INSECTICIDAL ACTIVITY OF N-FORMYLLOLINE

D.L. Dahlman[1], M.R. Siegel[2] and L.P. Bush[3]

[1]Dept. Entomology, [2]Dept. Plant Pathology, [3]Dept. Agronomy
University of Kentucky, Lexington, KY 40546-0091 USA

ABSTRACT

Several insect species were sensitive to N-formylloline, a pyrrolizidine alkaloid found in significant concentrations in tall fescue (*Festuca arundinacea* L.) infected with *Neotyphodium coenophialum* (= *Acremonium coenophialum*) Glenn, Bacon *et* Hanlin. Topical or injected doses were toxic in a dose dependent manner. Acute doses resulted in quick incapacitation; lower doses caused behavioral changes, uncoordinated movements, tremors, etc. Insects tested included green bug, bird cherry-oat aphid, large milkweed bug, spined soldier bug, American cockroach, house fly, face fly, cat flea and Japanese beetle.

KEYWORDS

Tall fescue, N-formyl loline, *Acremonium coenophialum*, insects
Acronyms: EI = endophyte-infected

INTRODUCTION

It is widely accepted that endophytes are common inhabitants of grasses. Forage and turf varieties of endophyte-infected (EI) perennial ryegrass and EI tall fescue are resistant to many insects naturally exposed to EI plant material (Dahlman *et al*., 1991; Breen, 1994). Here we report on the insecticidal activity of N-formylloline, a pyrrolizidine alkaloid found in significant concentrations in EI tall fescue, but not found in endophyte-free plants.

MATERIALS AND METHODS

Tests were conducted with bird cherry-oat aphid, *Rophalosiphum padi* (L.); greenbug, *Schizaphis graminum* (Rondani); large milkweed bug, *Oncopeltus fasciatus* (Dallas); spined soldier bug, *Podisus maculiventris* (Say); American cockroach, *Periplaneta americana* (L.); insecticide susceptible strains of house fly, *Musca domestica* L. and face fly, *Musca autumnalis* De Geer; cat flea, *Ctenocephalides felis* (Bouché) and Japanese beetle, *Popillia japonica* Newman.

For the test of sprayed plants, 2-3 individual plants of tall fescue (endophyte-free, Mustang variety) were carefully separated into ramets and placed in assay cages described by Siegel *et al*. (1990). Ramets were sprayed to run off with 1.2 ml and allowed to dry before placement of cages and 50 alate aphids. Trypan blue (0.1%) was sprayed on test ramets using identical conditions. After drying, the dye was washed from the ramet and the concentration estimated by its absorption at 590 nm. We estimate that 2-4% of the total material was held on the ramet.

The loline preparation contained 180 mg of N-formylloline/ml in 3.67 M formic acid. All controls contained the same amounts of formic acid even though dilutions were so large that effects of formic acid were negligible. Loline was provided to milkweed bug nymphs via cotton pad containing water (Johnson *et al*., 1985). A microapplicator was used for all topical applications and injections. Glass petri dishes (15 cm dia) were treated with acetone solutions of N-formyl loline prior to placement of individual adult cockroaches. Twenty-five cat flea eggs were placed on 5 cm dia filter paper discs which had been treated with the acetone solutions of test chemicals. The discs were held in 5 cm dia glass petri dishes at 80% relative humidity for 30 days, after which the number of adult fleas was determined.

RESULTS AND DISCUSSION

A marked increase in mortality of newly hatched milkweed bugs was observed between N-formylloline concentrations of 7.5 and 30 µg/ml. The insecticide, methomyl, was 10-fold more toxic than either acephate or carbaryl when evaluated using the same experimental conditions. We estimate that N-formylloline was 30-50 times less toxic than methomyl.

Topical and injected applications of N-formylloline to adult milkweed bugs produced dose-dependent responses ranging from almost immediate paralysis to no effect. Intermediate responses were typified by spasmodic and uncoordinated movements. Greater than 90% were killed or incapacitated with topical doses ž2.5 µg within the first 24 h (Table 1). Response to injected N-formylloline was less acute even though the bugs were rapidly knocked down (Table 1). Perhaps the N-formylloline is either rapidly metabolized and/or sequestered, thus reducing the absolute amount of available toxicant.

Schizaphis graminum was more sensitive to N-formylloline than *R. padi*. The 3 day LC_{50} was 9.3 µg /ml for *S. graminum* while the LC_{50} for *R. padi* was >37.5 µg/ml (Table 2). Based on our dye solution procedure, we estimate the approximate LC_{50} of N-formylloline for *S. graminum* and *R. padi* to be 0.37 and 1.5 µg/ml, respectively.

