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The influence of temperature and vapour pressure deficit on conidia germination and germ tube production in an Australian *Podosphaera xanthii* isolate

Zaiton Sapak · Paul Melloy · Elizabeth J. Minchinton · Moin U. Salam · Victor J. Galea

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Abstract Powdery mildew of cucurbits, caused by *Podosphaera xanthii*, is an economic constraint in cucurbit production worldwide. This study examined the influence of temperature and vapour pressure deficit (VPD) on the rate of conidial germination and the formation of germ tubes in an Australian *P. xanthii* isolate 12 – 48 h after inoculation. Two experiments were prepared by inoculating cucumber, cv. crystal salad, leaf-discs using a spore settling tower. The first experiment incubated inoculated cucumber leaf-discs at eight temperatures between 8 and 35 °C under saturated vapour pressure (SVP), the second compared 18 VPD conditions between 0.038 and 1.797 kPa, in six humidity chambers (33% – 99% relative humidity) and three temperatures (22 °C, 25 °C, 28 °C). Leaf-discs were cleared, stained and microscopically inspected for conidial germination and the number of germ tubes. The optimal temperature for germination was 28ºC at SVP, where more than 50% of conidia had germinated by 12 h, and 85% by 48 h. Fewer germinated conidia were recorded after 12 h at other temperature treatments between 17 °C and 31 °C. The germination percentage and germination rate were significantly lower when vapour pressure was between 0.13 and 2.5 kPa, with germination in response to VPD varying by approximately 10%, indicating difficulty associating conidial germination to VPD above 0.13 kPa. Germ tube production was highest between 25 ºC and 28 ºC at the lowest VPD treatment at near SVP, with more than 50% of the germinated conidia producing at least three germ tubes. Germination and formation of germ tubes significantly reduced when VPD increased. *P. xanthii* conidia were able to produce a primary germ tube under relatively dry conditions, such as 2.53 kPa, but these results show infection would be less likely and require longer incubation. This study provides the first crucial step in simulating powdery mildew infections on cucurbit plants and may lead to a model capable of providing risk forecasts or fungicide management decision support tools.

Z. Sapak
Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM), Jasin Campus, Merlimau, 77300 Melaka, Malaysia
e-mail: zaiton3338@uitm.edu.my

Z. Sapak · P. Melloy (✉) · V. J. Galea
School of Agriculture and Food Sciences, The University of Queensland, Gatton, QLD 4343, Australia
e-mail: p.melloy@uq.edu.au

V. J. Galea
e-mail: v.galea@uq.edu.au

E. J. Minchinton
Department of Jobs, Precincts and Regions, 475 Mickleham Road, Attwood, VIC 3047, Australia

M. U. Salam
Food and Agriculture Organization of the United Nations (FAO), FAO Representation in Bangladesh, House 74, Road 18, Block J, Banani, Dhaka 1213, Bangladesh
e-mail: moinsalam1@gmail.com

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Introduction

Powdery mildew, caused by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, is a constraint for cucurbit production worldwide. In Australia, during the 2020–21 season, growers produced 430 million tonnes of cucurbit crops with a production value of $495.5 million AUD (Horticulture Innovation Australia, 2022). Powdery mildew impacts economic returns for growers through quantitative and qualitative yield losses of up to 30 to 50% (El-Naggar et al., 2012; Sapak et al., 2017).

Inoculum for primary infection by *P. xanthii* occurs from wind dispersed ascospores, produced by cleistothecia following sexual recombination, or asexual conidia produced on a wide range of alternative hosts. In Australia reports of sexually compatible strains of *P. xanthii* are uncommon (Letham & Priest, 1989; Parbery, 1990) and the co-occurrence of two compatible mating types in the field is rare. In addition, primary infection by ascospores on some cucurbit species may not be possible (McGrath, 1994; Smith, 1970) making their role in causing the disease unclear. Due to a wide potential host range and lack of detail for which pathogen isolates are capable of infecting alternative hosts, it seems researchers are currently resigned to the assumption that *P. xanthii* conidial inoculum is ubiquitous.

High conidial germination rates on a susceptible host can result in greater powdery mildew severity if environmental conditions are also conducive for colonisation and sporulation (Jarvis et al., 2002; Trecate et al., 2019). Temperature and relative humidity (RH) are cited as the two most influential environmental variables in powdery mildew epidemics (Jarvis et al., 2002; Sitterly, 1978; Suleiman et al., 2016). Other variables such as air turbulence, light radiation and the structural and chemical properties of the host leaf have also shown to influence infections (Itagaki & Shibuya, 2018; Yarwood, 1957).

The asexual lifecycle can be simplified into the following stages; germination, germ tube elongation, germ tube branching, infection, colonization, candle production, and dispersal (Jarvis et al., 2002). Following germ tube initiation and elongation, appressoria form in an attempt to pierce the host epidermal cells with a penetration peg. Successful penetration of the host cell wall by the pathogen leads to the formation of haustoria, which it uses to obtain nutrients while extending its mycelial mass over the leaf surface (Green et al., 2002). Haustoria are often difficult to observe using microscopy and therefore observations of germinated conidia with secondary germ tubes as indicators of successful infection are used as a proxy for successful parasitism (Trecate et al., 2019). The time between germination and appressoria development can occur rapidly in powdery mildews. Nair (1962) described *E. graminis* producing appressoria within 4 h of conidia germination, followed by successful penetration of the wheat host 6 – 8 h later. It is the time between conidia germination and colonisation when RH or vapour pressure deficit (VPD) is suspected to have the greatest influence on successful infection (Aust & Hoyningen-Huene, 1986; Reuveni & Rotem, 1974; Yarwood, 1957). The survivability of conidia has been reviewed as highly variable between species and isolates (Yarwood et al., 1954). The conidia of some powdery mildew species survive longer in humid environments, while other species in drier environments, showing that specific host and pathogen studies are needed to understand disease infection and establishment (Nagy, 1976).

