STUDIES ON SELECTED ALKALOIDS IN THE FESCUES OF INTEREST IN THE PRODUCTION OF NEW CULTIVARS

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Charles Ellington Ainsworth Carrington

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ABSTRACT

Carrington, Charles Ellington Ainsworth, M.Sc., The University of Manitoba, May 1980. Studies on Selected Alkaloids in the Fescues of Interest in the Production of New Cultivars.

Major Professor: Kenneth W. Clark, Department of Plant Science

Quantitative and qualitative methods of fescue alkaloid analysis were established. Thirty-four meadow fescue cultivars and two *Festuca* x *Lolium* hybrids were established in a completely randomized design on a clay soil in Winnipeg, Manitoba in the summer of 1978, and qualitatively screened for alkaloid content the same year. A comparison of the loline content observed with that of two samples determined by an independent researcher to be "low" loline percent and "high" loline percent, showed an intermediate level with no apparent variation in all plants checked. Perloline concentration varied from "zero" to "very high". Perlolidine was not identified. The same plants were quantitatively screened for perloline content in the summer of 1979 on a regrowth basis. Perloline levels ranged from 0-6051.2 μg/gm dry matter. There were no significant variations in perloline content of plants within a cultivar; however, there was a significant variation in perloline concentration in plants between cultivars.

INTRODUCTION

Meadow fescue (Festuca elatior L.) and tall fescue (Festuca arundinacea Schreb.) are forage grasses with considerable potential for high productivity. Both grasses have similar morphological characteristics and were introduced into North America by the early European settlers. Both species have produced luxuriant fall pastures in those areas to which they are adapted. Wheeler (1950) suggests that the potential of meadow fescue for forage has not been fully appreciated, since it is capable of remaining green right up to the time that the ground freezes during winter. Bush and Buckner (1973) suggest that the chemical composition of tall fescue is equal to that of other forage grasses in areas where it is adapted.

For soils in Manitoba with ample moisture and good drainage, meadow fescue is recommended for pasture and seed production. Moreover, meadow fescue was a component of the most recommended hay and pasture mixtures in Manitoba since it is productive with alfalfa after bromegrass has ceased growth. But, in the mid 1970's meadow fescue was deleted from the list of recommended mixtures in Manitoba. There are two possible reasons: a decline in the availability of seed and the unwillingness of many planters to accept the existing varieties. In Europe, the use of Canadian synthetic varieties of meadow fescue has also declined because of slower recovery and higher alkaloid levels relative to other established varieties.

Considerable work on fescue alkaloids has been done by researchers in the United States. This was initiated in 1944 as workers sought the cause of "fescue foot" in cattle grazing toxic tall fescue pastures. Though, probably not the cause of "fescue foot", these alkaloids have also been linked to some forms of fescue toxicity. Gentry (1969) found 11 alkaloids in fresh tall fescue grass; Yates (1963) found nine alkaloids in cured tall fescue hay. These findings have led to further research into factors affecting the concentration of alkaloid compounds, their inheritance and their relationship to the poor performance of animals grazing tall fescue pastures.

In fescues, perloline is thought to be the most important alkaloid. Others of physiological importance are loline and perlolidine. However, the concentration of the last two alkaloids necessary for any significant pharmacological activity is much greater than that for perloline. Bush et al (1970 and 1972) found that perloline had a marked inhibitory effect on in vitro digestion and they suggest that the primary site of action of perloline is on rumen bacteria. This, thus, resulted in a decrease in volatile fatty acid production.

This study at the University of Manitoba was intended to establish a quantitative method for the estimation of fescue alkaloids and to use this method to assess satisfactorily the potential of the fescues and fescue x ryegrass hybrids.

LITERATURE REVIEW

Description of Meadow Fescue and Related Species

The Fescues

Meadow fescue (*Festuca elatior* L.) and tall fescue (*Festuca arundinacea* Schreb.) were introduced into North America from Europe by the early settlers. Both grasses have similar morphological characteristics and as a result confusion existed as to the differences between them. Before their identification as separate species in 1950, tall fescue was usually regarded as a variety of meadow fescue and was called *Festuca elatior* var. *arundinacea*.

