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WINTER PREVALENCE OF OBLIGATE APHID PATHOGEN *Pandora neoaphidis* MYCOSIS IN THE HOST *Myzus persicae* POPULATIONS IN SOUTHERN CHINA: MODELING DESCRIPTION AND BIOCONTROL IMPLICATION

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ABSTRACT

Pandora neoaphidis overwintering had been investigated by monitoring its prevalence in *Myzus persicae* populations in open fields. Cabbage plants in field plots were weekly taken after mycosis initiation, to count and examine the living and dead aphids infected by *P. neoaphidis*. Based on the field data, infection levels (*I*) varied with field temperature (T), relative humidity (RH) and aphid count (numbers of living aphids per plant, *N*) over days (*D*), fitting well to the modified logistic equation $I=0.91/[1+\exp(8.5+(2.0H_TH_{RH}-20.2NI_0)D)]$ ($r^2=0.897$), where H_T indicated daily hours of low temperature (<4°C), H_{RH} daily hours of high air humidity (>90% RH) and I_0 primary infection level. The model demonstrated the abiotic and biotic factors influencing *P. neoaphidis* mycosis development in winter, and also verifies the fungal overwintering by infecting available host aphids without a resting stage. Ultimately, *P. neoaphidis* mycosis reduced 81.4% of aphid populations, presenting great potential for biocontrol.

Key words: Entomopathogenic fungi, Aphid, Epizootic modeling, Entomophthorales, Microbial control.

INTRODUCTION

Green peach aphid (*Myzus persicae* Sulzer), a worldwide euryphagous pest of crucifer vegetables and ornamental plants, infests cool-season crops such as *Brassica oleracea* in southern China even in the wintertime (2). Its natural enemy *Pandora* (=*Erynia*) *neoaphidis* (Remaudière & Hennebert) Humber in the order of Entomophthorales widely disseminated via host flight is recorded from over 70 species of aphids on annual and perennial crops and weeds in temperate regions (10, 14). This entomopathogenic fungus actively discharges numerous conidia from mycosed dead aphids to infect nearby hosts and frequently prevails over aphid cohorts in nature, thus *P. neoaphidis* is considered as a promising agent for aphid control (8, 14, 15).

Many species of entomophthoralean fungi have limited epizootic potential, because of frequently forming resting spores *in vivo* particularly under harsh surroundings as in winter (16, 23, 24). Resting spores forcibly interrupt infection cycles and stay quiescence for months or years irrespective of host availability (7). *P. neoaphidis* proves lacking this type of spore, and probably overwinters by either leaving conidia on humid and cool topsoil layers or forming thick-walled conidia or hypha within cadavers

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in situ or reproducing in alternate hosts (4, 5, 11, 13, 17). Continuously invading available hosts in winter favors controlling aphids on cool-season crops which are widely cultivated in southern China, but whether this is the prior overwintering mode and the detailed performance of *P. neoaphidis* affecting host population dynamics are unclear for integrated pest management.

Accordingly, the modeling description and analysis on the seasonal occurrence of *P. neoaphidis* are required for evaluating the fungal potential in controlling aphid populations. But modeling studies on mycosis prevalence of Entomphthorales in the host populations are scarce, especially in semi-natural habitats or open fields, due to the effects of the biotic and environmental factors and their interactions are complicated in natural epizootic development (9, 14, 19). Epizootic outbreak necessitates high relative humidity (> 90% RH) lasting several days, due to fungal sporulation, conidial germination and infection requiring near-saturated air humidity (12, 22). High (> 30°C) or low (< 4°C) temperatures inactivate most Entomophthorales, and in between, temperature significantly influences the span of infection cycle period, e.g., *P. neoaphidis*-killed aphids appear in 4.2, 6.9 and 13.6 days after maintained under fluctuating summer, autumn and winter temperatures in UK respectively (1, 12). Light regime probably contributes to death of mycosed hosts occurring in late afternoon for mycosis diffusion on humid nights (12), and ultraviolet radiation otherwise shortens fungal survival duration (6). Besides, host-related factors such as host density and other biotic factors including fungal infection level and actions of contaminated predators affect mycosis transmission, due to the efficiency of transmission dependent on contact probability of infectious conidia and healthy hosts (18, 20).

The present study aimed to reveal *P. neoaphidis* prevalence potential in winter for aphid control. This was achieved by describing the fungal dynamics in *M. persicae* populations with a modified logistic model based on field data. In the model, the associations between *P. neoaphidis* epizootic development and the abiotic (field temperature and relative humidity) and biotic (host density and primary infection level) factors were analyzed.