Relative humidity (RH) is the percentage of water vapour held in the air (VP) relative to the potential water holding capacity at saturation (SVP, Eq. (1)). Vapour pressure deficit (VPD) is calculated as the difference between the actual atmospheric vapour pressure (VP) and saturated vapour pressure (SVP), Eq. (2). Both measurements are determined by temperature, which defines SVP (Allen et al., 1998). VPD is described as a more appropriate variable, compared to relative humidity, in host-parasite studies as it has a better linear correlation with evapotranspiration compared to RH which is non-linear (Anderson, 1936; Jarvis et al., 2002). For example, air with 80% RH at 15 °C has a VPD of 1.36 kPa, however air with 80% humidity at 25 °C has almost twice the drying force with a VPD of 2.53 kPa. It is therefore essential for experiments evaluating the effect of humidity and temperature in plant pathology to use VPD. For ease,

hereafter we will use VPD in place of references to RH, unless where the specific use of RH is necessary.

$$SVP = 10^{\left(\frac{T\times 7.5}{T+237.3}+0.786\right)} \times 0.1 \quad (1)$$

$$VPD = SVP - SVP \times (RH \div 100) \quad (2)$$

Studies have reported that powdery mildew conidia germinate in a higher proportion when vapour pressures are at, or very near, saturation, and when saturated conditions are prolonged, especially in the first 24 h (Jarvis et al., 2002; Reuveni & Rotem, 1974; Trecate et al., 2019; Yarwood, 1957). However, some studies have reported no difference between moisture treatments, these tend to be studies which have not included saturated vapour pressure (SVP) conditions (100% RH), or make the first observation of germinating conidia after 24 h (Hural, 1986; Jarvis et al., 2002; Xu & Butt, 1998). For example, *in vitro* germination studies by Hashioka (1937) and Cheah et al. (1996) reported no conidial germination on glass slides at RH of < 99% and < 94% respectively, while Reuveni and Rotem (1974) observed low germination at RH conditions 50–55% and the highest germination at 80 – 85% with prolonged leaf moisture (up to 24 h).

Following the successful infection and development of a haustorium, usually after 24 h (Trecate et al., 2019), differences in VPD, were observed to not influence powdery mildew colony growth and the subsequent production of conidiophores (Grove & Boal, 1991; Jarvis et al., 2002; Nagy, 1976; Yarwood, 1957).

The influence of temperature on the germination of powdery mildew conidia and its subsequent development is less controversial in the literature compared to VPD, and the differences between conidial germination in many cases can be attributed to the specific pathogenic strain and host interaction (Nagy, 1976; Yarwood et al., 1954). In cucurbit hosts, temperature optima for conidial germination range between 25- 28ºC (Cheah et al., 1996; Hashioka, 1937; Reuveni & Rotem, 1974; Trecate et al., 2019). However, *P. xanthii* isolates found in Queensland, Australia, may have broader preferences for temperature and humidity between 10-35ºC (MacManus & Akem, 2008; Sapak et al., 2017). Prior studies investigating the impact of VPD on infection and the establishment of powdery mildew have aided a granular understanding of their role during infection. In order to calibrate the Powdery Mildew of Cucurbits Simulation (POMICS) model (Sapak et al., 2017) for the impact of VPD and temperature on disease establishment a finer scale of environmental variables need to be studied.

Detailed infection studies of *P. xanthii* collected from Queensland, or elsewhere in Australia, have yet to report ideal conditions for powdery mildew epidemics. Therefore we undertook infection studies on a local cucurbit powdery mildew isolate in controlled environment facilities to understand the influence of temperature and VPD on the rate of disease establishment by observing the success of conidial germination and germ tube production in the first 48 h of infection. The results from this work will inform the POMICS model that can be used by cucurbit growers and agronomists as a decision support tool to manage the disease.

Materials and methods

Source of leaf-discs and inoculum Infection experiments were undertaken on seedlings of susceptible cucumber variety 'Crystal Salad' (Terranova Seed Pty Ltd, NSW Australia) grown to a 2—3 true leaf stage in free draining pots, 9 cm in diameter under ambient glasshouse conditions at The University of Queensland Gatton Campus. A wild-type *P. xanthii* isolate was sampled from the Queensland Department of Agriculture and Fisheries research station Gatton during 2008 and serially passaged on cucumber 'Crystal Salad' plants in a separate glasshouse to generate a continuous source of *P. xanthii* inoculum. The growth media comprised 1 cubic metre 0–10 mm composted bark (Basset Barks, Sunshine Coast, QLD) mixed thoroughly with 2 kg / m^3 Osmocote Plus (8–9 months release, Scotts Australia Pty Ltd, NSW), 1 kg Osmocote Plus (3 – 4 months release), 1.2 kg MoisturAid granular wetting agent (Yates Australia, NSW), 1.2 kg Dolomite (Mudgee Dolomite and Lime Pty Ltd, NSW), and 1.3 kg Osmoform 4-month release (Scotts Australia Pty Ltd, Australia). Seedlings were irrigated twice daily for 15 min using an automated drip system.

Inoculation Cucumber leaves were sampled at dusk between 1800 – 1900 h. Leaves sampled at this time of day have higher carbohydrate levels than those collected earlier (Fig. 1a) and thus are considered more suitable for use in the leaf-disc based studies (Amand & Wehner, 1995; Yarwood, 1946). Two fully expanded leaves from 4-week-old plants were excised in the laboratory at room temperature (22 ± 2 °C), rinsed with sterile distilled water to remove dirt and unwanted particles, then gently dried on filter paper. Once dried, the leaf-discs (22 mm diameter) were excised using a cork borer, then placed adaxial side up on sponge inserts soaked with sterile distilled water within 30 mL McCartney bottles (Fig. 1b). The leaf-discs were secured in place beneath caps modified to have a 20 mm diameter hole, exposing the adaxial leaf-disc surface (Fig. 1c). Once secured, the leaf-discs were placed at the bottom of a spore settling tower and inoculated in groups of 12. The spore settling tower was constructed from a flanged Perspex cylinder (100 cm in height, 40 cm diameter, and 20 mm thick). For each inoculation run fifteen infested cucumber leaf-discs 22 mm in diameter were placed on a platform suspended at the top of the spore settling tower below the air inlet valve. Conidia were dislodged from the infested leaves by first slowly applying a vacuum of -20 kPa to the spore settling tower, then sharply breaking the seal to produce a consistent rush of air across the infested leaf surface. The dispersed conidia were left to settle on the exposed leaf-discs, mounted in McCartney bottles, placed on the base plate of the settling tower. Conidial density on the leaf-disc surface was estimated by applying Stoke's law of sedimentation as suggested by Reifschneider and Boiteux (1988). Stoke's Law of sedimentation predicts the settling velocity and unit density of smooth spheres with diameters between about 1 and 70 µm. This method achieved a suitably even distribution of *P. xanthii* conidia which have a size of 24 –28 × 58–72 µm. A subset of four leaf-discs were assessed immediately following inoculation to confirm the estimated spore densities were consistent between inoculation replicates and that no pre-germinated conidia were introduced to the leaf-discs at inoculation.

