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Alkaloid Toxins in Endophyte-Infected Grasses

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ABSTRACT Grasses infected with clavicipitaceous fungi have been associated with a variety of diseases including classical ergotism in humans and animals, fescue foot and summer syndrome in cattle, and ryegrass staggers in sheep. During the last decade it has been recognized that many of these fungal infections are endophytic; a fungal endophyte is a fungus that grows entirely within the host plant. Inspection of field collections and herbarium specimens has revealed that such infections are widespread in grasses. The chemistry associated with these grass-fungal interactions has proved to be interesting and complex, as each grass-fungal pair results in a unique "fingerprint" of various alkaloids, of which some are highly toxic to herbivores. In many cases the presence of an endophyte appears to benefit the plant by increasing drought resistance, or by increasing resistance to attack by insects, thus improving the overall survivability of the grass. This review will focus on alkaloids that have been reported in endophyte-infected grasses.

Key Words: Fungal endophytes, Symbiosis, Ergot alkaloids, *Claviceps, Acremonium, Balansia,* Loline alkaloids, Fescue, Ryegrass, *Lolium, Festuca, Hordeum*

INTRODUCTION

Fungal parasites of grasses and cereals have been related to human disease since early in recorded history, and several accounts of such events appear in the Old Testament [Aaronson, 1989]. The most commonly recognized problem has been ergot [particularly *Claviceps purpurea* (Fries) Tulasne or *C. paspali* Stevens and Hall], which infects at least 35 genera of grasses including many used as human food, such as wheat (*Triticum*), rye (*Secale*), barley (*Hordeum*), and oats (*Avena*). Low levels of ergot alkaloids have been detected in commercial flour [Scott and Lawrence, 1980; Porter et al., 1987] and in bread and other baked goods [Scott and Lawrence, 1982]. Ergot alkaloids from either infected grain or fungal cultures produce a variety of mammalian pharmacological effects, i.e., increased or decreased blood pressure, vasoconstriction, uterotonic activity, action on the central nervous system such as hypothermia, and altered secretion of pituitary hormones. Ergot sclerotia have yielded drugs useful for treating headache, senile cerebral insufficiency, hypertension, infertility, and parkinsonism. Ergotism has been reviewed elsewhere [Berde and Schild, 1978; Burger, 1931; Ninomiya and Kiguchi, 1990].

Economic losses due to poisoning of livestock grazing on grasses infected with clavicipitaceous fungal endophytes, particularly those of the tribe Balansieae, have spurred research on the associated toxins in recent years. Research emphasis has been on tall fescue, *Festuca arundinaceae*, infected with *Acremonium coenophialum* in the United States and on perennial ryegrass, *Lolium perenne*, infected with *A. lolii* in New Zealand. However, many other endophyte-infected grasses are known to occur [Bacon et al., 1986; Latch et al., 1987; Saha et al., 1987; Clay, 1991; Siegel et al., 1987, 1990; Wilson et al., 1991, 1992; White, 1987].

ANIMAL TOXICITY

Alkaloid toxins found in endophyte-infected grasses are responsible for a variety of maladies of grazing animals including disorders such as "fescue foot" and "ryegrass staggers" [Siegel et al., 1990]. Cattle production losses attributed to endophyte-infected tall fescue have been related to ergot-type alkaloids (mainly ergovaline) and possibly to the loline group of alkaloids [Bush et al., 1992; Powell and Petroski, 1992]. Losses in sheep and cattle, from ingestion of endophyte-infected perennial ryegrass, are associated with tremorgenic alkaloids [Lauren and Gallagher, 1982; Steyn and Vleg-

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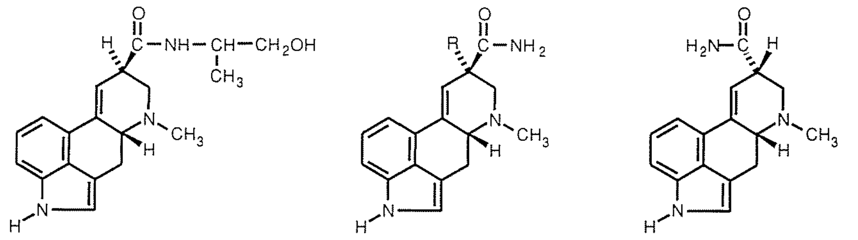
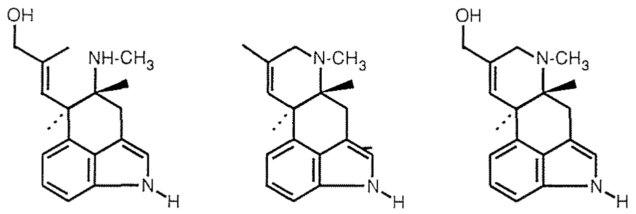


