TRYPTAMINE: LEVELS IN PASTURAGE
IMPLICATED IN
BOVINE PULMONARY EMPHYSEMA

by

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ABSTRACT

Reed canary grass (Phalaris arundinacea L.) (R.C.G.) was found to be the only species, of seven common pasture species, in Interior B.C. wet meadows containing significant quantities of the tryptamine alkaloids. The distribution and concentration of tryptamines within the plant and throughout the season, on a number of R.C.G. meadows both in the Interior and at the Coast developed under very different soil, climate and water regimes, was then accorded attention. Tryptamines were confined to the uppermost leaf blades. Certain of the meadows studied had long been associated with the occurrence of bovine pulmonary emphysema (BPE); the occurrence, concentration and disappearance seems to be somewhat consistent with the occurrence, severity and disappearance of BPE. The effect of nitrogenous fertilizers on tryptamine levels, nitrogen uptake and dry matter yields of R.C.G. was intimately related to the kind of nitrogenous fertilizer and soil water regime. Yields of dry matter, total N levels and tryptamine concentration were differentially related to source and amount of fertilizer; ammonium sources, especially on glei soils, and at high rates, favoured tryptamine production in R.C.G.

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1. INTRODUCTION

Non-protein nitrogenous constituents in certain forages have often been implicated in digestive disturbances in domestic stock, (Sullivan and Garber, 1947). Among the anti-quality nitrogenous constituents implicated are the tryptamines, particularly those occurring in species of the canary grass genus (Phalaris) (Culvenor et al., 1964; Moore et al., 1966; and Simons, 1970). Since there appears to be grounds for believing that a relationship exists between tryptamines, and other compounds with an indole-nucleus, and a condition designated on the ranges of western North America (Carlson et al., 1972) as bovine pulmonary emphysema (BPE), the decision was made to study their occurrence and distribution in the range forages of British Columbia. It soon became apparent that tryptamines were likely to be the most important of the indole compounds associated with the condition and that a single species, reed canary grass (Phalaris arundinacea L.) was the most important carrier. The study became then, largely, a study of tryptamines in reed canary grass.

Bovine pulmonary emphysema (BPE) is an acute, or less frequently, chronic condition characterized by rapid development of varying degrees of dyspnoea (Hyslop, 1969). A wide variety of other names have been used for this disease (Clapp, 1962; Maki, 1963), names which caused a lot of confusion. Blood (1962) considered that justification existed for grouping all the forms as one disease. BPE is now widely used, although it describes a pathological condition rather than a disease.

It is sometimes difficult to distinguish BPE from other pulmonary conditions but it is now recognized as occurring importantly in a variety

of forms in all western range states and provinces in North America and in other parts of the world (Hyslop, 1969; Clapp, 1962; Brink, 1964; Maki, 1963).

Although, the condition has doubtlessly occurred in B.C. for many years*, BPE has only recently been recognized as a condition in range cattle, occurring fairly commonly. (Brink, 1964; Clapp, 1962, 1963, 1964). The disease is economically important in causing death of cattle, in crippling them as the result of disease chronicity, and in wasting forage. The death losses are hard to determine and vary markedly from area to area (Hyslop, 1969; Maki, 1963; Moulten et al., 1963).

Now known to be pasture-mediated, the condition with few exceptions develops mainly in the autumn when livestock are moved from dry range to the green, lush aftermath of low wetland meadows which in many instances, early in the season, are flooded. Possibly because of its dramatic onset, the acute form is diagnosed more frequently than the chronic form. It develops in two to seven days after the herd has been placed on the lush pasture; generally no new cases occur after ten days.

The BPE, which is probably cosmopolitan in distribution, affects both dairy and beef cattle of all ages and sexes but occurs more frequently, and most severely, in the adult wet cows with a good capacity for feed consumption. However, outbreaks in young calves and bulls have been observed occasionally (Conway, 1967; Ehret and Pienaar, 1967; Omar and Knich, 1966; Maki, 1963).

In Europe and the eastern parts of North America, Channel Island cattle appear to be somewhat more susceptible to the chronic form than Holsteins but the acute form was most common in Holsteins, Angus, Shorthorns

*(see section 5.7)

and Herefords. BPE occurs quite frequently in Hereford cattle and cross-bred stock maintained under range conditions in North America (Hyslop, 1969; Blood, 1962).

The condition, at one time believed to be due to nitrate poisoning or other causes, is now thought to be induced, not by NO_3 , but by other non-protein nitrogenous compounds. Dickinson et al. (1967) and Carlson et al. (1968, 1972) found the condition could be reproduced by feeding farm animals tryptophan and 3-methylindole. Similarly, Gallagher et al. (1964) demonstrated the toxicity of closely related indole alkaloids, particularly tryptamines, to sheep.

The presence of indole ring compounds in the plants of suspected pastures in B.C. has not been demonstrated. It is relevant to note that tryptamines in Phalaris tuberosa and P. arundinacea in Australia and the United States cause a condition known as "Phalaris staggers" and to cause death in sheep and cattle (Gallagher et al. 1964, 1966, 1967; Simpson et al., 1969). Phalaris arundinacea is a common component of BPE pastures in B.C.

The impetus for the research reported in this thesis came from the widespread occurrence and potential for use of reed canary grass in B.C. Further impetus came from early leads provided by field and chemical studies in B.C. and, of course, from important findings by scientists in Australia and the United States.

If then, the indications are that BPE is associated with reed canary grass (and perhaps other species) and with the tryptamine alkaloids, the distribution and concentration of the tryptamines in the developing plant and the influence of extrinsic factors such as fertility on them should be determined.

The objectives of the study became:

1. To ascertain the presence or absence of tryptamine alkaloids in certain plant species present on our interior ranges.
2. To determine the distribution and concentration of tryptamines in various parts of the reed canary grass plant over the growing season.
3. To study the influence of maturation and season during vegetative development on the dry matter yield and concentration of total N and tryptamines in the reed canary grass plant.
4. To determine the influence of nitrogenous fertilization on the yield, and on the total N and tryptamines of the plant and to define, if possible, some of the inter-relationships.

2. LITERATURE REVIEW

2.1 Literature relating to BPE

There is large literature on pulmonary conditions in the bovine in North America and other parts of the world. Only the literature related to the condition known on the ranges of western U.S.A. and Canada as BPE are reviewed.

2.1.1 Early history of BPE

Bovine pulmonary emphysema has been recognized as a naturally occurring disease of cattle for over 100 years (Maki, 1963). The condition has been reported by Delalande et al., (1830) in France; by Michels (1865) in Belgium; by Gruter (1883) in Germany; by Seekles (1941) in Netherlands; by Minnet (1948) in India; by Escartin Foncilles (1955) in Spain; by Pardini (1956) in Italy; by Rainey (1958) in Tasmania; by Fankhauser and Luginbuhl (1960) in Switzerland; by Begg and Whiteford (1948), Maclean (1948) and Barker (1948) in Great Britain; by Butler (1940) and Railsback (1945, 1948) in United States; and by Schofield (1924, 1941, 1944) in Canada. These reports have been followed by additional reports in various countries and many workers have reviewed the earlier work from time to time (Ammann, 1939; Foucher, 1960; Jarrett et al., 1954; Schofield, 1948; O'Donoghue, 1960; Klussendorf, 1954; Gibbons, 1956, 1962; Goodman, 1956; Pierson, 1956; Moulton et al., 1961; Tucker, 1963; and Maki, 1963). In addition, three conferences, (1959, 1963, 1965) on pulmonary emphysema were held at Sheridan, Wyoming (U.S.A.) reviewing the importance, epizootiology, clinical symptoms, histopathology, etiology and experimental work being done in Canada and United States.

Pulmonary emphysema, although existing in British Columbia for many years, first came to the attention of the B.C. Department of Agriculture in 1958, when an outbreak occurred in the Tranquille farm beef herd. This herd suffered again from this disease in 1959, 1960 and 1961 (Clapp, 1964). In addition, there were frequent reports from other areas, as far north as Quesnel, as far south as the United States border, and as far east as certain areas in the Kootenays (Cunningham, 1965).

A wide variety of names* have been used for this disease. Many authors have regarded these names, a cause of confusion for laymen, as synonymous. As late as 1965 (ABE Sympos. Laramie, Wyoming) there was lack of acknowledgement that BPE was a specific entity which resulted in an emphysematous lung. However, scientists have agreed to use a common name and consider BPE a specific entity as the constancy of post mortem findings is remarkable (Maki, 1963). The B.C. bovine pulmonary emphysema committee defined the disease as: "BPE is a disease of bovines which results in an emphysematous lung and occurs when cattle are removed from dry range lands to green succulent pastures in the fall of the year" (Clapp, 1962-1964).

2.1.2 Symptoms

The first symptoms may be noted as early as 2 to 10 days after a change in management or of pasture. In Europe and Canada there is often a history, perhaps coincidental, of recent calving, though calves of

* fog fever, bog fever, skyline disease, Luyat disease, meadow mould, moonlight disease, atypical interstitial pneumonia, bovine asthma, acute alveolar emphysema and edema, aftermath disease, panthers and lungers disease (Clapp, 1962; Maki 1963).

affected dams usually remain normal (Hyslop, 1969).

In some outbreaks cattle may be found dead without premonitory symptoms. More frequently clinical signs tend to develop suddenly, breathing becoming rapid, a characteristic severe expiratory dyspnoea, with a more or less obvious "rocking" motion and a distinct respiratory grunt, with little or no rise in temperature, presence of copious frothy salivation and occasional bleeding from the nose. Other symptoms are increased heart rate, pounding pulse, appetite loss and depressed rumination.

As the disease progresses, the head and neck become extended. The animal breathes by mouth, assumes a "humped" stance and is reluctant to move. Exercise aggravates the condition and even slight exertion or excitement may prove fatal. Death may occur from asphyxia in 12 hours to 3 days or the chronic form may supervene (Hyslop, 1969; Cunningham, 1965).

BPE is reported by all investigators except Railsback (1945) to be a non-contagious condition. The macroscopic and microscopic pathology involved and the clinical symptoms have been summarized nicely by Tucker, (1959).

2.1.3 Causes

Causal agents of the condition are not known. A variety of agents has been suggested in the past, e.g. exposure to nitrogen dioxide (Seaton, 1958); hydrogen sulphide (Hull, 1965); virus (Jarrett et al., 1957); mouldy hay, sweet potatoes or other fodders (Jenkins and Pepys, 1965; Gibbons, 1962; Van Gils, 1951 b); toxins of bacterial origin (Schofield, 1948); endotoxins (Rhoades et al., 1967); lungworms (Jarrett et al., 1957);

inhalation of pollen, (Blood, 1962); toxic factors in some plants (Peterson, 1965); a black fungus, Rhizoctonia leguminicola, growing on red clover (Rainey et al., 1965); histamines (van Gils, 1951 b); high barley rations (Omar and Kinch, 1966); excessive amounts of turnip tops, alfalfa, kale and (especially) rape (Cote, 1944; Scholfield, 1948) or other cruciferous plants, such as cress; peppermint and thyme (Priouzeau, 1954 a, b); drinking of water infested with blue green algae (Clapp, 1962 and Railsback, 1945); alkali water (Goodman, 1956; Pierson, 1956); allergic reactions (Wictor, 1952; Quin, 1961); ingestion of spiders or gossamer (Begg and Whiteford, 1948); eructation of gases from the rumen (Moulton et al., 1963); and nitrate poisoning (Clapp, 1962). Many of these agents have been dismissed as facts were not consistent with their involvement.

An association between the appearance of BPE and the movement of stock from dry pastures to areas of relatively lush growth (particularly from mountain ranges to low land pastures) has been a subject of comment by numerous authors (Barker, 1948; Gibbons, 1962; van Gils, 1951 b; Schofield, 1948; Tucker and Maki, 1962; Delalande et al., 1830; Blood, 1962). It is also true for south central B.C. and intermountain states of the U.S.A. In most instances, it appears that, when meadows are implicated in the condition, a hay cut has been removed and a succulent aftermath is providing pasturage (Maki, 1963; Clapp, 1962; Brink, 1964). Various authors have reported that the disease could regularly be produced experimentally in cattle by placing them onto wetland lush pastures after they had grazed on dry land grasses during the summer (Bourd, 1953; Tucker and Maki, 1962; Clapp, 1962, 1963, 1964). Leslie (1949) noted an apparent relationship between high protein consumption and "fog fever".

Salisbury et al. (unpublished work), from their mycological study of wetland pastures of B.C. ranges, where BPE occurs, suggested that indole ring compounds e.g. tryptophan or other related compounds in high concentration with or without fungus might be the causative agent for BPE. Dickinson et al. (1967) and Carlson et al. (1968, 1972) have been able to produce BPE in livestock (cattle and goats) by rumen and intravenous placement of indole compounds and believe that metabolic products of tryptophan are importantly involved in producing the condition. They found that larger doses of tryptophan (0.57 to 0.70 g. per kilogram body weight) are needed to produce the condition as compared to 3-methyl indole (0.2 g. per kg. body weight). Gallagher et al. (1964) demonstrated that small doses (0.1 mg/kg body wt.) of 5-methoxy tryptamine produces many "Phalaris-staggers" symptoms in sheep and little larger doses (1.5 to 2 mg/kg body wt.) may prove fatal. As large doses of tryptophan are needed, and because parenteral inoculation of tryptophan does not elicit the condition, it is apparent that tryptophan is, at the most, a precursor involved in a complex sequence of biochemical processes (Carlson et al., 1968). The metabolic relatives of tryptophan, particularly tryptamines seem to play a more important role.

Zwoll (1973), in his report, mentions the occurrence of three "wetland" grass species, viz. Kentucky blue grass (Poa pratensis), common rush (Juncus effusus), and Nebraska sedge (Carex nebraskensis) as common to all three inciting pastures in substantial amounts and were grazed extensively by the cattle immediately after turn out. Congener species, or strains, occur on wetland pastures in B.C. but have not been directly associated with occurrence of BPE (Brink, 1964). In B.C. the sedge, Carex rostrata

and reed canary grass (Phalaris arundinacea) were associated with outbreaks of BPE (Anon, 1963; Clapp, 1963). No toxic compounds have been isolated from Carex rostrata but reed canary grass was found to contain eight alkaloids and some of them quite toxic (Audette et al., 1969).

2.1.4 Treatment

Treatment of sick animals has been unsuccessful. Drugs such as epinephrine, atropine, adrenalin, antihistamines, methylene blue and various bacterins have not altered the course of the disease (Begg and Whiteford, 1948; Schofield, 1948; Gibbons, 1962; Hyslop, 1969; and Maki, 1963). Cows with mild cases probably recover spontaneously. Prevention of BPE by feeding hay or straw and moving cattle to lush pasture for a few hours at a time initially and by modifying other management factors have been suggested and have been successful in some cases (Schofield, 1948; Hyslop, 1969).

2.2 Relevant literature on alkaloids

The literature on alkaloids is large and only relevant papers are reviewed.

2.2.1 Definition and occurrence of alkaloids in Phalaris spp.

The term "alkaloid" stems from "alkali-like", which refers to the usually chemically basic nature of alkaloid compounds. No precise definition exists in the literature to include all substances that scientists place within the alkaloid category. However, Pelletier (1970) provides a summary of the chemical, pharmacological and botanical properties that

must be considered when classifying a compound as an alkaloid. An alkaloid usually has the following properties: (1) chemically basic; (2) nitrogen-containing; (3) of plant origin; (4) complex molecular structure (usually with N atom involved in a heterocyclic ring); and (5) significant pharmacological activity.

Until now eight alkaloids have been reported in reed canary grass (P. arundinacea L.); the first research into the alkaloids of the grass came from Australian workers. Wilkinson (1958) reported finding one indole alkaloid, tryptamine (5-methoxy-N-methyl tryptamine), and one phenol alkaloid, hordenine (p-hydroxyphenethyl dimethylamine) in this species. Culvenor et al., (1964) found N, N-dimethyltryptamine (DMT) and 5-methoxy N, N-dimethyltryptamine (5 MeO-DMT) to be the major basic constituents of Phalaris tuberosa, with bufotenine (5-hydroxy-N, N-demethyltryptamine) and other uncharacterized indole derivatives also present. These authors found up to 0.3% gramine (3-dimethylaminomethyl indole) as the major base in reed canary grass, while Moore et al., (1966) found 400 to 800 mg/100g gramine in unpalatable cultivars. Oram and Williams (1967) found that DMT, 5-MeO-DMT and bufotenine were common to several diverse strains of Phalaris tuberosa and their concentration in the herbage varied widely between strains. Oram (1970) also reported the difference in the type of major tryptamine alkaloids in three different Phalaris tuberosa strains.

Audette et al., (1969) in Canada reported that hordenine was a major component in three reed canary varieties. Gramine, 5-methoxy-N methyl-tryptamine and a new alkaloid, 2,9-dimethyl-6-methoxy-1,2,3,4-tetrahydro-B-carboline were identified. Audette et al. (1970) again reported the presence of 2,9-dimethyl-6-methoxy-1,2,3,4-tetrahydro-B-carboline. Shannon

and Leyshon (1971) initially challenged the structure proposed for the above alkaloid by Audette et al. and concluded that it should be 2-methyl-6-methoxy-tetrahydro-B-carboline. However, Audette et al. (1970) confirmed the earlier structure and composition for the first mentioned alkaloid in addition to the last mentioned in reed canary grass (cited as a personal communication by Shannon and Leyshon in a footnote added to their paper). Alkaloid, 2-methyl-6-methoxy-tetrahydro-B-carboline was also found in reed canary grass by another research group of Purdue University (cited by Marten, 1973). Frahn and Keefe (1971) reported the presence of 2-methyl-1,2,3,4-tetrahydro-B-carboline, 6-methoxy-2-methyl-1,2,3,4-tetrahydro-B-carboline and 6-methoxy-2,9-dimethyl-1,2,3,4-tetrahydro-B-carboline in Phalaris tuberosa.

Two tryptamines common to reed canary grass and Phalaris tuberosa were reported in reed canary grass by several research teams: N, N-dimethyl tryptamine (DMT) and 5-MeO-N, N-dimethyl tryptamine (5-MeO-DMT) were reported in reed canary grass by Barnes et al. (1971); Simons and Marten (1971); Williams et al. (1971); and Woods and Clark (1971 a). Another tryptamine (N-monomethyl tryptamine) was reported in reed canary grass by Williams et al. (1971) and Woods and Clark (1971 a).

Bufotenine and 2-methyl-1,2,3,4-tetrahydro-B-carboline which occur in Phalaris tuberosa have not been reported in reed canary grass. Oram (1970) in a survey of 33 strains of 14 Phalaris species, found none of these species entirely free of tryptamines; two other Gramineae, barley (Hordeum vulgare L.) and giant reed grass (Arundo donax L.) contained gramine and donaxine respectively; the great majority of the simple alka-

loids are confined to the dicotyledonous plants (Snieckus, 1968; U.S.D.A. Technical Bull., 1961). Culvenor et al. (1964) and Moore et al. (1966) point to the (circumstantial evidence) association of "palatability" of reed canary strains and their content of a series of simple indole alkalamines; Vose (1959) and Heath and Hughes (1962) cited conflicting evidence concerning the palatability of reed canary pastures. Throughout the years, researchers and farmers have labeled reed canary grass a distinctly unpalatable species when compared to other cool season forage grasses (Davies, 1925; Beaumont et al., 1933; Rogler, 1944; Marten and Donker, 1968). However, extremely little sound evidence exists to document the real significance of palatability differences among forage species. The few controlled studies that have attempted to document the significance of unpalatability of reed canary grass have given contradictory evidence (Blakeslee et al., 1956; Hubbard and Nicholson, 1968). Since the discovery of toxic alkaloids in reed canary grass, palatability for sheep has been negatively associated with the concentration of total basic alkaloids (Simons, 1970; Simons and Marten, 1971; Williams et al., 1971; Marten et al., 1973). This relationship also holds true for cattle (Marten, 1973). Indeed lack of palatability is the most frequently cited reason why reed canary grass has not become a leading forage in its area of adaptation.

Regardless of the true significance of the existence of alkaloids and low relative palatability in reed canary grass, sizeable genetic differences are known to exist with these factors (Woods and Clark, 1971 b; Barnes et al., 1970). Considerable work on the breeding and selection of reed canary grass is in progress for new cultivars low in tryptamine alkaloids since the discovery by Woods and Clark (1971 b) that the presence of tryptamine alkaloids in reed canary grass is controlled by a single dominant gene.

2.2.2 Possible role of indole alkaloids of Phalaris spp. in toxicity to animals

P. arundinacea and P. tuberosa are valuable components of improved pastures in many parts of the world (Marten and Heath, 1973). In the cool temperate and high latitudes, P. arundinacea is used and in the vegetative stage was found to range in crude protein from 20 to 27% in British Columbia (Goplen et al., 1963). However, under conditions not yet fully defined these species may be unpalatable, cause weight loss and even cause death of animals. Phalaris tuberosa has been reported to cause incoordination ("Phalaris staggers") and/or death in sheep and cattle (MacDonald, 1942; Le Soeuf, 1948; Lee and Kuchel, 1953; Milne, 1955; Watson, 1956; Moore et al., 1961, 1966; Gallagher et al., 1964) depending on the location (Lee et al., 1956) and stage of growth (Gallagher, 1966). Gallagher et al. (1964, 1966, 1967) have described three syndromes; a peracute, an acute, or chronic in sheep grazing toxic pastures containing P. tuberosa. Simpson et al. (1969) reported the occurrence of subacute and chronic disease in sheep grazing pastures containing a high proportion of P. arundinacea, which had not previously been incriminated in natural outbreak of intoxication in Australia. Two of the tryptamine alkaloids that occur in reed canary grass and Phalaris tuberosa (DMT and 5-MeO-DMT) have been linked to the above diseases in sheep grazing Phalaris tuberosa (Culvenor et al., 1964; Gallagher et al., 1964, 1966). Gallagher et al., (1966) showed that oral, intravenous, and subcutaneous administration of gramine, DMT, and 5-MeO-DMT to sheep, guinea pigs, rats, and mice would induce all of the nervous and cardiac symptoms exhibited in the acute and peracute diseases; 5-MeO-DMT being the most potent in producing central nervous system disorder, followed by 5-HDMT and DMT. They also reported that the tryptamines interfere with the pharma-

cological function (muscle contraction, cardiac activity, and brain function) of the closely related compound, serotonin. The tryptamine alkaloids inhibit, competitively, the initial step in the breakdown of serotonin by the enzyme, monoamine oxidase, which normally regulates the concentration of serotonin and other amines in the central nervous system (Gallagher et al., 1966). They further reported that the yellow-brown pigment noted in "Phalaris staggers" was derived from indoles.