Meadow Fescue

Meadow fescue is a hardy short-lived grass attaining a height of 37.5 - 75 cm or even more on extremely fertile land. Although it prefers a heavy, moist soil, it performs well on light soils, or those with moderate water supply, if adequately fertilized and managed intensively. Wheeler (1950) suggests that for wet soils few grasses are better adapted. Meadow fescue does not propagate by root stocks; it does not produce a very heavy sod. Nevertheless, it has excellent seed producing characteristics.

In his research, Wheeler found that the species was first planted on a farm in Johnson County, Kansas. It has since been grown in areas of New England, North Atlantic and the Central States, and in the

Southern States on a smaller scale. Meadow fescue is of marked significance in Eastern Kansas, Nebraska, Missouri, Indiana and many agricultural regions of Western Canada; because of its ability to withstand more severe winter conditions, it is better suited than tall fescue.

Meadow fescue is also recognized in Western Europe as an excellent pasture grass. The chief factor which has so far limited its wide usage in Canada and the United States is its high susceptibility to leaf rust, *Puccinia* spp. In regions where it was established, its primary use was for seed production, which was sold in Europe.

Studies in the United States and Canada have shown that meadow fescue is a high yielding pasture species in those areas where it thrives best. Wheeler (1950) states that meadow fescue is especially suited for pasture because it is usually ready for grazing in early spring and continues growing late into the fall; however, it remains green until the ground is frozen. In Canada, meadow fescue is used primarily in combination with bromegrass and alfalfa. After bromegrass has produced a lush growth in spring and early summer, it rarely makes any significant fall growth. It is, therefore, during this period, with no competition and shading from bromegrass, that meadow fescue makes sufficient regrowth and provides the grass component in the mixture.

Tall Fescue

Tall fescue is a deep-rooted, long-lived perennial bunch grass with short underground stems. Its thick stands produce an even sod if kept mowed or grazed. It is well adapted to poor winter drainage; it has deep penetrating roots where soils are well drained. Cowan (1967) reported that in Klameth Falls, Oregon, a variety of tall fescue known as Alta has thrived on alkaline soil with pH of 9.5. Additional reports by other researchers also cite instances where tall fescue is known to thrive on soils with a pH of 4.7. This adaptability of tall fescue to acid, alkaline and even saline soils has made its use widespread throughout the United States and Canada. Buckner and Cowan (1975) have found, however, that although tall fescue is widely adapted, it grows best in the transition zone separating the Northern and Southern regions of the United States; an area where most cool and warm season grasses are not well adapted.

Bush et al (1973) points out that seed production of tall fescue in the United States increased from 10,900 Kg in 1940 to 31.3 million Kg in 1970; suggesting a greater demand for this crop in the United States. Research by Templeton and Taylor (1966) show that yield from well-fertilized pure stands and tall fescue legume mixtures are approximately 7-9 tonnes/ha in the United States. The increased use of tall fescue has been attributed not only to the seeming resistance of tall fescue to rust, but also to its wide adaptation to various climatic and soil conditions along with other valuable agronomic qualities.

Chemical Composition

Bush and Buckner (1973) state that a comparison of tall fescue with other grasses grown in the South Eastern United States indicates that the chemical composition of tall fescue is equal to that of other forages.

Further research has shown that it is possible to alter significantly the chemical composition of fescue by management practices. Hojjati et al (1977) cite references by Jones (1974) in which it is suggested that an increase in N fertilization usually causes an initial rise in protein levels, which decreases as the season advances. Fribourg et al (1978) found that N fertilizer not only increases N but also NO_3-N in plant tissue. Balasko (1977) suggests that reducing the amount of senesced tissue by increased N fertilization, will result in a reduction of leaching action of rain that would readily remove soluble sugars and fructosans from senesced forage, thereby retaining most of the soluble carbohydrate content.

Digestibility

Reports indicate that the digestibility of meadow and tall fescue is comparable to that of other forage grasses. Allinson (1971) has found that in controlled environmental chambers, long days and high temperatures, contributed to the lowering of in vitro cellulose digestibility of tall fescue plants harvested after 8 and 19 wks. Smith (1977) also cites literature which suggests that both long days and high temperatures significantly lowered the percent of in vitro dry matter digestibility (IVDMD). Balasko (1977) further suggests that by judicious use of fertilizer treatments and cutting management, quality tall fescue forage for winter grazing can be achieved.

