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# Activity of Entomophthoran Fungal Isolates (Zygomycetes) against *Nilaparvata lugens* and *Sogatodes orizicola* (Homoptera: Delphacidae)

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A total of 48 isolates of three species of entomophthoran fungi (*Erynia delphacis*, *Erynia* (*Zoophthora*) *radicans*, and an undescribed species of *Entomophaga*) were tested against adult *Sogatodes orizicola* and *Nilaparvata lugens* (Homoptera: Delphacidae). Only *E. delphacis* was infective to both insects, though *S. orizicola* was infected to some extent by all three pathogens. *N. lugens* was consistently less susceptible to these fungi, which exhibited longer incubation times in this insect. With one exception, fungi from non-*N. lugens* and, especially non-delphacid hosts, were not infective or only weakly infective to *N. lugens*; some of these isolates were moderately or strongly infective to *S. orizicola*, and one was the most infective to this insect, killing 91% of the sample. No consistent differences were found among planthopper morphs with regard to susceptibility to the fungi. However, macropterous males died significantly earlier than macropterous females which, in turn, died significantly earlier than brachypterous females. © 1988 Academic Press, Inc.

KEY WORDS: *Erynia delphacis*; *Erynia radicans*; *Entomophaga* sp.; entomophthoran fungi; *Sogatodes orizicola*; *Nilaparvata lugens*; infectivity of fungi.

## INTRODUCTION

The brown planthopper, *Nilaparvata lugens*, is one of the most serious pests of rice in Asia (Dyck and Thomas, 1979). Though the use of resistant cultivars has reduced the problem, *N. lugens* has shown the ability to develop biotypes which can grow on previously resistant varieties (Kenmore, 1980; Heinrichs et al., 1986), and the overall threat remains.

Problems of resistance to insecticides by *N. lugens*, and environmental problems arising from the application of insecticides to aquatic environments, place a priority on the development of ecologically sound control methods. *N. lugens* is susceptible to a range of fungal pathogens (Soper, 1985), and because of the warm humid environment in which rice grows, fungi are potentially important control agents. The dried-mycelium process developed by McCabe and Soper (1985) offers the further prospect of development of a commercial mycoinsecticide. Trials with hyphomycete fungi against *N. lugens* in the Philippines have shown considerable promise (Rombach et al., 1986).

This paper describes the preliminary screening of entomophthoran isolates against *N. lugens* and the (American) rice delphacid *Sogatodes orizicola*.

## MATERIALS AND METHODS

*Fungal isolates.* A total of 48 isolates from three species of fungi, *Erynia delphacis*, *Erynia* (Zoophthora) *radicans*, and an undescribed globose-spored species of *Entomophaga*, were examined (Table 1). The isolates were collected from *N. lugens* and other Homoptera in Asia, Australia, and South America over a number of years and were stored in liquid nitrogen at the U.S. Department of Agriculture, Agriculture Research Service, Entomopathogenic Fungi (ARSEF) Collection, Ithaca, New York.

