Effect of factors on conidium germination of *Botrytis cinerea in vitro.*

Salem Nassr[1] and Radwan Barakat[2]*

[1,2] Department of Plant Production and Protection, Faculty of Agriculture Hebron University, P.O.Box 40, Hebron, Palestine

ABSTRACT

Botrytis cinerea is a necrotrophic fungal plant pathogen distributed worldwide. The early stages of epidemiology namely spore germination is a topic of great interest among researchers. In the current study, the effect of various physical, chemical and nutritional factors on germination of *B. cinerea* conidia were studied *in vitro*. Results showed that there was no particular influence of spore age (5-14 days) on germination in 10 mM fructose. In addition, germination-self-inhibition was found to be associated with increased spore concentrations (above 4.5×10^5 conidia/ml) without significant differences between fungal isolates. When setting different pH values in the medium, conidial germination of *B. cinerea* was impaired by pH values below 6 and above 8. However, germination of *B. cinerea* was strongly enhanced (>90% after 24 hours) in the presence of sugars (i.e. fructose, sucrose and glucose) at concentrations above 100 mM, whilst the cations (Ca^{2+}, Mg^{2+}, K^{+}, and Fe^{2+}) had no visible influence on conidial germination at a wide range of concentrations (0.001-1mM). With other additives and in the presence of inorganic nitrogen forms (i.e. NH4 and NO3), conidial germination responded similarly with no particular influence on germination, whilst germ tube growth and elongation increased progressively with increasing concentrations of both N-forms.

Key Words: B. cinerea, conidial germination, early event, germ tube

1. INTRODUCTION

B. cinerea Pers. ex. Fr. is the causal agent of gray mold. The name of the sexual stage or teleomorph is *Botryotinia fuckeliana* (de Bary) Whetzel, but the ascocarps are rarely observed under field conditions (Polach and Abawi 1975). *B. cinerea* is a filamentous, heterothallic Ascomycete exhibiting great variability in mycelial growth rate, conidial germination, pathogenicity, incidence of sporulation structures, production of sclerotia, and resistance to anti-*Botrytis* chemicals (Grindle 1979; Lorbeer 1980; Di Lenna et al. 1981; Kerssies et al. 1997). The early events of plant's infection by plant pathogenic fungi are essential for disease initiation and progress. Such early events (adhesion, conidial germination, and formation of external infection structures) were intensively studied lately on *B. cinerea* throughout several studies (Doehlemann et al. 2006; Klimple et al. 2002; Schumacher et al. 2008).

Conidial germination of *B. cinerea* is induced by different physical and chemical signals, including the presence and quality of nutrients in particular sugars such as fructose (Kosuge and Hewitt 1964; Blakeman 1975). Conidial germination in most filamentous fungi requires the presence of low-molecular-mass nutrients such as sugars, amino acids and inorganic salts (Carlile and Watkinson 1994). Along with germination and after conidial adhesion, different mucilages are secreted and assist in anchoring of the germ tube and appressorium to the host surface. Several groups of proteins have been suggested to assist in germ tube and appressorium attachment and to mediate the exchange of early signalling between the fungus and the plant (Prins et al. 2000).

Conidia of *B. cinerea* are typically nutrient-dependent; they do not readily germinate in sterile water, and they usually require an exogenous input of nutrients for germination. In addition, it has been proposed that nutrient-dependent conidia of phytopathogenic fungi may use germination-stimulating compounds from a host plant as an alternative chemical cue when nutrient concentrations are too low for conidial germination (Filonow, 2002). In addition, diverse carbon sources (mono- and disaccharides, acetate) are effective at low concentrations (10 mM) to induce germination in *B. cinerea*. Rich media such as malt extract induced rapid germination and early germ tube

* Tel: 02 2220995 ext. 153 Fax: 02 2229303 Email: radwanb@hebron.edu

branching. Induction of conidial germination by nutrients, in particular sugars, is well known in saprotrophic fungi (Osherov and May, 2000). The mechanism of nutrient sensing by *B. cinerea* conidia is unknown. As diverse sugars and acetate induce germination with similar efficiency, it appears unlikely that nutrient sensing occurs by plasma membrane proteins (Forsberg and Ljungdahl, 2001). Conidia are also able to germinate on inert artificial surfaces; various amino acids plus sugars efficiently induced germination of conidia, while mineral salts such as ammonium and phosphate were effective only in the presence of low concentrations of sugars (Blakeman, 1975). On cuticular surfaces, however, dry-inoculated conidia can germinate at high humidity in the absence of liquid water (Prins *et al.,* 2000). Surface hydrophobicity, together with surface hardness, is well known to induce germination of *B. cinerea* conidia in the absence of nutrients (Osherove and May, 2000). The current study has illustrated the effect of such several physical and chemical factors on germination of *B. cinerea* conidia.

2. METHODS

2.1 FUNGAL ISOLATES AND COMMERCIAL CULTURE MEDIUM

B. cinerea wild type isolates used throughout this study were provided by the Plant Protection Research Center (PPRC) fungal collection at Hebron University. The first isolate, (PBC1) was isolated from infected beans (*Phaseolus vulgaris* L.) growing under greenhouse in Hebron. The second isolate, (PBC3) was isolated from infected grape berries (*Vitis vinefera L.*) growing in an open field in Hebron. Following isolation, the two isolates were grown on PDA medium and kept at 20±1 ºC under continuous light.

After 12 days, and when cultures sporulated, 5mm mycelium plug from each isolate culture was taken and placed in a fresh PDA culture plate; 24 hours later, one freely emerging conidium was transferred into another plate to get monosporic cultures. The monosporic cultures were grown on PDA medium amended with 10% (w/v) homogenized bean leaves. Plates were then kept under continuous light in an incubator at 20±1 $^{\circ}$C for the coming experiments.