Ergonovine R=H, Lysergic acid amide Isolysergic acid amide
R=OH, 8-Hydroxylysergic Acid Amide



Chanoclavine-1 Agroclavine Elymoclavine

Fig. 1. Clavine alkaloids and simple lysergic acid derivatives.

gaar, 1985; Weedon and Mantle, 1987; Miles et al., 1992]. These grazing maladies have been estimated to cause annual economic losses to farmers amounting to several hundred million dollars. However, endophyte infection appears to benefit these grasses by conferring increased resistance to insect attack [Clay et al., 1985; Clay and Cheplick, 1989; Clement et al., 1992; Riedell et al., 1991; Rowan and Tapper, 1989; Eichenseer et al., 1991] and the insect resistance may well be due to compounds other than those primarily responsible for animal toxicity.

Note that forage grasses may be additionally contaminated with sclerotia of *Claviceps* species and that such contamination may be a significant factor in animal toxicity studies conducted under field conditions. The endophytes considered in this review produce some of the same compounds found in ergot sclerotia and include the clavine alkaloids, lysergic acid derivatives, and ergopeptine alkaloids (Figs. 1, 2). Other types of alkaloids are discussed including some that are produced solely by the fungi (lolitrem B, Fig. 3) or solely by the grass (such as perloline, Fig. 4 [Yates, 1983]), and others that appear only in infected plants but that have not been identified in fungal cultures, such as the lolines (Fig. 5). Additional alkaloids are noted in Figures 5, 6, and 7. The toxic nature and contribution of each alkaloid must be fully understood before solutions to the animal health problems or before their full potential as insect protectants can be realized.

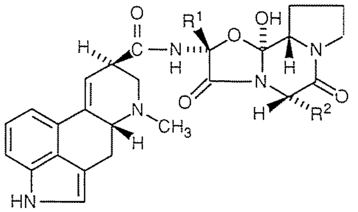
Some of the important grasses and their respective endophytes are listed in Table I. Table II lists many of the commonly reported alkaloids and grasses from which they have been isolated. Analytical methods commonly utilized to detect and quantitate the alkaloidal toxins are summarized in Table III.

The overall strategy for solving problems associated with alkaloid toxins has been to isolate suspected toxins using a variety of chromatographic techniques, elucidate structures using modern instrumental techniques such as NMR and X-ray crystallography, develop methods for the quantitative analysis of individual alkaloids present in grass samples, and determine if the isolated alkaloids are, in fact, actually the causative agents for the observed animal toxicity by feeding pure compounds to animals. It is anticipated that biotechnological techniques to alter the grass, the endophyte, or the animal via the rumen microbial digestive flora may ultimately be used to solve toxicity problems once the chemistry and toxicology of the toxins are fully understood.

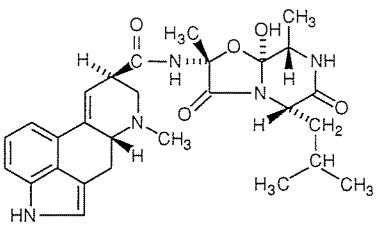
Endophyte-infected grasses survive better than endophyte-free grasses [Siegel et al., 1987] because of increased insect pest or herbivore resistance and drought tolerance. Alkaloid toxins isolated from grasses are being considered as potential sources of new insect control agents [Riedell et al., 1991], and as precursors for new pharmaceuticals.

TALL FESCUE

Tall fescue, *Festuca arundinaceae* Schreb., is the only perennial cool-season grass that is long lived and high yielding and can be grown in much of the transition zone between cool and warm humid regions [Sanchez, 1987].



