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Effects of temperature, relative humidity and age of conidia on the incidence of spotting on gerbera flowers caused by Botrytis cinerea

J. SALINAS[1], D.C.M. GLANDORF[1], F.D. PICAVET[1] and K. VERHOEFF[2]

[1] Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, the Netherlands
[2] Directorate for Agricultural Research, P.O. Box 59, 6700 AB Wageningen, the Netherlands

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Abstract

In recent years, spotting of ray florets of gerbera flowers has become an important problem. This type of small necrotic lesions may occur before, but especially shortly after harvesting the flowers. *Botrytis cinerea* was easily isolated from such lesions.

Inoculation with *B. cinerea* only gave typical necrotic lesions, when dry conidia were dusted on the flowers with a short period of high rh after inoculation. At 18-25 °C a high rh for at least 5 hours was necessary. Rotting of ray florets and receptacles by *B. cinerea* occurred when inoculated flowers were kept wet for a few days.

Spots consist of one to several necrotic, usually epidermal cells. A single conidium could give rise to a necrotic lesion after germination. Germination of conidia and lesion formation occurred between 4 and 25 °C; at 30 °C, germination and lesion formation did not occur. Between 18 and 25 °C, many lesions became visible within 1 day after inoculation; at 4 °C it took 2 to 3 days before lesions could be seen. If kept dry, conidia of *B. cinerea* remained ungerminated on ray florets of gerbera flowers and could be removed from the ray florets. Within 1 day at high rh, germination occurred and lesions were produced. Conidia of *B. cinerea*, stored dry, were able to survive much longer than the lifetime of a gerbera flower. Even after storage at room temperature for up to 14 months, some conidia were able to germinate in vitro and on ray florets and induce the formation of lesions. Addition of gerbera pollen diffusate stimulated germination and lesion formation.

Additional keywords: symptomatology, local lesions, viability of conidia.

Introduction

Species of the genus *Botrytis* are known as a group of fungi which cause destructive plant diseases on a wide variety of plants. Within the genus a number of species are specialized on certain plants, e.g. *B. tulipae* on tulip and *B. allii* on onion. *B. cinerea*, on the other hand, can cause diseases in many different plants. Infection takes place through wounds, via decaying or dead plant tissue or by direct penetration into the undamaged host (Verhoeff, 1980). Such infections may lead to rapidly spreading lesions, to a very limited necrosis, e.g. ghost spot on tomato fruits (Verhoeff, 1970), or to quiescent or latent infections, e.g. in flowers and fruits of strawberry (Powelson, 1960) and grape (McClellan and Hewitt, 1973).

With increasing frequency during recent years, small necrotic lesions have been observed on petals of several glasshouse-grown ornamental plant species, such as rose and gerbera. The lesions on ray florets of gerbera bear a strong resemblance to the spotting of chrysanthemum florets caused by *B. cinerea* (Taylor and Muskett, 1959). Although we are not aware of any scientific report on this disease on gerbera, it is commonly assumed that the symptoms are caused by *B. cinerea*; the disease is called 'smet' or 'pokken' in the Netherlands (Bakker, 1986; Verberkt, 1986) and 'picotte' in France (Marlot, 1984). Under certain conditions the small lesions may develop into spreading ones causing the whole flower to rot; these lesions may become covered with profusely sporulating *B. cinerea* (Verberkt, 1986).

In preliminary experiments, we could readily isolate *B. cinerea* from spotted gerbera flowers, induce the same symptoms after inoculation of flowers with this pathogen and re-isolate the fungus from spots on inoculated florets, thus confirming that *B. cinerea* is responsible for the spotting in gerbera flowers (Verhoeff and Salinas, unpublished).

In practice, lesions are sometimes found before the flowers are harvested, but more often lesions appear after harvest, especially during storage and transport. Considerable losses result from these infections as flowers bearing lesions are not accepted by the market. In 1986, approximately 250 ha of glasshouse area was used for the production of gerbera flowers in the Netherlands, which is about 7% of the total area used for the production of cut flowers. Therefore, the disease is an economically important one. In order to understand the aetiology of the disease, with the aim of developing effective control measures, research was started to investigate the principal factors that may be involved in the incidence of the disease. In this paper, results are given of experiments on symptomatology, the development of lesions under different external conditions and the viability of conidia in relation to the period of keeping gerbera flowers.

Materials and methods

Inoculum and flowers. All experiments were done with isolate Bc-12 of *B. cinerea* Pers.: Fr., obtained from an infected gerbera flower growing in a glasshouse. The fungus was kept as described before (Salinas and Schot, 1987).