The third isolate used was B05.10 which is a universal known strain. It was derived from the wild-type isolate SAS56 by treatment with benomyl for haploidization (Quidde *et al.,* 1999). This putative haploid wild type isolate B05.10 was provided by the lab. of Prof. P. Tudzynski (University of Munster, Germany).

2.2 CONIDIAL CONCENTRATION

The influence of conidial concentration on germination assays of *B. cinerea* isolates was assessed in a 24 well Sarstedt microtitre plate (Sarstedt, Newton. USA), according to (Doehlemann, 2006). Two plates of PDA medium amended with 10% w/v homogenized bean leaves were inoculated with 100 µl of conidial suspension (1×10^6 conidia/ml) from PBC3, PBC1 and B05.10 isolates. The inoculum was spread over the surface of the medium with the aid of a glass rod. After 11 days, conidia were harvested from each plate by 10 ml of sterile distilled water. Conidia were then filtered through a Nytex membrane to remove traces of mycelia and placed in a sterile plastic vial for each isolate.

Spore suspension was then washed three times with 10 ml of SDW and centrifuged (IEC Centra-CLD) for 3 minutes at 3000 rpm. The concentration of the conidial suspension was determined by a haemocytometer and diluted to the final concentrations of 4×10^5, 2.5×10^4, 5×10^3 and 2.5×10^3 conidia/ml. Spherical glass coverslips - 15mm (Roth, Karlsruhe, Germany) were placed in the bottom of each well of the 24-welled microtitre plate. A 25 µl of each concentration were placed in the bottom of the well to which 475 µl of 10mM D-Fructose solution were added to reach a final volume of 500 µl and according to (Doehlemann , 2006). Plates were then incubated in the dark at 20°C±1 and conidial germination counted after 5 hours of incubation. Each treatment consisted of 4 replicates (wells) and 100 randomly selected conidia were counted in each of the 4 wells under an inverted microscope. A conidium was considered as germinated when the germ tube was visible.

2.3 AGE OF CONIDIA

The influence of conidial age on germination of *B. cinerea*-isolate B05.10 conidia was assessed. The isolate B05.10 was grown on plates containing potato dextrose agar (PDA) amended with 10% homogenized bean leaves. Four plates of PDA medium were inoculated with 5 mm mycelium plug from a newly growing mycelium (two days old), and incubated at 21 $^{\circ}$C and continuous light.

Conidia were then harvested after 7, 9, 10, 12, and 14 days with 10 ml of SDW, and filtered through a Nytex membrane to remove traces of mycelia.
Spore suspensions were then washed three times with 10 ml of SDW and centrifuged (IEC Centra-CLD) for 3 minutes at 3000 rpm; supernatant was discarded each time. Conidial concentrations were then determined with the aid of a haemocytometer [Tiefe Depth Protondeur 0.200 mm] and fixed at 2.5×10^4 Conidia/ml. Spherical glass coverslips (15mm, Roth, Karlsruhe. Germany) were placed on each well of the 24-welled microtitre plate. Conidia (25 µl of each age) were placed in the bottom of the well. Fructose was prepared and suspended in liquid Gamborg B5 basal salt mixture (GB5) (Duchefa Biochem. BV, Haarlem, The Netherlands; Art: G0209.0050) to reach a final concentration of 10 mM. After that, 475 µl of the 10mM fructose+GB5 solution were added to reach a final volume of 500 µl. Sarstedt plates were then incubated in the dark at 20±1 ºC.
Using the same selected conidial ages, germination was monitored on a hydrophobic surface; polypropylene film was placed at the surface of a glass slide. Slides were then placed on a moist filter paper inside closed sterile petri dishes. Conidial suspension was prepared from the isolate B05.10 and fixed at a concentration of 1×10^5 Conidia/ml. The surfaces were then inoculated with 4 separate droplets of conidial suspension 25 µl each and then placed in an incubator. A completely randomized design was used, each treatment consisted of 4 replicates (wells); germinated spores were counted out of 100 randomly selected spores under an inverted microscope.

2.4 MICROCLIMATE PH

The influence of microclimate pH on germination of *B. cinerea*, isolate B05.10 was determined in 1mM fructose solution. Fructose solutions were prepared and adjusted to pH ranges starting from 3, 4, 5, 6, 7, 8, 9 and up to 10 using 1M NaOH and 1M HCl. Conidia of *B. cinerea* (B05.10) were harvested from 10 days old sporulating cultures grown previously on (PDA+Beans) medium with SDW and conidial concentration was fixed at 2.5×10^4 conidia/ml. Spherical glass coverslips were placed in the bottom of each of the 24 wells of the Sarstedt microtitre plate. After that, 25 µl spore suspension was placed in the middle of each well and 475 µl of Fructose solution were added to reach a final volume of 0.5 ml. A completely randomized design was used with 3 replicates for each treatment. Numbers of germinated conidia were recorded after 5 hours.

2.5 SUGARS

The role of carbon sources in conidial germination of *B. cinerea* was investigated using three sugars: Fructose, Glucose and Sucrose in 5 molar concentrations 1µM, 10 µM, 100 µM, 1mM and 10mM. Sugar solutions were prepared in DW and sterilized in the autoclave for 30 minutes at 127°C. *B. cinerea* was grown on PDA+10% beans (w/v) and incubated at 21°C and continuous light for ten days. Spore suspensions from the isolates B05.10, PBC3 and PBC1 were prepared using SDW and adjusted to a final concentration of 2.5×10^4 conidia/ml. Spherical glass coverslips were placed in the bottom of each of the 24 wells of the Sarstedt microtitre plate. Spore suspension (25µl) was placed in the middle of each well and 475 µl of each sugar treatment were added to reach a final volume of 0.5 ml. A completely randomized design was used with 4 replicates for each treatment. Numbers of germinated conidia were recorded after 5 and 25 hours.

