Neth. J. Pl. Path. 77 (1971) 6–13

Germination of powdery mildew conidia in vitro on cellulose membranes

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Accepted 16 October 1970

Abstract

Conidia of several powdery mildews were found to germinate to a high and reproducible percentage on 25 μm thick cellulose membranes, laid on modified Czapek Dox agar in Petri dishes. Germination on 45 μm thick, uncoated cellulose, coated cellulose, cellulose acetate and collodion membranes always was lower and more irregular than on 25 μm thick cellulose. Probably, the water permeability of the membranes is of importance in causing a humidity gradient favourable for germination of powdery mildew conidia.

Introduction

Conidia of powdery mildew fungi germinate and form appressoria on the surfaces of dry glass slides, incubated in a water-saturated atmosphere in the dark. In this way Zaracovitis (1964) succeeded in obtaining germination percentages of at least 80% of most of the powdery mildew species studied. Many other investigators also reported germination on glass slides but most of them found only low, variable germination percentages (Clayton, 1942; Delp, 1954; Drandarevski, 1969; Hashioka, 1937; Hirata, 1967; Koopmans, 1959; Manners and Houssain, 1963; Yarwood, 1957). Also at our laboratory poor germination of powdery mildew conidia on glass slides was obtained, probably due to water condensation or dew formation around the spores. Therefore, germination of powdery mildew conidia on membranes laid on agar was investigated. Germination of powdery mildew conidia on membranes has earlier been reported by Dickinson (1949).

Materials and methods

The powdery mildew conidia used were obtained from *Erysiphe betae* on beet, from *E. graminis* ff. spp. *avenae*, *hordei* and *tritici* on, respectively, oat, barley and wheat, from *E. pisi* on peas and from *Sphaerotheca fuliginea* on cucumber. The infected plants were maintained under greenhouse conditions at 18–22 °C. During winter additional light was given from 4.00 p.m. until midnight. In order to obtain only young viable conidia, old conidia were removed by blowing them away every one or two days.

Unless otherwise stated, a modified Czapek Dox agar was used which contained 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 1.05 g $MgSO_4.7H_2O$, 0.02 g $FeSO_4.7H_20$,

30 g sucrose, 1 g Difco yeast extract and 10 g agar per liter distilled water. The pH of this medium was 6.4 (Williams et al., 1966).

The following cellulose membranes of N.V. Papierindustrie Van Straten en Boon at Den Dolder, the Netherlands, were used: PT 300 and PT 600 (uncoated cellophane, thickness 25 and 45 μm respectively), MSAT 300 and MSAT 400 (cellophane coated with nitrocellulose, thickness 27 and 32 μm respectively) and MXXT 300 and MXXT 400 (cellophane coated with polyvinylidene chloride, thickness 28 and 35 μm respectively). Cellulose acetate membranes were made by pipetting 1.0, 2.5, and 5.0 ml of a 1% solution in acetone-ethylacetate (9 :1) in Petri dishes (diam. 5 cm). Collodion membranes were made in the same way using a 10% collodion solution (BDH, in acetone) in ether-ethanol (1:1). The resulting thicknesses of the cellulose acetate membranes were 4, 10, and 20 μm and of the collodion membranes 3, 7, and 14 μm respectively.

Germination tests were performed by dusting conidia on the membranes laid on agar in Petri dishes (diam. 5 cm). The Petri dishes were incubated upside down in the dark at 20 °C or at room temperature. A piece of filter paper with glycerine was laid in the cover of the Petri dish, to prevent any water condensation on the membrane. Germination percentages of conidia of beet, cucumber and pea powdery mildew were determined after 44 hours, those of conidia of powdery mildews on cereals after 20 hours. All experiments were carried out in duplo. Unless otherwise stated, germination of 250 conidia was assessed and length of 20 germ tubes (appressoria) selected at random was measured. Tests on the significance of difference between estimates have been made graphically using binomial probability paper No. 31.298 of Codex Book Company, Inc. (Ferguson, 1960). Conidia were considered to be germinated when a germ tube of at least 5 μm length was present. No experiments were performed during December till March because of low germination of barley powdery mildew conidia in this time of the year.

