

# Chapter 6

## Thermotolerance of Fungal Conidia



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**Abstract** Conidia of entomopathogenic fungi (EF) are the propagules most frequently used in arthropod biocontrol programs. This anamorphic form is essential for the infection process, including spore germination, penetration, vegetative growth, conidiogenesis and dissemination. Most EF are mesophilic and can develop between 10 and 40 °C, but optimal growth is between 25 and 35 °C. Abiotic factors, especially temperature (high or low) can determine their viability, virulence and success or failure of infection process. Temperature has the highest impact on conidial stress inhibiting metabolic processes, such as decreased morphogenesis during germination, protein denaturation and membrane disorganization. Several studies show that some strains of *Beauveria* spp., *Metarhizium* spp., and *Isaria* spp. exhibit conidial survival even when grown at high temperatures, indicating a relationship between conidial thermotolerance and their geographical isolation origin. Moreover, the high variability in fungal thermotolerance is also dependent of the culture media composition and growth condition. EF that grow at high temperatures do not grow at low temperatures and *vice versa*. Moreover, when growth conditions are not set at optimal temperatures, EF development is affected and their effectiveness in biological control programs of arthropods is reduced. Thermal stress directly impacts on fungal strains ability to target arthropods and their environmental activity performance. The screening for fungal strains with a higher thermotolerance and the improvement on conidial formulations may aid in optimizing the conditions for biocontrol agent application.

**Keywords** Temperature · Conidia · Germination · Biological control

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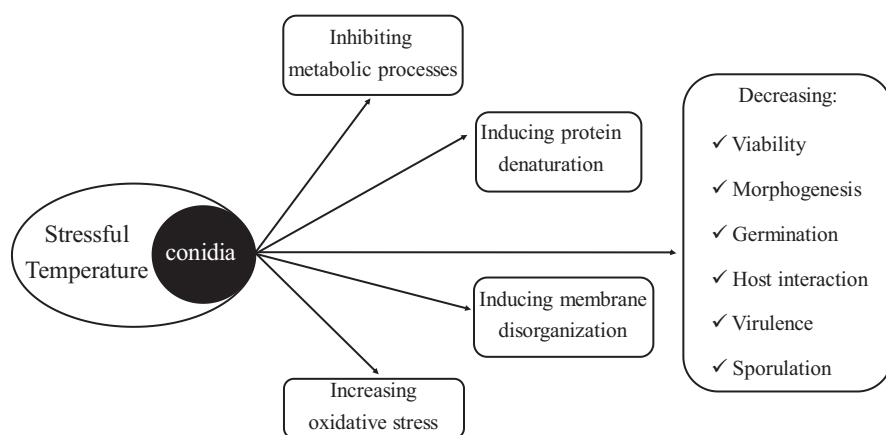
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## 6.1 Introduction

Entomopathogenic fungi (EF) are responsible for epizootics that often regulate insect pest populations. The genera *Metarhizium* (Hypocreales: Clavicipitaceae), *Beauveria* (Hypocreales: Cordycipitaceae), and *Isaria* (Hypocreales: Cordycipitaceae) are the fungi most frequently used in biological control programs. The asexual spore, named conidium, is an anamorphic and primary form essential in the life cycle of many filamentous fungi (Osherov and May 2001). Mostly, entomopathogenic fungal infection starts with the attachment of conidia to the insect cuticle, and then progresses to conidial germination, penetration, vegetative growth (as hyphal bodies), conidiogenesis and, finally, dissemination (Lacey et al. 2001; Pedrini 2018).

Temperature is an abiotic factor that influences all fungal development stages, from primary processes such as biochemical and cellular reactions in conidia to overall infection and fungus-host interaction (Zimmermann 1982; Cabanillas and Jones 2009) (Fig. 6.1). Generally, three thermal conditions are considered for fungal development, i.e., low (below 11 °C), intermediate (11–28 °C) and high temperatures (above 28 °C) (Vidal et al. 1997). Under this classification, EF are considered mesophilic because they develop well between 10 and 40 °C, with optimal growth between 25 and 35 °C (Crisan 1973; Fargues et al. 1997; Vidal et al. 1997; Dimbi et al. 2004). *Metarhizium rileyi* (= *Nomurea rileyi*) presented optimum growth at 25 °C (Ignoffo et al. 1976). Similar studies with *Metarhizium* spp. strains other than *M. rileyi* showed optimum growth between 11 and 35 °C (Ouedraogo et al. 1997), whereas *B. bassiana* strains grew over a wider temperature range, from 8 to 35 °C, exhibiting optimal growth at temperatures as low as 20 °C and as high as 30 °C (Fargues et al. 1997).