The overall quality of forage has been found to be lower under conditions of excessive growth, whether this growth was a result of time of accumulation or of favourable environmental conditions. This has been attributed to a higher ratio of senesced to live tissues. Beaty et al (1978) found that green forage averaged 70.9% digestibility while that of dead forage averaged 42.4%, thus the amount of dead forage significantly altered feed quality. Watson et al (1978) state that the digestibility of tall fescue exhibits seasonal variations with the lowest levels resulting from conditions of high temperatures and long days.

Description of *Lolium* and *Lolium* x *Festuca* Hybrids

Lolium

The ryegrasses (*Lolium* spp.) are considered to be bunch grasses. There are two main *Lolium* species used for forage in North America. These are perennial ryegrass and Italian or annual ryegrass. Perennial ryegrass (*Lolium perenne* L.) originated in Southern Europe, North Africa and Southeast Asia. It is believed that it was first cultivated for forage in England about 1677. It grows to a height of approximately 90 cm. The origin of Italian ryegrass (*Lolium multiflorum* Lam.) is uncertain. However, it was grown in the meadows of northern Italy in the 13th century. It reaches a height of 130 cm.

These ryegrasses are commonly used for hay and pasture in Australia, New Zealand, the British Isles and the temperate regions of Western Europe and the United States. Since they are not very winter hardy, they grow best west of the Sierra Nevada, the Cascade Range and in the southern humid areas of the United States (Frakes, 1973), but their use also extend northward along the Atlantic Coast. He concludes that Italian is distinguishable from perennial ryegrass by having attached awns, the annual or biennial habit of growth, leaf blades rolled in young shoots, a wider leaf blade, more florets per spikelet and taller mature plant height. Frakes (1973) also suggests that the ryegrasses can be grown on a wide range of soil types. However, if extended low temperatures, drought and poor fertility are characteristic of the area to be seeded, ryegrasses may not be the most desirable species.

Lolium x *Festuca* Hybrids

Attempts are being made by researchers to incorporate genetic material from the highly nutritious *Lolium* spp. (ryegrass) to the hardy *Festuca* spp. (fescues). This is desirable since the fescues are capable of surviving in a wider range of temperatures than do the ryegrasses. Since the fescues make luxuriant fall pastures such an accomplishment will help to enhance their pasture potential.

To date, hybridization of *Lolium* x *Festuca* has been accomplished both in the United States and some areas in Europe. The hybrids tend to be infertile but Buckner *et al* (1961) found that in tall fescue x ryegrass hybridization, doubling the chromosome number with colchicine restores fertility. However, meiosis is not stable and chromosomes are eliminated during successive generations; as a result, progress in intergeneric hybridization by conventional grass breeding techniques is relatively slow. Hill and Carnahan (1962) found that *Lolium* x *Festuca* hybridization was generally more successful when *Lolium* was the maternal parent.

Buckner *et al* (1961) found that the F_1 *Lolium* x *Festuca* hybrids have foliage closely resembling that of the ryegrass parent. However, morphologically there was considerable variation among plants. They reported that F_1 hybrids of perennial ryegrass x tall fescue survived for 9 years and that of Italian ryegrass x tall fescue survived at least 6 years, thus indicating that the hardiness of the fescues are transmitted to the F_1 hybrids. A comparison of the palatability and vigor of the F_1 hybrids with that of Ky31 showed that 37 of the 101 hybrids were superior in palatability and 53 were more vigorous. They also found that all 11 F_1 plants of Italian ryegrass x tall fescue were more vigorous and palatable than Ky31.

Genetics of Meadow Fescue, Tall Fescue, Ryegrass and Ryegrass x Fescue Hybrids

Meadow Fescue

Poehlman (1959) described varieties of meadow fescue with the diploid chromosome number 2n = 14, tetraploid chromosome number 2n = 28, hexaploid chromosome number 2n = 42 and decaploid chromosome number 2n = 70. Malik and Thomas (1967) suggest that in the formation of *Festuca elatior* complex, three species were probably involved, of which *Festuca pratensis* was one.

