Discovery of *Fusarium solani* as a naturally occurring pathogen of sugarbeet root maggot (Diptera: Ulidiidae) pupae: Prevalence and baseline susceptibility

Ayanava Majumdar a, Mark A. Boetel a,*, Stefan T. Jaronski b

a Department of Entomology, North Dakota State University, Fargo, ND 58105, USA
b USDA-ARS, Northern Plains Agricultural Research Laboratory, Sidney, MT 59270, USA

Received 19 September 2006; accepted 10 May 2007
Available online 18 May 2007

Abstract

The fungus *Fusarium solani* (Mart.) Sacc. was discovered as a native entomopathogen of the sugarbeet root maggot, *Tetanops myopaeformis* (Röder), in the Red River Valley of North Dakota during the 2004 sugarbeet production season. This is the first report of a native pathogen affecting the pupal stage of *T. myopaeformis*. Forty-four percent of larvae collected from a field site near St. Thomas (Pembina Co.) in northeastern North Dakota during May and June of 2004 were infected with the entomopathogen. The mean LC$_{50}$ of *F. solani*, assessed by multiple-dose bioassays with laboratory-reared pupae, was $1.8 \times 10^6$ conidia/ml. After isolation and confirmation of pathogenicity, a pure isolate of the fungus was deposited in the ARS Entomopathogenic Fungal Collection (ARSEF, Ithaca, NY) as ARSEF 7382. Symptoms of *F. solani* infection included rapid pupal tissue atrophy and failure of adults to emerge. Transverse dissections of infected pupae revealed dense hyphal growth inside puparia, thus suggesting fungal penetration and pathogenicity. Mycelia emerged from pupae after host tissues were depleted. Exposure of older pupae to lethal concentrations caused rapid mortality of developing adults inside puparia. A second, more extensive field survey was conducted during the 2005 cropping season, and *F. solani* infection was observed in root maggots at most locations, although at lower levels (1–10%) of prevalence than in 2004. Aberrant timing or amounts of rainfall received could have caused asynchrony between pathogen and host during the second year of the experiment.

© 2007 Elsevier Inc. All rights reserved.

Keywords: *Tetanops myopaeformis; Fusarium solani; ARSEF 7382; Pathogenicity; Insect pathogen; Entomopathogenic fungus; Prevalence; Pupal bioassay

1. Introduction

The sugarbeet root maggot, *Tetanops myopaeformis* (Röder), is the most important insect pest of sugarbeet in the Red River Valley (RRV) of North Dakota and Minnesota. High infestations of this pest in the RRV are typically managed by using synthetic chemical insecticides, and up to two or three applications per growing season are not uncommon. Heavy reliance on these materials could eventually lead to the development of insecticide-resistant root maggot strains. Therefore, alternative control tools are needed to minimize producer dependence on conventional chemical insecticides.

Research on alternative *T. myopaeformis* control strategies utilizing microbial agents, particularly insect-pathogenic fungi, has met with some success. A native fungus, *Syngliocladium tetanops* Hodge, Humber, and Wozniak, was isolated from field-collected *T. myopaeformis* larvae and shown to be pathogenic to the insect (Hodge et al., 1998; Wozniak, 1999; Wozniak and Smigocki, 2001). Jonason et al. (2005) compared *Metarhizium anisopliae* (Metsch.) Sorok. and *Beauveria bassiana* (Balsamo) Vuillemin, and also evaluated several *M. anisopliae* strains for infectivity to *T. myopaeformis* larvae. They observed greater virulence against *T. myopaeformis* larvae in *M. anisopliae* than in *B. bassiana*, and found *M. anisopliae* isolate
ATCC 62176 (MA1200) to result in the highest larval mortality among several isolates tested. Campbell et al. (2000, 2006) tested *M. anisopliae* for potential as a root maggot mycoinsecticide under field conditions, and achieved encouraging results. They suggested that *T. myopaeformis* control via entomopathogenic fungi could be improved by using more virulent strains. While most research has focused on fungal control of *T. myopaeformis* larvae, the available body of literature lacks information regarding pathogen surveys on other life stages of the insect. This key insect pest could be especially vulnerable to infection during the pupal stage because pupae, being incapable of locomotion, are unable move through soil and rub off infective units of a pathogen prior to penetration.

