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Title: SUBTERRANEAN CLOVER YIELD AND NUTRIENT CONTENT AS INFLUENCED BY SOIL MOLYBDENUM STATUS

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During the Spring of 1969, 47 surface soil samples (0 to 15 cm) as well as foliage samples of subterranean clover were collected from 32 selected western Oregon pastures, in an effort to determine their molybdenum status and to evaluate the use of anion exchange resin method as a means of assaying available molybdenum. The five most acid soils were limed to 80 percent base saturation. Greenhouse experiments were established during September, 1969, to evaluate the effect of molybdenum fertilization of subterranean clover yield and nutrient content.

Highly significant yield and total nitrogen responses of subterranean clover were obtained following molybdenum fertilization. Greatest percent yield as well as percent total nitrogen responses to applied molybdate occurred at the lower soil anion exchangeable molybdenum concentrations.

Statistically significant correlation coefficients were obtained

between anion exchangeable soil molybdenum and percent yield response to applied molybdate in the greenhouse studies (r = -0.743); anion exchangeable soil molybdenum and plant nitrogen concentrations in field subterranean clover samples (r = 0.875); anion exchangeable soil molybdenum and percent total nitrogen response to applied molybdate in the greenhouse studies (r = -0.560); anion exchangeable soil molybdenum and plant molybdenum concentrations in field clover samples (r = 0.861); anion exchangeable soil molybdenum and soil pH (r = 0.861).

Subterranean Clover Yield and Nutrient Content as Influenced by Soil Molybdenum Status

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SUBTERRANEAN CLOVER YIELD AND NUTRIENT CONTENT AS INFLUENCED BY SOIL MOLYBDENUM STATUS

INTRODUCTION

Increased attention in Oregon to improved legume pastures and forages like clover and alfalfa have revealed not infrequent legume response to applied molybdate.

Yield and nitrogen responses in legumes have been reported from molybdenum application in many countries and from many regions in the United States on soils of low pH. Unfortunately, there appears to be a rather small margin between forage plants deficient in molybdenum and those with molybdenum levels sufficiently high to be toxic to livestock. In general, levels of 10 to 20 ppm molybdenum in plant material is considered toxic to grazing animals and interferes with copper utilization by ruminants.

Reliable soil available molybdenum analyses are difficult to obtain. This is due to a number of circumstances including the very small amounts of molybdenum present in the soil as well as needed by the plant and numerous interferences in the chemical soil fusion procedure.

Anion exchange resins have been used successfully to determine the amount of phosphorus that is readily available in the soil. Since molybdenum and phosphorus are both anions and molybdenum soil chemistry is known to be comparable, similar reactions could be expected to occur in the soil. It would seem plausible that anion exchange resins could be used in determining available molybdenum in soils too.

The widespread problems of molybdenum deficiency and toxicity in different parts of Oregon prompted research aimed at finding a reliable method for determining plant available molybdenum. Undoubtedly, a satisfactory procedure for assaying potentially responsive soils to applied molybdenum and at the same time identifying likely toxic levels of soil molybdenum would prove most helpful. The objectives of present investigations were:

1. To examine the use of anion exchange resin as a method of predicting soil available molybdenum.
2. To determine nitrogen, molybdenum, and copper concentrations of subterranean clover as influenced by applied molybdenum to greenhouse and field soils.
3. To evaluate by means of greenhouse studies subterranean clover response to applied molybdenum on selected soils.
4. To correlate soil anion exchangeable molybdenum with plant nitrogen and molybdenum status together with percent yield response.
5. To study the relationship between soil pH and anion exchangeable molybdenum.

LITERATURE REVIEW

Subterranean clover yield and nutrient content as influenced by soil molybdenum status has been reviewed under the following heads:

Factors affecting available molybdenum.

- Soil reaction.
- Iron, aluminum, manganese, and titanium.
- Sulfur and phosphorus.
- Mineralogy and degree of weathering.
- Drainage status.
- Plant species.

Critical molybdenum values and deficiency symptoms in plant.

Legume response to molybdenum application.

Methods, forms, amounts, and frequency of molybdenum application.

Methods of molybdenum analysis.

Factors Affecting Available Molybdenum

Soil Reaction

Among the various factors that control molybdenum availability, soil reaction plays an important part in two ways: first, on the availability of molybdenum itself (Jones, 1957; deMooy, 1970) and second, on the availability of other elements, which in turn affect the molybdenum uptake in the plant.

The chemistry of molybdenum somewhat parallels that of phosphorus especially as its availability is influenced by soil pH (Barshad, 1951; Reisenauer, 1968; Tisdale and Nelson, 1966). Barshad (1951) observed that under the conditions he defined, the increase in adsorption of molybdate anions by clays would seem to indicate that molybdate anions ($MoO_4^{=}$:$HMoO_4^{-}$) exchange with OH^- ions of the clays in the same manner as phosphate ions (Stout, 1939); namely,

$$\text{Clay}\left\}\begin{matrix}OH\\OH\\OH\\OH\end{matrix}\right. + 2MoO_4^{=} \rightleftarrows \text{Clay}\left\}\begin{matrix}MoO_4\\MoO_4\end{matrix}\right. + 4\ OH^-$$

Jones (1957) studying molybdate sorption by soils observed that when molybdate was added to a soil solution, increasing the pH, a decreased molybdate adsorption by the soil resulted (Figure 1).

<u>Iron, Aluminum, Manganese, and Titanium</u>

The availability of molybdenum to plants in some acidic soils may be increased by raising pH of the soil. This relation to pH is analogous to that of phosphate which is held in insoluble form by iron and aluminum, and suggests that molybdenum may be similarly held in soils (Jones, 1956, 1957; Tisdale and Nelson, 1966).

At low pH, iron, aluminum, titanium, and manganese are readily available and they form insoluble compounds of molybdenum (Jones, 1956, 1957; Reisenauer, Tabikh, and Stout, 1962). Anderson

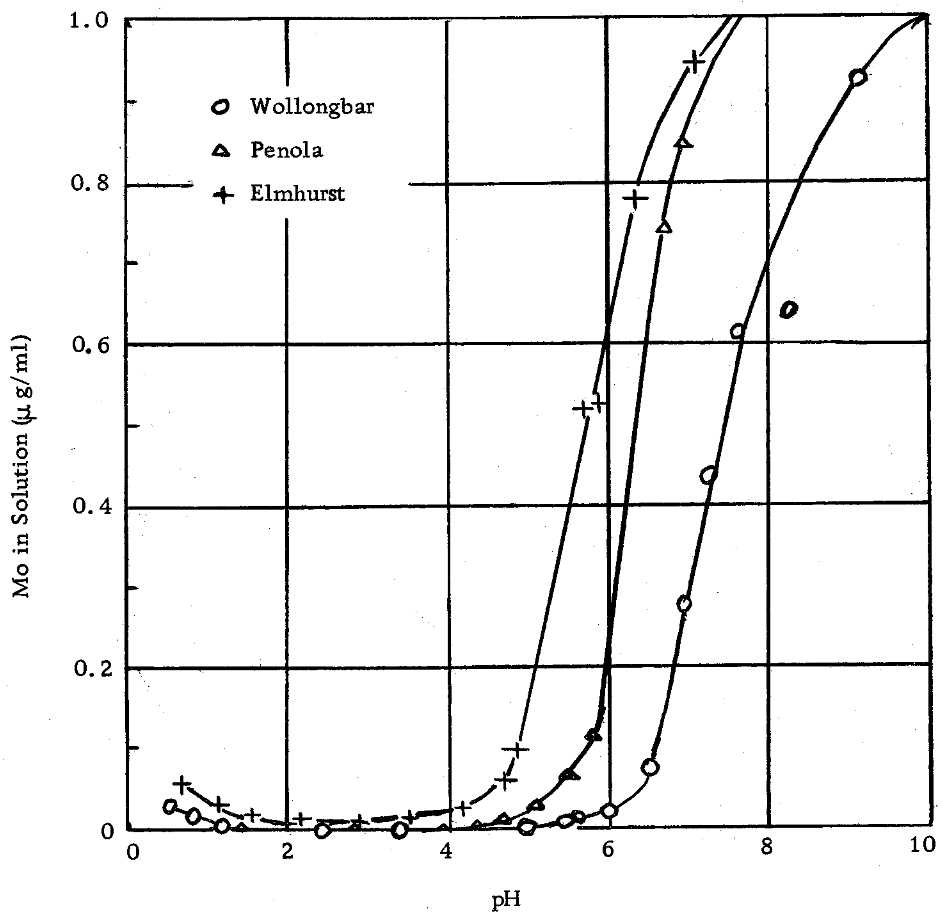

Figure 1. Effect of pH on the sorption of molybdate by soils.

and Oertel (1946) found a high concentration of molybdenum unavailable to plants in a soil which contained large amounts or ironstone gravel. High concentrations of molybdenum may occur in ironstone concretions (Stanfield, 1935; Oertel and Prescott, 1944). Williams and Moore (1952) found a correlation between the concentration of soil iron and the availability of the molybdenum present in normal soil. It is of interest that the first responses of clover to molybdenum in Australia (Anderson, 1942) and New Zealand (Davies, 1952) were obtained on ironstone soils with unusually high total molybdenum contents (Anderson, 1956a).

Reisenauer et al., (1962) while studying molybdenum reactions with soils and the hydrous oxides of iron, aluminum, and titanium observed that sorption of molybdate by $Fe_2O_3 \cdot xH_2O$ is accompanied by a stoichiometric release of two hydroxyl ions and one molecule of water.

Millikan (1948) observed antagonism between molybdenum and manganese. Anderson and Spencer (1950a) found that the application of heavy dressings of manganese sulfate to soil considerably decreased the concentration and amount of molybdenum in the plants and induced molybdenum deficiency. This induced molybdenum deficiency inhibited the utilization of nitrate nitrogen in nonlegumes and inhibited nitrogen fixation in clover, and in each case was corrected by the application of molybdenum. Such effects have also been observed by other workers (Anderson and Arnot, 1953; Millikan, 1951; Mulder, 1954).

Application of lime helps in precipitating iron, aluminum and manganese and at the same time releases molybdate ions by replacing absorbed $MoO_4^{=}$ with OH^- ions (Wells, 1956; Jones, 1957). The increase in the supply of available molybdenum to the plant due to liming, has been due not to molybdenum present in the lime (Anderson and Oertel, 1946; Evans, Purvis, and Bear, 1951) but rather to the effect of hydroxyl ions in replacing molybdate ions on the clay, or to some other mechanism affecting the availability of molybdenum in the soil.

Sulfur and Phosphorus

It has been observed that heavy application of phosphatic fertilizers will increase the molybdenum uptake by plants. Heavy applications of sulfates, on the other hand, have a depressing effect, because a high concentration of $SO_4^{=}$ may be expected to replace some of the adsorbed $MoO_4^{=}$ (Stout *et al.*, 1951; Tisdale and Nelson, 1966).

The sulfate ion exerts a depressing action on molybdenum uptake (Stout *et al.*, 1951; Mulder, 1954), an effect attributed by Stout *et al.* (1951) to competition of similarly sized and charged ions. Plant (1951) observed that "whiptail" of cauliflower can be induced by gypsum. It should be noted that sulfate also increased the response to molybdenum where it corrects sulfur deficiency in the plants (Anderson and Spencer, 1950b). Anderson and Spencer (1950a) found that application of heavy dressings of manganese sulfate to soil considerably decreased the concentration and amount of molybdenum in the plant, and induced molybdenum deficiency. It has been further shown by Mulder (1954) that the manganese and sulfate ions are both involved in reduced molybdenum uptake. Increasing the amounts of either manganese or sulfate applied to the soil increased the amount of molybdenum that had to be applied to correct molybdenum deficiency in the plant.