MATERIALS AND METHODS

Aphid and plant culture

Myzus persicae colonies were cultured for later field colonization, from the beginning of vigorous apterae collected in fields in September 2007. All the colonies were maintained on cabbage plants in a growth chamber at 25°C under a photoperiod of 12 h light and 12 h dark. Meanwhile, cool-season vegetable crops of cabbage (*Brassica oleracea*) were planted for infestation of *M. persicae* in the field experiment. Cabbage seeds germinated on layers of water-saturated filter paper at 20°C and light/dark 12h:12h, and then the seedlings were planted in walk-in 0.5-mm-mesh cages (2×2×2 m) standing in a field of the university campus farm for six weeks before transferred into open field plots.

Aphid infestation for observation

Healthy cabbage plants with four or five leaves were transferred into field plots in 20-cm plant spacing in late October. Four square field plots located separately on the university campus farm (119.729° E, 30.258° N) were prepared as replicates. Successively, five vigorous apterae of *M. persicae* with visually similar size taken from plants in the chamber were colonized onto each plant in the plots, to form a defined aphid population. The aphid cohorts infesting on the plants were used for monitoring natural *Pandora neoaphidis* prevalence level. Throughout the experimental period, all plants were grown conventionally, and no insecticide or fungicide was used.

Sampling and counting

P. neoaphidis mycosis seasonally occurs in this experimental planting zone, probably initiated by infected host

immigrants or air-borne conidia (3, 14). Sampling started as soon as white-tan dead aphids held tightly to the plants by rhizoids were observed. On each weekly sampling occasion, 12 plants were taken arbitrarily from the four plots (3 per each) and transferred to laboratory for counting living and dead aphids per plant. Temperature and relative humidity (RH) in the field were recorded two-hourly using an electronic hydrothermometer (Zheda Electric Apparatus, Inc., Hangzhou, Zhejiang, China) placed at the ground level.

Examining and evaluating the level of *P. neoaphidis* infection

All dead aphids on the 12 plants were separately maintained on coverslips for 12 hours at 20°C and 100% RH, for examining mycelial outgrowth and sporulation. To figure out the proportion of *P. neoaphidis*-killed aphids in cadavers, conidia ejected from mycosed cadavers were microscopically examined, and the identity of *P. neoaphidis* was confirmed based on the description of Humber (10). Besides, to figure out the *P. neoaphidis*-infected proportion in living aphids, thirty living aphids per plant (instars 3-4 without symptoms of infection and other diseases) were arbitrarily selected (total 360 capita examined on each sampling), and monitored for six days at 20°C and 12h light:12h dark using leaf discs; cadavers and conidia were examined as above. Leaf discs that need neither leaf changes nor aphid transference during the aphid monitoring period, were prepared by embedding the upside blades of detached cabbage within 1.5% agar in 90-mm Petri dishes and exposing the undersides for aphid raising (3).

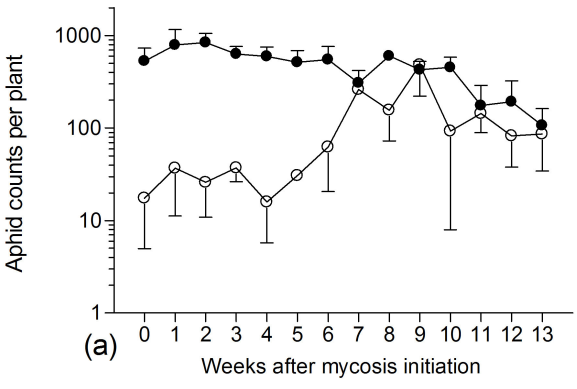
Modeling description for winter prevalence of *P. neoaphidis*

The abiotic/biotic factors of low-temperature duration per day (weekly mean hours of <4°C, denoted as H_T hereafter), high-RH (>90% RH) duration per day (H_{RH}), aphid count (number of living aphids per plant, N) and the primary infection level (computed from the first-sampling data, I_0) regulating infection level (I) trend over the days (D) after mycosis initiated were investigated. Before modeling analysis, values of H_T, H_{RH}, N and D were log (x+1) transformed and those of I_0, I were arcsine square-root transformed for reducing heterogeneity of variances. I–D observations were then fitted to a modified logistic equation $I=K/[1+\exp(a+(b_1H_TH_{RH}+b_2NI_I)D)]$, the binomial $b_1H_TH_{RH}+b_2NI_I$ presenting the increase rate of I over D, K a theoretically maximal I, a an intercept for the equation. The modeling analysis was conducted using an updated version of DPS software (21).

