Epizootiological Modeling of *Pandora neoaphidis* Mycosis Transmission in *Myzus persicae* Colonies Initiated by Primarily Infected Alates

Chun Chen and Ming-Guang Feng

Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310029, People’s Republic of China, and Institute of Applied Entomology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, Zhejiang 310029, People’s Republic of China

Received 25 September 2004/Accepted 14 January 2005

*Pandora neoaphidis* transmission was monitored within progeny colonies initiated by infected *Myzus persicae* alates individually flown for 1 to 5 h. Mycosis progress in the colonies was well fitted ($r^2 = 0.97$) to a modified logistic or Gompertz model that included their flight distance, postflight survival time, premycosis fecundity, and primary infection rate as influential variables.

Mycoses caused by aphid-pathogenic Entomophthorales such as *Pandora neoaphidis* play important roles in natural control of aphids worldwide (10, 14, 16, 20, 21, 24). Most of these fungi may survive adversity as resting spores in soil, whereas for *P. neoaphidis*, the most prevalent aphid pathogen, no resting spores have been discovered (9, 17). Although the resting spores may potentially initiate infection in aphids (18, 19, 22), it is difficult to understand the general phenomenon that *P. neoaphidis* is predominate in global aphid epizootics. Aphids are highly able to disperse themselves by hovering flight over vegetation or by passive flight with winds in wide ranges (23). This ecological strategy enables holocyclic or anholocyclic species to readily locate suitable hosts (5). A hypothesis that *P. neoaphidis* could be widely dispersed with the flight of their alates was thus proposed (15) and has been proven by recent findings that ca. 30% of thousands of air-trapped alates bear several species of fungal pathogens, including primarily *P. neoaphidis* (3, 7, 11). Infected alate usually die from mycosis after a few days of infection development inside host hemocoel (6, 12, 13). Whether or not infected alates during the limited latent period are capable of flying for dispersal and then surviving for colonization and reproduction is of primary interest for understanding the process of mycosis transmission via contagious infection of progeny individuals in contact with the cadavers of infected mother alates or the spores actively discharged from them. Computer-monitoring simulated flight experiments with infected alates may help to measure those capabilities (4, 11).

As a holocyclic species in temperate areas or an anholocyclic species in tropical or subtropical zones, the green peach aphid *M. persicae* is globally distributed and is able to infest over 40 different plant families (2). These features make *M. persicae* an ideal host model for insight into the process of mycosis transmission in aphid populations across vegetation. In this study, variables describing flight and postflight colonization capabilities of *M. persicae* alates infected by *P. neoaphidis* were measured by means of numerous batches of simulated flights.

Two epizootiological models were fitted to describe the development of their progeny colonies and within-colony mycosis transmission.

**Flight, colonization, and mycosis transmission by infected alates.** Vigorous *M. persicae* alates (≥2 days old) from caged plants at 20 to 23°C and a 12 h:12 h light-dark cycle were exposed to a 1-h spore shower from sporulating mycelial mats of *P. neoaphidis* F98028 (8) and then were assumed to have received primary infection. Maintained overnight under moist conditions for infection development, the alates were individually fixed by their abdomens on a channel mill (11) with a dip of water-soluble arabic glue and then flown for 1 to 5 h. The flight distance of each alate during a given period was recorded automatically in a computer by a signal receptor with data collected to a photoelectronic sensor on the channel mill where the alate flew circumferentially as its wings vibrated (11). Immediately after flight, the alates were gently removed from the mills by dipping water onto the abdomen to dissolve the glue and then were individually reared under the same regimen for 14 days so as to initiate new colonies on detached cabbage leaves in petri dishes. Each leaf with hairy roots from a petiole stimulated by prior treatment with 0.1% naphthalene acetic acid may support an aphid colony for at least 2 weeks. In this system, aphids were allowed to freely colonize both leaf sides, and the infected alates initiating new colonies were considered the only source of primary infection.

