

Variable performance of bird cherry-oat aphid on *Neotyphodium* – infected wild tall fescue from Tunisia

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Abstract

The extent of *Neotyphodium* based resistance in wild fescue to bird cherry-oat aphid (*Rhopalosiphum padi*) was determined by quantifying densities of this aphid on a series of *Neotyphodium*-infected (E+) and uninfected (E-) tall fescue entries. Little or no aphid survival was observed on E+ ‘Kentucky 31’ tall fescue and three E+ wild tall fescue accessions (16079, 15978, 16075) from Tunisia; however, three E+ Tunisia accessions (16036, 16044, 16085) supported populations of *R. padi*. These results suggest that wild tall fescue from Tunisia harbour diverse *Neotyphodium* endophytes. They also support earlier observations by entomologists that the magnitude of insect (including aphids) resistance in E+ grasses varies with the host genotype and *Neotyphodium* strain involved in the interaction.

Keywords: *Neotyphodium* endophyte, tall fescue, *Rhopalosiphum padi*, host plant resistance

Introduction

Much information on the extent and nature of grass–*Neotyphodium* endophyte–insect interactions has been generated since the first reports of endophyte based resistance to phytophagous insects in the early 1980s. By the start of the 21st century, researchers had characterised the outcome of many interactions between grasses, endophytes, and insects. For example, they have shown that both insect deterrence and toxicity result from the production of specific alkaloids by grass–endophyte associations, and that the host grass genotype or species and the *Neotyphodium* strain involved in the interaction affect the expression and the type of insect resistance (e.g. Breen 1994; Clement *et al.* 1994; Popay & Rowan 1994; Saikkonen *et al.* 1998; Popay & Bonos 2005).

Illustrative of the diversity of responses by plant-feeding insects to *Neotyphodium*-infected (E+) grasses are the results from studies involving the bird cherry-oat aphid (BCOA), *Rhopalosiphum padi* (Homoptera: Aphididae), and E+ tall fescue (*Festuca arundinacea*), E+ perennial ryegrass (*Lolium perenne*), and E+ wild barley (*Hordeum* spp.). This aphid, a common pest of grasses, has been widely used in aphid–grass endophyte studies. Several studies have documented feeding deterrence and/or antibiosis (reduced survival and reproduction) to BCOA in cultivars of E+ tall fescue (Johnson *et al.* 1985; Latch *et al.* 1985; Eichenseer *et al.* 1991; Eichenseer & Dahlman 1992; Breen 1993; Schardl & Phillips 1997; Bultman & Murphy 2000; Bultman & Bell 2003; Bultman *et al.* 2004). Loline alkaloids produced by these E+ cultivars are responsible for BCOA resistance (Siegel *et al.* 1990; Eichenseer *et al.* 1991; Wilkinson *et al.* 2000). By contrast, Christensen and Latch (1991) found that some *Neotyphodium*–tall fescue associations from southern Spain and Algeria did not deter BCOA, and Clement *et al.* (2001) discovered that the reproduction and development of this aphid was unaffected by two E+ wild tall fescue accessions from Tunisia.

More recently, the sensitivity of BCOA to novel associations of tall fescue cultivars and “safe or nontoxic endophytes” has been studied. These new associations have been developed to overcome the production of ergot alkaloids by the “wild-type” strain of *Neotyphodium coenophialum* that is commonly associated with tall fescue pastures in southeastern U.S (Bouton *et al.* 2002). In general, tall fescue cultivars infected with nontoxic strains (AR542 and AR502) were not as effective in suppressing BCOA population growth as the more common associations involving the wild-type strain (Bultman *et al.* 2004; Hunt & Newman 2005).

Johnson *et al.* (1985) and Latch *et al.* (1985) reported that BCOA was not deterred by *Neotyphodium* in perennial ryegrass. In subsequent research, however, some *Neotyphodium*–perennial ryegrass associations, relative to E- plants, deterred BCOA (Breen 1993). More recently, Meister *et al.* (2006) found that BCOA densities were reduced on all E+ perennial ryegrass cultivars over the course of 36 day assays. These variable results reinforce Breen’s (1993) view that the magnitude of the negative effects of the perennial ryegrass endophyte on BCOA preference behaviour and survival varies with specific plant genotype–endophyte associations. Also, Clement *et al.* (2001) found that wild barley endophytes do not reduce the survival of BCOA.