The total tryptamine alkaloids concentration of toxic *P. tuberosa* has been found to be as high as 0.3% of dry matter; and the concentrations in toxic pastures examined have invariably been higher than in non-toxic *P. tuberosa* as cited by Gallagher et al., (1966) (Moore, Williams and Chia - unpublished data). Circumstantial evidence has led to the association of *Phalaris* toxicity with the concentration of indole alkaloids. However, Oram (1970) presented experimental evidence refuting the certainty that the acute disease and sudden death disease of sheep occur with high concentrations of the alkaloids in grazed pasture.

Van Arsdell et al. (1954) and Audette et al. (1969, 1970) report adverse physiological effects in livestock grazing reed canary grass. Audette et al. (1969, 1970) reported "alkaloidal-type lesions" in cattle livers. A condition of nervous disorder and death of sheep grazing rorpha grass (a *Phalaris* hybrid introduced from South Africa) was observed in Florida (Ruelke and McCall, 1961). Marten (1973) cited diarrhea in lambs grazing pure stands of reed canary grass while no ill effects were noted with smooth brome grass (*Bromus inermis* L.) or orchard grass (*Dactylis glomerata* L.) in two successive years (Marten and Jordan - unpublished data). However, no diarrhea occurred in dairy heifers grazing reed canary grass as the sole diet in a 4-year pasture trial located in the same area (Marten and

Donker, 1968). From this, Marten (1973) concluded that sheep may be more susceptible than cattle to metabolic disturbances induced by the alkaloids of reed canary grass, which is in keeping with the Australian finding that "Phalaris staggers" is far more common in sheep than in cattle. Woods and Clark (D.L. Woods and K.W. Clark, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba - personal communication) have also observed severe purging of sheep and some cattle loss grazing reed canary grass, especially on high tryptamine-containing plants. Similarly Shetland ponies grazing reed canary grass were observed to have a behaviour similar to that of sheep and cattle suffering from "Phalaris staggers" by Robert in Minnesota (cited by Arne Hovin, Department of Agronomy, (Plant Genetics), University of Minnesota, St. Paul, Minnesota - personal communication). The ill effects of perfoliate to livestock grazing perennial rye grass (Lolium perenne L.) and tall fescue (Festuca arundinacea Schreb) have been reported by Yates (1962), Aasen et al. (1968) and Bush et al. (1972).

Although overt toxicity of reed canary grass to grazing ruminants has seldom been reported and never proved in B.C., reed canary grass forms an important component of most interior B.C. pastures incriminated in pulmonary emphysema outbreaks during the early sixties (Brink et al., 1964). Surprisingly, Kentucky bluegrass (Poa pratensis L.), common rush (Juncus effusus) and Nebraska sedge (Carex nebraskensis) have been recorded as the major species present on the BPE pastures in Minnesota by Zwoll (1973). Although in B.C. these species have not been associated with BPE on the Interior pastures, congener wetland species or strains do occur. Dickinson et al. (1967) and Carlson et al. (1968, 1972) demonstrated the reproduction of BPE by rumen and intravenous placement of indole compounds and suggested that indole derivatives may be involved in the etiology of pulmonary emphysema in

animals. Reed canary grass contains indole alkaloids, some of which are very toxic to the livestock (Marten, 1973; Gallagher et al., 1964).

It has generally been noted in B.C. that the incidence of BPE decreases after a good rainy summer or after a killing frost. This may be due to the disappearance of alkaloids from plant tissue by leaching, migration, physiological degradation, transformation and particularly by exhalation of indole alkaloids which are volatile (Mothes, 1960).

2.2.3 Influence of maturity, season and genetics on reed canary grass alkaloids

The concentration of alkaloids in plant parts differs in different plant species and so does their site of synthesis (Mothes, 1955). The ontogenetic pattern (variation) in the concentration of alkaloids in plants has been known for years, and these changes have been used as the basis for harvesting alkaloid-containing (drug) plants of commercial value. It has generally been recognized that alkaloids are present in most actively metabolizing tissues of the plant, although exact sites of synthesis in a plant are difficult to determine (Mothes, 1960). Alkaloids in reed canary grass are confined largely to the leaf blades, although they have been reported in all other parts of the plant in minor amounts (Marten, 1973). Simon and Marten (1971) from their preliminary trials revealed that the highest alkaloid concentrations in reed canary grass were in the younger (or upper) foliage, while older foliage and lower stems (stubble) had lower concentrations. High levels of indole alkaloids have been reported in very young vegetative regrowth as compared to mature plant (Barnes et al., 1971; Simons, 1970; Gallagher et al., 1966). Simons (1970) found that alkaloid concentration (% dry wt.) of reed canary grass in the field declined about 40% during the 30-day period from the

succulent stage to anthesis stage. Similar findings have been reported by Frelich (1972) where the mean alkaloid concentration declined at least 50% from two-week old vegetative regrowth to five week-old regrowth for four clones grown under controlled environments. Gentry et al. (1969) found that immature leaves of tall fescue contained 44% more perloline and 61% more total alkaloids than mature stems; a higher concentration of perloline and total alkaloids occurred in the roots than in the shoots in rapidly growing pasture plants. The findings of Woods and Clark (1971 b) appear to be at variance with the above results; they found increased alkaloid concentration from June to August in the early growth of reed canary grass in Manitoba. This may be due to the difference in sampling procedure adopted by the latter authors. Later maturing clones have been observed frequently to contain more alkaloids than earlier maturing clones although their overall vigor and yield potential are often inferior (Simons, 1970; Marten, 1973). Oram (1970) found higher levels of total tryptamine alkaloids in autumn than in winter in two Phalaris strains. Gentry et al. (1968, 1969) found that alkaloid content of herbage from tall fescue, managed as pasture, increased gradually from early spring (March) until a peak was reached in early August and then declined during September and October. The ontogenetic pattern for alkaloids in germinating seedlings is rather inconsistent (Demaree and Tyler, 1956; Mothes, 1955, 1960).

Aasen et al. (1968) reported a diurnal fluctuation in alkaloids in 14 day-old seedlings of rye grass and found the alkaloid content (perloline) to be higher in the afternoon than in the morning. Gallagher et al. (1966) also reported a large diurnal variation in alkaloid content, in P. tuberosa building up to a maximum in the early hours of the morning and falling off

rapidly in the afternoon and suggested that pastures should be grazed in the afternoon when the toxicity will be much lower. Frelich (1972) on the other hand, did not find any difference in alkaloid concentration in samples of established reed canary regrowth at the end of the dark period or after eight hours of light and concluded that photo-period has no effect on the concentration of alkaloids.

Bowden and Marion (1951) reported from their studies that gramine is elaborated from tryptophan in the tips of barley (Hordeum vulgare L.) leaves, the concentration of gramine increases from the base to the tip of the leaf, remains constant for the first ten days after germination, and disappears after one month. Results of Minnesota studies (Frelich, 1972; Marten, 1973) imply that the indole alkaloids in reed canary grass may be largely synthesized in the leaf blades.

In mown grass hay, the tryptamine alkaloids break down rapidly and mowing has been suggested as a means of avoiding P. tuberosa toxicity in sheep (Gallagher et al., 1966).

2.3 Relevant literature on the effects of soil fertility and fertilizers on nitrogen content and yield

2.3.1 Retention of nitrogenous fertilizers in soils

Almost all the N. absorbed from soil by rooted green plants is in the form of NO_3^- or NH_4^+ ions. Generally plants are capable of absorbing either of these ions and utilizing them in nitrogen metabolism. However, the form in which they absorb N depends upon the soil and climatic conditions under which crop plants grow and innate qualities of plants. In well aerated soils, the oxidation of ammonium to nitrate is so rapid that ammonium seldom

persists; thus, nitrate is the form normally available to plants.

Stevenson (1964) reported that soils contain fixed ammonium, i.e. ammonium ions are held in the lattice structure of silicate minerals; waterlogged soils or other soils in which conditions are such as to restrict the supply of oxygen tend to accumulate ammonium ions. He further stated that for reasons not clear, grassland soils contain rather high levels of exchangeable ammonium.

Nitrogen is mainly applied to the soils through various nitrogenous fertilizers either in the oxidized form as nitrates or in the reduced form as ammonium salts. Ammonium is rapidly oxidized to nitrate through microbiological processes in warm, moist and well aerated soils but in cold, dry or unaerated soils ammonium will remain unoxidized (Pesek, 1964).

Sabey et al. (1956) demonstrated that ammonium from ammonium sulphate, broadcasted in the field was oxidized within a period of few weeks even when applied in the fall on Iowa soils when soil temperatures were relatively low and decreasing. These authors further reported that besides favourable temperature, the presence of nitrifying bacteria, moisture and oxygen are needed for nitrification to take place. Gasser and Iordanov (1967) while comparing the effects of ammonium sulphate and calcium nitrate on barley, wheat and oats reported from their soil analysis data that all ammonium nitrogen had been nitrified 46 days after sowing. Mengel (1969) from his field studies on wheat, reported that soil analysis revealed that five weeks after the N application about 25% of the supplied NH_4^+ was converted to nitrate; eight weeks later about half the NH_4^+ had been oxidized to NO_3^- . Pesek (1964) stated that the main difference in the fertilization process, between the nitrate fertilizers and the ammonia, ammonium or urea fertilizers

is the time taken before the nitrogen in the latter group is nitrified and becomes subject to denitrification. This period may be long or short, depending on soil conditions and, to some extent, the actual source. Hence there may be differences between N-fertilizers due to differential nitrate losses from nitrate and other sources.

Sauchelli (1964) stated that if nitrogen is applied to a soil as nitrate, it penetrates the soil quite rapidly after a rain, because NO_3 is not absorbed by the colloidal complex (clay and humus) of soil. Thus excessive rainfall will carry off considerable NO_3 nitrogen and move it in the drainage water or it may be lost by denitrification under waterlogged conditions. Similar findings have been reported by Bates and Tisdale (1957) and by Raney (1960). However, attractive forces exist between the ammoniacal-N and the colloidal complex, and nitrogen applied as ammoniacal-N penetrates the soil less rapidly and drainage losses are reduced; on the other hand ammoniacal-N may also be fixed chemically or biologically.

Fertilizer trials have shown that urea is inferior in effecting increased yields to ammonium sulphate when applied as a top dressing on grass sods, but not when incorporated into soil (Stevenson, 1964). Major inefficiency in the use of urea results from losses of ammonia which may occur as a result of hydrolysis on a moist soil surface which then dries out (Jackson and Burton, 1962). These potential losses are magnified by the presence of some plant material, high temperature, low exchange capacity of soils, high rates of urea application, high pH and, especially, calcareous conditions (Volk, 1959). Volk and Sweat (1955) reported that some soils exhibit weak but unspecified attractive forces for urea, causing it to move through a soil column more slowly than the water in which it is dissolved.

Broadbent (1951) reported that urea does not persist long in warm moist soils and that it is rapidly hydrolyzed to ammonium by urease. Beacher and Wells (1960), however, reported that if incorporated into the soil, urea should be a superior source of nitrogen, where water percolates through soils, and where soils are water-logged after application or even in poorly drained soils.

Ammonium sulphate, like anhydrous ammonia and urea, provides ammonium ions which are held against the action of percolating water (Pesek, 1964). It is most effective under conditions of waterlogged or poorly drained soils (Beacher and Wells, 1960). Because it carries an acid radical, ammonium sulphate may be superior as a source of N under conditions where lowering of the pH even more than it is, lowered by other acid nitrogen sources is desirable or where the sulphur is needed (Stephens, 1960). On the other hand, this extra acid forming potential may be a serious cause of inefficiency over an extended period of usage (Pesek, 1964). Stability of ammonium sulphate will be greater than urea in the same type of soils and losses from ammonium sulphate, with one exception, (alkaline environment) may seem to be far lower than losses from urea, applied to the same soil (Black, 1968).

Ammonium nitrate, unlike anhydrous ammonia and urea, is a neutral salt and does not cause a sharp increase in pH in the region of application. Large ammonia losses from ammonium nitrate have not been reported (Pesek, 1964). The ammonium part of the fertilizer is retained on the colloidal complex of the soil but the nitrate part will move with water. If aeration is poor, half of the nitrogen in this carrier is subject to denitrification (Schwartzbeck et al., 1961). This fertilizer should perform best in well-aerated soils where top-dressing is required and precipitation not excessive

(Burton and Jackson, 1962). With high water percolation and normally water-logged conditions ammonium nitrate tends to be less effective than the other ammonium sources.

Kresge and Satchell, (1960) from their studies of nitrogenous fertilizers applied at 50, 150 and 300 pounds of nitrogen per acre as ammonium nitrate, ammonium sulphate, urea or calcium cyanamid on Hagerstown silt loam (pH 6.3) soil, found that significant amounts of ammonia were lost from calcium cyanamid and urea at all rates of applications but none or very little from ammonium nitrate or ammonium sulphate at any rate. The pH of soils receiving the highest rate of urea and highest rate of calcium cyanamid were above neutral (pH 7.6 and 8.1 respectively) while those receiving ammonium nitrate and ammonium sulphate went down in pH (5.9 and 6.0 respectively) by the end of the experiment.

Sodium nitrate, calcium nitrate and calcium cyanamid are also neutral salts causing little alteration of soil reaction. In the long term they have a tendency to increase soil pH. Since they supply nitrate, they have serious limitations for utilization where regular or continuous water-logging of soil occurs (Pesek, 1964).

Pesek (1964) states that agronomic efficiency within and among sources of N varies with rate of application, time of application, placement, environment and crop, and that physical and chemical properties of the sources specify the conditions under which they may be employed and how they must be used for optimum effectiveness.

2.3.2 Effect of soil fertility and N-nutrition on yield and total N in the plant

Soil fertility affects the total N content, the protein content and

also the yield of plants. There is little evidence that soil type apart from its nutrient content has much influence on plant composition (Fagan and Davies, 1937).

Increasing levels of N tend to increase total N, protein content and yield of many cereals and forages and highly significant correlations between yield and percentage N have been reported (Hera, 1969; Tyner, 1946; Goswami and Willcox, 1969; Harms and Tucker, 1973; Mengel, 1969).

Van Ryswyk et al. (1973) reported a yield range of 2380 kgs. to 5730 kgs. dry matter per hectare between fertilized and unfertilized native sedge hays of interior B.C. On the basis of provincial average (1938-1947), it was found that nitrogen gave yield increase of 1.63 tons, potash 0.16 per acre of pasture herbage in B.C. No figures are available for phosphorus, although a substantial increase also occurred from the use of phosphorus. Similar results also have been reported for forage crops under irrigated conditions. (Illustration stations' reports 1921-1953).

Dean and Clark (1972) while studying nitrogen fertilization of reed canary grass and its effects on production and mineral content in Manitoba found strongly linear relations ($P=0.01$) for dry matter and crude protein with 'nitrogen'. A significantly positive correlation was also noted with nitrogen for K, Mn, Fe, Cu, and Zn in various cuts, whereas Mo showed a significantly negative correlation in one cut.

Since Liebig several investigators have examined uptake and utilization problems of the nitrogen forms, reduced $-N(NH_4^+)$ and oxidized $-N(NO_3^-)$. Both soil nitrogen and that applied as fertilizer, as has been noted earlier, are taken up almost wholly by the plant in the form of NH_4^+ and NO_3^- ions. Fried et al. (1965) while working with excised rice roots found that NH_4^+ ions uptake, depending upon pH, can be three to ten times greater than that of

NO_3^- ions. They also found that NO_3^- ion uptake is much more hindered at low temperature than that of NH_4^+ ions. Likewise, Zsoldos and Jozsef (1969), while reviewing the work of ammonium and nitrate ion uptake by plants, report that the ammonium nitrogen uptake proceeds at a higher rate at low temperature, compared with other ions. Generally ammonium ions are more readily synthesized into protein because the reduction of the nitrate is avoided; this effect has been shown to increase protein levels in Italian rye grass by Nowakowski and Cunningham (1966). These authors, from their green house studies with NH_4^+ and NO_3^- -N nutrition, also found that the yield with NH_4^+ -N increased from 0 to 500 ppm but the yields with NO_3^- -N were highest at 200 ppm and decreased with increasing NO_3^- -N. Yields with NH_4^+ -N at 200 ppm were higher than with NO_3^- -N, irrespective of light intensity. With full daylight and up to 500 ppm of added N as NH_4^+ -N or NO_3^- -N, the percent N in the grass increased almost linearly. At any particular level of N, the grass receiving NO_3^- -N had a higher total soluble-N (non-protein N) in the case of NO_3^- -N as compared to NH_4^+ -N. Species, however, differ in their abilities to utilize NH_4^+ and NO_3^- ions (Pate, 1969). Mengel (1969) while reviewing the work on N sources states that in numerous field experiments both nitrate and ammonia as N sources gave equal yields in soil pH range of 6 to 7. In water culture, however, nitrate nutrition was superior to ammonia.

Barker and Bradfield (1963) found that at pH 6.5 corn plants made more growth on NH_4^+ compounds than on NO_3^- compounds and that the role of potassium is regulatory in the utilization of N. Bekmukhamedova reported (1961) that dry weight and all the essential amino acids including tryptophan were higher in corn plants supplied with ammonia nutrition than with nitrate nutrition.

Gasser and Iordanov (1967), found that the average dry matter produced by three crops (wheat, oats and barley) with fertilized N was slightly more with nitrate-N than with ammonium N in the later stages of growth and more N was taken up from nitrate than from ammonium.

Burton and Jackson (1962) from five years fertilizers study on Coastal bermuda grass using ammonium nitrate, ammonium sulphate, ammonium nitrate solution, anhydrous ammonia, urea-ammonium nitrate solution and urea, reported relative hay yields of 100.0, 96.2, 98.3, 94.0, 92.3 and 81.3 and relative nitrogen recoveries of 100.0, 98.6, 95.2, 96.4, 86.1 and 74.0, respectively. In sand culture, Coastal bermuda grass utilized ammonia and nitrate nitrogen equally well.

Jackson and Burton (1962) from another 2 years' study found that average forage yields of Coastal bermuda grass with surface-applied urea were 15% smaller than those resulting from surface-applied ammonium nitrate on a Tifton loamy sand. While sub-surface applications of urea and ammonium nitrate were both inferior to surface applications of ammonium nitrate; they did not differ significantly from each other. The urea actually resulted in slightly more forage than did ammonium nitrate applied in this manner.

Stephens (1960) from his study in Ghana, on a slightly acid soil in the savannah zone, concluded that ammonium sulphate was the most effective fertilizer, giving significantly higher yields than urea or cyanamid.

2.4 Effect of N-nutrition on alkaloids

Nutritional conditions greatly influence the alkaloid concentration and total alkaloid content in plants. In general good cultural conditions promote good yields of alkaloids. Early work on external influences on alkaloid

synthesis in plants has been reviewed by Mothes (1955). Researchers have devoted considerable attention to the influence of nutrients, especially nitrogen (N), on the concentration of alkaloids in plants. Numerous workers have stressed adequate sources of nitrogen for alkaloid production in Claviceps (Ergot), Papaver, Nicotiana, Hyoscyamus, Lupinus (Mothes, 1960).

The total amount of N available to a plant has been shown to increase the alkaloid content within a plant. Dimitrijevic (1960) found increased alkaloidal production with increasing N in Atropa belladonna. Bennet (1963) obtained up to 30-fold increases in perloine content of nitrogen-fertilized perennial rye grass grown in the greenhouse. De Maggio (1961) reported that an increase in the N content in a NPK fertilizer increased alkaloid production in Datura stramonium. Gentry et al. (1968, 1969) reported substantial increases in the perloine (and other alkaloids) concentration of tall fescue in the field by applying up to 220 kg N/ha.