Results

Germination on 25 μm thick cellulose membranes. Germination percentages of conidia of *E.graminis* ff. spp. *avenae*, *hordei* and *tritici* always were higher than 80%. Mostly, they varied between 88 and 95%. Sometimes even 98% of the conidia of these species germinated. Average germination percentages of conidia of *E.betae* and *E.pisi* were 85% and of *S.fuliginea* 75%. On germination conidia of *E.betae* and *E.pisi* produced short germ tubes, terminating in conspicious, lobed appressoria. Appressoria of various forms of *E.graminis* were club-shaped; after two days the appressoria often were laterally branched or elongated at the end, and the branches often were curved or sickle-shaped. Conidia of *S.fuliginea* produced forked germ tubes, but no appressoria. Germination of barley and cucumber powdery mildew conidia is shown in Fig.1. In Table 1 germination percentages of conidia of *E.graminis* f.sp. *hordei* and *S.fuliginea* at various densities of the conidia on the membrane are shown. There appeared to be no significant relation between the density and germination percentage of the conidia.

Effect of different agar media on germination. The effect of the composition of the agar on germination was studied by determining germination percentages and length of germ tubes of *E.graminis* f.sp. *hordei* and *S.fuliginea* on 25 μm thick cellulose mem-

Table 1. Germination of conidia of *E.graminis* f.sp. *hordei* and *S.fuliginea* in relation to density of conidia on 25 μm thick cellulose membranes.

E.graminis f.sp. *hordei*			*S.fuliginea*	
conidia/cm²	percentage of germination[1]	length of germ tubes (μm)	conidia/cm²	percentage of germination[1]
420	93	46	450	74
2080	89	45	1410	75
3470	88	44	3170	75
5280	89	44	4980	79
6940	87	46	6760	76

[1] No significant difference at the P = 0.05 level.

Tabel 1. Kieming van conidiën van E.graminis f.sp. hordei en S.fuliginea bij verschillende dichtheden van de conidiën op 25 μm dikke cellulosemembranen.

branes laid on water agar, potato-dextrose agar, cherry agar and modified Czapek Dox agar. The results of these experiments are given in Table 2. Germination percentages of both fungi were not significantly influenced by the type of agar medium used. On all media the characteristic club-shaped and forked germ tubes were observed. In the case of barley powdery mildew the agar medium did influence the growth of the germ tubes. On water agar growth of germ tubes stopped after one day, while growth on potato dextrose agar and modified Czapek Dox agar still

Fig. 1. Germination of conidia of *E.graminis* f.sp. *hordei* (left) and *S.fuliginea* (right) on 25 μm thick cellulose membranes, laid on modified Czapek Dox agar.

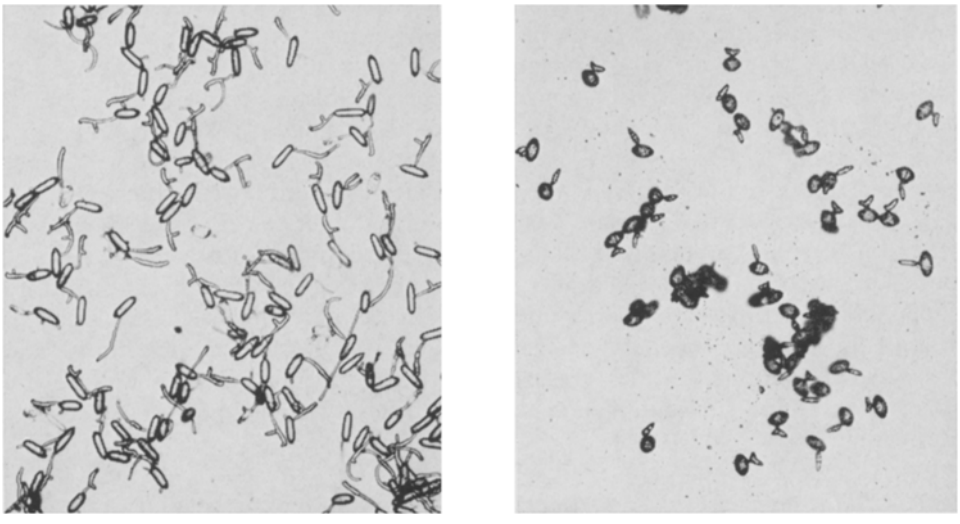

Fig. 1. Kieming van conidiën van E.graminis f.sp. hordei (links) en S.fuliginea (rechts) op 25 μm dikke cellulosemembranen, gelegd op gewijzigde Czapek Dox agar.

