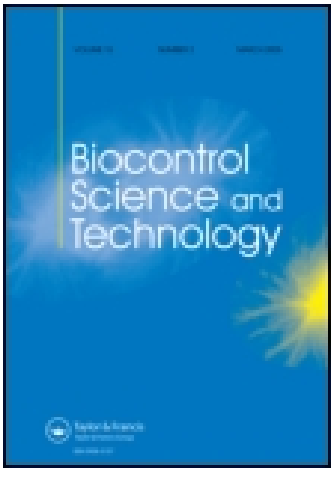

Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbst20

Factors affecting sporulation and germination of Pandora nouryi (Entomophthorales: Entomophthoraecae), a pathogen of Myzus persicae (Homoptera: Aphididae)

Wei Li [a b c], Wei-An Xu [d], Cheng-Fa Sheng [b], Hong-Tuo Wang [b] & Wei-Jian Xuan [b]

[a] Department of Life Science and Technology, Ocean University of China, Qingdao, P.R. China

[b] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China

[c] Graduate School of the Chinese Academy of Sciences, Beijing, P.R. China

[d] College of Life and Science, SUZOU University, Suzhou, P.R. China

Published online: 18 Jan 2007.

To cite this article: Wei Li, Wei-An Xu, Cheng-Fa Sheng, Hong-Tuo Wang & Wei-Jian Xuan (2006) Factors affecting sporulation and germination of Pandora nouryi (Entomophthorales: Entomophthoraecae), a pathogen of Myzus persicae (Homoptera: Aphididae), Biocontrol Science and Technology, 16:6, 647-652, DOI: 10.1080/09583150500532840

To link to this article: http://dx.doi.org/10.1080/09583150500532840

Biocontrol Science and Technology, 2006; 16(5/6): 647–652

SHORT COMMUNICATION

Factors affecting sporulation and germination of *Pandora nouryi* (Entomophthorales: Entomophthoraecae), a pathogen of *Myzus persicae* (Homoptera: Aphididae)

WEI LI[1–3], WEI-AN XU[4], CHENG-FA SHENG[2], HONG-TUO WANG[2], & WEI-JIAN XUAN[2]

[1]*Department of Life Science and Technology, Ocean University of China, Qingdao, P.R. China,* [2]*State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China,* [3]*Graduate School of the Chinese Academy of Sciences, Beijing, P.R. China, and* [4]*College of Life and Science, SUZOU University, Suzhou, P.R. China*

(Received 8 November 2004; returned 7 February 2005; accepted 29 October 2005)

Abstract

Pandora nouryi discharged large numbers of primary conidia between 8 and 25°C from cadavers on the surface of water-agar. At 8°C conidial discharge lasted for 120 h, but most conidia were produced within 48 h when temperature was >15°C. Saturated humidity alone was not enough to allow for sporulation to occur freely and where RH <95%, no conidia were discharged. Light did not affect the pattern of conidial production nor the total number of conidia. Germination percentages of conidia on the surface of water-agar were 40 and 66% at 8 and 30°C, respectively, and were significantly lower than that at 15–25°C where germination was >95%. Conidia on leaves germinated well when RH >74%, while no germination occurred when RH <100% on cover slips. All eight insecticides tested entirely inhibited conidial germination at recommended doses (R), in particular, both the organophosphorus pesticides Lorsben (chlorpyrifos) and the organochlorine pesticides Thiodan (endosulfan) completely inhibited conidial germination even at 0.2R dose.

Keywords: Myzus persicae, Pandora nouryi, *humidity, temperature, light regime, insecticides, sporulation, conidial germination*

Pandora nouryi (Remaudière & Hennebert) Humber is an obligate pathogen of aphids (Gustafsson 1965). In China, it was first documented in 1988 in Shandong province

Correspondence: Cheng-fa Sheng, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, P.R. China. E-mail: shengcf@ioz.ac.cn

First published online 27 February 2006

ISSN 0958-3157 print/ISSN 1360-0478 online © 2006 Taylor & Francis
DOI: 10.1080/09583150500532840

(Wang et al. 1988; Li 2000), in combination with *Pandora neoaphidis* and several other entomophthoralean species, inducing epizootics in *Myzus persicae* (Sulzer) (Homoptera:Aphidiae) that killed 30–80% of aphid populations in some vegetable fields during spring and autumn. Similar proportions of diseased aphids were found infected with *P. nouryi* (48.3%) or *P. neoaphidis* (47.6%) (Wang et al. 1988; Li et al. 2004b). Obviously, *P. nouryi* plays a critical role in depressing the population of aphids in this region. However, there is little information on the effects of environmental and artificial factors on *P. nouryi* biology which may be related to ecological fitness and epizootic potential. This paper presents studies on the pattern of sporulation of *P. nouryi* from cadavers of *M. persicae*, and conidial germination at different temperatures, humidities, and insecticide treatments. The results are briefly discussed with respect to the implication for the population dynamics and epidemiology of *P. nouryi*.