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TABLE 1

List of Isolates Screened against *Sogatodes orizicola* and *Nilaparvata lugens*

| ARSEF No. | Fungus species | Host insect | Country of origin |
|---|---|---|---|
| 331 | *Entomophaga* sp. | *Cicadetta puer*[a] | Australia |
| 365 | *Entomophaga* sp. | *Cicadetta puer* | Australia |
| 743 | *Entomophaga* sp. | Cicadellidae, unknown sp. | Brazil |
| 791 | *Entomophaga* sp. | CPL[b] | Brazil |
| 134 | *E. delphacis* | BPH[c] | Japan |
| 478 | *E. delphacis* | GLH[d] | Philippines |
| 458 | *E. delphacis* | GLH | Philippines |
| 459 | *E. delphacis* | GLH | Philippines |
| 460 | *E. delphacis* | GLH | Philippines |
| 461 | *E. delphacis* | GLH | Philippines |
| 575 | *E. delphacis* | BPH | Indonesia (Lombok) |
| 579 | *E. delphacis* | BPH | Indonesia (Lombok) |
| 581 | *E. delphacis* | BPH | Indonesia (North Sumatra) |
| 593 | *E. delphacis* | WBPH[e] | Indonesia (Sulawesi) |
| 603 | *E. delphacis* | WBPH | Indonesia (Sulawesi) |
| 657 | *E. delphacis* | BPH | China (Nanjing) |
| 660 | *E. delphacis* | ZZLH[f] | China (Nanjing) |
| 664 | *E. delphacis* | BPH | China (Changsha) |
| 665 | *E. delphacis* | BPH | China (Wuhan) |
| 666 | *E. delphacis* | BPH | China (Wuhan) |
| 667 | *E. delphacis* | BPH | China (Wuhan) |
| 668 | *E. delphacis* | BPH | China (Nanjing) |
| 669 | *E. delphacis* | BPH | China (Wuhan) |
| 670 | *E. delphacis* | BPH | China (Hangzhou) |
| 671 | *E. delphacis* | BPH | China (Guangzhou) |
| 672 | *E. delphacis* | BPH | China (Nanjing) |
| 673 | *E. delphacis* | BPH | China (Nanjing) |
| 674 | *E. delphacis* | BPH | China (Nanjing) |
| 676 | *E. delphacis* | BPH | China (Wuhan) |
| 682 | *E. delphacis* | BPH | China (Nanjing) |
| 686 | *E. delphacis* | BPH | China (Nanjing) |
| 698 | *E. delphacis* | BPH | China (Nanjing) |
| 1124 | *E. delphacis* | BPH | Japan |
| 1132 | *E. delphacis* | BPH | Japan |
| 1133 | *E. delphacis* | BPH | Japan |
| 1238 | *E. delphacis* | Cicadellidae, unknown sp. | Brazil |
| 1264 | *E. delphacis* | BPH | Japan |
| 1730 | *E. delphacis* | BPH | India (Hyderabad) |
| 1731 | *E. delphacis* | BPH | India (Hyderabad) |
| 685 | *E. radicans* | BPH | China (Wuhan) |
| 789 | *E. radicans* | CPL | Brazil |
| 790 | *E. radicans* | CPL | Brazil |
| 1125 | *E. radicans* | BPH | Japan |
| 1136 | *E. radicans* | BPH | Japan |
| 1236 | *E. radicans* | CPL | Brazil |
| 1263 | *E. radicans* | Cicadellidae, unknown sp. | Japan |
| 1500 | *E. radicans* | Cicadellidae, unknown sp. | Brazil |
| 1590 | *E. radicans* | Cicadellidae, unknown sp. | Brazil |

[a] Homoptera: Cicadidae.
[b] Cowpea leafhopper, *Empoasca kraemeri* (Homoptera: Cicadellidae).
[c] Brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae).
[d] Green leafhopper, *Nephotettix* sp. (Homoptera: Cicadellidae).
[e] Whitebacked planthopper, *Sogatella furcifera* (Homoptera: Delphacidae).
[f] Zig-zag leafhopper, *Recilia dorsalis* (Homoptera: Cicadellidae).





Some had been cultured on agar media for extensive periods before acquisition by ARSEF.

*Preparation of fungi.* The fungi were transferred as needed from liquid nitrogen storage to Sabouraud's dextrose agar supplemented by 1% yeast extract (SDAY). Once growing, they were transferred to shaking cultures in Sabouraud's dextrose medium supplemented with 1% yeast extract (SDY). All cultures were grown at room temperature (ca. 25°C). To prepare inoculum for bioassays, flasks containing SDY were inoculated with ca. 5% v/v of a well-grown shaking culture of the appropriate isolate. After 2–3 days, mats of mycelium were produced by suction filtering 400–600 ml of culture (depending on the extent of growth) through two layers of filter paper in an 11.5-cm Buchner funnel. The mats (prepared late afternoon or early evening) were placed on wire screens (ca. 1-cm grid) overnight at 100% RH. Two mats were prepared at one time for each isolate.

Sporulation usually commenced within 18 hr, but sometimes took longer. If sporulation did not occur, the mats were incubated for 6–24 hr (usually overnight) at 15°–18°C. This cold treatment was found to stimulate sporulation in some isolates. If no sporulation occurred after cold treatment, new mats were prepared and kept overnight at 15°–18°C from the outset.

For bioassays on *N. lugens*, the fungi were shipped from Ithaca to Ciba–Geigy Ltd., Basel, Switzerland, as slant cultures on SDAY and maintained on SDAY at 15°C until needed.

