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# Quantitative assessment of the infection rate of the entomophthoraceous fungus, *Zoophthora anhuiensis* against the green peach aphid Myzus persicae*

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**Abstract:** A two-step method was developed to quantitatively assess the infection rate of the entomophthoraceous fungus, *Zoophthora anhuiensis* (Li) Humber, on the green peach aphid, *Myzus persicae* (Sulzer). Firstly, a standard time-dose-mortality relationship, established by modeling data from bioassay 1 at varying conidial dosages (0.4 – 10.4 conidia/mm$^2$) of *Z. anhuiensis* F97028, was used to yield an estimate of expected mortality probability at a given dosage. Secondly, bioassay 2 was conducted by simultaneously exposing six ≤4-day-old nymphal colonies to a shower of *Z. anhuiensis* conidia at each of four dosages (resulting from exposures of 0.3 – 8.0 min). Subsequently, the colonies were separately immersed in a 0.1% chlorothalonil solution for 0.5 min to disinfect all surviving conidia on the host integument from 1 – 12 h after exposure under temperature treatments of 15 and 20℃, respectively. The infection rate during a specific period from the end of the exposure to the immersion was then estimated as the ratio of the observed mortality over the expected mortality probability at a particular dosage. The results showed that the infection of *M. persicae* from *Z. anhuiensis* was highly rapid with little difference between aphid colonies maintained at 15 and 20℃ before being immersed in the fungicidal solution after exposure. The first 6-hour period after exposure was most crucial to successful infection of the fungus with the infection rate greatly depending on conidial dosages. It took ≤1 h to infect >50% of the aphids at a dosage of >1.5 conida/mm$^2$ and >90% at >50 conidia/mm$^2$.

**Key words:** Zoophthora radicans, Myzus persicae, Infection rate, Dose dependence, Time-dose-mortality relationship, Conidia disinfection

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## INTRODUCTION

The entomophthoraceous fungus, *Zoophthora anhuiensis* (Li) Humber, is an aphid-specific pathogen only known from China. This fungal pathogen plays an important role in the natural control of aphid populations in the middle-lower reach of the Changjiang River during autumn and winter months (Li, 1986; Feng et al., 1995). Based on its virulence to the green peach aphid, *Myzus persicae* (Sulzer), the pathogen is a promising fungal agent for aphid control (Feng et al., 1998). The fungus grows and sporulates well *in vitro* at 10 – 20℃ (best at 15℃) but poorly over 25℃ (Li and Feng, 2000). At the appropriate temperature, illumination increases the level of sporulation, whereas temperature affects the rate of sporulation (Li and Feng, 2000). The estimates of $LT_{50}$ at a high conidial dosage (79 – 90 conidia/mm$^2$) of the fungus decreased from 8.4 days at 10℃ to 3.4 days at 25℃ on *M. persicae* (Liu et al., 2000), indicating that temperature affects the developmental rate of *Z. anhuiensis in vivo*. However, the rate of infection that occurs from conidial attachment through cuticular penetration is unclear.

Entomophthoraceous species may successfully infect their insect hosts within a few hours (Milner, 1997). There are different methods to estimate the proportions of insects infected by fungal pathogens within a specific period of

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time. Previously, the rates of entomophthoraceous infections were estimated mostly by counting germinated conidia in samples, fixed at varying time intervals, under scanning or transmission electronic microscope (Nadeau et al., 1996; Wraight et al., 1990). Glare and Milner (1991) measured the time required for the infection of *Pandora neoaphidis* (Remaudière & Hennebert) Humber to aphids by washing off conidia from aphids or transferring aphids from 100% RH to 70% RH at varying time intervals after inoculation. The lower relative humidity was assumed to inactivate the conidia. In the present study, a new method on basis of fungal disinfection by a fungicide at a specific time was developed to quantitatively assess fungal infection rates during a specific time period and used to estimate infection rate of *Z. anhuiensis* against *M. persicae*.

## MATERIALS AND METHODS

### Fungal isolate and inocula preparation

The isolate used in this study was *Z. anhuiensis* F97028, isolated from an *M. persicae* cadaver collected in Hangzhou, Zhejiang Province in November 1997. The isolate was maintained on slants of OS-SDAY at 3℃ in the dark, and subcultured every half year (Feng and Xu, 2001).