P. nouryi was isolated in 2002 from *M. persicae* cadavers at Tai'an, Shandong province, and maintained on SEA (80% Sabouraud dextrose agar plus 20% egg yolk, a modified medium mentioned by Papierok and Hajek (1997)) at 4°C in darkness. Before experimention, the fungus was passed three times through *M. persicae*. Cultures of *M. persicae* were maintained on rape plants, *Brassica napus* L. (cultivar Jingyou 1), at 20°C in a 12:12 light:dark (L/D) photoperiod. In the experiment, 8–10-day-young adult aphids (the total age from first instar) were used.

A humid chamber was constructed using two plastic pots (Doberski 1981). A small pot of 100 mL capacity contained holes for ventilation and was placed inside a bigger pot of about 300 mL. On one end of a thin copper thread, a small section of 2% water-agar was placed approximately 6 mm from the bottom of the smaller pot. The other end of the copper wire was fixed in the center of the humid chamber. A cadaver of *M. persicae* less than 4 h old was placed on the water–agar and a microscope slide (18 × 18 mm) was placed below the cadaver to collect primary conidia. About 100 mL of distilled water was poured into the big pot in order to obtain 100% humidity. The humid chambers were then placed in different temperature incubators at 8, 15, 20, 25, and 30°C with 12 L/12 D photophase. Microscope slides were replaced every 6 h until no conidia were discharged. The method of evaluating conidia numbers described by Milner (1981) was used. The primary conidia discharged on microscope slides were stained with lactophenol cotton blue. The number of the primary conidia produced by each aphid at each time interval was estimated from a count of 10 random microscope fields (×200 magnification) per slide. The area of the view field and the discharge area were measured. The primary spore count was then multiplied by the appropriate factor to give the total number. In these calculations it was assumed that the discharge area was circular. At each temperature level there were five replications.

Three photophases 24 L, 12L/12D and 24 D were used to test the effect of light on sporulation at 20°C. Light source was provided by three 30-W lights. Otherwise, conditions were the same as described above. Five cadavers were replicated at each photophase. Five different humid conditions (51, 74, 90, 95, and 97.5% RH) were adjusted obtained in aqueous glycerol solutiona (Doberski 1981) in humid chambers at 20°C. Distilled water provided 100% RH. Slides supporting the cadavers were placed on the bottom of the small pot over the glycerol solution and were replaced every 24 h. Humid chambers were equilibrated for 12 h at 20°C before trials were conducted. The humidity was measured with a humidity meter (Humidity Temperature Meter Center 313, Taiwan) to ensure required humid conditions were attained in the humid chambers. Eight cadavers were replicated at each humidity treatment.

Plugs of *P. nouryi* were removed from the growing margin of Petri dish cultures (15 days old) and individually placed into Petri dishes. The Petri dishes supporting the sporulating plugs of *P. nouryi* were inverted over 2% water–agar plates for about 15 min. The plates had previously been placed in incubators at the test temperatures for 24 h. For verification of germination, autoclaved cover slips were placed on the showered conidia. The plates were then incubated at a different temperature under 12 L/12 D and there were five replications at each temperature. For qualitative observation of the effect of different substrates at different humidities, primary conidia were incubated on the surface of autoclaved cover slips and leaves of rape plants, *Brassica napus* L. (cultivar Jingyou 1), laid on filter paper in tightly lidded Petri dishes at 20°C. The filter papers were soaked with appropriate glycerol solutions to give the appropriate RH. The petioles of leaves were wrapped in sterile cotton balls saturated with Hoagland–Snyder nutrient solution and fresh film to avoid evaporation of the nutrient solution. These leave were then washed with distilled water and air dried. Cover slips were placed about 3 mm above the leaves to collect secondary conidia discharged. Primary conidia (P) and their moulage (the empty primary conidia and mucus) (M) and primary conidia growing germ tubes (G) on the surface of leaves were transferred to slides using a transparent adhesive plaster (5 mm wide) and stained with lactophenol cotton blue. There were four replicates at each RH. Conidial germination was evaluated by counting 150–300 conidia at $\times 400$ magnification. Percentage conidial germination was calculated by the following formula: $(M+G)/(P+M+G) \times 100$.