Many *Fusarium* species are well-known pathogens of plants, insects, and humans. Although the systematic description of this genus is unresolved, *Fusarium* is unique because some isolates of the common phytopathogenic species (e.g., those associated with root rot diseases) also can infect subterranean insects. More than 13 *Fusarium* species are pathogenic to insects, and the group has a host range that includes Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera (Humber, 1992). Gupta et al. (1991) isolated the toxin beauvericin, a cyclosporine, from *Fusarium*. Those authors showed that beauvericin can kill 50% of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), larval test populations at a 663-ppm dose. Beauvericin also is lethal to dipteran insects such as mosquitoes and blow flies (Tanada and Kaya, 1993). Venugopal et al. (1989) observed that epizootics of *Fusarium* caused mortality levels that were equal to or greater than predators and parasitoids in populations of the whitefly, *Bemisia tabaci* (Gennadius). Epizootics were suggested as being favored by high humidity, low temperatures, and availability of whitefly hosts; however, actual weather parameters were not presented in that report.

According to Humber (1992), *Fusarium solani* (Mart.) Sacc. is infectious to pupae of the dipteran family Anthomyiidae; however, this pathogen has not been previously reported as infecting *T. myopaeformis* at any stage of its life cycle. Observations reported herein focus on detection, isolation, and prevalence of *F. solani* in *T. myopaeformis* pupae collected from several field sites where sugarbeet had been grown the previous season. The objectives of research were to determine prevalence of *F. solani* epizootics via field surveys, describe disease progression in *T. myopaeformis* pupae under laboratory conditions, and quantify baseline susceptibility of pupae to *F. solani*.

2. Materials and methods

2.1. Prevalence study—2004

A field site near St. Thomas (Pembina Co.) in northeastern North Dakota was selected for collections of *T. myopaeformis* pupae that had overwintered as larvae. The site was chosen based on the known occurrence of high *T. myo-

paeformis* infestations in the vicinity during the 2003 season. The field had been planted to sugarbeet in the year that preceded our collections, and potatoes were grown the year before sugarbeet. Spring wheat was growing on the site in 2004 during pupal collections. Major collections (*n* ≥ 100) of *T. myopaeformis* pupae were made on 21 May, 27 May, 2 June, and 4 June in 2004. Procedures for collecting and handling pupae were based on the methodology of Lacey and Brooks (1997). Field-collected pupae were collected in clean plastic bags (Ziploc, S.C. Johnson & Son, Racine, WI) and kept in plastic coolers to prevent physical stress during transportation. About 1200 pupae were collected in this first survey. Pupae were surface-sterilized with 1% sodium hypochlorite and three sterile water washes as per the procedures of Lacey and Brooks (1997). Live pupae were individually transferred to 37-ml plastic soufflé cups (Solo Cup Co., Highland Park, IL) using soft sterile stainless steel forceps, and were incubated at 25 ± 1 °C for 15 d. Pupae were provided high humidity inside cups by adding a few strips of moist filter paper before capping. The 15-d interval allowed healthy uninfected pupae to complete development and emerge as adult flies inside the cups. Presence/absence of fungal infection was assessed on all dead pupae at the end of the incubation period by using a stereomicroscope at 35× magnification. Pupae infected with *Fusarium* developed profuse white mycelial growth, and often remained adhered to the plastic surface of the cup by hyphal growth. Dead pupae not showing external growth of the fungus were transferred onto SDAY medium and incubated at 25 ± 1 °C for 5–7 d to allow time for fungal outgrowth.