2.6 SALT CATIONS

The role of the cations, Ca^{2+}, Mg^{2+}, and Fe^{2+} in conidial germination of *B. cinerea* was investigated. Ca ($CaCl_2$), Mg ($MgCl_2$), and Fe (FeSO4.7H2O) were prepared into 6 concentrations (0.001M 0.01M 0.1M 1mM 10mM and 1M). Solutions were prepared in distilled water and sterilized in the autoclave for 30 minutes at 127°C. *B. cinerea* was grown on (PDA+10% beans) and incubated at 21°C and continuous light for ten days. Conidial suspensions from the isolates B05.10 and PBC3 were harvested by SDW. Conidia were then filtered through Nytex membrane and washed three times to remove traces of mycelium. The concentration was adjusted to a final concentration of 1×10^3 conidia/ml. Spherical glass cover slips were placed in the bottom of each of the 24 wells of the Sarstedt microtitre plate. Spore suspension (25 µl) was placed in the middle of each well and 475 µl of each treatment were added to reach a final volume of 0.5 ml. A completely randomized design was used with 4 replicates for each treatment. Numbers of germinated conidia were recorded after

40 hours of incubation at 21°C. At the same time, the average germ tube length of 10 random germinated conidia (replicates) was recorded.

2.7 INORGANIC NITROGEN FORMS (NH4 AND NO3)

The effect of the nitrogen forms, $NH4^+$ and $NO3^-$ on conidial germination of *B. cinerea* was studied. The procedure is the same as that of the previous section. NH4 (NH4Cl), NO3 (NaNO3) was used as source of the cations. The spore concentration was set to 1×10^3 conidia/ml. A completely randomized design was used with 4 replicates for each treatment. Numbers of germinated conidia were recorded after 25 hours of incubation at 21°C. At the same time, the average germ tube length of 10 random germinated conidia (replicates) was recorded.

2.8 STATISTICAL ANALYSIS

The data of all experiments were analyzed statistically using analysis of variance (one way ANOVA) and Fisher least significant difference (LSD) test with the aid of (Sigma stat 2.0 for Windows® statistical package program, SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1 THE EFFECT OF CONCENTRATION OF CONIDIA ON GERMINATION OF *B. CINEREA* CONIDIA

The influence of spore concentration of *B. cinerea*-isolates B05.10, PBC1 and PBC3 on conidial germination was determined in 10mm fructose solution (Fig. 1). Results showed that conidial germination rates decreased with increasing spore concentrations. The highest germination rate was recorded at the spore concentration (2.5×10^3 conidia/ml) for all isolates. Generally, there was no significant difference in germination rates between the three *B. cinerea* isolates. it was evident that the three isolates responded similarly in which germination rates decreased with increasing spore concentrations (Figure 1 and 2: c, d, and e).

3.2 THE EFFECT OF AGE OF CONIDIA ON GERMINATION OF *B. CINEREA* CONIDIA

Spore age could be another factor involved in early conidial germination in fungi. The influence of conidial age of *B. cinerea* (B05.10) on germination percentage was investigated. No significant difference in germination percentages was found between different conidial ages in sugar amended with Gamborg' B5- salt mixture (GB5). Conidial germination percentages, however, were significantly reduced in older conidia (67% after 14 days) compared to younger conidia (91% after 5 days) when germination was tested on a hydrophobic surface (polypropylene) (see table 1 and 2).

3.3 THE EFFECT OF MICROCLIMATE PH ON GERMINATION OF *B. CINEREA* CONIDIA

The influence of microclimate pH on germination of *B. cinerea* conidia was assessed on Sarstedt plates. *B. cinerea* conidia were able to germinate well at ph values ranging from 6-8; the highest germination rate was obtained at pH 7. However, B05.10 conidia germinated poorly at pH= 3 and 10. The experiment was repeated twice. Data on the average germination rates in different microclimate pH are presented in Figure 3 and 4.

3.4 THE EFFECT OF SUGARS ON GERMINATION OF *B. CINEREA* CONIDIA

The influence of the sugars (Fructose, Sucrose and Glucose) on conidial germination of *B. cinerea* was tested in various concentrations (Fig.5 and 6). Results showed that germination of conidia was stimulated in sugars in various proportions according to various concentrations compared to SDW. Sucrose was the best in inducing conidial germination even after 5 hpi only recording 87% compared to glucose 18% and fructose 59%. Two sugars (sucrose and glucose) have induced high germination rates (>90%) after 24 hours of incubation at the highest concentration used (10mM) . The concentration (100 µM) was the breaking point for most sugars to induce significant increases in conidial germination.

3.5 THE EFFECT OF CATIONS ON GERMINATION OF *B. CINEREA* CONIDIA

The cations Ca^{2+}, Mg^{2+}, K^{+}, and Fe^{2+} had no influence on conidial germination of *B. cinerea* isolates (B05.10 and PBC1) at the relatively low concentrations used (0.001-1mM). At 10mM concentration, however, Fe reduced germination dramatically. At higher concentrations (>10mM), all cations showed toxicity and totally inhibited conidial germination. Concerning germ tube elongation, only Fe was able to enhance germination at low concentrations, but as concentration increased germ tube elongation decreased until totally inhibited at high concentrations (>10mM). All the other cations (Ca^{2+}, Mg^{2+} and K^{+}), however, showed no influence on germ tube elongation at all concentrations tested (Fig. 7)

3.6 THE EFFECT OF INORGANIC NITROGEN FORMS ON GERMINATION OF *B. CINEREA* CONIDIA

The effect of NH4 and NO3 on germination of *B. cinerea* (B05.10 and PBC1) conidia and germ tube lengths was investigated (Fig. 8). Inorganic nitrogen forms had no influence on germination percentages of *B. cinerea* isolates at all concentrations tested. However, germ tube length growth was dramatically influenced by both nitrogen forms positively; germ tube length increased by almost 99% at the highest concentration of NH4 (1M) compared to the control (SDW). NH4 form of nitrogen enhanced germ tube growth to a larger extent than NO3 form of N for both *B. cinerea* isolates. Both *B. cinerea* isolates responded almost similarly in respect to percentage germination and germ tube growth.