For obtaining a steady inoculum, the following procedure was used. Conidia were transferred to PDA plates and incubated for 3 days at 21 °C in darkness. Pieces of agar bearing mycelium were then taken and transferred onto sterilized leaves of gerbera plants and incubated at 21 °C under continuous light; the fungus sporulated heavily under these conditions. Conidia were taken from these cultures and inoculated onto X-medium plates and the developing mycelium was subsequently transferred to other X-medium plates which were incubated for 8 to 10 days at 21 °C under continuous light. Conidia were collected dry by means of a suction pump and kept as they were sampled in flasks in darkness until used.

In experiments in which longevity of conidia played a role, the sampling date was counted as day zero.

Flowers of gerbera cv. Rebecca (*Gerbera jamesonii* obtained from P. Schreurs B.V., Amsterdam), which are susceptible to *B. cinerea*, were used in the experiments. Flowers were collected 6 to 14 days after opening, depending on the season. The peduncles were cut 10 to 15 cm below the flower receptacle and placed in flasks with sterilized water.

Inoculation. For inoculation, conidial suspensions or dry conidia were used. In the former case, conidia were suspended in ultrapure (UP) water, in a solution of 0.11 M glucose, or 0.11 M glucose + 0.067 M K_2HPO_4/NaH_2PO_4 buffer (pH 5.0) in UP water, or in gerbera pollen diffusate. Pollen diffusate was prepared according to the method of Chu Chou and Preece (1968). Pollen grains were collected from gerbera flowers and were kept at room temperature for 16 hours at a concentration of 10^5 ml^{-1} in UP water. After centrifugation (10 min, 2000 *g*) the supernatant was collected and sterilized for 20 min at 121 °C.

Inoculations were done by placing three droplets of 5 μl each of a conidial suspension on each ray floret of a gerbera flower or by spraying a conidial suspension (10^4 or 10^5 conidia ml^{-1}) on the flower. When dry conidia were used, inoculations were done in an inoculation box (Fig. 1). The conidia were blown from the flasks into the inoculation box by means of air, coming from a pipetting balloon, through a tube with a diameter of 3 mm. At the end of the tube a fine mesh (0.1 mm) metal sieve was fixed in order to improve conidial dispersion. Each time after pressing the balloon, a cloud of conidia entered the inoculation box and descended slowly onto the flowers. After about 20 min, most of the conidia had settled on the flowers to be inoculated. Dispersion of the conidia as well as the number that had landed on the petals was determined by direct light microscopy of inoculated ray florets or by counting the number of conidia per cm^2 landed on glass slides placed between and on the flowers at inoculation time. By using 1 mg dry conidia, 1.1 (± 0.2) × 10^3 conidia per cm^2 were found on the ray floret surface. This was used as the standard inoculum.

The inoculated flowers were kept in an incubation chamber either at 50-70% (low) rh or at 100% (high) rh at different temperatures.

Lesions were counted using a dissecting microscope fitted with a 6.25 mm^2 ocular raster. For each ray floret, the numbers of lesions in three raster fields were determined.

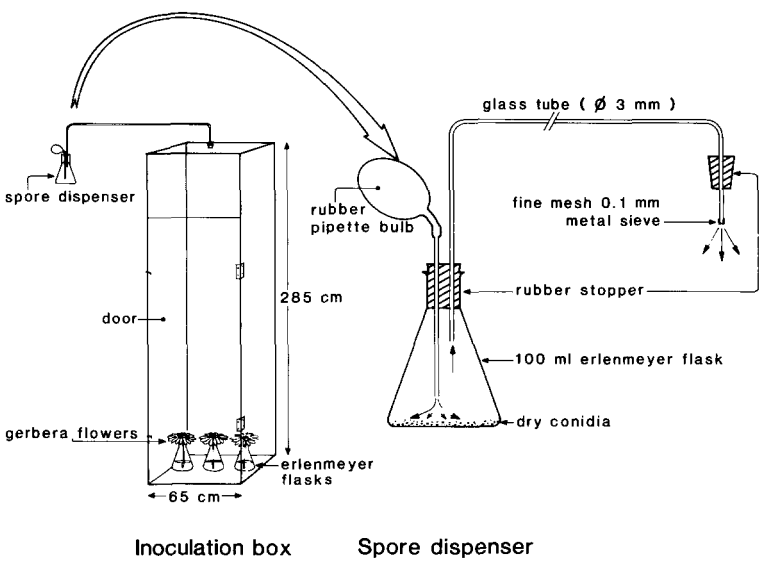

Fig. 1. Schematic representation of the inoculation box, used in the experiments.

