Behavior and Performance of *Diuraphis noxia* (Homoptera: Aphididae) on Fungal Endophyte-Infected and Uninfected Perennial Ryegrass

S. L. CLEMENT,[1] D. G. LESTER,[1] A. D. WILSON,[1] AND K. S. PIKE[2]

J. Econ. Entomol. 85(2): 583-588 (1992)

ABSTRACT The behavior and performance of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), on fungal endophyte-infected and endophyte-free perennial ryegrass, *Lolium perenne* L., was investigated in the laboratory and field. Aphids did not select endophyte-free over endophyte-infected leaf sheaths and stem segments in petri dish preference tests. Similarly, the probing behavior of *D. noxia* on endophyte-free and infected ***L. perenne*** did not vary in laboratory tests. However, compared with aphid counts on endophyte-free plants, counts on infected plants were significantly lower on the first day and thereafter in laboratory population growth tests. Numbers of *D. noxia* were higher on endophyte-free plants than on infected plants of *L. **perenne*** in replicated field plots. The results are discussed in relation to the possible mechanisms of resistance involved, our knowledge of the performance of other cereal aphids on endophyte-infected grasses, and the use of endophytic fungi as aphid biocontrol agents.

KEY WORDS Insecta, Russian wheat aphid, fungal endophyte, plant resistance

THE PRESENCE OF FUNGAL ENDOPHYTES, *Acremonium* spp. (Clavicipitaceae: Belansiae) in perennial ryegrass, ***Lolium perenne*** L., and tall fescue, ***Festuca arundinacea*** Schreb., has been linked to reduced herbivory of infected plants by grazing livestock and insects (Clay 1988). The basis for this resistance is probably associated with the production of alkaloids by fungal endophytes in infected plants. These chemicals can act as toxicants to livestock and insects, or feeding deterrents to insects (Clay 1988). Fungal endophyte infection in tall fescue also might be associated with increased plant vigor and drought tolerance (De Battista et al. 1990). These discoveries have stimulated much research on the nature of the relationships between endophytic fungi and animal, insect, and host plant performance (Siegel et al. 1987, Bacon & Siegel 1988, Clay 1989).

Several species of insects are negatively affected by endophyte-infected grasses (Clay 1989, Mathias et al. 1990), including the cereal aphids *Rhopalosiphum padi* (L.) (Latch et al. 1985; Johnson et al. 1985; Siegel et al. 1985, 1990), R. *maidis* (Fitch) (Buckley et al. 1991), ***Schizaphis graminum*** (Rondani) (Johnson et al. 1985, Siegel et al. 1990), and ***Diuraphis noxia*** (Mordvilko) (Clement et al. 1990b, Springer & Kindler 1990, Wilson et al. 1991). ***D. noxia***, commonly called the Russian wheat aphid, has caused considerable damage to wheat and barley in the United States since its discovery in Texas in 1986 (Peairs 1989, Pike et al. 1991).

This report is a continuation of our study of endophyte-enhanced resistance in grasses to ***D.*** noxia (Clement et al. 1990b). We present data on the host preference, probing behavior, and rate of population growth of ***D. noxia*** on endophyte-infected and endophyte-free plants of perennial ryegrass in the laboratory, and the abundance of the aphid on these two plant types in field plots. We also compare our findings with published accounts on the performance of other cereal aphids on grasses containing endophytic fungi. In view of the potential application of endophytic fungi as biocontrol agents against phytophagous insect pests (Clay 1989), studies such as this one are needed to elucidate the association between grass endophytes and insect performance.

Materials and Methods

Aphids used in laboratory tests were from a colony established from aphids collected in a ***Hordeum*** spp. germplasm nursery near Pullman, Wash., in summer 1988. The colony was maintained on 'Steptoe' barley, ***Hordeum vulgare*** L., in 15-cm pots in a Percival environmental control chamber (Percival Manufacturing Company, Boone, Iowa) at 22 ± 1°C and a photoperiod of

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

[1] Plant Germplasm Introduction and Testing Research Unit, USDA–ARS, 59 Johnson Hall, Washington State University, Pullman, Wash. 99164.

[2] Washington State University, Irrigated Agricultural Research and Extension Center, Rt. 2, Box 2953-A. Prosser, Wash. 99350.

14:10 (L:D). The culture plants were replaced every 10–14 d to avoid aphid overcrowding. Laboratory tests were conducted in 1989 in a separate Percival chamber maintained under similar temperature and photoperiod conditions.