4. DISCUSSION

The ability of fungi to adhere to and germinate on leaves and other substrata is well documented and is thought to represent an important early event in plant-microbe interactions (Braun and Howard 1994; Jones 1994). Spore germination in *B. cinerea* follows a developmental sequence of spore swelling, localized outgrowth of the germ tube and subsequent polarized growth of the new hyphae. It was noted that, conidial germination rates of *B. cinerea*-isolates decreased with increasing spore concentrations without significant differences between isolates. At concentrations above 4×10^{5} conidia/ml, conidia were unable to germinate and appeared in clots. Sharrock, et al. 2001 found that conidia of *B. cinerea* exhibit a self inhibition strategy during germination at high concentrations (1×10^{6} conidia/ml) or more. It is assumed that at high concentrations, conidia tend to produce specific germination and/or growth inhibitors regardless of the richness of the substrate. Furthermore, several germination-self-inhibitors in other fungal species such as *Puccinia, Uromyces, Colletrotrichum, Dictyostelium, Fusarium* and *Aspergillus* were investigated and reports showed that these inhibitors can be volatile or non-volatile (Allen 1955; Bacon et al. 1973; and Barrios-Gonzales et al. 1989). It was also concluded that self-inhibitors can affect other fungal processes, such as prevention of appressorium induction which make conidial germination unlikely to occur.

Spore age could be another factor involved in early conidial germination in fungi. It was found that conidial germination was significantly reduced in older conidia (67% after 14 days) compared to younger conidia (91% after 5 days) when germination was tested on a hydrophobic surface (Polypropylene). However, no difference was noticed when spores germinated in Fructose and GB5. This suggests that nutritional factors may mask the effect of age and older conidia can germinate as well as younger conidia if the growth substrate was supplied with appropriate nutritional source. Using different germination conditions, Shirashi et al., 1988 found that young *Botrytis* conidia, in general, germinated well at 20°C compared to old conidia.

As for pH, conidial germination was significantly impaired at high and/or low values (below 6 and above 8). Conidia germinated well at pH ranging from 6-8 with the highest germination rate at pH=7. In this direction, fungi very often can dynamically alter the local pH to fit its enzymatic arsenal, with the level of pathogenicity being related to the efficiency of the pH change. (Prusky et al. 2001). Generally, *B. cinerea* is classified among acidic fungi (Prusky and Yakoby, 2003) and similar to other pathogenic fungi, such as *Penicillium expansum*, *P. digitatum, P. italicum*, and *Sclerotinia sclerotiorum* that use tissue acidification in their attack (Vautard and Fevre, 2003). This investigation, however, was restricted to the conidial germination in vitro. The ability of *B. cinerea* to germinate at various pH values emphasizes the previous findings stating that *Botrytis* spp. change the medium or site pH to facilitate the enzymatic activities.

Nutritional supplements, namely sugars are considered rich nutrients; germination of *B. cinerea* conidia was stimulated in the three different sugars (fructose, sucrose and glucose) at various concentrations compared to the control (SDW). Almost all sugars have induced full germination

(100%) after 24 hours of incubation at the highest concentration used (10mM) knowing that the concentration (100 µM) was the (-breaking point-) for all sugars to induce significant increase in conidial germination. Sugars at relatively low concentrations (i.e. 10mM) induced early swelling of conidia and enhanced early germ tube branching. In this direction, it has been shown that Fructose induced germination of *B. cinerea* conidia more efficiently than any other monosaccharide (Blakeman, 1975). Germination induction by sugars was concentration dependent, and fructose was more effective than glucose. Similarly and among sugars, fructose has been pointed out as the best inducer of germination in *B. cinerea*, being more effective than glucose and other hexoses or disaccharides (Blakeman, 1975). One explanation for the particular important activity of fructose in conidial germination could be that this sugar is preferentially taken up by a fructose-specific transport system. This is surprising since glucose is usually the most efficient hexose not only as a nutrient, but also as a signalling compound (Doehlemann et al. 2005). Using almost the same protocol for germination, Doehlemann, et al. 2006 found similar results after incubation for 24 hours. Induction of conidial germination by nutrients, in particular sugars, is well known in saprotrophic fungi (Osherov and May 2000). In rich media, most fungi germinate quickly, including phytopathogens such as *Fusarium solani, Colletotrichum graminicola* and *Colletotrichum gloeosporioides* (Ruan et al. 1995; Chaky et al. 2001; Barhoom and Sharon, 2004).

The mechanism of sugar sensing by *B. cinerea* conidia is unknown. As diverse sugars and acetate induce germination with similar efficiency, it appears unlikely that nutrient sensing occurs by plasma membrane proteins (Forsberg and Ljungdahl, 2001).

