THE VIRULENCE OF THE ENTOMOPHTHORALEAN FUNGUS *PANDORA DELPHACIS* TO THE BROWN PLANTHOPPER, *NILAPARVATA LUGENS*[1)]

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Abstract The entomophthoralean fungus, *Pandora delphacis* (Hori) Humber, is a pathogen frequently causing epizootics of the brown planthopper, *Nilaparvata lugens* Stål, in rice growing areas of China. Two isolates (F95127 and F95129) of the fungus obtained from mycosis-killed planthoppers collected in Zhejiang Province were bioassayed for their virulence to *N. lugens*. For inoculation, batches of 4- or 5-instar-old nymphs from a laboratory population were exposed to time-varying spore shower from sporulating plates of fungal mass produced in Sabouraud liquid medium, resulting in 8 and 6 dosages for F95129 and F95127, respectively. The nymphs treated at each dosage were maintained on rice plants at 25°C and a photophase of 12L : 12D in growth chamber (nearly 100% RH) and were examined daily for mortality. The resulting data were well fitted to time-dose-mortality model, generating parameters of time and dose effects for estimation of virulence indices. The LD_{50} values 6—8 d after exposure were 327, 122 and 46 conidia/mm^2 for F95129 and 409, 133 and 74 conidia/mm^2 for F95127, respectively. The LT_{50} estimated for F95127 was 7.6 d at 95 conidia/mm^2 whereas that for F95129 decreased from 6.6 d at 172 conidia/mm^2 to 5.6 d at 525 conidia/mm^2. Based on these virulence indices and comparison of the slopes for dose effects from the model, the two isolates of *P. delphacis* had moderate virulence to *N. lugens* with insignificant difference to each other.

Key words *Pandora delphacis*, *Nilaparvata lugen*, virulence, time-dose-mortality analysis

1 INTRODUCTION

The brown planthopper, *Nilaparvata lugen* Stål, is one of the most devastating rice pests in China and southeastern Asian countries (Li *et al.* 1996). The entomophthoralean fungus, *Pandora delphacis* (Sori) Humber, is documented as an important pathogen naturally infecting planthoppers (Melissa and James 1987, Feng *et al.* 1995, 1996a, Li 1984). Cai (1986) investigated sporulation of the fungus cultured in vitro. A recent study has shown the effect of varying photophases on the growth and sporulation pattern of the fungal mass produced in liquid culture (Xu and Feng 1998). However, little effort has been made to demonstrate the potential of the fungus for use in microbial control since it was first described in Japan (Hori 1906). This paper presents bioassay data to describe time-dose-mortality relationships between two *P. delphacis* isolates and *N. lugens* and to evaluate the virulence of the fungal pathogen against the insect species.

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2 MATERIALS AND METHODS

2.1 Sources of isolates

Two *P. delphacis* isolates, F95127 and F95129, were isolated from naturally infected *N. lugens* cadavers collected in Hangzhou, Zhejiang Province (Feng *et al.* 1995) and maintained on SEMA (80% Sabouraud dextrose agar, 11.5% fresh milk, 8.5% egg yolk) at 15℃ and a photoperiod of 12L : 12D by subculture at intervals of about 20 d. Fungal masses from 7-d plate culture at 25℃ were used to initiate liquid culture in 100-ml flask containing 30-ml Sabouraud dextrose broth (SDB; 4% glucose, 1% peptone, 1% yeast extract plus 20μg PSN antibiotics), shaken at (28±1)℃ in dark (150 r/min). The 24-h liquid culture was then transferred into 80-ml SDB in 200-ml flask for multiplication for another 24 h at the same regime. Consequently, each 20 ml of the resulting liquid culture was poured onto a 90-mm Petri dish containing agar only and excessive water was removed using filter paper. Incubated at 25℃ and 12L : 12D for 24 h, fungal mats on the plates became sporulating and was ready for use in bioassay.

2.2 Planthoppers

A laboratory population of *N. lugens* were maintained on the plants of *Oryza sativa* L. (cv: TN1) in meshed plots in greenhouse at (30—35)℃ and 12L : 12D. Nymphs including 4—5 instars were randomly collected from the meshed plots to receive inoculation for bioassay.