RESULTS AND DISCUSSION

Field observation

Myzus persicae colonies were well established on plants, living aphid density reaching the peak of average 840 aphids per plant on Dec. 23 and successively declining to the lowest point of 107 aphids per plant on Mar. 17 (Figure 1 a). Sampling started on Dec. 16 when *M. persicae* killed by *Pandora neoaphidis* were first observed, six weeks after the colonization by *M. persicae*. In the subsequent weeks, the peak of numbers of cadavers per plant was 482 recorded on Feb. 10 (Figure 1 a). Weather-wise, daily mean temperature and RH in the field fluctuated from −1.1 to 16.9°C (average 7.2°C) and from 54 to 99.8% RH (average 82.4% RH), respectively. Daily duration (≥ 10 hours) of >90% RH occurred in eight weeks and the weekly mean span of <4°C never exceeded 16.3 hours per day (Table 1), these environmental conditions favoring the occurrence and prevalence of *P. neoaphidis* mycosis.



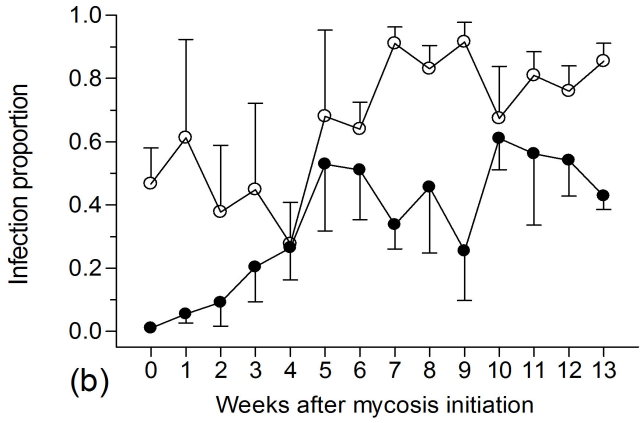


Figure 1. Field sampling for evaluating *Pandora neoaphidis* mycosis prevalence in *Myzus persicae* populations in winter. (a) The counts of living *M. persicae* (●) and dead aphids (○) per plant. (b) The proportions of *P. neoaphidis*-infected living aphids (●) and mycosed cadavers (○) examined on each sampling. Error bar: SD.

Table 1. Field observation of mycosis occurrence of *Pandora neoaphidis* in *Myzus persicae* populations in winter.

Sampling date [a]	Weekly mean ± SD		No. aphids examined [d]	Infection level [e]
	Low-temp duration (hrs) [b]	High-RH duration (hrs) [c]		
2007				
Dec. 16	4.0±3.5	7.1±3.6	569	0.024
Dec. 23	8.0±7.1	6.0±4.0	802	0.076
Dec. 30	7.7±7.8	12.9±9.6	670	0.100
2008				
Jan. 6	8.0±8.1	8.6±9.6	806	0.217
Jan. 13	16.3±5.5	12.3±9.2	550	0.265
Jan. 20	11.4±7.5	5.4±2.8	727	0.534
Jan. 27	10.6±9.2	9.1±8.7	1110	0.521
Feb. 3	4.0±4.8	16.3±5.8	3495	0.542
Feb. 10	0.0±0.0	14.6±8.8	2233	0.523
Feb. 17	0.6±1.5	9.4±8.4	6153	0.577
Feb. 24	1.1±2.3	20.9±5.6	1477	0.630
Mar. 3	0.9±1.6	24.0±0.0	2087	0.654
Mar. 10	1.1±2.3	14.9±8.9	1348	0.605
Mar. 17	1.7±2.4	11.7±8.8	1397	0.620

[a] Sampling began when mycosis was first observed in fields and ended with crop harvest.
[b c] Daily hours of low temperature (<4°C) or high relative humidity (>90% RH).
[d] The weekly examined aphids included cadavers and ca. 360 visually healthy living aphids collected from the 12 plants.
[e] *P. neoaphidis* infection level was computed based on the proportions of *P. neoaphidis*-killed and living-infected aphids.

Assessing *Pandora neoaphidis* prevalence level in fields

The proportions of cadavers killed by *P. neoaphidis* mycosis fluctuated in a range of 0.277-0.915, and the infected proportions in living aphids spanned from 0.001 to 0.610 (Figure 1 b). Based on these proportions, the weekly infection level of *P. neoaphidis* was computed as the sum of fungus-infected and -killed ones divided by the total of living and dead aphids, in a range of 0.024-0.654 (Table 1). Infection level increased slowly during the first four weeks, mostly attributing to the slight initial level of mycosis and environmental conditions. And the level soon over 0.5 meant the fungus-affected *M. persicae* in excess of mycosis-free ones in populations later. Finally, *P. neoaphidis*-killed cadavers accounted for 81.4% of the 18,384 cadavers collected, greater

than that of dead aphids parasitized by Hymenoptera or killed by other causes, i.e., *P. neoaphidis* mycosis contributed to 81.4% of the reduction of aphid population irrespective of migrants, and was the key factor acting on local aphid dynamics in winter.