Tall Fescue

By contrast, Webster and Buckner (1971) state that tall fescue is a bivalent-forming auto-allohexaploid. Malik and Thomas (1967) also reported that tall fescue species had tetraploid (2n = 28) chromosome levels and decaploids (2n = 70) chromosome levels. Malik and Thomas (1967) who were sampling a collection of fescues from the French Alps, found one plant which contained the triploid chromosome number (2n = 21). They postulated that this was possibly a hybrid between *Festuca pratensis* (2n = 14) and *Festuca arundinacea* var. Glaucescens (2n = 28), because it was morphologically similar to artificial triploids.

Ryegrass and Ryegrass x Fescue Hybrids

Perennial ryegrass and Italian ryegrass are believed to be selfincompatible but they can be crossed readily with each other. Both perennial and Italian ryegrasses have chromosome numbers of 2n = 14. Hybrids between diploid ryegrasses and auto-tetraploid ryegrasses with 2n = 28, also occur.

Chromosome numbers of ryegrass x fescue hybrids will vary depending on whether the parental material is meadow fescue x ryegrass or tall fescue by ryegrass.

Alkaloids of Fescue and Ryegrass

Definition of Alkaloids

Alkaloids, as defined by Pelletier (1970), are a group of compounds that have the following properties: 1) chemically basic; 2) nitrogen containing; 3) of plant origin; 4) significant pharmacological activity and 5) complex molecular structure. However, Robinson (1974) suggests that it is no longer justified to consider alkaloids as of plant origin only, since several animal products have all the necessary characteristics of alkaloids. Many authorities have reserved the name alkaloids for nitrogen compounds with complex molecular structure. Instead, simple amines are regarded as "protoalkaloids". Culvenor (1970) suggests that although alkaloids do not seem to play a role in plant nutrition or development, they may serve an ecological function.

Occurrence

The occurrence of "fescue foot" and poor animal performance of cattle grazing fescue pastures has motivated researchers to analyze the alkaloid content of this genus. Perloline was found to be the major alkaloid of fescue; two other alkaloids of lesser importance are loline and perlolidine. Perloline was first discovered by workers studying abnormal pigments present in the basal shoots of ryegrass (Lolium perenne L.), which they suspected of causing facial eczema in sheep (Grimmett and Melville, 1943). White and Reifer (1945) also found perloline in reasonable concentrations in Festuca.

The alkaloids identified in fescues represent two widely divergent structures - perloline and loline (Figures 1 and 2, respectively). Normally, the alkaloids of a plant are members of the same chemical group and probably share a common biogenetic pathway. Since this is not the case in fescue, it is difficult to postulate a common precursor for perloline and loline biogenesis.

Biosynthesis of Perloline, Perlolidine and Loline

Perloline and perlolidine are members of the quinoline group of alkaloids and are the only two known natural products with a diaza-phenanthrene ring structure. Culvenor (1973) cites references which suggest a possible biosynthesis pathway for perloline. He postulates that perloline originates from tryptamine (assuming loss of C_2), a C_3 unit and an aromatic ring. The tryptamine and dihydroxyphenylalanine units combine to form a 2-arylquinoline derivative in which the 2-aryl group is subsequently transferred to the nitrogen (Figure 3).

Since the chemical structure of perlolidine is as shown in Figure 4, then it is justifiable to speculate that perlolidine follows basically the same biosynthetic pathway as perloline. One can conceive that loss of the veratrole moiety will result in the formation of perlolidine (Figure 5).

FIGURE 1. Structure of perloline.

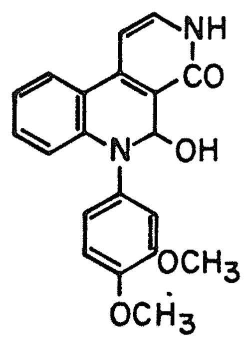

NH
CO
N
OH
OCH3
OCH3

FIGURE 2. Structure of loline.

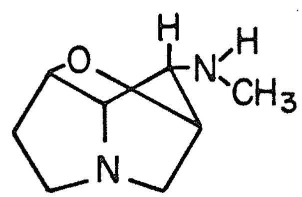

H
O
N
H
CH3
N

FIGURE 3. Suggested pathway for biosynthesis of perloline. Reproduced from Culvenor (1973).