Conidial germination. Germination in vitro was studied using conidial suspensions in distilled water, in gerbera pollen diffusate, in ray floret exudate (see below) or in a solution of 0.11 M glucose + 0.067 M K_2HPO_4/NaH_2PO_4 buffer (pH 5.0). For each treatment, three droplets of 40 μl, each containing 10^4 or 10^5 conidia ml^{-1}, were incubated at 21 °C for 20 hours in darkness.

Exudate of gerbera ray florets was prepared by ultrasonication of 20 g gerbera ray florets in 200 ml UP water. After filtration and centrifugation (10 min, 2000 *g*), the supernatant was sterilized for 20 min at 121 °C.

Light microscopy. For light-microscopical studies of the fungus on ray florets, three methods were used. Ray florets were fixed, cleared and stained with lactophenol-aniline blue according to the method of Erb at al. (1973) and mounted on microscope slides. By this procedure it was possible to distinguish the well stained fungal structures on the outer epidermal surface from those inside host cells, which were not stained. In this way, germination, penetration and fungal growth in the host could be followed. The procedure was not suitable for determining percentages of conidial germination in vivo, as conidia could be washed away from the ray floret surface during the various steps of this procedure.

For counting geminated and ungerminated conidia, two other methods were used. Whole ray florets were coated with a thin layer of 25% polystyrene in toluene (w/v) (Lingappa and Lockwood, 1963). After about one to two hours, the toluene had evaporated and the remaining polystyrene film was stripped away, stained in basic fuchsin (2%, w/v) in water for 1 hour, washed in water and mounted in 50% glycerol for microscopic examination. Germination of conidia could now easily be determined. With the second method, ray florets were incubated in lactophenol with 0.01% aniline blue for 1 hour and mounted in 50% glycerol.

Attachment of conidia of B. cinerea to ray florets. For assessing the attachment of ungerminated conidia to the ray florets after a period of low rh the following procedures were used. Inoculated flowers were immersed in water with 0.1% Tween 80, shaken vigorously for 3 min and washed in water, or inoculated flowers were shaken for 30 sec in 70% ethanol and subsequently rinsed with UP water. After both treatments the ray florets were observed microscopically to count conidia remaining on them. The flowers were incubated at high rh to check for possible lesion development.

All experiments were repeated at least four times, unless mentioned otherwise.

Results

Symptoms on gerbera flowers in practice. The symptoms on gerbera flowers obtained from various growers were examined visually. Most commonly, small, light to dark-brown lesions ('spotting') were found on ray florets of gerbera flowers (Fig. 2A). Sometimes, however, whole ray florets or a large part of them were water-soaked, light or dark-brown and partly rotten (Fig. 2B). The receptacle could also show infection by *B. cinerea*, the fungus being visible as sporulating mycelium on its surface. The symptoms were found before and after flower harvest. Local lesions were most frequently observed one or more days after flower harvest. Infection of the receptacles was usually seen when flowers had been wet for some time. Rotting of ray florets occurred close

Fig. 2. Small, necrotic lesions (spotting) (A) on ray florets and brown partly rotten ray florets of a gerbera flower (B).

to the receptacle or when local lesions were close to each other.

Microscopically, necrotic lesions consisted of one to eight necrotic or dark-brown epidermal cells. With larger lesions, brown mesophyll cells were also found. Usually one, sometimes more, germinated condidia of *B. cinerea* were found at the site of necrotic cells. In water-soaked tissue, hyphae of *B. cinerea* could be seen.

Comparison of inoculation methods. The results obtained by using different methods of inoculation are shown in Table 1. It appeared that only inoculation with dry conidia led to the development of the typical necrotic lesions, as found in practice and described above (Fig. 2A). After inoculation the rh had to be kept at 100%. When inoculated flowers were kept at low rh (50-70%) no symptoms appeared, not even after three weeks of incubation.

The number of lesions appearing at high rh varied with the density of the inoculum. Using a high density of conidia, the necrotic spots were so numerous, that whole ray florets collapsed within 20 hours of incubation. Inoculation with conidial suspensions led to the appearance of different types of symptoms: smaller and larger necrotic lesions, partial rotting of ray florets or of the whole flower, or even no symptoms at all. When nutrients had been added, rotting was the most common symptom.

