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Relative humidity, temperature, and the equilibrium moisture content of conidia of *Beauveria bassiana* (Balsamo) Vuillemin: a quantitative approach

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Abstract

Isotherms for conidia of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin were determined at nine constant temperatures between 10° and 50°C using saturated salt solutions. Seven different mathematical models were compared to quantify the effect of temperature and relative humidity on the equilibrium moisture content of conidia. A seven-term polynomial regression model with moisture content expressed on the fresh weight basis (R^2= 0.998, 103 *d.f.*) was the best and provided no systematic deviation between observed and fitted moisture contents. This model will be a component of a GIS to determine the potential utility of *B. bassiana* to control grain borers in grain stores throughout the tropics.

Keywords: Beauveria bassiana (Balsamo) Vuillemin; Conidia; Isotherm; Equilibrium moisture content; Relative humidity

1. Introduction

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has considerable potential for the control of the larger grain borer (*Prostephanus truncatus* Horn) of maize (*Zea mays* L.) and cassava (*Manihot esculenta* Crantz) (Smith *et al*., 1999). *P. truncatus* was introduced accidentally into East Africa in the late 1970s and is regarded as a devastating pest (Farrell *et al*., 1996). *B. bassiana* can also control other grain borers such as *Sitophilus oryzae* L., *Rhyzopertha dominica* F. and *Tribolium castaneum* Herbst (Rice and Cogburn, 1999).

Conidia of entomopathogenic fungi must retain high viability and virulence for effective biological control (McClatchie *et al*., 1994). The conidia must, therefore, have predictable shelf lives. To control the larger grain borer in stored maize grain conidia survival needs to be predicted until application (during which period environment can be controlled to a greater or lesser extent), but also subsequently within grain stores (where environment is unlikely to be controlled, and for which the precise conditions will vary with location).

The prediction of conidia survival is now possible using equations driven by temperature, even if temperature fluctuates, and either conidia moisture content or equilibrium relative humidity (Hong, Ellis and Moore, 1997; Hong *et al*., 1998, 1999). The use of conidia moisture content is more accurate than equilibrium relative humidity in these equations (Hong *et al*., 1997, 1998). However, environmental information will tend to comprise relative humidity. First, in a grain store, it is possible to obtain real time measurement of relative humidity. Secondly, modelling based on geographical information systems (GIS) and the like will utilise temperature and relative humidity data from meteorological records. The objective of this investigation was thus to determine isotherms (equilibrium relations between relative humidity and moisture content) for conidia of *B. bassiana* at different temperatures and then identify the most suitable model to quantify these relations.

2. Materials and methods

All investigations were conducted using conidia of a commercially-grown isolate of *B. bassiana* (JABB ID 9908-1330, JABB of the Carolinas Inc., USA). Conidia moisture content at receipt was 11.9% (w.b.) in equilibrium with 55% r.h. The equilibrium relative humidity of conidia was determined at 20°C using a Humidat IC1 (Novasina, Zürich), previously calibrated at 11, 55 and 90% r.h. Conidia were sealed in an aluminium foil packet and stored at 0°C for 14 d before the investigation began.

2.1.Duration to equilibrium

About 1 g of conidia were placed in a dish (4 cm diameter, 1 cm high) to a depth of 0.7 cm. Four dishes with conidia were initially placed in a desiccator over a saturated solution of potassium nitrate (KNO_3) (93% r.h.) at 20°C in order to raise conidia equilibrium relative humidity to about 93%. Another four dishes were placed in a desiccator over a saturated solution of sodium hydroxide (NaOH) (7.5% r.h.) at 20°C in order to reduce conidia equilibrium relative humidity to about 7.5%. Once the conidia reached each target relative humidity, the four dishes with conidia at 93% r.h. were placed in two desiccators (i.e. two dishes as two replicates in each desiccator) over a saturated solution of sodium hydroxide, and the four dishes with conidia at 7.5% r.h. were placed in two desiccators over a saturated solution of potassium nitrate. One pair of desiccators (one with sodium hydroxide and one with potassium nitrate solution) were placed in incubators maintained at 10°C, and the other pair in incubators maintained at 50°C. The equilibrium relative humidity of the conidia was determined at frequent intervals (1 h to 1 d, depending upon temperature).