The origin or form of N has been found to exert quite variable effects on alkaloid concentration. Nickolic (1960), using three sources of nitrogen, found that total content of alkaloids in tubers of Aconitum divergens plants receiving ammonium nitrate, sodium nitrate, or ammonium carbonate, as the sole source of N, was the highest from an ammonium nitrate source and the lowest from an ammonium carbonate source. Cromwell (1937) found ammonium sulphate increased the percentage of alkaloids in Atropa belladonna whereas ammonium nitrate did not. Similar results have been reported by Dawson (1938) for Nicotine in tobacco. Alove (1961) reported that ammonium sulphate favoured alkaloid synthesis in the roots of A. belladonna, while sodium nitrate tended to favour alkaloid production in the leaf. Michna and Szwadiak (1964) obtained a higher yield of morphine when they used ammonium sulphate

rather than calcium nitrate as the sole source of N. These results suggest that $\text{NH}_4\text{-N}$ enhances alkaloid synthesis more often than $\text{NO}_3\text{-N}$. James (1950) suggested that $\text{NH}_4\text{-N}$ is probably used directly in alkaloid synthesis, while $\text{NO}_3\text{-N}$ functions more in overall growth of the plant. The information regarding the influence of nitrogen on the concentration of alkaloids in reed canary grass came mainly from Australian and United States workers. Unpublished studies by Moore, Williams and Chia (cited by Gallagher et al., 1966) under controlled environmental conditions have shown that high soil N has a strong influence on the concentration of tryptamine alkaloids in P. tuberosa. Gallagher et al. (1966) observed that the higher the N content of the soil, the higher will be the alkaloid concentration, and suggested that heavy applications of nitrogenous fertilizers to Phalaris pastures are to be avoided. He further stated that greatest mortalities of sheep occurred on most fertile pastures. Moore and Hutchings (1967) observed neurological disorders among sheep grazing on Phalaris tuberosa that had been growing on soil high in nitrate-N (10 to 23 ppm), and theorized that a possible relationship may have existed between nitrate and the concentration of tryptamine alkaloids within the plants. Moore et al. (1967) obtained conflicting results when they applied nitrogen to Phalaris in nutrient cultures. Total tryptamine alkaloids increased linearly at relative nitrate levels of 1, 10 and 100 under an 8-hour day length. However, grass at the lowest nitrate level contained more alkaloids than at higher levels under a 16-hour day length. This would implicate light as a factor in alkaloid accumulation. Simons (1970) reported that responses to nitrogen on alkaloid concentration in Phalaris arundinacea were varied. An increase in N (from 0 kg N/ha. to 200 kg N/ha.) increased alkaloids in only one of four genotypes

and he concluded that some clones respond to fertilizer treatments more than others. In a similar study, Simons (1970) noted that a characteristically low alkaloid-containing genotype was unaffected by N supply (0 kg/ha. to 100 kg N/ha.) when compared to an initially high alkaloid-containing genotype. Frelich (1972) from greenhouse solution culture study found that the mean concentration of alkaloids in P. arundinacea supplied with 300 ppm N was 31% and 26% greater ($P < .05$) than that with 100 ppm N for the first and second harvest respectively (averaged across four genotypes and four N sources).

Marten, Simons and Frelich (1974) from field and greenhouse studies reported that up to 240 kg/ha. of N (NH_4NO_3 and $(\text{NH}_4)_2\text{HPO}_4$) did not change the alkaloid concentration of reed canary grass growing in a nitrogen-deficient peat soil in a Minnesota field study; however, about 390 kg/N caused a 40% increase in total basic alkaloid concentration. Simons (1970) from greenhouse studies found that 120 kg/ha. of N to nitrogen-deficient peat and mineral soils resulted in a 54% increase in alkaloid concentration in only two of six reed canary grass genotypes; also one genotype increased in alkaloid concentration by 38% when it received only 60 kg/ha. of nitrogen.

Frelich (1972), from greenhouse solution-culture study, reported that the source of nitrogen had a greater influence on alkaloid concentration on four genotypes of reed canary grass than did amount. The mean concentration of alkaloids in plants grown in solutions containing only NH_4^+ as the sole source of N was higher than the concentration in those plants supplied with N in the form of urea, NH_4^+ plus NO_3^- , or only NO_3^- by 27, 32 and 57% for the first harvest, and 24, 31 and 55% for the second harvest respectively. When

averaged over three N rates and two harvests, overall total basic alkaloid concentrations (% dry wt. of grass) by N sources were: ammonium chloride, 0.42; urea, 0.34; ammonium nitrate, 0.32; and sodium nitrate, 0.27. From these results, Frelich suggested, the form of N-fertilizer applied to soil could conceivably increase the concentration of alkaloids in P. arundinacea if soil conditions favour ammonium - N accumulation over nitrate - N.

In addition to N-nutrition, the effect of other environmental factors has been reviewed by Simons (1970), Frelich (1972), Marten (1973) and Marten et al. (1974). All the work until now on reed canary grass indicates that, although alkaloid concentration is changed by nutrition, alkaloid type remains unaffected.

3. MATERIALS AND METHODS

3.1 Field experiments

Two meadows with a BPE association of many years were chosen for this study in 1972, the Pond meadow, Tranquille, and the Whitecroft meadow, upper Louis Creek, B.C. As the study progressed, meadows and stands more accessible than the Pond and Whitecroft meadows were added to the study, viz., on the polder meadow, Pitt Meadows, and at the University Farm, Vancouver, B.C. where, at least in places, reed canary grass grew in pure stands.

Tranquille meadows ca. 1100 feet elevation are located west of Kamloops on the north shore of the Thompson River. The beef herd belonging to the B.C. Government farm is brought here from dry summer range during October or November each year and is left to graze on the aftermath after hay has been removed until a severe frost kills the vegetation. According to old records BPE has a morbidity of 12 to 14% on these meadows (Clapp, 1962). The actual area in use consists of about 200 acres. This has been fenced and further fenced into two areas which are referred to as the 'pond-area' and the 'non-pond area'. The 'pond-area' features a shallow pond which is spring fed and occupies about five acres. The area immediately surrounding the pond is largely established in scouring rush (Equisetum fluviatile L.), water parsnip (Sium suave Walt.), wild mint (Mentha canadensis L.) and false dragon-head (Physostegia spp.) as minor components. There is also a goodly supply of willows, a wooded area and 7 - 8 acres of pasture land where reed canary grass (Phalaris arundinacea L.) and beaked sedge (Carex rostrata Stokes) predominate.

The companion area, that is the 'non-pond' area, consists entirely of common pasture species, couch grass (Agropyron repens L. Beauv.), reed canary grass (Phalaris arundinacea L.) and beaked sedge (C. rostrata Stokes) as the major components.

These meadows are inundated by June and July from floodwaters of the Thompson river, notably after plant growth has started; the regional climate is continental and semi-arid and the soils are slightly acidic gleisols (Prof. L. Lavukulich, Dept. of Soil Science, University of B.C. - personal communication).

A nearby dry range at 1500 feet elevation, the so-called Curry field, supplied forage for chemical analysis; the cattle come off this hilly dry range to Tranquille meadows in the fall. The soils are mainly loams to gravelly sandy loams developed on drift (with a distinctive glacial landform generally covered by one to five feet of colluvium) and are rapidly drained. In forested regions, the colluvium may be more than 5 feet thick. On the grassland region, a fine sandy loam to silt loam capping (4 to 10 inches thick) and thought to have been deposited by slopewash and/or wind action, overlies the drift (preliminary soil map, C.D.A., 1971). Blue bunch wheatgrass (Agropyron spicatum (Pursh.) Scribn. and Smith) and crested wheatgrass (Agropyron cristatum (L.) Gaertn.) were the major plant species in this area.

The Whitecroft meadow, ca. 3000 feet elevation is about 25 miles northeast of Kamloops. The soils of this site are again acidic gleisols and are associated with a narrow valley subject to capricious air drainage.

Pitt Meadows are about 12 miles northeast of Port Coquitlam. The soils are on flood plain and the greater part of the land is dyked against floods from the Pitt, Alouette and Fraser Rivers. The soils are acidic gleisols

having silty clay to clay loam parent material. The water table rises with considerable freedom during the wet season and remains high up to the months of May and June. Low places are filled with water from a few inches to several feet. The soils are poorly drained in this area (Holland et al., 1957). Reed canary grass (P. arundinacea L.) is the predominant species but other species such as Yorkshire fog (Holcus lanatus L.), Kentucky bluegrass (Poa pratensis L.), timothy (Phleum pratense L.), Canada reed grass (Calamagrostis canadensis L.), beaked sedge (Carex rostrata Stokes) and alsike clover (Trifolium hybridum L.) are common.

At the University of B.C. Farm, the climate is humid-maritime and the soils, at one time supporting climax or second growth forest, are well drained, slightly acidic sandy outwash and till, are frequently plowed and often designated as the Alderwood series.

3.1.1 Dry matter yields, tryptamines and total N in certain plant species over the growing season

The purpose of this study was to obtain information on the presence and concentration of tryptamines in reed canary grass and the effect of vegetative development and season on them. Although, from earlier work and from our own pre-screening, it became clear that reed canary grass was the only species with notable amounts of tryptamines, other species were included to make sure of the presence or absence of these compounds at any stage or time in the growing season. In addition, dry matter yields and total N content of the species were determined.

This trial was started at Tranquille meadow, and Whitecroft meadow, after the water receded from these meadows. Three samplings were made at Tranquille on August 22, September 22 and October 14, 1973. Only one

sampling was made at Whitecroft meadow on September 22, 1973. Sampling dates bracket the main period of BPE occurrence from late August to early September. Tranquille meadow species were green until the last sampling date, when all but a few basal shoots were killed by frost. On the other hand, by September 22, parts of the Whitecroft meadow, subject to capricious air drainage patterns, had been severely frosted and parts had escaped frost. One day before sampling, one cow was reported dead due to BPE at this site. Well before the first sampling date, the two dry range species in the Curry field had cured.

For ease of comparison, sampling and presentation of results, the areas were divided into two management regimes termed "unmown" and "mown".

3.1.1.1 Unmown

Seven species, viz. scouring rush (Equisetum fluviatile L.), water parsnip (Sium suave Walt.) from the 'pond-area'; couch grass (Agropyron repens L.), beaked sedge (Carex rostrata Stokes) and reed canary grass (Phalaris arundinacea L.) from the 'non-pond' area; blue bunch wheatgrass (Agropyron spicatum (Pursh.) Scribn. and Smith) and crested wheatgrass (Agropyron cristatum (L.) Gaertn.) from Curry field; beaked sedge (Carex rostrata Stokes) and couch grass (Agropyron repens L.) from Whitecroft meadow were sampled.

There was some development and growth of scouring rush and water parsnip between the first and second sampling but no major growth difference was recorded in the other five species. By the time of third sampling, most species had stopped growing, some lodging was present in scouring rush and water parsnip had turned yellow on Tranquille meadow. The two dry range

species, blue bunch wheatgrass and crested wheatgrass, had cured well before the first sampling date and no difference was observed during sampling except that the new growth had started from the base by the time of third sampling. At Whitecroft meadow both the species were slightly chlorotic by sampling time.

Samples for dry matter yields were collected from meter squares after randomly placing a meter quadrat in the unmown areas. The plants were clipped at the ground level and all the vegetative growth was collected. For tryptamines, the samples were collected on the outside of each yield quadrat, clipped at ground level and divided into two more or less equal parts, viz. a lower "half" and an upper "half". Total nitrogen was determined on yield as well as tryptamines samples. The results are given in Section 4.1.1.

3.1.1.2 Mown (Aftermath)

In this study, the young aftermath was sampled after a hay cut had been removed. Reed canary grass (P. arundinacea L.), beaked sedge (C. rostrata Stokes) and couchgrass (A. repens L.) from Tranquille meadow and Whitecroft meadow were sampled. There was a continuous vegetative growth during the sampling period and Tranquille meadow species were green until the last sampling date, when all but a few basal shoots were killed by frost. Whitecroft meadow had been severely frosted but some parts had escaped frost by the sampling date.

The procedure of sampling was similar to that used in Section 3.1.1.1 except that no partitioning was done into lower and upper as the aftermath material was not tall enough. The results of the study are given in Section 4.1.2.

3.1.2 Distribution and concentration of tryptamines and total N in various parts of the grass over the growing season

This study was undertaken to determine the concentration and distribution of tryptamines and total N in different parts of reed canary grass and to assess variation in tryptamines and N attributable to maturity and season. Pure stands of reed canary grass were chosen at Tranquille meadow and at the University of B.C. Farm.

At Tranquille meadow, a reed canary grass stand not grazed by livestock growing close to a fence was chosen. Three samplings were done during the growing period, on August 28, September 22 and October 14, 1973. The grass remained green except for basal leaves which became brown and dry due to advance in maturity and growth until the last sampling date. The plants were sampled randomly, cut from the base and partitioned immediately into: (a) 4-upper blades, (b) lower blades, (c) upper stem half with sheaths and (d) lower stem half with sheaths.

At the University Farm, a mature stand of reed canary grass in a nursery established in 1970 was chosen for the study. Sampling again was done thrice during the growing period, on June 23, July 28 and October 20, 1973. The grass plants were randomly dug out with the roots with the help of a shovel and partitioned immediately into: (a) roots and rhizomes, (b) lower stem half, (c) upper stem half, (d) leaf sheaths, (e) 4-upper blades, (f) lower blades and (g) inflorescence. In case of root and rhizome samples, the soil was gently washed off with water immediately after their collection. No effort was made to separate roots and rhizomes. The results are given in Section 4.2. The grass was at the flowering stage at the time of the first sampling but was mowed shortly after in the last week of June, 1973. Therefore, the second and third samplings were taken from

the aftermath. At the time of the second sampling the grass was only at the 4-leaf stage, so no other parts could be collected except the 4-upper blades and the roots. No sampling was done during the month of August and the grass was allowed to grow until the third sampling date, October 20, when the grass was about three feet tall. All parts of the grass were collected and sampled except, of course, the inflorescence because the grass did not flower a second time.

3.1.3 Studies of N-nutrition of field grown reed canary grass

This study was done to determine the effect of N-nutrition under field conditions on the concentration of tryptamines, total N and dry matter yield of reed canary grass and how they are affected by maturity and season. The practical necessity and importance of this study arose (a) because of the wide use of nitrogenous fertilizers on B.C. pastures and (b) because results of greenhouse nutritional studies on reed canary grass in Minnesota had suggested applications to field conditions, particularly under acidic, poorly drained or waterlogged conditions in soils similar to those commonly occurring in fields of reed canary grass in B.C.

Three locations were chosen, viz. Tranquille meadows, Pitt Meadows and University of B.C. Farm.

3.1.3.1 The effect of 4-rates of ammonium sulphate fertilizer on the concentration of tryptamines and total N

This study was conducted on the Tranquille meadows. A pure stand of reed canary grass was chosen, which had been cut for hay on August 23; the fertilizer was applied on the surface immediately after the hay was removed,

namely on August 28. Ammonium sulphate fertilizer was used in this case as it contains an "acidic radical" in addition to an ammonium ion considered to be a source of N which is readily incorporated into alkaloids by Minnesota workers (Marten, 1973). Three rates of ammonium sulphate fertilizer, namely 50, 100 and 200 pounds of N per acre were used with "no-fertilizer" as a control. Four rates of N (the equivalents of 0, 50, 100 and 200 pounds N per acre) with two replicates were the main plots and two "sampling dates" (September 22 and October 14, 1973), the sub-plots in a split plot design. Each ultimate or sub-plot was 6 feet by 6 feet. The grass was 4 inches to 6 inches tall at the time of first sampling and 6 inches to 9 inches tall by the second sampling date. The grass was randomly clipped at the ground level from each sub-plot at the sampling dates. The results are presented in Section 4.3.1.

3.1.3.2 Effect of 5-rates of ammonium nitrate on the concentration of tryptamines, total N and on dry matter yields

An area of 60 feet by 60 feet of pure, developmentally well advanced reed canary grass was chosen at Pitt Meadows for this study shortly after the recession of the annual flood. In this trial ammonium nitrate, because it contained both ammonium and nitrate ions, was used. Two samples were collected randomly of the 4-upper leaves for analysis for tryptamines and total N on June 19, 1973. In addition, two samples for dry matter yields were also randomly collected on the same date, using a meter square quadrat frame, by clipping all the above-ground growth. The stand was mowed and hay removed on June 20, 1973.

The experiment was set up on June 21, 1973 as a split-plot design with 5 rates of N (the equivalents of 0, 50, 100, 200 and 300 pounds N per acre).

with two replicates as the main plots and three "harvesting dates" (August 26, September 29 and October 29, 1973) as sub-plots. Yield samples, obtained by randomly placing a meter quadrat frame in each sub-plot, were collected on the first sampling date only, when all above-ground vegetative growth was clipped. The 4-uppermost leaf blades were collected randomly for tryptamines analysis on each sampling date from the uncut grass (not harvested for yield) area in each sub-plot. Total N content was separately determined in both types of samples, i.e. samples collected for yield and for tryptamines. The results are presented in Sections 4.3.2 and 4.3.2.1.

3.1.3.3 Effect of rate and source of nitrogen fertilizer on the concentration of tryptamines, total N and on dry matter yield of reed canary grass

This trial was undertaken at two places: (a) Pitt Meadows and (b) the University of B.C. Farm. The purpose of this trial was to determine the effect of different forms and types of nitrogenous fertilizers, as well as of different rates, on the concentration of tryptamines, on total N and on dry matter yield. In addition the effect of maturity and season was monitored through sequential sampling over the growing period.

Five nitrogenous fertilizers, viz. ammonium sulphate, ammonium nitrate, urea, cyanamid and sodium nitrate at five rates of each (the equivalents of 0, 50, 100, 200 and 300 pounds N per acre) were used.

(a) At Pitt Meadows, a pure stand of reed canary grass was chosen and an area of 60 feet by 30 feet was earmarked for the trial. This was on a slightly higher site than that of the trial referred to under Section 3.1.3.2. Five samples, each obtained on June 18, 1973, were for tryptamines analysis and for yield and were treated in much the same manner as those in

Section 3.1.3.2. The stand for this trial was mowed and hay removed on June 20, 1973.

On June 21, 1973, the trial was set out on the newly mown surface as a split-split plot design with five sources of N with two replications as the main plots, five rates of N as sub-plots and three "harvesting dates" (August 18, September 28, and October 28) as sub-sub-plots (ultimate plots). The sampling procedure for samples of dry matter yield, tryptamines, and total N was similar to that described in Section 3.1.3.2. The results of the study are given in sub-sections of Section 4.3.3.1.

(b) At the University Farm, the experiment was set out as a split plot design with five sources of N as the main plots and five rates of N as sub-plots. The sources and rates of N used, and the dimensions of the area under the experiment were the same as described in part (a).

A fine seed bed was prepared and the fertilizers were broadcast over each sub-plot at the specified rates and incorporated into the soil surface, using a rake, on July 16, 1973. On the same date the plot was seeded with reed canary grass seed at the rate of 12 pounds per acre. The plot was immediately sprinkler irrigated and an effort was made to bring the soil moisture in the plot up to field capacity. This procedure was used to maintain soil moisture at field capacity through the growing season. The grass germinated 7 to 10 days after the sowing date. As a lot of weeds came up, two hand weedings were done in the third week of August and the first week of September.

The grass was sampled for dry matter yields and for tryptamines' analysis on October 20, 1973. For dry matter yields, the procedure was similar to that described in previous sections but for tryptamines' analysis,

the shoots from the top three inches of the plant were clipped randomly from each sub-plot. The results of the study have been presented in sub-sections of Section 4.3.3.2.

3.2 Handling and preparation of samples

As tryptamines are volatile in nature, two different procedures were used in handling and preparation of the samples for dry matter yields and for tryptamines' analysis.

3.2.1 Procedure for samples for dry matter yield and total N

Samples collected for dry matter yields were put in jute sacs and dried in the pot-hole-tunnel drier at a temperature of 60°C until a constant weight was attained. This was generally achieved after 48 hours of drying. The dry matter weight of each sample was recorded after weight uniformity was obtained. Later each sample was ground in a hammer mill and a representative sub-sample drawn from the pulverized material. The sub-sample was again ground in a Wiley mill to pass a 40-mesh screen and stored in a plastic bag at room temperature until needed for the determination of total N.

3.2.2 Procedure for samples for tryptamines and total N

The samples after collection in the field were immediately freeze-dried (lyophilized) in a box of dry ice and brought to the University of B.C., where they were stored in a cold room at approximately -36°C until needed. Each sample was cut into smaller pieces with a pair of scissors in its frozen state and freeze-dried at 27°C for 24 hours. Then each sample

was powdered in a Wiley mill through a 2-mm. screen and stored in a glass bottle until needed for analysis of tryptamines and total N content.

3.3 Analytical procedures

3.3.1 For total N in plant tissue

Total nitrogen was determined in dry powdered plant material by the modified semi-micro Kjeldahl procedure of Nelson and Sommers (1973) which also takes into account the nitrate-N, if present, in the plant samples.

3.3.2 For indole alkaloids

Tryptamines in the principal species and plant parts were determined qualitatively by thin-layer chromatography using the method of Woods and Clark (1971a) and quantitatively using the method of Seher (1961). Seher (1960) found a positive relationship between the concentration (quantity) and the spot size of antioxidants in thin-layer chromatography and used it for his quantitative analysis. He reported a linear relationship existed up to a certain range concentration after which the relationship became curvilinear. Later (1961) he successfully used this linear relationship for quantitative analysis of tocopherols in both paper and thin-layer chromatography. In the case of thin-layer chromatography the reproducibility was quite high ($\pm 5\%$).

3.3.2.1 Reagents

1. 95% ethyl alcohol (ethanol)
2. Methyl alcohol (Methanol)
3. Ammonia

Reagents (continued)

4. Concentrated hydrochloric acid (HCl)
5. Chloroform
6. 2N sulphuric acid
7. Sodium chloride
8. 40% W/v sodium hydroxide
9. Ehrlich's reagent (0.7 g. p-dimethylaminobenzaldehyde dissolved in 150 ml. concentrated HCl, made to 250 ml. with distilled water. This spray reagent should be at least a week old before use).
10. Xanthyrol (0.1 g. xanthyrol in 100 ml. of 95% ethanol:concentrated HCl 95:5, freshly prepared).
11. Standard solutions of alkaloids in 95% ethyl alcohol:
 - DMT (N, N-dimethyl tryptamine)
 - 5-MeO-DMT (5-methoxy-N, N-dimethyl tryptamine)
 - N, Methyl tryptamine
 - 5-MeO-N-methyl tryptamine
 - Tryptamine hydrochloride

Solvent systems used were methanol: strong ammonia solution (29% NH_3) 7:1, and methanol: concentrated HCl 9:1; on silica gel G plates (Merck: 250 mm., 20 x 20 cm.).

3.3.2.2. Extraction of the crude alkaloid fraction from plant tissue

Powdered grass, 5 grams, was Soxhlet-extracted for 16 hours with 95% ethanol and the liquid was taken to dryness under vacuum. The residue was dissolved by shaking alternately with 50 ml. of 2N sulphuric acid and 50 ml.

chloroform (two lots of each). The pigmented chloroform was discarded and the aqueous layer saturated with sodium chloride, and then made to above pH 11 with 40% W/v sodium hydroxide (20 ml. sufficient). The aqueous layer was then extracted for alkaloids, with increments, with 50 ml. chloroform. The solution was evaporated to dryness under vacuum, the residue was made to 1 ml. in chloroform.