2.3 Bioassay

The 4- or 5-instar nymphs were placed in a 90-mm Petri dish lined with a layer of filter paper. Because the nymphs are highly mobile, a nutrient solution provided by Dr. Qiang Fu (China National Rice Research Institute, Hangzhou 310006) was used to moisten the filter paper for resting the nymphs for probing and feeding during the following inoculation. Each plate with sporulating fungal mat was inverted onto each dish containing about 100 nymphs. As a result, the nymphs were exposed to the shower of spores (primary conidia) forcibly discharged from the plate. To measure the spore dosage the nymphs received, one 20-mm glass coverslip was placed at the center of each dish to receive spores discharged from the plate and was rotated at 90° at each quarter of the exposure time. After the exposure the dosage was determined by microscopically counting the numbers of spores from five fields of the coverslip (0.785 mm^2 per field). Eight dosages ranging from 0.6 to 1 186 spores /mm^2 were obtained by controlling the length of exposure time (1—60 min) and 122 nymphs not receiving spore shower for inoculation were included as control.

The planthoppers after exposure to each dosage were then maintained at 30℃ and 12L : 12D on 15-d-old rice seedlings in two glass cylinders (200 mm in length and 21 mm in diameter) containing 15-ml rice nutrient solution (Yoshida 1976). Seedlings in each cylinder were fixed by wrapping sponge near the roots. This system could sustain the planthoppers for at least 10 d with no need for change of food plants. The planthopper nymphs treated with dosage were daily observed for mycosis and cadavers, whenever found, were removed from cylinders and examined for fungal infection by mounting slides under microscope after overnight incubation in moist chamber.

2.4 Data analysis

A time-dose-mortality modeling technique (Feng *et al.* 1996, 1998; Nowierski *et al.* 1996, Robertson and Preisler 1992) was used to analyze the resulting time-dose-mortality data of *P. delphacis* for *N. lugens*. Assuming that a bioassay includes I dosages and J times of observation, the cumulative mortality probability, p_ij, caused by the dose $d_i(i = 1, 2, \cdots, I)$ at the time of the jth observation time $t_j(j = 1, 2, \cdots, J)$ can be depicted as follows:

$$p_{ij}=1-\exp[-\exp(\tau_j+\beta \log_{10}(d_i))], \quad (1)$$

where β is the slope to describe the dose effect, and τ_j is the parameter(s) for the time effect of d_i during the period from start to the jth observation, $(t_1, t_2, \cdots, t_{j-1}, t_j)$. Note that p_{ij} is a time-dependent (time cumulative) variable. However, Eq. 1 cannot be fitted directly to the bioassay data because the binomial variable, p_{ij}, for modeling does not satisfy the requirement for independence of time. To guarantee that the observed mortality probability is independent of time, the true mortality which occurred at d_i at the interval (t_{j-1}, t_j) must be considered. This true mortality, q_{ij}, was called conditional mortality probability (Robertson and Preisler 1992) and expressed below:

$$p_{ij}=1-\exp[-\exp(\gamma_j+\beta \log_{10}(d_i))], \quad (2)$$

where β is equal to that in Eq. 1 and γ_j describes the time effect of d_i at the interval (t_{j-1}, t_j). Because of the independence between the time intervals, Eq. 2 was used to fit the data by approaching the binomial reponse variable to the maximum likelihood equation (Feng *et al.* 1996, Nowierski *et al.* 1996, Robertson and Preisler 1992), resulting in $\hat{\gamma}_j$ and $\hat{\beta}$. Then, $\hat{\tau}_j$ were calculated using the formula (Robertson and Preisler 1992):

$$\hat{\tau}_j=\ln\left(\sum_{k=1}^{J} e^{\hat{\gamma}_j}\right). \quad (3)$$

The processes, including modeling, estimation of time-and dose-effect parameters for both conditional and cumulative models, test for goodness of fitness, and estimation of lethal dose and time effects (LD_{50} and LT_{50}) using the parameters, were conducted using DPS Data Processing System software (Tang and Feng 1997).