Insecticides (Table I) were tested at the rate of the manufacture's recommended concentration for field operation (R) and 5-fold lower than R concentration (0.2R). Evaluation of insecticide effect on conidial germination was as described in our previous study (Li et al. 2004a).

Number of conidia and percentage data were transformed by l g and sqrt (arcsin), respectively, before conducting significant test with one-way ANOVA and GLM-Univariate Analysis of variance (SPSS Inc. 1999). Before multiple comparisons were made, homogeneity of variances was tested by using Levene's test. Dunnett's T_3 was used when heterogeneousness occurred between variables ($P < 0.05$), otherwise LSD was used.

Table I. Effect of insecticides at 20% recommended field dose on conidial germination of *P. nouryi*.

Treatments	6 h 0.2R	12 h 0.2R	24 h 0.2R
Control	6.62±1.14a	51.42±3.14a	88.49±1.75a
Esfenvalerate (Sumi-alpha)	2.17±0.83b	33.80±2.97b	64.45±3.05b
Deltamethrin (Decis)	1.23±0.45bc	17.55±2.31c	45.51±3.15c
Fenpropathrin (Meothrin)	5.46±1.04a	36.54±4.29b	69.12±4.08b
Cyhalothrin (Kung Fu)	0.26±0.14c	12.95±3.93c	32.32±4.96c
Carbosulfan (Marshal)	5.32±0.76a	38.49±4.95b	72.94±1.92b
Chlorfluazuron (Atabron)	0.79±0.39bc	11.12±2.26c	39.40±5.16c
Chlorpyrifos (Lorben)	0	0	0
Endosulfan (Thiodan)	0	0	0

Note: Means (±SE) at two concentrations of one check time (6, 12, or 24 h) followed by the same letter were not significantly different ($P < 0.05$). All insecticides totally inhibited germination at the full recommended field dose. All insecticides which totally inhibited germination other doses are excluded from this table and the data analysis.

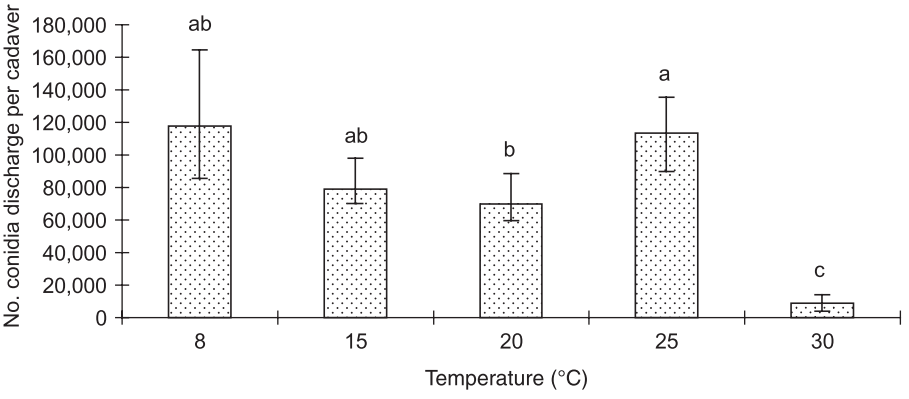

Figure 1. Mean and rang (max and min) conidia production per cadaver (different letters show significant diffrence between temperatures at 0.05 level).

Our results showed that large numbers of conidia could be produced between 8 and 25°C from *M. persicae* cadavers and that there was a significant effect of temperature on the output of primary conidia ($F_{(4,20)} = 74.98$, $P < 0.001$) (Figure 1). The discharge rate increased as temperature increased in the range of 8–25°C (Figure 2). Conidial discharge at 8°C was prolonged 6–12 h after incubation, and cadavers discharged conidia within 6 h. The longest duration of sporulation occurred at 8°C and conidial discharge could be detected after 120 h. Little sporulation occurred at 30°C and no conidia were discharged after 36 h. Peak sporulation between 15 and 25°C appeared at 12–18 h after incubation and few conidia could be detected after 48 h.