Application of sulfate and superphosphate fertilizers have resulted in depressed molybdenum uptake by plants and an increase in manganese uptake (During _et al._, 1960; Widdowson, 1966; Reisenauer, 1963; Walker, Adams, and Orchiston, 1955).

Mineralogy and Degree of Weathering

Mineralogy and degree of soil weathering and leaching affects the total and available molybdenum content of the soil.

Kline (1956) observed that igneous rocks are high in molybdenum content, while sedimentary and metamorphic rocks are low in molybdenum. Massey, Love, and Bailey (1967) found a good correlation between soil molybdenum and soil series.

Wells (1956) observed that molybdate ion retention power of a soil is associated with the amount of amorphous hydroxides of iron and aluminum, which in turn are low in lightly weathered soils and increase with the degree of weathering, and that concomitantly the molybdenum becomes less available. Fieldes and Swindale (1954) expressed the possibility of $MoO_4^=$ retention by the metahalloysite shown to be present in moderately and strongly weathered yellow-brown earths.

The increase in availability of molybdenum sometimes observed on podzolized soils is due to the removal of the hydrous oxides by leaching (Davies, 1956; Widdowson, 1962). The amount of total molybdenum, however, decreases as the soil becomes more leached (Grigg, 1960; Davies, 1956).

High molybdenum levels are known to occur on peat or muck soils (Davies, 1956; Dune and Jones, 1948; Gammon, Fiskel, and Mourkides, 1955; Kline, 1956; Walsh, Neenan, and O'Moore, 1952; Johnson, 1966). Other soils which exhibit high molybdenum content have been classified as alkaline soils, and poorly drained soils with a high water table and surface organic accumulation (Dye and O'Harra, 1959; Kubota _et al._, 1961; Kubota, Lemon, and Allaway, 1963.

Drainage Status

The molybdenum uptake by plants is influenced by soil drainage status. In general, low molybdenum levels have been observed in forage plants from naturally well-drained soils in Nevada (Kubota *et al.*, 1961), New Hampshire and Nevada (Kubota, Lemon, and Allaway, 1963), and Oregon (Kubota *et al.*, 1967).

Both high water table and surface organic matter accumulation, common soil characteristics of poorly drained soils, affect the molybdenum levels in plants (Kubota *et al.*, 1961). In England (Ferguson, Lewis, and Watson, 1943) the "teart" area of the molybdeniferous lower lias geological formation appears to be predominantly poorly drained (Lewis, 1943). The organic soils in Florida Everglades comprise a molybdenum toxic area for cattle where the problem is accentuated by very low copper contents in the vegetation (Kretschmer and Allen, 1956).

Plant Species

Several investigators (Barshad, 1948, 1951; Lewis, 1943; Robinson and Edgington, 1948; Kubota *et al.*, 1961) have noted large variations in molybdenum content among different plant species.

Barshad (1948) found that among different species, the variation depended on the amount of water-soluble molybdenum in the soil, the time of the year the plant was grown, the age of the plant, and the plant part. Barshad (1951) further observed that after an initial increase, the molybdenum content of the plants, with a few exceptions, steadily decreased with each harvest.

Kubota _et al._ (1961) demonstrated that alfalfa contains about two-thirds as much molybdenum as does the clover, and sedge about one-sixth as much.

It is well known that concentration of molybdenum is different in different plant tissues (Anderson, 1956a). Higher concentrations of molybdenum were found in the interveinal areas of leaves (Stout and Meagher, 1948), as compared to the stem (Barshad, 1948; Evans, Purvis, and Bear, 1950), in the nodules of legumes rather than in the roots, stems, and leaves (Mulder, 1948), and legume seeds (Bertrand, 1939; Meagher, Johnson, and Stout, 1952).

Critical Molybdenum Values and Deficiency Symptoms in Plant

Critical Molybdenum Values

Critical plant composition value of a nutrient has been defined as the nutrient level in the plant below which a growth stress may be expected to occur and these values have been used successfully for correcting nutrient deficiencies in plants (Melsted, Motto, and Peck, 1969; Johnson, 1966).

Plant analyses can be a powerful tool in the diagnosis of yield depressions. Some deficient and adequate or critical levels of molybdenum in plant tissue to predict the growth rate have been reviewed in Table 1.

Table 1. Mo concentration of legumes as an index to predict growth rate.

Plant	Tissue sampled	Stage of sample	Range in dry matter (μg/g)		Reference
			Deficient	Critical or Adequate	
Alfalfa	Upper stem cutting	Early flower stage	--	0.50	Melsted, Motto, and Peck (1969)
Alfalfa	Top of the half plant	--	--	0.30 to 0.50	James, Jackson, and Harward (1968)
Alfalfa	Leaves	10 percent in bloom	0.28	0.40 to 0.50	Reisenauer (1956)
Alfalfa	Whole plant	--	--	0.50	Stout _et_ _al_. (1951)
Alfalfa	Leaves	--	--	0.50	Evans and Purvis (1951)
Alfalfa	Leaves	Pre-bloom	0.10	0.30 to 0.60	Evans, Purvis, and Bear (1950)
Soybean	Upper stem cutting	Early flower stage	--	0.50	Melsted _et_ _al_., (1969)
Beans (Phaseolus spp.)	Tops	8 weeks old	--	0.40	Johnson _et_ _al_. (1952)

A variation in the tabulated critical values has been observed due to the differences in plant part analyzed, stage of sampling, variation in soil and climatic conditions, and other various antagonistic effects.

Molybdenum Deficiency Symptoms

Subterranean clover when grown on atmospheric nitrogen shows typical nitrogen deficiency symptoms when molybdenum supply is inadequate for the requirement of the Rhizobium. The foliage turns pale green or yellow and stunted, the blade margins fade or scorch, curl inward, and become limp; the petioles often turn red or brown and finally become severely chlorotic as molybdenum deficiency becomes more severe (Johnson, 1966; Nelson and Barber, 1964; Anderson, 1956b; Evans, 1956; Hewitt, 1956; Johnson, Pearson, and Stout, 1952).

Legume Response to Molybdenum Application

In the United States, most of the molybdenum deficiencies reported have occurred on low pH soils of the Atlantic and Gulf Coasts. Responses to molybdenum application in terms of increase in yield and nitrogen content of leguminous crops have been observed in 19 states, i.e., Connecticut, Delaware, Florida, Georgia, Hawaii, Idaho, Illinois, Indiana, Iowa, Minnesota, Nebraska, Nevada, New

Jersey, New York, Oregon, South Carolina, Texas, Washington, and Wisconsin (deMooy, 1970; Albrigo, Szafranek, and Childers, 1965; McVickar, Bridges, and Nelson, 1963; Lavy and Barber, 1963; Rubins, 1956).

First biological importance of molybdenum was reported by Bortels (1930, 1937) when he observed that it was essential for nitrogen fixation by bacteria growing symbiotically with legumes of pea, soybean, and red clover. He grew these plants in sand cultures and observed significant increases in nitrogen fixation and growth from the addition of traces of molybdenum to the culture solutions.

In the United States, molybdenum deficiencies have been recorded by Evans, Purvis, and Bear (1950) when they proved that leguminous plants need molybdenum when grown on either atmospheric or nitrate nitrogen. Molybdenum deficiency has been observed to be responsible for inhibition of symbiotic nitrogen fixation in clover (Anderson and Spencer, 1950a).

Significant responses to molybdenum application in subterranean clover have been observed on soils where phosphorus and sulfur have been applied. No soil responded to molybdenum where neither phosphorus nor sulfur had been added (McLachlas, 1955).

Methods, Forms, Amount, and Frequency of Molybdenum Application

Methods of Molybdenum Application

Molybdenum can be successfully applied in a number of ways. It can be mixed with superphosphate as molybdenum trioxide or sodium molybdate at 1 1/2 pounds of molybdenum trioxide per 2240 pounds of superphosphate. This mixture provides for a dressing of about two ounces of molybdenum per acre, depending upon the amount of superphosphate applied (Anderson, 1956a). Molybdenum solutions may be sprayed directly onto the foliage of plants. Stewart and Leonard (1953) recommended this procedure for the treatment of "yellow-spot" of citrus. It has also been used to treat cauliflower plants in the seedbed (Waring et al., 1948). Molybdenum is readily absorbed by the leaves and translocated in plants (Meagher, Johnson, and Stout, 1952). It can also be applied by mixing with the seed (deMooy, 1970; Boswell and Anderson, 1969; Parker and Harris, 1962).

Forms of Molybdenum Application

Anderson (1956a) noted that sodium molybdate, ammonium molybdate, and even the less soluble molybdenum trioxide were about equally effective as source. Molybdenite (molybdenum sulfite ore) was not satisfactory.

Amount of Molybdenum Application

The amount of molybdenum required per acre to correct deficiencies may vary with the crop, the soil, the method of application, and other factors such as pH, phosphate, sulfate, iron and manganese status of soil (Anderson, 1956a; Johnson, 1966). In general, the amount of molybdenum needed to correct deficiency is very small. The usual rates are in the order of few ounces per acre (Anderson, 1942, 1956a; Johnson, 1966). A few instances where 2 to 3 pounds of molybdenum were required are special cases (Anderson, 1956a; Johnson, 1966).

Frequency of Molybdenum Application

Anderson (1956a) observed that a single application of molybdenum at 2 ounces per acre remained effective for several years. Mulder (1954) concluded that acid soils, as well as those containing ironstone, fixed molybdenum strongly and required more frequent applications. Johnson (1966) noted that foliar analysis for molybdenum should serve as a guide to the need for additional fertilization.

Methods of Molybdenum Analysis

Current interest in the role of molybdenum in plant nutrition has stimulated critical study of analytical procedures for the determination of this element in soils and plants by a number of investigators (Dick and Bingley, 1947; Grigg, 1953; Johnson and Arkley, 1954; Marmoy, 1939; Piper and Beckwith, 1951).

Jackson (1958) and Reinsenauer (1965) have described reliable methods for determining total soil molybdenum.

The use of ammonium oxalate to extract available molybdenum has been widely used (Grigg, 1953). However, Grigg (1960) himself indicated that the acid-oxalate is not reliable for diagnosing deficiencies as it exacts a portion of the ironbound molybdenum, which is not available to plants.

Nichols (1960) observed that bio-assay procedure gives a fairly good indication of the available molybdenum, but it is not reliable for low levels (less than 1.0 μg/g) of molybdenum. Lavy and Barber (1964) have shown that Aspergillus niger method gives values very similar to labile molybdenum in the soil measured by the isotopic equilibrium technique. The amount removed by eight displacements, 0.0117 μg/100g of soil, was close to 0.0128 μg initially present as available molybdenum. This method was also used by Follett and Barber (1967).

Gupta and Mackay (1965) developed a new procedure for the determination of exchangeable molybdenum in podzol soils.

Anion exchange resins have been used successfully to determine the amount of readily available phosphorus in the soil (Williams, 1965;

Saunders, 1964; Saunders and Metelerkamp, 1962; Cooke and Hislop, 1963; Sheard and Caldwell, 1955; Amer et al., 1955). Since phosphorus and molybdenum are both anions, similar reactions could be expected to occur in the soil. This indicates that anion exchange resins could be useful in determining available molybdenum in soils.