Preparation of fungal inocula for conidia shower began from recovery of a preserved slant on an SEMA (80% Sabouraud dextrose agar, 11.5% fresh milk, and 8.5% egg yolk) plate at 15℃ and 12:12 (L:D) for 2 weeks. The resulting fungal colony was then transferred into 15 ml of Sabouraud dextrose broth (SDB: 1% peptone, 4% dextrose, and 1% yeast extract) plus 0.2% sesame oil in a 50-ml Erlinmeyer flask. After shaking (120 rpm) at 20℃ for 2 days, the liquid culture was poured into 80 ml SDB in a 250-ml flask for another 2-day incubation. Every 10 ml of the resulting liquid culture was then poured onto a 150-mm plate containing 2% agar only, and excessive water was removed gently with filter paper. After incubating at 20℃ and 12:12 (L:D) for 2 days, mycelia mats on the plates became well sporulated and were ready for use.

### Aphid stock

A laboratory population of *M. persicae* was maintained on plants of the Chinese cabbage, *Brassica oleracea* L., in meshed cages at ambient temperature and light. Every five vigorous apterae (two or three days after last ecdysis) taken from the population were moved onto a cabbage leaflet and placed in a 65-mm Petri dish. The Petri dish was cushioned with two layers of cotton yarn and one layer of filter paper saturated with Hoagland-Snyder nutrient solution (van Emden, 1972). The edge of each leaflet was brushed with a 0.1% α-naphthy acetic acid solution to stimulate outgrowth of short hairy roots, which encourages nutrient uptake and keeps the leaflet fresh for more than 10 days. The apterae were allowed to freely produce viviparae on the leaflets at 20℃ and 12:12 (L:D) for 2 days and then removed, leaving 52 – 86 nymphs per leaflet. Reared at the same regime for another 2 days, the resulting second- or third-instar nymphs were ready to be inoculated via conidia shower.

### Bioassay 1 for time-dose-mortality relationship

To establish a standard time-dose-mortality relationship for *Z. anhuiensis* F97028 against *M. persicae*, seven nymphal colonies established on the leaflets were separately exposed to a shower of the primary conidia from the sporulating fungal mats. This was achieved by inverting the plates onto the leaflets in Petri dishes and rotating each dish 90° (in a horizontal plane) each quarter of exposure time to enssure uniform exposure. To determine conidia dosages, 20-mm square coverslips were placed beside the nymphal colonies to collect the discharged conidia, which were then counted from nine fields (0.785 $mm^2$ per field) under a microscope (Feng and Johnson, 1991; Feng et al., 1998). Different dosages (0.4 – 10.4 conidia/$mm^2$) were obtained by controlling the time of exposure (0.3 – 4.0 min). After exposure, the nymphs on the leaflets were maintained in the growth chamber at the regime of 20℃ and 12:12 (L:D). Nymphs were examined daily and mortality was recorded daily for 7 days. One colony, consisting of 58 nymphs not exposed to a conidia shower, was included as a control in the bioassay.

### Bioassay 2 for assessment of infection rate

Every six colonies of nymphs (on leaflets)

were simultaneously exposed to a conidia shower at each of four dosages from low to high, resulting from exposure times from 0.3 – 8.0 min. These were then maintained at 15℃ or 20℃ under a photophase of 12:12. Each colony consisted of 21 – 31 nymphs. Subsequently, the six colonies on leaflets at each dosage were separately immersed for 0.5 min in a 0.1% chlorothalonil solution 1, 2, 4, 6, 8, and 10 h (maintained at 20℃) or 1, 2, 4, 6, 10, and 12 h (maintained at 15℃) after exposure respectively. This treatment was known to kill all conidia that survived on the surface of the aphid integument at the time of immersion. Thus, the time interval from exposure to immersion was considered as the time length of effective infection. After immersion, all nymphal colonies on leaflets were maintained at the regime of 20℃ and 12:12 (L:D) and observed daily for aphid mortality for 7 days.

A preliminary assay was undertaken to select an appropriate fungicide that was lethal to *Z. anhuiensis* conidia, but harmless for *M. persicae* nymphs. Among several fungicides tested at various concentrations, a solution containing 0.1% chlorothalonil was found to meet the requirement. It had no effect on the development of 71 first-instar nymphs of *M. persicae* into apterae but was entirely inhibitory to germination of *Z. anhuiensis* conidia with no conidia germinating within 24 h after treatment. Thus, the 0.1% chlorothalonil solution was chosen for use in the treatments.

