no indication that the methyl esters of fatty acids were translocated or metabolized. The meristematic and differentiating cells of axillary buds are destroyed when in contact with these fatty acid materials (*7*), whereas physiologically more mature cells are not damaged under most conditions.

The occurrence of a wide variety of fatty acids in the tobacco plant is well established (*6*). In addition, many fatty acids are found in tobacco seeds (*4*). The methyl esters of fatty acids, therefore, are not completely foreign compounds to tobacco plants.

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NITRATE IN FORAGE

Influence of Stage of Growth and Soil Nitrogen on Nitrate Content of Herbage of Alfalfa, Red Clover, Ladino Clover, Trefoil, and Bromegrass

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Alfalfa accumulated nitrate in high amounts at young stages of growth. Red and ladino clover contained less nitrate than alfalfa. Per cent nitrate increased in these legumes as soil nitrogen was increased. The nitrate content of birdsfoot trefoil usually was never more than a trace. Bromegrass contained large amounts of nitrate when soil nitrogen was high. Per cent nitrate decreased with advance in maturity in species where it was high in the young stages of growth.

THE nitrate (NO_3) content of plants is of particular importance since consumption of forage containing excessive amounts can cause injury to livestock. Species, stage of maturity, and soil nitrogen level are important factors influencing nitrate accumulation.

Numerous investigators have studied nitrate accumulation in corn, sorghums, cereal grains, tobacco, vegetables, and weed species. Few studies have been conducted on perennial forage species, especially legumes, which have been regarded as the least likely to store nitrate. Crawford and Kennedy (*4*) listed alfalfa among the nonaccumulators. However, Olson and Whitehead (*9*) reported that alfalfa and other forage legumes contained "appreciable amounts of nitrate" but gave no supporting data. Case (*3*) reported that alfalfa contained 0.11 to 0.66% KNO_3 during a year when corn contained 8.04% KNO_3. Tucker *et al.* (*13*) found 1.22% KNO_3 in alfalfa and 4.48% KNO_3 in pigweed growing together in the same field. Kretschmer (*6*) found much less nitrate in legumes than in oats and ryegrass. Wilson (*15*) measured the expressed sap of 56 plant species collected from a variety of locations and conditions and found that legumes sometimes contained more nitrate than grasses.

Nitrate poisoning of livestock and nitrogen dioxide silage gas formation have been observed from grass-legume silage, as well as from corn silage, in certain years in drought areas of Wisconsin. Peterson *et al.* (*10*) reported that each of two 10- × 30-foot silos filled with alfalfa and equipped with drain basins gave off visible fumes of NO_2 gas. High concentrations of gas were detected from each silo.

No data were found regarding the nitrate content of perennial forage legumes at various stages of growth or when fertilized with nitrogen. Some data are available for perennial forage grasses. Carey, Mitchell, and Anderson (*2*) found that the nitrate content of bromegrass increased markedly with increasing rates of application of NH_4NO_3 fertilizer. Ramage (*11*) reported that orchardgrass harvested in the very early spring contained 0.08, 0.22, and 2.23% KNO_3 when fertilized with 100, 200, and 400 pounds of N per acre, respectively.

This paper provides information on the nitrate content of alfalfa, medium red clover, ladino clover, birdsfoot trefoil, and smooth bromegrass as influenced by stage of growth and soil nitrogen level.

Material and Methods

Forage samples were from three separate experiments. One set of samples was obtained during 1955 and 1956, and was used also for other analyses (*1*, *7*, *14*). Details of the experiment and data on plant heights and hay yields have been reported (*14*). The herbage samples came from plots seeded to Vernal alfalfa (*Medicago sativa* L.), Canadian Common smooth bromegrass (*Bromus inermis* Leyss.), a mixture of these two species, Wisconsin Common medium red clover (*Trifolium pratense* L.), and Wisconsin Common ladino clover (*Trifolium repens* L.). Samples were obtained during 1955 from the first crop beginning in early spring and from the second crop following cutting on June 15, and at each of six stages of growth. This was repeated during 1956.