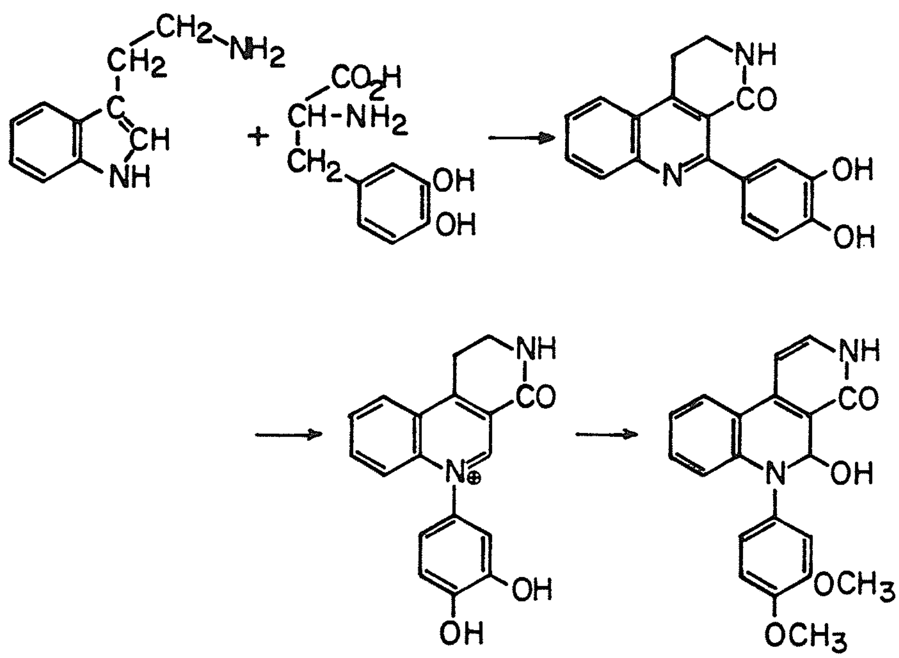

CH2
NH2
CH2
C
CH
NH
+
CO2H
CH-NH2
CH2
OH
OH
NH
CO
N
OH
OH
NH
CO
N⊕
OH
OH
NH
CO
N
OH
OCH3
OCH3

FIGURE 4. Structure of perlolidine.

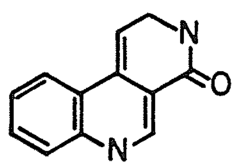
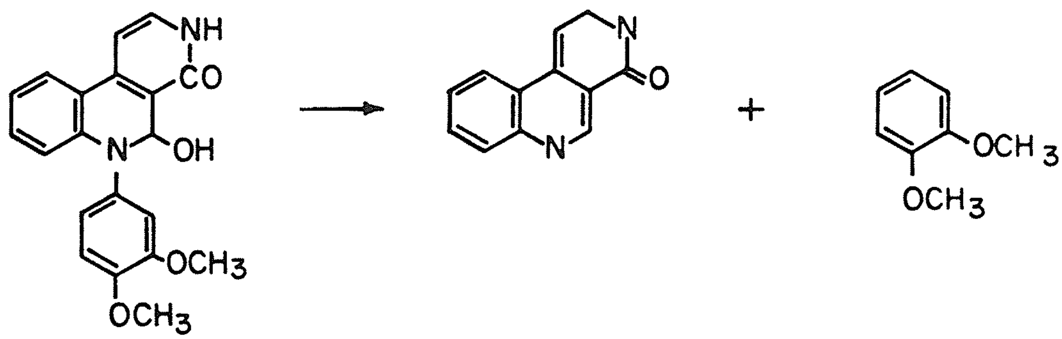


FIGURE 5. Possible formation of perlolidine.


