments that every bulb was easily attacked at the damaged area if it was dipped at once in spore suspension and planted in moist vermiculite (Table 2). On the other hand, if the wounded bulbs were first planted in moist vermiculite and then dipped in a spore suspension 2–3 days afterwards, they did not get attacked (Fig. 5). However, if the wounded bulbs were stored in a usual storage room at 17 °C and 70 % RH for 6 or 12 days, they became heavily attacked after contamination followed by planting. This indicates that not only the age of the wound is important, but that the conditions during storage also influence the susceptibility of the tissue towards *P. corymbiferum*.

The supposition that conditions in storage influence the period during which wounded tissue remains susceptible to infection was proved by storing damaged bulbs at different temperatures and the same relative humidity in climate chambers. The bulbs were damaged by means of carborundum powder and stored for varying numbers of days at 17 ° or 9 °C. After storage they were contaminated with spores and planted in moist vermiculite. The results, in Table 3, show that at a temperature of 17 °C, just 10 % of the wounds became infected after 2 days and none after 4 days. At 9 °C, on the other hand, the infection after 2 days was still 70 % and after 4 days 40 %. This means that the wounded tissue remained susceptible to infection by *P. corymbiferum* for a longer period at 9 ° than at 17 °C.

In another experiment the damaged bulbs were stored at 9 °C, but at different relative humidities. These bulbs, also, were contaminated with spores after storage and there-

Fig. 5. Artificially damaged bulbs which were contaminated immediately after wounding or after the wounded bulbs were kept during 1 or 2 days in humid vermiculite

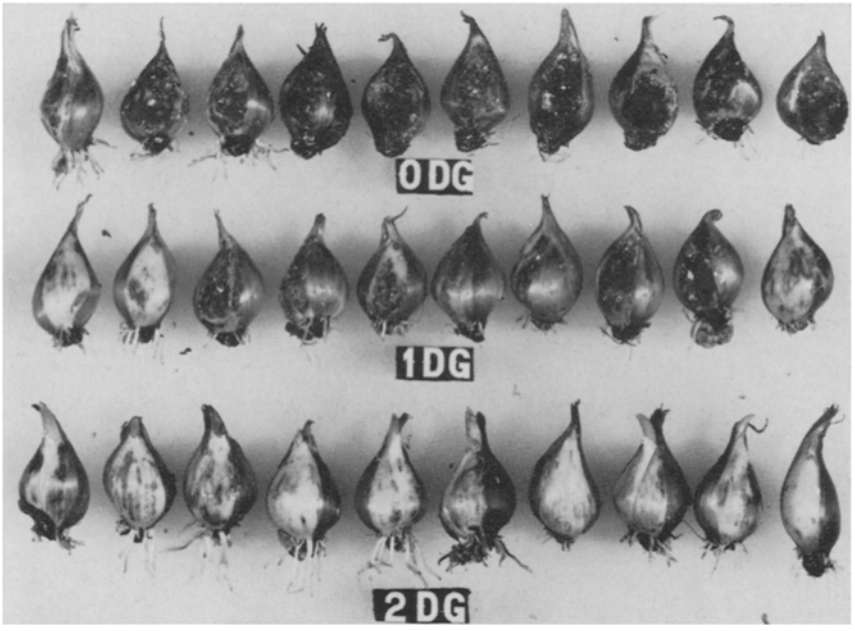


Fig. 5. Kunstmatig verwonde bollen die direct na de verwonding werden besmet of nadat de beschadigde bollen 1 of 2 dagen in vochtige vermiculiet werden bewaard

Table 3. Effect of storing artificially damaged bulbs at different temperatures on the rate of attack by *P. corymbiferum*. R.H. 95%. After storage, bulbs contaminated with spores and planted.

Number of days between damaging with carborundum powder and contamination	*Percentage of diseased bulbs*	
	at 17°C	*at 9°C*
1 day	30	95
2 days	10	70
4 days	0	40
7 days	0	10

Tabel 3. Het effect van een bewaring van kunstmatig beschadigde bollen bij verschillende temperaturen op de mate van aantasting door P. corymbiferum uitgedrukt in percentages zieke bollen. Rel. vochtigheid 95%. Na de bewaring besmet en geplant.

Table 4. Effect of relative humidity during the storage period on infection by *P. corymbiferum*, using artificially damaged bulbs. Temperature 9 °C. Storage period 4 days. After storage, bulbs contaminated with spores and planted.

Relative humidity (%)	*Percentage of diseased bulbs*
99	0
75	77
50	93

Tabel 4. Het effect van de relatieve vochtigheid tijdens de bewaring op de mate van aantasting door P. corymbiferum bij kunstmatig beschadigde bollen, uitgedrukt in percentages aangetaste bollen. Temperatuur 9°C. Bewaarperiode 4 dagen. Na de bewaring besmet en geplant.

after planted in vermiculite (Table 4). This experiment shows that only at the highest relative humidity the wounds became unsuitable for infection by *P. corymbiferum* within 4 days at 9 °C.