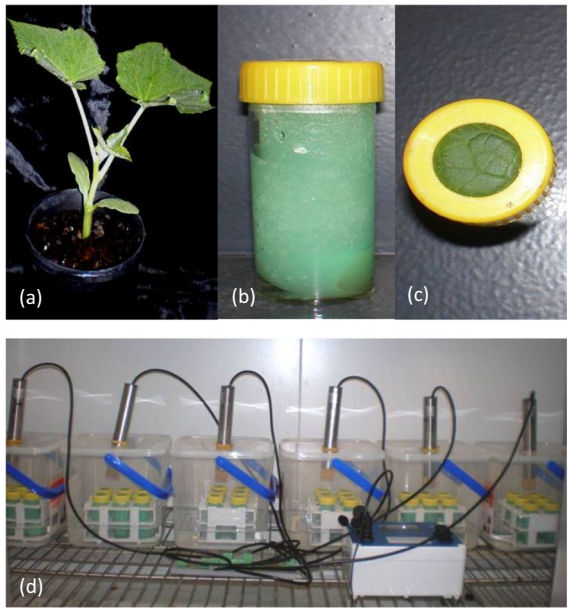

Fig. 1 Four-week-old cucumber seedlings cv. Crystal Salad used for the detached leaf method. (**a**) Two fully expanded young cucumber leaves selected for leaf-disc harvest. (**b**) Side view of McCartney bottle, internal sponge and cap and (**c**) top view of a leaf-disc exposed through opening in cap. (**d**) Incubation of inoculated cucumber leaf-discs in humidity chambers placed in an illuminated constant temperature incubator

Temperature and VPD controlled experiments were undertaken using 5 L airtight plastic containers containing 500 mL distilled water or saturated salt solutions to achieve the targeted humidity levels (Table 1) (Winston & Bates, 1960). SVP and VPD were calculated using Eqs. 1 and 2 described by Murray (1967). A miniature (4 cm diameter; 12 V) fan attached to the underside of the lid circulated air to ensure uniform temperature and VPD conditions. The humidity chambers were placed in an illuminated constant-temperature incubator (Astra Panels Salisbury, Brisbane, Australia) in a completely randomised layout. Calibrated temperature and humidity sensors (Monitor Sensors Pty. Ltd., Caboolture, Australia) logged conditions in the chambers every 15 min and three Grolux® growth fluorescent tubes (Osram Sylvania®, Australia) were programmed to emit a light intensity of 2.0 Wm^{-2} s^{-1} at the leaf level on a 14 / 10 h light–dark cycle, similar to Australian summer field growing conditions.

Effect of temperature on conidial germination and germ tube development at saturated VPD The effect of temperature on conidial germination and germ tube production were compared in eight treatments, 8, 17, 19, 22, 25, 28, 31, and 35 °C (± 2 °C)

Table 1 Saturated salt solutions used to give different levels of relative humidity at each of five temperatures adapted from Winston and Bates (1960)

Saturated salt solution	Relative humidity (%)						
Temperature (°C)	10	15	20	25	28‡	30	35
Potassium hydrogen phosphate (KH_2PO_4)	98.0	97.0	96.5	96.0	95.0	93.5	93.2‡
Potassium chloride (KCl)	88.0	86.5	85.0	85.0	85.0	84.5	83
Sodium chloride (NaCl)	76.0	76.0	76.0	75.5	75.5	75.5	75.5
Calcium nitrate ($Ca(NO_3)_2.4H_2O_4$	57.5‡	56.0	55.5	50.5	50.0	47.0	43‡
Magnesium chloride ($MgCl_2.6H_2O$)	34.0	34.0	33.0	32.5	32.5	32.5	32.5

‡ Approximated

with a saturated target VPD of 0 kPa, which varied between 0.02 to 0.04 kPa. Conidial germination of *P. xanthii* were assessed at 12, 24, 36, and 48 h after inoculation, by randomly sampling two leaf-discs from each humidity chamber. A similar number were sampled to assess germ tube development following leaf clearing. The experiment was repeated once.

Conidial germination was observed by removing conidia from the leaf-disc surfaces using a 4 × 2 cm piece of adhesive tape (Amsalem et al., 2006). The tape was then mounted on a glass slide over a drop of Lactophenol cotton blue stain. Prepared slides were observed under an Olympus compound light microscope (40x) and 100 conidia were assessed per leaf-disc. Conidia with a primary germ tube with a length of at least half the diameter of the conidia were considered germinated (Lacy, 1994). Germ tubes were classified as primary, secondary or tertiary according to their relative length.

Germ tube formation was observed on cleared leaf-discs under a compound microscope (40x). The leaf-discs were cleared following the method described in Liberato et al. (2005), and mounted on glass slides with a drop of 85% lactic acid under a coverslip. Leaf-discs inspected at time periods 12, 24 and 48 h for germ tube formation were discarded due to either insufficient germ tube formation (12 and 24 h) or excessive hyphal growth (48 h). At 36 h, for each leaf-disc, 100 conidia were randomly assessed by counting germ tubes. Four leaf-discs were assessed for conidial density immediately following inoculation to confirm no pre-germinated conidia were introduced to the leaf-discs at inoculation. This experiment was repeated once.