Table 2. Germination of conidia of *E.graminis* f.sp. *hordei* and *S.fuliginea* laid on 25 μm thick cellulose membranes on different agar media.

Medium	*E.graminis* f.sp. *hordei*			*S.fuliginea* (percentage of germination[1])
	percentage of germination[1]	length of germ tubes (μm)		
		after 1 day	after 2 days	
water agar	90	41	40	77
cherry agar	89	44	54	82
potato dextrose agar	87	57	65	78
modified Czapek Dox agar	89	51	66	83

[1] No significant difference at the P = 0.05 level.

Tabel 2. Kieming van conidiën van E.graminis f.sp. hordei en S.fuliginea op 25 μm dikke cellulosemembranen, gelegd op verschillende agar media.

continued by the formation of lateral or terminal branches on the appressoria. Growth of germ tubes on cherry agar (pH 6.0) also continued after one day, but to a lesser extent than on potato dextrose and modified Czapek Dox agar. Conidia did not germinate on cherry agar pH 4.0. Because of its growth-stimulating effect on appressoria of barley powdery mildew conidia and its known composition, modified Czapek Dox agar was used in all other experiments.

Effect of different membranes on germination. The influence of type and thicknes of membranes was studied, using cellulose, coated cellulose, cellulose acetate and

Table 3. Germination of conidia of *E.graminis* f.sp. *hordei* and *S.fuliginea* on different membranes laid on modified Czapek Dox agar.

Type of membrane	Coating	Thickness (μm)	*E.graminis* f.sp. *hordei*		*S.fuliginea* (percentage of germination)
			percentage of germination	length of germ tubes (μm)	
cellulose		25	94	56	68
cellulose		45	15	16	44
cellulose	nitrocellulose	27	63	14	5
cellulose	nitrocellulose	32	56	14	1
cellulose	polyvinylidene chloride	28	26	12	0
cellulose	polyvinylidene chloride	35	29	9	0
cellulose acetate		4	48	38	56
cellulose acetate		10	19	33	38
cellulose acetate		20	8	31	27
collodion		3	23	8	4
collodion		7	19	6	5
collodion		14	15	6	0
without membrane		–	57	18	7

Tabel 3. Kieming van conidiën van E.graminis f.sp. hordei en S.fuliginea op verschillende membranen, gelegd op gewijzigde Czapek Dox agar.

collodion membranes. Experiments were performed with *E.graminis* f.sp. *hordei* and *S.fuliginea*. Results are given in Table 3. Both barley and cucumber powdery mildew conidia showed highest germination percentages on 25 μm thick cellulose membranes. Besides, the germ tubes of barley powdery mildew conidia on 25 μm thick cellulose showed the typically club-shaped appressoria, while they were thin, small and undifferentiated on other membranes. In these cases usually a varying number of undifferentiated germ tubes per conidium was present. Germination of barley mildew conidia on agar without membranes and that on cellulose acetate (4 μm) resembled each other in producing mostly one long undifferentiated germ tube besides the smaller ones.

Effect of combinations of membranes on germination. In order to investigate the influence of relative humidity on spore germination, combinations of membranes in one Petri dish were made by covering half of the agar with a 25 μm, and the other half with a 45 μm cellulose membrane or by leaving half of the surface uncovered. The experiments were carried out using conidia of *E.graminis* f.sp. *hordei* and *S.fuliginea*. In each experiment germination of 100 conidia was estimated. Results are given in Table 4. No significant differences in germination compared to those of conidia on the corresponding membranes alone were observed. In all cases barley powdery mildew conidia formed appressoria on the 25 μm cellulose membranes and only thin, undifferentiated germ tubes on the 45 μm cellulose membranes. On agar without membranes usually one long differentiated germ tube and several shorter ones were formed.

Discussion

High germination percentages of conidia of a number of powdery mildew fungi were obtained, by dusting young viable conidia on 25 μm thick cellulose membranes laid

Table 4. Germination of conidia of *E.graminis* f.sp. *hordei* and *S.fuliginea* on combinations of 25 and 45 μm thick cellulose membranes in a Petri dish.