An injection of as little as 0.78 µg N-formylloline/adult (ca. 16 ppm) produced an immediate and predictable response in the spined soldier bug, a predacious homopteran. The insect extended its proboscis, then its wings, tilted from side to side for a few seconds, then fell over. Most were immobile within a few minutes and >90% were dead within 24 h.

Adult American cockroaches placed in glass petri dishes coated with N-formylloline responded within 4-6 h with uncoordinated and jerky movements, ultimately lying on their backs and twitching. Affected insects did not recover. Ten µg/cm^2 caused 73% mortality at 24 h while 5 µg/cm^2 gave 27% mortality. No mortality was observed with acetone alone or with 8.88 µg/cm^2 formic acid, the amount present in the highest concentration of N-formylloline used in the test.

A dose of 50 µg/cm^2 caused 95% mortality with the cat flea but 5 µg/cm^2 only caused 15% mortality. When filters originally treated at the higher dose were re-evaluated for residual effects 48 days later, only 15% corrected mortality was obtained. Thus there was no significant residual effect.

Topical application of 10 and 20 µg N-formylloline/Japanese beetle larvae (165 and 330 ppm, respectively) caused 60-70% mortality but a dose of 5 µg had no effect.

Topical applications of 10 µg N-formylloline/house fly (ca. 645 ppm) caused less than 20% mortality after 24 h. A topical dose of 8 µg/face fly caused 56% corrected mortality after 24 h.

Several species of insects from different orders were sensitive on N-formylloline and similar symptoms of toxicity were observed. Insects were quickly incapacitated, although not immediately killed by acute doses. At lower doses the insects demonstrated various behavioral changes, uncoordinated movements, tremors, etc.

A substantial amount of additional work will be required before the mode of action and practical use of N-formylloline will be elucidated. Meanwhile, it should be emphasized that N-formylloline, as well as other toxic alkaloids produced either by the endophyte or the plant in response to endophyte infection, makes a significant contribution to the success of the plant in resisting insect attack. Therefore, elimination of endophyte from potential forage crops to render them more favorable for large animal consumption may also remove sources of insect resistance.

REFERENCES

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. **18:** 265-267.

Breen, J.P. 1994. *Acremonium* endophyte interactions with enhanced plant resistance to insects. Ann. Rev. Entomol. **39:**401-423.

Dahlman, D.L., H. Eichenseer and M.R. Siegel. 1991. Chemical perspectives on endophyte-grass interactions and their implications to insect herbivory. Pages 227-252 *in* P. Barbosa, V.A. Krischik and C.G. Jones, eds. Microbial mediation of plant-herbivore interactions. Wiley, N.Y.

Johnson, M.D., D.L. Dahlman, M.R. Siegel, L.P. Bush, G.C.M. Latch, D.A. Potter and D.R. Varney. 1985. Insect feeding deterrents in endophyte-infected tall fescue. Appl. & Envir. Microbiol. **49:** 568-571.

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

Paper 96-08-115 of the Kentucky Agricultural Experiment Station, Lexington, KY.

Table 1
One day *Oncopeltis fasciatus* adult response to topical and injected applications of N-formylloline.[a]

Topical Conc. (µg/bug)	% Mortality[b]	Injected Conc. (µg/bug)	% Mortality[c]
10.00	97.5	25.00	95
7.50	97.5	12.5	38
5.00	90.0	6.25	28
2.50	92.5	3.12	33
1.00	57.5	1.56	15
0.50	40.0	1.04	8
0.25	0	0.78	3

[a] 40 adult insects/treatment.
[b] Values adjusted for morbidity and mortality in water control (Abbott,1925). No mortality in control during test.
[c] Values adjusted for mortality in water control (Abbott, 1925).

Mortality in water injected controls after 1 day = 2.5%.

Table 2
Three day mortality of *Schizaphis graminum* and *Rophalosiphum padi* on endophyte-free tall fescue ramets sprayed with N-formylloline.[a]

	Corrected Percent Mortality[c]	
Loline Conc. (µg/ml)[b]	*R. padi*	*S. graminum*
150.0	87.5	no data
75.0	72.5	89.2
37.5	32.6	71.3
18.7	13.2	57.4
9.3	2.5	52.3
4.7	no data	21.6

[a] Six replications/treatment, approximately 50 aphids/replication
[b] Each plant received 1.2 ml of solution, sprayed to run off, 2-4% of which was estimated to remained on the plant.
[c] Values adjusted for mortality in control (Abbott, 1925). Mortality in control for *S. graminum* = 9.9%, for *R. padi* = 3.5%.