

# Variable Effects of Grass–*Neotyphodium* Associations on Cereal Leaf Beetle (Coleoptera: Chrysomelidae) Feeding, Development and Survival<sup>1</sup>

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**Abstract** Although cereal grains are the preferred food plants of the cereal leaf beetle, *Oulema melanopus* (L.), several other graminoid species are acceptable feeding hosts of larvae and adults of this chrysomelid beetle. In view of the potential for expanding the use of diverse endophytic fungi (*Neotyphodium* Glenn, Bacon and Hanlin) to protect forage and cereal grasses from insect pests, more information on the effect of *Neotyphodium*-infected (E+) grasses on the behavior and performance of the most important graminoid pests, including *O. melanopus*, is required. In feeding and oviposition choice experiments, adult *O. melanopus* fed readily on E+ and uninfected (E-) plants of wild tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire) and alpine timothy (*Phleum alpinum* L.), while exhibiting a feeding preference for E- over E+ plants of one tall fescue accession. In larval survival and development experiments, low survival on E+ plants of alpine timothy and one tall fescue accession (averaged 3.75 - 12.5%) was not linked to slow development of surviving larvae because developmental periods were similar on E- (averaged 11.31 - 12.73 days) and E+ (11.33 - 11.7 days) plants. Larval mortality was 100% on E+ plants of tall fescue from Morocco. Thus, *O. melanopus* feeding and survival is significantly reduced on some E+ wild grasses. Our results also expand our knowledge of the antiinsect properties of fungal endophytes in diverse grasses for possible use in protecting forage and cereal grass cultivars from *O. melanopus* and other important pests.

**Key Words** cereal leaf beetle, temperate grasses, *Neotyphodium* endophytes, host plant resistance

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Cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), is an adventive Palearctic insect in North America where it was first reported in Michigan in 1962, with populations now established in eastern regions of North America from Ontario to Alabama and in northwestern regions from Utah and Montana to Washington and southern British Columbia (Haynes and Gage 1981, Morrill et al. 1992, Olfert et al., 2004, Buntin et al. 2004). In Europe and North America, the beetle is a well-known pest of cereal crops, particularly wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) (CAB International 2002).

Several C<sub>3</sub> perennial grasses are acceptable feeding hosts of *O. melanopus*, e.g., tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire), orchard grass (*Dactylis*

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*glomerata* L.), timothy (*Phleum pretense* L.), and *Phalaris* spp. (e.g., Hodson 1929, Wilson and Shade 1964, 1966, Rao 2003, Clement et al. 2009). However, only 3 laboratory studies have quantified cereal leaf beetle feeding, development, and mortality on perennial grasses in the U.S. Wilson and Shade (1964, 1966) showed that several perennial grasses were acceptable food plants; however, larvae usually gained less weight and had longer developmental periods on perennial grasses than on small grains. In the third study, both fungal endophyte (*Neotyphodium*)-infected (E+) and uninfected (E-) tall fescue (cultivar Kentucky-31) plants were acceptable adult feeding and larval development hosts, but larval mortality was significantly greater on E+ plants (Clement et al. 2009). *Neotyphodium* (Clavicipitaceae) fungi in temperate grasses are linked to increased plant fitness, such as greater drought tolerance and resistance to insect and mammalian herbivores. This resistance is the result of endophyte synthesis of secondary metabolites, some functioning as insect (mainly loline and peramine alkaloids) and mammalian (lolitrem and ergot alkaloids) toxins (Bacon and Siegel 1988).

There are, as well, few definitive reports of *O. melanopus* feeding on field plants of perennial grasses. Grant and Patrick (1993) observed adult feeding on fescue in Tennessee, and Rao (2003) observed a high adult infestation in a new planting of tall fescue in northwestern Oregon. Clement et al. (2009) recorded cereal leaf beetle feeding on *Agrostis*, *Dactylis*, *Elymus*, *Lolium*, and *Phalaris* accessions, including both E- and E+ tall fescue, in grass seed regeneration nurseries in southeastern Washington.