On non-inoculated flowers and on flowers treated with water or nutrients but without conidia, necrotic lesions developed on rare occasions after incubation for some time at 100% rh, if flowers were used that had been grown especially for these experiments. On flowers obtained from growers, numerous lesions sometimes developed after incubation at 100% rh.

As inoculation with dry conidia, followed by a period of 100% rh, led to the appearance of the same symptoms as found in practice, all routine inoculations were subsequently done by this method.

At room temperature, the first necrotic lesions were seen after 7 hours of incubation at 100% rh (Table 2). However, germination started after 4 hours under these conditions. Furthermore, after 5 hours of incubation at 100% rh, followed by 24 hours at low rh, necrotic lesions also developed.

The many microscopic observations revealed that one conidium could cause a lesion.

Table 1. Comparison of methods of inoculation with *B. cinerea* and the development of the typical small necrotic lesions, after incubation for 7 days at high or low rh.

Method of inoculation	Symptoms	
	100% rh	50-70% rh
application of dry conidia	typical necrotic lesions[1]	no symptoms
application of dry conidia followed by spraying with some UP water	typical necrotic lesions[1]	no symptoms
spraying with UP water followed by the application of dry conidia	typical necrotic lesions[1]	no symptoms
spraying conidial suspension in water	A[2]	no symptoms
application of droplets of conidial suspension in water	A	A
application of droplets of conidial suspension in water supplemented with various nutrients	B[2]	C[2]
spraying with UP water, or application of UP water droplets with or without various nutrients (controls)	no symptoms	no symptoms

[1] Already present after incubation for 1 day.
[2] A) different types of symptoms (as described in the text), depending on inoculum concentration; B) rotting of the whole ray floret; C) different types of symptoms (as described in the text) and necrotic circles at the border of the droplets.

On all lesions one or more germinated conidia were always present, but not all germinated conidia were associated with a necrotic lesion. Germ tubes of conidia were mostly very short; less than 1% of the germ tubes were longer than 20 μm.

Microscopically, lesions appeared within 7 hours of incubation at high rh; usually, one or two epidermal cells were necrotic. Prolongation of the incubation period at high rh led to larger lesions, with six to eight epidermal cells being necrotic. Also some mesophyll cells showed a dark discolouration.

Effect of temperature on conidial germination and lesion formation. The effect of the incubation temperature on germination of conidia and on lesion formation was also studied. Inoculated flowers were kept in incubation boxes at different temperatures and every day two ray florets of each of four different flowers were taken to follow germination and lesion formation. The results are given in Table 3. The highest percentage of germination and the highest numbers of lesions were found when flowers were incubated at room temperature, i.e. between 18 and 25 °C. At 30 °C conidia did not germinate and no lesions were formed even after 7 days of incubation. At 4 °C, germination and lesion formation took place between the second and the third day of incubation, while at the other temperatures tested, one day was sufficient for both phenomena. Two to three days were necessary to obtain maximum germination percentages and maximum lesion formation at 15, 20 and 25 °C and room temperature; at 4 °C four days of incubation were necessary.

Table 2. Germination of conidia and appearance of lesions on gerbera flowers after inoculation with dry conidia of *B. cinerea* and incubation at room temperature for different periods at high rh[1].

Incubation period (h) at 100% rh	Germination of conidia (%)[3]	Necrotic lesions cm^{-2}	
		incubation at 100% rh only[4]	incubation at 100% and 50-70% rh[2,4]
1	0	0	0
3	0	0	0
4	1.8 ± 0.6	0	0
5	3.5 ± 0.1	0	12 ± 2.1
6	6.5 ± 0.9	0	50 ± 10.5
7	8.2 ± 1.0	43 ± 20.1	ND[5]
8	8.7 ± 2.5	57 ± 10.0	ND
10	11.8 ± 1.5	59 ± 8.7	ND
12	17.9 ± 0.5	95 ± 12.7	ND
24	30.8 ± 1.5	106 ± 20.6	120 ± 20.5

[1] Results from one experiment; the experiment was repeated six times.
[2] Incubation at 100% rh for the specified period was followed by incubation at 50-70% rh for 24 h.
[3] Each point is the average of the values obtained from 12 ray florets (30 conidia per ray floret) from four different flowers.
[4] Each point is the average of the values obtained from 12 ray florets from four different flowers.
[5] ND: not determined.