*Test insects. S. orizicola* were reared on rice (cv "Star Bonnet" or "M201") seedlings at 25°–26°C in cages. The rice was germinated in flats of soil in the greenhouse. Initially, the rice was grown at about 26°C, but it was later found that better results were obtained at 28°–30°C. The rice was used 15–35 days after planting at 26°C or 10–25 days after planting when grown at the higher temperature. *N. lugens* was reared in the Ciba–Geigy insect-rearing facility in a similar manner to *S. orizicola*, but both rice and insects were grown at 26°C.

*Infection of insects.* For each fungal isolate, two sets of approximately 60 adult insects (of mixed sex and morph, but excluding brachypterous males) were collected by aspiration or directly into test tubes. They were then transferred to 9-cm Petri dishes containing cut rice leaves on water agar and confined under a gauze screen (ca. 1-mm grid).

One fungal mat was placed over each Petri dish to allow the forcibly discharged conidia to fall through the gauze onto the insects. The mats were turned through 90° at varying intervals, depending on the rate of sporulation, to provide for even distribution of conidia. The number of conidia present on the agar around the leaf pieces was assessed with the aid of a stereomicroscope. When the dose had reached 90–120 conidia/mm$^2$, the mat was removed. This required from 5 min to several hours, depending mainly on the fungal isolate, but also on the batch. However, it was impractical to expose the insects for more than about 3 hr because the discharge of secondary conidia at that time made accurate counts impossible. Therefore, because of poor sporulation, some isolates were tested at lower doses, often with only a single sample of insects, and some could not be tested against one or both insects. Poor growth or sporulation was taken as a valid criterion for rejection of these isolates from further consideration. In particular, *Entomophaga* sp. and some Japanese isolates of the other species which had been cultured on agar media over a long period, sporulated poorly and were not infective.

Sometimes, due to variation in sporulation rates over time, higher than desired doses were obtained. When time and the supply of insects permitted, new samples were treated. Otherwise the original samples were kept and the assay repeated only if more than 50% of the insects were infected at the high dose.

After treatment, the hoppers were held in the Petri dishes at 100% RH for 24 hr and then transferred to test tubes containing 5–7 rice seedlings each. If the transfers were made immediately after showering, very low rates of infection were observed. Three to eight (usually six) insects were held in each tube. The tubes were checked and any dead hoppers removed daily for 1 week. If there were no external signs of disease, cadavers were macerated in aceto–orcein and examined under the microscope for the presence of hyphal bodies.

Initially, one isolate, ARSEF 579 (chosen because it was the first isolate tested which was infective to *S. orizicola*, and had been used in development of the bioassay), was run each time as a standard, without uninfected controls. For about half the *S. orizicola* and all the *N. lugens* tests, uninfected controls were run as well.

*Data analysis.* Control mortality was very variable, averaging 28.5% for *S. orizicola* and 35.0% for *N. lugens*. This is above the limit of 20% regarding as acceptable in insecticide studies by Swaroop (1966), but could not be avoided given the conditions required to maximize fungal infectivity.

Among the treated insects, mortality in the absence of any detectable disease varied widely even among apparently similar samples collected at the same time, possibly due to differences among the rearing cages from which the insects were obtained. Fungal infections, however, could often be detected by the microscopical detection of hyphal bodies in squash preparations even in the absence of external mycelium. This led to the recognition of "overt" infections in which external mycelium was present, and "cryptic" infections in which only internal hyphal bodies could be found. Presumably, the fungi were not the primary cause of death in the latter, through the stress imposed by the infection may have contributed to mortality. Cryptic infections were seen most commonly in *N. lugens*, for which they were recorded separately. However, for *S. orizicola*, cryptic infections were not recorded separately, but most occurred on the first day after infection, and all infections detected on that day were cryptic. Therefore the numbers of infected *S. orizicola* recorded 1 day after exposure were used as an approximate estimate of cryptic infections.

Because of these problems, no attempt was made to correct for control mortality using such methods as Abbott's formula. Infection levels were calculated as percentages of both the total sample treated ("raw" % infection) and the total after subtraction of insects dying without detected infections ("adjusted" % infection) (Table 2). Although unorthodox, this approach did allow some compensation for highly variable nondisease mortality and provided low and high estimates of infection, between which the "true" value can be assumed to lie.

In addition to the above, only two replicates were used for most isolates, so statistical analyses were not used in the process of selection of promising isolates for further consideration. The isolates were instead ranked on the basis of both measures of infection level for each host species, with double weight being given to performance against *N. lugens*, since this is the ultimate target. From the resulting list of promising isolates, a small number were chosen from a range of geographic origins for further study. This final criterion was used in the hope of maximizing the range of ecological adaptation within the group.