Membrane combination	*E.graminis* f.sp. *hordei*		*S.fuliginea* (percentage of germination[1])
	percentage of germination[1]	length of germ tube (μm)	
25 μm cellulose/45 μm cellulose	$85^{a}/22^{b}$	40/34	$68^{d}/49^{e}$
25 μm cellulose/no membrane	$82^{a}/57^{c}$	46/105	$69^{d}/10^{f}$
no membrane/45 μm cellulose	$71^{c}/31^{b}$	92/38	$19^{f}/51^{e}$
control 25 μm cellulose	88^{a}	42	76^{d}
control 45 μm cellulose	25^{b}	28	43^{e}
control without membrane	63^{c}	101	14^{f}

[1] Letters indicate groups of germination percentages which differed significantly at the P = 0.01 level. Groups a, b, d and e combine germination percentages which did not differ significantly at the P = 0.05 level and groups c and f at the P = 0.01 level.

Tabel 4. Kieming van conidiën van E.graminis f.sp. hordei en S.fuliginea op combinaties van 25 en 45 μm dikke cellulosemembranen.

on agar. The morphological characteristics of the germ tubes and appressoria are in agreement with descriptions of Zaracovitis (1965) on germination of powdery mildew conidia on glass slides. Germination of conidia of *Erysiphe graminis* on membranes with formation of appressoria has earlier been reported by Dickinson (1949). In this study collodion membranes containing paraffin wax and double membranes with paraffin overlaying gelatin were used. The membranes were mounted in modified Van Tieghem cells with a drop of distilled water below the membrane. Germination percentages were not mentioned but in case of double membranes it was observed that the wax layer was usually penetrated and that at the tip of a penetration tube a haustorium-like, bilobed swelling was formed.

Penetration and haustoria formation of germinated conidia on 25 μm thick cellulose membranes was never observed in our experiments. Yet, there seems to have been an uptake of nutrients because the growth of appressoria and its side branches was stronger on modified Czapek Dox and potato dextrose agar, than on water agar.

The type of membrane greatly influenced germination. Highest germination percentages were obtained with 25 μm thick cellulose membranes. Thicker cellulose membranes resulted in lower germination percentages. The same results were observed with cellulose acetate and collodion membranes, indicating that the water permeability of the membranes and/or the relative humidity in the Petri dishes might be of importance. This assumption was strenghened by the observation that a coating of 25 μm thick cellulose membranes with nitrocellulose or polyvinylidene chloride, which rendered the membranes less water permeable, resulted in relatively low germination of barley powdery mildew conidia and practically no germination of cucumber powdery mildew conidia. Combination of two membranes in a Petri dish each covering half of the agar did not influence germination compared to that of conidia on the corresponding membrane alone. Obviously, the relative humidity in the Petri dishes is of minor importance. Hence, the water permeability of the membranes may be decisive. This can be explained by assuming a gradient of humidity present just above the surface of a water permeable membrane, ranging from 100% R.H. on the membrane to the R.H. in the Petri dish. This situation, combined with the absence of liquid water could created optimal humidity conditions for germination of powdery mildew conidia and is probably best imitated when 25 μm thick cellulose membranes are employed. The permeability of the other membranes is probably too low to create a favourable humidity gradient; the permeability of cellulose acetate (4 μm), on the other hand may be too high. This assumption is based on the observation that germination of barley powdery mildew conidia in the latter instance is similar to that on agar without membranes, in which case low and irregular germination is caused by direct contact of the conidia with liquid water in the agar. A gradient of humidity above water permeable membranes might resemble the humidity gradient present on a transpiring leaf and might explain high germination percentages in vivo in spite of a low R.H. of the environment (Ramsey et al., 1938; Yarwood and Hazen, 1944). On germination in vitro on glass slides no gradient of humidity is present. Therefore the glass slides have to be incubated in a water-saturated atmosphere, to assure high germination percentages (Zaracovitis, 1964).