2.2. Determination of isotherms

Two samples (each of 1 g) of conidia of *B. bassiana* were stored in each of 110 desiccators maintained at nine temperatures (10, 15, 20, 25, 30, 35, 40, 45 and 50°C) in combination with 10-14 different relative humidities between 5.5 and 96%. Relative humidity was controlled by saturated salt solutions (Table 1). Note that temperature affects the relative humidity provided by saturated salt solutions. This effect can range from negligible (e.g. zinc chloride) to substantial (e.g. sodium bromide) (Table 1). Each sample of conidia (1 g) was placed in a dish within a desiccator above the saturated salt solution, as described above, and moisture content determined at equilibrium, normally after 7 to 14 d exposure. These durations were determined following the results of the initial investigation (above). Conidia moisture content was determined gravimetrically by drying two replicates of 1 g each in a mechanically-ventilated oven at 103 ± 2°C for 17 h. Moisture content was calculated on both the dry weight basis (M_D, d.b.) and fresh weight basis (M, w.b.). The isotherms at all nine temperatures were then quantified by seven different mathematical models developed originally for isotherms of seeds (Table 2).

In order to compare the models fitted, the standard error of fitted moisture contents (*SEMC*) (Chen and Clayton, 1971; Ajibola, 1986; Tagawa *et al.*, 1993) and mean relative percentage error (E, %) (Mazza *et al.*, 1990; 1994; Jayas and Mazza, 1991) were calculated as follows:

$$E\ (\%) = (100/n)[\Sigma(M_o - M_f)/M_o]$$

$$SEMC = [\Sigma(M_o - M_f)^2/df]^{1/2}$$

where n is the number of observations, df is degrees of freedom of the fitted model, M_o and M_f are observed and fitted moisture content, respectively.

3. Results and discussion

3.1. Duration to equilibrium

The duration to equilibrium between conidia moisture content and relative humidity was shorter with desorption than with absorption, and not surprisingly shorter at 50°C than at 10°C (Fig. 1). In general, it took about 6 h and 6 d for the equilibrium relative humidity of conidia to decline from 93 to 7.5% at 50°C and 10°C, respectively, and about 2 d and 7 d for equilibrium relative humidity to increase from 7.5 to 93% at 50°C and 10°C, respectively.

3.2. Isotherms

The equilibrium moisture content of conidia decreased the warmer the temperature at a given relative humidity and the lower the relative humidity at a given temperature (Fig. 2). For example, equilibrium moisture contents of conidia stored over saturated solutions of magnesium chloride ($MgCl_2$) at 10° (34% r.h.) and 50°C (31.5% r.h.) (Table 1) were 8.4 and 5.8%, respectively, but over saturated solutions of sodium chloride at 10° (75.5% r.h.) and 50°C (74.5% r.h.) they were 19.8 and 19.5% moisture content, respectively (Fig. 2). Note that above about 50% r.h. the effect of temperature on equilibrium moisture content at a given relative humidity diminished such that at 85% r.h. and above no effect of temperature was discernible.

Each of seven models (Table 2) was fitted to the 110 observations, using GENSTAT (GENSTAT Committee, 1997), in order to determine which best quantified the equilibrium relation between conidia moisture content and relative humidity at different temperatures. However, prior to this it was necessary to determine the precise terms to apply within the polynomial models (6 and 7 in Table 2). The complete models tested were

$$M_D = a + bR + cT + dR^2 + eRT + fR^3 + gR^2T + hT^2 + iT^3 + kRT^2$$

$$M = a + bR + cT + dR^2 + eRT + fR^3 + gR^2T + hT^2 + iT^3 + kRT^2$$

where M and M_D are conidia moisture contents (%) expressed on the fresh weight basis (w.b.) and dry weight basis (d.b.), respectively; R is relative humidity (%), T is temperature (°C), and a, b, c, d, e, f, g, h, i, k are constants (Fang *et al.*, 1998).