Effect of VPD on conidial germination and germ tubes at three temperature regimes The influence of VPD on conidial germination and germ tube production was evaluated at three temperatures of 22, 25, and 28 °C ± 2 °C, and four relative humidity values (Table 2). Each temperature and relative humidity treatment provided an independent VPD treatment. Each VPD treatment chamber contained 20 leaf replicates. Four leaf replicates were sampled at 12, 24, 36 and 48 h after inoculation to assess spore germination using the sticky-tape method. The remaining four were sampled at 36 h to assess germ tube formation in-situ following leaf clearing and staining (Liberato et al., 2005).

Data analysis All data were analysed in R version 4.2.0 (R Core Team, 2022). Germination experiments were analysed with binomial generalized additive models (gam) using the R package `mgcv` (Wood, 2017). Data from temperature range experiments fitted germination to infection period using a thin plate spline consisting of three knots, and temperature using penalized cubic splines specifying shrinkage consisting of 6 knots. In the VPD germination experiments the gam which best fit the data used a thin plate

Table 2 Vapour pressure deficit and temperature treatments for assessing the portion of germinated conidia and number of germ tubes

Relative humidity	Vapour pressure deficit at three temperatures		
	22 °C	25 °C	28 °C
99%	0.026	0.032	0.038
95%	0.132	0.158	0.189
85%	0.397	0.475	0.567
75%	0.661	0.792	0.945
55%	1.190	1.425	1.701
32%	1.798	2.154	2.570

All VPD values given in units of kilopascals (kPa)

spline on infection period with 3 knots, temperature treatment as a linear term and penalized cubic splines fit to VPD with 4 knots.

Ordinal logistic regression, using the `polr` function from the R package MASS (Venables & Ripley, 2002), was used to estimate germ tube production in the temperature and VPD experiments. The ordinal logistic regression can be described as follows,

$$logit(P(Y \leq j)) = \beta_{j0-n_1x_1\cdots-n_px_p} \quad (3)$$

where Y is the ordinal response variable (germ tube number), with j categories (0, 1, 2, 3 germ tubes). β is a predictor variable such as temperature, VPD or incubation time; n is a vector of coefficients with length p, and x is a vector of predictor values. This regression method was used because the response variable can be defined as an ordered classification of different conidial germination states. Four levels were defined as: 0 = non-germinated conidia, 1 = germinated conidia with 1 germ tube, 2 = germinated conidia with two germ tubes; and 3 = germinated conidia with three or more germ tubes. In the temperature range experiment, germ tube production was fitted to temperature with a basis spline containing four knots at 8, 23, 28 and 35 °C. In the VPD range experiment, germ tube production fit a VPD predictor using a basis spline with a knot at 0.5 kPa and two boundary knots, temperature and experimental replicate were specified as linear predictors. A complete description of statistical methods, code and results can be found at https://paulmelloy.github.io/P_xanthii_titlepage.html.

Results

Effect of temperature on conidial germination and germ tube formation at saturated VPD Microscopic observation of four inoculated leaf-discs immediately following inoculation confirmed no pre-germinated conidia were introduced to the leaf-discs, and conidial density ranged between 66 and 71 per cm^2. *P. xanthii* conidia germinated between 17—31 °C, with the highest germination percentage at 28 °C across all sampling periods (Fig. 2). No germination was observed in temperature treatments 8 °C and 35 °C at any time points. Despite a significant interaction between temperature and incubation time ($P < 0.001$), the best fitting model showed temperature ($P < 0.001$) and infection time ($P < 0.001$) as non-linear additive effects. The temperature germination model estimated optimal conidial germination between 27 °C and 28 °C with an R^2 of 0.971 and Akaike information criterion (AIC) value of 560.5. The data showed ~ 50% of conidia produced germ tubes within 12 h of inoculation. This proportion increased by another 10% 24 h after inoculation and reached 85% at 48 h. Germination at temperatures between 22 °C and 25 °C (Fig. 2) were on average slower than the 28 °C treatment; after 24 h approximately 48% and 52% of the conidia germinated respectively, and approximately 75% to 80% germinated after 48 h. Germination was significantly slower at temperatures lower than 22 °C or greater than 29 °C regardless of incubation times ($P < 0.001$). At 17 °C, < 10% of conidia had germinated after 12 h, and only 25% had germinated after 48 h (Fig. 2).

Observations of the cleared leaf-discs with a microscope after 36 h of incubation revealed that temperature had a significant effect on germ tube production ($P < 0.001$) (Fig. 3a). Tertiary germ tube production peaked between 25 °C and 28 °C, with a mean 49—53% of conidia producing at least three germ tubes respectively. Although there were fewer conidia with tertiary germ tubes in the 22 °C treatment, the proportion of secondary germ tubes were higher, resulting in a mean 63% of conidia with two or more germ tubes in the 22, 25 and 28 °C temperature treatments. An ordinal logistic regression of germ tube production showed significant differences between temperature treatments ($P > 0.001$) (Fig. 3b). Only 4.5% of germinated conidia had produced tertiary germ tubes at 19 °C, with no tertiary germ tubes observed at 17 °C and 31 °C. Observations made during germ tube assessments noted the 25—28 °C treatments contained more primary and secondary germ tubes with elongated hyphae. These elongated hyphae also established multiple penetration sites in the host tissues via appressoria along their hyphae. In comparison, 19 °C and 31 °C treatments showed primary and secondary germ tubes were consistently shorter after 36 h of incubation.