Longevity of conidia of B. cinerea. The effects of storage at different temperatures on longevity of conidia of *B. cinerea* were studied. Gerbera flowers were inoculated with conidia that had been stored dry at three different temperatures, and were kept at 100% rh for 20 hours. Conidia stored for up to 8 weeks were able to germinate and induce the formation of lesions on ray florets (Fig. 3). Conidia stored at 4 °C showed highest germination percentages following the first 4 weeks of storage, which was correlated with the highest number of lesions. On the other hand, conidia stored at 25 °C no longer germinated and no lesions were produced after three weeks of storage.

Longevity of conidia kept as dry spores at 4 and 25 °C was also tested in vitro, using water or water with different nutrients as a germination medium. The results from germination tests in water (Table 4) indicate that longevity was retained better at 4 °C than at 25 °C. However, when nutrients were added to the germination medium hardly any differences could be found between the two storage temperatures; even after 8 weeks of storage, more than 50% of the conidia were still able to germinate within 20 hours at 20 °C. Germination seemed to be better at the lower concentration tested. As conidia did not germinate in water and germinated very poorly on gerbera flowers after 8 weeks of storage, an experiment was done to test wether such conidia were no longer pathogenic

Table 3. Effect of incubation temperature on germination of conidia (G, in %) and on lesion formation (L, in numbers per cm^2) on gerbera ray florets, inoculated with dry conidia of *B. cinerea* and kept at 100% rh[1].

Incubation period (days)	Incubation temperature											
	room temp.		4 °C		15 °C		20 °C		25 °C		30 °C	
	G	L	G	L	G	L	G	L	G	L	G	L
1	34 ± 8[2]	99 ± 15[3]	0	0	4 ± 1	2 ± 1	18 ± 2	53 ± 20	30 ± 8	100 ± 25	0	0
2	55 ± 10	198 ± 12	1 ± 0.5	3 ± 1	29 ± 12	99 ± 12	37 ± 10	120 ± 16	34 ± 15	105 ± 15	0	0
3	54 ± 14	199 ± 21	8 ± 1	29 ± 6	43 ± 12	116 ± 20	44 ± 18	140 ± 40	41 ± 10	111 ± 24	0	0
4	52 ± 8	202 ± 30	43 ± 10	165 ± 19	41 ± 6	107 ± 19	41 ± 12	147 ± 25	36 ± 12	118 ± 12	0	0
7	53 ± 6	180 ± 18	46 ± 15	–[4]	41 ± 8	108 ± 18	45 ± 8	130 ± 20	40 ± 6	117 ± 18	0	0

[1] Results of one experiment; the experiment was repeated at least four times.
[2] Each figure is the average of the values obtained from 8 ray florets (40 conidia per ray floret) from four different flowers.
[3] Each figure is the average of the values obtained from 8 ray florets from 4 different flowers.
[4] The ray florets were almost completely necrotic.

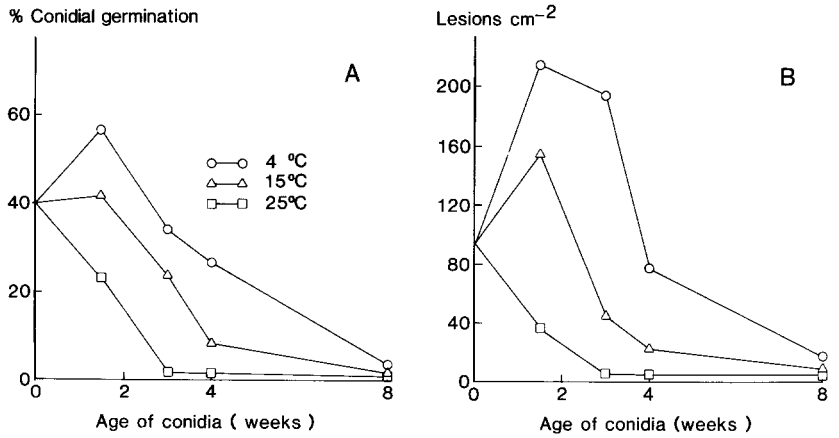

Fig. 3. Survival of conidia, stored at 4, 15 and 25 °C at low rh. Longevity was tested by inoculating gerbera flowers with the stored conidia and determining germination of conidia (A) and number of lesions formed (B) on ray florets after 20 hours at high rh. Each point is the average of the values obtained from 12 ray florets from four different flowers; the experiment was repeated four times.

Table 4. Effect of storing dry conidia of *B. cinerea* at two temperatures for 8 weeks on germination (%) in vitro (after incubation for 20 h at 20 °C) in different media at two conidial concentrations (viz. 10^4 and 10^5 ml^{-1})[1].