	R^1	R^2
Ergotamine	Me	$PhCH_2$
Ergosine	Me	i-Bu
β-Ergosine	Me	sec-Bu
Ergovaline	Me	i-Pr
Ergostine	Et	$PhCH_2$
Ergoptine	Et	i-Bu
β-Ergoptine	Et	sec-Bu
Ergonine	Et	i-Pr
Ergocrystine	i-Pr	$PhCH_2$
α-Ergocryptine	i-Pr	i-Bu
β-Ergocryptine	i-Pr	sec-Bu
Ergocornine	i-Pr	i-Pr



Ergobalansine

Fig. 2. Ergopeptine alkaloids.

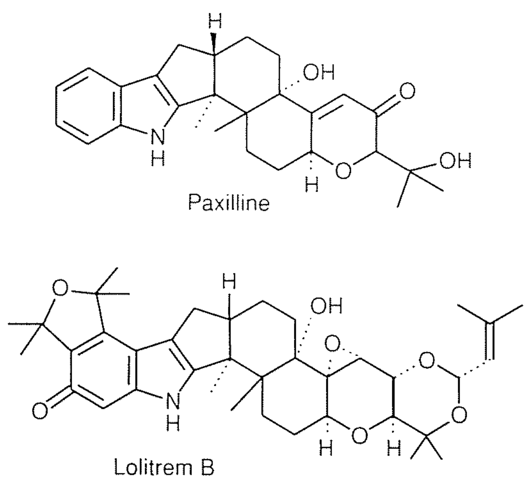


Fig. 3. Paxilline and lolitrem B (tremorgenic alkaloids).

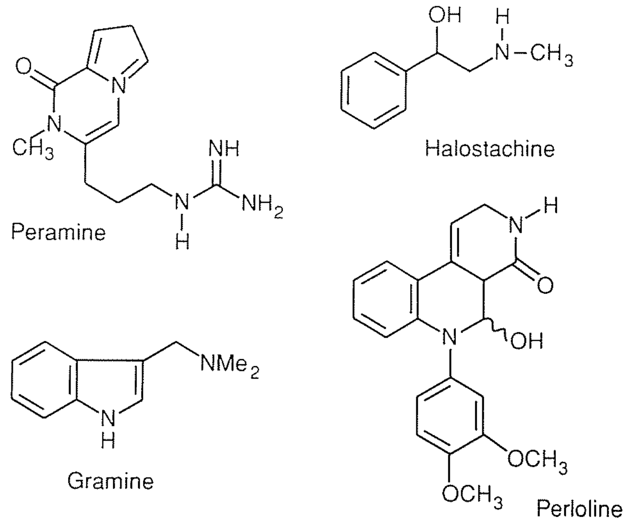
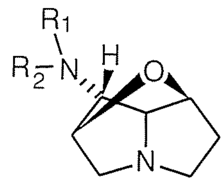


Fig. 4. Peramine, halostachine, gramine, and perloline.



	R_1	R_2
Norloline	H	H
Loline	H	CH_3
N-Methyl Loline	CH_3	CH_3
N-Formyl Norloline	H	HCO
N-Acetyl Norloline	H	CH_3CO
N-Formyl Loline	CH_3	HCO
N-Acetyl Loline	CH_3	CH_3CO

Fig. 5. Loline (saturated pyrrolizidine) alkaloids.

An estimated 35 million acres are planted in tall fescue in the United States as forage for cattle and sheep. The cultivar, Kentucky-31 tall fescue, germinates easily, adapts to a wide range of soils, tolerates heat, drought, and resists insects. Unfortunately, cattle grazing on tall fescue can be affected by a variety of disorders such as gangrene of the extremities (fescue foot), hard masses of fat deposits that cause digestive and calving problems (fat necrosis), and a summer condition of heat intolerance, rough coat, poor weight gain, excessive salivation, lower milk production, and reduced pregnancy rate (summer slump syndrome).

The observations that fescue toxicosis was linked to the presence of an endophyte [Bacon et al., 1977] and that alkaloid toxins were produced by the association of the endophyte and grass, and the detection of ergopeptine alkaloids by tandem mass spectrometry [Plattner et al., 1983; Yates et al., 1985] are milestones along the way of resolving the problems associated with grazing endophyte-infected tall fescue (EITF).