3.3.2.3. Chromatography method for identification and quantification of tryptamines

Methanol: strong ammonia solution 7:1 on silica gel plates with Ehrlich's reagent as spray were mainly used in the procedure. The main reason for this was that the development of a chromatogram was faster with this solvent than with methanol:HCl 9:1, and the colour reaction by Ehrlich's reagent was brighter and longer-lasting as compared to xanthydrol. Other standard solutions except tryptamine hydrochloride, were used for identification purpose only. The R_f's and colour reactions are given in Table I.

In the beginning of the experiment, a positive relation was found between the concentration of tryptamine hydrochloride solution and its spot size. Effort was made to find a range of linearity for this relationship. After preliminary experiments, it was found that a linear relationship existed up to 8 µg per spot (10 µl) of tryptamine hydrochloride solution after which it became curvilinear. The thin layer system is quite sensitive, the lower limit for tryptamine hydrochloride being 0.04 to 0.08 µg per spot in a mixture with a grass extract (Woods and Clark, 1971 a). Standards containing .5, 1, 3, 5 and 7 µg per spot tryptamine hydrochloride

Table I: Rf values and colours produced by reference compounds

Compound	<u>Rf value</u>		<u>Colour produced</u>	
	HCl solvent on silica	NH ₃ solvent on silica	Ehrlich's reagent	Xanthydrol reagent
Tryptamine hydrochloride	.90	.60	Blue-grey	Purple
5-MeO-N-methyl tryptamine*	.80	.50	Royal blue	Blue
N, Methyl tryptamine*	.80	.50	Blue-grey	Purple
N, N-Dimethyl-5- MeO tryptamine*	.65	.70	Royal blue	Blue
N, N-Dimethyl tryptamine*	.65	.70	Blue-grey	Purple

* Not distinguishable by any of these characteristics from the compounds found in reed canary grass samples, and co-chromotographing with them.

were prepared and used for quantitative analysis in this study. Quantities were usually above the lower level.

Ten microliters (μ l) of the alkaloid extract were spotted on silica gel (G) plate at widely spaced intervals, and equal volumes of each series of standard solutions of different concentrations of tryptamine hydrochloride were placed in the gaps on the plate. Then the chromatogram was developed in an ascending system unidirectionally up to a length of 15 cms. Each chromatogram was then taken out of the developing tank and allowed to sit for five minutes to dry off the solvent. Each chromatogram was sprayed with Ehrlich's reagent and left in the dark for 30 minutes for development of colours without heating. After the colours developed on the chromatogram, it was put on a light plate and areas of spots determined by laying a sheet of millimeter graph paper on the chromatogram, outlining the spot, and counting the square (Fig. 1). Areas were then plotted against weights, and the weight corresponding to area of the tryptamines was read from the graph. As the area varies according to experimental conditions, a separate graph for each set of experimental runs was prepared. The concentration of tryptamines in this procedure was calculated on the assumption of 37% extraction recovery (Woods and Clark, 1971 a). So the actual amounts present in the grass samples may be considerably more.

As the screening system did not separate the unmethoxylated compounds from the 5-methoxy compounds, it was not possible to conclude that the unmethoxylated compounds were absent. However, the spots were usually of the royal blue colour characteristic of the 5-methoxy compounds, and it was, therefore, concluded that the compounds usually present included the 5-methoxy derivatives and that as many as four tryptamines might be present. Moreover,

Figure 1: Chromatogram illustrating the method for
determining tryptamines in grass extract

Solvent - Methanol: strong ammonia (7:1)

Key: 2, 3, 4, 5 and 6 different loads of pure tryptamine
hydrochloride (.5, 1, 3, 5 and 7 $\mu\text{g}/\text{spot}$ respectively);
1 and 7 duplicate sample of grass extract.

the main purpose of the study was to make some estimation of the concentration of tryptamines rather than their number. In nearly all cases the difference between the presence or absence of tryptamines was marked, but occasionally samples having tryptamines present in only small quantities were found. These were recorded as "trace" quantities.

4. RESULTS AND OBSERVATIONS

4.1 Dry matter yields, tryptamines, and total N of certain plant species over the growing season

4.1.1 For plots not mown

Dry matter yields as g/m^2 , total nitrogen as % dry wt. and tryptamines as $\mu\text{g/g}$ dry wt. are presented in table II-A. All pasture species except reed canary grass lacked tryptamines at all sampling dates. In reed canary grass, tryptamines occurred in both upper and lower parts of the plant in the samples collected August 28; by September 22, they occurred only in the upper parts of the fast maturing shoots and by October 14, with tops frosted, tryptamines were not recorded. With the advance of season and maturity the concentration of tryptamines in reed canary grass decreased. Also tryptamines concentration in the upper parts (including actively growing tissues) was higher than the lower parts of reed canary grass.

Dry matter yields were generally higher in the early emerging stands at the first sampling, but the late emerging stands caught up and in subsequent sampling their dry matter yields were equal to or even higher than those of early emerging stands, probably because soil moisture became limiting in the early emerging hence higher and drier sites.

The total nitrogen in the upper parts (wherever partitioned) was much higher than in the lower parts in all pasture species, with the exception of two dry-range species. This difference was much higher at the time of first sampling but was reduced in the second and third samplings. In the two dry-range species, where regrowth from the base had already started, the total N

Table II-A: Dry weight and single determinations of total nitrogen and tryptamines for "unknown" plants of several species sampled on three dates in 1973 from two wet meadows and one dryland range, Kamloops, B.C.

Species	Site	Drying procedure	SAMPLING DATE								
			August 28			September 22			October 14		
			dry wt. g/m ²	Total N (% dry wt.)	Tryptamines µg/g dry wt.	dry wt. g/m ²	Total N (% dry wt.)	Tryptamines µg/g dry wt.	dry wt. g/m ²	Total N (% dry wt.)	Tryptamines µg/g dry wt.
<u>Equisetum</u> <u>fluviatile</u>	Late emerging, Tranquille meadow	a.d.	425.6	1.86	-	524.0	1.70	-	501.1	1.20	-
		f.d.-u	-	2.40	0.0	-	1.83	0.0	-	1.46	0.0
		f.d.-l	-	1.50	0.0	-	1.19	0.0	-	1.17	0.0
	Early emerging, Tranquille meadow	a.d.	525.2	1.94	-	512.4	1.65	-	526.5	1.23	-
		f.d.-u	-	2.39	0.0	-	1.75	0.0	-	1.19	0.0
		f.d.-l	-	1.56	0.0	-	1.25	0.0	-	1.03	0.0
<u>Sium</u> <u>suave</u>	Late emerging, Tranquille meadow	a.d.	673.5	1.79	-	770.3	1.41	-	701.4	1.09	-
		f.d.-u	-	1.97	0.0	-	1.61	0.0	-	0.57	0.0
		f.d.-l	-	1.32	0.0	-	1.50	0.0	-	0.51	0.0
	Early emerging, Tranquille meadow	a.d.	712.7	1.87	-	750.8	1.47	-	542.4	1.06	-
		f.d.-u	-	1.98	0.0	-	1.61	0.0	-	0.67	0.0
		f.d.-l	-	1.07	0.0	-	1.51	0.0	-	0.55	0.0
<u>Agropyron</u> <u>repens</u>	Early emerging, Tranquille meadow	a.d.	1051.4	0.93	-	-	-	-	584.2	1.02	-
		f.d.-u	-	1.44	0.0	-	-	-	-	0.73	0.0
		f.d.-l	-	0.56	0.0	-	-	-	-	0.58	0.0
<u>Carex</u> <u>rostrata</u>	Early emerging, Tranquille meadow	a.d.	543.5	1.78	-	-	-	-	-	-	-
		f.d.-u	-	2.37	0.0	-	1.67	0.0	-	0.84	0.0
		f.d.-l	-	1.65	0.0	-	1.15	0.0	-	0.67	0.0
<u>Phalaris</u> <u>arundinacea</u>	Early emerging, Tranquille meadow	a.d.	1121.8	1.68	-	-	-	-	-	-	-
		f.d.-u	-	1.59	88	-	1.12	64	-	0.68	0.0
		f.d.-l	-	0.68	42	-	0.53	0.0	-	0.33	0.0

Cont.

Table II-A: cont.

<u>Agropyron</u> <u>cristatum</u> and A. <u>spicatum</u>	Dryland Curry field	a.d.	63.2	0.53	-	-	-	-	-	-	-
		f.d.-u	-	0.54	0.0	-	0.50	-	-	0.31	0.0
		f.d.-l	-	0.54	0.0	-	0.51	-	-	0.55	0.0
<u>Agropyron</u> <u>spicatum</u>	Dryland Curry field	a.d.	55.9	0.59	-	-	-	-	-	-	-
		f.d.-u	-	0.52	0.0	-	0.0	-	-	0.49	0.0
		f.d.-l	-	0.60	0.0	-	0.0	-	-	0.55	0.0
<u>Carex</u> <u>rostrata</u>	Sub-irrigated, Whitcroft ranch	a.d.	-	-	-	692.5*	1.14*	-	-	-	-
C. rostrata, A. repens with other minor spp.	Sub-irrigated, Whitcroft ranch	a.d.	-	-	-	392.2*	1.10*	-	-	-	-

a.d. - air dried
f.d. - freeze dried
u - upper half
l - lower half

tryptamines were determined for freeze dried samples only

* leaf tips frozen

was a little higher in the lower parts than in the upper parts in the samples collected on October 14.

With the advance of season and maturity a decline was noted in the total N and dry matter yield of many species.

4.1.2 For plots mown (Aftermath)

Dry matter yields as g/m^2 , total N as % dry wt. and tryptamines as $\mu\text{g/g}$ dry wt. are given in table II-B. In this trial similar results are presented to that in Section 4.1.1. Again total N patterns are much the same as would be expected and explained in the previous section. The dry matter yield increased up to September 22 after which there was no increase. Tryptamines in very young aftermath reed canary grass harvested August 28 were very high and although they declined with advancing season and maturity they remained high until the last sampling. At Whitecroft meadow where sampling was done on plots frosted and not frosted of reed canary grass aftermath separated by only a few feet, high tryptamines levels were recorded in the aftermath not frosted, whereas in frosted materials tryptamines were not found (Figure 2).

4.2 The distribution and concentration of tryptamines and total N in various parts of the grass over the growing season

Two sites which differed greatly in soil and climate were chosen for this study (see Section 3.1.2). Tryptamines and total N were determined for different parts of reed canary grass; the results of these trials, at the University of B.C. Farm and at Tranquille meadows, are presented in tables III and IV.

At the University of B.C. Farm, where the plants were partitioned into

Table II-B: Dry weight and single determinations of total nitrogen and tryptamines for "aftermath" of several species sampled on three dates in 1973 from two wet meadows, Kamloops, B.C.

Species	Site	Drying procedure	SAMPLING DATE								
			August 28			September 22			October 14		
			dry wt. g/m ²	Total N (% dry wt.)	Tryptamines µg/g dry wt.	dry wt. g/m ²	Total N (% dry wt.)	Tryptamines µg/g dry wt.	dry wt. g/m ²	Total N (% dry wt.)	Tryptamines µg/g dry wt.
<u>Carex</u> <u>rostrata</u>	Tranquille meadow	a.d.	30.0	1.79	-	63.2	1.78	-	59.7	1.39	-
		f.d.	-	2.62	0.0	-	2.56	0.0	-	2.09	0.0
<u>Phalaris</u> <u>arundinacea</u>	Tranquille meadow	a.d.	49.5	2.56	-	139.5	2.45	-	172.0	2.10	-
		f.d.	-	3.32	170	-	2.84	110	-	2.55	74
<u>Agropyron</u> <u>repens</u>	Tranquille meadow	a.d.	51.8	2.06	-	141.3	1.97	-	141.8	1.83	-
		f.d.	-	2.97	0.0	-	2.75	0.0	-	1.65	0.0
<u>P. arundi-</u> <u>acea</u>	Whitecroft ranch	f.d.	-	-	-	-	2.60*	0.0*	-	-	-
	Whitecroft ranch	f.d.	-	-	-	-	3.60	130	-	-	-
<u>A. repens</u>	Whitecroft ranch	f.d.	-	-	-	-	3.13	0.0	-	-	-
<u>C. rostrata</u>	Whitecroft ranch	f.d.	-	-	-	-	1.70	0.0	-	-	-

a.d. - air dried
f.d. - freeze dried

tryptamines were determined for freeze dried samples only

* leaf tips frozen

54a.

Figure 2: Nitrogen and tryptamines in contiguous frosted and unfrosted samples of reed canary grass, Whitecroft Meadows, September 22, 1973.

Table III: Total nitrogen and tryptamines in reed canary grass parts
sampled at two plot locations, three dates at the University of B.C.

Plant part	SAMPLING DATE					
	June 6, 1973*		July 28, 1973**		October 20, 1973**	
	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.
Root and rhizome	0.86 0.76	0.0 0.0	0.88 0.79	0.0 0.0	0.92 0.92	0.0 0.0
Stem:						
lower half	0.22 0.23	0.0 0.0	- -	- -	0.26 0.28	0.0 0.0
upper half	0.83 0.84	0.0 0.0	- -	- -	0.37 0.38	0.0 0.0
Leaf blades:						
lower	1.79 1.81	0.0 0.0	- -	- -	2.07 2.08	40 trace
uppermost four	2.83 2.80	150 145	3.76 3.80	188 200	2.81 2.80	120 128
Leaf sheaths	1.03 0.98	0.0 0.0	- -	- -	0.90 0.91	0.0 0.0
Inflor- escences	1.42	0.0	- -	- -	- -	- -

* Flowering, (before first cutting)

** Vegetative (aftermath)

Table IV: Single determinations of total nitrogen and tryptamines in parts of unmown reed canary grass after early summer flooding, Tranquille Meadow, B.C.

Plant part	SAMPLING DATE					
	August 28, 1973		September 22, 1973		October 14, 1973	
	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.
Stem and sheath:						
lower half	0.44	0.0	0.49	0.0	0.41	0.0
upper half	0.69	0.0	0.57	0.0	0.48	0.0
Leaf blades:						
lower	2.39	trace	1.87	trace	1.49	0.0
uppermost four	2.73	116	2.20	70	1.68	44

different components (table III), tryptamines were mainly found in the uppermost four-leaf blades. In lower leaf blades the tryptamines were usually absent or present in trace amounts. Tryptamines were not found at any time in roots and rhizomes, upper or lower stem halves, leaf sheaths or inflorescences (Figure 3). Total N again was as expected, higher in the younger and actively metabolizing tissues than the older or more mature tissues. The data record a decline with advancing season in tryptamines and in total N in upper leaves and some other parts; it is to be noted that the four-leaf sample is somewhat arbitrary and that in late season some of the lower leaves of four are likely senescing. The data recorded are for two separate samplings and show the high degree of repeatability of the chemical determinations.

The data recorded in table IV for Tranquille meadows substantiate the results recorded in table III. High levels of tryptamines were recorded in the younger plant material (aftermath) collected on July 28.

4.3. Studies of N-nutrition of reed canary grass

It is of practical value to know something of the relationship of fertilizer nitrogen, to know the level of total nitrogen and the level of nitrogenous tryptamines in reed canary grass, particularly in view of the fact that great variability in the palatability of reed canary grass has been noted by ranchers and farmers after fertilizer applications have been made.

4.3.1 The effect of four rates of ammonium sulphate fertilizer on the concentration of tryptamines and total N in reed canary grass over the growing season

The data from this trial are presented in table V; the averages given

58a.

Figure 3: Nitrogen and tryptamines in parts of reed canary grass, averaged over three dates, University of B.C. Farm.

Table V: Total nitrogen and tryptamines in reed canary grass from Tranquille meadows sampled on two dates following one post-flooding application of ammonium sulfate on August 28, 1973

Ammonium Sulphate lbs. N/acre	SAMPLING DATE				SEASONAL MEANS	
	September 22, 1973		October 14, 1973		Total N (% dry wt.) $\bar{x}$ Ns	Tryptamines $\mu\text{g/g}$ dry wt. $\bar{x}$ Ts
	Total N (% dry wt.) $\bar{x}$ N	Tryptamines $\mu\text{g/g}$ dry wt. $\bar{x}$ T	Total N (% dry wt.) $\bar{x}$ N	Tryptamines $\mu\text{g/g}$ dry wt. $\bar{x}$ T		
0	2.98	108.0	2.76	77.0	2.87 c ¹	92.5 D
50	3.33	112.0	3.07	79.0	3.20 bc	95.3 C
100	3.36	127.0	3.00	90.5	3.33 ab	108.8 B
200	3.99	146.0	3.31	105.5	3.65 a	125.8 A
All-treatment means	3.49 a ²	123.1 A	3.03 b ²	88.0 B	-	-

Duncan's multiple range test. 1 & 2. Means within a group followed by the same letter(s) are not statistically significant at the 5% level of probability.

for total N and tryptamines in the table are from two variates. Seasonal and treatment means and Duncan's multiple range test are also given. The analysis of variance is given in Appendix 8.1, table I.

The uptake of nitrogen applied in the ammonium form on the acidic gleisol was a nearly linear reflection of fertilizer rate and all the treatment means were significantly different ($P < .05$); tryptamine concentration closely paralleled total N concentration and only slight declines in levels occurred by the time the season ended, around October 14. Significant declines were also noted in total N and tryptamine levels between the first and second sampling dates. These findings confirmed previous work by Barnes *et al.* (1971), Gallagher *et al.* (1966) and Simons (1970) with Phalaris species.

4.3.2 The effect of five rates of ammonium nitrate on the concentration of tryptamines, total N, and on dry matter yields of reed canary grass

Data for total N and tryptamines, and dry matter yields and total N for the samples collected before the setting up of the fertilizer trial are presented for a mature stand of reed canary grass in table VI-A and VI-B, respectively; these provide some information about the nature of the stand.

The results for the trial for total N and tryptamines, and dry matter yields and total N are summarized in table VII-A and VII-B, respectively. The data represents the average values of two replications in each case. Seasonal means, treatment means and Duncan's multiple range tests are given. The analysis of variance for total N and tryptamines, dry matter yields and total N data are given in Appendix 8.2, table II and III.

Initially tryptamine and total N concentrations appeared to be simply related to fertilizer rates, an observation recorded earlier by Frelich (1972);

Table VI-A: Total nitrogen and tryptamines in the uppermost four blades of reed canary grass, harvested June 19, 1973, Pitt Meadows, B.C.

Sample No.	Total N (% dry wt.)	Tryptamines $\mu\text{g/g}$ dry wt.
1	2.94	95
2	2.84	101

Table VI-B: Dry matter yield and total nitrogen of first cut hay, reed canary grass, harvested June 19, 1973, Pitt Meadows, B.C.

Sample No.	Dry matter yield g/m^2	Total N (% dry wt.)
1	934.4	1.73
2	977.5	1.62

Table VII-A: Effect of an application of ammonium nitrate fertilizer, June 21, 1973, on total nitrogen and tryptamines in uppermost four blades, of reed canary grass, grown at Pitt Meadows, B.C.

Ammonium Nitrate lbs. N/acre	SAMPLING DATE						SEASONAL MEANS	
	August 26		September 29		October 29		Total N (% dry wt.) $\bar{x} N_s$	Tryptamines $\mu\text{g/g dry wt.}$ $\bar{x} T_s$
	Total N (% dry wt.)	Tryptamines $\mu\text{g/g dry wt.}$	Total N (% dry wt.)	Tryptamines $\mu\text{g/g dry wt.}$	Total N (% dry wt.)	Tryptamines $\mu\text{g/g dry wt.}$		
	$(\bar{x} N)$	$(\bar{x} T)$	$(\bar{x} N)$	$(\bar{x} T)$	$(\bar{x} N)$	$(\bar{x} T)$		
0	2.75	172.0	2.49	123.5	2.05	105.0	2.43 e ¹	133.5 C
50	2.89	169.5	2.68	124.0	2.39	109.0	2.65 d	134.2 C
100	3.10	174.5	3.00	124.5	2.74	111.5	2.95 c	136.8 BC
200	3.46	183.5	3.25	135.0	2.94	110.0	3.21 b	142.8 B
300	3.93	200.5	3.55	142.0	3.25	107.5	3.57 a	150.0 A
All treatment means	3.22 c ²	180.0 C	2.99 b ²	129.8 B	2.67 a ²	108.6 A	-	-

Duncan's multiple range test. 1 & 2

Means within a group followed by the same letter(s) are not statistically significant at 5% level of probability.

Table VII-B: Effect of an application of ammonium nitrate, June 21, 1973,
on dry matter yield and total nitrogen for reed canary grass,
Pitt Meadows, B.C.; harvest date, August 26, 1973

Ammonium Nitrate lbs. N/acre	Dry Matter Yield g/M^2 $\bar{x}$ dry matter	Total N (% dry wt.) $\bar{x}$ N
0	153.1 d ¹	1.44 D
50	165.0 d	1.55 CD
100	197.1 c	1.70 C
200	227.2 b	2.09 B
300	309.3 a	2.40 A

Duncan's multiple range test.

¹ Means within a group followed by the
same letter(s) are not statistically
significant at 5% level of probability.

but as the season's end approached (third sampling) rate of fertilizer application continued to be reflected in the total N concentration while tryptamine concentration declined to a uniform level under all rates of ammonium nitrate application. Significant decline in the levels of total N and tryptamine was noted for three harvests (averaged over five N-rates and two replicates); while in case of seasonal means, significant differences were noted between all treatments for total N but not so for tryptamine; where significant differences were noted only at higher fertilizer rates (table VII-A).

Dry matter yield and total N (table VII-B) also showed increases with increasing rates of applied N from 0 to 300 pounds per acre, but significant differences were noted at higher levels of applied N only.