**Fig. 6.1** Effect of non-optimal temperatures for fungal growth in various processes of conidia life cycle

Thermotolerance is defined as the ability to withstand relatively hot (or cold) conditions. Viability in thermotolerance above 40 °C was observed in several strains of *Metarhizium* spp. (Hedgecock et al. 1995; Li and Feng 2009). However, the growth of some strains of *Beauveria* spp. was inhibited after 2 h at 45 °C. On the other hand, cold activity was reported for both *Beauveria* spp. and *Metarhizium* spp., *B. bassiana* being able to grow at temperatures as low as 5 °C and some *M. anisopliae* strains at 8 °C, due to their cold adaptation (McCammon and Rath 1994; Croos and Bidochka 2001; Fernandes et al. 2008; Santos et al. 2011).

This physical factor is very important to regulate all development processes, since the beginning of germination up to conidial sporulation (Edelstein et al. 2005; Keyser et al. 2014) (Fig. 6.1). Thus, the screening of fungal strains thermotolerant to high and low temperatures, together with the molecular and genetic characterization, and the investigation on formulations to increase fungal performance are key factors to distinguish strains with high potential to be used in biological control programs of arthropods (Dillon and Charnley 1990; Fernandes et al. 2010; Oliveira et al. 2018).

## 6.2 Thermal Characteristics and Geographical Origin

Interactions between different environmental abiotic factors, spore germination and other physiological traits in fungi were early reported by Gottlieb (1950). EF are ubiquitous in soils worldwide, from the Arctic to the tropics (Zimmermann 2007), thus different thermal behavior in fungi isolated from different geoclimatic origins and/or from diverse hosts might be expected. In fact, *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*) strains from tropical or subtropical origin (Cuba, USA, India, Nepal, and Pakistan) demonstrated high tolerance to upper limits (optimal development at 32–35 °C) than European strains (optimal growth at 25–28 °C). Conversely, European strains isolated from temperate areas are able to grow at 8 °C and show to be more tolerant to low temperature than those fungal strains originated from tropical or subtropical regions (Vidal et al. 1997; Fargues and Bon 2004).

Some studies reported no thermal variability between *B. bassiana* strains isolated from temperate or tropical areas from Europe, Africa, Asia and America (Fargues et al. 1997, Devi et al. 2005, Rangel et al. 2005, Borisade and Magan 2014). Although *M. anisopliae* strains from the tropical region (Africa) were susceptible to germinate at 15 °C (Dimbi et al. 2004), *M. anisopliae* isolates from Ontario (Canada) showed large variation in both growth rate and conidial production at temperatures between 8 and 22 °C. This latter study shows that *M. anisopliae* isolated from forested areas were cold-active, while the isolates from agricultural areas showed an ability for growth at high temperatures and resilience to UV exposure (Bidochka et al. 2001).

Regarding cold activity, fungal strains isolated far from the equator presented higher relative germination under cold conditions than strains originated from near the equator. *Beauveria bassiana* isolated from higher latitudes were cold-active,

however, there was not a similar correlation for heat (Fernandes et al. 2008). Thermal characteristics and geographical origins coincide with conditions during natural epizootics between EF and hosts. Persistence in the environment indicates certain adaptation as it was reported for *Isaria* spp., which showed to be well adapted to semiarid region (Cabanillas and Jones 2009). Finally, the specific pathogen *M. acridum*, virulent against desert locusts, is able to tolerate temperatures up to 42 °C, and thus to avoid the behavioral fever developed by host in an attempt to stop the development of the fungus, i.e., a successful strategy to avoid infection by thermosensitive EF such as *B. bassiana* (Elliot et al. 2002).

### 6.3 Culture Conditions and Conidial Thermotolerance

The production of conidia by EF is influenced mostly by culture conditions (viz., temperature, pH, water activity, aeration) and media composition (viz., carbon and nitrogen sources, metal ions) (Hallsworth and Magan 1996; Ibrahim and Jenkinson 2002; Ying and Feng 2006; Rangel et al. 2008). Elevated temperatures reduce spore viability, growth, germination and virulence (Anderson and Smith 1972; McCammon and Rath 1994; Inglis et al. 1996; Rangel et al. 2010; Tumuhaise et al. 2018). It is possible, however, to optimize both conidial production and thermotolerance by efficient culture conditions and/or supply of culture media (Ouedraogo et al. 1997; Cabanillas and Jones 2009; Kim et al. 2010c). Accordingly, *Isaria* spp. showed greater tolerance (from 20 to 30 °C) after growing on Sabouraud Dextrose Agar Yeast extract (SDAY) than fungi grown on Sabouraud Maltose Agar (SMA) (Cabanillas and Jones 2009). Submerged cultures of *I. fumosorosea* grown in Sabouraud Dextrose Broth (SDB) were able to develop well from 20 to 34 °C, showing optimal growth at 28 °C. In solid fermentations, however, these strains grew optimally at 25 °C (Esther et al. 2013). Although the excess of heat on conidia causes unviability in the derived fungal propagules, it is possible to improve thermotolerance from 26 to 30 °C by increasing the sucrose content in the culture media (McClatchie et al. 1994). Conidia of *M. robertsii* produced on potato, dextrose, agar and yeast extract (PDAY) medium, containing low concentrations of salicylic acid, demonstrated increased tolerance to heat (Rangel et al. 2012). Millet grain was used as a substrate to produce conidia by *B. bassiana* and *M. anisopliae*, potentially enhancing conidial thermotolerance of fungi grown on a massive production system (Kim et al. 2011).