The most suitable polynomial model was selected by determining, iteratively, whether or not the inclusion of each of the ten model parameters shown above enhanced model fit significantly ($P = 0.05$). This analysis, using GLIM (Baker and Nelder, 1978), showed that seven-term models were appropriate ($P < 0.005$, 103 d.f., $R^2 = 0.994$ and 0.998 for M_D and M, respectively (Table 2). These seven terms (Table 2) were precisely the same as those seven selected by Fang *et al.* (1998) to quantify the effect of temperature and relative humidity on the equilibrium moisture content of seeds of six species.

Comparison of the fit of the seven models showed clearly that the equilibrium moisture contents of conidia of *B. bassiana* stored at relative humidities between 5.5 and 96% in combination with temperatures between 10 and 50°C were best quantified by the polynomial regression model with moisture content expressed on the fresh weight basis (Table 3, Fig. 3). The full model (with s.e.) is

$$\begin{array}{l} M = 2.426 + 0.338R - 0.035T - 0.0071R^2 - 0.00081RT + 0.000077R^3 + 0.000013R^2T \\ \quad\;\; (0.322) \quad (0.023) \quad (0.009) \quad (0.0005) \quad\quad (0.00045) \quad\quad (0.000003) \quad\quad (0.000005) \end{array}$$

and was the only model fitted which did not result in systematic deviation of observations from fitted values (compare Fig. 3g with Figs 3a-f).

The observations, and hence the model fitted, capture the full range of diurnal and seasonal variation in environment applicable to the role of *B. bassiana* in controlling grain borers in grain stores in the tropics. For example, at Niamey (Niger) during January – March ambient relative humidity is as low as 28 and 11% at 6.00 am and at noon, respectively, and temperature is about 15 and 37°C, respectively, while during July – September, relative humidity is 82 and 60%, and temperature is 33 and 23°C, respectively (Anon., 1958).

The application of GIS combined with meteorological data and equations which describe biological responses to environment has already been shown to map the loss of seed viability during storage in Africa (Tucker and New, 1997) and to estimate the populations of *P. truncatus* in rural maize stores in Benin (Meikle *et al.*, 1998). Similarly, the model developed here provides the missing component which will enable the survival of conidia of *B. bassiana* to be estimated in grain stores throughout Africa. More generally, model (7) may also be useful when evaluating the potential of biological control by other entomopathogenic fungi.