The median time to death ($LT_{50}$) was calculated for samples of 15 or more insects from a regression of logit transformation of cumulative percentage infection against log time in days. Since cryptic infections were most likely a result of infected insects dying prematurely from other causes, only overt infections were used in these calculations. For *S. orizicola*, this meant excluding infections recorded on day 1 (an approximation of cryptic infections), while for *N. lugens* the actual figures for overt infections were used.



TABLE 2

PERCENTAGE INFECTION OF *Sogatodes orizicola* AND *Nilaparvata lugens* BY ENTOMOPHTHORAN FUNGI

| | *S. orizicola* | | | | *N. lugens* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | % Infected | | | | % Infected | |
| ARSEF No. | *n* | Dose (conidia/mm²) | Raw | Adjusted | *n* | Dose | Raw | Adjusted |
| *Entomophaga* sp. | | | | | | | | |
| 331 | 61 | 114 | 34 | 48 | 58 | 10 | 0 | 0 |
| 365 | 119 | 93 | 23 | 30 | 68 | 46 | 2 | 3 |
| 743 | 55 | 165 | 0 | 0 | 114 | 99 | 0 | 0 |
| 791 | 101 | 62 | 47 | 82 | NT[a] | | — | — |
| *E. delphacis* | | | | | | | | |
| 134 | 55 | 65 | 24 | 37 | 33 | 94 | 1 | 2 |
| 458 | 108 | 100 | 45 | 53 | 121 | 114 | 0 | 0 |
| 459 | 93 | 103 | 33 | 39 | 111 | 109 | 0 | 0 |
| 460 | 105 | 90 | 2 | 3 | 110 | 121 | 0 | 0 |
| 461 | 107 | 102 | 14 | 15 | 100 | 116 | 1 | 1 |
| 478 | 117 | 105 | 50 | 63 | 115 | 112 | 3 | 5 |
| 581 | 114 | 97 | 28 | 58 | 103 | 95 | 7 | 21 |
| 593 | 120 | 114 | 91 | 100 | 122 | 111 | 1 | 1 |
| 603 | 132 | 127 | 83 | 96 | 120 | 97 | 8 | 14 |
| 664 | 108 | 105 | 40 | 42 | 122 | 103 | 17 | 22 |
| 666 | 113 | 108 | 70 | 85 | 114 | 105 | 45 | 59 |
| 668 | 115 | 101 | 51 | 56 | 61 | 90 | 16 | 20 |
| 671 | 113 | 87 | 25 | 29 | 116 | 95 | 1 | 1 |
| 672 | 116 | 98 | 46 | 50 | 120 | 99 | 24 | 35 |
| 673 | 106 | 92 | 53 | 55 | 113 | 104 | 7 | 14 |
| 682 | 117 | 94 | 47 | 54 | 119 | 98 | 52 | 68 |
| 686 | 98 | 94 | 50 | 59 | 112 | 105 | 24 | 34 |
| 698 | 230 | 95 | 68 | 84 | 119 | 101 | 30 | 37 |
| 1124 | 126 | 94 | 2 | 2 | 141 | 113 | 0 | 5 |
| 1132 | NT | | — | — | NT | | — | — |
| 1133 | NT | | — | — | 120 | 109 | 1 | 2 |
| 1238 | 231 | 104 | 53 | 75 | 135 | 97 | 1 | 2 |
| 1264 | NT | | — | — | NT | | — | — |
| 1730 | 114 | 94 | 36 | 39 | 105 | 105 | 51 | 72 |
| *E. radicans* | | | | | | | | |
| 685 | 63 | 1039 | 0 | 0 | NT | | — | — |
| 789 | 106 | 93 | 0 | 0 | 60 | 68 | 0 | 0 |
| 790 | 104 | 93 | 2 | 3 | NT | | — | — |
| 1125 | 119 | 90 | 0 | 0 | 120 | 103 | 0 | 0 |
| 1136 | NT | | — | — | NT | | — | — |
| 1236 | 107 | 93 | 0 | 0 | 120 | 106 | 0 | 0 |
| 1263 | NT | | — | — | 108 | 59 | 0 | 0 |
| 1500 | 122 | 103 | 10 | 23 | 118 | 92 | 0 | 0 |
| 1590 | 116 | 91 | 3 | 6 | 113 | 108 | 0 | 0 |

*Note*. Isolates for which detailed information is given in Table 3 have been excluded. Conidial dose is average of replicates. For explanation of raw and adjusted % infection, see text.