*Metarhizium acridum* grows on agar medium and produces both aerial and microcycle conidia. Tolerance of both propagules was compared at 40–45 °C, showing that microcycle conidia were more heat resistant than normal aerial conidia (Zhang et al. 2010). Microcycle conidiation is defined as a process in which the germination of spores directly produces the formation of conidia, without the intervention of an intermediate mycelial growth. This microcycle conidiation can be induced by manipulation of environmental conditions, especially culture conditions

that are stressful to fungi (Hanlin, 1994; Bosch and Yantorno 1999; Zhang et al. 2010; Jung et al. 2014).

A relationship between thermotolerance and hydrophobicity can be traced. Employing a siloxane-mediated conidial collection method based on hydrophobicity, it is possible to classify conidia from *B. bassiana* and *M. anisopliae* into two groups with different thermotolerance (Kim et al. 2010b). Similar results were observed for species with hydrophobic conidia such as *B. bassiana*, *M. brunneum*, *M. robertsii*, *M. anisopliae* and *I. fumosorosea*, which were more thermotolerant than species with hydrophilic conidia such as *Tolypocladium cylindrosporum*, *T. inflatum*, *Simplicillium lanosoniveum*, *Lecanicillium aphanocladii*, *Aschersonia placenta* and *A. aleyrodis* (Souza et al. 2014). The sugar content (types and concentrations) used as carbon source in culture media for conidial production may affect both conidial thermotolerance and hydrophobin-like or formic-acid-extractable (FAE) protein content (Ying and Feng 2004), (see Sect. 6.5).

## 6.4 Effect of Abiotic Factors on Conidial Germination

The interaction of abiotic factors (temperature, humidity, light, pH) are important to germination, dispersion, and development of fungal conidia (Glare et al. 1986; Jaronski 2010; Osherov and May 2001; Oliveira et al. 2018). Water is fundamental to start conidial germination. However, the interaction between water and temperature can reduce the viability and/or limit conidial viability of some EF, e.g., *B. bassiana*, *M. anisopliae*, *I. farinosa* and *I. fumosorosea* require humidity and optimum temperature conditions to their development (Hallsworth and Magan 1999; Devi et al. 2005; Borisade and Magan 2014).

Humidity and temperature are key factors in activation of metabolic pathways allowing the nutrients mobilization required for conidial germination, but are also important during storage periods, longevity and persistence of quiescent conidia (Daoust and Roberts 1983; Dillon and Charnley 1990, Bouamama et al. 2010). These factors also affect physiological interactions between the host and pathogen (Walstad et al. 1970; Luz and Fargues 1997; James et al. 1998). The ultraviolet (UV) radiation (UV-A and UV-B) also affects conidial germination of *M. acridum* and *M. robertsii*, limiting entomopathogenic fungal development, however increasing thermotolerance by accumulating trehalose and mannitol (Braga et al. 2001; Pereira-Junior et al. 2018; Rangel and Roberts 2018).

## 6.5 Thermal Effects and Metabolic Processes

EF have mechanisms to overcome and circumvent thermal effects (Rangel et al. 2010; Tseng et al. 2011; Keyser et al. 2014), triggering signal transduction and metabolic pathways that synthesize the molecules that will ultimately protect the

fungal cells from damage caused by heat exposure (Farrell and Rose 1967; Ying and Feng 2004; Zhang et al. 2011; Liao et al. 2014; Wang et al. 2017, 2018). Among them, fungal proteins of hydrophobic nature associated to cell walls are often linked with thermal protecting functions. Hydrophobins are small proteins important for fungal growth and development (Wösten and Vocht 2000). Hydrophobin-like or formic-acid-extractable (FAE) proteins were studied in aerial conidia of *B. bassiana* and *I. fumosorosea* based on thermotolerance. FAE proteins provide hydrophobicity to conidia, exhibiting different composition between *B. bassiana* and *I. fumosorosea*. For both fungi, conidial viabilities decreased after exposure to heat stress (48 °C for up to 150 min), perhaps as a result of different conidial structure related to FAE proteins (Ying and Feng 2004). Zhang et al. (2011) characterized structurally the cell wall carbohydrates in *B. bassiana*, and demonstrated that targeted gene knockouts lacking  $\beta$ -1,3-glucanosyltransferase destabilize the cell wall and decreased germination after 1 to 4 h of heat shock at temperatures >40 °C.