Two seed lots of 'Repell' ryegrass (provided by R. Welty, USDA-ARS, Corvallis, Oreg.) and seed of two Plant Inventory (PI) accessions from the Western Regional Plant Introduction Station, Pullman, Wash., were selected as sources of endophyte-infected and uninfected plants of perennial ryegrass. Microscopic examination of stained seed (n = 150 per lot or PI line) using methods described by Wilson et al. (1991) revealed endophyte infection levels of 91% and 0% in the two seed lots of 'Repell', and 99% (PI 462339) and 0% (PI 462340) in the two PI lines. The two PI lines originated from the same natural population of L. perenne in a 60-yr-old pasture near Auckland (Mangere District), New Zealand. Plants were grown from seed in a greenhouse under natural conditions of sunlight and temperatures of 21–30°C, and were fertilized weekly with Peters 20-20-20 (N/P/K) (W. R. Grace & Company, Fogelsville, Pa.).

Leaf sheaths and sections of vegetative stems for laboratory experiments were removed from the lower portions of tillers from 6-mo-old endophyte-infected and uninfected plants. Infection status of plants was determined using microscopic methods described by Wilson et al. (1991).

Host Preference Tests. Tests were conducted by scattering 200 apterous aphids, previously starved for 2 h, over 3-5 cm sections of plant samples of endophyte-free (PI 462340) and infected (PI 462339) ryegrass on water-moistened filter paper in plastic 15-cm petri dishes. Each petri dish was sealed with Parafilm and the number of resting and feeding aphids on each plant sample was recorded 18 h later. The aphids wandering around in the petri dish also were counted, but these counts were disregarded in the analysis.

Two tests were conducted, each with six petri dishes (replicates). In test 1, six leaf sheath segments (three from an endophyte-free and three from an infected plant) were arranged in two rows (three segments per row), with alternating segments from endophyte-free and infected plants. Segments were 2.5 cm apart. Test 2 was conducted in a similar fashion, except that vegetative stems were used. Similar experimental methods were used by Johnson et al. (1985) and Siegel et al. (1985) to show that *R. padi* preferred endophyte-free over endophyte-infected tall fescue after 18 h.

Behavioral Observations. To measure the probing responses of on endophyte-free (PI 462340) and infected (PI 462339) ryegrass, we compared the behavior of individual aphids on 5-cm-long sections of vegetative stems from both types of plants. Alate aphids were starved 4-5 h before they were transferred individually with a camel's-hair brush to the edge of a stem in a 15-cm glass petri dish. Parameters of aphid behavior observed were: time to first probe or stylet penetration; number of probing bouts; time spent probing; and time spent walking and resting on the plant and in the petri dish. We selected these parameters based on the work of Givovich et al. (1988) on cowpea aphid behavior. Each aphid was observed with a binocular microscope for 1200 s. The behavior of 15 individual females was recorded on stems from each plant type.

Population Growth Tests. Seed was germinated in moist vermiculite in 500-ml beakers in a Stults seed germinator (Stults Scientific Engineering Corporation, Springfield, Ill.) (20 ± 2°C; photoperiod of 14:10 [L:D]; ≈90% relative humidity). Small plants (6-21 d old) were transferred to potting soil in white plastic Supercells (Ray Leech Conetainers, Canby, Oreg.) (3.8 by 20.6 cm) placed in holding racks positioned over trays filled with water. For each test, 15 late-instar to adult apterous aphids were transferred with a camel's-hair brush to the base of each plant. Clear plastic tubes (3.6 by 30 cm), capped with nylon organdy screen and tightly inserted into the Supercells, confined the aphids.

In test 1, aphids were transferred to 16 plants (8-wk-old) of 'Repell', eight of which were endophyte-infected and eight endophyte-free. The occurrence of the ryegrass endophyte *Acremonium lolii* Latch, Christensen & Samuels was verified by microscopically examining leaf sheaths from plants before the test. In test 2, aphids were transferred to 10 plants (1-wk-old) of PI 462339 (endophyte-infected) and to 10 plants (1-wk-old) of PI 462340 (endophyte-free). Two weeks after test 2 was completed, microscopic examination of leaf sheath samples confirmed the presence of the ryegrass endophyte in aphid-free plants and absence of the endophyte in aphid-infested plants. Each test lasted 7 d during which the number of live aphids on each plant was recorded daily.