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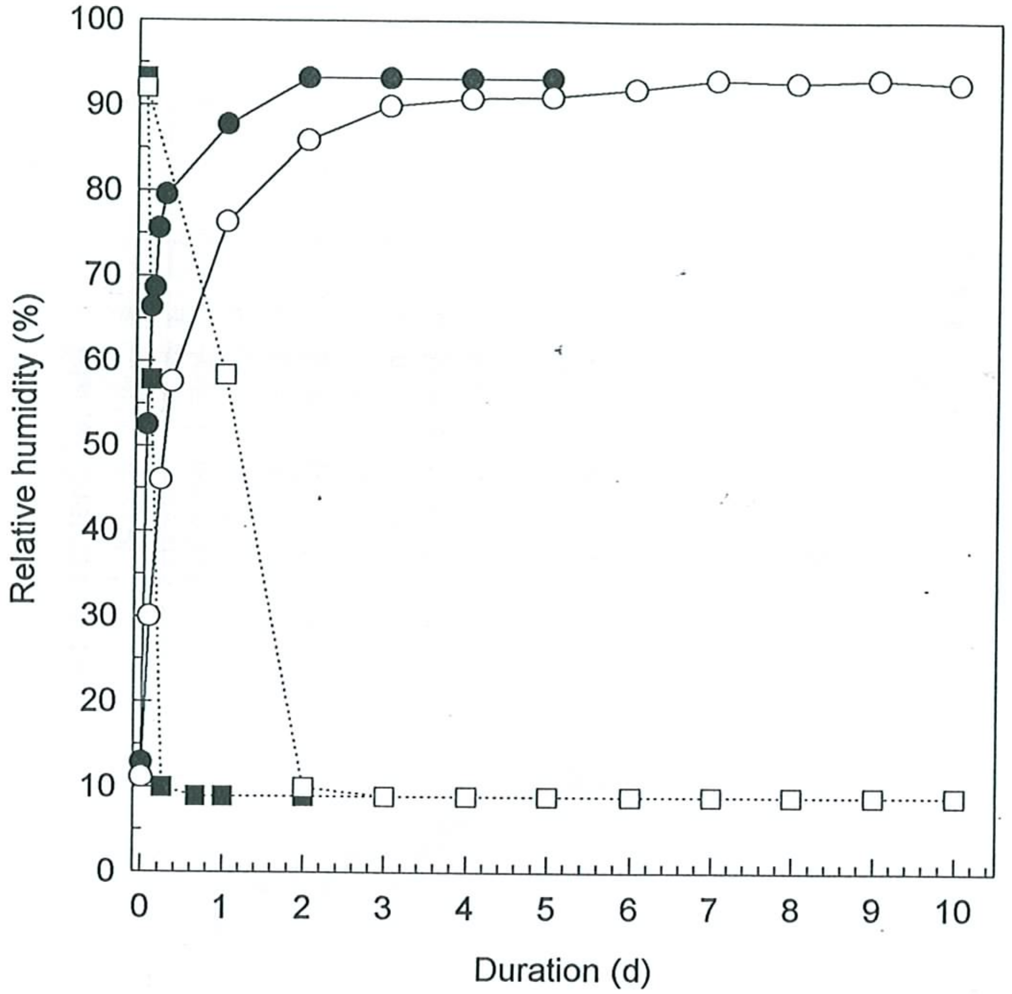