**Data analysis**

The data from bioassay 1 were analyzed using the following time-dose-mortality model:

$$P_{ij} = 1 - \exp[-\exp(\tau_j + \beta \log 10(d_i))]$$

ok!

where $P_{ij}$ represents the cumulative mortality probability caused by dose $d_i$ ($i = 1, 2, \cdots, I$) at the time $t_j$ ($j = 1, 2, \cdots, J$), $\beta$ is the slope describing the dose effect, and $\tau_j$ is the parameter(s) for the time effect of $d_i$ during the period from start to the $j$th observation. For procedures of modeling, computation of $\beta$ and $\tau_j$, and the test for goodness of fit we refer the reader to Nowierski et al. (1996), Feng et al. (1996, 1998), and Feng and Poprawski (1999). The modeling was performed using DPS data processing system software (Tang et al., 1997), generating the estimates of $\beta$ and $\tau_j$ for computing an expected cumulative mortality probability at a given time for a particular dosage of conidia.

Percent mortality in bioassay 2 was calculated by determining the number of aphids killed on each leaflet by *Z. anhuiensis* following a conidia shower and immersion in 0.1% chlorothalonil at infection times and temperatures from 1 – 10 h at 20℃ or 1 – 12 h at 15℃. The mortality values observed from the aphid colonies at each dosage were then compared to the expected cumulative mortality probability given by the model at the same dosage. The ratio of the observed cumulative mortality, attributed to infection during a specific period of time before immersion, over the expected cumulative mortality probability was considered to be the infection rate at the time interval after exposure.

## RESULTS

**Time-dose-mortality modeling**

Initial aphid mortality, attributed to infection by *Z. anhuiensis*, occurred on day 2 after exposure to the conidia shower at most of the dosages (Table 1). The mortality then dramatically increased on days 4 – 6. At day 7 the mortality ranged from 45.3% at 0.4 conidia/mm$^2$ to 95.5% at 10.4 conidia/mm$^2$. All cadavers exhibited typical *Zoophthora* symptoms (i.e., yellowish in colour and fungal outgrowths visible). There was no control mortality due to fungal infection.

The data in Table 1 fit the time-dose-mortality model well based on the Hosmer-Lemeshow test for homogeneity of modeling ($C = 6.49$, $df = 8$, $P = 0.12$) (Nowierski et al., 1996; Feng and Poprawski, 1999). The modeling generated the estimates of the parameters of $\beta$ and $\tau_j$. The parameter for dose effect, $\beta$, was estimated to be 1.469, which was similar to the dose effect estimates of some chemical pesticides (1.2 – 4.5) (Preisler and Robertson, 1989). The cumulative time effect parameter, $\tau_j$, was estimated to be –3.58, –2.57, –1.71, –0.65, –0.13, and 0.17 for days 2 – 7, respectively. Based on the estimates of $\beta$ and $\tau_j$, the expected cumulative mortality probability of *M. persicae*

**Table 1 The number of daily surviving *M. persicae* individuals after exposure to conidial shower of *Z. anhuiensis* at varying dosages and cumulative mortality on day 7 in bioassay 1**

| No. conidia $mm^{-2}$ | No. aphids surviving on the *j*th day after conidial shower | | | | | | | Mortality due to infection (%) |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | |
| 0.4 | 86 | 86 | 82 | 77 | 69 | 58 | 47 | 45 |
| 0.8 | 52 | 49 | 46 | 41 | 28 | 23 | 17 | 67 |
| 1.3 | 67 | 64 | 61 | 55 | 41 | 27 | 18 | 73 |
| 1.7 | 78 | 75 | 66 | 57 | 32 | 17 | 12 | 85 |
| 2.0 | 54 | 51 | 47 | 42 | 28 | 14 | 10 | 82 |
| 2.5 | 73 | 70 | 67 | 61 | 29 | 13 | 5 | 93 |
| 10.4 | 67 | 61 | 53 | 28 | 5 | 4 | 3 | 96 |

as a function of the dosage of *Z. anhuiensis* conidia and the length of time after exposure was then computed for the following comparison.