With this in mind Olsen (1967) developed a method to extract molybdenum from soil with an anion exchange resin. The amount of molybdenum extracted with this procedure gave a positive linear relationship between the amount extracted with pH. Ross (1969) observed that this method holds some promise as a measure of expected plant growth and plant nitrogen from exchangeable soil molybdenum. However, they further emphasized the need for detailed study of this method on an increased number and kind of soils.

EXPERIMENTAL METHODS AND MATERIALS

Forty seven surface soil samples (0 to 15 cm) were collected during May, 1969, from 32 selected western Oregon hill soils (Table 2). A map of western Oregon is provided which locates the sampling sites (Figure 2). These soils represented different stages of development on residual basalt and sedimentary shales as well as alluvial deposits. The soils represented a range in pH, base status, organic matter content, drainage status and fertility (Tables 5, 6, and 7). Foliage samples of subterranean clover were taken from plants growing at each site as the soils were sampled.

While all soils were acid, they ranged from very strongly acid pH 4.95 to slightly acid pH 6.15. The average rainfall of these sites ranged from 80 to 155 cm per annum.

Soil samples were stored in plastic bags in a cool room prior to laboratory analysis and greenhouse studies. Soils were air dried and sieved through a two centimeter sieve in preparation for the greenhouse studies. A representative subsample weighing approximately one Kg was taken from each soil sample for the laboratory study. These subsamples were ground in a mortar and pestle, sieved through a 2 mm sieve and stored in a refrigerator until chemical analysis was performed.

The subterranean clover samples were dried in a forced air

Table 2. Location of Sampling Sites

Sample No.	Location	County	Treatment
1a	Cannon	Coos	Check
1b	Cannon	Coos	Limed in field
1c	Cannon	Coos	Limed in lab
2a	Rydell	Douglas	Check
2b	Rydell	Douglas	Limed in lab
3a	Bambridge	Douglas	Check
3b	Bambridge	Douglas	Mo
3c	Bambridge	Douglas	Limed in lab
4a	John Wahl	Curry	Check
4b	John Wahl	Curry	Limed in field
5a	Hiatt	Washington	Check
5b	Hiatt	Washington	Limed in lab
6a	Sletto	Marion	Check
6b	Sletto	Marion	Limed in lab
7	Schacht	Marion	Check
8a	Harold Knapp	Curry	Check
8b	Harold Knapp	Curry	Mo + Limed in field
9a	Thorton	Yamhill	Check
9b	Thorton	Yamhill	Limed in field
10a	Zimmerman	Marion	Check
10b	Zimmerman	Marion	Limed in field
11a	Beckley	Douglas	Check
11b	Beckley	Douglas	Limed in field
12a	Ingram	Coos	Check
12b	Ingram	Coos	Mo
13a	Tschantz	Yamhill	Check
13b	Tschantz	Yamhill	Limed in field
14a	Geaney	Coos	Check
14b	Geaney	Coos	Mo
15	Simmons	Polk	Check
16a	Langdon	Douglas	Check
16b	Langdon	Douglas	Mo
17a	Holland	Coos	Check
17b	Holland	Coos	Limed in field
18	Humpherey	Marion	Check
19	Oberg Bros.	Yamhill	Check
20a	John Bjerg	Curry	Check
20b	John Bjerg	Curry	Mo
21	McGuire	Polk	Check
22a	Carmen	Coos	Check

Table 2. (Continued)

Sample No.	Location	County	Treatment
22b	Carmen	Coos	Mo
23a	Mast	Coos	Check
23b	Mast	Coos	Mo
24	Hartley	Marion	Check
25a	Russell	Douglas	Check
25b	Russell	Douglas	Mo
26	Fard	Marion	Check
27	Mosher	Douglas	Check
28	Rice Dillard	Douglas	Check
29	Canyonville	Marion	Check
30a	Gross	Coos	Check
30b	Gross	Coos	Mo
31	Goslin	Washington	Check
32	Stanford	Douglas	Check

Note:

1. Five most acid soils (samples Nos. 1c, 2b, 3c, 5b, and 6b) were limed in the laboratory to 80 percent base saturation for greenhouse studies.
2. Only plant samples were collected from 11b and 23b sampling sites.
3. Only soil samples were collected from 16b; 17a, b; 20a, b; 25b; 28 sampling sites.
4. Soil sample numbers 1b; 3b; 5a; 8b; 10a; 12b; 14a, b; 16b; 20b; 22b; 25b; 30b; 31 were brought later on from the field. So they were not included in the greenhouse studies.

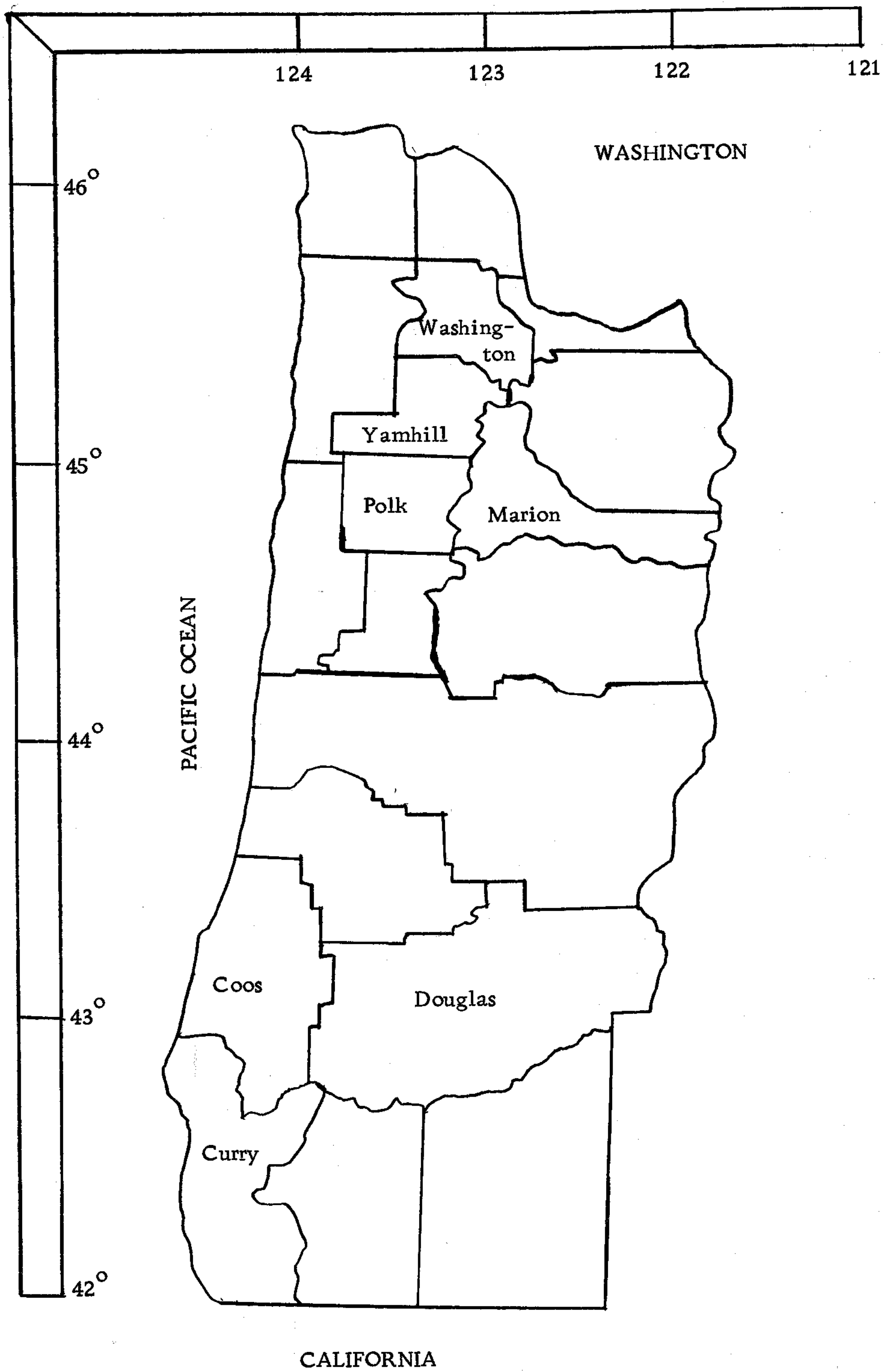

Figure 2. Map of western Oregon.

oven for about 48 hours at about 75°C ± 5°C. Plant samples were then ground in a Wiley Mill and stored until plant chemical analysis was conducted.

Greenhouse Experiments

Experimental Design and Treatments

A completely randomized design was utilized and the data analyzed as a factorial. The treatments included two rates of molybdenum, check and a rate of molybdenum equivalent to 0.444 Kg/hectare. There were three replications per treatment.

All soils were uniformly treated with a blanket application of P, K, S, and B as indicated in Table 3. All the nutrients were applied in solution form. The five most acid soils were limed to 80 percent base saturation. These five soils were also included in the greenhouse study unlimed.

The entire experiment was conducted in one quart plastic cottage cheese pots. Small holes were made in the bottom of each pot to facilitate drainage. Air dry 2 mm sieved soil (908 g) was weighed into appropriately labelled pots. A blanket fertilizer was added in each pot as a nutrient solution to provide P, K, S, and B at rates indicated in Table 3. Pots were then brought to near field capacity with distilled water before sowing subterranean clover.

Table 3. Amount and source of nutrients applied to the soils in the greenhouse experiments.

Nutrient	Equivalent rate of nutrient (kg/hectare)	Source of nutrient	Amount of source added (g/4 litters)	Nutrition solution added (cc/pot)
P	90.0	KH_2PO_4 + $NaH_2PO_4 \cdot H_2O$	50.707 13.437	20
K	90.0	KH_2PO_4	50.707	
S	90.0	$CaSO_4 \cdot 2H_2O$	78.307	
B	5.6	$Na_2B_4O_7 \cdot IOH_2O$	7.995	20
Mo	0.444	$Na_2MoO_4 \cdot 2H_2O$	0.181	20

Subterranean clover (Mount Baker variety) seed was inoculated with NZ-29 rhizobium culture and was sown into the pots on September 8, 1969. The seed was mixed into the soil and distilled water was sprinkled onto the top of each pot at regular intervals to maintain moisture near field capacity. Pots were thinned in an effort to keep uniform number of plants in each pot. Molybdenum was applied to each pot as a solution of sodium molybdate (Table 3). Pots were maintained near field capacity for the duration of the study.

Greenhouse lighting consisted of daylight fluorescent lamps spaced at 10 cm intervals and maintained at 30 cm above the plant tops. Red spectra lighting was provided by parallel strings of 100 watt incandescent lamps, one lamp every two feet. Aluminum foil was fixed on top of lamps to increase efficiency of lighting. The greenhouse temperature was controlled at 27° C (80° F) during day and 18° C (65° F) during the night.

The first harvest was taken on October 18, 1969 and the second and final harvest on November 29, 1969. Plant samples from each harvest were dried, weighed and ground as previously noted. Ground plant samples from the three replications were then bulked for plant analysis.

Laboratory Analysis

Soil Analysis[1]

Routine soil analysis was carried out in the laboratory using the Standard Oregon State University soil analysis methods listed in Table 4.