2.2. Prevalence study—2005

An expanded follow-up survey was conducted during June 2005 in Pembina and Walsh Counties of North Dakota, with the aim of screening a large number of pupae from multiple locations. This prevalence study was conducted at 10 commercial fields in four northeastern North Dakota townships that typically have moderate to high *T. myopaeformis* infestations. About 2400 pupae were collected and individually examined for *Fusarium* infection using the previously described process. Field histories of collection sites were obtained from the cooperating sugarbeet producers. Field coordinates and elevations were determined using global positioning system hardware (Garmin International Inc., Olathe, KS). Soil temperature was recorded at a depth of 5 cm during the time of collection using a pocket thermometer (Cooper-Atkins, Middlefield, CT).

2.3. Field collection of larvae for pupal bioassays

The test insect, *T. myopaeformis*, cannot be reared from egg to pupa in the laboratory because there is no established artificial diet for larvae. Therefore, to conduct bioassays, third-instar larvae were collected from
Infested fields in late summer (i.e., July and August). Field-collected larvae were surface-sterilized with a 1% sodium hypochlorite solution and rinsed twice with distilled water, then placed into moistened silica sand (Unimin Corporation, Le Sueur, MN), and maintained at 5 ± 1°C for six months to allow completion of diapause. Post-diapausal larvae were placed onto moistened filter paper at 25 ± 1°C in sterile plastic petri plates to stimulate pupation. About 75% of larvae pupated within the first 72 h of exposure to warm temperatures. This rearing and handling technique provided sufficient numbers of pupae at a synchronized state of development for testing.

2.4. Pathogenicity tests to satisfy Koch’s postulates

Healthy pupae, reared from field-collected larvae as previously described, were artificially inoculated by topically applying a suspension (>1 × 10^6 conidia/ml) of the fungus. Sterile water was topically applied to the control pupae in the same manner. Ten fungus-inoculated pupae were individually transferred to soufflé cups by using soft sterile stainless steel forceps. Cups were covered with transparent lids before inoculating pupae. A few pieces of sterile filter paper were moistened with distilled water and placed into each cup to provide high humidity. Cups were inverted to ensure moisture retention during the incubation period. Pupae were incubated in cups for 15 d at 25 ± 1°C and a 12:12 (light:dark) h photoperiod. Profuse conidiophores with conidia in slime heads, as well as the shape and septation of macro- and microconidia, suggested that the fungus belonged to the Fusarium genus. Prolific fungal growth on the exterior of pupae 15 d after treatment (DAT) confirmed mycosis. Failures of adults to emerge coincided with shrinkage of pupal tissues, and were restricted to fungus-treated insects. Tissue shrinkage was visible at 5–7 DAT under a stereomicroscope by using 35× magnification. Abundant microconidia were produced in slime heads. Successful development to adulthood was limited to the control pupae. No contamination from saprophytic microorganisms was detected in infected cadavers. The fungus was reisolated from artificially inoculated pupae and maintained on SDAY medium. Microscopic reexamination of microconidia and conidiophores again suggested that the fungus was a Fusarium species. This also indicated that the isolate was virulent against T. myopaeformis pupae as per Koch’s postulates of infectious diseases. The fungus was deposited in the ARS Entomopathogenic Fungal Collection (ARSEF, Ithaca, NY) where it was accessioned as ARSEF 7382 and confirmed to belong to the Fusarium genus (R.A. Humber [Curator, ARSEF], personal communication). A follow-up evaluation at the Fusarium Research Center, Pennsylvania State University, confirmed the isolate as F. solani (Mart.) Sacc. (J.H. Juba, D.M. Geizer, personal communication).