**Infection rate**

In bioassay 2, the results of *M. persicae* colonies at 15℃ on leaflets immersed in 0.1% chlorothanil solution for infection times for 1 – 10 h after exposure are given in Table 2. The cumulative mortality on day 7 varied greatly among the colonies treated with the 0.1% chlorothalonil solution at different lengths of time after exposure though they received the same dosage of conidia. At the dosage of 0.7 conidia/$mm^2$, the mortality observed was 13.6, 29.6, 45.5, 57.1, 56.7, and 58.1% in the colonies immersed 1, 2, 4, 6, 8, 10 h after exposure, respectively. Compared to the expected cumulative mortality of 61.4% estimated at the same dosage for the same day, the mortality observed apparently increased with the length of time from exposure to immersion. The infection rate was thus estimated as 22.1% within 1 h after exposure, 48.2% within 2 h, 74.1% within 4 h, and reached 93.0% within 6 h but increased little after 6 h. Also, effective infection occurred by

**Table 2 Observed cumulative mortality (OCM) of *M. persicae* on day 7 at four conidia dosages of *Z. anhuiensis* in bioassay 2 (maintained at 20℃ for 1 – 10 h before being immersed in 0.1% chlorothalonil solution) in comparison with expected cumulative mortality (ECM)**

| No. conidia $mm^{-2}$ | Infection time (h) | No. Aphids tested | OCM (%) | Infection rate (%) | No. conidia $mm^{-2}$ | Infection time (h) | No. Aphids tested | OCM (%) | Infection rate (%) |
|---|---|---|---|---|---|---|---|---|---|
| 0.7 | 1 | 22 | 13.6 | 22.1 | 9.0 | 1 | 24 | 66.7 | 67.4 |
| | 2 | 27 | 29.6 | 48.2 | | 2 | 26 | 84.6 | 85.5 |
| | 4 | 22 | 45.5 | 74.1 | | 4 | 27 | 88.8 | 89.7 |
| | 6 | 28 | 57.1 | 93.0 | | 6 | 22 | 90.9 | 91.8 |
| | 8 | 30 | 56.7 | 92.3 | | 8 | 21 | 90.5 | 91.4 |
| | 10 | 31 | 58.1 | 94.6 | | 10 | 29 | 100.0 | 101.0 |
| ECM | | | 61.4 | | ECM | | | 99.0 | |
| 1.8 | 1 | 30 | 46.7 | 57.2 | 49.9 | 1 | 31 | 90.3 | 90.3 |
| | 2 | 25 | 44.0 | 53.9 | | 2 | 21 | 100.0 | 100.0 |
| | 4 | 30 | 53.3 | 65.2 | | 4 | 22 | 100.0 | 100.0 |
| | 6 | 31 | 77.4 | 94.7 | | 6 | 26 | 100.0 | 100.0 |
| | 8 | 30 | 80.0 | 97.9 | | 8 | 27 | 100.0 | 100.0 |
| | 10 | 25 | 80.0 | 97.9 | | 10 | 28 | 100.0 | 100.0 |
| ECM | | | 81.7 | | ECM | | | 100.0 | |

> 50% within 1 h and > 90% within 6 h at 1.8 – 9.0 conidia/mm$^2$, and reached 90% within 1 h at 49.9 conidia/mm$^2$. The data from bioassay 2 of the colonies maintained at 15℃ before immersion (Table 3) show a tendency similar to those at 20℃. Under both temperature treatments, the infection of *Z. anhuiensis* to *M. persicae* was highly rapid. The first 6 hour period after exposure was found to be most crucial to successful infection of *M. persicae* by *Z. anhuiensis* with the infection rate greatly depending on conidia dosage. No more than one hour was needed for > 50% infection above 1.5 conidia/mm$^2$ and for > 90% infection above 50 conidia/mm$^2$.