Total soil molybdenum was determined colorimetrically by the thiocyanate method (Johnson and Arkley, 1954) after being wet ashed with perchlonic acid (Reisenauer, 1965).

Anion exchangeable soil molybdenum was assayed on a twenty-five gram soil sample which had been ground to pass 60 mesh sieve. The soil was placed in a 250 ml erlenmeyer flask containing 100 ml of double distilled water and 10 g of moist anion exchange resin (Dowex 1-X4, J. T. Baker Chemical Co.). The soil solution was

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[1]The author is grateful to the Soil Testing Laboratory, Oregon State University, for routine soil chemical analysis.

Table 4. Summary of the methods used for the routine soil chemical analyses.

Test or determination	Reference	Notes on the method
pH	Jackson, 1958	1:2 soil-water suspension was used to determine the pH of the soil samples.
Total nitrogen	Assoc. of Official Agri. Chem. Ed. 8. 1955	Total nitrogen was determined by the Kjeldahl method.
Available phosphorus	Olsen *et al.*, 1954 (modified method)	On 1:10 soil to extract ratio using 0.5 N sodium-bicarbonate, adjusted to pH 8.5, extracted P was determined by the molybdenum blue colorimetric procedure using the Bausch and Lomb Spectronic 20 spectrophotometer.
Exchangeable bases (K, Ca, Mg)	Schollenberger & Simon 1945	On a single extraction (1:10 soil to extractant ratio) using 1 N ammonium acetate adjusted to pH 7.0; the exchangeable bases were determined on a flame emission spectrophotometer.
CEC	Schollenberger & Simon 1945	CEC was determined with 1 N ammonium acetate buffered at pH 7.0.
Organic matter	Walkley & Black, 1934 Walkley, 1947	Organic matter was determined as easily oxidizable material using 0.1 N $KMnO_4$ to titrate the soil $K_2Cr_2O_7$ solution.

shaken on a wrist action shaker overnight to ensure reaction with the anion exchange resin. The resin was then washed on a 60 mesh screen to remove most of the soil. It was then transferred to a glass column with one cm inside diameter. Thereupon the column was leached with 50 ml of 2M NaCl. Anion exchangeable molybdenum in the leachate was determined colorimetrically (Reisenauer, 1965).

Plant Analysis

Total nitrogen was determined according to the method given in Table 4.

Plant samples were prepared for copper and molybdenum analysis by wet ashing with perchloric acid (Jackson, 1958). Copper was determined on a Perkin Elmer, Model 303, atomic absorption spectrometer. Molybdenum was determined colorimetrically (Reisenauer, 1965).

Statistical Analysis

Treatment effect on clover yield (combined greenhouse harvest) and nitrogen uptake were statistically assessed by the analysis of variance technique employing a factorial design model (Snedecor and Cochran, 1967). To evaluate the levels of significance, Fisher's "F" test and LSD values at 0.05 and 0.01 probability levels were also calculated.

RESULTS AND DISCUSSION

Soil Chemical Analysis

Soil test results from surface 15 cm of soil obtained at each field sampling sites are presented in Tables 5, 6, and 7.

Total Nitrogen, Organic Matter, and Available Phosphorus

In reference to Table 5, it has been observed that total nitrogen (dry weight basis) ranged from 0.119 to 0.489 percent, organic matter from 2.81 to 15.20 percent, and available phosphorus from 1.0 to 12.4 ppm.

In general, higher levels of nitrogen were associated with higher organic matter content. Soils that had been limed appeared to contain more anion exchangeable soil Mo (Table 7) than their unlimed counterparts. Several other workers have made similar observations (Barshad, 1951; Evans, Purvis, and Bear, 1951; Anderson, 1956a).

On account of the soil phosphorus levels being low in these soils, they were treated with phosphorus before planting. According to Anderson (1956a), Anderson and McLachlan (1951), and McLachlan (1955), responses to molybdenum application can only be observed when phosphorus deficiency of soil has been corrected.

Table 5. Soil chemical analysis for total nitrogen, organic matter, and phosphorus.

Sample No.	TN (%)	OM (%)	Phos. (ppm)	Sample No.	TN (%)	OM (%)	Phos. (ppm)
1a	0.337	8.22	2.8	16a	0.214	5.84	2.1
2a	0.243	5.19	1.4	17a	0.302	9.09	2.1
3a	0.268	6.87	1.0	17b	0.235	6.82	3.8
4a	0.450	15.20	1.4	18	0.260	7.41	4.1
4b	0.364	11.36	1.4	19	0.213	4.81	5.5
6a	0.260	6.87	1.4	20a	0.330	8.01	2.1
7	0.389	8.22	1.0	21	0.239	5.36	2.8
8a	0.489	13.15	1.0	22a	0.197	5.30	2.8
9a	0.273	4.71	1.0	23a	0.175	6.87	2.1
9b	0.313	6.33	4.1	24	0.189	3.52	4.8
10b	0.272	6.17	1.4	25a	0.274	4.98	1.0
11a	0.289	7.03	1.4	26	0.217	6.98	2.8
12a	0.152	4.60	1.4	27	0.119	2.81	4.8
13a	0.301	6.98	4.8	28	0.164	3.52	2.1
13b	0.313	7.14	2.8	29	0.341	10.00	9.0
15	0.218	4.87	1.4	30a	0.189	5.14	3.5
				32	0.246	9.31	12.4

Note:
1. Refer Table 2 for details of sampling sites.
2. Five most acid soils (sample Nos. 1c; 2b; 3c; 5b; 6b) were limed. So they were not included in this table for routine soil chemical analysis.
3. No soil samples were collected from 11b and 23b sampling sites.
4. Soil sample Nos. 1b; 3b; 5a; 8b; 10a; 12b; 14a,b; 16b; 20b; 22b; 25b; 30b; 31 were brought later on from the field. So they were not run for routine soil chemical analysis.

Exchangeable Cations and Cation Exchange Capacity

Data in Table 6 indicated the following ranges of exchangeable bases, potassium 0.24 to 1.49, calcium 2.00 to 22.80, and magnesium 0.70 to 15.60 me/100 g of soil. The cation exchange capacity of the soils under study varied from 11.30 to 49.60 me/100 g of soil.

pH, Total Molybdenum, and Anion Exchangeable Molybdenum

Fifty two soils (including check, lime, and molybdenum treatments) were included in the study. Their pH values varied from 4.95 to 7.10. Total molybdenum ranged from 0.36 to 0.95 μg/g, and anion exchangeable molybdenum varied between 0.36 to 2.10 μg/100 g of soil (Table 7).

Soils 17a, 17b, and 28, which indicated exceptionally high amounts of soil anion exchangeable molybdenum (20·32, 31·86, and 70·40 μg/100 g soil) were not included in any correlation studies involving molybdenum.

Soil anion exchangeable molybdenum was highly correlated with soil pH in the non-molybdenum treated soils (Figure 3). The sample correlation coefficient, r = 0.803 was obtained from the regression equation:

$$y = -2.618 + 0.642X$$

where y = soil anion exchangeable molybdenum (μg/100g)

Table 6. Soil chemical analysis for the exchangeable cations.

Sample No.	me/100g K	Ca	Mg	CEC	Sample No.	me/100g K	Ca	Mg	CEC
1a	1.03	4.4	2.3	25.0	16a	0.50	7.8	5.9	23.9
2a	0.86	3.8	2.7	20.1	17a	1.49	7.8	2.6	28.5
3a	0.37	2.8	1.3	19.5	17b	0.90	5.0	1.8	22.0
4a	0.52	2.2	1.0	33.4	18	1.47	9.6	4.1	29.7
4b	0.50	4.4	1.1	27.4	19	0.88	12.5	5.0	25.9
6a	0.78	6.8	3.0	38.7	20a	0.60	7.8	3.4	24.6
7	0.42	8.4	4.1	35.5	21	0.85	19.8	9.1	36.3
8a	0.64	2.0	0.7	30.3	22a	0.66	6.6	4.6	23.6
9a	0.62	6.2	1.8	27.5	23a	0.82	5.6	1.5	29.8
9b	0.56	11.7	2.0	29.1	24	1.18	12.8	5.0	23.2
10b	0.56	15.8	3.7	34.3	25a	0.43	12.4	6.4	30.9
11a	0.64	4.1	1.8	20.6	26	0.84	8.7	2.4	23.3
12a	0.52	4.8	2.3	26.5	27	0.28	5.3	3.3	11.3
13a	0.24	5.0	2.5	24.5	28	0.26	7.1	4.6	16.4
13b	0.28	7.7	2.9	23.4	29	0.24	15.8	7.1	26.2
15	0.39	6.6	2.3	21.6	30a	0.70	5.6	2.7	19.6
					32	0.86	22.8	15.6	49.6

Note: 1. Refer Table 2 for details of sampling sites.
2. Five most acid soils (sample Nos. 1c; 2b; 3c; 5b; 6b) were limed. So they were not included in this table for routine soil chemical analysis.
3. No soil samples were collected from 11b and 23b sampling sites.
4. Soil sample Nos. 1b; 3b; 5a; 8b; 10a; 12b; 14a, b; 16b; 20b; 22b; 25b; 30b; 31 were brought later on from the field. So they were not run for routine soil chemical analysis.

Table 7. Soil chemical analysis for pH, total molybdenum, and anion exchangeable molybdenum.

Sample No.	pH (1:2)	Exch. Mo (μg/100g)	Total Mo (μg/g)	Sample No.	pH (1:2)	Exch. Mo (μg/100g)	Total Mo (μg/g)
1a	4.95	0.50	0.44	14a	5.30	0.75	0.30
1b	6.15	1.60	0.50	14b	5.30	0.85	0.26
1c	6.90	1.76	0.42	15	5.35	0.96	0.81
2a	5.05	0.54	0.42	16a	5.40	0.96	0.75
2b	6.75	1.36	0.50	16b	5.40	1.04	0.72
3a	5.15	0.64	0.44	17a	5.45	20.32	3.16
3b	5.10	1.76	0.46	17b	5.85	31.86	10.30
3c	6.80	2.00	0.44	18	5.50	0.80	0.77
4a	5.15	0.36	0.40	19	5.50	0.50	0.40
4b	5.25	1.20	0.46	20a	5.50	1.00	0.49
5a	5.15	0.90	0.46	20b	5.80	1.36	0.55
5b	7.00	1.36	0.38	21	5.55	1.4	0.70
6a	5.15	0.36	0.40	22a	5.55	0.90	0.36
6b	7.10	2.10	0.42	22b	5.80	1.16	0.42
7	5.20	0.70	0.44	23a	5.60	0.76	0.42
8a	5.20	0.80	0.53	24	5.65	0.90	0.54
8b	5.50	1.00	0.50	25a	5.65	0.90	0.28
9a	5.20	0.40	0.50	25b	5.65	1.10	0.26
9b	5.50	0.70	0.63	26	5.75	1.54	0.61
10a	5.20	0.80	0.36	27	5.80	0.84	0.28
10b	5.40	1.36	0.46	28	5.80	70.40	9.60
11a	5.25	0.80	0.46	29	5.80	1.10	0.50
12a	5.25	0.70	0.34	30a	5.80	1.20	0.36
12b	5.25	2.00	0.30	30b	5.75	1.26	0.38
13a	5.25	0.54	0.95	31	5.95	0.84	0.54
13b	5.50	0.90	0.75	32	6.15	2.00	0.42

Note: 1. Refer Table 2 for details of sampling sites.