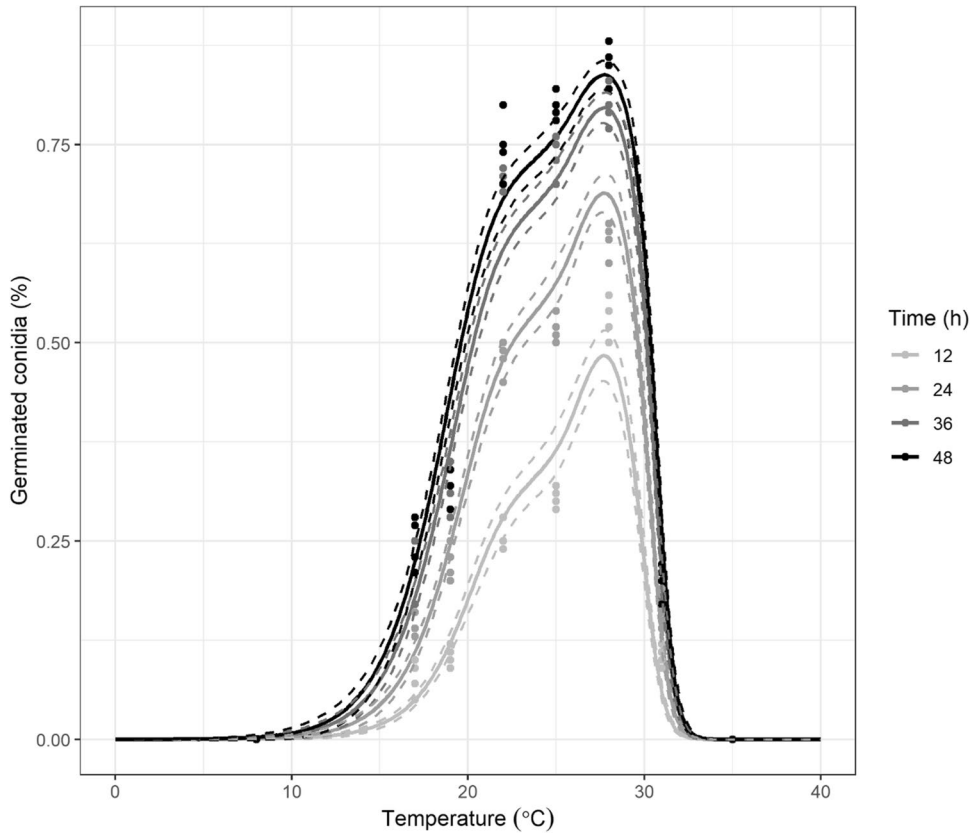

Fig. 2 The effects of temperature on conidial germination at four incubation times. Each dot shows the raw observation data of the percentage germinated conidia from 100 conidia. The solid trend line shows the mean predicted model estimates. The dotted line indicates the 95% confidence intervals around the mean

Effect of VPD on conidial germination and germ tube formation at three temperatures VPD had a significant non-linear effect on *P. xanthii* conidia germination ($P<0.001$). The best fit gam model ($R^2=0.958$) included a non-linear term for incubation time ($P<0.001$) and a linear term across the three temperatures ($P<0.001$). The highest conidial germination was obtained at VPD values close to saturation (0.026 kPa) at the three tested temperatures, 22 °C, 25 °C and 28 °C (Fig. 4). Germination significantly dropped when the VPD increased ($P<0.001$), irrespective of the temperature or incubation time. A gam model testing the interaction between VPD and temperature, while significant, was not a better fit than a model without interaction between variables VPD, temperature and infection period. Germination seemed to be less affected by VPD in treatments over 0.5 kPa, and these drier treatments may have been more affected by temperature. The warmer treatments of 25 °C and 28 °C, showed a slight rise in conidial germination between 1.5 and 2 kPa after 24 h (Fig. 4).

VPD also had a significant effect on the development of germ tubes ($P<0.001$). Germinated conidia with tertiary germ tubes were most prevalent in the lowest VPD treatments, with the warmer treatments producing a greater proportion of tertiary germ tubes ($P=0.001$) (Fig. 5a). Differences between the repeated experiments were significant ($P<0.001$) yet the three experimental replicates showed similar trends.

As in the germination experiments, drier treatments with a VPD greater than 0.038 kPa showed significantly fewer germinated conidia ($P<0.001$) (Fig. 5a). Of the germinated conidia in the VPD treatments 0.132 kPa or greater, conidia with a single germ tube were in a higher proportion than