4.3.2.1 The possible relationship between total N and tryptamine levels

The data (table VII-A) provide an interesting relationship between the total N and tryptamine levels of plant tissue. When tryptamine levels (seasonal means) are plotted against the total N in the plant tissue corresponding to five N-rates, it appeared (Figure 4) that the total N content initially increased more with very little change in tryptamine levels at lower levels of applied N, but with the increase in N-rate, both the entities increased more or less linearly.

4.3.3 The effect of N-source and N-rate on total N, tryptamines and dry matter yields of reed canary grass

Two sites, viz. Pitt Meadows and University of B.C. Farm were chosen for the trials (Section 3.1). The influence of five N-sources at five rates on the concentration of tryptamines, dry matter yields and total N in the

Figure 4: Tryptamine concentration in the uppermost four blades of reed canary grass as a reflection of plant nitrogen concentration following the post-flooding application of ammonium nitrate, Pitt Meadows.

plant tissue was determined. The percent total N was determined separately in samples collected for tryptamines and dry matter yield because of a hypothesis that a relationship might exist between total N and other two entities, particularly the N-containing tryptamines. Sequential sampling at Pitt Meadows, provided information on the effect of season and maturity on total N content and tryptamine levels in the plant tissue.

4.3.3.1 At Pitt Meadows

Data for total N and tryptamines, dry matter yield and total N for mature reed canary grass collected before the fertilizer trial was laid out, are presented in table VIII-A and VIII-B; an indication is given of the levels of the above components in the stand prior to fertilization.

Data from the fertilizer trial on total N and tryptamines, dry matter yield and total N are presented in table IX and XIII, respectively and are the average of two replications in each case. Source means, treatment means and harvest means and Duncan's multiple range test are also given. The analysis of variance for total N, tryptamines, dry matter yield and total N are given in Appendix 8.3, tables IV, V and VI.

4.3.3.1.1 The effect of N-source on total N and tryptamines

The N-source main effect is significant ($P \leq .05$) for both total N and tryptamines (Appendix 8.3, tables IV and V). The differences between mean N totals for the several N-sources are significant except those between urea and ammonium nitrate (table IX). Simple linear regression, in which different rates of applied N from different N-sources constituted the independent variable and the total N content the dependent variable, shows that the

Table VIII-A: Total nitrogen and tryptamines in the uppermost four blades of reed canary grass, harvested June 18, 1973, Pitt Meadows, B.C.

Sample No.	Total N. (% dry wt.)	Tryptamines $\mu\text{g/g}$ dry wt.
1	2.35	68
2	2.51	80
3	2.36	80
4	2.38	72
5	2.70	82

Table VIII-B: Dry matter yield and total nitrogen of first cut hay, reed canary grass, harvested June 18, 1973, Pitt Meadows, B.C.

Sample No.	Dry matter yield g/m^2	Total N (% dry wt.)
1	737.1	1.09
2	680.4	0.88
3	538.7	0.89
4	623.7	0.91
5	546.9	1.24

Table IX: Effect of an application of five kinds of fertilizer applied at five rates June 21, 1973 on the total nitrogen and tryptamines of the uppermost four leaves of reed canary grass, Pitt Meadows, B.C. harvested on three dates in the season, 1973.

Rate lbs. N/acre	Date of Sampling	SOURCE of NITROGEN										Treatment Means (All Sources)	
		Cynamid		Amm. Nitrate		Urea		Amm. Sulphate		Sod. Nitrate			
		Total N (% dry wt) $\bar{x}$ N	Tryptamines μ g/g dry wt. $\bar{x}$ T	Total N (% dry wt) $\bar{x}$ N	Tryptamines μ g/g dry wt. $\bar{x}$ T	Total N (% dry wt) $\bar{x}$ N	Tryptamines μ g/g dry wt. $\bar{x}$ T	Total N (% dry wt) $\bar{x}$ N	Tryptamines μ g/g dry wt. $\bar{x}$ T	Total N (% dry wt) $\bar{x}$ N	Tryptamines μ g/g dry wt. $\bar{x}$ T	Total N (% dry wt) $\bar{x}$ N _T	Tryptamines μ g/g dry wt. $\bar{x}$ T _T
0	Aug. 18	2.59	168.0	2.54	171.0	2.57	176.0	2.52	171.0	2.60	174.0	2.31 e ¹	136.1 C
	Sep. 28	2.39	125.5	2.30	131.0	2.35	127.5	2.27	126.0	2.37	128.5		
	Oct. 28	2.09	106.0	2.00	110.0	2.04	111.0	2.0	109.0	2.05	107.0		
50	Aug. 18	2.80	169.0	2.64	179.0	2.65	178.0	2.87	173.5	2.79	177.0	2.52 d	138.2 BC
	Sep. 28	2.50	128.5	2.53	127.5	2.45	126.5	2.59	125.5	2.69	128.0		
	Oct. 28	2.14	104.0	2.29	115.0	2.29	118.0	2.32	111.5	2.25	111.5		
100	Aug. 18	2.94	169.0	2.98	174.0	3.06	179.0	3.15	174.5	2.90	183.0	2.72 c	139.1 BC
	Sep. 28	2.61	129.5	2.83	130.5	2.69	131.0	2.97	127.0	2.66	130.0		
	Oct. 28	2.28	107.5	2.46	113.5	2.40	117.0	2.60	110.5	2.37	111.0		
200	Aug. 18	2.99	171.0	3.58	176.0	3.46	189.0	3.70	192.5	3.40	172.5	3.07 b	141.2 B
	Sep. 28	2.73	122.0	3.19	131.5	3.15	124.0	3.35	138.5	2.99	131.5		
	Oct. 28	2.49	112.5	2.78	116.5	2.73	109.5	2.99	123.0	2.54	108.0		
300	Aug. 18	2.96	175.5	3.79	196.5	3.69	206.0	4.00	221.5	3.62	174.5	3.30 a	148.2 A
	Sep. 28	2.89	125.0	3.52	128.0	3.42	132.5	3.67	152.5	3.12	130.0		
	Oct. 28	2.68	108.0	3.07	112.0	3.01	115.5	3.31	133.0	2.83	112.5		
Source Means (All Dates)		2.60 c ²	134.7 A	2.83 d ²	140.8 CD	2.79 d ²	142.7 BC	2.95 b ²	146.0 B	2.74 a ²	138.6 D	-	-

MEANS DATEWISE (All treatments and all sources)

Date of Sampling	Total N (% dry wt.)	Tryptamines μ g/g dry wt.
Aug. 18	3.07 c ³	179.6 C
Sep. 28	2.81 b	129.5 B
Oct. 28	2.48 a	112.5 A

Duncan's multiple range test. 1 & 2 & 3.
Means within a group followed by the same letter(s) are not statistically significant at 5% level of probability.

highest responses were given by plants receiving N in the form of NH_4^+ and lowest by those receiving NO_3^- -N, and that those receiving N from both sources are intermediate (Figure 5-A).

The mean concentration of tryptamines (averaged over three harvests and five N-rates) in plants receiving ammonium-N is significantly higher ($P < .05$) than those receiving nitrate -N.

Simple linear regression, with N-rates from different N-sources independent, and tryptamines levels in the plant tissue dependent, showed that plants supplied N from ammonium sulphate gave the highest response in tryptamine level, particularly at higher levels of applied N, and those receiving N from sodium nitrate gave the lowest response; those receiving N from ammonium nitrate fertilizer were intermediate (Figure 5-B). Earlier work by Frelich (1972) also showed that ammonium sources gave higher yields of indole alkaloids than nitrate sources in reed canary grass grown in solution culture.

4.3.3.1.2 The effect of N-rate on total N and tryptamines

The effects of N-rate on total N and tryptamines are significant ($P < .05$) (Appendix 8.3, tables IV and V). With increasing levels of applied N, the mean total N in the plant tissue (averaged over five N sources, two replications and three harvests) increased almost linearly and significant increases were recorded with each increase in level of applied N. For tryptamines, however, little increase was noted at the lower levels of applied N; significant increases occurred only at 200 and 300 pounds of applied N per acre. These results are in keeping with the earlier work on reed canary grass reported by Marten (1973) where substantial increases in total indole alkaloids occurred with quite high levels of N applications.

Figure 5: Simple linear regression showing the effect of increasing rates of three kinds of fertilizer on (A) total nitrogen and on (B) tryptamines, in the uppermost four blades of reed canary grass, Pitt Meadows (points determined from regression equations).

■—■—■ (NH₄)₂SO₄
 ●—●—● NH₄NO₃
 ▲—▲—▲ NaNO₃

4.3.3.1.3 The effect of season and maturity on total N and tryptamines

With the advance of season and growth, significant declines in mean total N and mean tryptamines levels (averaged over five N-sources, five rates and two replications) were recorded (table IX).

4.3.3.1.4 N-source x rate interaction for total N and tryptamines

The interactions between N sources and N-rates for total N and for tryptamines are significant ($P \leq .05$) (Appendix 8.3, tables IV and V). Although, increases in mean total N and mean tryptamine levels started with the rising levels of applied N, significant differences at a particular level of applied N from different N-sources for these two entities, and, particularly, for tryptamines, occurred only at higher levels of N-application (table X).

4.3.3.1.5 N-source x harvesting date (D) interaction for total N and tryptamines

The interaction between N-sources and harvesting dates is not significant for total N but is significant ($P \leq .05$) for tryptamines (Appendix 8.3, tables IV and V). At the time of first harvesting, significant differences occurred in the mean tryptamines levels (averaged over five N rates and two replications) among plants receiving N from three fertilizers (ammonium sulphate, urea and cyanamid) and also from those receiving N from the other two fertilizers; significant differences in tryptamine levels did not occur between plants receiving N from ammonium nitrate and sodium nitrate. At the time of second harvest, ammonium sulphate was the only N-source giving a significant increase in the levels of tryptamines over other N-sources; by

Table X: Effect of nitrogen sources and nitrogen rates on total nitrogen and tryptamines of the uppermost four leaves of reed canary grass, averaged over two reps and three harvests, Pitt Meadows, B.C.

Source of Nitrogen	NITROGEN RATE (LBS. PER ACRE)									
	<u>0</u>		<u>50</u>		<u>100</u>		<u>200</u>		<u>300</u>	
	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.
Cynamid	2.36 mno ¹	133.2 f	2.48 klm	133.8 ef	2.61 hijk	135.3 def	2.74 fgh	135.2 def	2.84 ef	136.2 def
Ammonium nitrate	2.28 o	137.3 def	2.49 klm	140.5 cdef	2.76 fg	139.3 cdef	3.18 c	141.3 cde	3.46 b	145.5 bc
Urea	2.32 o	138.2 cdef	2.46 lmn	140.8 cdef	2.71 fghi	142.3 cd	3.11 c	140.8 cdef	3.37 b	151.3 b
Ammonium sulphate	2.26 o	135.3 def	2.60 ijkl	136.8 def	2.91 de	137.3 def	3.35 b	151.5 b	3.66 a	169.0 a
Sodium nitrate	2.34 no	136.5 def	2.58 jke	138.8 cdef	2.64 ghij	141.3 cde	2.97 d	137.3 def	3.19 c	139.0 cdef

Duncan's multiple range test.

¹ Means followed by the same letter(s) within a group are not statistically significant at 5% level of probability.

by the time of the third harvest, significant differences occurred in levels of tryptamines of plants given ammonium and nitrate N-sources but not of plants within a source itself (table XI).

4.3.3.1.6 N-rate x harvesting date interaction for total N and tryptamines

The interactions between N-rate and harvesting date are significant ($P \leq .05$) for both total N and tryptamines (Appendix 8.3, table IV and V). Significant differences in mean total N (averaged over five N sources and two replications), however, occurred at each level of applied N at all harvests; significant declines in mean total N were recorded at each N-rate with the advance in season and in growth. In plant samples given the two highest N rates (200 and 300 pounds N per acre) mean tryptamines levels were significantly different from each other and also significantly different from the other three lower N rates at first harvesting time; for subsequent harvests the differences in mean tryptamines levels occurred in plants receiving the lowest and the highest N rates only (table XII). For mean total N on the other hand, as expected on a priori grounds, significant declines occurred with the advance in season; however, significant differences were recorded at each harvest between plants receiving different N-rates. Figures 6-A and 6-B depict very clearly, in a comparative way, these declines in total N and tryptamine levels in plants at three rates of applied N (0, 100 and 300 pounds N per acre).

4.3.3.1.7 N-source x rate x harvesting date interaction for total N and tryptamines

The interactions between N-sources, rate and harvesting date are non-significant for total N but significant for tryptamines (Appendix 8.3, table IV)

Table XI: Effect of nitrogen sources on tryptamines of uppermost four leaves of reed canary grass, harvested on three dates during the season, averaged over two reps of five rates of nitrogen, Pitt Meadows, B.C.

Source of Nitrogen	Tryptamines, $\mu\text{g/g}$ dry wt.		
	<u>Harvesting Date</u>		
	August, 1973	September, 1973	October, 1973
Cynamid	170.5 d ¹	126.1 f	107.6 i
Ammonium nitrate	179.3 e	129.7 f	113.4 gh
Urea	185.6 c	128.3 f	114.2 g
Ammonium sulphate	186.6 b	133.9 a	117.5 g
Sodium nitrate	176.2 e	129.6 f	110.0 hi

Duncan's multiple range test.

¹ Means followed by the same letter(s) are not statistically significant at 5% level of probability.

Table XII: Effect of nitrogen rates on total nitrogen and tryptamines of uppermost four leaves of reed canary grass, harvested on three dates during the season, averaged over two reps of five sources of nitrogen, Pitt Meadows, B.C.

Harvesting Date	NITROGEN RATE (LBS. N PER ACRE)									
	0		50		100		200		300	
	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.	Total N (% dry wt.)	Tryptamines µg/g dry wt.
Aug. '73	2.56 k ¹	172.0 c	2.75 j	175.3 c	3.00 i	175.9 c	3.42 h	180.2 b	3.61 g	194.8 a
Sept. '73	2.34 f	127.7 e	2.55 k	127.2 e	2.75 j	129.6 de	3.08 e	129.5 de	3.32 d	133.6 d
Oct. '73	2.04 c	108.6 h	2.26 b	112.0 gh	2.42 a	111.9 gh	2.70 j	114.0 fg	2.98 i	116.2 f

Duncan's multiple range test.

¹ Means followed by the same letter(s) within a group are not statistically significant at 5% level of probability.

Figure 6: The interaction between rates of nitrogen fertilizers and harvesting dates for (A) total nitrogen and (B) tryptamines, in the uppermost four blades of reed canary grass, Pitt Meadows.

■—■—■ 300 LBS N PER ACRE
 ●—●—● 100 LBS N PER ACRE
 ▲—▲—▲ 0 LBS N PER ACRE

4.3.3.1.8 The effect of N-sources on dry matter yield and total N

The main effects of N-sources on dry matter yield and total N are significant ($P \leq .05$) (Appendix 8.3, table VI). Dry matter yields (averaged over five N rates and two replications) were significantly higher for plants supplied with ammonium sources of N than for those supplied with nitrate-N; ammonium sulphate gave the highest yields of all the fertilizers (table XIII). For total N a similar pattern was recorded (described in Section 4.3.3.1.1). Similar trends are usual and are reported by other workers (Nowakowski and Cunningham, 1966; Barker and Bradfield, 1963; Stephens, 1960).

4.3.3.1.9 The effect of N-rates on dry matter yield and total N

The effects of N-rates on dry matter yields and total N are significant ($P \leq .05$) (Appendix 8.3, table VI). The mean dry matter yields and mean total N in plant tissue (averaged over five N-sources and two replications) increased significantly with increasing rates of N application (table XIII). These results are in keeping with the previous findings by many workers who obtained significant positive correlations between yield and percentage N with increasing rates of N applications in cereals and forages (Tyner, 1946; Hera, 1967; Goswami and Willcox, 1969; Mengel, 1969; Harms and Tucker, 1973) and in reed canary grass (Dean and Clark, 1973). In most instances yield increased linearly with increasing N until very high levels of N are applied.

4.3.3.1.10 N-source x rate interaction for dry matter yield and total N

The interactions between N-sources and rates are significant ($P \leq .05$) for both dry matter yield and total N (Appendix 8.3, table VI). The mean dry matter yields and total N of reed canary grass did not differ much

Table XIII: Effect of an application of five kinds of fertilizer applied at five rates on June 21, 1973 on dry matter yields and total nitrogen for reed canary grass, Pitt Meadows, B.C.; harvest, August 18, 1973

Rate lbs. N/ acre	SOURCE of NITROGEN										Treatment Means (All Sources)	
	Cynamid		Amn. Nitrate		Urea		Amn. Sulphate		Sod. Nitrate		Dry Matter (% dry wt.) g/M ² x D.M. _T x N _T	
	Dry Matter	Total N	Dry Matter	Total N	Dry Matter	Total N	Dry Matter	Total N	Dry Matter	Total N		
	(% dry wt.)	(% dry wt.)	(% dry wt.)	(% dry wt.)	(% dry wt.)	(% dry wt.)	(% dry wt.)	(% dry wt.)	(% dry wt.)	(% dry wt.)		
	$\frac{g}{M^2}$	$\bar{x}$ N	$\frac{g}{M^2}$	$\bar{x}$ N	$\frac{g}{M^2}$	$\bar{x}$ N	$\frac{g}{M^2}$	$\bar{x}$ N	$\frac{g}{M^2}$	$\bar{x}$ N		
	x D.M.	x N	x D.M.	x N	x D.M.	x N	x D.M.	x N	x D.M.	x N		
0	276.1	1.96	266.8	1.93	273.5	1.94	268.0	1.90	278.3	1.94	272.5 e ¹	1.93 E
50	294.9	2.05	306.7	2.07	302.0	2.07	303.9	2.08	302.45	1.96	302.0 d	2.05 D
100	309.1	2.10	329.9	2.43	342.8	2.23	346.8	2.38	332.7	2.03	332.2 c	2.23 C
200	365.1	2.32	371.7	2.84	390.9	2.54	395.2	2.87	370.9	2.61	378.7 b	2.63 B
300	406.4	2.55	377.1	3.08	424.6	2.78	453.1	3.29	298.8	2.75	392.0 a	2.89 A
Source Means	330.3 d ²	2.19 C	330.4 d ²	2.47 AB	346.7 c ²	2.31 BC	353.4 b ²	2.50 A	316.6 a ²	2.26 C	-	-

Duncan's multiple range test. 1 & 2

Means within a group followed by the same letter(s) are not statistically significant at 5% level of probability.

between sources at lower rates of N applications; but with increasing rates of N they generally differed significantly between ammonium and nitrate-N sources (table XIV). Regression analysis with N-rates independent and dry matter yields dependent (Figure 7-A) and N-rates independent and total N dependent (Figure 7-B), for three N-sources, showed that there were no clear cut differences among N-rates for dry matter yields or total N at lower N-rates but significant differences occurred at higher N-rates; ammonium sulphate gave the highest response, sodium nitrate the lowest, and ammonium nitrate an intermediate response. Both dry matter yields and total N increased almost linearly with increasing rates of N from all N-sources.

4.3.3.2 At University of B.C. Farm

Results from the fertilizer trial for total N and tryptamines, dry matter yields and total N are presented in tables XV and XVI. Source means, treatment means and Duncan's multiple range tests are given. The analyses of variance for total N, tryptamine, dry matter yields and total N are given in Appendix 8.4, tables VII and VIII.

4.3.3.2.1 The effect of N-source on total N and tryptamines

The N-source main effects are significant ($P < .05$) for both total N and tryptamines (Appendix 8.4, table VII). The mean total N (averaged over five N-rates) was significantly higher in plants supplied with N from the nitrate source than in those supplied with N from the ammonium sources. The mean total N content was highest in plants supplied with sodium nitrate, followed in order by those supplied with ammonium nitrate, ammonium sulphate, urea and

Table XIV: Effect of nitrogen sources and nitrogen rates on dry matter yield and total nitrogen of reed canary grass, averaged over two reps, Pitt Meadows, B.C.

Source of Nitrogen	NITROGEN RATE (LBS. N PER ACRE)									
	<u>0</u>		<u>50</u>		<u>100</u>		<u>200</u>		<u>300</u>	
	Dry Matter Yield g/m ²	Total N (% dry wt.)	Dry Matter Yield g/m ²	Total N (% dry wt.)	Dry Matter Yield g/m ²	Total N (% dry wt.)	Dry Matter Yield g/m ²	Total N (% dry wt.)	Dry Matter Yield g/m ²	Total N (% dry wt.)
Cynamid	276.1 klm ¹	1.96 j	294.9 jkl	2.05 ij	309.1 ij	2.10 ij	365.1 fg	2.32 gh	406.4 bc	2.55 ef
Ammonium nitrate	266.8 m	1.93 j	306.7 j	2.07 ij	329.9 hi	2.43 efg	371.7 ef	2.84 c	377.1 def	3.08 b
Urea	273.5 lm	1.94 j	302.0 j	2.07 ij	342.8 gh	2.23 hi	390.9 cde	2.54 ef	424.6 b	2.78 cd
Ammonium sulphate	268.0 lm	1.90 j	303.9 j	2.08 ij	346.8 gh	2.38 fgh	395.2 cd	2.87 c	453.1 a	3.29 a
Sodium nitrate	278.3 klm	1.94 j	302.5 j	1.96 j	332.7 h	2.03 j	370.9 ef	2.61 de	298.8 jk	2.75 cd

Duncan's multiple range test.

¹ Means followed by the same letter(s) within a group are not statistically significant at 5% level of probability.

Figure 7: Simple linear regression showing the effect of increasing rates of three kinds of fertilizer on (A) dry matter yield and on (B) total nitrogen, reed canary grass, Pitt Meadows (points determined from regression equations).