This research is a continuation of our studies on the effect of different grass–endophyte associations on the feeding and performance of important insect pests of forage and cereal grasses (e.g., Clement et al. 1990, 1994, 2001, 2005, 2007). Herein, we present data on *O. melanopus* adult feeding and larval performance on E+ PI (plant inventory) grass accessions (tall fescue and alpine timothy, *P. alpinum* L.) from the gene bank at the USDA, ARS Western Regional Plant Introduction Station (WRPIS), Washington State Univ., Pullman, WA. The WRPIS grass collection harbors diverse *Neotyphodium* strains for basic research and for combining with tall fescue and other grasses for improved abiotic and biotic tolerance and resistance (Latch et al. 2003, Clement et al. 2001, 2009). In view of the potential application of using endophytic fungi from different grasses to protect cereals (wheat, barley) from insect pests (Marshall et al. 1999, Clement et al. 2005, Caradus 2009), studies like this are needed to generate a comprehensive assessment of the effects of diverse grass–*Neotyphodium* associations on the behavior and performance of the most economically important cereal pests.

## Materials and Methods

**Plants, endophyte isolation, and insects.** Two tall fescue accessions (PI 598863 from Morocco; PI 619480, Tunisia), one alpine timothy accession (PI 619539, Russian Federation), and barley (cv. UC 937) were evaluated in a series of experiments. One E+ and one E- plant of each accession were grown from seed germinated in an environmental chamber (22°C and 14L:10D) and pruned, subdivided, and repotted (15-cm pots filled with commercial potting soil) several times before they were split as 1-3-yr-old plants to provide E+ and E- vegetative propagules (test plants) for larval feeding and survival/development experiments. By obtaining replicate test plants from one E+ and one E- plant, we reduced possible effects of plant genotype on beetle performance. Fescue and timothy test plants, before their use in experiments, and 'source plants' were maintained in a greenhouse (13–33°C, natural light) where they were watered as needed and fertilized biweekly (0.6 g Peter's 20–20–20 per liter H<sub>2</sub>O).

Each PI test plant had 2 - 3 new tillers (each with 3 - 4 leaves) when used in experiments. Barley was directly seeded into commercial potting soil, and potted plants were maintained under similar greenhouse conditions.

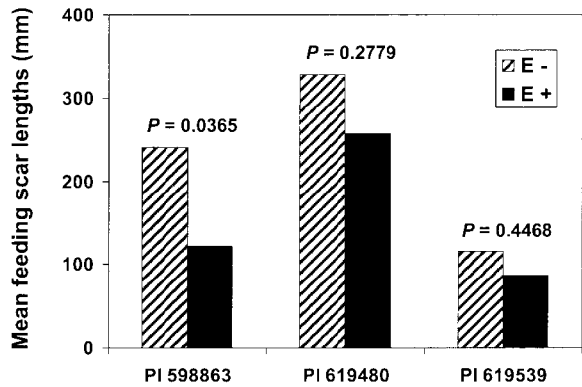
Five to 6 wks after PI test plants from E+ and E- 'source plants' were rooted in pots, the endophyte status of each plant was determined by isolating *Neotyphodium* fungi on potato dextrose agar (PDA) supplemented with streptomycin sulfate and tetracycline hydrochloride (50 µg each per ml of PDA). Following procedures in Clement et al. (2001), basal stem sections (~1 cm) from at least 2 tillers per plant were surface disinfected and placed on PDA in sealed Petri dishes and incubated in a laboratory (complete darkness, room temperature). Petri dishes were examined for mycelial growth from plant tissue at 2 - 3 day intervals for 45 d. A PI test plant was scored E- if *Neotyphodium* mycelia did not appear during this period of time. This time-consuming isolation method generates reliable data on the presence of viable *Neotyphodium* in plant tissue. The fungi in the E+ accessions were confirmed as *Neotyphodium* (PI 598863, PI 619480) and *Neotyphodium*-like (PI 619539) from published descriptions of colonies on agar and conidia (Latch et al. 1984, White and Morgan-Jones 1987), although their exact identity (strain/species) has not been established.