2.5. Bioassays for baseline susceptibility

Baseline susceptibility of T. myopaeformis pupae to F. solani ARSEF 7382 was investigated using a pure culture of the fungus. The pathogen was subcultured twice after initial isolation to obtain a pure culture for use in the bioassays. There was no previously established procedure for conducting bioassays on T. myopaeformis pupae. Therefore, an assay method that uses T. myopaeformis pupae, raised from field-collected larvae, was developed. Conidia were washed off the cultures using sterile distilled water at seven days after inoculation of the SDAY medium. Fresh stock suspensions were prepared for each bioassay. Conidia were counted using a hemacytometer (Improved Neubauer, Hauser Scientific, Horsham, PA), and by employing the procedure of Goettel and Inglis (1997). Viability was estimated at the onset of bioassays by inoculating SDAY medium with the conidial suspension using the procedures of Goettel and Inglis (1997), and incubating at 25 ± 1°C in continuous darkness for 48 h. Conidia were considered viable if the length of the germ tube exceeded twice the length of propagules. Profuse germ tube growth was observed within 48 h of surface inoculation. The germination test was repeated for each stock suspension to increase accuracy of viability assessments. Average viability of conidia was 98%.

Ten pupae were surface-treated with 1% sodium hypochlorite for 1 min, rinsed three times with sterile water, and dried in the air stream of a laminar-flow hood. Insects were aligned in double rows on two sheets of moistened sterile filter paper and spaced 1.5 cm apart to prevent over-exposure. Pupae were incubated at 25 ± 1°C and 12:12 (light:dark) h for 1–2 d to allow preconditioning for testing. Moisture facilitates metamorphosis, and the process is visible at 30× magnification under a stereomicroscope. Pupae that appeared healthy were used for bioassays. Consistent emergence of healthy adults from control pupae confirmed that this procedure provided healthy test subjects for the assays.

Conidia were produced by culturing F. solani on SDAY medium, and washing conidia off the substrate with sterile water to obtain a stock suspension. Aliquots were prepared from the stock suspension by diluting with sterile distilled water. Conidia concentrations in aliquots were confirmed by using a hemacytometer. Eight treatments, 2 × 10^4, 1 × 10^5, 3 × 10^5, 5 × 10^5, 7 × 10^5, 1 × 10^6, 1.5 × 10^6, and 2.8 × 10^6 conidia/ml, were applied in 30-µl aliquots to the test pupae. This volume provided adequate coverage of the entire puparium. Pupal dimensions averaged 7.0 mm long by 2.1 mm wide. Each conidial suspension was shaken vigorously before application to ensure uniform dosage delivery. Control pupae were treated with sterile distilled water. Treatments were replicated eight times, and 10 pupae were used per treatment in each replicate. The entire experiment was conducted twice.

Test concentrations were applied using a micropipette (Pipetman, Gilson, France) equipped with sterile disposable
tips. Excess solution was absorbed by filter paper immediately underlying each test pupa. Petri dishes containing test pupae were sealed with Parafilm “M” (Pechiney Plastic Packaging, Menasha, WI) to reduce moisture loss; however, to allow for pupal respiration, a small (~2 mm diam.) hole was made at the edge of each lid by using a hot needle. Pupae were then incubated at 24 ± 1 °C and 12:12 (light:dark) h for 5–6 d. This facilitated fungus establishment while allowing visual observations of disease progression. Pupae were subsequently transferred to clean 37-ml plastic soufflé cups using soft sterile forceps to minimize physical injury to developing pupae. To maintain humidity in cups during the test, three or four strips of moistened filter paper were placed in each, and the cups were then closed with fitted plastic lids. Cups were inverted and incubated for 10 d under previously described conditions. Pupae were transferred to SDA medium following the incubation period. Mycosis was confirmed by microscopic examination and isolation of F. solani on the culture medium. Infected pupae were also examined microscopically via median transverse sectioning to determine the extent of penetration and internal growth by the fungus.

2.6. Statistical analyses

Mortality data from bioassays were initially analyzed for homogeneity of variance (Levene’s test for Equality of Variances) via the independent samples t-test using SPSS version 12.0.1 as per the procedures of Dytham (1999). Control mortality was corrected for by using Abbott’s formula (1925), and the median lethal concentration and dose (i.e., LC<sub>50</sub> and LD<sub>50</sub>, respectively) were calculated per Finney (1964). Estimates were calculated separately for each bioassay by using log-transformed values for treatments. Data transformation improved the fit of the regression line and clearly indicated a dose–mortality relationship. A χ<sup>2</sup> goodness-of-fit test was then used to evaluate the fit of the regression line.