Duration of sporulation was much longer at low temperature than at high temperature, which may be an adaptive response to the fluctuation of *M. persicae* populations under different environmental conditions. Liu (1991) reported that the temperature range for development of *M. persicae* was 5–29°C and fecundity and reproductive rate increased as temperature increased. Therefore, in order to increase chances of encounter with the host when the aphid population density was very low during the cool season, the fungus stayed in cadavers and sporulates for a longer

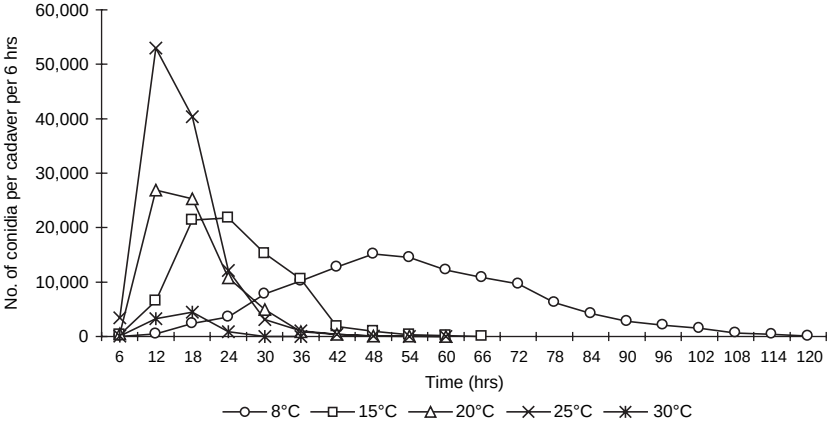

Figure 2. Sporulation of *P. nouryi* from cadaver of *M. persicae* at different temperature.

period. At higher temperatures when high densities of host exist, however, conidia in cadavers are discharged in a short period and spread rapidly in the host population.

Light did not affect the pattern of conidial discharge, nor total production of primary conidia (the average numbers were 63,000, 63,258 and 58,370 at 12 L, 12 L/12 D and 12 D, respectively) from cadavers at 20°C ($F_{(2,12)} = 0.157$, $P = 0.857$), but light could slightly stimulate the rate of discharge. *Entomophthora thaxteriana* (Wilding 1971) and *Conidiobolus coronatus* (Callaghan 1969) showed well-marked periodicity with most conidial discharge during the light regime. But Glare et al. (1986) found that there were no significant differences between the time-course patterns and total conidial output from *M. persicae* cadavers killed by *Zoophthora phalloides* held under continuous light or continuous dark. Our results further confirmed that there are large differences between species in the degree of their response to light (Wilding 1971).

The results of our study indicated that 100% RH did not satisfy the requirement for full sporulation from cadavers of *M. persicae* infected with *P. nouryi*, and existence of more moisture conditions (for example, in our experiment the cadavers fixed over the surface of water–agar obtained more moisture because of the evaporation of water in water–agar) was necessary for free discharge. At 20°C, the variability in discharge among cadavers was large at 100% RH and the average duration was greatly extended for 96–120 h. The production of conidia per cadaver was 12,485 (3545, 29,861) and was significantly fewer than that of cadavers on the water–agar surface ($F_{(1,11)} = 25.87$, $P < 0.001$). Six of eight cadavers could only discharge primary conidia within 24 h after aphid death at 97.5% RH and the average number of conidia was 8721 (1976, 21,343). At 95 and 90% RH, none of conidia were discharged, but conidiophores were detected on the surfaces of six out of eight cadavers. Fungi in cadavers at 74 and 51% RH failed to emerge through the insect cuticle. Butt et al. (1990) suggested that pseudocystidia of *Entomophthora* species would help trap a layer of moist air over the body of the infected insect and so prolong the period during which spores were discharged. Scarcity of pseudocystidia in *P. nouryi* (Gustafsson 1965) may explain our observation.

Germination was >95% at 15–25°C after 24 h when conidia were discharged on the surface of 2% water–agar, but 40 and 66% at 8 and 30°C, respectively. When RH was above 74%, conidia on surfaces of leaves could germinate (9%) after 24 h and the rate of germination increased when RH increased; at 100% RH germination rate reached 94%. However, there was no conidial germination when RH <100% on cover slips; even at 100% RH percentage of germination was only 0.85%. No germination was found when the conidia on cover slips under humid conditions of RH <100% for 6 h were replaced on the surface of water–agar. Our data suggest that the surfaces conidia attach to can affect conidial germination. Previous studies demonstrated that the nature of the surface has significant effects on conidial survival (Furlong & Pell 1997; Griggs et al. 1999).