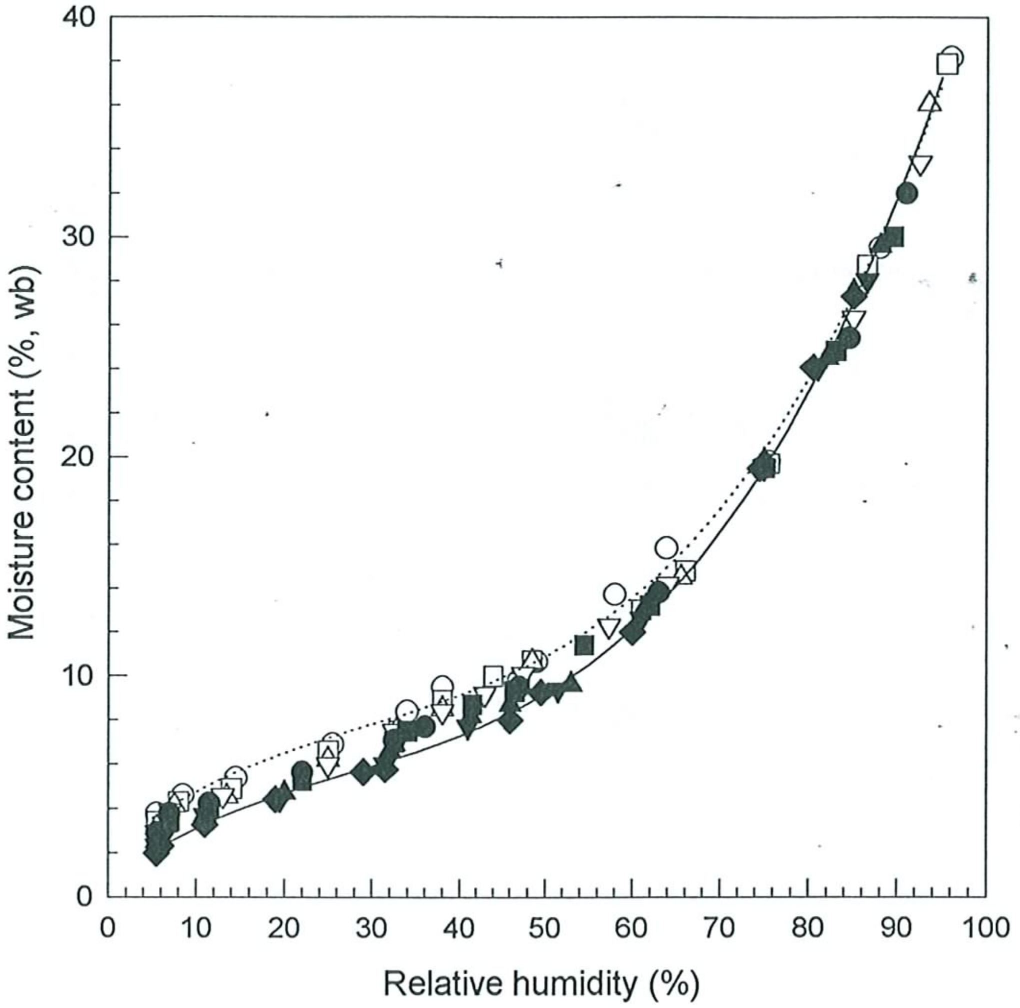
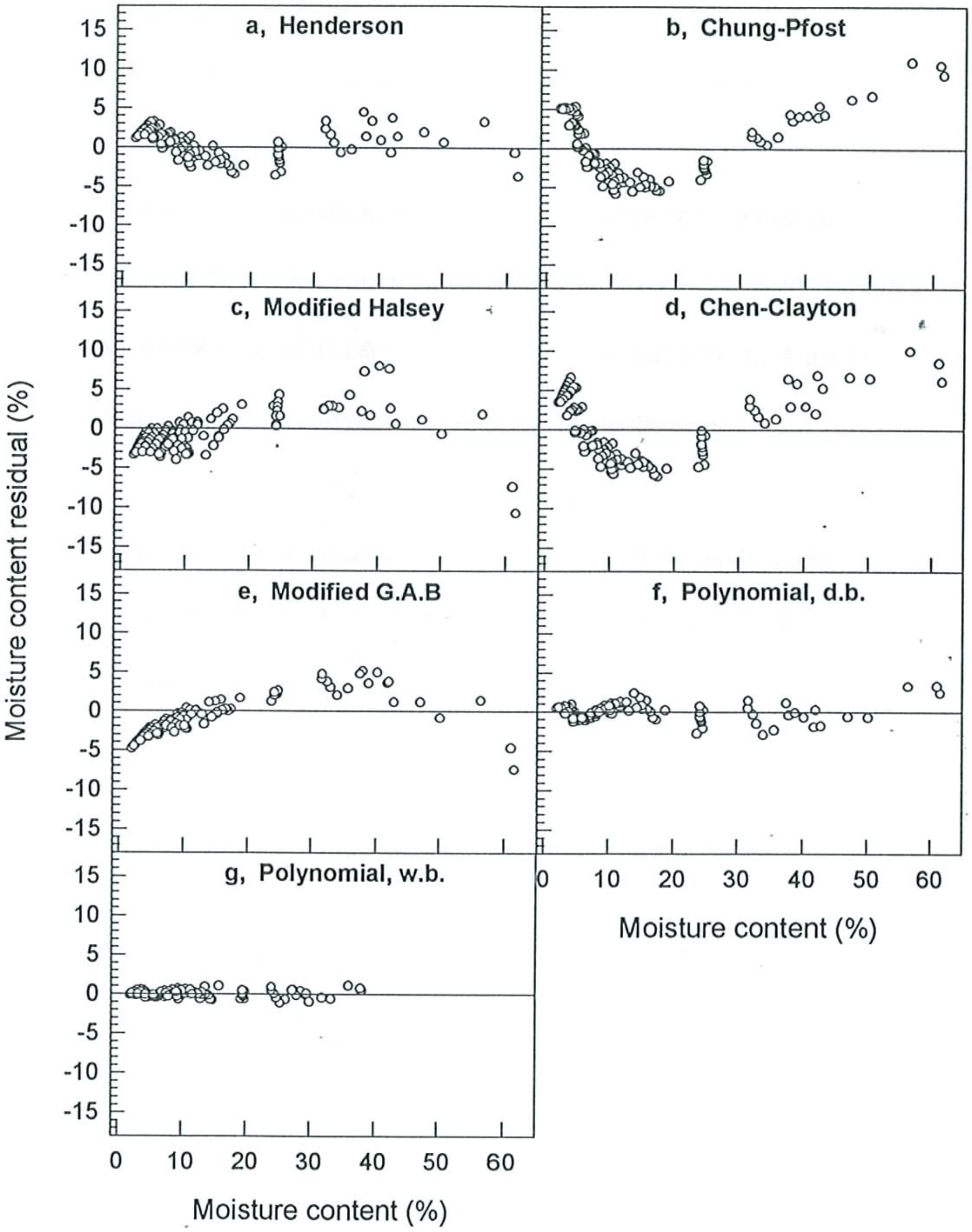