Field Studies. During the 3rd wk of April 1989, ll-wk-old greenhouse-grown plants of 'Repell' perennial ryegrass were transplanted into two plots (3 by 10 m) at the research station of the Western Regional Plant Introduction Station, Central Ferry, Wash. This area of southeastern Washington has experienced very high populations of *D. noxia* (Clement et al. 1990a, Pike et al. 1991). A completely randomized design with four replications of each treatment (row of endophyte-free or infected plants) was used in each plot. Each 2-m row contained eight plants. Interrow and interplant spacing were 1.5 and 0.45 m, respectively. Plots were 150 m apart.

One plot was maintained under dryland conditions and one was irrigated weekly with over-

Table 1. Distribution of *D. noxia* on sections of leaf sheaths (test 1) and vegetative stems (test 2) of endophyte-infected (+) and endophyte-free (-) plants of perennial ryegrass

Test 1						Test 2					
Replicate	Type of sample	Observed frequency	G	df	Significance"	Replicate	Type of sample	Observed frequency	G	df	Significance"
1	+	33	0.06	1	NS	1	+	23	16.36	1	***
	-	35					-	59			
2	+	14	0.29	1	NS	2	+	34	1.38	1	NS
	-	17					-	25			
3	+	18	0.03	1	NS	3	+	18	6.70	1	**
	-	19					-	37			
4	+	3	5.78	1	*	4	+	14	0.04	1	NS
	-	12					-	15			
5	+	21	0.02	1	NS	5	+	29	3.17	1	NS
	-	20					-	17			
6	+	2	1.33	1	NS	6	+	42	0.84	1	NS
	-	5					-	34			
Pooled			1.45	1	NS	Pooled			2.10	1	NS
Heterogeneity			6.06	5	NS	Heterogeneity			26.38	5	***
Total			7.51	6	NS	Total			28.48	6	***

"Expected distributions are based upon a 50:50 distribution of aphids on infected and endophyte-free plant samples. NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

head sprinklers delivering ~20 mm per application. The vegetation around the dryland plot was cultivated barley and a mixture of five species of perennial grasses in a larger field plot. The irrigated plot was next to an open field and a large nursery of grass germplasm. Natural colonization of the plots was the only source of aphids.

The first, third, and fifth plants within each row were collected during the 1st wk of August, for a total of 12 plants per treatment per plot. Microscopic examination of leaf sheaths from sampled plants confirmed the presence of viable endophytic fungi in the 12 plants removed from infected rows in the nonirrigated plot, whereas endophyte was detected in 13 plants from the irrigated plot, including one plant from an endophyte-free row. Each plant was placed in a labeled plastic bag (40 by 31 cm) as it was collected, and transported in a cooled ice chest to a laboratory, where it was immediately placed in a Berlese funnel for 72 h. Aphids that separated from the plant in the bag were removed with a camel's-hair brush and preserved in a vial filled with 70% ethanol, along with the aphids extracted within the Berlese funnel. All immature and adult *D. noxia* were counted.

Analyses. The observed frequency of aphids on endophyte-free and infected plant material in preference tests was compared with the expected ratio (50:50) using a replicated G test (Sokal & Rohlf 1981). Time budget data (behavioral observations) and aphid count data from field tests were analyzed by analysis of variance (SAS Institute 1985). Results of population growth tests were analyzed with SAS-GLM repeated-measures analysis of variance (SAS Institute 1987, p. 602-606). All data were transformed by $\log_{10}$ or $\log_e (x + 1)$ if data included zeroes, before statistical analysis was done. Untransformed means are reported here.

Results

Results of preference tests showed that highly variable numbers of aphids settled on plant material after 18 h, generally much less than the 200 individuals initially released in each petri dish (Table 1). Most aphids were wandering around in petri dishes. In test 1, no significant differences were observed in the number of aphids on endophyte-free versus infected leaf sheaths in five of six replications. In test 2, aphid distributions were not significantly different between endophyte-free and infected vegetative stems in four of six replications. The heterogeneity G test was significant for test 2 because aphid distributions deviated significantly from expectation (50:50 ratio) in two replicates (Table 1). This large heterogeneity G indicates that departures from expectation were not in a uniform direction; that is, aphids did not consistently prefer one type of plant material over the other.