The objective of this study was to quantify the development of BCOA populations on several E+ wild tall fescue accessions from Tunisia, thereby expanding earlier research that characterised the variable performance of this aphid on a limited number of E+ accessions from this North African country (Clement *et al.* 2001). Additionally, this research improves our overall understanding of the impact of wild grass–endophyte associations on the behaviour and survival of phytophagous insects.

Materials and Methods

Aphids, plants, and endophyte status

Aphids were obtained from a laboratory colony reared on ‘Stevens’ wheat (*Triticum aestivum*) in a growth chamber (21 ± 2°C, 14 h light:10 h dark). This colony was initiated with progeny of 12 alates from a BCOA colony in the Department of Entomology, Washington State University, Pullman, Washington, in April 2003.

Six tall fescue accessions were evaluated, seed of which was originally collected from wild plants in Tunisia in 1994 (Cunningham *et al.* 1997) and stored in the seed bank at the USDA-ARS Western Regional Plant Introduction Station, Pullman, Washington, USA. Additionally, ‘Kentucky 31’ tall fescue was evaluated in one experiment, with seed (E+ and E-) provided by T. Phillips, University of Kentucky, Lexington, Kentucky, USA. The Tunisia accessions are identified as tall fescue in the GRIN database (Genetic Resources Information Network; <http://www.ars-grin.gov/npgs>) of the U.S. National Plant Germplasm

System. Taxonomic classification of the Tunisia accessions was confirmed via morphological examination of greenhouse grown plants by the Intermountain Herbarium (Utah State University, Logan, Utah, USA). This examination suggests that accession 16044 might include hybrids between tall fescue and meadow fescue (*F. pratensis*). All accessions were hexaploids ($2n=6x=42$) as determined by examination of stained root tips during mitosis and flow cytometry comparisons. Based on flow cytometry, using propidium iodide stain and *Pisum sativum* ('Little Marvel') as a standard, the genome size of the Tunisia material is 14.30 ± 0.14 pg/2C, a value within the range of the genome sizes (12.10 – 17.86 pg/2C) of 35 hexaploid tall fescue populations in Italy (Ceccarelli *et al.* 1992). However, the Tunisia accessions appear to have a smaller genome than the 17.92 pg/2C we observed for 'Kentucky 31' tall fescue. Taxonomy and ploidy status of Tunisia accession 16085 was not determined.

Seed was germinated in September 1999 (Tunisia accessions) and December 2004 ('Kentucky 31') in accordance with procedures in Clement *et al.* (2001). Germinated seeds were sown in separate 15-cm pots containing a commercial soil mix, maintained in a glasshouse (15 – 30°C ; natural light), and fertilised bi-weekly with 0.60 g of Peter's 20-20-20 in 1 L of water. These potted plants were pruned, subdivided, and repotted several times before we split them to obtain replicate E+ and E- clones for aphid assays. The E+ and E- status of each 'source plant' for experimental clones was determined in 2000 (Tunisia accessions) and 2005 ('Kentucky 31') using methods in Clement *et al.* (2001).

Aphid assays

Six plants provided replicate clones for the 2003 assay. Each 4-year-old E+ (16079, 16075, 16036, 16044, 16085) and E- (16079) source plant (Table 1) was split to obtain 10 vegetative propagules, each with 3–4 tillers, for rooting in plastic cone-tainers (40-mm diam at the top tapering to 28-mm at the bottom by 20.5-cm tall) containing a commercial soil mix. Cone-tainers were placed in a holding rack on a glasshouse bench, and watered as needed. Two wk after transplanting to cone-tainers, we removed 1–2 growing tillers from each clone to isolate stem sections on potato dextrose

agar (methods in Clement *et al.* 2001) to confirm their E+ and E- status. After another 2 wk, when each 4-wk-old cone-tainer plant had re-growth tillers and leaves, we removed all senescing plant tissue and infested each plant with 25 adult apterous BCOA. By removing senescing tissue, we controlled for leaf age, which can influence BCOA survival and reproduction (Eichenseer *et al.* 1991). Clear plastic vented tubular cages (36-mm diam by 30-cm tall), capped with nylon organdy screen, were tightly inserted into cone-tainers to confine the aphids. This assay was conducted in a glasshouse (15.3 – 28.3°C ; natural light).