—■— (NH₄)₂SO₄
 —●— NH₄NO₃
 —▲— NaNO₃

Table XV: Effect of five sources of nitrogen applied at five rates on total nitrogen and tryptamines in the upper parts of summer sown, autumn harvested reed canary grass, totem field, University of British Columbia

Rate lbs. N/acre	SOURCE of NITROGEN											
	Cynamid		Urea		Amm. Nitrate		Sod. Nitrate		Amm. Sulphate		Treatment Mean (All Sources)	
	Total N	Tryptamines	Total N	Tryptamines	Total N	Tryptamines	Total N	Tryptamines	Total N	Tryptamines	Total N	Tryptamines
	(% dry wt.)	µg/g dry wt.	(% dry wt.)	µg/g dry wt.	(% dry wt.)	µg/g dry wt.	(% dry wt.)	µg/g dry wt.	(% dry wt.)	µg/g dry wt.	(% dry wt.)	µg/g dry wt.
0	3.08	37	3.02	36	3.16	36	3.11	38	3.09	38	3.09 d ¹	37.0 A
50	3.24	38	3.29	38	3.39	39	3.40	38	3.36	39	3.34 d	38.4 A
100	3.39	40	3.69	40	3.95	35	4.09	42	3.81	42	3.79 c	39.8 A
200	3.94	36	3.94	39	4.39	36	4.60	40	4.02	48	4.18 b	39.8 A
300	3.81	39	4.34	42	4.80	40	5.03	39	4.47	57	4.49 a	43.4 A
Source Means	3.49 c ²	38.0 B	3.67 c ²	39.0 B	3.94 ab ²	37.2 B	4.05 a ²	39.4 B	3.75 bc	44.8 A	-	-

Duncan's multiple range test.

¹ & ² Means within a group followed by the same letter(s) are not statistically significant at 5% level of probability.

Table XVI: Effect of five sources of Nitrogen applied at five rates on dry matter yields and total nitrogen of summer sown, autumn harvested reed canary grass, totem field, University of British Columbia

Rate lbs. N/acre	SOURCE of NITROGEN										Treatment Means (All Sources)	
	Cynamid		Urea		Amm. Nitrate		Sod. Nitrate		Amm. Sulphate		Dry Matter Yield g/M ²	Total N (% dry wt.)
	Dry Matter Yield g/M ²	Total N (% dry wt.)	Dry Matter Yield g/M ²	Total N (% dry wt.)	Dry Matter Yield g/M ²	Total N (% dry wt.)	Dry Matter Yield g/M ²	Total N (% dry wt.)	Dry Matter Yield g/M ²	Total N (% dry wt.)		
0	124.4	2.37	118.5	2.44	121.5	2.35	125.8	2.38	130.0	2.24	124.0 e ¹	2.36 C
50	150.5	2.40	155.0	2.46	160.4	2.49	165.5	2.48	158.0	2.44	157.9 d	2.45 C
100	229.9	2.54	242.5	2.56	286.0	2.64	255.2	2.70	463.0	2.58	293.9 c	2.60 B
200	322.4	2.56	411.4	2.64	437.3	2.76	387.8	2.84	651.8	2.69	442.1 b	2.70 B
300	368.0	2.63	587.1	2.86	603.4	2.96	492.3	3.08	731.8	2.84	556.5 a	3.87 A
Source Means	237.6 b ²	2.50 C	302.9 b ²	2.59 ABC	321.7 b ²	2.64 AB	285.3 b ²	2.70 A	426.9 a ²	2.56 BC	-	-

Duncan's multiple range test.

¹ & ² Means within a group followed by the same letter(s) are not statistically significant at 5% level of probability.

cynamid. The earlier work on barley by Michael et al. (1965) is supported (cited from Mengel, 1969).

The mean tryptamine content (averaged over five N-rates) was significantly higher in plants supplied with ammonium sulphate than in plants supplied N from other N-sources; no significant differences in tryptamine levels occurred in plants supplied with ammonium nitrate, urea, sodium nitrate or cynamid (table XV).

4.3.3.2.2 The effect of N-rate on total N and tryptamines

The effect of N-rates is significant ($P < .05$) for total N but non-significant for tryptamines (Appendix 8.4, table VII). With increasing rates of applied N, total N in the plant tissue increased almost linearly (table XV).

4.3.3.2.3 The effect of N-source on dry matter yield and total N

The N-source main effects are significant ($P < .05$) for both dry matter yields and total N (Appendix 8.4, table VIII). The mean dry matter yields (averaged over five N-rates) were significantly higher in plants receiving ammonium sulphate than in those receiving other N-sources; no significant differences in mean yields occurred in plants receiving N from the other four fertilizers (table XV). The earlier work by Stephens (1960), who found that ammonium sulphate was the most effective fertilizer in giving higher yields over other N-sources, is supported. For total N, similar results were obtained (described in Section 4.3.3.2.1).

4.3.3.2.4 The effect of N-rate on dry matter yield and total N

The effects of N-rates are significant ($P < .05$) for both dry matter

yields and total N (Appendix 8.4, table VIII). The mean dry matter yields and mean total N (averaged over five N-sources) of reed canary grass increased almost linearly with increasing rates of applied N (table XVI).

5. DISCUSSION

5.1 Presence or absence of tryptamines in important plant species of the BPE meadows

Reed canary grass was the only species (of ten important grasses and forbs screened) in the Tranquille and Whitecroft meadows containing notable quantities of tryptamines (Section 4.1.1 and 4.1.2). Snieckus (1968) observed that the great majority of the simple alkaloids are found in dicotyledonous plants so that the occurrence of these alkaloids in reed canary grass, a monocotyledonous plant, is by no means singular; it offers speculation on possible departures from the usual in nitrogen metabolism. The observation receives some support from the review of alkaloid containing plants, U.S. Dept. of Agriculture Tech. Bull. 1961.

5.2 The distribution and concentration of tryptamines in various plant parts, and at different stages of growth of reed canary grass

Results (Section 4.2) showed clearly that tryptamines were almost wholly present and at all times in the uppermost four leaf blades, while the lowermost leaves did not contain tryptamines or contained them only in trace amounts at all times. Tryptamines were not recorded in leaf sheaths, inflorescences, stems and roots-and-rhizomes. These results are in accord with the studies of Minnesota workers (Marten, 1973) who reported that alkaloids in reed canary grass are confined largely to the leaf blades. This hypothesis is in keeping with the finding of Bowden and Marion (1951) who found that gramine in barley leaves is elaborated from tryptophan. Unanswered is the matter of the role tryptamines play in rapidly growing, metabolically active, photosynthetic tissues.

Higher levels of tryptamines were recorded in the aftermath (Section 4.1.2, tables II-A and II-B; Section 4.3.2, tables VI-A, VII-A; Section 4.3.3.1, tables VIII-A and IX) than the mature grass. Moreover, tryptamines were found in much higher concentrations in the upper halves of reed canary grass than the lower halves or even absent (Section 4.1.1, table II) with the advance of season and growth. Also with the advance of maturity (growth) and season the concentration of tryptamines in reed canary grass declined significantly. The above trends were noted in reed canary grass at Tranquille Meadows, Whitecroft meadows, Pitt Meadows and at the University of B.C. Farm, irrespective of the wide variations in soil and climatic factors. These results agree with the earlier work on Phalaris and on other grass species (Gallagher et al., 1966; Gentry et al., 1969; Simons, 1970; Marten, 1973). The above results suggest that tryptamine levels, largely irrespective of variations in the soil and climate, are an expression of growth in active chlorophyllous tissue; Mothes (1955, 1960) and Marten (1973) suggested this earlier. Perhaps studies with chlorotic or achlorophyllous tissues might aid in determining tryptamine association with leaf growth per se or indirectly with the photosynthetic process.

The reduction of tryptamines with plant maturity probably indicates that some other plant compounds were accumulating at a rate faster than tryptamines, thereby creating a dilution effect. Nowacki (1963) suggested that the variation in lupine alkaloids during development might be the result of nonenzymatic transformation. Regardless of the cause, it must be noted that considerable variation in the tryptamines concentration of reed canary grass does occur among the plant parts and also within the plant part itself during development.

5.3 The effect of N-nutrition on tryptamines

Nitrogen nutrition plays an important role in increasing the concentration of tryptamines in reed canary grass. Both N-source and N-rate gave significant ($P \leq .05$) increases in tryptamine levels. When different sources were compared (Section 4.3.3) it was found that N-source, and not N-rate, played the most important role in increasing the concentration of tryptamines in reed canary grass. The mean concentration of tryptamines in plants supplied N from ammonium sulphate was higher than concentration in those plants supplied N from urea, ammonium nitrate, sodium nitrate and cyanamid. Plants given N in the form of NH_4^+ gave the highest increases in tryptamines levels and those given N in the form of NO_3^- gave none or very little increases in tryptamines, while those given N in forms of NH_4^+ and NO_3^- both gave intermediate response. Moreover, significant increases in tryptamines levels occurred only with relatively quite high levels of applied N. These results are in agreement to those of Frelich (1972) and Marten (1973) where they found that ammonium sources gave higher yields of indole alkaloids than nitrate sources and significant increases occurred only at quite high levels of applied N to reed canary grass. Actually ammonium sulphate was the only single source which gave notable increases in tryptamine levels in reed canary grass at all the sampling dates. With urea or ammonium nitrate, notable increases in tryptamine levels in reed canary grass were found in the first and second samplings, and in first sampling respectively. At any sampling time, no increase or very little was recorded in tryptamine levels in plants given cyanamid or sodium nitrate. Similarly no increase or very little increase was recorded in tryptamine levels in second and third samplings, and in third sampling, in plants given ammonium nitrate or urea respectively.

The increases in tryptamines occurred in all youthful chlorophyllous tissue as it was produced; their relative level of increase declined with the advance of season (Section 4.3.3.1, table IX). At Pitt Meadows where ammonium nitrate was the only N source in one of the trials, notable increases in tryptamine levels occurred in the first two samplings only (Section 4.3.2, table VII-A) and none or very little in the third one.

From trials with fertilizers three pertinent points became apparent:

(1) significant increases occurred in tryptamines in plants supplied N from NH_4^+ sources while no increase, or very little, was recorded in plants supplied N from NO_3^- sources; (2) with the advance in season, the relative increases among different N-rates within a single source declined; and (3) significant increases in tryptamines concentration in reed canary grass occurred only with relatively high rates of applied N.

The greater increase in the concentration of tryptamines in plants supplied with NH_4^+ than those supplied with NO_3^- source is probably due to the maintenance of N in the NH_4^+ form in the soils under wetland and poorly drained conditions and more direct use of NH_4^+ in tryptamine (alkaloid) formation. The urea-N undoubtedly was partially available to the plant in NH_4^+ form ($\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} - (\text{NH}_4)_2\text{CO}_3$), and it, along with ammonium sulphate, caused greater tryptamine concentration in the grass than did sodium nitrate or cyanamid. However, N from urea-N resulted in mean tryptamine concentration little, but not (statistically) significantly, higher than that from ammonium nitrate-N. This may be due to some nitrification of $(\text{NH}_4)_2\text{CO}_3$, thereby supplying both NH_4^+ and NO_3^- to the plant.

The decline in relative increases in tryptamine concentration among different N-rates within a NH_4^+ -N source with the advance of season may be explained on the basis of some nitrification taking place and thereby depletion

of NH_4^+ -N in soil under wetland conditions with the passage of time and, also partly due to advance in growth, thereby giving dilution effect. Also the difference in response of different fertilizers in increasing tryptamines from one harvesting date to another may be due to a difference in their nitrification rate under wetland conditions. Ammonium sulphate, due to its acidic properties, might have maintained its NH_4 -N longer, particularly under wetland or poorly drained soils as were in these studies (Section 3.1); it gave the highest increases in tryptamines in plants at all harvests at Tranquille meadows and at Pitt Meadows. This was also true at the University Farm where soils are well drained but slightly acidic; ammonium sulphate was the only N source (among the five fertilizers) which gave notable increases in tryptamine concentration in reed canary grass. Quite interesting results were noted in the case of ammonium nitrate applied on the same date under slightly different soil conditions (Section 3.1.3.3) at Pitt Meadows; in one trial, it gave notable increases in tryptamine concentrations in reed canary grass for the first two harvests (Section 4.3.2, table VII-A) whereas in the second trial (Section 4.3.3.1, table IX) notable increases in tryptamines were recorded for the first harvest only. This again seems to be due to difference in nitrification rates under different soil conditions as suggested by Pesek (1964). Little increase in tryptamine concentration in plants supplied with nitrate sources wherever recorded may be due to NH_4^+ ions present in soils, particularly under grassland soils as reported by Stevenson (1964).

The synthesis of many alkaloids is known to begin with amino acids. For example, the immediate precursor to the alkaloids gramine and tryptamine found in reed canary grass is the amino acid tryptophan (O'Donovan and Leete, 1963). As suggested by these investigators and also as suggested by Frelich (1972),

there are two probable routes by which tryptophan may be involved in the formation of these particular alkaloids; one, directly following its synthesis and/or two, indirectly through protein synthesis and subsequent protein breakdown to tryptophan. All of the essential amino acids including tryptophan have been reported in higher concentration in plants supplied with $\text{NH}_4\text{-N}$ than those with $\text{NO}_3\text{-N}$ (Bekmukhamedova, 1961).

The direct use of $\text{NH}_4\text{-N}$ for tryptamines (alkaloids) formation is probably the result of several factors: (1) a readily-available supply of ammonia to the plant under wet, poorly drained acidic soils (particularly grassland soils); (2) a large reserve of ammonia in the plant; (3) a continual replenishment of the plant's supply through rapid uptake by the roots; and (4) an excessive build-up of tryptophan beyond the amount that can be used in protein synthesis. Due to the last reason it also becomes easy to understand why significant increases in tryptamines levels in reed canary grass occur at relatively higher levels of applied N.

On the other hand there may be less direct incorporation of $\text{NO}_3\text{-N}$ in tryptamines (alkaloids) because: (1) nitrates are only slowly reduced to ammonia in the plant, and (2) any nitrate that is reduced to ammonia is promptly used in amino acid synthesis and subsequently protein synthesis. Thus, $\text{NO}_3\text{-N}$ incorporation into tryptamines (alkaloids) may occur mostly indirectly with the main pathways going into protein synthesis and degradation; on the other hand more of $\text{NH}_4\text{-N}$ incorporated into tryptamines (alkaloids) may be directly by way of tryptophan.

There is no biochemical information indicating whether the indirect or direct route of alkaloid formation generally occurs more frequently. However, investigators have shown that N supplied to Atropa, Nicotiana and

Papaver in the form of ammonium salts favoured alkaloid synthesis when compared to nitrates (Section 2.4).

These results, if extrapolated to other wetland meadows having the same soil and climatic conditions in British Columbia or other areas, indicate that the form of N applied to the soil could conceivably make a substantial difference in the levels of tryptamines and other alkaloids in reed canary grass and thereby in its toxicity to the livestock. Therefore, it may be recommended that reed canary grass commonly growing under wetland conditions should not be fertilized with " NH_4^+ fertilizers".

5.4 The effect of N-nutrition on dry matter yield and total N

Significant increases ($P \leq .05$) were recorded in dry matter yields and total N of reed canary grass for N-sources as well as for N-rates (Section 4.3). These findings are in keeping with the earlier work on reed canary grass (Goplen et al., 1972). Both dry matter yield and total N increased almost linearly with increasing rates of applied N, which again is in agreement with the earlier work on cereals and forages and also on reed canary grass (Section 2.3.2). When different sources were compared among each other, it was found that both dry matter yields and total N were higher in plants supplied with NH_4 -N source than those supplied with a NO_3 -N source under wetland conditions (table XIII). These results are in agreement with those reported previously by other workers (Nowakowski and Cunningham, 1966; Barker and Bradfield, 1963; Stephens, 1960). Under well drained conditions at the University of B.C. Farm, dry matter yields were still higher in those plants supplied with NH_4 -N than those with NO_3 -N but total N in the plants was higher in the latter group. The difference in total N in the two different

situations may be due to: (1) leaching and loss of nitrate N under wetland conditions; (2) the dilution effect of higher yields with $\text{NH}_4\text{-N}$; and (3) the over-riding second effect by the first one under the wetland situations.

5.5 The possible relationship of total N and tryptamines in the plant

With increasing rates of applied N both total N and tryptamines in the plant increased almost linearly. At lower levels of applied N, total N increased relatively more than tryptamines in the plant tissue, but at higher levels of applied N both components increased almost linearly (Section 4.3.2). The positive influence of N-nutrition on the concentration of tryptamines (alkaloids) and the positive inter-relationship between total N and tryptamines (alkaloids) within a plant seems understandable when one considers Robinson's (1968) partial definition of an alkaloid ("a group of naturally occurring organic compounds containing nitrogen") and James (1950) suggested role of ammonium-N and nitrate-N in plants ("ammonium-N is probably used directly in alkaloid synthesis by plants, whereas nitrate-N is more functional for overall growth"). In addition basicity is common property of alkaloids, which is traceable to the presence of ammonium groups. Therefore, any alteration of plant's N-nutrition should very likely affect its concentration of alkaloids.

5.6 The effect of frost on total N and tryptamines

In reed canary grass, touched by frost at the Whitecroft meadow, there was in a matter of two days at most a decline of over 30 percent in total N and also a total disappearance of tryptamines; on the other hand plants only a few feet away, which were not hit by the frost, contained quite high levels of total N and tryptamines (Section 4.1.2). No tryptamine was recorded in

reed canary grass at Tranquille meadows in samples collected on October 14 only a few days after frost.

According to Pate (1969), nitrogen, especially among other nutrient elements is retrieved with noticeable efficiency; through progressive analyses he showed that as much as 70 to 90 percent of leaf protein is withdrawn before a leaf dies or abscisses. The disappearance of tryptamines after a killing frost from the plant tissue may be due to one or many of the factors mentioned earlier (Section 2.2.2). It cannot be stated what happens to tryptamines after a killing frost; further research would be profitable. It might be remarked that much has been learned about the metabolism of alkaloids, but their functions in the plant, if any, are still largely unknown.

5.7 Relationship between alkaloids in reed canary grass and BPE

Reed canary grass (Phalaris arundinacea L.) is a circumboreal species but as a native species it occurred only sparingly in B.C. Introduction of European strains has led to the widespread occurrence in all areas of the province with a developed agriculture. It may be of consequence that BPE has been identified as a condition only in recent decades in western Canada and in the intermountain states. It is a matter of record (Brink - personal communication) that Tisdale and Brink established the first plantings of reed canary grass in the Tranquille meadows in the mid 1930's, that T.W. Willis as director of the C.D.A research station in the mid 1940's extended the plantings and that, in recent years, reed canary grass is established over all parts of (i.e. most) the meadows to which it is adapted. It is also to be noted that BPE does not occur in the Chilcotin area of B.C. where reed canary grass does not occur. Although this kind of evidence may be spurious, observations of this nature may be helpful in clearing up some of the obscurities associated

with the seemingly erratic occurrence of the condition.

One of the earlier observations relating to the occurrence of BPE in B.C. was the fact that cases did not occur after frost struck the meadows (several reports to the B.C. Minister of Agriculture). Can it be dismissed, merely as coincidence, that tryptamines disappear from reed canary grass tips soon after they are frosted? Can it be dismissed that BPE develops on the "young" aftermath pasturage of reed canary grass when tryptamine levels are high and not commonly on older pasturage? High levels of soil and plant fixed-nitrogen, also, it is observed, tend to be associated with higher tryptamine levels in the plant and also with BPE incidence.

All the above factors and the results of this study constitute evidence of an association between tryptamines in reed canary grass and the occurrence of BPE on our Interior B.C. pastures. However, this does not rule out the possible involvement of other indole compounds and particularly tryptophan which may be present in other grass species, and active work is underway in this direction (Zowell, 1973). Conclusive evidence for the association can only be suggested by feeding experiments with livestock. Also research is needed in rumen physiology on the relationship of the rumen flora and fauna of ruminants to alkaloid assimilation. It is significant that putrescine is absorbed to pass through the rumen walls*. It cannot be overlooked that tryptamines are somewhat volatile and could be included in eructed gasses and taken into the animal lung. Also it cannot be overlooked that BPE occurs seldomly in animals pasturing on fairly succulent forages at the Coast or the Interior and that a sharp change from dry to succulent forage as a common associated condition is probably associated with a sharp change in rumen flora and fauna.

6. SUMMARY

The research literature records that pulmonary emphysema, a moderately serious, common detrimental condition occurring in the bovines of the Interior of B.C. and of the Intermountain U.S.A. can be induced by "feeding" tryptophan and probably tryptamines or related compounds. Tryptamines are known to occur in reed canary grass, a widely used forage, especially in our area. Furthermore, reed canary grass is a common component of meadows associated with BPE. Although cause of BPE cannot be directly assigned to tryptamines in reed canary grass, without animal feeding trials, a study of distribution of tryptamines in species in the BPE meadows and of influences modifying their concentration with the intent of strengthening the case for association is warranted and is reported here.

On all meadows studied, throughout the growing season, reed canary grass was the only species with notable concentrations of tryptamines; tryptamines were not found in six other common meadows species. Tryptamines and total N recorded were much higher in the upper halves of reed canary grass than those in the lower halves. Tryptamines promptly disappeared from reed canary grass after sharp frost. Also the levels of total N and tryptamine found in younger plants/aftermath was much higher than those in the older/mature reed canary grass.

In reed canary grass tryptamines were mainly found in the uppermost four leaf blades while lower leaf blades contained none or very minor amounts of tryptamines. Tryptamines were not found in inflorescences, leaf sheaths, stems, roots-and-rhizomes on any sampling date. Tryptamine levels declined notably with the advance of season and maturity.