Trehalose is a disaccharide that accumulates in fungi during stress situations, such as adverse growth conditions, heat, and hyperoxidative shock. Thus, along with other polyols these molecules are known as stress metabolites (Van Laere 1989; Fillinger et al. 2001; Liu et al. 2009). Polyols accumulation is associated with thermotolerance by helping in the stabilization of structure (and function) of proteins and enzymes at high temperatures (Kim and Lee 1993). Accumulation of glycerol, erythritol, arabinol, mannitol, and trehalose in conidia of *M. anisopliae*, *B. bassiana*, and *I. farinosa* under different culture age (up to 120 days), temperature (5–35 °C) and pH (2.9–11.1) were reported by Hallsworth and Magan (1996). Also, high accumulation of both trehalose and mannitol were observed in abiotic stressed conidia of *M. acridum*, suggesting they are part of a mechanism that the fungus uses to attain its high tolerance to UV-B radiation and heat (Rangel and Roberts 2018).

The heat stress also triggers the production of toxic reactive oxygen species (ROS), favoring oxidative stress in fungal propagules (Zhang and Feng 2018). Catalase is an antioxidant enzyme characterized in *B. bassiana* (Pedrini et al. 2006) that showed to be an important regulator of conidial thermotolerance (Wang et al. 2013). The relationship between oxidative stress and elevated culture temperature also was reported for *Aspergillus niger* (Bai et al. 2003). The heat shock proteins (HSPs) are also associated with tolerance to heat: overexpressing the gene encoding for HSP25 in *M. robertsii* increased fungal growth under heat stress either in nutrient-rich medium or on insect wings, and also enhanced the tolerance of heat shock-treated conidia to osmotic stress (Liao et al. 2014).

## 6.6 Conidial Formulation and Thermotolerance

Formulations preserve the viability of conidia exposed to environmental stresses, improving the efficiency of fungal propagules in microbial control (Faria and Wraight 2007). Conidial formulations based on oil or oil-in-water emulsions are investigated because the combination of conidia with oils improved their

performance against heat stress (Malsam et al. 2002; Mendonça et al. 2007; Barreto et al. 2016; Paixao et al. 2017; Oliveira et al. 2018).

Oil-based formulations of *M. anisopliae* s.s. were used to improve both germination and appressorium production in conidia used for tick control (Barreto et al. 2016; Alves et al. 2017), and also to protect conidia against the effect of solar radiation (Alves et al. 1998) and high temperatures (McClatchie et al. 1994). Conidia of *M. anisopliae* s.l. and *M. robertsii* formulated on either vegetable or mineral oils were more tolerant to heat stress than those either unformulated or formulated on carboxymethylcellulose gel (Paixao et al. 2017). *M. anisopliae* and *B. bassiana* viability also increases when fungi are formulated in emulsifiable oil (Oliveira et al. 2018), and vegetable oil improved both performance and thermotolerance of *B. bassiana* (Kim et al. 2010a). Thus, formulation is considering a very important tool to manage heat stress on conidia.

## 6.7 Conclusion

Temperature is a key factor that limits survival of entomopathogenic fungal conidia used in biological control programs. As detailed in this chapter, most of the investigations in this area have concentrated in: (i) fungal screening for thermotolerance, based on geographical origin of the strains (McCammon and Rath 1994; Morley-Davies et al. 1996; Fargues et al. 1997; De Croos and Bidochka 1999; Devi et al. 2005), (ii) test of tolerance to low or high temperature (Fernandes et al. 2008; Paixão et al. 2017), (iii) appropriate culture medium for conidial production (Hallsworth and Magan 1999; Cabanillas and Jones 2009; Esther et al. 2013), (iv) formulations to increase conidia thermotolerance and protection (Hedgecock et al. 1995; Barreto et al. 2016; Paixão et al. 2017), and (v) biological/molecular characteristics and mechanisms that mediate stress tolerance (Liu et al. 2009; Fernandes et al. 2010; Rangel et al. 2018). On the basis of the literature available, we can conclude that EF are promising tools against many arthropods (Zimmermann 2007; Faria and Wraight 2007). However, additional research is still needed mostly in both screening of thermotolerant strains and formulation types of fungal propagules, to circumvent the negative effects of abiotic factors that potentially limits their efficacy, thus improving the use of EF in biological control programs.

## References

- Alves, R. T., Bateman, R. P., Prior, C., & Leather, S. R. (1998). Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. *Crop Protection*, 17, 675–679.
- Alves, F. M., Bernardo, C. C., Paixão, F. R., Barreto, L. P., Luz, C., Humber, R. A., & Fernandes, É. K. (2017). Heat-stressed *Metarhizium anisopliae*: Viability (in vitro) and virulence (in vivo) assessments against the tick *Rhipicephalus sanguineus*. *Parasitology Research*, 116, 111–121.