3 RESULTS

3.1 An overview to the bioassay

Listed in Table 1 are the numbers of the planthoppers surviving during 10 d after exposure to spore shower at varying spore dosages of the isolates F95127 and F95129, respectively. The cumulative mortality observed on day 10 after exposure ranged from 27.4 % to 60.7% for F95127 and 41.3% to 82.3% for F95129, increasing as the dosage increased.

3.2 Modeling, estimation of parameters, and goodness of fitness

The time-dose-mortality data for the two isolates were separately fitted to Eq. 2, generating parameters $\hat{\beta}$ and $\hat{\gamma}_j$ (j = the jth day after exposure) that describe conditional dose and time effects for the two isolates acting on *N. lugens*. Then, the $\hat{\gamma}_j$ values were used to estimate cumulative time effects, τ_j for Eq. 1 (j = the number of days after exposure) based on Eq. 3. All parameters estimated for the dose and time effects of the two isolates on *N. lugens* are listed in Table 2.

Table 1 The numbers of *N. lugens* nymphs daily surviving after exposure to spore shower of the two *P. delphacis* isolates F95127 and F95129 at varying spore dosages and cumulative mortality probability observed on day 10.

Dose	No. nymphs	No. planthoppers daily surviving after exposure										Observed mortality
conidia/mm^2	treated	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	probability
Isolate F95127												
0.6	62	59	58	56	54	52	51	50	48	46	45	0.27
6.4	113	110	107	102	95	91	89	87	84	82	77	0.32
7.4	78	74	71	66	60	56	53	50	47	45	43	0.45
12.0	87	82	76	71	65	61	57	54	50	47	47	0.46
64.2	103	94	89	82	75	65	61	58	55	51	45	0.56
94.5	84	77	71	65	58	50	46	43	39	36	33	0.61
Isolate F95129												
2.4	92	88	87	84	81	76	73	68	64	60	54	0.41
9.4	95	92	91	87	84	79	74	69	61	54	53	0.44
33.4	93	86	84	81	76	68	60	54	47	46	46	0.51
79.5	86	81	78	74	65	58	52	47	41	41	38	0.56
172.2	93	90	84	80	70	63	54	46	40	40	39	0.58
524.8	63	56	52	49	42	36	31	25	20	20	19	0.70
619.6	81	71	63	55	46	41	34	30	25	23	23	0.72
1186.2	51	42	36	30	22	17	14	12	10	9	9	0.82

The slope, $\hat{\beta}$ for the conditional dose effect of F95127 (0.516 ± 0.119) was slightly larger than that for F95129 (0.478 ± 0.064) but this difference was not significant ($t = 0.0536$, $P = 0.9581$). This indicates that the two isolates had similar dose effect on *N. lugens*. The parameters estimated for the time effects also varied in a similar pattern. During the 10-d period after exposure the cumulative dose effect, $\hat{\tau}_j$ (estimated from $\hat{\gamma}_j$ using Eq. 3), increased from -3.434 to -1.118 for F95127 and from -3.552 to -1.043 for F95129. This is visual when the values of the conditional and cumulative mortality probability simulated using $\hat{\beta}$, $\hat{\gamma}_j$, and $\hat{\tau}_j$ in Table 2 were plotted against the number of days after exposure to spore shower and the logarithms of spore dosages (Fig. 1). Therefore, the time-dose-mortality relationships of the two isolates with the insect species tested were similar.

Table 2 Parameters of dose and time effects estimated for conditional and cumulative time-dose-mortality models by fitting the data from bioassaying the *P. delphacis* isolates F95127 and F95129 against *N. lugens* nymphs at 30℃ and 12L:12D