A cereal leaf beetle laboratory colony, which supplied neonate larvae and adults for experiments, was started with adults collected April through May 2008 in organic and commercial wheat fields near Connell, WA (46°42'N, 118°51'W) and maintained on potted barley plants in laboratory (21 - 23°C) cages (39h × 33w × 43d cm).

**Feeding and oviposition experiments.** Three paired choice-experiments were conducted to record the feeding and oviposition preferences of adult *O. melanopus* for E+ and E- plants. Six mating adults (3♂ and 3♀) were starved for 4 h then placed on the lid of a plastic Petri dish on the top of an inverted 15-cm pot placed between one E+ and one E- plant in an acrylic cage (51 × 51 × 51 cm). There were 9 (PI 598863 experiment) or 10 (PI 619480 and PI 619539 experiments) replicate cages randomly positioned on a greenhouse (15.6 - 26.7°C, natural light) bench. After 24 h, all adults were removed, and plants were taken to a laboratory to measure the length of adult feeding scars on leaves (Fig. 1) and to count eggs on each plant.

**Larval development and mortality experiments.** Three experiments recorded larval development (number of days for neonate larvae to reach the fourth instar) and larval survival on E+ and E- test plants. For each growth chamber experiment (21 - 25°C, 35 - 70% RH, and 16L:8D), 10 E+ and 10 E- plants of one PI accession were arranged in accordance with a completely randomized design and positioned so leaves from different plants would not touch. Each plant was infested with 8 neonates (2 - 8 h postegg hatch), which were transferred with a fine-hair brush from infested barley leaves removed from the colony and placed in a Petri dish to monitor egg hatch. We recorded the number of larvae surviving to the fourth instar and the number of days for each neonate larva to reach this stage on each test plant. We determined the instar by measuring head capsule widths (Hoxie and Wellso 1974) with a dissecting microscope. Similar methods, procedures, and growth chamber conditions were followed to record larval survival and development to the fourth instar on barley plants ( $n = 5$ , 3 wks-old and each with 2 - 3 tillers).

**Statistical analyses.** Data from 5 experiments were analyzed separately by one-way analysis of variance (ANOVA). Larval survival data (%) were transformed using the arcsine transformation to satisfy ANOVA assumptions with pretransformed means presented here (SAS Institute 2006). Data from one survival and development experiment (PI 598863) were not statistically analyzed because mortality was 100% on E+ plants



**Fig. 1. Comparison of cereal leaf beetle adult feeding damage on endophyte-infected (E+) and uninfected (E-) tall fescue (PI 598863, PI 619480) and alpine timothy (PI 619539) accessions.**

(Table 1). Low egg counts did not permit statistical comparisons between numbers of eggs laid on E+ and E- plants in experiments.

**Results and Discussion**

*Neotyphodium* cultures on agar developed only from tiller sections of experimental plants derived from E+ source plants of the three PI accessions. Thus, cereal leaf beetle data from accessions are associated with either E+ or E- plants.

**Table 1. Comparison of cereal leaf beetle larval survival and development on endophyte-infected (E+) and uninfected (E-) alpine timothy (PI 619539) and tall fescue (PI 619480, PI 598863) accessions, and cultivated barley.**