Observations of aphids did not demonstrate significant behavioral differences between alates on endophyte-free and endophyte-infected vegetative stems. For example, times to first test probes on endophyte-free and infected plant material averaged 170.2 ± 65.27 s ($\bar{x}$ ± SEM) and 134.8 ± 62.21 s, respectively. These mean values were not significantly different from one another ($F = 0.06$; df = 1, 28; $P = 0.81$). Likewise, the number of aphid probing bouts on endophyte-free (2.93 ± 1.21) and infected (4.4 ± 1.04) ryegrass ($F = 2.7$; df = 1, 28; $P = 0.11$) and average times spent probing on endophyte-free (105.4 ± 56.43 s) and infected (153.2 ± 62.1 s) plant material ($F = 2.84$; df = 1, 28; $P = 0.10$) were not statistically significant. Aphids spent most of their time in nonprobing activity; mean times spent resting or walking around on plant surfaces and in petri dishes ranged from 1,046.8 ± 62.1 s

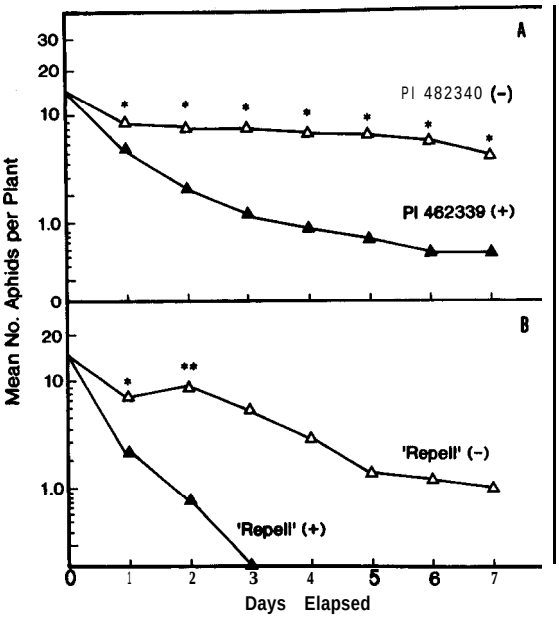

Fig. 1. Population trends of *D. noxia* on perennial ryegrass plants with (+) and without (-) fungal endophyte. (A) PI accessions. (B) 'Repell'. Comparisons within each time period significant at $P = 0.05$ (*) or $P = 0.01$ (**) (ANOVA [SAS Institute 19851).

for aphids exposed to infected stems to 1,094.6 ± 56.43 s for aphids exposed to endophyte-free stems ($F = 0.25$; df = 1, 28; ***P = 0.62).***

In both population growth tests, aphid numbers declined on endophyte-infected and endophyte-free plants, but the rate of population decline was more pronounced on infected plants after 7 d. No live aphids were found on infected 'Repell' ryegrass after 3 d and counts averaged < 1 aphid on infected PI 462339 ryegrass after 4 d (Fig. 1). The repeated-measures analysis revealed significant differences in aphid mortality among endophyte-infected and uninfected plants and over time on these two plant types (Table 2). Also, there was a significant time × treatment interaction in both tests (Table 2), indicating that the effect of time on aphid mortality was different for the two treatments.

D. noxia averaged 0.23 aphids (n = 3 aphids) on 13 endophyte-infected plants and 4.82 (n = 53 aphids) on 11 endophyte-free plants in the nonirrigated plot. These mean values were significantly different from one another ($F = 18.13$; df = 1, 22; $P = 0.0003$). Low populations of ***D. noxia*** in the irrigated plot did not permit a statistical comparison of aphid counts between the two treatments, but more aphids were found on endophyte-free plants ***(n*** = 19 aphids) than on infected plants ***(n*** = 1 aphid). Three of the four aphids found on infected plants in both plots were alates, indicating recent colonization rather than aphid reproduction on these plants. To the best of our knowledge, this is the first report that compares aphid population levels on endophyte-free and infected graminaceous plants in replicated field plots. ***D. noxia*** accounted for <1% of the total number of aphids recovered from each plot.