2. No soil samples were collected from 11b and 23b sampling sites.

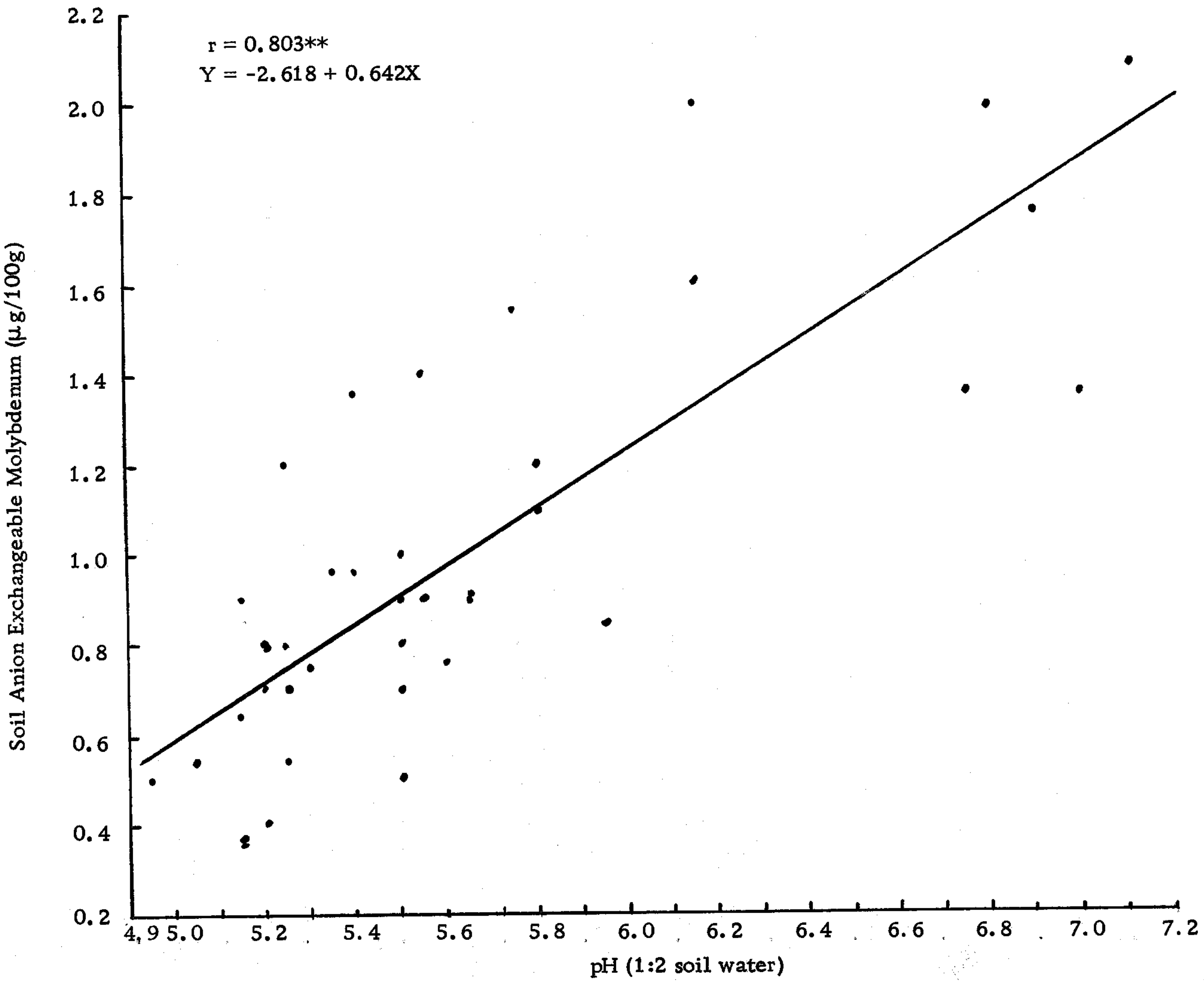

Figure 3. Relationship between soil pH and soil anion exchangeable molybdenum in non-molybdenum treated soils.

and x = pH (1:2 soil water)

This correlation suggests that molybdenum availability increases as the pH increases from 4.95 to 7.10 (Table 6). These results are in agreement with other workers (deMooy, 1970; Reisenauer, 1968; Tisdale and Nelson, 1966; Jones, 1957; Mulder, 1954; Barshad, 1951; Evans, Purvis, and Bear, 1951).

Total soil molybdenum ranged from 0.36 to 0.95 μg/g (Table 7). Only three soil samples contained 3.16, 10.30, and 9.60 μg Mo/g soil. Similar results were obtained by Kubota et al. (1967) for Oregon soils. No apparent relationship has been observed between either pH and total molybdenum or total molybdenum and soil anion exchangeable molybdenum. Johnson (1966) and Robinson et al. (1951) reported that total amounts may not indicate adequate plant available molybdenum. However, Kubota et al. (1967) have indicated that total molybdenum content of soil is an index of molybdenum content of legumes.

Chemical Analysis of Field Subterranean Clover

Molybdenum

Data in Table 8 indicate that field subterranean clover plant samples contained from traces to 1.23 μg/g molybdenum. Similar range has been reported for alfalfa and soybean by a number of

Table 8. Molybdenum, copper, and nitrogen concentration of field subterranean clover samples.

Sample No.	Mo (µg/g)	Cu (µg/g)	N (%)	Sample No.	Mo (µg/g)	Cu (µg/g)	N (%)
1a	0.24	11.8	2.61	14a	0.14	21.0	2.78
1b	1.23	8.3	3.63	14b	0.25	10.0	2.92
2a	0.29	8.3	2.63	15	0.20	8.3	2.72
3a	0.33	9.0	2.50	16a	0.54	7.5	3.47
3b	1.00	7.0	3.36	18	0.23	9.4	2.82
4a	0.10	15.3	2.50	19	0.22	10.0	2.75
4b	0.40	7.6	3.19	21	0.60	7.0	3.13
5a	0.15	10.5	3.11	22a	0.41	10.0	3.05
6a	0.07	10.8	2.48	22b	0.69	9.4	3.16
7	0.18	11.8	2.90	23a	0.13	12.9	2.62
8a	0.16	16.0	2.77	23b	0.67	4.7	3.04
8b	0.17	7.6	3.05	24	0.14	5.3	2.89
9a	0.00	8.3	2.69	25a	0.17	5.7	2.94
9b	0.12	8.3	2.81	26	0.60	13.0	3.21
10a	0.28	9.4	2.85	27	0.12	12.9	2.72
10b	0.56	9.2	3.33	29	0.54	11.3	3.33
11a	0.14	11.8	2.65	30a	0.39	7.0	3.11
11b	0.17	10.0	2.94	30b	0.44	4.1	3.39
12a	0.21	6.0	2.65	31	0.17	9.4	2.89
12b	0.80	6.0	3.50	32	1.14	10.5	3.61
13a	0.19	25.3	2.73				
13b	0.21	18.8	2.97				

Note: 1. Refer Table 2 for details of sampling sites.
2. No plant samples were available at 16b; 17a, b; 20a, b; 25b; 28 sampling sites.

investigators (Boswell and Anderson, 1969; Melsted et al., 1969; James et al., 1968; Reisenauer, 1956).

The correlation between soil anion exchangeable molybdenum and plant molybdenum concentration in field samples gave an r value of 0.861 (Figure 4). The high correlation so obtained suggests that the anion exchangeable molybdenum status of soils under study considerably influenced the plant molybdenum concentration. Evans et al. (1950) have reported that there was close relationship between the quantity of molybdenum applied in the nutrient solution and the molybdenum content of alfalfa tissue. Kubota et al. (1967) observed a linear association between total soil molybdenum and molybdenum concentration of legumes ($r = 0.962$). Gupta (1969) noted that the application of molybdenum or lime increased the molybdenum content of alfalfa tissue up to the highest level of applied molybdenum or lime.

The high correlation between anion exchangeable molybdenum and plant molybdenum certainly suggests that the anion resin is capable of assaying plant available molybdenum.

Copper

The copper concentration in the field samples (Table 8) ranged from 4.10 to 25.30 μg/g. Copper concentration decreased with an increase in molybdenum content of plant following the application of

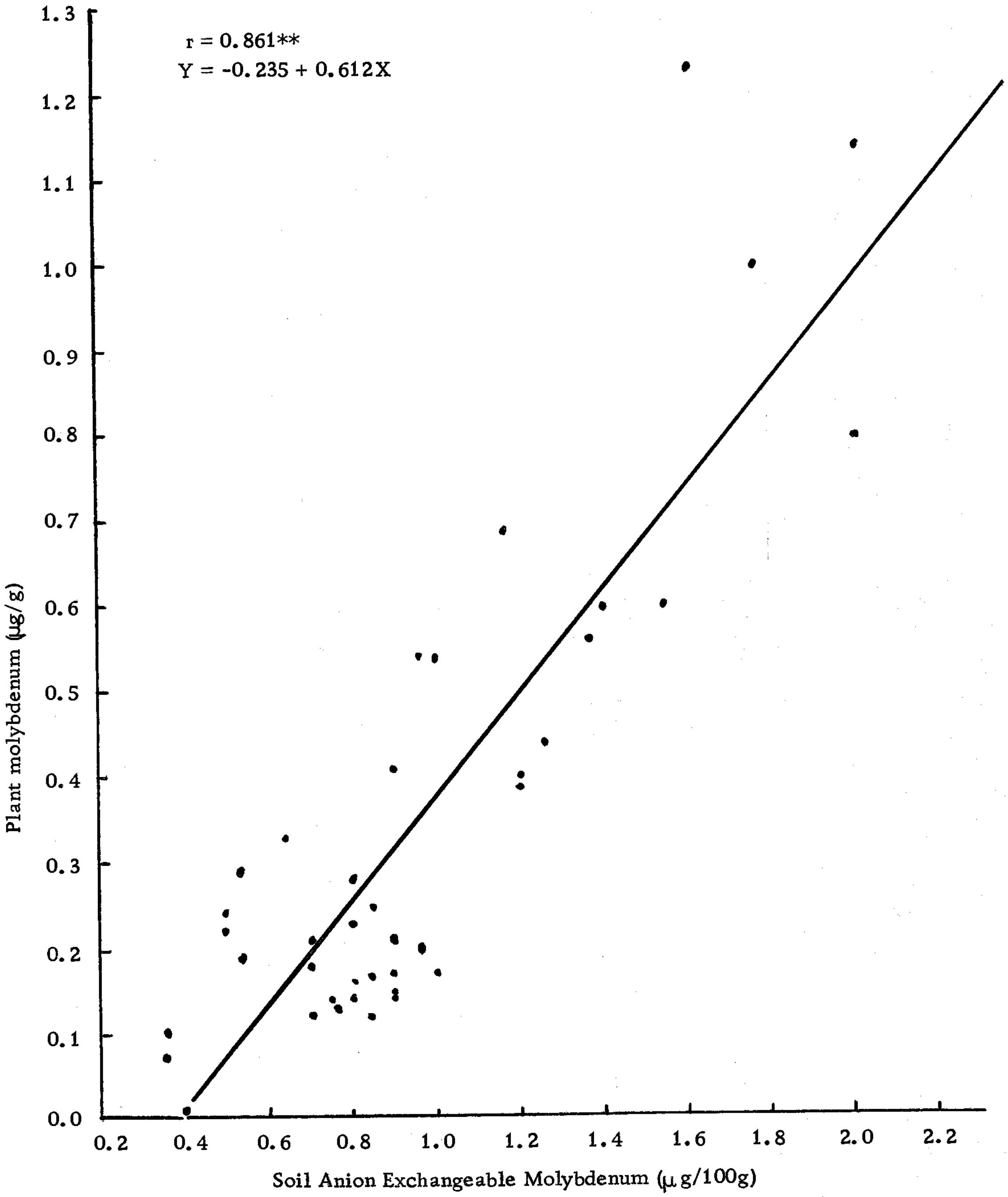

Figure 4. Relationship between soil anion exchangeable molybdenum and plant molybdenum from field samples.

either lime or molybdenum to the soil. In all but 6 instances, copper concentration of the field sampled clover plants was above the critical copper level of 6.70 μg/g suggested by Nelson, Berger, and Andries (1956). Several other investigators (Durings, 1967; Rubota et al., 1967; Dick, 1956) have reported that the copper availability to the animal might possibly be limited by a high pasture molybdenum concentration and called it Mo-induced Cu deficiencies.