- Anderson, J. G., & Smith, J. E. (1972). The effects of elevated temperatures on spore swelling and germination in *Aspergillus niger*. *Canadian Journal of Microbiology*, 18, 289–297.
- Bai, Z., Harvey, L. M., & McNeil, B. (2003). Elevated temperature effects on the oxidant/antioxidant balance in submerged batch cultures of the filamentous fungus *Aspergillus niger* B1-D. *Biotechnology and Bioengineering*, 83, 772–779.
- Barreto, L. P., Luz, C., Mascarin, G. M., Roberts, D. W., Arruda, W., & Fernandes, É. K. (2016). Effect of heat stress and oil formulation on conidial germination of *Metarhizium anisopliae* ss on tick cuticle and artificial medium. *Journal of Invertebrate Pathology*, 138, 94–103.
- Bidochka, M. J., Kamp, A. M., Lavender, T. M., Dekoning, J., & De Croos, J. N. (2001). Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: Uncovering cryptic species? *Applied and Environmental Microbiology*, 67, 1335–1342.
- Borisade, O. A., & Magan, N. (2014). Growth and sporulation of entomopathogenic *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa* and *Isaria fumosorosea* strains in relation to water activity and temperature interactions. *Biocontrol Science and Technology*, 24, 999–1011.
- Bosch, A., & Yantorno, O. (1999). Microcycle conidiation in the entomopathogenic fungus *Beauveria bassiana* bals. (vuill.). *Process Biochemistry*, 34, 707–716.
- Bouamama, N., Vidal, C., & Fargues, J. (2010). Effects of fluctuating moisture and temperature regimes on the persistence of quiescent conidia of *Isaria fumosorosea*. *Journal of Invertebrate Pathology*, 105, 139–144.
- Braga, G. U., Flint, S. D., Miller, C. D., Anderson, A. J., & Roberts, D. W. (2001). Both solar UVA and UVB radiation impair conidial culturability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae*. *Photochemistry and Photobiology*, 74, 734–739.
- Cabanillas, H. E., & Jones, W. A. (2009). Effects of temperature and culture media on vegetative growth of an entomopathogenic fungus *Isaria* sp. (Hypocreales: Clavicipitaceae) naturally affecting the whitefly, *Bemisia tabaci* in Texas. *Mycopathologia*, 167, 263–271.
- Crisan, E. V. (1973). Current concepts of thermophilism and the thermophilic fungi. *Mycologia*, 65, 1171–1198.
- Croos, J. N., & Bidochka, M. J. (1999). Effects of low temperature on growth parameters in the entomopathogenic fungus *Metarhizium anisopliae*. *Canadian Journal of Microbiology*, 45, 1055–1061.
- Croos, J. N., & Bidochka, M. J. (2001). Cold-induced proteins in cold-active isolates of the insect-pathogenic fungus *Metarhizium anisopliae*. *Mycological Research*, 105, 868–873.
- Daoust, R. A., & Roberts, D. W. (1983). Studies on the prolonged storage of *Metarhizium anisopliae* conidia: Effect of temperature and relative humidity on conidial viability and virulence against mosquitoes. *Journal of Invertebrate Pathology*, 41, 143–150.
- Devi, K. U., Sridevi, V., Mohan, C. M., & Padmavathi, J. (2005). Effect of high temperature and water stress on in vitro germination and growth in isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. *Journal of Invertebrate Pathology*, 88, 181–189.
- Dillon, R. J., & Charnley, A. K. (1990). Initiation of germination in conidia of the entomopathogenic fungus, *Metarhizium anisopliae*. *Mycological Research*, 94, 299–304.
- Dimbi, S., Maniania, N. K., Lux, S. A., & Mueke, J. M. (2004). Effect of constant temperatures on germination, radial growth and virulence of *Metarhizium anisopliae* to three species of African tephritid fruit flies. *BioControl*, 49, 83–94.
- Edelstein, J. D., Trumper, E. V., & Lecuona, R. E. (2005). Temperature-dependent development of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson in *Anticarsia gemmatilis* (Hübner) larvae (Lepidoptera: Noctuidae). *Neotropical Entomology*, 34, 593–599.
- Elliot, S. L., Blanford, S., & Thomas, M. B. (2002). Host–pathogen interactions in a varying environment: Temperature, behavioural fever and fitness. *Proceedings of the Royal Society of London B: Biological Sciences*, 269, 1599–1607.
- Esther, C. P., Erika, A. S., María, M. C. R., & de la Torre, M. (2013). Performance of two isolates of *Isaria fumosorosea* from hot climate zones in solid and submerged cultures and thermotolerance of their propagules. *World Journal of Microbiology and Biotechnology*, 29, 309–317.