| Entry and origin                | Endophyte status              | Mean ± SD      |                    |
|---------------------------------|-------------------------------|----------------|--------------------|
|                                 |                               | Survival (%)   | Development (days) |
| PI 619539<br>Russian Federation | E-                            | 18.75 ± 16.93  | 12.73 ± 2.40       |
|                                 | E+                            | 12.50 ± 15.59  | 11.70 ± 1.77       |
|                                 | <i>F</i> values ( <i>df</i> ) | 0.84 (1, 18)ns | 1.35 (1, 23)ns     |
| PI 619480<br>Tunisia            | E-                            | 16.25 ± 14.49  | 11.31 ± 1.03       |
|                                 | E+                            | 3.75 ± 11.86   | 11.33 ± 2.31       |
|                                 | <i>F</i> values ( <i>df</i> ) | 6.94 (1, 18)*  | 0.00 (1, 14)ns     |
| PI 598863<br>Morocco            | E-                            | 22.50 ± 15.37  | 11.39 ± 1.65       |
|                                 | E+                            | 0              | —                  |
| Barley                          | E-                            | 55.0 ± 20.92   | 10.22 ± 1.11       |

\* *P* = 0.0168; ns, not significant.

Although beetles fed readily on all E+ and E- plants, they preferentially ( $P = 0.0365$ ) fed on E- over E+ plants of PI 598863 (Fig. 1). The numbers of eggs laid in the feeding and oviposition experiments were: 9 (E+) and 1 (E-) on PI 598863; 25 (E+) and 9 (E-) on PI 619480; and 7 (E+) and 16 (E-) on PI 619539. Thus, these wild grasses are acceptable, if not superior, oviposition hosts.

The average number of days for larvae to reach the fourth instar differed little among those feeding on E+ and E- plants of PI 619480 and PI 619539. Mean days to the fourth instar on E- and E+ plants of PI 619480 and on E- and E+ plants of PI 619539 were not significantly different ( $P = 0.9758$  and  $0.2569$ , respectively) (Table 1).

Although average larval survival rates were low (<22.5%) in all experiments, the strong protective effects of E+ tall fescue against *O. melanopus* larvae were readily apparent, exemplified by little or no (0 - 3.75%) larval survival on E+ plants of PI 598863 and PI 619480. Average survival percentages on E+ (12.5%) and E- (18.75%) plants of alpine timothy (PI 619539) were not statistically different ( $P = 0.3728$ ). Interestingly, low larval survival on E+ plants of alpine timothy and one tall fescue accession (PI 619480) was not linked to slow development of surviving larvae on these plants (Table 1). By contrast, the developmental period of surviving *O. melanopus* larvae on E+ Kentucky-31 tall fescue was significantly extended, compared with the average development time of larvae on E- plants of this cultivar (Clement et al. 2009). We are unaware of other reports detailing the absence of extended developmental times by surviving early-stage insects on E+ plants.

Compared with *O. melanopus* performance on the wild grass accessions, larval survival was higher (averaged 55%) and larvae developed faster (averaged 10.22 days) on barley plants. This result is consistent with reports that cereals are superior to forage grasses as *O. melanopus* food plants (Wilson and Shade 1964, 1966).

These results document and extend the phenomenon of variable effects of E+ grasses on the behavior and performance of phytophagous insects (see Breen 1994 and Clement et al. 1994) by showing that cereal leaf beetle adult feeding and larval survival rates were markedly different on E+ plants of wild grass accessions. These different outcomes were likely related to differences in alkaloid types and concentrations produced by the different wild grass–*Neotyphodium* associations.

The results herein bring the number of economically-important cereal pests adversely affected by one or more grass–*Neotyphodium* association to 5 species (from *Diuraphis noxia* (Mordvilko), *Metopolophium dirhodum* (Walker), *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), and *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae) [Clement et al. 1994, 2001, 2005, 2007]). It may be possible to exploit this knowledge to “vaccinate crop grasses against pests” (Clay 1990) if, for example, research successfully addresses the potential accumulation of mammalian toxins in seeds and possible incompatibility between modern cereal cultivars and natural or genetically-modified endophyte strains (Clement et al. 1994). Finally, the existence of endophytes in the wild relatives of wheat and barley could facilitate the development of cereal cultivars with endophyte-based pest resistance traits (Wilson et al. 1991, Marshall et al. 1999, Caradus 2009).

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