3. Results

A total of 3600 pupae collected during the two field surveys were individually screened in this study. In 2004, F. solani prevalence fluctuated from 4% to 80% among pupal collection dates (Table 1). The average prevalence level of F. solani on T. myopaeformis pupae that year was 44%. In 2005, prevalence of F. solani (Table 2) ranged from <1% to 7% at St. Thomas, 6% at Lodema, and <1% at Cavalier. A 15-d incubation period was sufficient to allow mycelial egress from cadavers of infected field-collected pupae.

Major characteristics of the F. solani growing on SDA medium included white aerial mycelia, light yellow growth toward the center as the colony matured, and numerous mucoid structures containing scattered conidia. Slime droplets were clearly visible at the terminal end of conidiophores. Conidial characteristic of F. solani (Humber, 1997) were abundant in slime heads, and were distinguishable under a light microscope at 400× magnification. Microconidia were about 5–10 µm long, ovoid, and most abundant. Macroconidia were fusiform, 15 µm or more in length, curved, and few in number. Macroconidia appeared to become more numerous as colonies matured.

At five to six days after treatment, shrinkage of developing cuticular tissues inside puparia provided the first evidence of F. solani infection. The fungus also killed fully-formed adults inside puparia and prevented adult eclosion, thus indicating rapid (i.e., within 24–48 h) penetration and high virulence against the insect. Transverse sections of pupae (Fig. 1a), performed at about 10 DAT, revealed F. solani to have penetrated and developed inside the pupae. Mycelia emerged from dead pupae after complete tissue destruction and depletion of nutrients (Fig. 1b). The mycelia had abundant microconidia that could easily be scraped from the pupal surface using a sterile loop. Visibility of pupae was often obscured by dense growth of the fungus in advanced stages of infection. Additional conidiophores were formed singly or in groups near the end of this period.

Isolate ARSEF 7382 of F. solani was highly virulent to T. myopaeformis pupae in these laboratory bioassays. Methodology used for conducting bioassays was conducive to making both visual and pathological observations of disease progression. Complete (i.e., 100%) adult emergence in controls indicated the usefulness of preconditioning, and also demonstrated that healthy pupae were used in these bioassays.

Levene’s test generated a high homogeneity of variances (F = 0.013; df = 1, 16; P = 0.909) among the bioassays, indicating the suitability of an independent sample t-test for comparing the two groups of observations. The t-test indicated no significant differences (t = 0.013; df = 1, 16; P = 0.956) between mean mortality values from the two bioassays. Median lethal values and results from χ<sup>2</sup> goodness-of-fit tests from each assay are presented in Table 3. Since no information regarding the actual number of conidia penetrating treated pupae were collected, the bioassay results reported herein are provided in terms of conidial concentration. The median lethal concentration value

<table>
<thead>
<tr>
<th>Collection date</th>
<th>Pupae collected</th>
<th>Pupae infected by F. solani</th>
<th>Infected pupae (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 May</td>
<td>5</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>21 May</td>
<td>100</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>24 May</td>
<td>12</td>
<td>5</td>
<td>41</td>
</tr>
<tr>
<td>25 May</td>
<td>21</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>27 May</td>
<td>300</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>2 June</td>
<td>100</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>4 June</td>
<td>700</td>
<td>461</td>
<td>65</td>
</tr>
<tr>
<td>Total</td>
<td>1200</td>
<td>537</td>
<td>44</td>
</tr>
</tbody>
</table>