[a] Not tested due to poor growth or sporulation.

Overall speed of kill was compared for hosts, isolates, and host morphs (macropterous males (MM), macropterous females (MF), and brachypterous, females (BF) by the Kruskal–Wallis test (SAS Institute, Inc., Cary, North Carolina) using only

data for overt infections for *N. lugens* and data for day 2 or later for *S. orizicola* (Table 3). Pairwise comprisons between morphs within isolates were made using the Wilcoxon rank-sum test. Results of the latter are included in Table 3.

*Tests against N. lugens in Indonesia.* Three isolates of *E. delphacis*, ARSEF 657, 669, and 1731, were tested to a limited extent at the Ciba–Geigy Field Station at Cikampek, West Java, Indonesia, during January–February 1986 (ARSEF 669) and 1987 (ARSEF 657, 1731). The *N. lugens* were collected either from field cages or, for tests of ARSEF 1731 only, from field plots which had been artificially infested some weeks previously. Otherwise, the methods were as described above. ARSEF 669 was also tested against small (instars 1–2) and large (instars 3–5) *N. lugens* nymphs.

## RESULTS

Control mortalities were very variable and usually high, ranging from 2 to 57% (mean 28.5%, 11 samples) for *S. orizicola* and from 6% to 59% (mean 35.0%, 9 samples) for *N. lugens*.

The overall levels of infection obtained with most isolates against both insects are given in Table 2, and detailed data for the 11 promising isolates are given in Table 3. Only *E. delphacis* showed any potential in these tests. The 10 most promising isolates were ARSEF 575, 657, 660, 665, 667, 669, 670, 674, 676, and 1731. Detailed results for these isolates are listed in Table 3. Other isolates were not considered further, except for ARSEF 579, which was included because it was tested many times, providing a large sample for comparisons among morphs. Its relatively low ranking was due to poor performance against *N. lugens*, and sometimes against *S. orizicola*. This appeared to be due to one or two batches of the fungus which showed greatly reduced infectivity. Other samples of ARSEF 579 showed excellent activity against *S. orizicola*, so only samples with infection levels over 60% were included in the analysis of infection of morphs (Table 3), though the results for all replicates are given in Table 2.

ARSEF 685 is the only recent isolate of *E. radicans* from *N. lugens*. However, in preliminary studies, it sporulated very poorly at 25°C, but much better at 15°–20°C. It also tended to grow in solid clumps in shake-flask culture, and in this form it could not be prepared as mats for bioassays. It was tested against *S. orizicola* at a range of doses of both primary and capillary conidia, but was not infective even at extremely high doses (Table 2).

In general, *S. orizicola* was more susceptible to all three fungi (Table 2), and died significantly faster ($P < 0.05$) than *N. lugens* (Table 3). However, high control mortalities and the limited number of replicates made close comparisons of infection levels difficult. Also, reliable determination of virulence (measured as speed of kill) was hampered by occurrence of cryptic infections. These were especially common in *N. lugens* and occurred mainly in the first 2 days after infection.

Significant overall differences in virulence were observed among fungal isolates and host morphs. In general, *S. orizicola* males died faster than females, and MF were usually found to have died earlier than did BF. These differences were significant ($P < 0.05$) in 7 of 11 isolates for MM vs MF, and 8 for MM vs BF, but only 2 for MF vs BF, no doubt partly due to the smaller sample sizes for BF. There was a similar trend with *N. lugens*, but the differences between MM and MF were significant in only three isolates tested in the laboratory, as well as for ARSEF 1731 tested against field collected (but not laboratory reared) *N. lugens*. Too few *N. lugens* BF were tested for any conclusions to be drawn about them.