The second set of samples was obtained during 1959, 1960, and 1961, and was used also for other analyses (*8*, *12*). Details of the experiment and data on plant heights and hay yields have been given (*12*). Samples came from plots seeded to Vernal alfalfa, Dollard medium red clover, Oregon Common ladino

Table I. Per Cent Nitrate and Protein in Dry Herbage of Alfalfa, Red Clover, and Ladino Clover with Advance in Maturity during Two-Year Period of 1955–56

	Alfalfa				Red Clover			Ladino Clover[a]		
	Alfalfa Alone		Alfalfa in Brome							
Stage of growth	Nitrate	Protein	Nitrate	Protein	Stage of growth	Nitrate	Protein	Stage of growth	Nitrate	Protein
SPRING GROWTH (FIRST CROP)										
Vegetative	0.24	32.4	0.08	31.6	Vegetative	0.02	28.6	Vegetative	0.05	29.6
Prebud	0.11	26.5	0.03	27.2	Prebud	0.02	26.5	Prebud	0.05	28.2
Midbud	0.07	23.3	0.03	23.2	Late bud	0.06	20.5	Heads emerging	0.06	25.0
1/10 Bloom	0.04	17.9	0.02	18.5	Full bloom	0.02	15.9	Few blooms	0.06	21.8
Full bloom	0.06	15.8	0.01	16.1	Green seed pod	0.02	15.3	Full bloom	0.08	21.7
Green seed pod	0.08	14.3	0.03	14.1	Seed	0.04	14.6	Seed	0.14	21.4
SUMMER GROWTH (SECOND CROP)										
Vegetative	0.62	32.6	0.13	31.4	Vegetative	0.03	26.9	Light bloom	0.05	27.1
Bud	0.29	26.4	0.04	26.2	Prebud	0.04	24.5	Heavy bloom	0.04	24.9
1/10 bloom	0.09	21.1	0.03	22.6	1/10 Bloom	0.04	21.4	Seed and bloom	0.03	23.5
Full bloom	0.03	18.7	0.03	19.1	Full bloom	0.02	17.8	Seed	0.04	22.4
Green seed pod	0.04	17.7	0.03	17.1	Green seed pod	0.03	16.6	Vegetative and seed	0.15	23.4
Seed	0.10	15.9	0.03	16.6	Seed	0.06	16.8	Vegetative and seed	0.15	22.9

[a] Ladino clover sampled at same time as alfalfa, since stage of growth is difficult to determine in ladino. Condition most prevalent at time of cutting was noted.

Table II. Per Cent Nitrate and Protein in Dry Herbage of Four Legume Species with Advance in Maturity during 3-Year Period of 1959–61

	Alfalfa		Red Clover		Ladino Clover[a]		Birdsfoot Trefoil	
Stage of Growth	Nitrate	Protein	Nitrate	Protein	Nitrate	Protein	Nitrate	Protein
SPRING GROWTH (FIRST CROP)								
Prebud	0.18	26.8	0.03	27.9	0.10	26.4	0.01	22.7
Early bud	0.19	22.2	0.05	23.0	0.08	25.5	0.01	21.3
1/10 Bloom	0.15	18.2	0.06	18.3	0.11	23.7	0.01	19.8
Full bloom	0.10	15.5	0.04	13.9	0.07	20.5	0.01	17.0
Green seed pod	0.12	14.8	0.03	14.6	0.10	20.6	0.01	16.2
SUMMER GROWTH (SECOND CROP)								
Prebud	0.32	25.9	0.10	25.6	0.07	24.7	0	22.4
Early bud	0.21	23.1	0.06	23.7	0.09	22.9	0	22.2
1/10 Bloom	0.12	19.5	0.05	20.9	0.07	21.7	0	22.0
Full bloom	0.12	19.3	0.09	17.2	0.16	24.1	0.01	20.9
Green seed pod	0.17	17.5	0.10	16.7	0.27	23.2	0.01	18.8

[a] Ladino clover sampled whenever alfalfa reached these stages, since maturity cannot be definitely determined in ladino.

clover, and Empire birdsfoot trefoil (*Lotus corniculatus* L.). Samples were obtained from the first crop and from the second crop following cutting on June 15 ± 1 day, and at each of six stages of growth. Only the last five stages of growth were used in this study, since no herbage was available from trefoil at the first growth stage.

Ladino clover in both experiments was harvested on the same dates as alfalfa, because stages of growth in ladino are difficult to determine.