Suspected alkaloid toxins in EITF were isolated over the course of many years [Tookey and Yates, 1972; Yates et al., 1975; Davis et al., 1983; Petroski et al., 1989; Petroski and Powell, 1991]. Methods of analysis were developed for ergot alkaloids and ergopeptines [Yates and Powell, 1988], ergovaline [Rottinghaus et al., 1991], perloline [Lepom and Robowsky, 1985], and lolines

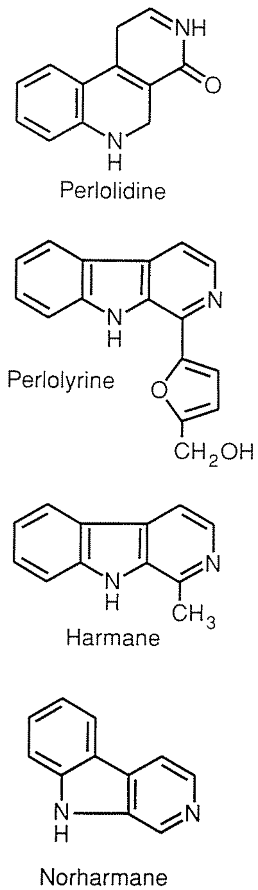


Fig. 6. Perlolidine, perlolyrine, harmane, and norharmane.

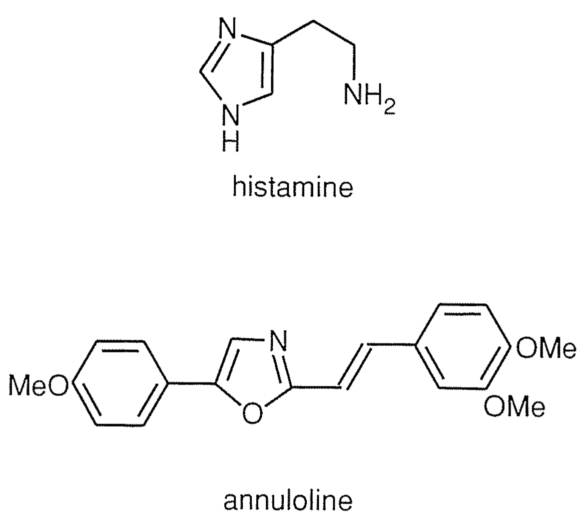


Fig. 7. Histamine and annuloline.

[Yates et al., 1990]. The loline group of pyrrolizidine alkaloids has recently been reviewed [Powell and Petroski, 1992].

Feeding pure compounds to animals, to accurately determine their toxicity, is a high-priority endeavor but is still difficult because the isolation of sufficient quantities of alkaloids for animal testing (ideally cattle or sheep) currently requires major resources [Petroski et al., 1989]. The isolation of minor ergot alkaloids from fescue is difficult [Petroski and Powell, 1990], particularly if quantities are required. Attempts are in progress to find larger-scale loline isolation protocols. Research is also being conducted to produce ergovaline in quantity by submerged fermentation.

Because of the reported insect pest resistance of EITF [Johnson et al., 1985], loline-type alkaloids were studied as potential agents for insect control [Riedell et al., 1991]. Several synthetic loline derivatives were as effective against greenbugs as nicotine sulfate, a commonly used insecticide. Ideally, a multicomponent, naturally derived insect pest control product could be developed for home use. Other uses for the alkaloids of EITF are also being explored.

PERENNIAL RYEGRASS

Perennial ryegrass staggers is a neurological disorder of grazing animals characterized by severe muscular spasms resulting in falls or drownings [Siegel et al., 1987]. A variety of alkaloids have been isolated from endophyte-infected perennial ryegrass, *Lolium perenne* L., as shown in Table II. Lolitrem B has been isolated from perennial ryegrass and is a tremorgenic compound which may play a role in ryegrass staggers [Gallagher et al., 1984]. The structure of lolitrem B is related to other known tremorgenic mycotoxins such as penitrems or janthitrems.

The alkaloid peramine has been associated with resistance of perennial ryegrass infected with *Acremonium lolii* to the Argentine stem weevil (*Listronotus bonariensis* Kuschel) [Rowan et al., 1986]. Methods for the detection and measurement of peramine in endophyte-infected grasses were developed [Tapper et al., 1989; Fannin et al., 1990], followed by refined isolation techniques [Rowan and Tapper, 1989], and ultimately a chemical synthesis of peramine was reported [Brimble and Rowan, 1990]. Chemical synthesis will enable peramine to be selectively labeled with stable or radioisotopes which will permit peramine metabolic fate studies and animal toxicity studies.