Overall, 328 alates survived ≥1 day after colonization, with initiated colonies consisting of ≥1 nymph, and allowed the process of mycosis transmission to be observed. All observations were grouped based on survival days and are shown in Table 1. Observed trends in the development of the grouped colonies and accompanying mycosis transmission over days are plotted in Fig. 1. On average, the alates flew for 2.9 ± 1.7 (range, 1 to 5) h or 2.6 ± 2.2 (0.01 to 10.2) km after infection, survived for 3.2 ± 0.9 days after colonization, and produced 5.3 ± 3.2 nymphs during their survival periods. Of those, 98.5% were mycosed on days 2 to 5 and 50% were mycosed on day 3. In each group, the rate of primary infection ranged from 0.083 to 0.5, varying with the numbers of nymphs left by the infected alates on their death days. Variations in flight time and distance did not differ significantly among the groups (P > 0.05). Those surviving longer after colonization tended to produce...
more nymphs, but their fecundities prior to death were not always significantly different from one group to another (Table 1).

Contagious infection occurred in most colonies and developed over days after their mother alates were mycosed (Fig. 1B). Thirty-eight colonies were entirely mycosed on day 7, and 56 were entirely mycosed on day 14. Overall, entirely or partially mycosed colonies were 13.1% on day 5, 45.7% on day 7, 76.2% on day 10, and 80.2% on day 14. Mycosis transmission tended to occur earlier, develop faster, and cause higher proportions of mycosed individuals in the colonies when their mother alates died earlier or produced fewer nymphs prior to death.

Modeling of progeny colony development. When both progeny colony increase and within-colony mycosis transmission were assumed to be affected by variables associated with the flight and postflight colonization of the infected alates and the primary infection rate at their death time, the number of live aphids per colony ($N_i$) over days ($T_i; i = 1, 2, \ldots, 14$) fit to the complex logistic model $N = K/\{1 + \exp[b_0 + (b_1D_r + b_2T_r + b_3P_{out} + b_4\ln(1 + N_{idd}) + b_5\ln(1 + b_6 + b_7T_r)]\} + b_5[1 + \exp(b_6 + b_7T_r)]$, where $N = \ln(N_i + 1), D_r = \text{flight distance (km)}, T_r = \text{survival time (days)}, P_{out} = \text{primary infection rate},$ and $N_{idd} = \text{number of nymphs produced by each infected mother alate on the death day}$. The parameter $K$ was interpreted as the maximal potential for the increase of progeny colony initiated by the infected alate, and $b_0$ was simply the intercept for the fitted curve. The expression $b_1D_r + b_2T_r + b_3P_{out} + b_4\ln(1 + N_{idd})$ was interpreted as the rate of increase of the colony over time, and the fitted curve was adjusted by $b_5[1 + \exp(b_6 + b_7T_r)]$. Based on weighted modeling (weight = number of infected alates in each group), the observations in Fig. 1A and Table 1 were well fitted to the model (Fig. 2A) ($r^2 = 0.97; F_{8, 90} = 354.5; P < 0.0001),$ yielding a colony increase rate of 0.05$D_r + 0.06T_r + 0.26P_{out} - 0.38\ln(1 + N_{idd})$ and a maximal colony potential of 57 $[= \exp(4.06) - 1]$ aphids. The fitted expression 0.74$[1 + \exp(-9.31 + 2.03T_r)]$ reflected well distinct trends of reproduction by the infected alates within the first week and then by the apterous adults developed from their progeny during the second week. Apparently, the rate of colony increase was de-

<table>
<thead>
<tr>
<th>Death day after flight</th>
<th>No. of alates mycosed</th>
<th>Flight time (h)</th>
<th>Flight distance (km)</th>
<th>Mean ± SD (per alate or progeny colony)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. of live aphids on:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>2.8 ± 1.0</td>
<td>2.7 ± 2.4</td>
<td>3.3 ± 2.3</td>
</tr>
<tr>
<td>3</td>
<td>164</td>
<td>2.9 ± 1.7a</td>
<td>2.6 ± 2.2a</td>
<td>5.1 ± 2.9b</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>2.9 ± 1.7a</td>
<td>2.4 ± 2.1a</td>
<td>6.5 ± 3.3b</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>3.6 ± 1.7a</td>
<td>3.2 ± 2.6a</td>
<td>8.6 ± 3.4a</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3.0 ± 0.0a</td>
<td>2.6 ± 0.6a</td>
<td>7.7 ± 2.3ab</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1.0 ± 0.0</td>
<td>1.4 ± 0.0</td>
<td>11.0 ± 0.0</td>
</tr>
<tr>
<td>Overall</td>
<td>328</td>
<td>2.9 ± 1.7</td>
<td>2.6 ± 2.2</td>
<td>5.3 ± 3.2</td>
</tr>
</tbody>
</table>