The third set of samples was obtained during 1963 from second harvest-year stands of Vernal alfalfa and Empire birdsfoot trefoil. On April 9, 1963, as growth was just beginning, areas of the legume stands were fertilized with 0, 200, and 400 pounds per acre of elemental N as NH_4NO_3. Herbage samples were harvested on May 10 when both species were still vegetative, and on May 23 when they were in early bud.

The three experiments were established on Miami silt loam soil on the University Hill Farms at Madison, Wis. The areas were limed and fertilized with P and K for the growing of alfalfa. All herbage samples were harvested to leave a $1^1/_2$- to 2-inch stubble.

Fresh herbage samples were dried in a forced-draft, hot-air dryer at about 145° F. Samples were ground in a Christy-Norris mill through a $^1/_{32}$-inch screen, thoroughly mixed, and stored for future use.

Nitrate was determined colorimetrically as nitrate nitrogen by the phenoldisulfonic acid method (*5*) and recalculated to equivalent nitrate (NO_3). Crude protein was determined as total nitrogen as outlined by AOAC, and the nitrogen values were multiplied by 6.25. Determinations were made on a dry weight basis (70° C.).

Results

Content in Legumes. Percentages of nitrate and protein in three legume species during 1955–56 are shown in Table I, and in four legume species during 1959–61 in Table II. Alfalfa grown alone contained the highest per cent nitrate at the young stages of growth in both experiments, followed in order by ladino clover, red clover, and birdsfoot trefoil. Alfalfa and red and ladino clover were similar in per cent nitrate at mature stages, and red and ladino clover were similar at all growth stages. Trefoil contained only a trace of nitrate.

Alfalfa grown alone was higher in per cent nitrate than alfalfa grown in association with bromegrass (Table I), particularly at the young growth stages. The reason for this difference is not clear, but part of the available soil N was no doubt absorbed by the bromegrass. The mixture was about 70 and 52% alfalfa during the spring and about 80 and 75% alfalfa during the summer of 1955 and 1956, respectively.

Per cent nitrate in alfalfa was higher during the summer than during the spring of both experiments (Tables I and II), and the difference was most marked at the young stages of growth. There was little difference between the spring and summer growths in red clover or trefoil, and little difference in ladino clover except for a higher per cent nitrate in the herbage sampled at the final two stages of growth during summer.

Alfalfa decreased in per cent nitrate from early growth to full bloom during both spring and summer, but it increased slightly at the seed stage. Red and ladino clover changed very little with advance in maturity, except for a slight increase in ladino during the mature stages of summer. Trefoil contained only a trace of nitrate at all growth stages.

Alfalfa, red clover, and ladino clover samples taken during the spring growth period of the two experiments matched growth stages. The data, therefore, were combined to provide a 5-year average (Table III). Differences among species, growth periods, and growth stages were the same as described above.

Only alfalfa contained concentrations of nitrate of 0.2% or higher, a concentration above which it is considered that ill effects could occur to livestock if the herbage were the only feed. This level was found at the young growth stages of alfalfa grown alone, particularly in second-crop herbage. A concentration approaching 0.2% also was found in ladino clover at the two most mature growth stages of the second-crop herbage.

Content in Bromegrass. Percentages of nitrate and protein in bromegrass during 1955–56 are shown in Table IV. Only traces of nitrate were found, except in the summer growth associated with alfalfa. Per cent protein was highest during summer and when grown with alfalfa.

Per cent nitrate in the bromegrass grown with alfalfa was very high at the youngest stages of growth during summer, but decreased rapidly as the grass matured (1.36 to 0.08%). Nitrate content was above or approached the 0.2% level in all but the most mature stage. The high nitrate content in the summer growth was apparently a response to N released from sloughed alfalfa nodules. The N became available to the summer growth because of the breakdown of the nodules with the advent of warm soil temperatures.

Trefoil Low in Nitrate. Per cent nitrate in birdsfoot trefoil during 3 years of analysis (Table II) was never more than a trace. The question arose as to whether trefoil would accumulate a significant amount of nitrate if the available N content of the soil were high. A preliminary trial in the greenhouse indicated that it probably does not accumulate nitrate, in contrast with many other forage plants. Herbage of Empire birdsfoot trefoil in the vegetative stage (5 inches tall) grown in the greenhouse contained only 0.16 and 0.05% nitrate when grown with O and 300 pounds per acre of N (as NH_4NO_3), respectively. Vernal alfalfa (8 inches tall) contained 0.32 and 2.18% of nitrate, and Dollard medium red clover (5 inches tall) contained 0.16 and 3.98%, respectively.