Nitrogen

The plant nitrogen concentration varied between 2.48 to 3.68 percent (Table 8). Reisenauer (1956) reported similar figures ranging from 2.63 to 3.61 percent in case of severe and non nitrogen-starvation symptoms in alfalfa respectively.

The correlation coefficient of 0.875 indicated a good relationship between soil anion exchangeable molybdenum and percent nitrogen in subterranean clover (Figure 5). A curvilinear relationship ($r = 0.815$) was found when plant molybdenum was correlated with percent nitrogen in subterranean clover plant (Figure 6). The role of molybdenum in the process of nitrogen fixation has been well documented (Bortels, 1930, 1937; Anderson, 1942; Anderson and Spencer, 1950a; Evans, 1956; Evans et al., 1950, 1951).

The increase in plant nitrogen associated with more soil available Mo undoubtedly affected the quality of subterranean clover

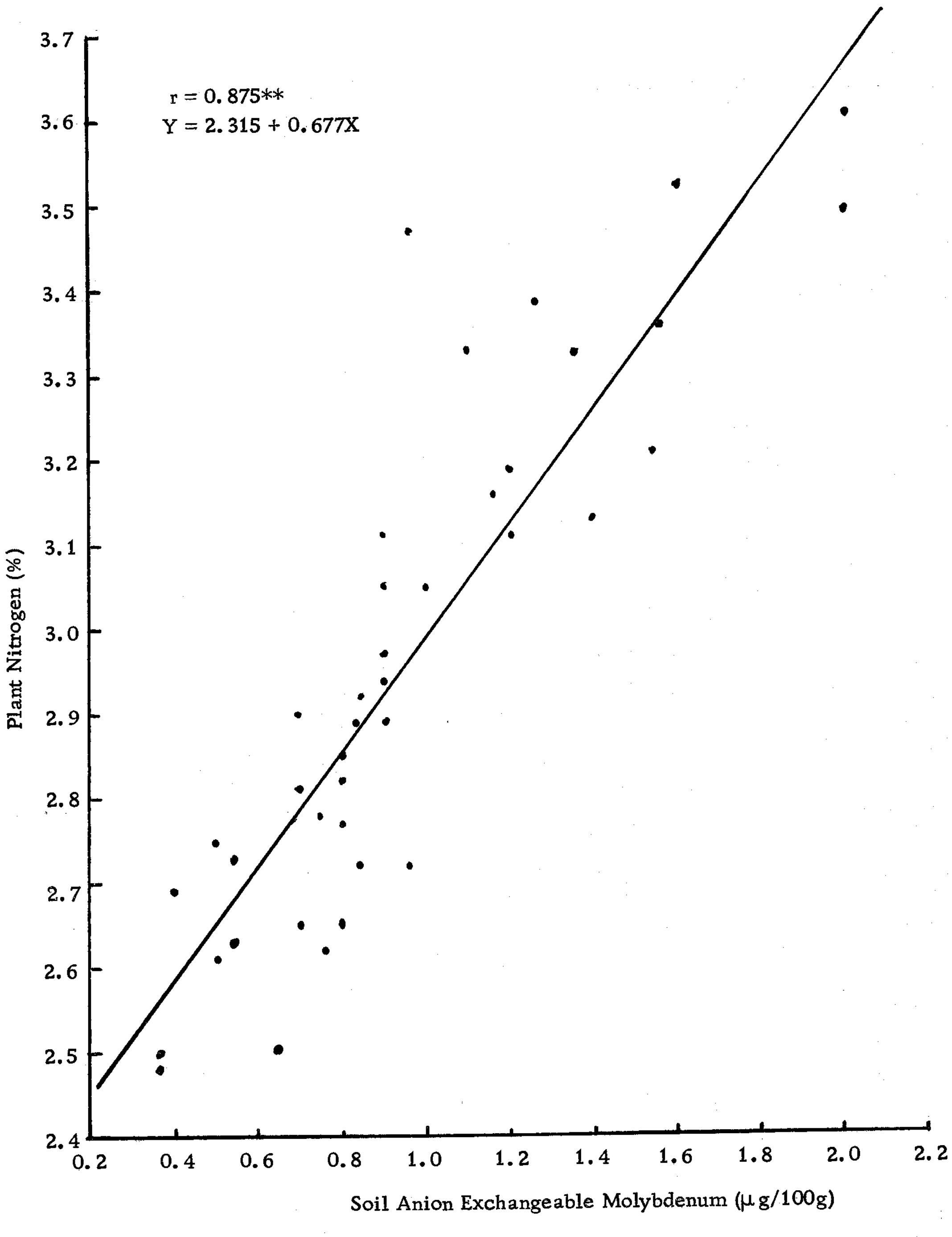

Figure 5. Relationship between soil anion exchangeable molybdenum and plant nitrogen from field samples.

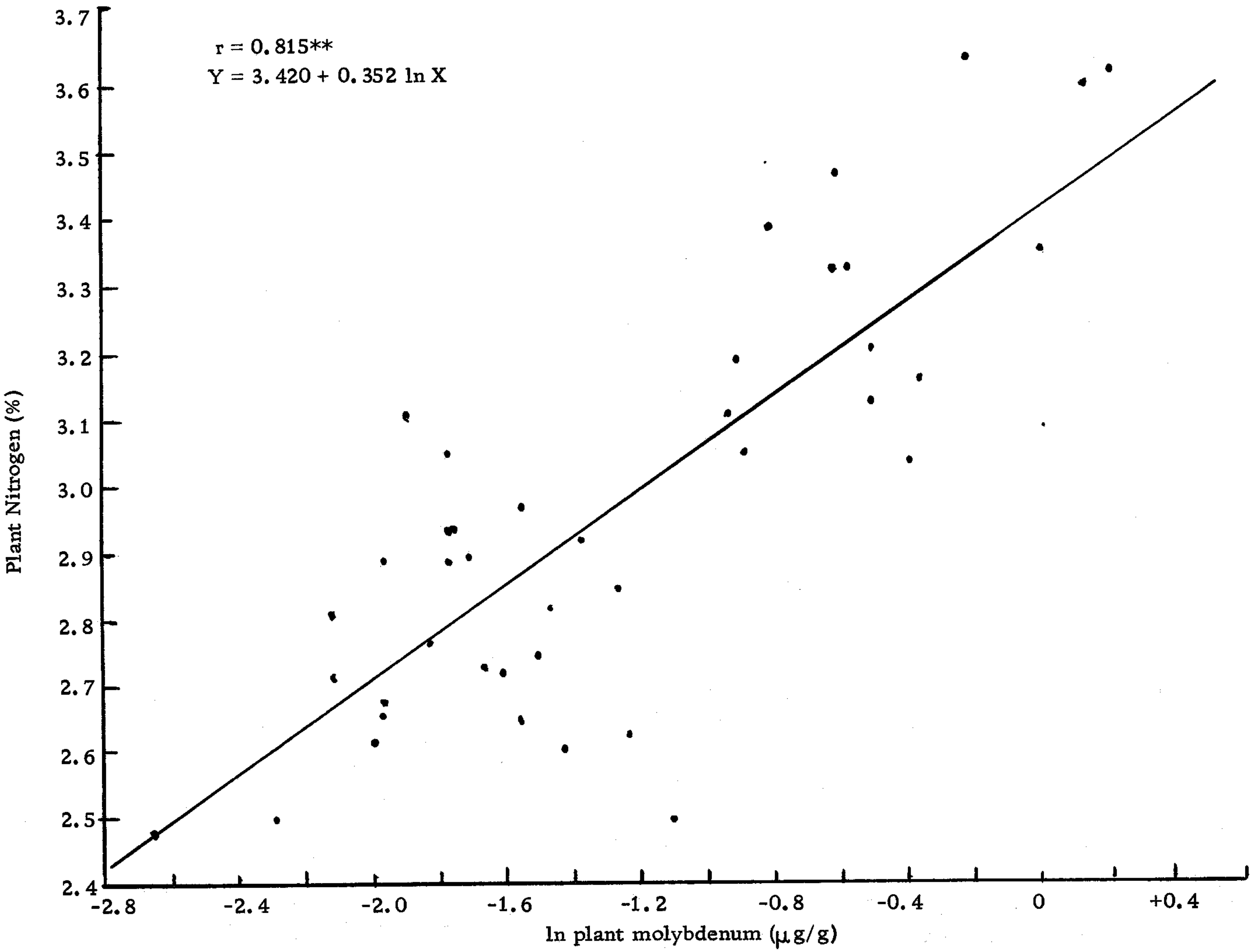

Figure 6. Relationship between plant molybdenum and plant nitrogen concentration from field samples.

as a source of plant protein. Upon converting the percent plant nitrogen to percent protein (percent protein = percent N x 6.25), subterranean clover protein content was increased 8.19 percent due to higher supplies of available soil molybdenum.

Greenhouse Experiments

Subterranean Clover Yield

The average total dry matter yield from the greenhouse experiments obtained from two harvests is presented in Table 9. The data reveals that subterranean clover yield varied from 3.263 to 7.388 g/pot for the non-molybdenum treatment and from 3.954 to 8.623 g/pot for the molybdenum treated soils. The mean clover yield for the non-molybdenum treated soils compared with molybdenum treated was calculated as 5.633 and 6.102 g/pot respectively.

An analysis of variance (Appendix Table 3) indicated the yield increases following molybdenum application were highly significant (F calculated = 54.50; F required = 6.81). A highly significant response to molybdenum application was observed in 31 of the 38 soils included in the experiments. Two soils gave a significant negative response, while only two gave no response to applied molybdenum. These results would indicate the vast majority of soils studied were molybdenum deficient. No explanation can readily be offered to

Table 9. Subterranean clover yield (dry matter/pot) and percent yield response over check.