Conditional mortality model, Eq. 2				Cumulative mortality model, Eq. 1			
Parameter[1)]	Mean	S.E	t[2)]	Parameter[1)]	Mean	var($\hat{\tau}_j$)	cov($\hat{\beta}$, $\hat{\tau}_j$)
Isolate F95127							
$\hat{\beta}$	0.516	0.119	4.335	$\hat{\beta}$	0.516	0.014	0.014
$\hat{\gamma}_1$	−3.434	0.179	19.228	$\hat{\tau}_1$	−3.434	0.032	−0.010
$\hat{\gamma}_2$	−3.629	0.204	17.775	$\hat{\tau}_2$	−2.834	0.024	−0.013
$\hat{\gamma}_3$	−3.343	0.301	11.100	$\hat{\tau}_3$	−2.363	0.029	−0.014
$\hat{\gamma}_4$	−3.109	0.240	12.953	$\hat{\tau}_4$	−1.975	0.028	−0.016
$\hat{\gamma}_5$	−3.107	0.207	14.979	$\hat{\tau}_5$	−1.695	0.026	−0.017
$\hat{\gamma}_6$	−3.606	0.232	15.560	$\hat{\tau}_6$	−1.557	0.025	−0.016
$\hat{\gamma}_7$	−3.737	0.276	13.558	$\hat{\tau}_7$	−1.450	0.023	−0.016
$\hat{\gamma}_8$	−3.446	0.242	14.251	$\hat{\tau}_8$	−1.323	0.023	−0.016
$\hat{\gamma}_9$	−3.559	0.256	13.877	$\hat{\tau}_9$	−1.221	0.021	−0.016
$\hat{\gamma}_{10}$	−3.437	0.201	17.117	$\hat{\tau}_{10}$	−1.118	0.021	−0.016
Isolate F95129							
$\hat{\beta}$	0.478	0.064	7.512	$\hat{\beta}$	0.478	0.004	0.004
$\hat{\gamma}_1$	−3.522	0.185	19.040	$\hat{\tau}_1$	−3.522	0.029	−0.009
$\hat{\gamma}_2$	−3.879	0.205	18.965	$\hat{\tau}_2$	−2.992	0.029	−0.009
$\hat{\gamma}_3$	−3.686	0.229	16.109	$\hat{\tau}_3$	−2.586	0.029	−0.010
$\hat{\gamma}_4$	−3.149	0.185	16.980	$\hat{\tau}_4$	−2.136	0.025	−0.009
$\hat{\gamma}_5$	−3.143	0.252	12.462	$\hat{\tau}_5$	−1.824	0.026	−0.009
$\hat{\gamma}_6$	−3.061	0.287	10.649	$\hat{\tau}_6$	−1.569	0.024	−0.008
$\hat{\gamma}_7$	−3.049	0.293	10.406	$\hat{\tau}_7$	−1.364	0.025	−0.009
$\hat{\gamma}_8$	−2.866	0.265	10.819	$\hat{\tau}_8$	−1.163	0.023	−0.008
$\hat{\gamma}_9$	−3.824	0.135	28.412	$\hat{\tau}_9$	−1.096	0.021	−0.008
$\hat{\gamma}_{10}$	−4.008	0.139	28.689	$\hat{\tau}_{10}$	−1.043	0.020	−0.008

1) The subscripts represent the number of the days (Eq. 1) or the ith day (Eq. 2) after exposure.

2) The t statistics were highly significant for all parameters estimated ($P < 0.0001$).