Modeling description

Winter epizootic development in *P. neoaphidis*-affected *M. periscae* populations over time after mycosis initiation was well fitted to the abiotic/biotic-factors-modified logistic model $I=0.91/[1+\exp(8.5+(2.0H_TH_{RH}-20.2NI_0)D)]$, ($r^2=0.897$, $F_{3,10}=28.99$, $P=0.0001$), all estimated parameters in modeling significantly (Student's t test: $P<0.05$ for all parameters). The estimate of K equaled to the value (0.62) of theoretically maximal I, approaching that of 0.65 observed in the fields. The factors and their interaction including field temperature-RH and aphid density-primary infection level shaped the trend of the *P. neoaphidis* epizootic development, and the rate of I over D was fitted as $2.0H_TH_{RH}-20.2NI_0$. Thus, the resultant model was biologically robust enough to describe the mycosis occurrence of *P. neoaphidis* in winter quantitatively in relation to the variables considered (Figure 2). Variables we did not measure included the influence of sunlight, host transference and other natural enemies, thus a complete explanation is impossible here, warranting further studies.

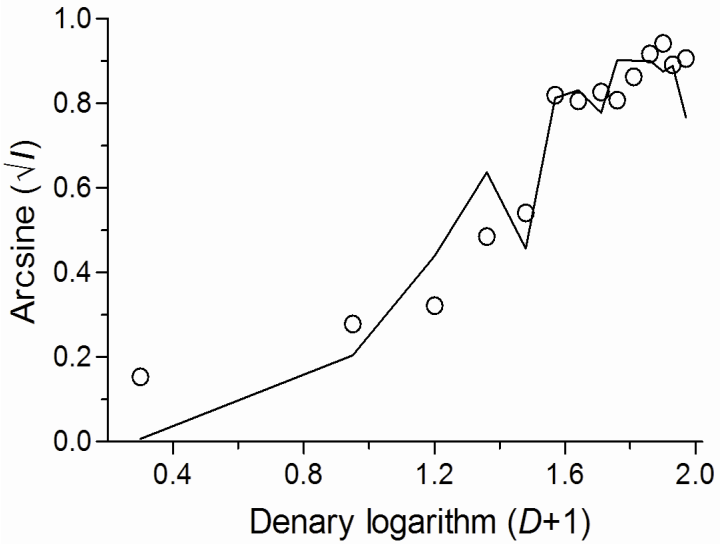


Figure 2. Trend of *Pandora neoaphidis* mycosis prevalence in *M. persicae* populations in winter. The curve describing infection level (I) trend was determined by a modified logistic equation $I=0.91/[1+\exp(8.5+(2.0H_TH_{RH}-20.2NI_0)D)]$ ($r^2=0.897$, $F_{3,10}=29.0$, $P=0.0001$), arcsine square-root transformations for estimates of primary infection level (I_0) and I, and log-transformations (x+1) for H_T (daily hours of low temperature <4°C), H_{RH} (daily hours of high air humidity >90% RH), N (number of living aphids per plant) and D (days after the first sampling date). The fitted parameters were estimated in modeling significantly (Student's t test: $P<0.05$ for all parameters). ○, the weekly computed infection level based on the *P. neoaphidis*-infected proportions in living aphids and dead aphids.

Concluding remarks and implications

Based on the results presented above, winter field temperatures in southern China are not consistently low enough (rarely below 0°C) to prevent the activities of *P. neoaphidis*, and infection continuously occurred in this field experiment, demonstrating the fungus tending to invade available hosts without a resting stage for overwintering. In the previous report (5), winter conditions in Switzerland (temperature constantly below 0°C) killing host pea aphids (*Acyrthosiphon pisum*) force *P. neoaphidis* to survive as primary spores in soil (a resting stage), and the fungus infects hosts recurred in spring. The distinct results attributes to the difference of host systems and environmental conditions, and also implies that *P. neoaphidis* prefers to infect hosts if available, i.e., active infection is the prior strategy of the fungal overwintering. But winter field conditions drastically prolong infection cycle period, four weeks required for infection level increasing from 0.024 to 0.534 in this study compared with 9 days for *Neozygites fresenii*-infected proportion in *Aphis Gossypii* rising from 0.12 to 0.75 in summer (1, 12). Considering

entomophthoralean epizootics always occurring too late to control pest populations below economic damage levels, mycosis development in winter is still vital for the timing of epizootic outbreaks (4, 14, 18, 20). That is, no pesticide is required to use on cool-season crops in winter, due to maintaining a high infection level of *P. neoaphidis* in fields favoring pest management in aphid-infested spring, in agreement with suggestions in some studies (1, 15) such as keeping plants in field margins to support fungal survival in alternate nonpest hosts between crop cycles for improving early season multiplication and dispersal to crop aphids.

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