- Fargues, J., & Bon, M. C. (2004). Influence of temperature preferences of two *Paecilomyces fumosoroseus* lineages on their co-infection pattern. *Journal of Invertebrate Pathology*, *87*, 94–104.
- Fargues, J., Goettel, M. S., Smits, N., Ouedraogo, A., & Rougier, M. (1997). Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. *Mycologia*, *89*, 383–392.
- Faria, M. R., & Wraight, S. P. (2007). Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*, *43*, 237–256.
- Farrell, J., & Rose, A. (1967). Temperature effects on microorganisms. *Annual Reviews in Microbiology*, *21*, 101–120.
- Fernandes, E. K., Rangel, D. E., Moraes, Á. M., Bittencourt, V. R., & Roberts, D. W. (2008). Cold activity of *Beauveria* and *Metarhizium*, and thermotolerance of *Beauveria*. *Journal of Invertebrate Pathology*, *98*, 69–78.
- Fernandes, É. K., Keyser, C. A., Chong, J. P., Rangel, D. E., Miller, M. P., & Roberts, D. W. (2010). Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. *Journal of Applied Microbiology*, *108*, 115–128.
- Fillinger, S., Chaveroche, M. K., Van Dijck, P., de Vries, R., Ruijter, G., Thevelein, J., & d'Enfert, C. (2001). Trehalose is required for the acquisition of tolerance to a variety of stresses in the filamentous fungus *Aspergillus nidulans*. *Microbiology*, *147*, 1851–1862.
- Glare, T. R., Milner, R. J., & Chilvers, G. A. (1986). The effect of environmental factors on the production, discharge, and germination of primary conidia of *Zoophthora phalloides* Batko. *Journal of Invertebrate Pathology*, *48*, 275–283.
- Gottlieb, D. (1950). The physiology of spore germination in fungi. *The Botanical Review*, *16*, 229–257.
- Hallsworth, J. E., & Magan, N. (1996). Culture age, temperature, and pH affect the polyol and trehalose contents of fungal propagules. *Applied and Environmental Microbiology*, *62*, 2435–2442.
- Hallsworth, J. E., & Magan, N. (1999). Water and temperature relations of growth of the entomogenous fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. *Journal of Invertebrate Pathology*, *74*, 261–266.
- Hanlin, R. T. (1994). Microcycle conidiation—A review. *Mycoscience*, *35*, 113–123.
- Hedgecock, S., Moore, D., Higgins, P. M., & Prior, C. (1995). Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. *Biocontrol Science and Technology*, *5*, 371–378.
- Ibrahim, L., & Jenkinson, P. (2002). Effect of artificial culture media on germination, growth, virulence and surface properties of the entomopathogenic hyphomycete *Metarhizium anisopliae*. *Mycological Research*, *106*, 705–715.
- Ignoffo, C. M., Garcia, C., & Hostetter, D. L. (1976). Effects of temperature on growth and sporulation of the entomopathogenic fungus *Nomuraea rileyi*. *Environmental Entomology*, *5*, 935–936.
- Inglis, G. D., Johnson, D. L., & Goettel, M. S. (1996). Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. *Biological Control*, *7*, 131–139.
- James, R. R., Croft, B. A., Shaffer, B. T., & Lighthart, B. (1998). Impact of temperature and humidity on host–pathogen interactions between *Beauveria bassiana* and a coccinellid. *Environmental Entomology*, *27*, 1506–1513.
- Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens. *BioControl*, *55*, 159–185.
- Jung, B., Kim, S., & Lee, J. (2014). Microcycle conidiation in filamentous fungi. *Mycobiology*, *42*, 1–5.
- Keyser, C. A., Fernandes, É. K., Rangel, D. E., & Roberts, D. W. (2014). Heat-induced post-stress growth delay: A biological trait of many *Metarhizium* isolates reducing biocontrol efficacy? *Journal of Invertebrate Pathology*, *120*, 67–73.