Regarding the addition of salt cations and from looking at the results, it was obvious that the tested cations (Ca^{2+}, Mg^{2+}, K^{+}, and Fe^{2+}) had no influence on conidial germination at a wide range of concentrations (0.001-1mM). However, at high concentrations (>10mM), germination declined sharply, especially with Fe^{2+} which suggests a level of toxicity induced at high concentrations. It is very likely that conidia before germination are not affected at low concentrations of cation availability in the growth substrate. However, after germination, germ tube growth becomes more sensitive to a wide range of cation concentrations in the growth media. Fe^{2+} seems to provide an important nutritional source for germ tube growth at low concentrations (0.001 M). Barakat and Almasri, 2009 (unpublished data) found that at high concentrations (i.e. 1M) all these cations inhibited germination of *Botrytis* conidia and the level of toxicity varied between isolates. Shirani and Hatta (1987), found that at the concentration (5×10^4 conidia/ml) conidial germination of *B. cinerea* was optimum (100%) in the presence of Ca^{2+} ($CaCl_2$) and was relatively high (66%) in Mg^{2+} ($MgSO_4$) at the concentrations (0.1-0.7 g/liter). Conidial germination responded almost similarly to nitrogen forms. While N-forms had no influence on germination, germ tube growth and elongation responded positively with increasing concentrations of both forms. This suggests that conidia may depend more on available energy inside the spore to germinate but after germination, germ tube growth greatly depends on nutritional elements available in the growth substrate.

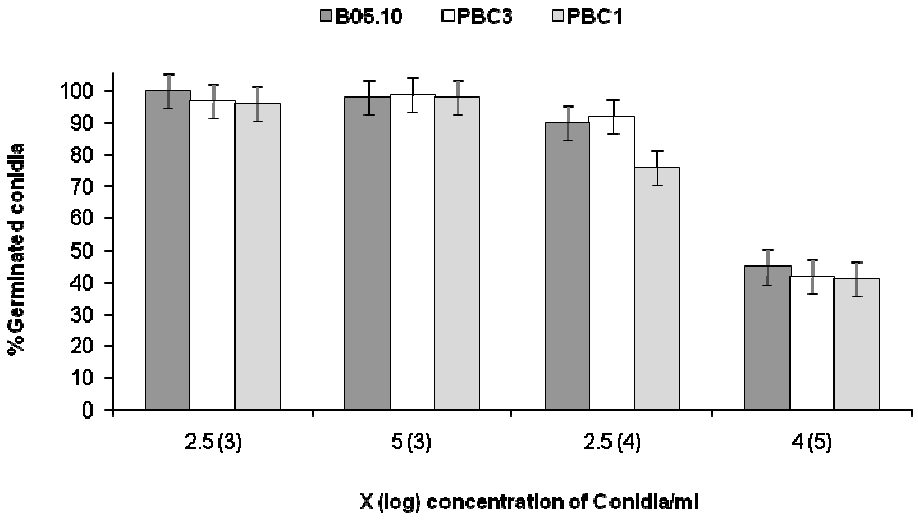


Figure 1. Effect of spore concentration on conidial germination rates of *B. cinerea* isolates grown on (PDA+10% bean leaves) medium and incubated in 10mM Fructose at 20±1 ºC under continuous light after 20 hours of incubation. (vertical bars represent LSD= 5.49, n=3).

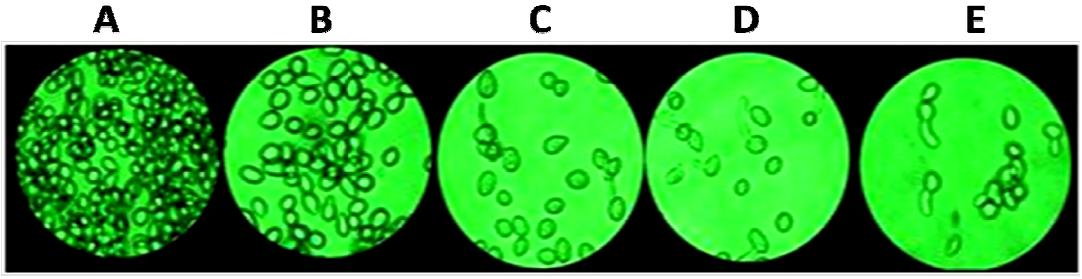


Figure2. *B. cinerea* (B05.10) conidial germination at different concentrations of conidia at 200X. Conidial concentrations: (A), 5×10^6 conidia/ml; (B), 1×10^6 conidia/ml; (C), 4×10^5 conidia/ml; (D), 2.5×10^4 conidia/ml and (E), 5×10^3 conidia/ml.

Table 1. Influence of conidial age on germination of *B. cinerea*-isolate B05.10 after 20 hours of incubation in 10 mM fructose solution+GB5

Age of B05.10 culture (days)	% Germination
5	97a
7	95a
10	96a
12	95a
14	93a

Means followed by the same letter in the same column are not significantly different (P= 0.064). GB5: Gamborgs B5-basic salt mixture.

Table 2. Influence of conidial age on germination of *B. cinerea* conidia isolate B05.10 after 20 hours of incubation on polypropylene surface.

Age of B05.10 culture (days)	% Germination
5	91 a
7	84 ab
10	92 a
12	78 bc
14	67 c

-Means followed by the same letter in the same column are not significantly different (LSD=11.309, n=4). GB5: Gamborgs B5-basic salt mixture.