In a field trial during the spring of 1963, Empire birdsfoot trefoil and Vernal alfalfa were fertilized with 0, 200, and 400 pounds per acre of elemental N (as NH_4NO_3). As shown in Table V, alfalfa accumulated significant amounts of nitrate when fertilized, but the nitrate content of trefoil remained low at each N level. Per cent protein in both species was not influenced greatly by the N fertilizer.

Table III. Average Percentages of Nitrate and Protein in Spring Growth of Alfalfa, Red Clover, and Ladino Clover during Five Years, 1955–56 and 1959–61

Stage of Growth	Alfalfa		Red Clover[a]		Ladino Clover[b]	
	Nitrate	Protein	Nitrate	Protein	Nitrate	Protein
Prebud	0.15	26.7	0.03	27.3	0.08	27.2
Early bud	0.15	22.7	0.06	22.0	0.08	25.3
1/10 Bloom	0.10	18.1	0.04	17.4	0.10	22.9
Full bloom	0.08	15.6	0.03	14.4	0.08	22.2
Green seed pod	0.10	14.6	0.03	14.6	0.12	20.9

[a] Wisconsin Common variety during 1955–56 and Dollard variety during 1959–61.
[b] Ladino clover sampled whenever alfalfa reached these stages, since maturity cannot be definitely determined in ladino.

Table IV. Per Cent Nitrate and Protein in Dry Herbage of Bromegrass during Two-Year Period of 1955–56

Stage of Growth	Bromegrass Alone		Brome in Alfalfa	
	Nitrate	Protein	Nitrate	Protein
Spring Growth (First Crop)				
Vegetative	0.02	19.5	0.01	23.4
Boot	0.01	16.6	0.01	19.6
Heads emerging	0	14.5	0.01	15.4
Heading	0	10.2	0.02	12.0
Seed in milk	0	8.8	0.05	9.6
Seed dough	0	7.4	0.02	9.4
Summer Growth (Second Crop)				
Vegetative	0.01	23.1	1.36	32.0
	0.01	19.3	0.69	26.7
	0.03	17.4	0.42	22.3
	0.01	15.5	0.19	18.1
	0.01	16.9	0.15	17.7
	0.01	14.9	0.08	17.0

Table V. Nitrate and Protein Percentages in Dry Herbage of Alfalfa and Birdsfoot Trefoil Following Spring Application of Nitrogen Fertilizer in 1963

Legume	N, Lb./Acre	Growth Stage and Date Sampled			
		Vegetative, May 10		Early Bud, May 23	
		Nitrate, %	Protein, %	Nitrate, %	Protein, %
Alfalfa	0	0.21	26.5	0.03	21.8
	200	0.53	26.5	0.05	20.1
	400	1.00	29.1	0.24	22.3
Trefoil	0	0.02	30.2	0.01	27.4
	200	0.09	31.6	0.02	25.9
	400	0.28	32.3	0.06	27.3

Discussion

Legumes accumulated significant amounts of nitrate under normal field conditions, as shown with alfalfa at the young stages of growth. Concentrations were high enough supposedly to be toxic if fed to livestock as the only feed. The N level of the soil was important, since alfalfa was shown to accumulate higher amounts of nitrate as the soil N level was increased.

Birdsfoot trefoil was unique among the four legumes studied, in that it never contained more than a trace of nitrate at any stage of growth. Also, it did not accumulate a high amount of nitrate when fertilized with a nitrogenous fertilizer. Trefoil apparently does not absorb excess levels of nitrate or the enzyme systems in the plant are such that excess N is accumulated not as nitrate but as some other form of N.

Bromegrass accumulated nitrate in significant amounts when the level of soil N was high. This occurred in association with alfalfa, since no fertilizer trials were conducted with bromegrass. Other investigations (*2*, *11*) have shown that perennial forage grasses increase in nitrate content with high rates of application of nitrogenous fertilizers.