- Kim, D., & Lee, Y. J. (1993). Effect of glycerol on protein aggregation: Quantitation of thermal aggregation of proteins from CHO cells and analysis of aggregated proteins. *Journal of Thermal Biology*, *18*, 41–48.
- Kim, J. S., Skinner, M., & Parker, B. L. (2010a). Plant oils for improving thermotolerance of *Beauveria bassiana*. *Journal of Microbiology and Biotechnology*, *20*, 1348–1350.
- Kim, J. S., Skinner, M., Hata, T., & Parker, B. L. (2010b). Effects of culture media on hydrophobicity and thermotolerance of Bb and Ma conidia, with description of a novel surfactant-based hydrophobicity assay. *Journal of Invertebrate Pathology*, *105*, 322–328.
- Kim, J. S., Je, Y. H., & Roh, J. Y. (2010c). Production of thermotolerant entomopathogenic *Isaria fumosorosea* SFP-198 conidia in corn-corn oil mixture. *Journal of Industrial Microbiology and Biotechnology*, *37*, 419–423.
- Kim, J. S., Kassa, A., Skinner, M., Hata, T., & Parker, B. L. (2011). Production of thermotolerant entomopathogenic fungal conidia on millet grain. *Journal of Industrial Microbiology and Biotechnology*, *38*, 697–704.
- Lacey, L. A., Frutos, R., Kaya, H. K., & Vail, P. (2001). Insect pathogens as biological control agents: Do they have a future? *Biological Control*, *21*, 230–248.
- Li, J., & Feng, M. G. (2009). Intraspecific tolerance of *Metarhizium anisopliae* conidia to the upper thermal limits of summer with a description of a quantitative assay system. *Mycological Research*, *113*, 93–99.
- Liao, X., Lu, H. L., Fang, W., & Leger, R. J. S. (2014). Overexpression of a *Metarhizium robertsii* HSP25 gene increases thermotolerance and survival in soil. *Applied Microbiology and Biotechnology*, *98*, 777–783.
- Liu, Q., Ying, S. H., Feng, M. G., & Jiang, X. H. (2009). Physiological implication of intracellular trehalose and mannitol changes in response of entomopathogenic fungus *Beauveria bassiana* to thermal stress. *Antonie Van Leeuwenhoek*, *95*, 65–75.
- Luz, C., & Fargues, J. (1997). Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana*, pathogenic to *Rhodnius prolixus*. *Mycopathologia*, *138*, 117–125.
- Malsam, O., Kilian, M., Oerke, E. C., & Dehne, H. W. (2002). Oils for increased efficacy of *Metarhizium anisopliae* to control whiteflies. *Biocontrol Science and Technology*, *12*, 337–348.
- McCammon, S. A., & Rath, A. C. (1994). Separation of *Metarhizium anisopliae* strains by temperature dependent germination rates. *Mycological Research*, *98*, 1253–1257.
- McClatchie, G. V., Moore, D., Bateman, R. P., & Prior, C. (1994). Effects of temperature on the viability of the conidia of *Metarhizium flavoviride* in oil formulations. *Mycological Research*, *98*, 749–756.
- Mendonça, C. G. D., Raetano, C. G., & Mendonça, C. G. D. (2007). Tensão superficial estática de soluções aquosas com óleos minerais e vegetais utilizados na agricultura. *Engenharia Agrícola*, *27*, 16–23.
- Morley-Davies, J., Moore, D., & Prior, C. (1996). Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to simulated sunlight and a range of temperatures. *Mycological Research*, *100*, 31–38.
- Oliveira, D. G. P., Lopes, R. B., Rezende, J. M., & Delalibera, I., Jr. (2018). Increased tolerance of *Beauveria bassiana* and *Metarhizium anisopliae* conidia to high temperature provided by oil-based formulations. *Journal of Invertebrate Pathology*, *151*, 151–157.
- Osharov, N., & May, G. S. (2001). The molecular mechanisms of conidial germination. *FEMS Microbiology Letters*, *199*, 153–160.
- Ouedraogo, A., Fargues, J., Goettel, M. S., & Lomer, C. J. (1997). Effect of temperature on vegetative growth among isolates of *Metarhizium anisopliae* and *M. flavoviride*. *Mycopathologia*, *137*, 37–43.
- Paixao, F. R. S., Muniz, E. R., Barreto, L. P., Bernardo, C. C., Mascarin, G. M., Luz, C., & Fernandes, É. K. (2017). Increased heat tolerance afforded by oil-based conidial formulations of *Metarhizium anisopliae* and *Metarhizium robertsii*. *Biocontrol Science and Technology*, *27*, 324–337.