OTHER COMMON GRASSES

Stipa robusta (= *Stipa vaseyi*) is a perennial grass found in certain areas of the southwestern United States. It is commonly known as sleepygrass, as horses

TABLE I. Endophyte-Infected Grasses

Plant code[a]	Grass	Endophyte	Reference
1.	*Andropogon virginicus*	*Balansia henningsiana*	Bacon et al., 1986
2.	*Calamagrostis epigeos*	Unknown	Kaczmarek et al., 1967
3.	*Cenchrus echinatus*	*Balansia obtecta*	Powell et al., 1990
4.	*Festuca arundinaceae*	*Acremonium coenophialum*	Bush et al., 1992
		A. lolii	
		A. starrii	
		Epichloë typhina	
		Phialophora sp.	
5.	*Festuca gigantea*	*Acremonium* sp.	Bush et al., 1992
6.	*Festuca pratensis*	*Acremonium uncinatum*	Bush et al., 1992
7.	*Festuca versuta*	Unknown	Yates et al., 1990
8.	*Hordeum* sp.	*Acremonium* sp.	Wilson et al., 1991
9.	*Lolium annuum*	Unknown	Karimoto et al., 1964
10.	*Lolium cuncatum*	Unknown	Yates and Tookey, 1965
11.	*Lolium perenne*	*Acremonium lolii*	Bush et al., 1992
		A. coenophialum	
		A. lolii	Rowan et al., 1986
		Epichloë typhina	Bush et al., 1992
12.	*Lolium temulentum*	*Acremonium* sp.	Culvenor, 1973
13.	*Poa autumnalis*	*Acremonium coenophialum*	Bush et al., 1992
14.	*Sporobolus poiretii*	*Balansia epichloë*	Bacon et al., 1986
15.	*Stipa robusta*	*Acremonium* sp.	Petroski et al., 1992

[a]Plant code numbers are correlated with the alkaloids reported in these species in Table II.

that ingest this grass may become profoundly somnolent or stuporous for periods of time lasting up to several days. Sleepygrass from the Sacramento and Sierra Blanca mountains of New Mexico is particularly noted for producing this effect in horses. Sleepygrass from this region is infected with an endophytic species of *Acremonium* closely related to the endophyte of tall fescue. In an attempt to determine its active principle(s), fractionation of a methanolic extract of endophyte-infected sleepygrass has yielded lysergic acid amide, isolysergic amide, 8-hydroxylysergic acid amide, ergonovine, chanoclavine-I, and N-formylloline [Petroski et al., 1992].

Sandbur grass, *Cenchrus echinatus L.,* is an annual species native to the tropics or subtropics. It is found in cultivated fields, pastures, abandoned fields, lawns, along roadsides, and along beaches. Animals avoid eating the seed heads because of burs. The plant is a serious weed pest because the burs contaminate hay and forages. An ethanol extract of sandbur grass infected with *Balansia obtecta,* a fungal endophyte of the Clavicipitaceae, yielded a novel peptide derivative of lysergic acid called ergobalansine [Powell et al., 1990]. Ergobalansine differs from other known ergopeptine alkaloids in that the characteristic proline residue has been replaced by an alanine residue. Nothing about the pharmacology of ergobalansine is known at this time. However, Clay et al. [1985] found that infected *Cenchrus* inhibited the development of fall armyworms.

Insect antifeedants and toxins from wild *Hordeum* species are being explored. Endophytic fungi have been found in *Hordeum* germplasm [Wilson, et al., 1992).

Quackgrass (*Agropyron repens* Rydb.) leaves behind a substance that kills alfalfa seedlings when the quackgrass weed itself is killed. The substance was identified as 5-hydroxyindoleacetic acid by Dr. Roger D. Hagin [Hays, 1992]. It is unknown if quackgrass is endophyte-infected. Clavine alkaloids [Bacon et al., 1986] have been found in *Balansia* infected grasses.