For *S. orizicola* infected with the isolates listed in Table 3, there was a significant negative correlation between percentage infection and median time to death ($r$ = −0.61, −0.69, −0.92 for MM, MF, BF,



TABLE 3

INFECTIVITY TOWARD THREE MORPHS OF *Sogatodes orizicola* AND *Nilaparvata lugens* OF 11 ISOLATES OF *Erynia delphacis*

| ARSEF No. | Morph | No. tested | % Infected | | Time to death (days) | |
|---|---|---|---|---|---|---|
| | | | Raw | Adjusted | Median | Mean ± SE |
| *Sogatodes orizicola* | | | | | | |
| 575 | MM | 43 | 77 | 92 | 2.6 | 2.7 ± 0.17$^{a}$ |
| | MF | 24 | 50 | 63 | 3.0 | 3.1 ± 0.29$^{a,b}$ |
| | BF | 46 | 57 | 70 | 3.5 | 3.8 ± 0.22$^{b}$ |
| 579 | MM | 314 | 80 | 92 | 2.6 | 2.8 ± 0.05$^{a}$ |
| | MF | 195 | 71 | 82 | 2.9 | 3.2 ± 0.07$^{b}$ |
| | BF | 60 | 67 | 71 | 3.3 | 3.5 ± 0.10$^{c}$ |
| 657 | MM | 34 | 88 | 100 | 2.1 | 2.2 ± 0.14$^{a}$ |
| | MF | 37 | 92 | 97 | 2.4 | 2.6 ± 0.15$^{a}$ |
| | BF | 43 | 65 | 80 | 3.1 | 3.3 ± 0.16$^{b}$ |
| 660 | MM | 134 | 90 | 94 | 2.0 | 2.3 ± 0.07$^{a}$ |
| | MF | 47 | 85 | 93 | 2.6 | 2.7 ± 0.12$^{b}$ |
| | BF | 53 | 72 | 75 | 2.8 | 3.0 ± 0.20$^{b}$ |
| 665 | MM | 46 | 61 | 85 | 2.7 | 2.9 ± 0.15$^{a}$ |
| | MF | 68 | 56 | 70 | 3.2 | 3.4 ± 0.15$^{b}$ |
| | BF | 6 | 50 | 75 | — | 3.5 — |
| 667 | MM | 49 | 78 | 95 | 2.3 | 2.4 ± 0.13$^{a}$ |
| | MF | 66 | 85 | 97 | 2.6 | 2.9 ± 0.11$^{b}$ |
| | BF | 8 | 63 | 100 | — | 2.7 ± 0.37 |
| 669 | MM | 54 | 81 | 90 | 2.2 | 2.3 ± 0.12$^{a}$ |
| | MF | 54 | 89 | 96 | 2.5 | 2.8 ± 0.16$^{b}$ |
| | BF | 4 | 50 | 100 | — | 3.5 — |
| 670 | MM | 65 | 69 | 83 | 2.4 | 2.8 ± 0.15$^{a}$ |
| | MF | 19 | 58 | 61 | 3.0 | 3.2 ± 0.24$^{a,b}$ |
| | BF | 43 | 44 | 51 | 3.8 | 3.7 ± 0.14$^{b}$ |
| 674 | MM | 58 | 84 | 88 | 2.7 | 2.9 ± 0.12$^{a}$ |
| | MF | 51 | 80 | 82 | 3.3 | 3.4 ± 0.13$^{b}$ |
| | BF | 6 | 83 | 83 | — | 3.5 ± 0.55 |
| 676 | MM | 48 | 71 | 85 | 2.4 | 2.6 ± 0.11$^{a}$ |
| | MF | 20 | 95 | 95 | 2.7 | 2.7 ± 0.13$^{a,b}$ |
| | BF | 56 | 66 | 84 | 2.9 | 3.0 ± 0.14$^{b}$ |
| 1731 | MM | 78 | 88 | 100 | 2.2 | 2.4 ± 0.11$^{a}$ |
| | MF | 45 | 82 | 95 | 2.5 | 2.8 ± 0.17$^{b}$ |
| | BF | 7 | 71 | 100 | — | 3.5 — |
| *Nilaparvata lugens* | | | | | | |
| 575 | MM | 59 | 63 | 90 | 4.0 | 3.9 ± 0.14$^{a}$ |
| | MF | 52 | 52 | 68 | 4.3 | 4.9 ± 0.37$^{a}$ |
| | BF | 8 | 38 | 50 | — | 3.5 ± 0.58 |
| 579 | MM | 152 | 33 | 48 | 4.0 | 4.1 ± 0.14$^{a}$ |
| | MF | 142 | 27 | 42 | 4.4 | 4.7 ± 0.16$^{b}$ |
| | BF | 31 | 13 | 29 | — | 4.5 ± 0.71 |
| 657 | MM | 72 | 61 | 69 | 3.2 | 3.3 ± 0.07$^{a}$ |
| | MF | 26 | 77 | 87 | 3.8 | 3.8 ± 0.23$^{a}$ |
| | BF | 17 | 71 | 71 | — | 3.5 — |
| 660 | MM | 107 | 54 | 73 | 3.5 | 3.6 ± 0.14$^{a}$ |
| | MF | 97 | 65 | 79 | 3.6 | 3.7 ± 0.13$^{a}$ |
| | BF | 29 | 36 | 59 | — | 3.5 ± 0.27$^{a}$ |
| 665 | MM | 43 | 70 | 77 | 3.8 | 3.9 ± 0.17$^{a}$ |
| | MF | 51 | 71 | 82 | 4.3 | 4.5 ± 0.19$^{b}$ |
| | BF | 14 | 36 | 56 | — | 4.3 ± 0.58 |
| 667 | MM | 78 | 38 | 46 | 3.0 | 3.2 ± 0.23$^{a}$ |
| | MF | 66 | 55 | 88 | 3.4 | 3.4 ± 0.13$^{a}$ |
| | BF | 29 | 45 | 59 | — | 3.4 ± 0.15$^{a}$ |