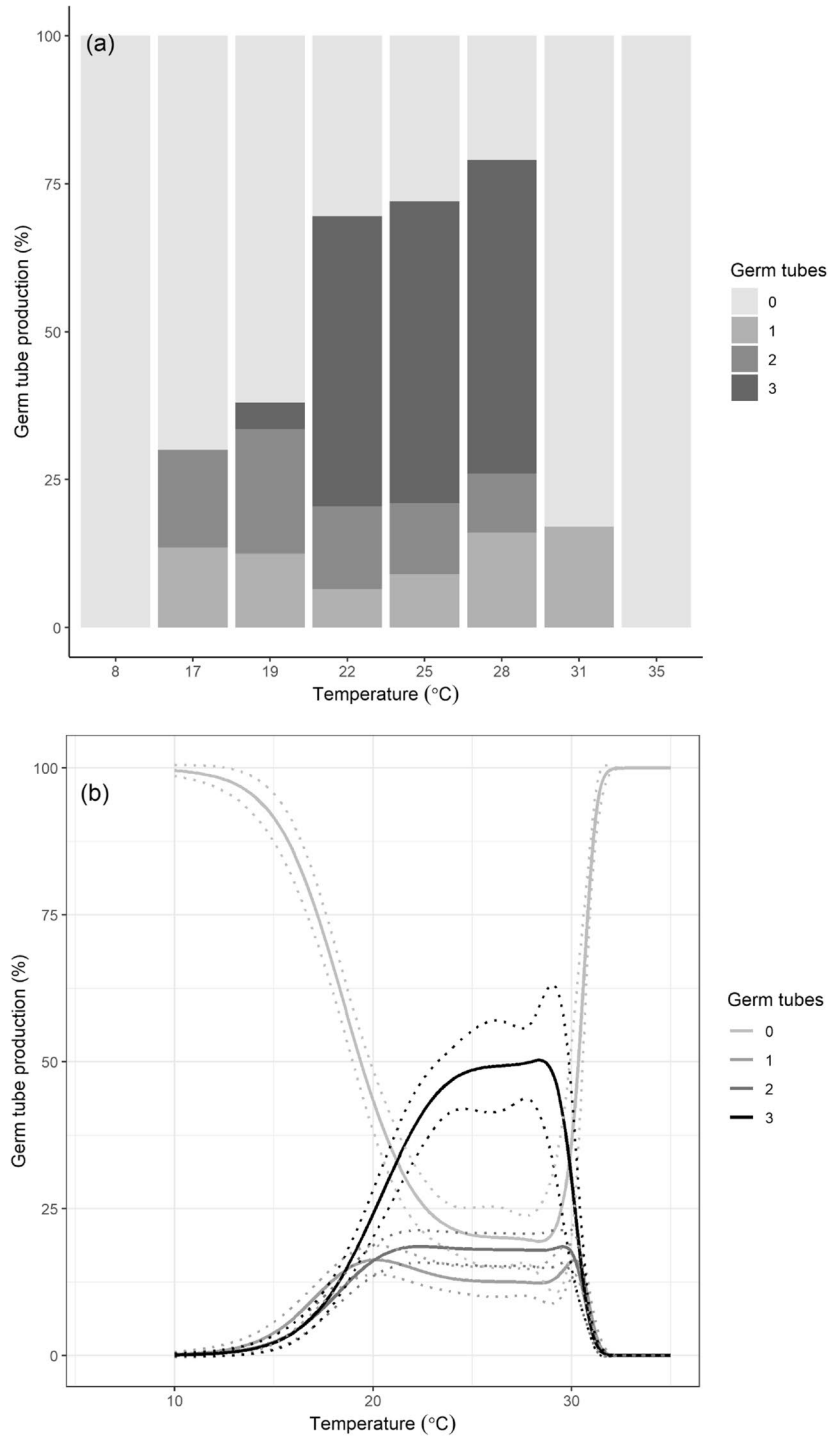

Fig. 3 (**a**) The mean *P. xanthii* germ tube formation as a percentage on cucumber leaf-discs at saturated atmospheric VPD values between approximately 0.02 kPa to 0.04 kPa at 36 h after inoculation. (**b**) Ordinal logistic regression model of germ tubes production over a range of temperatures. Solid line indicates the estimated mean. The dotted line indicates the upper and lower 95% confidence interval around the mean. Temperature was fitted to a b-spline with 4 knots

conidia with secondary or tertiary germ tubes. Secondary germ tubes were produced in all VPD treatments, although the percentage of conidia producing secondary germ tubes reduced significantly in drier VPD treatments equal to or greater than 0.132 kPa ($P < 0.001$). While very few tertiary germ tubes were observed in the driest VPD treatment (2.57 kPa), the pathogen was able to produce

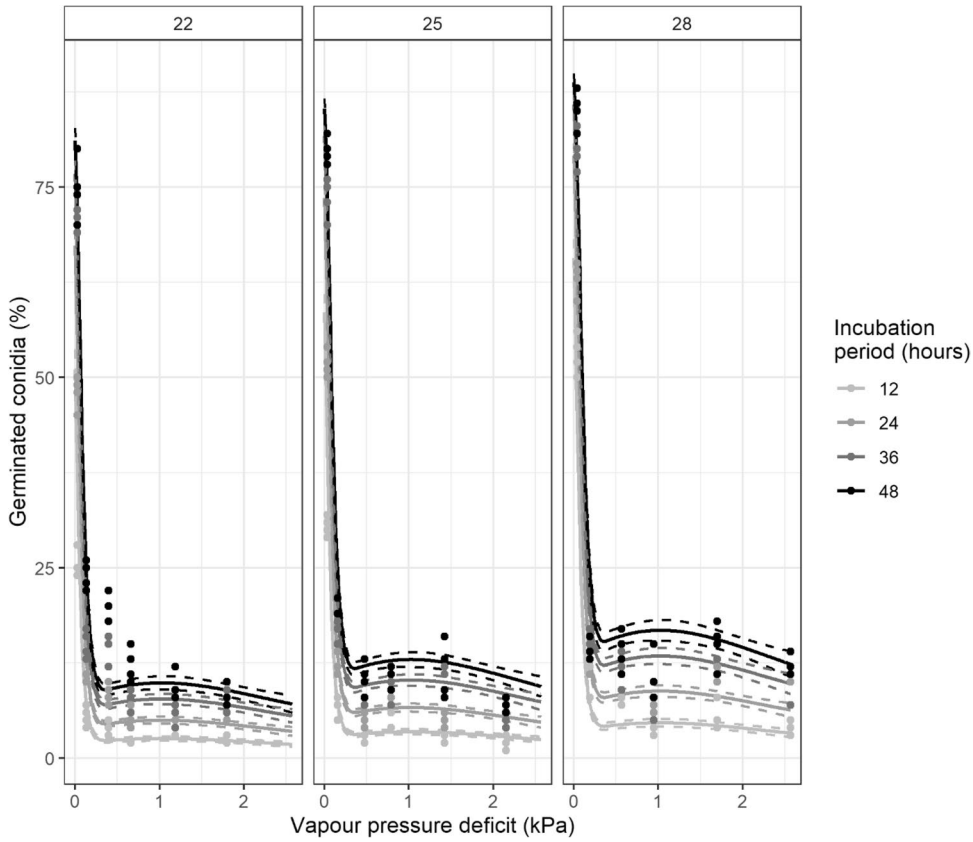

Fig. 4 The effects of atmospheric vapour pressure deficit (VPD) and temperature on conidial germination sampled after 12, 24, 36 and 48 h of incubation. Points represent percentage germination observations of approximately 100 conidia; solid trend lines show the mean germination percentage estimated by a generalized additive model. Dotted lines show the upper and lower 95% confidence intervals of each mean

secondary germ tubes and tertiary germ tubes in all treatments (Fig. 5a). The presence of appressoria were still seen after 36 h of incubation at 22 °C and 0.66 kPa (Fig. 6).

Cleared leaf-discs from the 28 ± 2 °C / 0.038 kPa treatment showed some *P. xanthii* conidia produced a forked primary germ tube after 12 h of incubation. A secondary germ tube would then emerge after 24 h from another part of the conidium. This secondary germ tube then elongated, with the attendant development of appressoria. The appressoria of *P. xanthii* developed without a specific shape, unlike other powdery mildew species, but hyphal widening could be used to differentiate the appressoria from the hyphae, as suggested by Boesewinkel (1977). From beneath the appressoria, penetration pegs developed and pierced the epidermal host cells. These infection structures could be observed after 24 h on cleared leaf tissues (Fig. 5). Once a parasitic relationship was established with the host, conidia were able to produce more germ tubes and branching hyphae, as observed after 36 and 48 h of incubation.