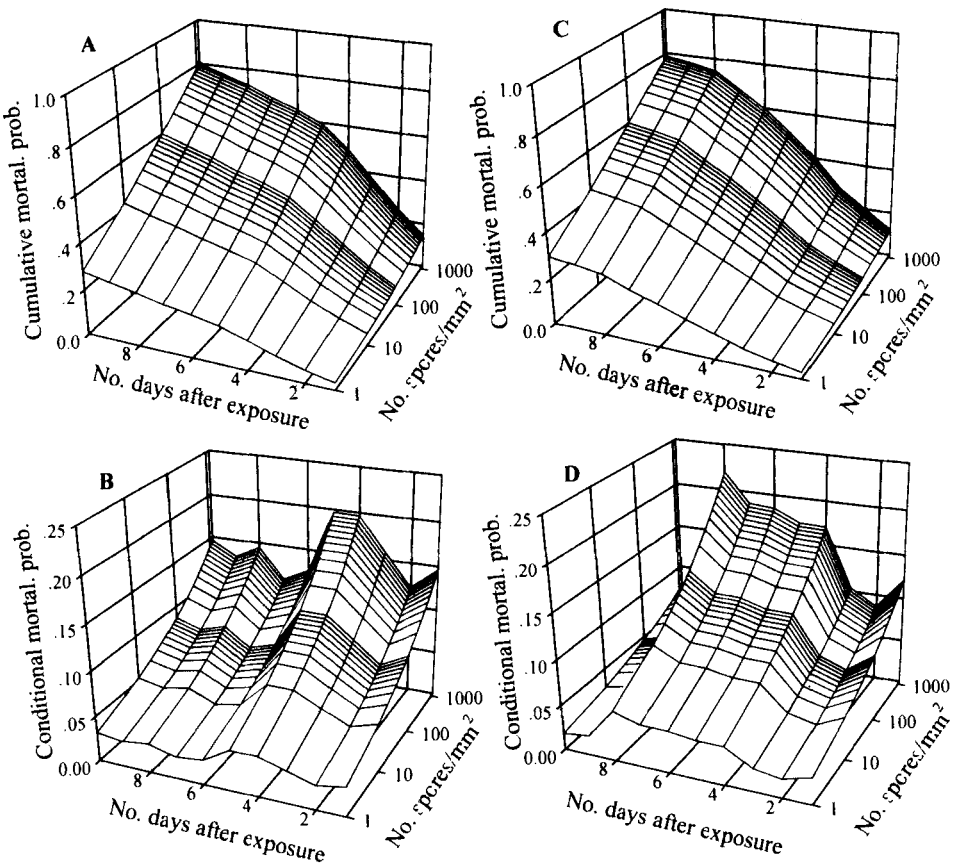

Fig. 1 The cumulative and conditional mortality probability of *P. delphacis* isolates F95127 (A and B) and F95129 (C and D) vs *N. lugens* nymphs.

The Hosmer-Lemeshow test (Nowierski *et al.* 1996) for the heterogeneity of the goodness of the fit for the binomial variable, q_{ij}, was not significant for F95127 ($\hat{C}=1.34$, $X^2_{(0.05)}=15.51$, df $=8$) and F95129 ($\hat{C}=10.14$, $X^2_{(0.05)}=15.51$, df $=8$), indicating an excellent fitness of the data to the model.

3.3 Indices of virulence

Virulence indices including LD_{50} and LT_{50} for the two isolates tested were computed on a basis of the estimates of $\hat{\beta}$ and $\hat{\tau}_j$ in Table 2 following Feng *et al.* (1996b), Nowierski *et al.* (1996), and Robertson and Preisler (1992). The LD_{50} values and associated standard errors are shown in Fig. 2. Based on the time-dose-mortality relationships determined by the parameters in Table 2, the LD_{50} values were a function of the time length after exposure to spore shower. On days 8—10 after the exposure, the LD_{50} values estimated were 71, 45, and 29 conidia/mm^2 for F95127 and 46, 33, and 26 conidia/mm^2 for F95129, respectively. The estimates of the LT_{50} values for F95129 were 9.0, 7.4, 6.6, and 5.6 d at

the dosages of 33, 80, 172 and 525 conidia/mm^2, respectively. Only two estimates of the LT_{50} values were obtained for F95127, i. e. , 7. 5 and 8. 2 d at 95 and 64 conidia/mm^2, respectively.

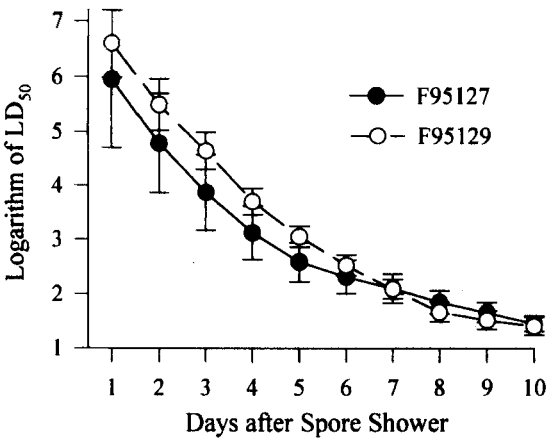

Fig. 2 The LD_{50} values (associated with standard errors in bars) of *P. delphacis* isolates F95127 and F95129 against *N. lugens* nymphs.