Relative humidity (%)
100
90
80
70
60
50
40
30
20
10
0
Duration (d)
0
1
2
3
4
5
6
7
8
9
10


Moisture content (%, wb)
Relative humidity (%)
0
10
20
30
40
0
10
20
30
40
50
60
70
80
90
100


a, Henderson
b, Chung-Pfost
c, Modified Halsey
d, Chen-Clayton
e, Modified G.A.B
f, Polynomial, d.b.
g, Polynomial, w.b.
Moisture content residual (%)
15
10
5
0
-5
-10
-15
0
10
20
30
40
50
60
Moisture content (%)

Captions to the figures

Figure 1. Progress of desorption (squares) and absorption (circles) for conidia of *B. bassiana* at 10° (open symbols) and 50°C (closed symbols).

Figure 2. Equilibrium relations between relative humidity and the moisture content of conidia of *B. bassiana* at 10 (○), 15 (□), 20 (Δ), 25 (∇), 30 (●), 35 (■), 40 (▲), 45 (▼) and 50°C (◆). The isotherms fitted at 10°C (...) and 50°C (—) are described by a polynomial regression model (see text).

Figure 3. A comparison of residuals (observed minus fitted conidia moisture content) for seven models (Table 2). Moisture content is expressed on the dry weight basis, except in g where it is on the fresh weight basis.

Table 1. Summary of the 110 environments at which the equilibrium moisture content of conidia of *B. bassiana* were determined. The relative humidity shown provided by each saturated salt solution at each temperature is from: [1] Vertucci and Roos (1993); [2] Fang *et al.* (1998); [3] Winston and Bates (1960); * by interpolation or extrapolation.