Storage temp. (°C)	Storage time (weeks)	Percentage germination in							
		ultrapure water		glucose/ phosphate[2]		ray floret exudate		pollen diffusate	
		10^4	10^5	10^4	10^5	10^4	10^5	10^4	10^5
4	0	0[3]	1	94	29	97	30	100	99
	1	33	2	94	70	100	30	100	99
	2	30	15	99	75	82	24	100	74
	3	19	20	99	79	74	39	99	69
	4	2	2	70	56	74	32	75	50
	8	1	1	70	50	60	20	60	20
25	0	1	2	96	43	96	35	99	99
	1	2	2	99	39	96	51	99	60
	2	1	1	99	91	70	20	66	67
	3	1	1	70	64	55	20	60	40
	4	0	0	74	36	70	21	64	35
	8	0	0	62	43	50	10	59	10

[1] Results from one experiment; the experiment was repeated twice.
[2] 0.11 M glucose + 0.067 M KH_2PO_4/Na_2HPO_4 buffer (pH 5.0).
[3] Each figure is the average of the values obtained from six 40-µl droplets of a conidial suspension; in each droplet 50 conidia were examined.

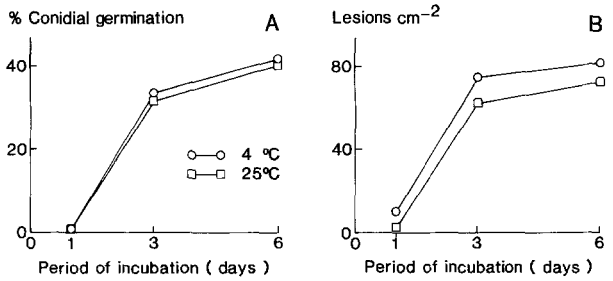

Fig. 4. Germination of conidia (A) and number of lesions cm^{-2} (B) on gerbera ray florets after 1, 3 and 6 days of incubation under high rh at 20 °C. Before inoculation the conidia had been kept dry at 4 or 25 °C for 10 weeks. Each point is the average of the values obtained from 12 ray florets from three different flowers.

to gerbera flowers or whether germination and penetration were only delayed. Conidia were stored for 10 weeks at 4 or 25 °C and then dusted onto gerbera flowers, incubation being at 100% rh. Again, conidial germination was very poor after 20 hours, but increased slowly after 3 and 6 days of incubation. Necrotic lesions also developed on the ray florets within this period (Fig. 4).

In Table 5, results are given of an experiment to determine the longevity of conidia lying on gerbera flowers at low rh. As the flowers started to deteriorate after 3 weeks, the experiment could not be extended beyond this period. Regardless of the period of storage at low rh, the conidia were equally able to germinate and induce lesion formation on the ray florets when placed under 100% rh for 20 hours. Samples taken from the flowers before placing them under high humidity conditions showed that under low rh no germination of conidia had taken place and no lesions had been formed. In vitro, conidia could survive at room temperature for a long period, as shown in Fig.

Table 5. Survival of conidia of *B. cinerea* dusted on gerbera flowers and kept at 50-70% rh for different periods and subsequently incubated at 100% rh for 20 hours[1].

Incubation period at low rh (days)	Germination of conidia (%)	Number of lesions cm^{-2}
0	30 ± 8[2]	96 ± 15[3]
4	32 ± 10	93 ± 18
7	25 ± 8	78 ± 12
11	19 ± 14	56 ± 20
14	20 ± 14	80 ± 24
18	25 ± 9	100 ± 15
21	29 ± 11	100 ± 14

[1] Results of one experiment; the experiment was repeated at least four times.
[2] Each figure is the average of the values obtained from 12 ray florets (30 conidia per ray floret) from four different flowers.
[3] Each figure is the average of the values obtained from 12 ray florets from four different flowers.