TABLE 3—*Continued*

| ARSEF No. | Morph | No. tested | % Infected | | Time to death (days) | |
|---|---|---|---|---|---|---|
| | | | Raw | Adjusted | Median | Mean ± SE |
| 669 | MM | 80 | 83 | 89 | 2.5 | 2.7 ± 0.14$^a$ |
| | MF | 30 | 80 | 100 | 3.9 | 4.0 ± 0.22$^b$ |
| | BF | 4 | 0 | 0 | — | — — |
| 670 | MM | 69 | 59 | 89 | 3.7 | 3.8 ± 0.17$^a$ |
| | MF | 49 | 53 | 67 | 3.8 | 4.1 ± 0.23$^a$ |
| | BF | 2 | 50 | 100 | — | 3.5 — |
| 674 | MM | 74 | 77 | 86 | 3.9 | 3.9 ± 0.11$^a$ |
| | MF | 41 | 71 | 88 | 4.1 | 4.0 ± 0.15$^a$ |
| | BF | 3 | 33 | 100 | — | 5.5 — |
| 676 | MM | 80 | 69 | 87 | 3.4 | 3.5 ± 0.12$^a$ |
| | MF | 31 | 68 | 95 | 3.7 | 3.8 ± 0.17$^a$ |
| | BF | 4 | 0 | 0 | — | — — |
| 1731 (lab.) | MM | 66 | 45 | 52 | 4.6 | 4.7 ± 0.18$^a$ |
| | MF | 46 | 48 | 56 | 4.5 | 4.5 ± 0.19$^a$ |
| | BF | 5 | 20 | 20 | — | 5.5 — |
| 1731 (field) | MM | 140 | 59 | 85 | 3.2 | 3.2 ± 0.11$^a$ |
| | MF | 160 | 46 | 72 | 3.6 | 3.7 ± 0.11$^b$ |

*Note*. For explanation of raw and adjusted % infection, see text. MM, macropterous males; MF, macropterous females; BF, brachypterous females.

$^{a-c}$ Means with same superscript are not significantly different. Absence of superscript (some BF) indicates that data were not analyzed due to small sample size. Comparisons were made within isolates only.

respectively, $P < 0.05$), but no such relationship existed for *N. lugens*.

Frequently, proportionately more males than females were infected (Table 3), but no significant differences were revealed by $\chi^2$ tests. Because of the high control mortalities and small number of replicates, more sophisticated analyses were considered inappropriate.

Data obtained in Indonesia using field cage-collected adult *N. lugens* infected with ARSEF 657 and 669 were marred by extremely high control mortalities (>50%), as well as background infections by *E. delphacis*, *Entomophaga* sp., and *Hirsutella citriformis* (Deuteromycotina: Hyphomycetes). Parasitism, mainly by Strepsiptera, but also by Dryinidae (Hymenoptera), was also very common. Up to five strepsipterans, but usually only one, were found in a single individual. In addition, mermithid nematodes were found in some hoppers tested with ARSEF 657. These problems did not occur with nymphs, but only 1% of small and 9% of large nymphs were infected, suggesting that immatures were less susceptible than adults.

The insects against which ARSEF 1731 was tested were collected from a field plot, where these problems were much less severe. Unfortunately, it was not possible to test other isolates against insects from that source. Only the data for ARSEF 1731 in Indonesian *N. lugens* are included in Table 3. However, this isolate killed field collected BPH significantly faster than laboratory-reared insects (Table 3).