SUMMARY

Fungal endophytes are of widespread occurrence in grass species and fungal-infected grasses often are toxic to herbivores, particularly to mammals and insects. Toxicity to herbivores is attributed to the presence of alkaloids. The toxic alkaloids are either produced by the fungus or by the plant in response to fungal infection. Each grass-fungal pair appears to result in a unique set of secondary metabolites that increases the overall survivability of the grass and has various adverse affects on herbivores. Fungi of the genus *Claviceps* are the most common and widely studied; however, *Acremonium* species are of considerable economic importance in tall fescue and perennial ryegrass.

Recognition that *Acremonium* endophytes also occur in certain wild *Hordeum* species that are resistant to the Russian wheat aphid may signal the discovery of new biocontrol agents for use against this and other impor-

TABLE II. Alkaloids Associated With Endophyte-Infected Grasses

Alkaloid	Plant code[a]	Reference
Loline alkaloids		
General	4	Petroski et al., 1989 (references for other grasses below)
Loline	6,11	Bush et al., 1992
N-acetylloline	7	Yates et al., 1990
N-formylloline	5,6	Bush et al., 1992
	7	Yates et al., 1990
	11,13	Bush et al., 1992
N-methylloline	7	Yates et al., 1990
Norloline		
N-acetylnorloline	7	Yates et al., 1990
N-formylnorloline		
Ergot alkaloids		
Ergonovine	1,14	Bacon et al., 1986
	4	Yates and Powell, 1988
Lysergic acid amide	4	Petroski and Powell, 1991
	15	Petroski et al., 1992
Isolysergic acid amide	15	Petroski et al., 1992
8-hydroxylysergic acid amide		
Chanoclavine-I	1,14	Bacon et al., 1986
	15	Petroski et al., 1992
Agroclavine	14	Bacon et al., 1986
Elymoclavine	1,14	Bacon et al., 1986
Ergopeptine alkaloids		
General	4	Plattner et al., 1983
		Yates and Powell, 1988 (references for other grasses below)
Ergotamine		
Ergosine	2	Kaczmarek et al., 1967
β-ergosine		
Ergovaline		
Ergostine		
Ergoptine		
β-ergoptine		
Ergonine		
Ergocrystine		
α-ergokryptine	2	Kaczmarek et al., 1967
β-ergokryptine		
Ergocornine	2	Kaczmarek et al., 1967
Ergobalansine	3	Powell et al., 1990
Other alkaloids		
Annuloline	9	Karimoto et al., 1964
Gramine	8	Culvenor, 1973
Halostachine	4	Davis et al., 1983
Histamine	11	Jeffreys, 1964
Harmane	4	Yates, 1962
Norharmane	4	Yates, 1962
Lolitrem B	11	Gallagher et al., 1984
Paxilline	11	Weedon and Mantle, 1987
Peramine	11	Rowan et al., 1986
Perloline	4,11	Yates et al., 1975
	12	Dannhardt and Steindl, 1985
Perlolyrine	11	Jeffreys, 1970
Perlolidine	11	Jeffreys, 1964

[a]Plant code numbers correspond to grass and associated endophyte listed in Table I.

TABLE III. Analysis Methods for Specific Alkaloids

Alkaloid type	Method	Reference
Saturated pyrrolizidine (lolines)	GC	Yates et al., 1990
	GC/MS	Takeda et al., 1991
Clavine alkaloids	MS	Porter et al., 1981
Ergot alkaloids and ergopeptines	MS/MS	Plattner et al., 1983
	MS/MS	Yates et al., 1985
	HPLC	Scott and Lawrence, 1980
	HPLC	Yates and Powell, 1988
Ergovaline	HPLC	Rottinghaus et al., 1991
Lolitrem B	HPLC[a]	Lauren and Gallagher, 1982
	HPLC	Gallagher et al., 1985
Peramine	HPLC	Tapper et al., 1989
	TLC	Fannin et al., 1990
Perloline	HPLC	Lepom and Robowsky, 1985

[a]HPLC for similar compounds.

tant insect pests (Clement, unpublished data). Further research on the chemistry of these unique *Acremonium* and *Balansia* grass-fungal interactions is expected to lead to methods to predict and control animal toxicity, to suggest leads for environmentally safe insect-control agents, and to provide new compounds useful as pharmaceuticals.

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