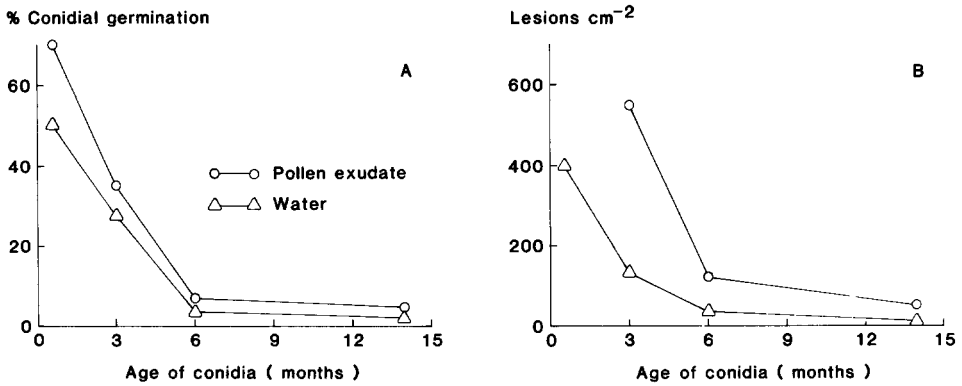

Fig. 5. Percentage germination of conidia (A) and number of lesions cm^{-2} (B) produced after 6 days of incubation at high rh at 20 °C. Before inoculation, conidia had been stored dry at room temperature for up to 14 months. Immediately after inoculation with 8 mg conidia, the inoculated flowers were sprayed with water or with pollen exudate. Each point is the average of the values obtained from 15 ray florets from five different flowers; the experiment was repeated twice.

5. After 6 months storage, about 3% germination occurred when conidia were dusted onto gerbera flowers and also approximately 40 lesions per cm^2 were formed after 6 days incubation. Addition of nutrients, by spraying the flowers with gerbera pollen diffusate immediately after inoculation, stimulated both phenomena.

Effect of nutrients on conidial germination and lesion formation. When testing the effect of different nutrients in vivo, it appeared that the percentage germination of conidia and the number of lesions were highest on inoculated flowers sprayed with gerbera pollen diffusate. Spraying inoculated flowers with ray floret exudate or with glucose gave no stimulation of the number of lesions compared with the water control (Table 6).

Table 6. Effect of spraying gerbera flowers with pollen diffusate, ray floret exudate or glucose immediately after inoculation with dry conidia of *B. cinerea*, on germination of conidia and development of lesions, after 20 hours of incubation at 100% rh[1].

Type of spray	Germination of conidia (%)	Number of lesions cm^{-2}
Distilled water (control)	49.4 ± 9.7[2]	182 ± 30[3]
Pollen diffusate	74.9 ± 10.2	287 ± 37
Ray floret exudate	50.6 ± 8.7	181 ± 48
0.11 M glucose	52.1 ± 8.0	167 ± 42

[1] Results from one experiment; the experiment was repeated at least four times.
[2] Each figure is the average of the values obtained from 9 ray florets (40 conidia per ray floret) from three different flowers.
[3] Each figure is the average of the values obtained from 9 ray from three different flowers.

Attachment of conidia to ray florets. Inoculated flowers kept for 1 to 14 days at low rh, were shaken in 0.1% Tween 80 for 3 min or in 70% ethanol for 30 sec. After placing them at high rh for 1 week no lesions appeared. Microscopic examination showed that no conidia were present on the shaken flowers.

Discussion

The results of the experiments, described in this paper, clearly indicate that the small necrotic lesions ('spotting') on ray florets of gerbera are caused by *B. cinerea*. Necrotic spots were formed when viable conidia of *B. cinerea* were able to germinate and, probably, germ tubes could penetrate into the ray florets. However, necrotic spots only appeared after inoculation with dry conidia under high humidity conditions, i.e. in the presence of a water film on the ray florets (Table 1). These conditions are similar to those described by Verhoeff (1970) for necrotic lesions on tomato fruits, caused by *B. cinerea*. One or more factors, necessary for early penetration from the germ tubes, may be present in too low concentrations when spore suspensions are used. Such factors could be the enzymes cutin esterase and cutinase. Cutin esterase is present in ungerminated conidia of *B. cinerea* and cutinase may be found during or after germination (Salinas et al., 1986). They demonstrated that under suitable conditions, *B. cinerea* produces one enzyme which is able to hydrolyze cutin. Research is in progress to discover whether cutin esterase causes some breakdown of the cuticle, upon which its products induce the production of cutinase.