Discussion

Although our results indicate that perennial ryegrass is not a good host for the Russian wheat aphid, they also show that endophyte-free ryegrass is far superior to endophyte-infected ryegrass as a host plant. Although the mechanism of resistance in endophyte-infected ryegrass is not apparent, one interpretation of the results of our population growth and field tests is that low aphid performance and abundance on infected ryegrass was the result of antibiosis effects or starvation. Expression of ***D. noxia*** resistance in endophyte-infected plants apparently required prolonged exposure to the plants. There was no evidence of antixenosis resistance in endophyte-

Table 2. Results of a repeated-measures analysis of variance with mean number of Russian wheat aphids per endopbyte-infected and uninfected ryegrass plant

Source	df	sum of squares	Mean square	*F*	*P*
Test 1: 'Repell' with vs. 'Repel1 without endophyte					
Effect of treatment	1				
Treatment	14	5.56	5.56	17.83	<0.001
Error		4.37	0.31	—	—
Effect of time					
Time	6	4.90	0.82	52.36	<0.0001
Time × treatment	6	1.27	0.21	13.62	<0.0001
Error	84	1.31	0.02	—	—
Test 2: PI 462339 with vs. PI 462340 without endophyte					
Effect of treatment					
Treatment	1	12.08	12.08	45.02	<0.0001
Error	18	4.83	0.27	—	—
Effect of time					
Time	6	3.60	.60	26.13	<0.0001
Time × treatment	6	1.03	.17	7.51	<0.001
Error	108	2.48	0.02	—	—

infected plants because the probing behavior of *D.* noxia was similar on plant material from endophyte-free and infected plants, and the aphid was not deterred by infected plant material in other laboratory tests. However, antixenosis might be responsible for the inability of perennial ryegrass (with or without endophyte) to serve as a good host of

All cereal aphid species are not adversely affected by endophyte-infected grasses. Furthermore, each aphid species is not affected the same way by a particular endophyte-infected host or by the presence of endophytes in different host species. For example, the behavior of ***Metopolophium dirhodum*** (Walker), ***Sitobion fragariae*** (Walker), and ***Macrosiphum (Sitobion) avenae*** (F.) was not influenced by the presence of endophyte in tall fescue or perennial ryegrass (Latch et al. 1985, Johnson et al. 1985). ***R. padi*** and ***S. graminum*** showed no preference for endophyte-free perennial ryegrass over endophyte-infected plant material, but both species showed strong preferences for endophyte-free tall fescue over infected plant material (Latch et al. 1985, Johnson et al. 1985, Siegel et al. 1985). By contrast, the feeding behavior of ***R. maidis*** was not influenced by the endophyte in tall fescue, but it preferred feeding on endophyte-free ryegrass over infected plants (Buckley et al. 1991). However, ***D. noxia*** is adversely affected by the presence of endophyte in both tall fescue and perennial ryegrass (Clement et al. 1990b). In addition to nonpreference as a mechanism of resistance in endophyte-infected tall fescue to ***R. padi***, a toxicant may also play a role in protecting infected tall fescue from this aphid (Johnson et al. 1985). Similarly, antibiosis may be responsible for the poor performance of ***D. noxia*** on endophyte-infected ryegrass in this study, but this hypothesis warrants further study.

Six species of aphids are responsible for most aphid-caused injury to small grains worldwide (Pike et al. 1991), and four of these species—namely ***R. padi, R. maidis, S. graminum,*** and now ***D.*** noxia-have been shown to be adversely affected by endophyte infection in at least one graminoid. At the present time, it is difficult to imagine how this knowledge could be used to protect small grains from aphid attack, unless fungal endophytes were used as biocontrol agents through their introduction into uninfected cereals by artificial inoculation. Indeed, Clay (1990) recently proposed that endophytes could be used to "vaccinate" crop grasses against pests. However, because mammalian toxicities have been associated with the production of alkaloids in endophyte-infected grasses (Porter et al. 1981), research would have to demonstrate that endophytic strains toxic to insects but innocuous to mammals could be produced (Clay 1989). Moreover, toxic alkaloids could not accumulate in the seeds of innoculated plants (Clay 1990). Our results may have implications for the future use of fungal endophytes as aphid biocontrol agents, provided that the stated problems can be overcome.

Acknowledgment

We thank R. Welty for providing seed of 'Repell' ryegrass., K. Dorschner and D. Hostetter for reviewing the manuscript, R. Pollock for technical assistance, and J. Hendrix for word processing. Research supported in part by a USDA-CSRS Western Regional IPM grant to K. S. Pike.

References Cited

Bacon, C. W. & M. R. Siegel. 1988. Endophyte parasitism of tall fescue. J. Prod. Agric. 1: 45-55.