Discussion

This study found temperature and VPD play significant roles in the germination rate and production of secondary and tertiary *P. xanthii* germ tubes on cucurbit leaves. Moderate temperatures of 22– 28 °C and a low VPD near saturation provided the optimal conditions for successful infection and are likely to accelerate powdery mildew infection and development early in the asexual lifecycle.

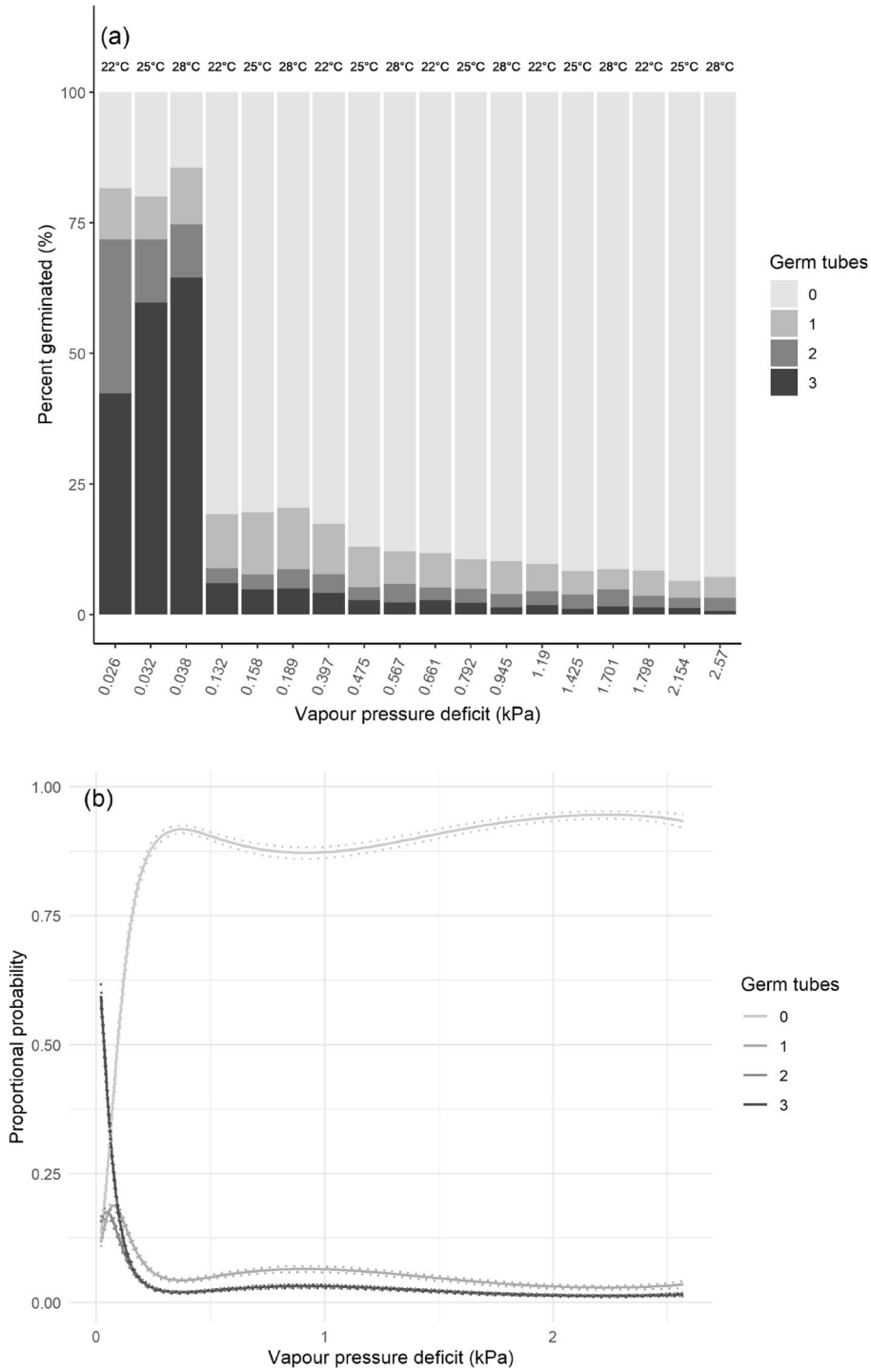

Fig. 5 (**a**) The effects of atmospheric vapour pressure deficit (VPD) at three difference temperatures of 22°C, 25°C and 28°C on formation of *P. xanthii* germ tubes on cucumber leaf-discs after 36 h of incubation. (**b**) Predicted mean germ tube production over a range of VPD values, and the 95% confidence intervals. Values were predicted using an ordinal logistic regression model, VPD was fit to a b-spline with 3 knots, the middle knot set to 0.5 kPa

These optimal (22– 28 °C), maximum (≤ 31 °C) and minimum (> 8 °C) germination temperatures are complimentary to earlier reports of *P. xanthii* on cucurbits (Cheah et al., 1996; Gupta et al., 2001; Hashioka, 1937; Trecate et al., 2019). For example, Hashioka (1937) and Trecate et al. (2019) both reported suppressed germination below 10 °C on cucurbit leaves, and no germination below 5 °C

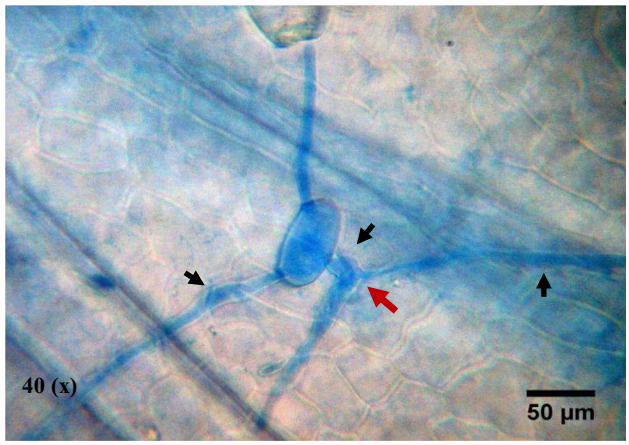

Fig. 6 Micrographs showing that germinated conidia stained with Lactophenol Cotton Blue at approximately 0.60 kPa vapour pressure deficit produce at least three germ tubes, with the presence of appressoria (indicated by black arrows) seen after 36 h of incubation at 22°C. The primary germ tube can be identified by the fork-shaped structure (indicated by the red arrow). The tertiary germ tube is normally shorter than the secondary germ tube. The appressoria of *P. xanthii* develop without forming any special shapes but can be recognized by a widening of the hyphae

(Hashioka, 1937). Studies reporting *P. xanthii* germination on glass slides, however, observed much higher minimum germination temperatures with either a trace amount, or no germinating conidia below 15—18 °C (Cheah et al., 1996; Hashioka, 1937; Nagy, 1976). This might be due to the VPD on the phylloplane likely being lower and more conducive for germination and/or the pathogen responding to the presence of the host.