Table 1 Prevalence of Fusarium solani isolate ARSEF 7382 in T. myopaeformis pupae at original detection site: St. Thomas, Pembina Co., North Dakota, 2004
(LC$_{50}$) from bioassay I was 1.70 · 10$^6$ conidia/ml. The LC$_{50}$ value for bioassay II was 1.84 · 10$^6$ conidia/ml. The fiducial limits of the two bioassays ranged from 0.64 · 10$^6$ to 4.78 · 10$^6$ conidia/ml. Fig. 2 provides a graphical representation of dose–mortality curves from the bioassays. Regression analyses generated $r^2$ values of 74.8 and 76.3 for bioassays I and II, respectively, thus demonstrating consistency of the results. The high $r^2$ values also validated that most of the mortality in the bioassays could be explained by the treatments. Regression equations were $Y = 3.266 + (0.64)X$ for bioassay I and $Y = 3.319 + (0.61)X$ for bioassay II.

4. Discussion

This is the first report of infection of *T. myopaeformis* by *F. solani*, and it was confirmed by conducting two field surveys and laboratory screening. Pupae of *T. myopaeformis* infected by *F. solani* were found at the original site on all collection dates in 2004. Infected pupae also were present in 2005, although the frequency of infected insects was much lower than was observed in 2004. Laboratory studies demonstrated high virulence of isolate ARSEF 7382 to *T. myopaeformis* pupae. The high seasonal prevalence of *Fusarium* infection observed at St. Thomas, ND could qualify it as a disease in an enzootic wave (Fuxa and Tanda, 1987); however, our prevalence surveys did not involve a multiyear investigation. Therefore, the long-term impact of *F. solani* on *T. myopaeformis* populations from year to year is yet to be determined. Sampling was discontinued after 4 June in 2004 because the majority of pupae in the field had eclosed and emerged from soil as adults. Nevertheless, information from collections beyond that date would not likely have been of practical relevance because most pupation and adult emergence should have occurred before then.

Soil moisture and temperature can be important regulators of biological events in the life cycles of soil-inhabiting insects and microorganisms. According to nine years (1995–2003) of available records from the North Dakota Agricultural Weather Network (NDAWN), average rainfall amounts for April, May, and June at St. Thomas were 17.4, 61.4, and 67.0 mm, respectively (Table 4). Rainfall

### Table 2

Survey of commercial fields for locating *Fusarium solani* epizootics in *T. myopaeformis* pupae, Pembina and Walsh Co., North Dakota, 2005

<table>
<thead>
<tr>
<th>Township</th>
<th>Collection site coordinates</th>
<th>Elevation (m)</th>
<th>Soil temperature (°C)</th>
<th>Previous crop</th>
<th>Pupae collected</th>
<th>Infected pupae</th>
<th>Infection rate (%)</th>
<th>Emergence failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Thomas</td>
<td>N 48.33, W 97.28</td>
<td>257</td>
<td>20</td>
<td>Potato</td>
<td>740</td>
<td>33</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>N 48.36, W 97.31</td>
<td>262</td>
<td>15</td>
<td>Bean</td>
<td>13</td>
<td>1</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>N 48.37, W 97.29</td>
<td>262</td>
<td>16</td>
<td>N/A*</td>
<td>70</td>
<td>2</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>N 48.37, W 97.32</td>
<td>262</td>
<td>15</td>
<td>N/A*</td>
<td>173</td>
<td>1</td>
<td>&lt;1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>N 48.41, W 97.27</td>
<td>261</td>
<td>17</td>
<td>Bean</td>
<td>153</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>N 48.71, W 97.27</td>
<td>261</td>
<td>19</td>
<td>N/A*</td>
<td>195</td>
<td>6</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>N 48.92, W 97.32</td>
<td>252</td>
<td>16</td>
<td>Potato</td>
<td>346</td>
<td>18</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Lodema</td>
<td>N 48.38, W 97.32</td>
<td>264</td>
<td>15</td>
<td>Potato</td>
<td>390</td>
<td>23</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Cavalier</td>
<td>N 48.49, W 97.38</td>
<td>265</td>
<td>16</td>
<td>Barley</td>
<td>300</td>
<td>2</td>
<td>&lt;1</td>
<td>14</td>
</tr>
<tr>
<td>Forest River</td>
<td>N 48.15, W 97.24</td>
<td>N/A*</td>
<td>N/A*</td>
<td>Bean</td>
<td>20</td>
<td>2</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2400</td>
<td>92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Not available.
amounts in 2004 were 43.2, 91.2, and 20.3 mm for the same months. Therefore, differences between the multiyear average and 2004 rainfall amounts were +25.8 mm (April), +29.8 mm (May), and –46.7 mm (June). Soil temperatures were in the range of 12–17 °C in May and June. The above-average rainfall in April and May of 2004, along with moderate soil temperatures, could have triggered the serendipitously discovered outbreak of *F. solani* reported herein.