The germination test described is a relatively simple one and, when necessary precautions are taken, provides a means to check the viability of powdery mildew conidia. The influence of nutrients or fungicidal compounds on germination and

growth of germ tubes (appressoria) can be assessed by mixing these chemicals through the agar. In this way, the effect of some systemic and non-systemic fungicides on germination has already been studied (De Waard, 1970). The physiology of powdery mildew fungi is difficult to investigate, because of their obligate-parasitic character. Studies on the physiology of germinated conidia as in the case of other obligate parasites (e.g. Van Etten, 1969) could be facilitated using the technique described in the present article.

Acknowledgments

The skilful assistance of Miss A.J. Kelholt and Miss L. Steendam is greatly appreciated. The author is indebted to Dr. A. Fuchs, Laboratory of Phytopathology at Wageningen, for continuous interest in this work.

Samenvatting

Kieming van conidiën van echte meeldauw schimmels op cellulosemembranen in vitro

Bij incubatie van conidiën van echte meeldauwschimmels op 25 μm dikke cellulosemembranen, gelegd op agar in Petri-schalen werden hoge kiemingspercentages verkregen. Kieming op 45 μm dikke, ongecoate cellulose, gecoate cellulose, celluloseacetaat- en collodionmembranen was altijd slechter en onregelmatiger dan op 25 μm dikke cellulosemembranen. Waarschijnlijk is de waterdoorlaatbaarheid van de membranen van belang voor de kieming door de vorming van een vochtigheidsgradient.

References

Clayton, C. N., 1942. The germination of fungus spores in relation to controlled humidity. Phytopathology 32:921–943.

Delp, C. J., 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44:615–620.

Dickinson, S., 1949. Studies in the physiology of obligate parasitism. IV. The formation on membranes of haustoria by rust hyphae and powdery mildew germ-tubes. Ann. Bot. 13:345–353.

Drandarevski, Ch. A., 1969. Untersuchungen über den echten Rübenmehltau *Erysiphe betae* (Vanha) Weltzien. II. Biologie und Klimaabhängigkeit des Pilzes. Phytopath. Z. 65:124–154.

Etten, J. L. van, 1969. Protein synthesis during fungal spore germination. Phytopathology 59:1060–1064.

Ferguson, J. H. A., 1960. Binomiaal waarschijnlijkheidspapier, een praktisch hulpmiddel. Landbouwk. Tijdschr., 's-Grav. 72:87–93.

Hashioka, Y., 1937. Relation of temperature and humidity ot *Sphaerotheca fuliginea* (Schlecht.) Poll. With special reference to germination, viability and infection. Trans. nat. Hist. Soc. Formosa 27:129–145.

Hirata, K., 1967. Notes on haustoria, hyphae and conidia of the powdery mildew fungus of barley, *Erysiphe graminis* f.sp. *hordei*. Mem. Fac. Agric. Niigata Univ. 6.

Koopmans, M. J., 1959. An in vitro evaluation of the toxicity of chemicals for Erysiphaceae. Meded. LandbHogesch. OpzoekStns Gent 24:821–827.

Manners, J. G. & Houssain, S. M. M., 1963. Effects of temperature and humidity on conidial germination in *Erysiphe graminis*. Trans. Brit. mycol. Soc. 46:225–234.

Ramsey, J. A., Butler, C. G. & Sang, J. H., 1938. The humidity gradient at the surface of a transpiring leaf. J. exp. Biol. 15:255–265.

Waard, M. A. de, 1970. Effect of systemic and non-systemic compounds on the in vitro germination of powdery mildew conidia. Meded. Rijksfac. Landbouwwet. Gent. In press.
Williams, P. L., Scott, K. J. & Kühl, J. L., 1966. Vegetative growth of *Puccinia graminis* f.sp. *tritici* in vitro. Phytopathology 56: 1418–1419.
Yarwood, C. E., 1957. Powdery mildews. Bot. Rev. 23:235–301.
Yarwood, C. E. & Hazen, W. E., 1944. The relative humidity at leaf surfaces. Am. J. Bot. 31:129–135.
Zaracovitis, C., 1964. Factors in testing fungicides against powdery mildews. The germination of the conidia in vitro. Annals Inst. Phytopath. Benaki, N.S., 6:73–106.
Zaracovitis, C., 1965. Attempts to identify powdery mildew fungi by conidial characters. Trans. Brit. mycol. Soc. 48:553–558.

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