Buckley, R. J., P. M. Halisky & J. P. Breen. 1991. Variation in feeding deterrence of the corn leaf aphid related to Acremonium endophytes in grasses. Phytopathology 81: 120.

Clay, K. 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69: 10-16.

1989. Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. Mycol. Res. 92: 1-12.

1990. Fungal endophytes of grasses. Annu. Rev. Ecol. Syst. 21: 275-297.

Clement, S. L., R. C. Johnson & K. S. Pike. 1990a. Field populations of the Russian wheat aphid (Homoptera: Aphididae) and other cereal aphids on cool-season perennial grass accessions. J. Econ. Entomol. 83: 846-849.

Clement, S. L., K. S. Pike, W. J. Kaiser & A. D. Wilson. 1990b. Resistance of endophyte-infected plants of tall fescue and perennial ryegrass to the Russian wheat aphid (Homoptera: Aphididae). J. Kans. Entomol. Soc. 63: 646–648.

De Battista, J. P., J. H. Bouton, C. W. Bacon & M. R. Siegel. 1990. Rhizome and herbage production of endophyte-removed tall fescue clones and populations. Agron. J. 82: 651-654.

Givovich, A., J. Weibull & J. Pettersson. 1988. Cowpea aphid performance and behavior on two resistant cowpea lines. Entomol. Exp. Appl. 49: 259-264.

Johnson, M. C., D. L. Dahlman, M. R. Siegel, L. P. Bush, G.C.M. Latch, D. A. Potter & D. R. Varney. 1985. Insect feeding deterrents in endophyte-infected tall fescue. Appl. Environ. Microbiol. 49: 568571.

Latch, G.C.M., M. J. Christensen & D. L. Gaynor. 1985. Aphid detection of endophyte infection in tall fescue. N.Z. J. Agric. Res. 28: 129–132.

Mathias, J. K., R. H. Ratcliffe & J. L. Hellman. 1990. Association of an endophytic fungus in perennial ryegrass and resistance to the hairy chinch bug (Hemiptera; Lygaeidae). J. Econ. Entomol. 83: 1640-1646.

Peairs, F. 1989. Economic impact of the Russian wheat aphid in the western United States; 1987–1988. Great Plains Agric. Council, Publ. no. 129.

Pike, K. S., D. Allison, L. K. Tanigoshi, R. F. Harwood, S. L. Clement, S. E. Halbert, C. M. Smith, J. B. Johnson, G. L. Reed & P. K. Zwer. 1991. Rus-

sian wheat aphid: biology, damage, and management. Pacific Northwest Ext. Publ. 371.

Porter, J. K., C. W. Bacon, H. G. Cutler, R. F. Arrendale & J. D. Robbins. 1981. Ergot alkaloid identification in Clavicipitaceae systemic fungi of pasture grasses. J. Agric. Food Chem. 29: 653-657.

SAS Institute. 1985. SAS user's guide: statistics. SAS Institute, Cary, N.C.

198'7. SAS user's guide: statistics. SAS Institute, Cary, N.C.

Siegel, M. R., G.C.M. Latch & M. C. Johnson. 1985. Acremonium fungal endophytes of tall fescue: significance and control. Plant Dis. 69: 179-183.

1987. Fungal endophytes of grasses. Annu. Rev. Phytopath. 25: 293-315.

Siegel, M. R., G.C.M. Latch, L. P. Bush, F. F. Fannin, D. D. Rowan, B. A. Tapper, C. W. Bacon & M. C. Johnson. 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response: J. Chem. Ecol. 16: 3301-3315.

Sokol, R. R. & F. J. Rohlf. 1981. Biometrv. Freeman, New York.

Springer, T. L. & S. D. Kindler. 1990. Endophyte-enhanced resistance to the Russian wheat aphid and the incidence of endophytes in fescue species, pp. 194-195. *In* S. S. Quisenberry & R. E. Joost [eds.], Proceedings of an international symposium, *Acremonium*/grass interactions, November 3, 1990, New Orleans, La. Louisiana Agricultural Experiment Station, Baton Rouge, La.

Wilson, A. D., S. L. Clement & W. J. Kaiser. 1991. Survey and detection of endophytic fungi in Lolium germplasm by direct staining and aphid assays. Plant Dis. 75: 169-173.

Received for publication 22 January 1991; accepted 28 October 1991.