Extremely saturated soil conditions in June of 2005, resulting from the unusually high rainfall (130.8 mm), could have been detrimental to *F. solani* or could have negatively affected host availability. MacRae and Armstrong (2000) observed that soil moisture levels exceeding 45% on a weight basis were detrimental to *T. myopaeformis* pupae. The high percentage of emergence failure observed in this study provides important information regarding the effect of environment on this insect’s biology. Temporal asynchrony between *Fusarium* and *T. myopaeformis* could have resulted in the low infection rates observed in 2005. Indirect effects from high soil moisture, such as increases in inhibitory soil microorganisms, also cannot be ruled out.

Low temperatures and high moisture have been shown to be suitable for *Fusarium* epizootics in other insects (Villacaros and Robin, 1989; Venugopal et al., 1989; Pandit and Tarannum, 2002). These conditions also would have coincided with the time *T. myopaeformis* larvae typically begin moving toward the upper 10 cm of the soil profile to pupate (Harper, 1962). In 2004, prevalence of *F. solani* in *T. myopaeformis* pupae was 15.2% in the two major collection dates of May, although the average rose to 43.9% in June. Field history could contribute to *F. solani* epizootics such as those observed in the prevalence surveys. For example, crop residue following certain crops such as potato, which is a known susceptible host to some *Fusarium* species, could preserve or result in increased inoculum levels of *F. solani* in soil. This is supported by our 2004 observations, in which *F. solani* was found infecting 44% of *T. myopaeformis* pupae in a field that had been previously planted to potato.

Phytopathogenicity of ARSEF 7382 is yet unknown. Therefore, further research is needed to determine the feasibility of the isolate in crop rotation schemes that include crops known to be susceptible to other species and isolates of this organism. Genetic diversity of the isolate we collected is also unknown. The fungus *F. solani* is known for its polymorphism based on genetic analysis (Brasileiro et al., 2004). It is possible that the fields used as collection sites in our study contained a diversity of isolates with

---

### Table 3

Responses of *Tetanops myopaeformis* pupae to *Fusarium solani* isolate ARSEF 7382 in two virulence bioassays

<table>
<thead>
<tr>
<th>Bioassay</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>Slope ± SE</th>
<th>$\text{LC}_{50}$ (conidia/ml)</th>
<th>Fiducial limits for $\text{LC}_{50}$</th>
<th>Calculated$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper</td>
<td>Lower</td>
</tr>
<tr>
<td>I</td>
<td>10.2</td>
<td>0.003</td>
<td>0.64 ± 0.14</td>
<td>$1.70 \times 10^6$</td>
<td>0.68 $\times 10^6$</td>
<td>4.23 $\times 10^6$</td>
</tr>
<tr>
<td>II</td>
<td>8.6</td>
<td>0.002</td>
<td>0.61 ± 0.15</td>
<td>$1.84 \times 10^6$</td>
<td>0.61 $\times 10^6$</td>
<td>5.34 $\times 10^6$</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>1.77 $\times 10^6$</td>
<td>0.64 $\times 10^6$</td>
<td>4.78 $\times 10^6$</td>
<td>7.24 $\times 10^7$</td>
</tr>
</tbody>
</table>

Slope and $\text{LC}_{50}$ values of bioassays I and II were not significantly different (two-tailed; $P = 0.956$).