Discussion

It appears from these experiments that the fungus can easily enter iris bulbs through fresh wounds, either made artificially with carborundum powder or occurring naturally as a result of the rupturing of tissue by the developing root tips. This is shown in Table 1 and 2. The fungus seems to be unable to enter through unwounded tissue.
Conditions during storage, after artificial wounding, greatly influenced the extent of the subsequent infection. At 17 °C and high relative humidity the susceptibility of the tissue decreased more rapidly than at 9 °C. At a low relative humidity the tissue remained susceptible.
This could not be proved in the same way for natural wounds in the rooting area, since these may occur over a prolonged period. It may be accepted, however, that the influence of the factors just mentioned is also valid here, considering the relation

between the appearance of roots and the disease rating (Table 1), which indicates that only fresh wounds are infected. At a low temperature the fresh wounds occur over a more extended period than at a higher temperature, because the roots develop more slowly; moreover it can be concluded from the results with artificially damaged bulbs that at a lower temperature level the ruptures in the parenchymatic tissue at the basal plate of the bulb remain longer susceptible. It must not be forgotten that a certain temperature also influences the rate of growth of the fungus. At this stage it may be assumed that the extent of attack by *P. corymbiferum* at the base of bulbous iris is mainly the resultant of the speed of initial root growth, susceptibility of the wound tissue and growth rate of the fungus.
According to Gäumann (1946) *P. corymbiferum* must be called an obligate wound parasite, but he disliked this term because the fungus does not live in wounds especially. For this reason it might be better to call this type of pathogen a wound-depending parasite.
Observations about the attack of apple and citrus by *Penicillium* spp. agree in some respects with the results of our experiments with bulbous iris.
In infection of citrus fruits by *P. digitatum* and *P. italicum*, the entrance of the fungus also occurs through fresh wounds (Green, 1932; Miyakawa, 1962). Moreover it was considered that at high humidity the fruits become less susceptible (Hopkins and Loucks, 1948). The influence of temperature and humidity were not fully determined.
For apple it was shown that *Penicillium expansum* enters the fruit through lenticels and wounds (Baker and Heald, 1934).
A further study of temporal susceptibility of wounded tissue of bulbous iris, may give additional information on the host–parasite relation.

Acknowledgments

The author is indebted to Miss G. G. Stelling for assistance. He thanks Miss dra. A. C. Stolk, Centraalbureau voor Schimmelcultures at Baarn, for the identification of a number of *Penicillium* isolates.

Samenvatting

Wonden als invalspoort voor Penicillium corymbiferum bij bol-iris

Bij bloembollen komen twee typen verwondingen voor, nl. beschadigingen die ontstaan ten gevolge van het verwerken van de bollen (transport enz.) en barstjes in het bolweefsel die worden gemaakt door de naar buiten dringende worteltoppen. De snelheid van wortelvorming wordt beïnvloed door bollen te planten in vermiculiet van verschillende vochtigheid of temperatuur. Bij een snelle ontwikkeling van de jonge wortels is de aantasting minder sterk dan bij een trage ontwikkeling (Tabel 1). Deze resultaten rechtvaardigen de veronderstelling dat de schimmel uitsluitend door wonden kan binnendringen. Deze veronderstelling werd bevestigd door proeven waarin bollen kunstmatig werden beschadigd met carborundum. Alleen de jonge wonden waren geschikt voor de schimmel. Oude wonden werden in vochtige vermiculite onvatbaar, doch in de bewaarcel bleef de vatbaarheid bestaan (Tabel 2).
In Tabel 3 en 4 wordt getoond dat de omstandigheden tijdens de bewaring de tijds-

duur beïnvloeden gedurende welke het weefsel van de wonde vatbaar blijft.
Er kan worden aangenomen dat de aantasting van de basis van de bol, welke niet eerder werd onderscheiden van de aantasting van mechanisch beschadigde plekken, de resultante is van de snelheid van wortelgroei (die bepaalt hoe lang nieuwe wonden ontstaan), de vatbaarheid van het wondweefsel en de groeisnelheid van de schimmel.

References

Baker, K.F. and Heald, F.D., 1934. An investigation of factors affecting the incidence of lenticel infections of apples by Penicillium expansum. State College of Washington, Agric, Exp. Stn, Bull. 298.

Gäumann, E., 1946. Pflanzliche Infektionslehre. Birkhauser, Basel.

Green, F.M., 1932. The infection of oranges by Penicillium. J. Pomol. 10: 184–215.

Hopkins, E.F. and Loucks, K.W., 1948. A curing procedure for the reduction of mold decay in citrus fruits. Univ. of Florida, Agric. Exp. Stn Bull. 450.

Miyakawa, T., 1962. Studies on the Penicillium rot of satsuma orange fruits. Tokushima Hort. Exp. Stn, Special Rep. 1.

Saaltink, G.J., 1965. Penicillium corymbiferum als parasiet van bol-iris. Meded. LandbHogesch. Opzoekstns Gent 30: 1652–1659.

Saaltink, G.J., 1966. Penicillium corymbiferum als gelegenheidsparasiet van bol-iris. Neth. J. Pl. Path. 72: 207.