The influence of N-rate and N-source on the concentration of tryptamines

and total N was tested at four locations under field conditions while the effect on dry matter yields and total N were recorded at only three out of the four locations. Both N-rate and N-source gave significant changes in dry matter yields, total N and tryptamines of reed canary grass at all the locations except at the University of B.C. Farm, where with N-rates significant increases were not recorded in tryptamine levels in the plant tissue. The mean concentration of tryptamines in plants supplied with ammonium sulphate was significantly ($P < .05$) higher than in those supplied with urea, ammonium nitrate, sodium nitrate and cyanamid. Plants given N in the form of NH_4^+ gave the highest increases in tryptamine concentration and in those supplied with N in the form of NO_3^- , no increase or very little in tryptamines were recorded, while in those supplied with both forms of N, intermediate responses were recorded. The great increase in tryptamines concentration in plants supplied with $\text{NH}_4\text{-N}$ compared to $\text{NO}_3\text{-N}$ was postulated to be the result of a more direct use of $\text{NH}_4\text{-N}$ for tryptamines formation. Significant increases in tryptamine concentration occurred only with high levels of applied N; this was thought to be due to utilization of N first in the compounds required by the plant for its sustained growth.

Initially tryptamine and total N concentrations appeared to be simply related to fertilizer rates, particularly for $\text{NH}_4\text{-N}$ sources; but as the season's end approached rate of application continued to be reflected in the total N while tryptamines concentration declined to a uniform level under all rates of applied N. Actually ammonium sulphate was the only single source which gave notable increases in tryptamines concentration at all the sampling dates; but even in this case notable declines occurred. Also some other N-sources gave inconsistent increases in tryptamine concentrations with the

advance in season. This was considered due to the slow nitrification of NH_4 -N sources under wetland and poorly drained condition and due to the difference in nitrification rates of different N-sources.

In all the trials notable declines were recorded in tryptamine and total N concentration in the plant tissue with the advance in season and maturity. A positive relationship was recorded between these two components in the plant tissue over the growing season, particularly in plants fertilized with ammonium-N sources.

The dry matter yields and total N increased linearly with increasing rates of applied N in all the trials. Under wetland and slightly acidic conditions higher yield and total N were recorded for reed canary grass supplied with ammonium-N sources than for grass supplied with nitrate-N sources; under well drained slightly acidic soils, dry matter yield was higher for plants given ammonium-N sources, while total N concentration was higher in plants supplied with nitrate-N sources.

The results of this study, and the earlier literature, provided circumstantial evidence for a close association of tryptamines in reed canary grass and the occurrence of BPE on B.C. pastures. It was also concluded that the toxicity/death loss of reed canary grass could conceivably be reduced and palatability improved through the wise use of fertilizers. There is a need for further feeding trials and for an exploration of the relationship between tryptamines or other indole alkaloids in reed canary grass and the rumen flora, especially at the time cattle are moved from dry ranges to lowland lush pastures.

REFERENCES CITED

- Aasen, A.J., C.C.J. Culvenor, E.P. Finnie, A.W. Kellock and L.W. Smith, 1968. Alkaloids as a possible cause of rye grass staggers in grazing livestock. *Austr. J. Agr. Res.*, 20:71-86.
- Alove, A.S., 1961. The effect of various forms of nitrogen and potassium on yield and alkaloid accumulation in belladonna. *Fiziol. Rast.*, 8:673-680. (Abstract) *Hort. Abstr.*, NO. 3526, 32:445, 1962.
- Ammann, K., 1939. Das Lungenemphysem des Pferdes und Rindes. *Archiv. f. Wissensch. w. Pract. Tiercheilkunde*, 74:348-401.
- Anon., 1921-1953. Progress reports by the division of illustration stations, Dominion Experimental Farms, Dominion of Canada, Dept. of Agr.
- Anon., 1961. U.S.D.A. Tech. Bull. No. 1234, Washington, D.C. (1961): Alkaloid-bearing plants and their contained alkaloids.
- Anon., 1963. 58th Annual Report, Dept. of Agr., B.C., pp. 52.
- Anon., 1963. Proc. Sympos. Acute Bovine Pulmonary Emphysema (ABPE). Univ. of Wyoming, July 12-13, 1963.
- Anon., 1971. Preliminary soil map for part of the Kamloops Research Station Rangeland. Prepared by C.D.A. Soil Survey, Vancouver, B.C., 1971.
- Audette, R.C.S., J. Bolan, H.M. Vijayanagar, R. Bilous and K. Clark, 1969. Phytochemical Investigation of Manitoba Plants II. A gas-liquid chromatographic screening technique for the identification of the alkaloids of Phalaris species. *J. Chromatog.*, 43:295-307.
- Audette, R.C.S., H.M. Vijayanagar, J. Bolan and K.W. Clark, 1970. Phytochemical investigation of Manitoba plants. I. A new indole alkaloid and associated alkaloids from Phalaris arundinacea. *Can. J. Chem.*, 48:149-155.
- Barker, A.V. and Richard Bradfield, 1963. Effect of potassium and nitrogen on the free amino acid content of corn plants. *Agron. J.*, 55:465-470.
- Barker, J.R., 1948. Physiological dysfunctions and metabolic disorders in grazing animals. Hypocalcaemia, hypomagnesaemia, hypophosphataemia and fog fever. *Rep. Proc. Conf. Grass and Animal Health*: 86-92, discussion: 93-110.
- Barnes, R.F., A.B. Simons and G.C. Marten, 1971. Evaluation of selected clones of Phalaris arundinacea. II. Indole alkaloid derivatives. *Agron. J.*, 63:507-509.
- Bates, T.E. and S.L. Tisdale, 1957. The movement of nitrate-nitrogen through columns of coarse textured soil materials. *Soil Sci. Soc. Amer. Proc.*, 21:525-528.

- Beacher, R.L. and J.P. Wells. 1960. Rice fertilizer studies 1952 to 1958. Ark. Agri. Exp. Sta. Bull. 620.
- Beaumont, A.B., R.E. Stitt and R.S. Snell. 1933. Some factors affecting the palatability of pasture plants. J. Amer. Soc. Agron., 25:123-128.
- Begg, H. and W.A. Whiteford. 1948. Acute interstitial pulmonary emphysema of bovines. Vet. Rec., 60:135.
- Bekmukhamedova, N.B., 1961. Synthetic activity of the root system of corn during ammonia and nitrate nutrition. Fiziol. Rast., 8:51-54.
- Bennet, W.D., 1963. A note on the effect of nitrate and phosphate on the perloline content of perennial rye grass. New Zeal. J. Agr. Res., 6:310-313.
- Black, C.A., 1968. Nitrogen, p. 405-557. In: Soil plant relationships. Black, C.A. (ed.), John Wiley and Sons, Inc., New York. London. Sydney.
- Blackeslee, L.Y., C.M. Harrison and J.F. Davis, 1956. Ewe and lamb gains on brome and reed canary grass pastures. Mich. Agr. Exp. Sta. Quart. Bull., 39:230-235.
- Blood, D.C., 1962. A typical interstitial pneumonia of cattle. Can. Vet. J., 3:40-47.
- Bourd, J. 1953. Contribution à l'étude de l'emphysème des regains chez les bovines. Thesis Vétérinaire, Toulouse, France (cited by Foucher, 1960).
- Bowden, K. and L. Marion, 1951. The biogenesis of alkaloids. IV. The formation of gramine from tryptophan in barley. V. Radio-autographs of barley leaves fed with tryptophan-B-C14. J. Chem., 29:1037-1042, 1043-1045.
- Brink, V.C. 1964. In report to the Deputy Minister, B.C. Dept. of Agri., Victoria, B.C., (Unpublished).
- Broadbent, F.E. 1951. Denitrification in some California soils. Soil Sci., 72:129-137.
- Burton, G.W. and J.E. Jackson. 1962. Effect of rate and frequency of applying six nitrogen sources on Coastal Bermuda grass. Agron. J., 54:40-43.
- Bush, L.P., J.A. Boling, G. Allen and R.C. Buckner. 1972. Inhibitory effects of perloline to remen fermentation in vitro. Crop Sci., 12:277-279.
- Butler, W.J., 1940. Pulmonary emphysema or asthema in cattle. Lederle Vet. Bull, 9:11.

- Carlson, J.R., I.A. Dyer and R.J. Johnson, 1968. The tryptophan-induced interstitial pulmonary emphysema in cattle. *Am. J. Vet. Res.* 29(10): 1983-1989.
- Carlson, J.R., M.T. Yokoyama and E.O. Dickinson, 1972. Induction of pulmonary emphysema in cattle and goats with 3-methyl-indole. *Science* 176:298-299.
- Clapp, J.B. 1962. Pulmonary emphysema project - Tranquille meadows, Kamloops; by livestock branch, B.C. Dept. of Agri.
- Clapp, J.B. 1963. Pulmonary emphysema project - Tranquille meadows, Kamloops; by livestock branch, B.C. Dept. of Agri.
- Clapp, J.B. 1964. Pulmonary emphysema project - Tranquille meadows, Kamloops; by livestock branch, B.C. Dept. of Agri.
- Conway, D.A. 1967. A typical interstitial pneumonia in calves. *Irish Vet. J.*, 21:86.
- Cote, F.T. 1944. Rape poisoning in cattle. *Can. J. Comp. Med.*, 8:38-41.
- Cromwell, B.T. 1937. Experiments on the synthesis of syoscyamine in Atropa belladonna. *Biochem. J.*, 31:551-559.
- Culvenor, C.C.J., R. Dal Bon and L.W. Smith. 1964. The occurrence of indole-alkylamine alkaloids in Phalaris tuberosa L. and P. arundinacea. *L. Austr. J. Chem.* 17:1301-1304.
- Cunningham, C.C. 1965. Acute Bovine pulmonary emphysema. Report B.C. Dept. Agri. Court House, Nelson, B.C. October 15, 1965.
- Davies, W. 1925. The relative palatability of reed canary grass. *J. Min. Agri.* 32:106-116.
- Dawson, R.F. 1938. Nitrogen nutrition and nicotine synthesis in tobacco. *Bot. Gaz.* 100:336-346.
- Dean, J.R. and K.W. Clark. 1972. Nitrogen fertilization of reed canary grass and its effects on production and mineral element content. *Can. J. Plant Sc.* 52(3):325-331.
- Delalande, Lecoq, Masure and Vigney. 1830. Obstruction du feuillet, observée sur les vaches laitières, vulgairement appelée dans nos campagnes empanchure, indigestion des regains. *Mémoires de la Société Vétérinaire du Calvados et de la Manche.* 1:109, 135 (cited by Foucher, 1960).
- De Maggio, A.E. 1961. Influence of various concentrations of nutrients on growth and alkaloid synthesis in Datura stramonium *J. Pharm. Sci.* 50:676-678.

- Demaree, G.E. and V.E. Tyler, Jr. 1956. The accumulation of Hordenine in the seedlings of Panicum miliaceum. J. Amer. Pharm. Ass. 45:421-423.
- Dickinson, E.O., G.R. Spencer and J.R. Gorham. 1967. Experimental induction of an acute respiratory syndrome in cattle resembling bovine pulmonary emphysema. The Vet. Rec. 80(16):487-490.
- Dimitrijevic, R. 1960. Belladonna as a cultivated plant. Arh. Poljopr. Nanke. 13(39):104-107. (Hort. Abstr. No. 4835. 31:614, 1962).
- Ehret, W.J. and J.G. Pienaar. 1967. An outbreak of atypical interstitial pneumonia of cattle. J.S. Afr. Vet. Med. Ass. 38:407.
- Escartin Foncilles, A. 1955. Enfisema pulmonar, histamina y antihistaminicos de sintesis. Veterinaria (Madrid). 19:545-550, 552-554. (Bibl. Agr. 20:60195, 1956).
- Fagan, T.W. and R.O. Davies. 1937. The nitrogen and mineral content of the produce of grassland. 4th Intern. Grassl. Cong., Gr. Brit. Rpt. 370-375.
- Fankhauser, R. and H. Luginbuhl. 1960. Chronic interstitial pneumonia and adenomatosis in cattle in the canton of Uri, Switzerland. Part I. Schweiz. Arch. Tierheilk. 102:47-58. (Vet. Bull. 31:512, 1961).
- Foucher, J. 1960. L'emphysème des regains dans le Marais Poitevin; essai de traitement. Thesis 45. Ecole Natl. Vét. Alfort. France. 76 pp.
- Frahn, J.L. and D.F. O'Keefe. 1971. The occurrence of tetrahydro-B-carbolines alkaloids in Phalaris tuberosa (Gramineae). Austr. J. Chem. 24:2189-2192.
- Frelich, J.R. 1972. Effect of environmental factors on indole alkaloids in reed canary grass (Phalaris arundinacea L.). Ph.D. thesis. University of Minnesota. 141 pp. (Order No. 73-10, 666). Univ. Microfilms, Ann Arbor, Michigan.
- Fried, M., F. Zsoldes, P.V. Vose and I.L. Shatokhin. 1965. Characterizing the nitrate and ammonium uptake process of rice roots by use of ^{15}N labelled ammonium nitrate. Physiol. Plant. 18:331-320.
- Gallagher, C.H., J.H. Koch and H. Hoffman. 1966. Diseases of sheep due to ingestion of Phalaris tuberosa. Austr. Vet. 42(Aug.):279-286.
- Gallagher, C.H., J.H. Koch and H. Hoffman. 1967. Deaths of ruminants grazing Phalaris tuberosa in Australia. Austr. Vet. J. 43(Nov.): 495-500.
- Gallagher, C.H., J.H. Koch, R.M. Moore and J.D. Steel. 1964. Toxicity of Phalaris tuberosa for sheep. Nature. 204 (Nov. 7):542-545.

- Gasser, J.K.R. and I.G. Iordanov. 1967. Effects of ammonium sulphate and calcium nitrate on the growth, yield and nitrogen uptake of barley, wheat and oats. *J. Agric. Sci.* 68:307-315.
- Gentry, C.E., L. Henson, R.C. Buckner and R.A. Chapman. 1968. Seasonal variation in and effect of nitrogen on the alkaloid content of tall fescue. *Proc. Assoc. Southern Agri. Workers, Inc.* Abstr. 65:53.
- Gentry, C.E., R.A. Chapman, L. Henson and R.C. Buckner. 1969. Factors affecting alkaloid content of tall fescue (Festuca arundinacea, Schreb.). *Agron. J.* 61:313-316.
- Gibbons, W.J. 1956. Pulmonary emphysema. *North Amer. Vet.* 37:98, 100.
- Gibbons, W.J. 1962. Bovine pulmonary emphysema. *Mod. Vet. Pract.* 43(8): 34-37.
- van Gils, J.H.J. 1951b. Acuut longemphyseem bij hit rund. Een pathologisch-anatomische studie. *Tijdschr. v. Diergeneesk.* 76:833-839. (Biol. abstr. 26:15887, 1952).
- Goodman, A.A. 1956. Bovine asthma. *North Am. Vet.* 37:850-852.
- Goplen, B.P., S.G. Bonin, W.E.P. Davis and R.M. MacVicar. 1963. Reed canary grass. *Can. Dept. Agri. Publ.* 805.
- Goswami, A.K. and J.S. Willcox. 1969. Effect of applying increasing levels of nitrogen to rye grass; amino acid composition of peptide and protein fractions, *Biblog. II. J. Sci. Fd. and Agric.* 20:596-599.
- Grüter, D. 1883. Lungen Emphysem bei Kühen. *Preussische Meth.* s40. (also *J. Brit. Vet. Med.* III, 1883, p. 83; cited by Amman, 1939).
- Harms, C.L. and B.B. Tucker. 1973. Influence of nitrogen fertilization and other factors on yield, prusic acid, nitrate and total nitrogen concentration of sudan grass cultivars. *Agron. J.* 65(1):21-26.
- Heath, M.E. and H.D. Hughes. 1962. Reed canary grass. pp. 243-250. In *FORAGES*, H.D. Hughes, M.E. Heath and D.S. Metcalfe (eds.). The Iowa State Univ. Press, Ames, Iowa, U.S.A.
- Hera, C. 1969. Soil and fertilizer nitrogen in specific plant components with emphasis on small grains. pp. 157-164. In *N-15 in Soil Plant Studies*. *Proc. Intern. Atomic Agency*, Vienna, 1971.
- Holland, W.D., A.L. Van Ryswyk and C.C. Kelley. 1957. Preliminary Rept. No. 1. Soil Survey of Pitt Meadows Municipality. *B.C.D.A., Kelowna, B.C.*, June, 1957.
- Hubbard, W.A. and H.H. Nicholson. 1968. Reed canary grass vs. grass-legume mixtures under irrigation as pasture for sheep. *J. Range Management.* 21:171-174.

- Hull, M.W. 1965. Hydrogen sulphide as rumen gas and its effects on lung. Proc. Sympos. on Acute Bovine Pulmonary Emphysema. Univ. of Wyoming. 1965.
- Hyslop, N. St. G. 1969. Development in Veterinary science - bovine pulmonary emphysema. The Can. Vet. J. 10(10):251-257.
- Jackson, J.E. and G.W. Burton. 1962. Influence of soil treatment and nitrogen placement on the utilization of urea nitrogen by Coastal Bermuda grass. Agron. J. 54:47-49.
- James, W.O. 1950. Alkaloids in the plant. pp. 16-86. In THE ALKALOID CHEMISTRY AND PHYSIOLOGY, Vol. 1, R.H.F. Manske and H.L. Holmes, eds.). Academic Press, Inc., New York, N.Y., U.S.A.
- Jarrett, W.F.H., W.I.M. McIntyre and G.M. Urquhart. 1953. Recent work on husk. "Cuffing pneumonia". Vet. Rec. 65:153-156.
- Jarrett, W.F.H., W.I.M. McIntyre and G.M. Urquhart. 1954. Husk in cattle. Vet. Rec. 66:665-676.
- Jarrett, W.F.H., W.I.M. McIntyre and G.M. Urquhart. 1957. The pathology of experimental bovine parasitic bronchitis. J. Path. Bact. 73:183.
- Jenkins, P.A. and J. Pepys. 1965. Fog fever precipitin (FLH) reaction to mouldy hay. Vet. Rec. 77:464-466.
- Klussendorf, R.C. 1954. Pulmonary emphysema. North Am. Vet. 35:745-746.
- Kresge, C.B. and D.P. Satchell. 1960. Gaseous loss of ammonia from nitrogen fertilizers applied to soils. Agron. J. 52:104-107.
- Lee, H.J. and R.E. Kuchel. 1953. The aetiology of "Phalaris staggers" in sheep. I. Preliminary observations of the preventive role of cobalt. Austr. J. Agric. Res. 4:88-89.
- Lee, H.J., R.E. Kuchel and R.F. Trowbridge. 1956. The aetiology of "Phalaris staggers" in sheep. Austr. J. Agric. Res. 7:333-334.
- Leslie, M.J.S. 1949. Fog fever. Vet. Rec. 61:228-229.
- Le Soeuf., H.D. 1948. Poisoning of sheep by Phalaris tuberosa. Austr. Vet. J. 24:12-13.
- Maclean, M.W. 1948. Acute interstitial pulmonary emphysema of bovines. Vet. Rec. 60:202.
- Maki, L.R. 1963. A review of the literature of the history and occurrence of bovine pulmonary emphysema. pp. 1-17. In Proc. Sympos. on Acute Bovine Pulmonary Emphysema. Laramie, Wyoming, July 12-13, 1963.

- Maki, L.R. and J.O. Tucker. 1965. Acute bovine pulmonary emphysema. Proc. Sympos. on Acute Bovine Pulmonary Emphysema. Univ. of Wyoming, 1965.
- Marten, G.C. 1973. Alkaloids in reed canary grass. pp. 15-31. In "Anti-quality components of forages". Crop.Sc. Soc. of America. No. 4, 1973.
- Marten, G.C., A.B. Simons and J.R. Frelich. 1974. Alkaloids of reed canary grass as influenced by nutrient supply. Agron. J. 66:363-368.
- Marten, G.C. and J.D. Donker. 1968. Determinationants of pasture value of Phalaris arundinacea L. vs. Bromus intermis Leyss. Agron. J. 60:703-705.
- Marten, G.C. and M.E. Heath. 1973. Reed canary grass. Chap. 25. In FORAGES, M.E. Heath, D.S. Metcalf and R.F. Barnes (eds.). 3rd. ed. The Iowa State Univ. Press, Ames, Iowa, U.S.A.
- Marten, G.C., R.F. Barnes, A.B. Simons and F.J. Wooding. 1973. Alkaloids and palatability of Phalaris arundinacea L. grown in diverse environments. Agron. J. 65:199-201.
- McDonald, I.W. 1942. A "staggers" syndrome in sheep and cattle associated with grazing on Phalaris tuberosa. Austr. Vet. J. 18:182-189.
- Mengel, K. 1969. Effects of Nitrogen sources on the chemical components in plants with emphasis on small grains. pp. 239-248. In N-15 in Soil-Plant Studies. Proc. Intern. Atomic Agency, Vienna, 1971.
- Michels. 1865. Pneumatose des marain dans l'espèce bovine. Annales de médecine vétérinaire. p. 193 (cited by Foucher, 1960).
- Michna, M. and J. Szwadiak. 1964. The effect of boron and two forms of nitrogen fertilizers on the yield and morphine content in capsules of the poppy variety. Niebieski, K.M. Biul. Inst. Rusl. leezn. 10:138-145. (Hort. Abstr. No. 1619, 36:188, 1966).
- Milne, J.A. 1955. The occurrence of 'Phalaris staggers' in sheep. New Zeal. Vet. J. 3:119-121.
- Minnet, F.C. 1948. Panting in cattle. J. Am. Vet. Med. Assoc. 113:545-550.
- Moore, R.M., G.W. Arnold, R.J. Hutchings and H.W. Chapman. 1961. Poisoning of Marino sheep on Phalaris tuberosa L. pastures. Austr. J. Sc. 24: 88-89.
- Moore, R.M., J.D. Williams and Joyce Chia. 1966. Effect of environmental factors on alkaloids in Phalaris tuberosa. Int. Grassl. Cong. Proc. 10th (Helsinki, Finland). 6:524-527. Academic Press, New York, U.S.A.
- Moore, R.M., J.D. Williams and Joyce Chia. 1967. Factors affecting concentrations of dimethylated indole alkylamines in Phalaris aquatica L. Austr. J. Biol. Sci. 20:1131-1140.