**Table 3 Observed cumulative mortality (OCM) of *M. persicae* on day 7 at four conidia dosages of *Z. anhuiensis* in bioassay 2 (maintained at 15℃ for 1 – 10 h before being immersed in 0.1% chlorothalonil solution) in comparison with expected mortality probability (ECM)**

| No. conidia mm$^{-2}$ | Infection time (h) | No. Aphids tested | OCM (%) | Infection rate (%) | No. conidia mm$^{-2}$ | Infection time (h) | No. Aphids tested | OCM (%) | Infection rate (%) |
|---|---|---|---|---|---|---|---|---|---|
| 0.8 | 1 | 28 | 3.6 | 5.7 | 6.9 | 1 | 27 | 55.6 | 56.7 |
| | 2 | 30 | 26.7 | 42.2 | | 2 | 28 | 75.0 | 76.5 |
| | 4 | 25 | 28.0 | 44.2 | | 4 | 21 | 76.2 | 77.7 |
| | 6 | 25 | 60.0 | 94.8 | | 6 | 31 | 87.1 | 88.8 |
| | 10 | 26 | 61.5 | 97.2 | | 10 | 25 | 92.0 | 93.8 |
| | 12 | 29 | 62.1 | 98.1 | | 12 | 20 | 90.0 | 91.7 |
| ECM | | | 63.3 | | ECM | | | 98.1 | |
| 1.6 | 1 | 24 | 45.8 | 57.6 | 54.8 | 1 | 30 | 100.0 | 100.0 |
| | 2 | 25 | 44.0 | 55.3 | | 2 | 22 | 100.0 | 100.0 |
| | 4 | 32 | 56.3 | 70.8 | | 4 | 25 | 100.0 | 100.0 |
| | 6 | 26 | 65.4 | 82.3 | | 6 | 26 | 100.0 | 100.0 |
| | 10 | 28 | 71.4 | 89.8 | | 10 | 26 | 100.0 | 100.0 |
| | 12 | 29 | 79.3 | 99.7 | | 12 | 28 | 100.0 | 100.0 |
| ECM | | | 79.5 | | ECM | | | 100.0 | |

Running head: Quantitative assessment of the infection rate of *Z. anhuiensis*

## DISCUSSION

All entomophthoraceous species are important insect pathogens and are characterized by their ability to rapidly infect hosts (Humber, 1989; Milner, 1997). Based on the results presented above, we demonstrated that infection of *Z. anhuiensis* to *M. persicae* was largely completed at 15 – 20℃ within 6 h after the attachment of the conidia to the integument with the infection rate being dose-dependent. In the previous reports, germination of *Z. radicans* (Brefeld) Batko conidia attached to the integument of *Empoasca fabae* (Harris) reached 58.9% within 2 h at 20℃ and 90% during a 14-h period (Wraight et al., 1990). On the wings of the black flies, *Simulium rostratum* (Walker) and *S. decorum* (Stone), 50% of the primary conidia of *Erynia conica* (Nowakowski) Remaudière & Hennebert germinated during the first 2-4 h and 70 – 80% within 9 h (Nadeau et al., 1996). The infection of *Pandora neoaphidis* (Remaudière & Hennebert) Humber to aphids also occurred within 2-4 h after inoculation and peaked within 6-8 h (Glare and Milner, 1991). Thus, the rate of *Z. anhuiensis* infection to *M. persicae* assessed with our method shows a trend similar to those in previous reports but relates to conidia dosages used.

Fungal infection starts from germination of conidia attached to the host integument because conidia germination may result in penetration into the integument. However, germination does not necessarily lead to successful infection. With this point in mind, fungal infection rates based

on counts of germinated conidia under scanning electronic microscope (Nadeau et al., 1996; Wraight et al., 1990) are virtually indirect estimates. In such an experimental system, moreover, statistical robust analyses are usually not possible due to the limited number of specimens usually associated with electronic microscopy. On the other hand, the washing or drying method introduced by Glare and Milner (1991) may allow for direct estimation of infection rates but hardly gurantees that all conidia are killed at time as specific as possible before germination.

The method developed for quantitative assessment of fungal infection rate in this study is more reliable and practicable for the following two reasons. Firstly, a fungicide that is fatal to fungal conidia but harmless to host insects can be chosen to disinfect the conidia on the host integument at the appropriate time required. This makes it feasible to precisely examine the effective infection of conidia during a specific period after exposure to the conidia shower at a given dosage. Secondly, a generalized time-dose-mortality relationship can be generated to give an expected mortality at the dosage and infection time concerned. This allows examination of the difference between 'normal' infection and time-specific infection at the dosage concerned. Hence, this method offers an improvement over previous methods used to quantitatively assess the rate of fungal infection.