Sample No.	Yield (g/pot) Treatment		Yield response (%)	Yield response (g/pot)	Sample No.	Yield (g/pot) Treatment		Yield response (%)	Yield response (g/pot)
	Check	Mo				Check	Mo		
1a	4.244	5.151	121.0	0.907**	13b	5.748	6.356	111.0	0.608**
1c	5.995	6.815	114.0	0.820**	15	5.645	6.162	109.0	1.517**
2a	5.365	6.161	115.0	0.796**	16a	7.888	8.623	109.0	0.735**
2b	6.202	6.485	105.0	0.283**	17a	5.907	6.245	106.0	0.338**
3a	4.444	5.136	115.6	0.692**	17b	4.627	5.438	117.0	0.811**
3c	6.208	6.702	108.0	0.494**	18	7.790	7.912	102.0	0.122NS
4a	3.825	4.342	114.0	0.517**	19	6.907	8.325	120.0	1.418**
4b	4.877	5.941	122.0	1.064**	20a	7.180	6.924	96.4	0.256**
5b	6.313	7.169	113.6	0.856**	21	7.383	6.915	94.0	-0.418**
6a	5.760	6.080	106.0	0.320**	22a	4.816	5.262	109.0	0.446**
6b	6.812	7.265	107.0	0.453**	23a	6.108	6.920	113.0	0.812**
7	5.734	6.453	112.5	0.719**	24	5.168	5.320	103.0	0.152*
8a	3.630	3.954	109.0	0.324**	25a	4.242	4.380	103.0	0.138*
9a	5.121	5.859	114.0	0.738**	26	7.073	6.983	99.0	-0.090NS
9b	5.038	5.654	112.0	0.616**	27	4.472	4.793	107.0	0.321**
10b	6.059	6.428	106.0	0.369**	28	4.120	4.385	106.4	0.265**
11a	6.300	6.451	102.4	0.151*	29	6.714	6.095	91.0	-0.619**
12a	5.594	5.804	104.0	0.210**	30a	5.382	6.362	118.0	0.980**
13a	5.692	6.268	110.0	0.576**	32	3.263	4.360	134.0	1.097**

Note:
1. Refer Table 2 for details of sampling sites.
2. Five most acid soils (sample Nos. 1c; 2b; 3c; 5b; 6b) were limed in the laboratory to 80 percent base saturation and were included in the greenhouse studies.
3. No soil samples were collected from 11b and 23b sampling sites.
4. Soil sample numbers 1b; 3b; 5a; 8b; 10a; 12b; 14a, b; 16b; 20b; 22b; 25b; 30b; 31 were brought later on from the field. So they were not included in the greenhouse studies.

account for the depression in clover yield on these two soils following Mo application.

Similar results have been recorded by deMooy (1970), James et al. (1968), Evans et al. (1950), and Anderson (1942) for soybean, alfalfa, and subterranean clover.

Percent Yield Response

Greater percent clover yield responses over check following molybdenum application occurred in soils where less Mo was extracted with anion exchange resin. The relationship between anion exchangeable soil molybdenum and percent yield response to applied molybdate was examined on 24 soils with pH $\leqslant$ 5.75 (Figure 7). The resultant correlation r = -0.743 demonstrated that the Mo extracted from soils by anion exchange resin is well correlated with clover yield responses in the greenhouse. When the exchangeable molybdenum and pH exceeded 1.54 μg/100g and 5.75 respectively, there appeared to be no similar correlation between anion exchange Mo and percent yield increase in clover.

Soil samples numbers 4b and 6a were not included in the afore mentioned correlation since plants grown on these soils were late discovered to be Cu deficient. High organic matter (11.36 percent) and low pH (5.25) likely contributed to the Cu deficiency apparent as classical symptoms in the leaves. The Cu deficient plants recovered

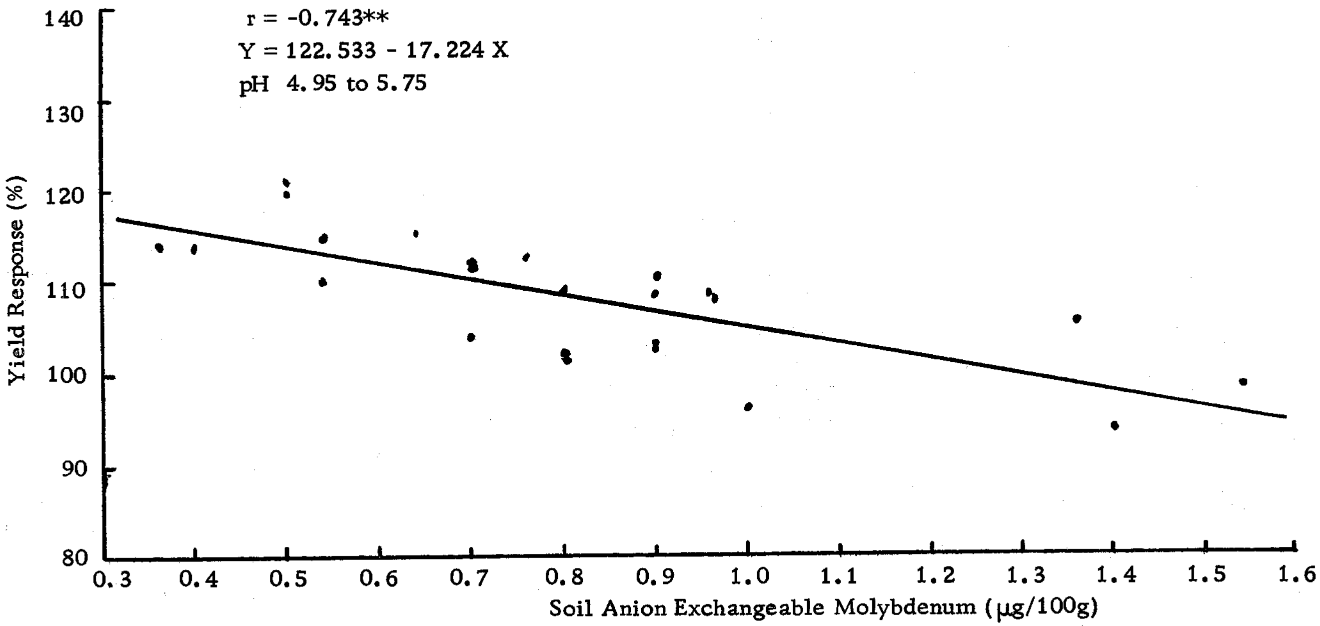

Figure 7. Relationship between soil anion exchangeable molybdenum and yield response in greenhouse studies.

when Cu was applied to the soil.

Subterranean clover which gave the greatest percent yield response over check to applied Mo occurred in soils with anion exchangeable molybdenum levels approaching 0.50 μg/100 g.

It is of interest to note that Lavy and Barber (1963) reported a relationship between the molybdenum content of the seeds from the untreated plot and yield response of soybeans due to molybdenum application. In their case, molybdenum response was related to soil pH and molybdenum concentration of 1.60 μg/g in untreated soybean seeds.

Plant Molybdenum

The molybdenum concentration of the plant material obtained from the greenhouse experiment is presented in Table 10. Molybdenum concentration of clover grown in non-Mo treated soils varied from 0.02 to 1.54 μg/g except 17b and 28. In molybdenum treated soils the clover Mo concentration ranged from 0.05 to 2.10 μg/g, except for two samples (17b and 28).

It is apparent from this data that plant molybdenum concentration was markedly increased following the application of molybdenum to the soils. Several research workers have reported such results (Evans *et al.*, 1950; Lavy and Barber, 1963; Boswell and Anderson, 1969; deMooy, 1970).

Table 10. Molybdenum concentration (μg/g) of greenhouse grown subterranean clover on molybdenum treated and untreated soils.

Sample No.	Treatment		Sample No.	Treatment	
	Check	Mo		Check	Mo
1a	0.11	0.16	15	0.28	0.34
1c	0.11	0.29	16a	0.27	0.50
2a	0.38	0.45	17a	1.54	2.10
2b	0.20	0.68	17b	22.80	23.40
3a	0.08	0.52	18	0.21	0.29
3c	0.30	0.60	19	0.21	0.30
4a	0.11	0.18	20a	0.13	0.22
4b	0.07	0.72	21	0.06	0.26
5b	0.39	0.50	22a	0.02	0.05
6a	0.05	0.27	23a	0.05	0.36
6b	0.33	0.65	24	0.06	0.20
7	0.25	0.49	25a	0.07	0.09
8a	0.03	0.16	26	0.04	0.20
9a	0.11	0.36	27	0.38	0.80
9b	0.16	0.31	28	11.10	13.59
10b	0.06	0.15	29	0.36	0.43
11a	0.28	0.41	30a	0.25	0.38
12a	0.06	0.08	32	0.42	0.72
13a	0.35	0.41			
13b	0.11	0.21			

Note:
1. Refer Table 2 for details of sampling sites.
2. Five most acid soils (sample Nos. 1c; 2b; 3c; 5b; 6b) were limed in the laboratory to 80 percent base saturation and were included in the greenhouse studies.
3. No soil samples were collected from 11b and 23b sampling sites.
4. Soil sample number 1b; 3b; 5a; 8b; 10a; 12b; 14a, b; 16b; 20b; 22b; 25b; 30b; 31 were brought later on from the field. So they were not included in the greenhouse studies.

That plant Mo concentration of greenhouse grown clover (Table 10) is much lower than clover obtained from the field (Table 8) is apparent from data obtained in these studies. James et al. (1968) have noted similar results.

Two plant samples contained relatively higher concentrations of molybdenum. The clover grown in non-molybdenum treated soils contained molybdenum concentrations of 11.10 and 22.80 μg/g while in molybdenum treated soils 13.59 and 23.40 μg/g. These high levels (10 to 20 μg Mo/g plant) have been considered by several workers to be toxic to grazing animals and may interfere with copper utilization by ruminants (Durings, 1967; Kubota et al., 1967; Dick, 1956; Barshad, 1948). Other researchers have associated these high Mo levels with the presence of high levels of total as well as exchangeable molybdenum in the soils they were grown in (Kubota et al., 1967; Davies, 1956; Barshad, 1951).

Plant Nitrogen

Data in Table 11 indicate that percent nitrogen in subterranean clover ranged from 2.10 to 3.27 percent in non-molybdenum treated soils. However, in molybdenum treated soils it varied from 2.54 to 3.61 percent. Total nitrogen fixed varied from 0.0936 to 0.2477 g/pot in non-molybdenum treatment and 0.1105 to 0.2742 g/pot in molybdenum treated soils.

Table 11. Clover nitrogen status as influenced by applied molybdenum.