- Moore, R.M. and R.J. Hutchings. 1967. Mortalities among sheep grazing Phalaris aquatica L. Austr. J. Exp. Agr. and Anim. Husb. 7:17-21.
- Mothes, K. 1955. Physiology of alkaloids. Ann. Rev. Pl. Physiol. 6:393-432.
- Mothes, K. 1960. Alkaloids in the plant. pp. 1-29. In THE ALKALOIDS, Vol. VI, R.H.F. Manske (ed.). Academic Press, New York, U.S.A.
- Moulton, J.E., C.E. Cornelius and B.I. Cobourn. 1963. Acute pulmonary emphysema in cattle. J. Am. Vet. Med. Ass. 142:133-137.
- Moulton, J.E., J.B. Harrold and M.A. Horning. 1961. Pulmonary emphysema in cattle. J. Am. Vet. Med. Assoc. 139:669-677.
- Nelson, D.W. and L.E. Sommers. 1973. Determination of total nitrogen in plant material. Agron. J. 65:109-112.
- Nickolic, R.T. 1960. Trials on growing Aconitum divergens. Acta. Pharm. Jugoslavica. 10:177-187. (Abstract) Biol. Abstr. Sect. D, 1961, 36, Abstr. 65, 855.
- Nowacki, E. 1963. Inheritance and biosynthesis of alkaloids in lupine. Genetica Polonica. 4(2):161-202.
- Nowakowski, T.Z. and R.K. Cunningham. 1966. Nitrogen fraction and soluble carbohydrates in Italian rye grass II. Effects of light intensity, form and level of nitrogen. J. Sci. Fd. Agric. 17:145-150.
- O'Donoghue, J.G. 1960. Bovine pulmonary emphysema. Canadian Vet. J. 1: 482-484.
- O'Donovan, D. and E. Leete. 1963. Biosynthesis of gramine: feeding experiments with tryptophan-B- (H^3, C^{14}) . J. Amer. Chem. Soc. 85:461-463.
- Omar, A.R. and D.A. Knich. 1966. Atypical interstitial pneumonia, a condition resembling fog fever, in young calves. Vet. Rec. 78:766-768.
- Oram, R.N. 1970. Genetic and environmental control of the amount and composition of toxins in Phalaris tuberosa L. pp. 785-788. In Int. Grassl. Congr. Proc. 11th. (Surfers' Paradise, Queensland, Australia).
- Oram, R.N. and J.D. Williams. 1967. Variation in concentration and composition of toxic alkaloids among strains of Phalaris tuberosa L. Nature. 213:945-947.
- Pate, J.S. 1969. Movement of nitrogenous solutes in plants. pp. 165-187. In N¹⁵ in Soil Plant Studies. Proc. Int. Atomic Energy Agency, Vienna, 1971.
- Pedini, B. 1956. Hemato-morphological and blood protein picture in cattle with emphysematus pneumolymphadenitis (cystic lymphogangliar pneumatosis). Soc. Ital. Sci. Vet. Atti. 10:567-571. (Bibl. Agr. 22:5753, 1958).

- Pellétier, S.W. 1970. The plant alkaloids - general information. pp. 1-10. In CHEMISTRY OF ALKALOIDS, S.W. Pelletier (ed.), Van Nostrand Reinhold Co., New York, Toronto, London and Melbourne.
- Pesek, John T., Jr. 1964. Comparing efficiency of nitrogen fertilizers. pp. 365-391. In FERTILIZER NITROGEN, ITS CHEMISTRY AND TECHNOLOGY, Vincent Sauchelli (ed.), ACS monograph No. 161, Reinhold Publishing Corporation, New York, London.
- Peterson, D.R. 1965. Bovine pulmonary emphysema caused by the plant Perilla frutescens. Proc. Sympos. on Acute Bovine Pulmonary Emphysema, Univ. of Wyoming.
- Pierson, R.E. 1956. Cow asthma or pulmonary emphysema increasing in Rocky Mountain area. Cattle Guard. 1:42.
- Priouzeau, M. 1954a. Intoxications alimentaires à retentissement pulmonaire, chez les bovidés. Rec. Méd. Veterinaire. 130:81-104. (Vet. Bull. 25: 156, 1955).
- Priouzeau, M. 1954b. La broncho-pneumonie emphysémateuse des bovidés. Rec. Méd. Vétérinaire. 130:223-231. (Vet. Bull. 25:2979, 1955).
- Quin, A.H. 1961. The syndrome of bovine pulmonary emphysema. Can. Vet. J. 2:80.
- Railsback, G.A. 1945. Idiopathic pulmonary emphysema of cattle. Vet. Med. 40:369-370.
- Railsback, G.A. 1948. Bovine pulmonary emphysema (panthers) in Arkansas cattle. Jen-Sal J. 31: September.
- Rainey, D.P., E.B. Smalley, M.H. Crump and F.M. Strong. 1965. Isolation of a salivation factor from Rhizoctonia leguminicola. Nature (London). 205:203.
- Rainey, J.W. 1958. A bovine respiratory disorder believed due to seed of wild white clover (Trifolium repens). Vet. Rec. 70:167-168, 169.
- Raney, W.A. 1960. The dominant role of nitrogen in leaching losses from soil in humid regions. Agron. J. 52:563-566.
- Rhoades, K.R., K.L. Heddleston and P.A. Rebers. 1967. Experimental hemorrhage septicemia: Gross and microscopic lesions resulting from acute infections and from endotoxin administration. Can. J. of Comp. Med. and Vet. Sci., Gardenvale, Que. 31(9):226-233.
- Robinson, Trevor. 1968. Alkaloids. Sci. Amer. 20:113-119.
- Rogler, G.A. 1944. Relative palatabilities of grasses under cultivation on the northern Great Plains. J. Amer. Soc. Agron. 36:487-496.

- Ruelke, O.C. and J.T. McCall. 1961. Evaluation of romphagrass for pasture. Agron. J. 53:406-407.
- Sabey, B.R., W.V. Bartholomew, R. Shaw and J. Pesek. 1956. Influence of temperature on nitrification in soils. Soil Sci. Soc. Amer. Proc. 20:357-360.
- Salisbury, P.J., P.L. Stovell and V.C. Brink. Fungi of autumn forage in wetland pasture of B.C. range areas - unpublished.
- Sauchelli, Vincent. 1964. The role of nitrogen in agriculture. pp. 1-9. In FERTILIZER NITROGEN, ITS CHEMISTRY AND TECHNOLOGY, Vincent Sauchelli (ed.), ACS monograph No. 161, Reinhold Publishing Corporation, Chapman and Hall Ltd., London.
- Schofield, F.W. 1924. Acute alveolar emphysema in calves. Ann. Rept. Ontario Vet. College. 40 pp.
- Schofield, F. 1941. Non-specific hemoglobinuria and acute pulmonary emphysema of cattle as essential enterotoxemias. J. Am. Vet. Med. Assoc. 99:28-32.
- Schofield, F. 1944. Acute bovine pulmonary emphysema. Ann. Rept. Ontario Vet. College, No. 29. 17 pp.
- Schofield, F. 1948. Acute pulmonary emphysema of cattle. J. Am. Vet. Med. Assoc. 112:254-259.
- Schwartzbeck, R.A., J.M. MacGregor and E.L. Schmidt. 1961. Gaseous nitrogen losses from nitrogen fertilized soils measured with infrared and mass spectroscopy. Soil Sci. Amer. Proc. 25:186-189.
- Seaton, V.A. 1958. Pulmonary adenomatosis in Iowa cattle. Am. J. Vet. Res. 19:600-609.
- Seekles, L. 1941. Beschouwingen naar aanleiding van functioneele stoornissen bij het paard, bekend als "grass sickness" en "forage poisoning", mede in verband met het vraagstuk der auto-intoxicatie bij groote huisdieren (paard en rund). Tijdschr. Diergeneesk. 68:109-131. (Biol. Abst. 20:20762, 1946).
- Seher, A. 1960. Eine neue Methode Zur qualitativen und quantitativen Analyse synthetischer Antioxydantien. Nahrung:466-478.
- Seher, A. 1961. Die Analyse von Tocopherolgemischen mit Hilfe der Dünnschicht-chromatographie. Mikrochim. Acta. 2:308-313.
- Shannon, P.V.R. and W.M. Leyshon. 1971. The structure and synthesis of a tetrahydro-B-carboline alkaloid from Phalaris arundinacea: Some new tetrahydro-B-carbolines. J. Chem. Soc. C16:2837-2839.
- Simons, A.B. 1970. Relationship of indole alkaloids to palatability of Phalaris arundinacea L. and influence of several factors on alkaloid concentration. Ph.D. Thesis. Univ. of Minnesota. 146 pp. (Order No. 71-18, 817). Univ. Microfilms, Ann Arbor, Mich. (Diss. Abstr. 32:39-B).

- Simons, A.B. and G.C. Marten. 1971. Relationship of indole alkaloids to palatability of Phalaris arundinacea L. A-ron. J. 63:915-919.
- Simpson, B.H., R.D. Jully and S.H.M. Thomas. 1969. Phalaris arundinacea as a cause of deaths and incoordination in sheep. New Zeal. Vet. J. 17:240-244.
- Snieckus, V. 1968. The distribution of indole alkaloids in plants. pp. 1-40. In THE ALKALOIDS, Vol. XI, R.H.F. Manske (ed.), Academic Press, New York, U.S.A.
- Stephens, D. 1960. Fertilizer experiments with phosphorus, nitrogen, and sulphur in Ghana. Emp. J. Exp. Agr. 28:151-164.
- Stevenson, F.J. 1964. Soil Nitrogen. pp. 18-39. In FERTILIZER NITROGEN, ITS CHEMISTRY AND TECHNOLOGY, Vincent Sauchelli (ed.), ACS monograph No. 161, Reinhold Publishing Corp., Chapman and Hall, Ltd., London, 1964.
- Sullivan, J.T. and R.J. Garber. 1947. Chemical composition of pasture plants with some reference to the dietary needs of grazing animals. Bull. No. 489, 61 pp., November, 1947. The Pennsylvania State College, School of Agriculture, Pennsylvania, U.S.A.
- Tucker, J.O. 1959. Pulmonary emphysema meeting minutes. Sheridan, Wyoming. Sept. 3-4, 1959. Mimeographed. Division of Vet. Sci., Univ. of Wyoming.
- Tucker, J.O. 1963. Acute pulmonary emphysema of cattle. Research - current status. Paper presented at the Intermountain Veterinary Medical Association meeting. Las Vegas, Nevada, January 25, 1963.
- Tucker, J.O. and L.R. Maki. 1962. Acute pulmonary emphysema of cattle I and II. Am. J. Vet. Res. 23:821, 824.
- Tyner, Edward H. 1946. The relation of corn yields to leaf nitrogen, phosphorus and potassium content. Soil Sci. Soc. Proc. 1946:317-325.
- Van Arsdell, W.J., G.A. Branaman, C.M. Harrison and J.F. Davis. 1954. Pasture results with steers on reed canary grass. Quart. Bull. Mich. Agr. Exp. Stn. 37(1):125-131.
- Van Ryswyk, A.L., W.A. Hubbard and J.E. Miltimore. 1973. Beef production potential and chemical composition of fertilized and unfertilized sedge hays grown on organic soil of interior B.C. Canada J. Anim. Sci. 53:181-186.
- Volk, G.M. 1959. Volatile losses of ammonia following surface application of urea to turf or bare soils. Agron. J. 51:746-749.
- Volk, G.M. and A.W. Sweat. 1955. Mobility of urea nitrogen applied to Florida soils. Proc. Soil Sci. Soc. of Florida. 15:117-123.
- Vose, P.B. 1959. The agronomic potentialities and problems of the reed canary grass, Phalaris arundinacea L. and P. tuberosa L. Herb. Abstr. 29:77-83.

- Watson, E.F. 1956. An outbreak of "Phalaris staggers" in sheep at Kojunup, Western Australia. J. Austr. Inst. Agric. Sci. 22:209-211.
- Wictor, C.E. 1952. Report of the Los Angeles County Livestock Dept., 1951-52, 56 pp. (Vet. Bull. 23:2729, 1952).
- Wilkinson, S. 1958. 5-Methoxy-N-Methyltryptamine: a new indole alkaloid from Phalaris arundinacea L. Chem. Soc. J. (London), Part II: 2079-2081.
- Williams, M., R.F. Barns and J.M. Cassidy. 1971. Characterization of alkaloids in palatable and unpalatable clones of Phalaris arundinacea L. Crop. Sci. 11:213-217.
- Woods, D.L. and K.W. Clark. 1971a. Variation in the content of tryptamines in clones of Phalaris arundinacea L. Crop. Sci. 11:121-122.
- Woods, D.L. and K.W. Clark. 1971b. Genetic control and seasonal variation of some alkaloids in reed canary grass. Can. J. Plant Sci. 5:323-329.
- Yates, G. Shelly. 1962. Toxicity of tall fescue forage: A Review. Economic Botany. 16:295-303.
- Zsoldos, F. and A. Jozsef. 1969. Ammonium and nitrate ion uptake by plants. pp. 81-89. In N-15 Soil-Plant Studies. Proc. Intern. Atomic Agency, Vienna, 1971.
- Zwoll, Vayne van. 1973. Relationship of range forage and livestock management practices to acute bovine pulmonary emphysema. Prog. Rept. 112 (Dec. 1973). Ore. State Univ. Extension Service.

APPENDIX 8.1

Table I: Analysis of variance (study 4.3.1) for total nitrogen and tryptamines in reed canary grass from Tranquille Meadows sampled on two dates following one post-flooding application of ammonium sulphate on August 28, 1973

Sources of Variation	df	S.S.	M.S.	F-test
<u>Total Nitrogen</u>				
Blocks (B) ¹	1	0.22326	0.22326	4.96
N-rate (R)	3	1.25200	0.41732	13.13*
Error A	3	0.09532	0.03177	0.71
Date (D)	1	0.84181	0.84181	18.72*
D x R	3	0.18787	0.06262	1.39
Error B	4	0.17987	0.04497	
Total	15	2.780133		
<u>Tryptamines</u>				
Blocks (B) ¹	1	85.5630	85.5630	1.57
N-rate (R)	3	2778.6900	926.2300	1270.26**
Error A	3	2.1875	0.7292	0.01
Date (D)	1	4935.0650	4935.0650	90.66**
D x R	3	54.6870	18.2290	0.33
Error B	4	217.7500	54.4375	
Total	15	8073.9425		

¹ The main effect N-rate was tested with the date x rate factor

* Significant ($P \leq .05$)

** Significant ($P \leq .01$)

APPENDIX 8.2

Table II: Analysis of variance (study 4.3.2) for total nitrogen and tryptamines in uppermost four blades of reed canary grass from Pitt Meadows sampled on three dates following an application of ammonium nitrate fertilizer, June 21, 1973

Source of Variation	df	S.S.	M.S.	F-test
<hr/>				
B1		<u>Total Nitrogen</u>		
Blocks (B) ¹	1	0.00085	0.0009	0.34
N-rate (R)	4	4.92047	1.2301	474.03**
Error A	4	0.01036	0.00259	1.03
Date (D)	2	1.53704	0.7685	305.37**
D x R	8	0.09179	0.0115	4.56*
Error B	10	0.02515	0.0025	
Total	29	6.58566		
<hr/>				
		<u>Tryptamines</u>		
Blocks (B) ¹	1	13.3333	13.3333	0.38
N-rate (R)	4	1157.4667	289.3667	17.54**
Error A	4	66.0000	16.5000	0.47
Date (D)	2	26891.4667	13445.7333	383.43**
D x R	8	720.5333	90.0667	2.57
Error B	10	350.0667	35.0667	
Total	29	29199.4667		

¹ The main effect N-rate was tested with the date x rate factor

* Significant ($P < .05$)

** Significant ($P < .01$)

APPENDIX 8.2

Table III: Analysis of variance (study 4.3.2) for dry matter yield and total nitrogen of reed canary grass, from Pitt Meadows, sampled on August 26, 1973 following an application of ammonium nitrate fertilizer, June 21, 1973

Source of variation	df	S.S.	M.S.	F-test
<u>Dry matter yield</u>				
Blocks (B)	1	62.5000	62.5000	0.60
N-rate (R)	4	38530.4800	9632.6200	92.80**
Error A	4	415.2000	103.8000	
Total	9	39008.1800		
<u>Total Nitrogen</u>				
Blocks (B)	1	0.00169	0.0017	0.23
N-rate (R)	4	1.28506	0.3213	44.24**
Error A	4	0.02906	0.0073	
Total	9	1.31581		

** Significant ($P < .01$)

APPENDIX 8.3

Table IV: Analysis of variance (study 4.3.3.1) for total nitrogen in uppermost four blades of reed canary grass from Pitt Meadows sampled on three dates following an application of five kinds of fertilizer applied at five rates, June 21, 1973

Source of Variation	df	S.S.	M.S.	F-test
N-source (S) ¹	4	1.9581	0.4895	108.38**
Error A	5	0.0226	0.0045	0.44
N-rate (R) ¹	44	19.4680	4.8671	475.61**
S x R	16	2.0522	0.1283	12.53**
Error B	20	0.2047	0.0102	1.47
Date	2	8.7624	4.3812	627.86**
D x S	8	0.0541	0.0068	0.97
D x R	8	0.1663	0.0208	2.98
D x R x S	32	0.3645	0.0114	1.63
Error C	50	0.3489	0.0070	
Total	149	33.4018		

¹ The main effects N-source and N-rate were tested with the date x source and date x rate interactions, respectively.

** Significant ($P \leq .01$)

APPENDIX 8.3

Table V: Analysis of variance (study 4.3.3.1) for tryptamines in uppermost four blades of reed canary grass from Pitt Meadows sampled on three dates following an application of five kinds of fertilizer applied at five rates, June 21, 1973

Source of Variation	df	S.S.	M.S.	F-test
N-source (S) ¹	4	2160.6673	540.1668	17.09**
Error A	5	158.0300	31.6070	1.04
N-rate (R) ¹	4	2594.3340	648.5835	21.31**
S x R	16	3348.9987	209.3124	6.88**
Error B	20	608.8000	30.4400	1.57
Date	2	121712.4140	60856.2070	3138.00**
D x S	8	547.4527	68.4316	3.53**
D x R	8	1189.9860	148.7483	7.67**
D x R x S	32	1212.4813	37.8900	1.95*
Error C	50	969.6660	19.3933	
Total	149	134502.8300		

¹ The main effects N-source and N-rate were tested with the date x source and date x rate interactions respectively.

* Significant ($P \leq .05$)

** Significant ($P \leq .01$)

APPENDIX 8.3

Table VI: Analysis of variance (study 4.3.3.1) for dry matter yield and total nitrogen of reed canary grass from Pitt Meadows sampled on August 18, 1973 following an application of five kinds of fertilizer applied at five rates, June 21, 1973

Source of Variation	df	S.S.	M.S.	F-test
<u>Dry Matter Yield</u>				
N-source (S) ¹	4	8559.1320	2139.7830	79.57**
Error A	5	134.4650	26.8930	0.25
N-rate (R)	4	101605.8200	25401.4550	235.48**
S x R	16	22773.4640	1423.3415	13.19**
Error B	20	2157.4000	107.8700	
Total	49	135230.2810		
<u>Total Nitrogen</u>				
N-source (S) ¹	4	0.7277	0.1819	8.51*
Error A	5	0.1070	0.0214	3.09*
N-rate (R)	4	6.5216	1.6304	235.61**
S x R	16	0.6420	0.0401	5.80**
Error B	20	0.1384	0.0069	
Total	49	8.1366		

¹ The main effect N-source was tested with the N rate x source factor

* Significant ($P < .05$)

** Significant ($P < .01$)

APPENDIX 8.4

Table VII: Analysis of variance (study 4.3.3.2) for the effect of five kinds of fertilizer applied at five rates on total nitrogen and tryptamines in uppermost four blades of summer sown, autumn harvested reed canary grass from totem field, University of B.C.

Source of Variation	df	S.S.	M.S.	F-test
<u>Total Nitrogen</u>				
N-source (S)	4	0.97438	0.24359	6.78**
N-rate (R)	4	6.66478	1.66619	46.36**
Error	16	0.57502	0.03599	
Total	24	8.21418		
<u>Tryptamines</u>				
N-source (S)	4	178.6400	44.6600	3.70*
N-rate (R)	4	113.4400	28.3600	2.35
Error	16	193.3600	12.0850	
Total	24	485.4400		

* Significant ($P < .05$)

** Significant ($P < .01$)

APPENDIX 8.4

Table VIII: Analysis of variance (study 4.3.3.2) for the effect of five kinds of fertilizer applied at five rates on dry matter yield and total nitrogen of summer sown, autumn harvested reed canary grass from totem field, University of B.C.

Source of Variation	df	S.S.	M.S.	F-test
<u>Dry Matter Yield</u>				
N-source (S)	4	97915.3840	24478.8460	5.16**
N-rate (R)	4	680466.1120	170116.5280	35.88**
Error	16	75861.7040	4741.3565	
Total	24	854243.2000		
<u>Total Nitrogen</u>				
N-source (S)	4	0.11302	0.02826	5.15**
N-rate (R)	4	0.82754	0.20689	37.73**
Error	16	0.08774	0.00548	
Total	24	1.02830		

** Significant ($P < .05$)

Figure 1: Proposed direct (I) and indirect (II) biosynthetic pathways of N incorporation into gramine (after pathway proposed for gramine in plants by O'Donovan and Leete, 1963), DMT, and 5-MeO-DMT in Phalaris arundinacea (c.f. Frelich, 1972).

Figure 1 (cont'd): Biosynthesis of gramine from tryptophan.
Entrance of N into side chain of gramine
occurs through a transamination reaction (III).

Figure 1 (Cont'd): Biosynthesis of DMT and 5-MeO-DMT
from tryptophan (after pathway proposed
by Leete, 1967).

Tryptophan **3-methyleneindoleine** **3-aminomethylindole** **3-dimethylaminomethylindole
(Gramine)**