Per cent nitrate decreased with advance in maturity in species in which it was high during the young stages of growth. This occurred in alfalfa and in bromegrass grown with alfalfa. Toxic nitrate concentrations are more likely to be present at young than at mature stages of growth.

Work with nonleguminous crops (*2*, *11*) has indicated that heavy applications of nitrogenous fertilizers are accompanied by both increased protein and increased nitrate contents. The current work indicates that there may be little relationship between the contents of protein and nitrate in legumes. The herbage of all four legumes was high in protein, but some legumes had a high nitrate content while others had little or no nitrate. Alfalfa and red clover, for example, were similar in per cent protein but alfalfa contained high levels of nitrate at the young growth stages in contrast to red clover.

Heavy applications of nitrogenous fertilizer to alfalfa and trefoil did not change greatly their per cent protein. However, per cent nitrate in alfalfa was increased while it was changed very little in trefoil.

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FERTILIZER RAW MATERIALS

Reactivity Range of Nonblended Florida Pebble Phosphate

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Phosphate rocks from different locations within the Florida fields showed small differences with respect to chemical composition, but rather large differences in physical properties. The rates of reaction with acid exhibited by the specimens appeared to be associated more with chemical than with physical characteristics. Consequently, the observed differences in reaction rates were considerably smaller than the differences between Florida rocks and those from other geographic deposits. A 35-year-old Florida rock used to calibrate a general scale of rock reactivity proved to be somewhat more reactive than the general run of rock now being mined in Florida.

Phosphate rocks from different geographic deposits vary widely in speed of reaction with acid. Differences in reactivities of commercial rocks have long been recognized by superphosphate manufacturers, though only recently has attention been given to the development of a scale of reactivity as a means of classifying phosphate from different sources (*6*). This scale was defined in terms of the relative reactivities of available specimens of rock from several fields. Although these specimens represented points on the reactivity scale, they did not necessarily typify specific varieties of rock, because rock from the same producing field may vary considerably.

The type specimen of Florida land pebble was found to have an intermediate reactivity. Since this variety of rock supplies 75% of the phosphorus in domestic rock sold or used by the producers, a survey was undertaken to determine its range in reactivity. The findings are summarized in this paper.

Selection and Procurement of Test Samples

The land pebble phosphate region in Florida, located in the west-central part of the state (Figure 1), is divided on the basis of phosphorus content into a northern, high-grade area and a southern, lower grade area (*4*). Current commercial mining activity is confined to the northern district, in Hillsborough and Polk Counties. This territory is subdivided into smaller areas containing rock deposits recognized by the mining community as differing in hardness, color, and grade from those in adjoining areas. Thus, five mining districts may be distinguished: (1) a small region east of Bartow, containing very soft ore; (2) a larger area east of Lakeland, comprised of moderately soft, high-grade ore appearing as mixed black and white pebbles; (3) another region of the same type of rock but of medium grade, west of Mulberry; (4) an area extending south from Mulberry, containing hard, dominantly black pebble, of medium to low grade; and (5) a large area extending from the west of Bartow southeast to Fort Meade along the Peace River and containing hard, yellowish rock, chiefly of concentrate size.

To provide general coverage of the Florida field, one mining company in each district was asked to furnish test samples in three size classifications, corresponding as closely as possible to coarse pebble, fine pebble, and flotation concentrates. Samples were to be from plant output before the blending of grades that is normally practiced in commerce to provide products of specified grade. The materials submitted and judged by producers to be typical of each variety of rock are listed in Table I and their major chemical composition is shown in Table II. Only one size of rock was received from district 1 because the producer contacted is now out of commercial production in that area.

To eliminate particle size as a variable, all samples were ground and/or screened to 100 to 150 mesh prior to testing.

Scope of Measurements

The survey includes measurement of those properties of a rock that are considered to influence its reactivity—carbonate, fluorine, iron, and aluminum contents, calcium-phosphorus ratio, pore size distribution, and surface area (*2, 6, 9*)—as well as estimation of the relative reactivity itself by known techniques: measurement of phosphorus solubility in solutions of neutral ammonium citrate, citric acid, and phosphoric acid (*3, 8*). The rate of solubilization in phosphoric acid is related directly to the manufacture of triple superphosphate; the analogous rate in sulfuric acid as in