Excessive temperatures of 35 °C prevented *P. xanthii* conidia germinating in the present study. These observations, under saturated conditions, again corroborated studies by Hashioka (1937) and Trecate et al. (2019) where almost no germination was observed at 34 °C and > 35 °C respectively. These similar temperature optima and ranges are surprising considering the differences in experimental design and spatial populations from where the pathogen was sampled in each study (Australia, Taiwan and the Czech Republic). These findings reinforce how uncontroversial the temperature response of *P. xanthii* infecting cucurbits under saturated vapour pressure are among researchers. The moisture requirements reported for *P. xanthii* germination and germ tube production on a cucurbit host, however, are often reported with less consistency between studies.

VPD played an important role in the speed at which *P. xanthii* conidia germinated and produced germ tubes in the present study. VPD treatments which were close to saturated (0.04—0.2 kPa), showed higher rates of conidial germination and germ tube production in the three temperature treatments tested (22, 25, 28 °C). These findings largely support other studies where moisture treatments close to saturation increased germination, infection and conidia survival in cucurbits (Cheah et al., 1996; Hashioka, 1937; Reuveni & Rotem, 1974).

In the present study the differences between germination and production of secondary and tertiary germ tubes in the two most saturated treatments (0.026 and 0.132 kPa; or 99 and 95% RH) at 22 °C were on average 36%. However, Hashioka (1937) reported minimal differences in conidial germination between their 100% and 94—97% RH treatments (temperature range 11 – 13 °C) after 1, 2, 4 and 6 days of incubation on cucurbit leaves. A comparison of these results may seem conflicting if RH is not converted to VPD, as the lower temperature reduces the dryness of the air or VPD. Hashioka's humidity treatments, 94—97% RH at 11 – 13 °C equate to a VPD range of 0.04 – 0.09 kPa. Another explanation for this difference might be the cooler temperatures in Hashioka's (1937) experiment aided in preserving the conidia, however, we contend it might be more likely a combination of the lower temperatures and VPD.

This study is the first to clearly define the role of VPD influencing the germination and infection

processes of *P. xanthii*, thereby clarifying the observations made by previous authors on the influence of relative humidity on cucurbit powdery mildews. Considering the effect of moisture in the air using RH alone, without the impact of temperature on drying force, may mask moisture effects on pathogen infections. Dry conditions were unable to prevent powdery mildew infection, since a small proportion of conidia were able to survive at high VPD conditions without the presence of free water. These results support those reported by Reuveni and Rotem (1974) and Hashioka (1937). The ability of some powdery mildew species to initiate the early formation of germ tubes under low relative humidity has also been discussed by Yarwood (1950), who suggested that species, such as *P. xanthii* (Syn. *Sphaerotheca fuliginea*), have more internal liquid in their vacuole to initiate germination under dry conditions without the presence of free water, which is required by other fungi for germination. In contrast, a study by Sivapalan (1994) showed free water could displace conidia before infection and cause abnormally germinating conidia unable to infect the host. Hashioka (1937) also noted similar observations of elongated germ tube growth in water droplets which alter its ability to infect the host.

The results presented here in this study show lower VPD resulted in greater infection but only describes half of the lifecycle. For rapid powdery mildew onset and increased severity, multiple infection cycles are required. Reuveni and Rotem (1974) show colonisation and dispersal of *P. xanthii* (Syn. *Sphaerotheca fuliginea*) conidia on squash leaves is negatively affected by humidity. If the optimum conditions for both infection and sporulation stages are ignored, such as reported by Whipps and Budge (2000) who maintained a constant relative humidity and temperature for eight weeks to evaluate powdery mildew (*Oidium lycopersici*) disease severity on tomato plants, a different conclusion might be reached to the optimum conditions for disease. Whipps and Budge (2000) thus concluded the optimum conditions for powdery mildew establishment on tomatoes was approximately 80% RH (0.439 kPa VPD) and 19 °C. Perhaps for powdery mildew in cucurbits, like this experiment, if conditions were held constant for whole infection cycles, including *P. xanthii* sporulation, a drier VPD optimum might be concluded. For plants in the field, however, the pathogen is undoubtedly tuned to the diurnal fluctuations in temperature and humidity, where a drier daytime VPD may facilitate pathogen sporulation and dispersal, while lower VPD and temperatures during the night preserve conidia and encourage germination and infection. For plants grown in commercial temperature-controlled glasshouses, however, these results are also highly relevant to managing powdery mildew. Periodic adjustment of air temperature or VPD may disrupt the pathogen lifecycle, lowering the risk of disease and requirements for chemical control.

In summary, the present study confirms the germination of *P. xanthii* conidia is constrained by temperature and VPD. Low (< 8 °C), high temperatures ≥ 35 °C and large VPD (> 0.2 kPa) provide less favourable conditions for pathogen conidial germination and result in the reduction of disease risk (or severity) on cucurbits. In contrast, moderate temperatures between 22 and 28 °C can enhance germination and formation of germ tubes which lead to infection structures such as appressoria, penetration pegs and haustoria. This information can be used in developing a disease model for powdery mildew of cucurbits. However, the influence of these two factors needs to be investigated under field conditions with the impact of diurnal fluctuations in mind.

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Data availability https://doi.org/10.6084/m9.figshare.21554712
https://github.com/PaulMelloy/PM_cucurbit_infection
https://paulmelloy.github.io/P_xanthii_titlepage.html

Declarations

Ethical statement This research did not involve any animal and/or human participants.

Competing interests The authors declare that they have no conflict of interests.

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