$F$ test ($P$) 0.75 (0.556) 0.46 (0.769) 16.48 (<0.001) 12.67 (<0.001) 8.52 (<0.001) 11.15 (<0.001) 0.88 (0.478)

* Means with different letters in each column differed significantly (Tukey’s HSDs, $P < 0.05$; df = 4, 21 for $F$ tests of the observations among groups 2 to 6).

FIG. 1. Observed trends in the development of progeny colonies (A) and within-colony mycosis transmission (B) individually initiated by Pandora neoaphidis-infected Myzus persicae alates that were allowed to colonize cabbage leaves for 14 days after simulated flight. Symbols indicate survival periods (days) of the infected alates after colonization. The first observation, marked with an asterisk for each case in panel B, represents the primary infection rate (proportion of the alate cadaver in its progeny colony). Error bars indicate standard errors.
pended upon the flight distance of the infected alates, their survival days after flight, the number of their nymphs prior to death, and the rate of primary infection at their death time.

Since contagious infection occurred soon after their deaths, death, and the rate of primary infection at their death time. As a result, the fitted Gompertz model depicted well the progress of mycosis transmission at the rate of 0.04Df – 0.02Ts + 1.34Pmi(0.12PmiN)y over the colonization days (Fig. 2B) (r² = 0.97; F₄, 93 = 679.8; P < 0.0001). Again, the variables Df, Ts, and Pmi were determinants for the transmission rate and thus the fluctuating Pmi. Interestingly, N was a prominent determinant for Pmi. This indicates that the mycosis transmission via contagious infection was density dependent.

Since both fitted models described well interactions between P. neoaphidis and M. persicae in the development of progeny colonies and mycosis transmission (Fig. 2), they may shed light into general mechanisms involved in the wide dispersal and local transmission of aphid mycoses caused by Entomophthorales. Our results clarify that the P. neoaphidis-infected alates were capable of flying for dispersing themselves, initiating progeny colonies on plants, and transmitting their primary infection to progeny through contact. This supports our recent reports from examination of air captures of several aphid species and simulated flight studies with infected Slibion avenae alates (3, 4, 11) and highlights the decisive role of alate-borne inocula in initiation of M. persicae mycosis.

The potential for dispersal of the Entomophthorales with their migratory flight is imaginable, because alates may passively fly with wind for over 1,000 km (23). Recently, 35 P. neoaphidis isolates derived from global aphid hosts have been found to share a uniform 1,100-bp size of the amplified internal transcribed spacer region (25). This could be indirect, molecular evidence for the source of the primary inocula of P. neoaphidis associated with migratory alates. Although active flight of the infected alates in the simulated flight system could never be as far as their potential passive flight with wind in nature, the flight distances of up to 10.2 km measured within 5 h in this study guarantee efficient dissemination of primary inocula among aphids across areas or vegetation. Upon successful colonization by infected alates on suitable plants, their primary infection can be contagiously transmitted within progeny colonies, as shown in this paper, and eventually across colonies if aphid populations develop in the field (14), because aphid colonies containing infected individuals hardly escape from the fate of an epizootic in suitable environments (8). Our findings would be revelatory for exploring pathogen-aphid interactions and for understanding mechanisms involved in natural control of aphids by the obligate fungal pathogens.

This study was jointly supported by the Natural Science Foundation of China (30430150), the National Frontier Research Program Project 973 (2003CB114203), the Special Fund for Graduate Study Programs in Chinese Universities (200203355041), and the Cheung Kong Scholars Programme, Ministry of Education, China.

REFERENCES
3. Chen, C., and M. G. Feng. 2004. Observation on the initial inoculum source...