For the 2006 assay, methods and procedures were similar to those for the 2003 assay. The performance of BCOA was quantified on replicate clones from six of the same source plants that provided experimental material for the 2003 assay. Four additional source plants representing 'Kentucky 31' (E+, E-) and accession 15978 (E+, E-) provided replicate clones for the 2006 assay. With the exception of 2-yr-old E+ and E-'Kentucky 31' source plants, all Tunisia source plants were 7-yr-old in 2006 (Table 1). Unlike 2003, this assay was conducted in a growth chamber ($21 \pm 2^\circ\text{C}$, 14 h light:10 h dark).

In both assays, cone-tainer plants were arranged in a completely randomised design in holding racks and the number of live aphids (alates + apterous forms) on each plant was recorded after 10 d. Results of the population growth tests, with the exception of zero data sets, were analysed by analysis of variance. Data were transformed by $\log_{10}(x + 1)$ if data sets included zeros, before statistical analysis was done. Untransformed means (\pm standard deviation) are reported (Table 1).

Results and Discussion

In both assays, *Neotyphodium* cultures on agar developed only from tiller sections of replicate clones derived from E+ source plants. Thus, all BCOA counts reported herein are associated with either E+ or E- clones.

The strong protective effect of E+ 16079 and E+ 15978 against BCOA in earlier research (Clement *et al.* 2001) was also evident in this study. Additionally, E+ 'Kentucky 31' suppressed all

Table 1 Density (no./plant) of *Rhopalosiphum padi* aphids after 10 days on replicate clones of *Neotyphodium*-infected (E+) and uninfected (E-) tall fescue plants.

Entries	Endophyte status	2003 ^a		2006 ^b	
		0 \pm SD ^c	Range	0 \pm SD ^c	Range
KY31	E +	–	–	0.0	–
KY31	E –	–	–	79.4 \pm 16.49a	53–105
16079	E +	4.1 \pm 4.75c	0–13	0.5 \pm 1.08d	0–3
16079	E –	87.5 \pm 22.11a	62–122	76.0 \pm 11.53a	64–97
15978	E +	–	–	3.5 \pm 2.88d	0–8
15978	E –	–	–	46.1 \pm 14.70b	23–59
16075	E +	0.0	–	0.0	–
16036	E +	34.4 \pm 10.32b	12–52	17.9 \pm 6.52c	9–27
16044	E +	37.9 \pm 16.66b	15–63	6.4 \pm 7.69d	0–22
16085	E +	40.6 \pm 16.10b	19–66	16.5 \pm 11.29c	4–37

^a n = 10

^b n = 7–10

^c Means followed by the same lowercase letter are not significantly different (P = 0.05).

development and survival of BCOA, as expected, and all E-clones of different entries supported good BCOA population growth (Table 1).

In 2003, E+ 16075 was resistant and three E+ accessions (16036, 16044, 16085) supported BCOA population growth. In 2006, E+ clones of 16075 were again strongly resistant to BCOA, while low aphid densities were recorded on E+ clones of 15978, 16036, 16044, 16079, and 16085. In both assays, aphid densities on the 'susceptible' E+ accessions were significantly ($P < 0.05$) lower than densities on all E- entries.

We cannot explain the higher level of aphid resistance conferred by *Neotyphodium* infection of 16036, 16044 and 16085 in 2006 (Table 1). Perhaps different experimental environments (glasshouse vs. growth chamber), via production of different alkaloid concentrations, influenced the outcome of the grass-endophyte-BCOA interactions in 2003 and 2006. Both assays were conducted with aphids from the same laboratory colony, thus the different density patterns cannot be attributed to the performance of different BCOA biotypes. Popay and Rowan (1994) first suggested that BCOA response to endophytes may be biotype-specific.

The results herein document and extend the phenomenon of variable effects of E+ grasses on insects by showing that some wild tall fescue-*Neotyphodium* associations provide a lower degree of resistance to BCOA than other associations involving E+ cultivars ('Kentucky 31') and E+ wild fescue populations (Table 1). Interestingly, Hunt and Newman (2005) raised the possibility that new combinations of 'safe and nontoxic' strains of *N. coenophialum* and tall fescue cultivars may not provide high levels of resistance to cereal aphids. Because Hunt and Newman (2005) focused on a Moroccan strain of *N. coenophialum* with 'Georgia' tall fescue, and our results are based on Tunisian strains of *Neotyphodium*, the possibility exists that the some North African strains may not endow new host cultivars with high levels of aphid resistance. The variable BCOA densities on E+ grasses in this study were likely related to differences in the alkaloid types and concentrations produced by the different Tunisia tall fescue-endophyte associations.

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