## References


Feng, M. G. and Johnson, J. B., 1991. Bioassay of four entomophthoralean fungi (Entomophthorales) against *Diuraphis noxia* and *Metopolophium dirhodum* (Homoptera: Aphididae). *Environ. Entomol.*, **23**: 338 – 345.

Feng, M. G., Hu, G. C. and Huang, S. W., 1995. Epizootics and associated fungal pathogens in Myzus persicae in southern China. *In*: Proceedings of the 1995 National Symposium on Biological Control, p. 161. Beijing, China.

Feng, M. G., Tang, Q. Y., Hu, G. C. and Huang, S. W., 1996. Susceptibility of seven species of aphids to a *Beauveria bassiana* isolate: analysis of time-dose-mortality model. *J. Basic Sci. Engin.*, **4**: 22 – 33 (in Chinese).

Feng, M. G. and Liu, C. L., Xu, J. H. and Xu, Q., 1998. Modeling and biological implication of time-dose-mortality data for the entomophthoralean fungus, *Zoophthora anhuiensis*, on the green peach aphid *Myzus persicae*. *J. Invertebr. Pathol.*, **72**: 246 – 251.

Feng, M. G. and Poprawski, T. J., 1999. Robustness for the processing of bioassay data of microbial control agents based on time-dose-mortality model. *Subtrop. Plant Sci.*, **51**: 38 – 40.

Feng, M. G. and Xu, Q., 2001. A simple method for routine maintenance and preservation of entomophthoraceous cultures. *J. Invertebr. Pathol.*, **77**: 141 – 143.

Glare, T. R. and Milner, R. J., 1991. Ecology of entomopathogenic fungi. *In*: Handbook of Applied Mycology, vol. 2, Humans, Animals and Insects (D.K. Arora, L. Ajello, and K.G. Mukerji, Eds.), pp. 547 – 612. Marcel Dekker, New York.

Humber, R. A., 1989. Synopsis of a revised classification for the entomophthorales (Zygomycotina). *Mycotaxon*, **34**: 441 – 460.

Li, H. P. and Feng, M. G., 2000. Effect of temperature and light on the spore production of the entomophthoralean fungus *Zoophthora anhuiensis*. *Mycosystema*, **19**: 348 – 353 (in Chinese).

Li, Z., 1986. *Erynia anhuiensis*, a new pathogen of aphids. *Acta Mycol. Sin.*, **5**: 1 – 6 (in Chinese).

Liu, C. L. and Feng, M. G., 2000. Impact of temperature and humidity combinations on development of mycosis in *Myzus persicae* with the entomophthoralean fungus, *Zoophthora anhuiensis*. *Acta Entomol. Sin.*, **43**: 380 – 387 (in Chinese).

Milner, R. J., 1997. Prospects for biopesticides for aphid control. *Entomophaga*, **42**: 227 – 239.

Nadeau, M. P., Dunphy, G. B., and Boisvert, J. L., 1996. Development of *Erynia conica* (Zygomycetes: Entomophthorales) on the cuticle of the adult flies *Simulium* rostratum and *Simulium* decorum (Diptera: *Simuliidae*). *J. Invertebr. Pathol.*, **68**: 50 – 58.

Nowierski, R. M., Zeng, Z., Jaronski, S. Dekgado, F. and Swearingen, W., 1996. Analysis and modeling of time-dose-mortality of *Melanoplus sanguinipes*, *Locusta migratoria migratorioides*, and *Schistocerca gregaria* (Othortera: Acrididae) from *Beauveria*, *Metarhizium* and *Paecilomyces isolates* from Madagascar. *J. Invertebr. Pathol.*, **67**: 236 – 252.

Preisler, H. K. and Robertson, J. L., 1989. Analysis of time-dose-mortality data. *J. Econ. Entomol.*, **82**: 1534 – 1542.

Tang, Q. Y. and Feng, M. G., 1997. Practical statistics and computer processing platform. China Agricultural Press, Beijing. 407 p (in Chinese).

Van Emden, H. F., 1972. Aphid technology. Academic Press, London and New York, 344 p.

Wraight, S.P., Butt, T.M., Galaini-Wraight, S., Allee, L., Soper, R.S. and Roberts, D.W., 1990. Germination and process of the entomophthoralean fungus *Erynia radicans* on the Potato leafhopper, *Empoasca fabae*. *J. Invertebr. Pathol.*, **56**: 157 – 174.