As is shown in Table 2, a period of 5 hours of 100% rh was sufficient to induce necrosis at room temperature. Additionally at other temperatures between 4 and 25 °C, necrotic spots were formed under conditions of high humidity. The results indicate that when ungerminated conidia of *B. cinerea* are present on gerbera flowers, necrotic spots can be formed when the flowers, after harvest, are kept in storage under cool and usually high rh conditions. Although the period between the start of germination and appearance of the first lesions was very short (3 hours), it appears that within this period formation of lesions can be prevented by dry conditions (Table 2). Alderman et al. (1985) concluded that dryness of the germ tube may be critical for the fungus and hinder penetration, but it is also possible that after germination a dry period may have a negative effect on the production and/or activity of cutinolytic enzymes necessary for the penetration of the cuticle (Kolattukudy, 1985). Conidia of *B. cinerea* are usually regarded as short-lived propagules but there is plenty of information which suggests that they possess a considerable ability of survival (Blakeman, 1980; Coley-Smith, 1980). The results presented here confirm this suggestion, as some conidia older than one year were still alive and also were able to infect gerbera flowers (Fig. 5). When ungerminated conidia lay on gerbera flowers they also remained infective (Table 5). Dry conidia of *B. cinerea* kept at different temperatures showed two common phenomena. First, their viability decreased as they became older; this is largely in agreement with the findings of Coley-Smith (1980). Second, there was a delay in their germination and subsequently in their infection. Freshly harvested conidia stored for 10 weeks, showed a delay in germination and subsequently more time was needed before lesions appeared (Fig. 4). The presence of pollen diffusate stimulated germination, as was found also by Chu Chou and Preece (1968) and, probably indirectly, penetration (Table 6). Microscopical studies showed that one conidium was sufficient to induce lesion formation. Therefore, condi-

tions during the period gerbera flowers are open, and especially conditions during transport and handling of the flowers, seem to be key factors in the incidence of necrotic spots on the flowers.

There are no indications for latent infections by *B. cinerea* on gerbera flowers. Under low rh conditions, conidia lay on the ray florets ungerminated and could also be removed from these. As soon as the rh becomes high, i.e. when a water film is formed, germination may start, leading to the formation of lesions.

It is interesting to note, that at temperatures of approximately 21 to 25 °C, the lesions remained limited. However, the fungus was not dead, at least not always, as has been found in preliminary experiments. The factors involved in penetration and also in preventing the development of a spreading lesion, might give valuable information for a breeding programme for less susceptible gerbera cultivars.

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Samenvatting

Invloed van temperatuur, relatieve vochtigheid en ouderdom van de conidiën op het optreden van smet op gerberabloemen veroorzaakt door Botrytis cinerea

De laatste jaren komt het verschijnsel van het optreden van kleine necrotische plekken ('smet') op de lintbloemen van gerberabloemen steeds vaker voor. Deze necrosen zijn waarneembaar vóór, maar vooral kort na de oogst. Uit de necrotische plekjes kon de schimmel *Botrytis cinerea* gemakkelijk geïsoleerd worden.

Inoculatie van gerberabloemen met *B. cinerea* leidde alleen tot de vorming van dergelijke lesies als gebruik werd gemaakt van droge sporen van de schimmel en als na inoculatie de relatieve luchtvochtigheid een aantal uren hoog was. Tussen 18 en 25 °C diende deze periode minimaal 5 uren te zijn. *B. cinerea* kon de bloemen ook geheel doen verrotten, vooral als de geïnoculeerde bloemen een aantal dagen bij 4 °C nat gehouden werden, bij een hogere inoculumdichtheid of na toediening van voedingsstoffen bij inoculatie van de bloemen.

De necrotische plekjes bestonden veelal uit één tot enkele bruingekleurde epidermiscellen. Een dergelijke necrose kon reeds door één spore worden veroorzaakt.

Kieming van de sporen en lesievorming traden op tussen 4 °C (de laagste temperatuur die in de proeven is gebruikt) en 25 °C. Bij 30 °C vond geen kieming plaats en werden geen lesies gevormd. Tussen 18 en 25 °C werden veel lesies zichtbaar binnen 24 uur na inoculatie; bij 4 °C duurde dat 2 tot 3 dagen. Mits de sporen droog bleven, konden zij in ongekiemde toestand op bloemblaadjes blijven liggen en konden ook zij weer gemakkelijk daarvan verwijderd worden. Zodra de luchtvochtigheid hoog werd gedurende een aantal uren, trad kieming op en werden lesies gevormd.

Sporen van *B. cinerea* konden veel langer in leven blijven dan gerberabloemen. Droog bewaard bij kamertemperatuur, bleek 1 tot 2% van de sporen zelfs na 14 maanden nog in staat te kiemen, zowel in vitro als op bloemblaadjes van gerbera, en de vorming van

lesies te induceren. Toediening van diffusaat van stuifmeel van gerbera stimuleerde de kieming en het optreden van lesies.

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