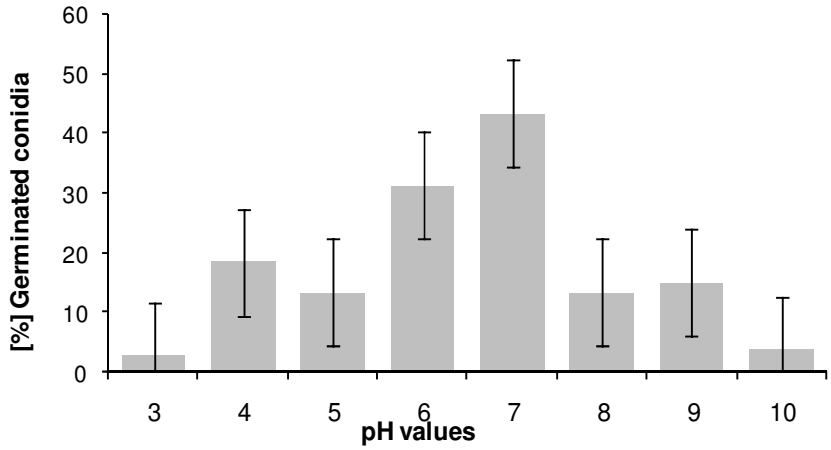


Figure 3. Influence of microclimate pH on conidial germination of *B. cinerea*-isolate B05.10 in 1mM fructose solution after 5 hours of incubation. (LSD = 9.020, n=3).

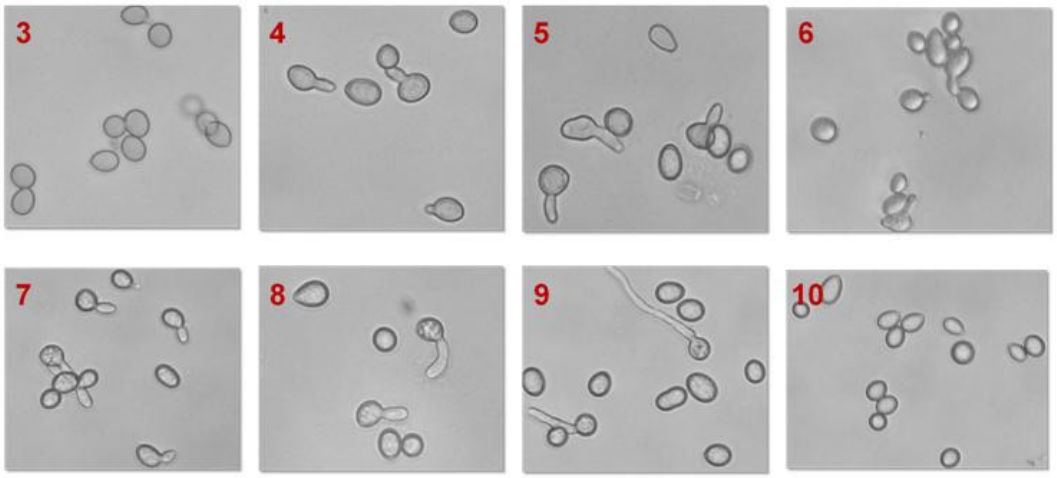
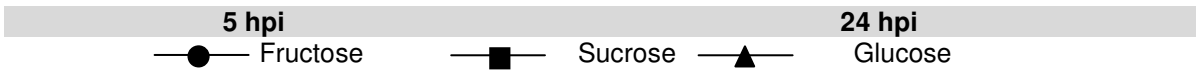
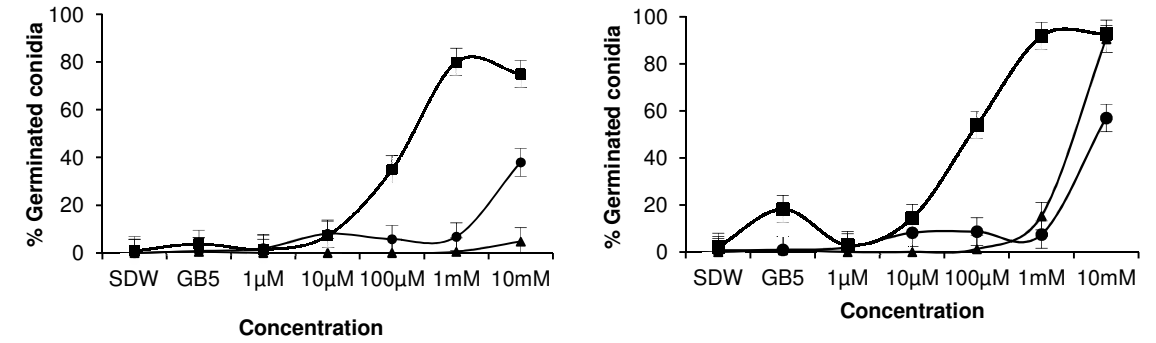


Figure 4. Conidial germination of *B. cinerea* –isolate B05.10 under different pH values in 1mM fructose solution at 200 X .



***B. cinerea* (B05.10)**



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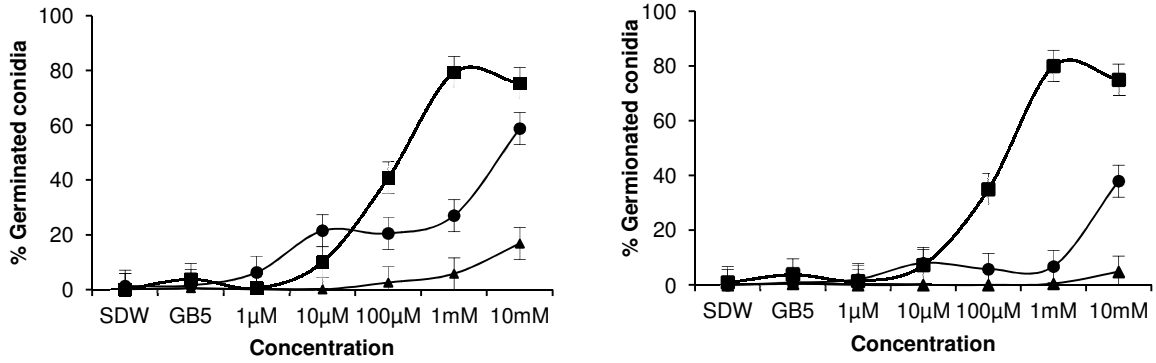