Based on the virulence indices obtained above, the two *P. delphacis* isolates tested were both moderately virulent to *N. lugens* compared with those of *P. neoaphidis* against cereal aphids (Feng and Johnson 1991).

4 DISCUSSION

The best way to inoculate insects with entomophthoralean fungi is to expose target insects to spore shower of these fungal pathogens, which are characteristic with their forcibly spore-discharging system. Though this bioassay system works well with relatively immobile insects such as aphids (Feng and Johnson 1991), problems occurred when it was used to deal with mobile insects. In the present study, the great mobility of *N. lugens* resulted in inconsistent exposure to spore shower, leading to a poor correlation between spore dosages determined and mortality data obtained. Many methods including cool shock and the use of carbon dioxide or ether could temporarily rest insects for easiness of inoculation but affect their physiological condition that may result in aberrant consequence (Feng and Johnson 1991, Wilding 1976). Therefore, we used a nutrient solution to moisten filter paper in Petri dishes where the planthoppers were maintained to receive the exposure. This modification to some degree reduced the mobility of the planthoppers and eased the inoculation because the planthoppers could suck the nutrient solution from the filter paper during the exposure.

The two *P. delphacis* isolates tested displayed moderate virulence to *N. lugens*, indicating that a potential of *P. delphacis* for use in control of planthoppers is relatively lim ited. However, an epizootic caused by *P. delphacis* in the field depends not only on its vir-

ulence but also on its biological adaptation to environment. Moisture and temperature have been well documented to affect the development of entomophthoralean epizootics (Melissa *et al.* 1987, Preisler *et al.* 1989, Shimazu 1977). We usually found cadavers of planthoppers killed by *P. delphacis* in late-cropped rice fields during late September and October but rarely in summer (unpublished data). While paddy fields usually have adequate moisture for spore germination and infection of entomophthoralean fungi, high temperature in summer may be an inhibitory factor to the development of *P. delphacis* epizootic because the optimum temperature for its growth ranges from (25—30)℃ (Shimazu 1977). On the other hand, in recent bioassays, *P delphacis* (isolate F95129) was found to kill the green peach aphid, *Myzus persicae*, much more rapidly than an isolate of *P. neoaphidis*, a well known aphid-specific pathogen (unpublished data). Moreover, *P. delphacis* is cultured in vitro far more easily than *P. neoaphidis*, yielding over 30 mg of dry mycelial mass per milliliter within 48 h of liquid culture with each milligram producing 3 or 4 $\times 10^5$ spores (Xu and Feng 1998). With these in consideration, the fungal species deserves more study for a candidate agent for control of aphids. Also, further study needs be directed to determine the effects of environmental factors on the development of a planthopper epizootic caused by *P. delphacis* and, thus, to more objectively evaluate the potential of the fungus for use in microbial control of planthoppers.

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飞虱虫疠霉对褐飞虱的毒力

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飞虱虫疠霉(*Pandora delphacis* (Hori) Humber)是我国稻区常见引发稻飞虱流行病的重要病原真菌。本研究用从稻飞虱虫尸上分离获得的两个飞虱虫疠霉菌株 F95127 和 F95129 分别对褐飞虱 (*Nilaparvata lugens* Stål) 进行了毒力测定。将 4—5 龄若虫曝露在液体培养所获的产孢菌丝平板下进行“孢子浴”接种，通过控制孢子浴时间获得系列接种剂量。各剂量处理的若虫分别饲养于生长培养箱中，温度 25℃，光照 12L：12D，相对湿度接近饱和，逐日检查死亡数。所得数据很好地拟合时间—剂量—死亡率模型，由此获得用于估计毒力指标的时间和剂量效应参数。接种后 6—8 d，F95129 和 F95127 的 LD_{50}分别为 327、122、46 孢子/mm² 和 409、133、74 孢子/mm²，F95127 在 95 孢子/mm² 剂量下的 LT_{50}为 7.6 d，而 F95129 的 LT_{50}则从 172 孢子/mm² 时的 6.6 d 下降到 525 孢子/mm² 时的 5.6 d。根据所测毒力指标和剂量效应参数的分析比较，两个飞虱虫疠霉菌株对褐飞虱均具有中等毒力，且彼此间无显著差异。