- Pedrini, N. (2018). Molecular interactions between entomopathogenic fungi (Hypocreales) and their insect host: Perspectives from stressful cuticle and hemolymph battlefields and the potential of dual RNA sequencing for future studies. *Fungal Biology*, *122*, 538–545.
- Pedrini, N., Juárez, M. P., Crespo, R., & de Alaniz, M. J. (2006). Clues on the role of *Beauveria bassiana* catalases in alkane degradation events. *Mycologia*, *98*, 528–534.
- Pereira-Junior, R. A., Huarte-Bonnet, C., Paixão, F. R., Roberts, D. W., Luz, C., Pedrini, N., & Fernandes, É. K. (2018). Riboflavin induces *Metarhizium* spp. to produce conidia with elevated tolerance to UV-B, and upregulates photolyases, laccases and polyketide synthases genes. *Journal of Applied Microbiology*, *125*, 159–171.
- Rangel, D. E., & Roberts, D. W. (2018). Possible source of the high UV-B and heat tolerance of *Metarhizium acridum* (isolate ARSEF 324). *Journal of Invertebrate Pathology*, *157*, 32–35.
- Rangel, D. E., Braga, G. U., Anderson, A. J., & Roberts, D. W. (2005). Variability in conidial thermotolerance of *Metarhizium anisopliae* isolates from different geographic origins. *Journal of Invertebrate Pathology*, *88*, 116–125.
- Rangel, D. E., Alston, D. G., & Roberts, D. W. (2008). Effects of physical and nutritional stress conditions during mycelial growth on conidial germination speed, adhesion to host cuticle, and virulence of *Metarhizium anisopliae*, an entomopathogenic fungus. *Mycological Research*, *112*, 1355–1361.
- Rangel, D. E., Fernandes, É. K., Dettenmaier, S. J., & Roberts, D. W. (2010). Thermotolerance of germings and mycelium of the insect-pathogenic fungus *Metarhizium* spp. and mycelial recovery after heat stress. *Journal of Basic Microbiology*, *50*, 344–350.
- Rangel, D. E., Fernandes, É. K., Anderson, A. J., & Roberts, D. W. (2012). Culture of *Metarhizium robertsii* on salicylic-acid supplemented medium induces increased conidial thermotolerance. *Fungal Biology*, *116*, 438–442.
- Rangel, D. E., Finlay, R. D., Hallsworth, J. E., Dadachova, E., & Gadd, G. M. (2018). Fungal strategies for dealing with environment-and agriculture-induced stresses. *Fungal Biology*, *122*, 602–612.
- Santos, M. P., Dias, L. P., Ferreira, P. C., Pasin, L. A., & Rangel, D. E. (2011). Cold activity and tolerance of the entomopathogenic fungus *Tolypocladium* spp. to UV-B irradiation and heat. *Journal of Invertebrate Pathology*, *108*, 209–213.
- Souza, R. K., Azevedo, R. F., Lobo, A. O., & Rangel, D. E. (2014). Conidial water affinity is an important characteristic for thermotolerance in entomopathogenic fungi. *Biocontrol Science and Technology*, *24*, 448–461.
- Tseng, M. N., Chung, P. C., & Tzean, S. S. (2011). Enhancing the stress tolerance and virulence of an entomopathogen by metabolic engineering DHN-melanin biosynthesis genes. *Applied and Environmental Microbiology*, *77*, 4508–4519, AEM-02033.
- Tumuhaise, V., Ekesi, S., Maniania, N. K., Tonnang, H. E. Z., Tanga, C. M., Ndegwa, P. N., Irungu, L. W., Srinivasan, R., & Mohamed, S. A. (2018). Temperature-dependent growth and virulence, and mass production potential of two candidate isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin for managing *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) on cowpea. *African Entomology*, *26*, 73–83.
- Van Laere, A. (1989). Trehalose, reserve and/or stress metabolite? *FEMS Microbiology Letters*, *63*, 201–209.
- Vidal, C., Fargues, J., & Lacey, L. A. (1997). Intraspecific variability of *Paecilomyces fumosoroseus*: Effect of temperature on vegetative growth. *Journal of Invertebrate Pathology*, *70*, 18–26.
- Walstad, J. D., Anderson, R. F., & Stambaugh, W. J. (1970). Effects of environmental conditions on two species of muscardine fungi (*Beauveria bassiana* and *Metarrhizium anisopliae*). *Journal of Invertebrate Pathology*, *16*, 221–226.
- Wang, Z. L., Zhang, L. B., Ying, S. H., & Feng, M. G. (2013). Catalases play differentiated roles in the adaptation of a fungal entomopathogen to environmental stresses. *Environmental Microbiology*, *15*, 409–418.

- Wang, J., Ying, S. H., Hu, Y., & Feng, M. G. (2017). Vital role for the J-domain protein Mdj1 in asexual development, multiple stress tolerance, and virulence of *Beauveria bassiana*. *Applied Microbiology and Biotechnology*, *101*, 185–195.
- Wang, Z., Zhou, Q., Li, Y., Qiao, L., Pang, Q., & Huang, B. (2018). iTRAQ-based quantitative proteomic analysis of conidia and mycelium in the filamentous fungus *Metarhizium robertsii*. *Fungal Biology*, *122*, 651–658.
- Wösten, H. A., & de Vocht, M. L. (2000). Hydrophobins, the fungal coat unravelled. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes*, *1469*, 79–86.
- Ying, S. H., & Feng, M. G. (2004). Relationship between thermotolerance and hydrophobin-like proteins in aerial conidia of *Beauveria bassiana* and *Paecilomyces fumosoroseus* as fungal biocontrol agents. *Journal of Applied Microbiology*, *97*, 323–331.
- Ying, S. H., & Feng, M. G. (2006). Medium components and culture conditions affect the thermotolerance of aerial conidia of fungal biocontrol agent *Beauveria bassiana*. *Letters in Applied Microbiology*, *43*, 331–335.
- Zhang, L. B., & Feng, M. G. (2018). Antioxidant enzymes and their contributions to biological control potential of fungal insect pathogens. *Applied Microbiology and Biotechnology*, *102*, 4995–5004.
- Zhang, S., Peng, G., & Xia, Y. (2010). Microcycle conidiation and the conidial properties in the entomopathogenic fungus *Metarhizium acridum* on agar medium. *Biocontrol Science and Technology*, *20*, 809–819.
- Zhang, S., Xia, Y., & Keyhani, N. O. (2011). Contribution of the *gas1* gene of the entomopathogenic fungus *Beauveria bassiana*, encoding a putative glycosylphosphatidylinositol-anchored  $\beta$ -1, 3-glucanase, to conidial thermotolerance and virulence. *Applied and Environmental Microbiology*, *77*, 2676–2684.
- Zimmermann, G. (1982). Effect of high temperatures and artificial sunlight on the viability of conidia of *Metarhizium anisopliae*. *Journal of Invertebrate Pathology*, *40*, 36–40.
- Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Science and Technology*, *17*, 879–920.