***B. cinerea* (PBC1)**

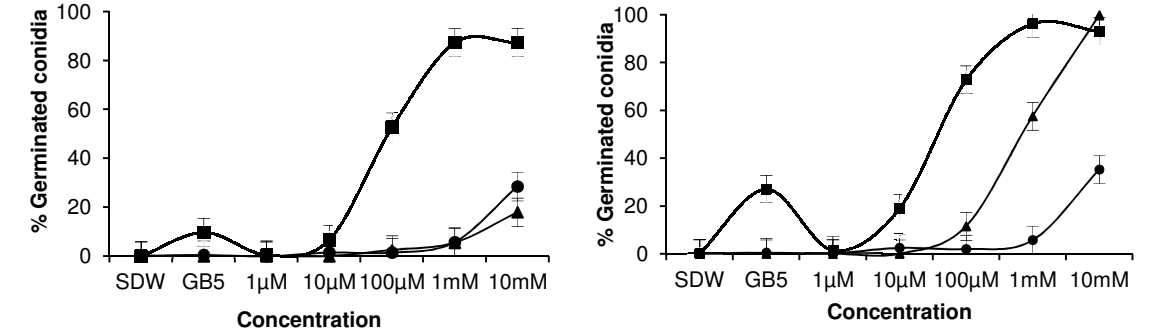

Figure 5. Influence of Fructose, Sucrose and Glucose solutions on germination of *B. cinerea* conidia. (LSD=10.168, n=4, p<0.001). Experiment was done after 5 and 24 hours of incubation in various concentrations at 20±1 ºC. SDW: SDW; GB5: Gamborg's B5 basic salt mixture; hpi: hours post inoculation.

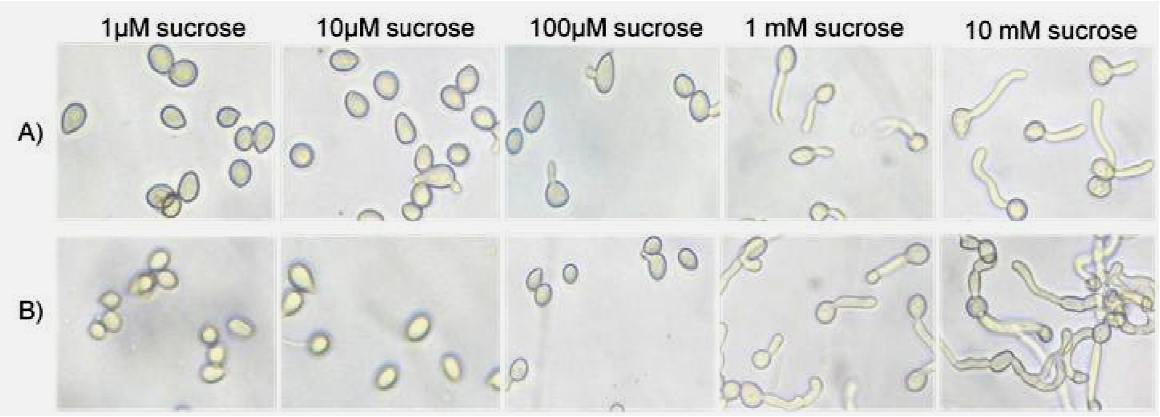

Figure 6. Conidial germination of *B. cinerea* (B05.10) in different concentrations of sucrose. A): after 5 and B): after 24 hours at 200X.