All test insecticides inhibited conidial germination entirely at recommended doses (Table I). Significant differences were detected at 6, 12, or 24 h ($F_{(6,49)} = 13.97$, $P < 0.001$; $F_{(6,49)} = 20.17$, $P < 0.001$; $F_{(6,49)} = 27.08$, $P < 0.001$) between insecticides at 0.2R dose. Percentages of germination were >64% on the surface of water–agar treated with the three insecticides esfenvalerate–fenpropathrin and carbosulfan after 24 h and adverse effects were significantly lower than that of other insecticides. Even at 0.2R dose, conidia treated with chlorpyrifos and endosulfan could not germinate after 24 h. Generally, effects of fungicides on entomopathogenic fungi are much more

obvious than that of insecticides (Majchrowicz & Poprawski 1993). However, the results of our study suggested that the negative impact of insecticides on epizootics of entomophthoralean fungi in aphid populations should not be overlooked.

Acknowledgements

We thank Dr Mark S. Goettel (Lethbridge Research Centre, Canada) for kindly reviewing this manuscript and his invaluable advice for improvement. This work was supported by the State Key Laboratory of Integrated Management of Pest Insects and Rodents (Grant No. Chinese IPM0501), Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China.

References

Butt TM, Beckett A, Wilding N. 1990. A histological study of the invasive and developmental processes of the aphid pathogen *Erynia neoaphidis* (Zygomycotina: Entomophthorales) in the pea aphid *Acyrthosiphon pisum*. Canada Journal of Botany 68:2153–2163.

Callaghan AA. 1969. Light and spore discharge in Entomophthorales. Transactions British Mycological Society 53:87–97.

Doberski JW. 1981. Compartive laboratory studies on three fungal pathogens of the elrn bark beetle *Scolytus scolytus*: effect of temperature and humidity on infection by *Beauveria bassiana, Metarhizium anisopliae*, and *Paecilomyces farinosus*. Journal of Invertebrate Pathology 37:195–200.

Furlong MJ, Pell JK. 1997. The influence of environmental factors on the persistence of *Zoophthora radicans* conidia. Journal of Invertebrate Pathology 69:223–233.

Glare TR, Milner RJ, Chilvers GA. 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.

Griggs MH, Vandenberg JD, Sawyer AJ. 1999. Effect of relative humidity on viability of primary conidia of *Zoophthora radicans*. Journal of Invertebrate Pathology 73:315–320.

Gustafsson M. 1965. On species of the genus *Entomophthora* Fres. in Sweden I. Classification and distribution. Lantbrukshögskolans Annaler 31:103–202.

Liu SS. 1991. The influence of temperature on the population increase of *Myzus persicae* and *Lipaphis erysimi*. Acta Entomologica Sinica 34:189–197. (In Chinese)

Li ZZ. 2000. Flora fungorum sinicorum. vol. 13. Entomophthorales. Beijing, China: Science Press. (In Chinese)

Li W, Wang XF, Sheng CF. 2004a. Impact of sixteen chemical pesticides on conidial germination of two entomophthoralean fungi: *Conidiobolus thromboides* and *Pandora nouryi*. Biocontrol Science and Technology 14:737–741.

Li W, Wang XF, Li ZH, Xu WA, Sheng CF. 2004b. Survey of entomophthoralean fungi in Shandong province. Entomological Knowledge 41:350–353. (in Chinese)

Majchrowicz I, Poprawski TJ. 1993. Effects *in vitro* of nine fungicides on growth of entomopathogenic fungi. Biocontrol Science and Technology 3:321–336.

Milner RJ. 1981. Patterns of primary spore discharge of *Entomophthora* spp from the blue green aphid, *Acyrthosiphon kondoi*. Journal of Invertebrate Pathology 38:419–425.

Papierok B, Hajek AE. 1997. Fungi: Entomophthorales. In: Lacey LA, editor. Manual of techniques in insect pathology. London: Academic Press. pp 187–212.

SPSS Inc., 1999. Release 10.0 Version for Windows.

Wang WM, Zhao J, Lu WH. 1988. A pathogenic fungus of aphids, *Erynia nouryi*. Study and Application of Entomogenous Fungi in China 1:153–155. (in Chinese)

Wilding N. 1971. Discharge of conidia of *Entomophthora thaxteriana* Petch from the pea aphid *Acyrthosiphon pisum* Harris. Journal of General Microbiology 69:417–422.