NH
CO
N
OH
OCH3
OCH3
N
O
N
+
OCH 3
OCH3

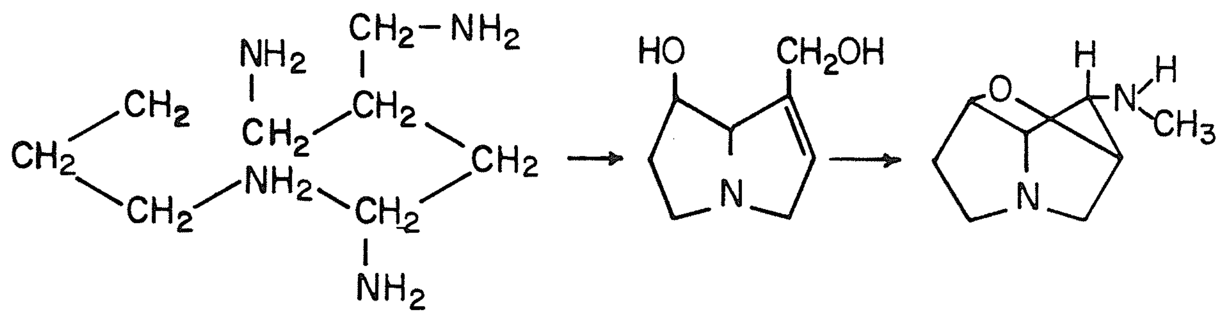
Biosynthesis of Loline

Loline is pyrrolizidine alkaloid which is composed of a pyrrolizidine ring. In addition it has a cyclic ether bridge and an attached secondary nitrogen (Figure 2). Bull et al (1968) suggest that contemporary studies indicate that the pyrrolizidine ring is built up from two 4-carbon units related to putrescine and ornithine as shown in Figure 6. Once the pyrrolizidine ring is formed, through further reactions the cyclic ether bridge is incorporated into the structure.

Distribution Within the Plant

Gentry et al (1969) reported the distribution of alkaloids in five strains of tall fescue, and the relationship of growth stage and growth conditions to alkaloid levels. They reported that the seeds of Ky31 and Kenwell varieties contain loline and two unknown alkaloids. Seeds of Alta, NK36 and Goar on the contrary possess no alkaloids. The alkaloid content of Ky31 and Alta seedlings increases over a period of 18 days from germination; most of the alkaloid is perloline. Perloline is the principal alkaloid in seedlings of all varieties. Ten-day-old seedlings of Ky31 and Kenwell contain six different alkaloids; while those of Alta, Goar and NK36 have only three. Total alkaloid levels of Ky31 are higher in the roots, shoots, stems and leaves than in the seeds and seedling heads. In all parts except seeds, perloline is the predominant alkaloid. The highest concentration is found in the roots of regrowth material and in the stems of plants at the dough stage. This presence of perloline in plant seedlings, but not in the seeds, indicates that the genetic template for perloline synthesis within the plant is present; but requires some form of catalyst either environmental or physiological,

FIGURE 6. Suggested biogenetic pathway of loline. Modified from Bull *et al*. (1968).


CH2 – NH2
NH2
CH2
CH2
CH2
CH2
CH2
CH2
NH2
CH2
NH2
HO
CH2OH
N
H
O
N
H
CH3
N

in order to stimulate perloline production.

Factors Affecting Alkaloid Levels in Plants

The alkaloid levels of plants, though genetically determined, fluctuate considerably due to environmental influence. This has been substantiated by researchers who found that some non-toxic plants grown successfully in certain parts of the world, are very toxic when grown in other regions (Waller and Nowacki, 1978). This toxicity has been attributed to an increase in alkaloid content; other factors have also been implicated in the cause of noted increases in alkaloid levels. The effect of these factors on alkaloid concentrations are not the same for all plants. It has been reported by Waller and Nowacki (1978) that one factor may alter the biosynthesis or degradation of alkaloids of various origins; this same factor will increase the alkaloid production in one species and decrease it in another species. Some factors which influence alkaloid content are: i) variety of grass; ii) season of the year; iii) rate and type of fertilizer treatment; iv) water and general climatic conditions and v) fungal infection.

Variety of Grass

It has been observed that certain varieties will differ in their levels of alkaloid content. This is quite conceivable since alkaloid content is genetically controlled. Cornelius *et al* (1974) have found that the progeny of high-perloline parents are significantly higher in perloline than was progeny of low perloline parents. Buckner *et al* (1973), working with *Lolium* x *Festuca* hybrids, have also found that by increasing the ploidy level, there was a subsequent increase in perloline content.