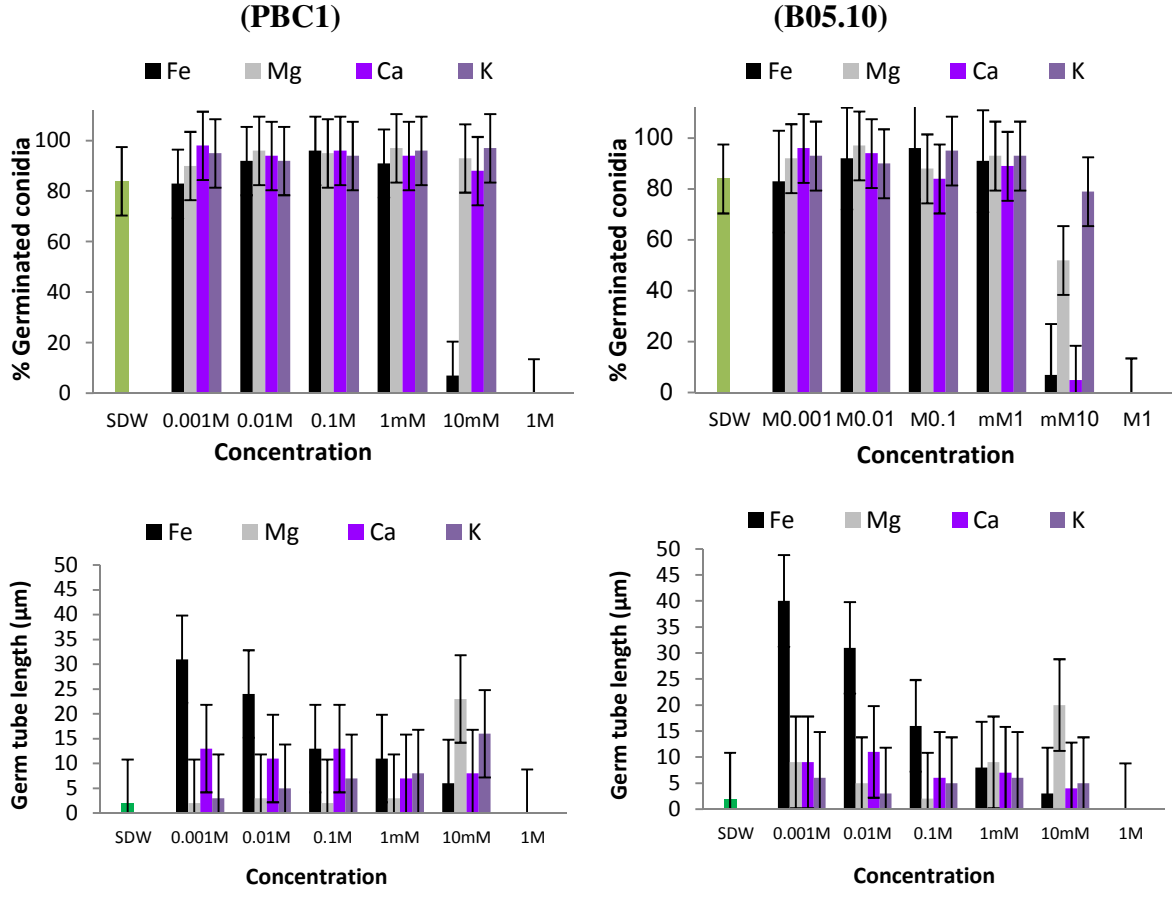

Figure 7. Influence of Ca^{2+}, Mg^{2+}, K^{+}, Fe^{2+} in various concentrations on conidial germination and germ tube elongation of *B. cinerea* after 40 hours of incubation. Conidial germination (LSD=13.527, n=4) ; Germ tube elongation (LSD=8.815, n=10).

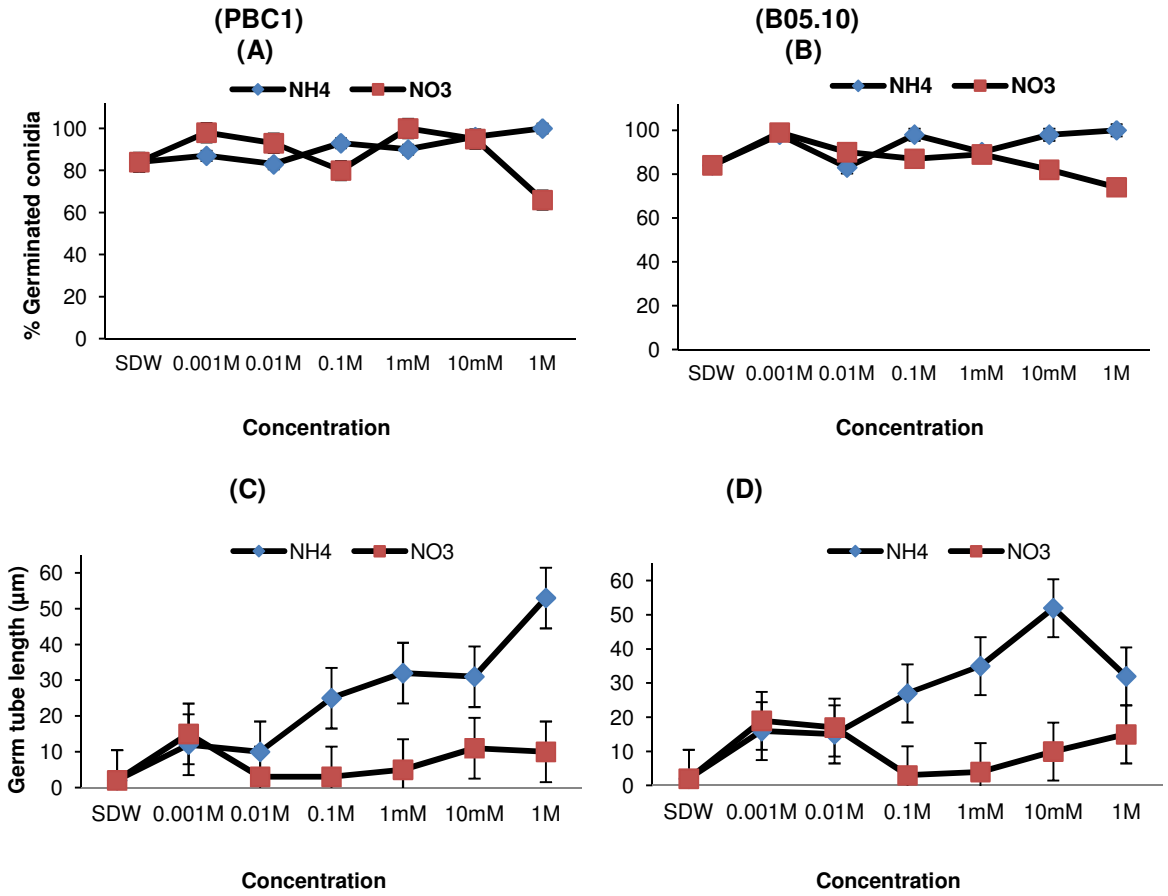

Figure 8. Influence of NH4 and NO3 in various concentrations on conidial germination and germ tube elongation of *B. cinerea* PBC1 (A, C), and B05.10 (B, D). Differences between means of germination percentages were not significant; bars in (B, D) represent the standard error of the mean with LSD=8.489.

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COMPETING INTERESTS

"Authors declares that no competing interests exist.".

AUTHORS' CONTRIBUTIONS

Authors may use the following wordings for this section: " 'Author 1' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author 2' managed the analyses of the study, read and approved the final manuscript."

CONSENT (WHERE EVER APPLICABLE)

NOT APPLICABLE

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

NOT APPLICABLE