A total of 640 pupae (i.e., eight treatments replicated eight times, with 10 pupae per treatment) were used in each bioassay.

$^a$ Mathematically calculated from empirical values following Finney (1964).

---

### Table 4

Comparison of 2004 and 2005 monthly totals and averages from the preceding 9 years for rainfall and precipitation, St. Thomas, ND

<table>
<thead>
<tr>
<th>Factor</th>
<th>1995–2003</th>
<th></th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>Average</td>
<td>2004</td>
<td>2005</td>
<td></td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>April</td>
<td>17.4</td>
<td>43.2</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>61.4</td>
<td>91.2</td>
<td>90.9</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>67.0</td>
<td>20.3</td>
<td>130.8</td>
</tr>
<tr>
<td>Soil temperature (°C)</td>
<td>April</td>
<td>3.3</td>
<td>7.0</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>11.9</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>19.2</td>
<td>17.0</td>
<td>19.0</td>
</tr>
</tbody>
</table>

Source: NDAWN, The North Dakota Agricultural Weather Network, North Dakota State University, Fargo.
differing infectivity and virulence to the insect; however, additional screening will be required to make such determinations because our testing was limited to isolate ARSEF 7382.

Time-dose response assays could not be conducted due to dense mycelial growth during incubation that obscured visual assessments of disease progression. Also, the immobility of T. myopaeformis pupae would have made behavioral observations difficult for testing this fungus. Therefore, dose–response assays were the most appropriate type of procedure for assessing virulence of F. solani in root maggot pupae. The adequacy of dose–response assays for establishing pathogenicity against immobile test subjects, such as insect eggs and pupae, was suggested by Shi and Feng (2004). Mortality of Fusarium-treated pupae in our study exceeded 80% at the highest dosage in each bioassay. Thus, these findings suggest a strong dose–mortality relationship for F. solani as an entomopathogen against T. myopaeformis pupae. Gopalakrishnan and Narayanan (1989) reported 100% mortality in the guava shield scale, Pulvinaria (= Chloropulvinaria) psidi Maskel, at 5 d after treatment with 4.8×10^6 conidia/ml of F. oxysporum isolate IMI 318632. Based on the nature of infection as described herein, it is hypothesized that F. solani isolate ARSEF 7382 causes mortality in SBRM pupae through a complex process that includes physical blockage of spiracles, enzymatic dissolution of the cuticle, and destruction of internal tissues.

This investigation provides important information regarding prevalence, baseline susceptibility, and disease progression of F. solani isolate ARSEF 7382 in T. myopaeformis pupae. Overall, the findings suggest that this pathogen has potential for use as a bioinsecticide for T. myopaeformis control. Future research efforts should include assessments of virulence and sublethal effects of infection on the survival and reproductive biology of T. myopaeformis adults. Additional study will be needed to determine the efficacy of this new isolate against other life stages of T. myopaeformis. The potential effects of F. solani isolate ARSEF 7382 on nontarget fauna also should be determined to assess its practicality as a potential bioinsectical organism.

Acknowledgements

We thank Dr. R.A. Humber of the U.S. Department of Agriculture—Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF, Cornell, University, Ithaca, NY), and J.A. Juba and Dr. D.M. Geiser of the Fusarium Research Center, Plant Pathology Department, Pennsylvania State University for identification and accession of fungal cultures. The assistance in disease surveys provided by R. Dregseth, C. Evensvold, C. Zander, B. McCamy, D. Miller, P. Burange, R.D. Adharapurapu, T. Wiener, V.R. Panavala, and Y. Horita are also greatly appreciated. This work was partially supported by a grant from the North Dakota State Board of Agricultural Research and Education. Valuable suggestions provided by Drs. G. Brewer, C. Windels, and H. Kuya, as well as those from the anonymous reviewers, are gratefully acknowledged.

References