Salt	Temperature (°C)								
	10	15	20	25	30	35	40	45	50
	Relative humidity (%)								
$ZnCl_2$	5.5*	5.5^2	5.5*	5.5^2	5.5*	5.5^2	5.5*	5.5*	5.5^2
NaOH	8.5*	8.0^2	7.5*	7.0^2	7.0*	7.0^2	6.6*	6.3*	6.0^2
LiCl	14.5*	14.0^2	13.5*	13.0^2	11.5*	11.5^2	11.0*	11.0*	11.0^2
$KC_2H_3O_2$	25.5*	25.0^1	25.0*	25.0^1	22.0^3	22.0^1	20.0^3	19.5*	19.0^1
$MgCl_2$	34.0^3	33.0^2	32.8*	32.5^2	32.5*	32.5^2	32.0^3	31.5^3	31.5^2
NaI	38.0*	38.0^1	38.0*	38.0^1	36.0^3	34.0^1	32.5^3	-	29.0^1
K_2CO_3	47.0^3	44.0^3	-	43.0^3	-	41.5^2	41.0*	41.0*	41.0^2
KNO_2	49.0*	48.5^1	48.5^3	47.5	47.0^3	46.5^1	46.0^3	-	-
$Mg(NO_3)_2$	58.0^3	-	-	-	-	-	-	-	-
NaBr	63.9^3	61.0^3	-	57.5^3	-	54.5^3	53.0^3	51.5^3	49.5^3
$NaNO_2$	66.0*	66.0^1	65.5^3	64.0^1	63.0*	62.0^1	61.5^3	61.0*	60.0^1
NaCl	75.5^2	75.5^2	75.0*	75.0^2	75.0*	75.0^2	75.0*	75.0*	74.5^2
KCl	88.0^3	86.5^3	85.0^3	85.0^3	84.5^3	83.0^3	82.0^3	81.0^3	80.5^3
KNO_3	96.0^3	95.5^3	93.5^3	92.5^3	91.0^3	89.5^3	88.0^3	86.5^3	85.0^3

Table 2. The seven mathematical models applied to quantify the isotherms of conidia of *B. bassiana* at 10-50°C. M_D and M are equilibrium moisture contents expressed on the dry weight basis (d.b.) and fresh weight basis (w.b.), respectively; RH and R are relative humidities expressed as decimal and percentage values, respectively; T_A and T are temperatures in °K and °C, respectively; a, b, c, d, e, f, g are constants. The references shown describe models for crop seed isotherms.

Model	Equation	Reference
(1) Henderson	$M_D = [\ln (1 - RH)/\text{-}a * T_A]^{(1/b)}$	Henderson (1952)
(2) Chung-Pfost	$M_D = (-1/b) * \ln[T_A * \ln RH/\text{-}a]$	Chung and Pfost (1967)
(3) Modified Halsey	$M_D = [-\exp(a * T_A + c)/\log RH]^{(1/b)}$	Iglesias and Chirife (1976)
(4) Chen-Clayton	$M_D = -1/(c * T_A^{d}) * \ln[\ln RH/\text{-}a * T_A^{b}]$	Chen and Clayton (1971)
(5) Modified Guggenheim-Anderson-de Boer (G.A.B.)	$M_D = a*b*(c/T)*RH/(1-b*RH)[(1- b*RH + b*(c/T)*RH)]$	Jayas and Mazza (1993)
(6) Polynomial regression (d.b.)	$M_D = a + bR + cT + dR^2 + eRT + fR^3 + gR^2T$	Fang *et al.* (1998)
(7) Polynomial regression (w.b.)	$M = a + bR + cT + dR^2 + eRT + fR^3 + gR^2T$	

Table 3. Comparison of the variance ratio (*F*), coefficient of determination (R^2), mean relative error (*E*), and standard error of fitted moisture content (*SEMC*) among seven models (Table 2) which quantify equilibrium relations between relative humidity and the moisture content of conidia of *B. bassiana* at 10° - 50°C.

Model	*F*	*d.f.*	R^2	*E* (%)	*SEMC*
(1) Henderson	6,309.3	2 & 108	0.981	14.9	1.70
(2) Chung-Pfost	1,312.2	2 & 108	0.913	17.1	4.08
(3) Modified Halsey	2,031.3	3 & 107	0.962	19.1	2.71
(4) Chen-Clayton	670.6	4 & 106	0.915	15.1	4.04
(5) Modified G.A.B.	2,026.1	3 & 107	0.962	31.8	2.71
(6) Polynomial regression (d.b.)	5,837.2	7 & 103	0.994	0.4	1.05
(7) Polynomial regression (w.b.)	19,326.8	7 & 103	0.998	0.1	0.43