## DISCUSSION

With the exception of ARSEF 660, which was isolated from the zig-zag leafhopper, *Recilia dorsalis*, all promising isolates originated from *N. lugens*. This isolate was collected along with many from *N. lugens* (D. W. Roberts, pers. Commun.), and it is possible that the insect from which it came was infected from *N. lugens*.

Of all the isolates tested, only one (ARSEF 1730) was substantially more infective to *N. lugens* than to *S. orizicola*. In contrast, many isolates were more infective to *S. orizicola* than to *N. lugens*, particularly isoaltes from sources other than *N. lugens*. In particular, ARSEF 593 and 603 (isolated from the white backed planthopper, *Sogatella furcifera*), which were the most infective isolates for *S. orizicola*, showed almost no activity against *N. lugens* (Table 2). Since *S. orizicola* is more susceptible and died more quickly from *E. delphacis* than did *N. lugens*, it is a better test insect than *N. lugens* for studies where specific target-related data are not required.

Shimazu (1977 and pers. commun.) found that only macropterous adults and large nymphs of *N. lugens* could be readily infected by *E. delphacis* in the laboratory, in contrast to the results obtained here. However, he used very high doses (ca. 1200 conidia/mm$^2$) and obtained only 20–30% infection in the susceptible insects, indicating that the isolate he used was only weakly pathogenic.

Most of the promising isolates came from Wuhan and Nanjing in China. However, ARSEF 1731, from Hyderabad, India, was very infective to *S. orizicola* and infected about half of the *N. lugens* tested, and ARSEF 670, from Hangzhou, China, infected about 60% of both insects. These were included, along with ARSEF 575 and 579 (from Indonesia), in the list of isolates to be tested further in order to broaden the potential range of ecological adaptations.

No tropical isolates of *E. radicans* are known from *N. lugens*. Since this species is the best studied with regard to production and formulation of dried mycelium (McCabe and Soper, 1985), such isolates should be actively sought for testing.

*Entomophaga* sp. has been found in *N. lugens*, but no isolates from that host have survived. Recent attempts at isolation in Indonesia, where this fungus was very common in *N. lugens*, were unsuccessful (D. G. Holdom, unpubl.).

Strain stability is an important problem with these fungi. The isolates of *Entomophaga* sp. sporulated well when first cultured but deteriorated as this study progressed. The Japanese isolates of *E. delphacis* had also been cultured for long periods before acquisition into ARSEF, and all were uninfective, or were only slightly infective, with some isolates sporulating poorly or not at all.

ARSEF 579, which was tested repeatedly, an initial high infectivity toward *S. orizicola*, but later this activity appeared to decline. Cultures derived from one cryopreserved sample in particular showed very poor activity. This isolate was initially added to the list of 10 selected for further study, but later dropped because of its apparent instability. Later, ARSEF 674 showed the same problem and had to be dropped from further study (D. G. Holdom, unpubl.).

Future studies with these fungi should take careful note of this problem. Ideally, large stocks of a standardized batch of an isolate should be frozen in liquid nitrogen and working cultures checked repeatedly and replaced as needed.

The results obtained with *N. lugens* nymphs infected with ARSEF 669 in Indonesia suggest that nymphs are less susceptible than adults, at least under laboratory conditions. This could be due to the smaller surface area of the insects, resulting in fewer conidial contacts, the removal of conidia by molting before penetration of the cuticle could occur, or to some internal resistance mechanism of the insect, or any combination of these factors. Infected nymphs were found in the field, though most infected insects there were adults.

This work has demonstrated that there is wide variation in both pathogenicity and virulence among isolates of *E. delphacis*, even those from the same host. While the relative values obtained are useful, absolute values should be used with care. The differences in the level of infection and speed of kill among morphs and stages within the same host, especially the low ap-

parent susceptibility of nymphs cast doubt on the potential of *E. delphacis* as a practical control agent for *N. lugens*. More work is needed to establish the extent of these differences. Further doubt about the value of this fungus stems from the apparent instability of isolates in terms of sporulation and pathogenicity. This aspect, too, needs further study to determine both the extent of the problems and possible solutions.

It is not clear whether the different results obtained with ARSEF 1731 against field and laboratory insects were due to differences in the fungus or the insects, but more work should be done to determine whether different populations of *N. lugens* differ in their susceptibility to fungal pathogens.

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