Sample No.	Nitrogen (%) Treatment Check	Nitrogen (%) Treatment Mo	TN (g/pot) Treatment Check	TN (g/pot) Treatment Mo	TN response (%)	TN response (g/pot)	Sample No.	Nitrogen (%) Treatment Check	Nitrogen (%) Treatment Mo	TN (g/pot) Treatment Check	TN (g/pot) Treatment Mo	TN response (%)	TN response (g/pot)
1a	2.73	2.80	0.1159	0.1442	124.4	0.0283**	15	2.51	2.85	0.1417	0.1756	124.0	0.0339**
1c	2.84	3.31	0.1703	0.2256	132.5	0.0553**	16a	3.14	3.18	0.2477	0.2742	110.7	0.0265**
2a	2.45	2.62	0.1314	0.1614	122.8	0.0300**	17a	2.77	2.83	0.1636	0.1767	108.0	0.0131 NS
2b	3.27	3.30	0.2028	0.2140	105.5	0.0152 NS	17b	2.50	2.54	0.1157	0.1381	119.4	0.0224*
3a	2.87	3.06	0.1275	0.1572	123.2	0.0297**	18	2.94	2.96	0.2290	0.2342	102.3	0.0052 NS
3c	3.09	2.97	0.1918	0.1990	103.8	0.0072 NS	19	3.14	3.20	0.2169	0.2664	122.8	0.0495**
4a	2.56	2.60	0.0979	0.1129	115.3	0.0150 NS	20a	2.96	3.16	0.2125	0.2188	103.0	0.0063 NS
4b	2.10	2.57	0.1024	0.1527	149.0	0.0503**	21	2.99	3.19	0.2207	0.2206	100.0	-0.0001 NS
5b	3.23	3.61	0.2039	0.2588	126.9	0.0549**	22a	2.37	2.61	0.1141	0.1373	120.0	0.0232*
6a	2.78	2.78	0.1601	0.1690	105.6	0.0089 NS	23a	3.06	3.22	0.1869	0.2228	119.2	0.0359**
6b	2.90	3.06	0.1975	0.2223	112.6	0.0248*	24	2.78	2.80	0.1437	0.1490	103.7	0.0053 NS
7	3.04	3.04	0.1743	0.1962	112.5	0.0219*	25a	2.71	2.57	0.1150	0.1126	98.0	-0.0024 NS
8a	2.79	2.82	0.1013	0.1105	110.0	0.0092 NS	26	2.64	2.73	0.1867	0.1906	102.1	0.0039 NS
9a	2.57	2.62	0.1316	0.1535	116.6	0.0219*	27	2.45	3.26	0.1096	0.1563	142.6	0.0467**
9b	3.24	3.27	0.1632	0.1849	113.3	0.0217*	28	2.48	2.91	0.1022	0.1276	125.0	0.0254*
10b	3.14	3.15	0.1902	0.2025	106.4	0.0123 NS	29	3.11	3.35	0.2088	0.2042	98.0	-0.0046 NS
11a	3.01	3.17	0.1896	0.2035	102.0	0.0139 NS	30a	2.90	3.08	0.1561	0.1959	125.5	0.0398**
12a	2.35	2.92	0.1315	0.1695	129.0	0.0380**	32	2.87	3.02	0.936	0.1317	140.6	0.0381**
13a	3.00	3.12	0.1708	0.1956	114.5	0.0248*							
13b	2.65	2.96	0.1523	0.1881	123.5	0.0358*							

Note:
1. Refer Table 2 for details of sampling sites.
2. Five most acid soils (sample Nos. 1c; 2b; 3c; 5b; 6b) were limed in the laboratory to 80 percent base saturation and were included in the greenhouse studies.
3. No soil samples were collected from 11b and 23b sampling sites.
4. Soil sample numbers 1b; 3b; 5a; 8b; 10a; 12b; 14a, b; 16b; 20b; 22b; 25b; 30b; 31 were brought later on from the field. So they were not included in the greenhouse studies.

A statistically significant difference in total nitrogen fixed by the clover plants was obtained (Appendix Table 4) between molybdenum and non-molybdenum treated soils.

For 23 of the 38 soils used in the study, subterranean clover nitrogen content (g N/pot) was significantly higher following molybdate application. In the remaining soils (except soil sample numbers 21, 25a, and 29), applied molybdate tended to give higher nitrogen content even though the increase was not significant. The effect of molybdenum in increasing plant N in legumes has been previously discussed (page 38).

Percent Total Nitrogen Response

An attempt was made to determine the correlation between soil anion exchangeable molybdenum and the percent total nitrogen response (Figure 8). The correlation ($r = -0.560$) so obtained suggested that the percent total plant nitrogen response from applied molybdenum was closely related to soil anion exchangeable molybdenum between 0.30 to 1.54 μg/100 g soil and within the pH range of 4.95 to 5.75.

Plant Copper

Molybdenum application slightly decreased copper uptake by the plant. The apparent antagonistic effect of Mo on plant Cu concentration,

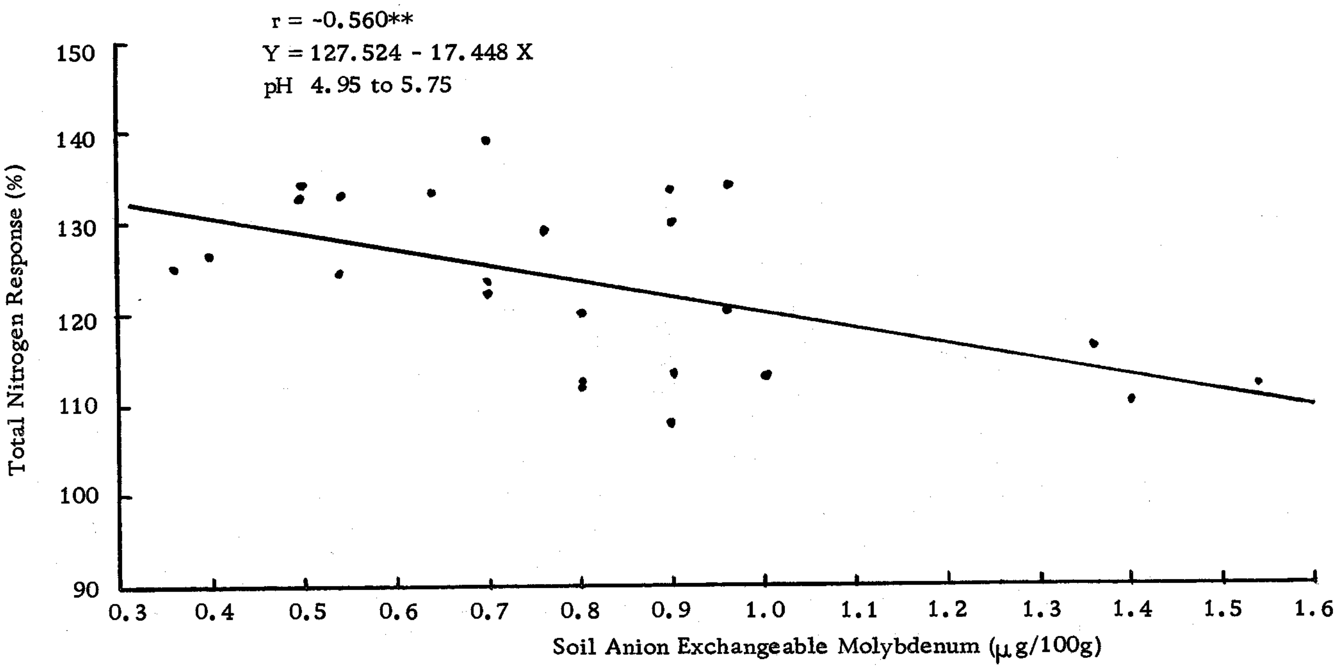

Figure 8. Relationship between soil anion exchangeable molybdenum and total nitrogen response in greenhouse studies.

Table 12. Copper concentration of greenhouse grown subterranean clover.

Sample	Cu (μg/g)		Sample	Cu (μg/g)	
	Treatment			Treatment	
No.	Check	Mo	No.	Check	Mo.
1a	9.4	7.5	15	10.9	9.8
1c	6.7	6.7	16a	11.9	11.2
2a	8.2	8.2	17a	9.4	8.2
2b	8.2	7.9	17b	9.0	8.2
3a	10.6	10.2	18	11.2	10.2
3c	8.2	9.5	19	11.3	10.9
4a	6.2	6.2	20a	9.2	8.2
4b	8.2	7.9	21	13.5	13.0
5b	9.4	9.4	22a	10.2	9.8
6a	10.0	10.0	23a	9.4	9.2
6b	8.8	8.6	24	13.3	10.5
7	9.4	8.2	25a	9.4	8.6
8a	9.2	8.2	26	9.4	9.2
9a	9.2	8.6	27	7.5	5.9
9b	9.2	6.7	28	8.3	6.8
10b	9.4	9.2	29	10.2	10.2
11a	11.0	8.5	30a	8.2	8.2
12a	9.2	6.2	32	11.2	8.6
13a	11.9	11.9			
13b	12.5	12.5			

Note: 1. Refer Table 2 for details of sampling sites.

2. Five most acid soils (sample Nos. 1c; 2b; 3c; 5b; 6b) were limed in the laboratory to 80 percent base saturation and were included in the greenhouse studies.

3. No soil samples were collected from 11b and 23b sampling sites.

4. Soil sample numbers 1b; 3b; 5a; 8b; 10a; 12b; 14a, b; 16b; 20b; 22b; 25b; 30b; 31 were brought later on from the field. So they were not included in the greenhouse studies.

though slight, can be seen in the data presented in Table 12. In most of the cases (except 3), copper concentration was well above the deficient range of 6.7 μg/g previously noted (page 38).

General Discussion

Anion exchangeable soil Mo has been shown to be correlated with clover response on Mo treated soils (Ross, 1969). In the studies reported herein, a good correlation has been established not only with anion exchangeable soil Mo and clover response to applied molybdate, but also with plant Mo as well as plant N concentrations.

A wide range of soils were included in these investigations which represented a range in parent materials, organic matter, degree of weathering, and soil pH. While no critical level of Mo for subterranean clover was established, it appeared that soils within the pH range 4.95 to 5.75 and which contained less than between 1.2 to 1.4 μg/100 g of soil anion exchangeable Mo responded to applied molybdate in the greenhouse experiments.

The higher amounts of anion resin extractable Mo with increasing soil pH provided indirect evidence that molybdate anions ($MoO_4^{=}$:$HMoO_4^{-}$) exchanged with OH^{-} ions on the clays. However, no relationship between total soil Mo and anion exchangeable soil Mo was observed.

Lower plant Mo and N concentrations in subterranean clover plant samples in the greenhouse studies as compared with field plant samples may possibly be attributed to confined root system in the pots and the purity of the chemicals used. James et al. (1968) have also reported similar results. In the greenhouse studies, no relationship was observed between anion exchangeable soil Mo and plant Mo or plant N or between plant Mo and yield.

There was no significant change in plant Cu concentration following Mo application to the soil. The slight decrease in plant Cu concentration following Mo application might well have been a dilution effect associated with increased plant growth. Mo treated soils produced clover somewhat higher in plant molybdenum concentration than untreated soils, there was little evidence of an imbalance in the copper-molybdenum plant nutritional status. Indeed, the common levels of plant Cu concentration ranged from 6.2 to 13.5 μg/g in greenhouse studies, while field samples ranged from 4.1 to 25.3 μg/g. This plant Cu level appears sufficiently high relative to the plant Mo concentration to likely present little chance of nutritional disorder in ruminants.

SUMMARY AND CONCLUSIONS

In the present studies, an effort was made to examine the use of an anion exchange resin as a method of predicting soil available molybdenum and furthermore to evaluate subterranean clover yield and nutrient content as influenced by soil molybdenum status of western Oregon hill soils.

Surface soil samples as well as clover foliage samples were collected from selected subterranean clover pastures during the spring of 1969. The greenhouse experiments were designed as a completely randomized design with factorial arrangement of treatments. The treatments used in these studies included check and the addition of molybdenum equivalent to 0.444 kg/hectare.

Apart from the routine soil chemical analysis, both total and anion exchangeable soil Mo were also determined. Dry matter yield from greenhouse experiments was recorded. Field and greenhouse grown subterranean clover plant samples were analysed for nitrogen, molybdenum, and copper.

Statistical analysis revealed that there was a significant increase in subterranean clover yield and total N resulting from Mo application in the greenhouse experiments.

When anion exchangeable soil Mo was correlated with a number of plant and soil characteristics:

1. A relationship between anion exchangeable soil Mo and percent yield response (r = -0.743) to applied Mo was established in greenhouse studies.

2. Anion exchangeable soil Mo and plant N concentration in field subterranean clover samples provided a correlation coefficient of 0.875.

3. Anion exchangeable soil Mo was correlated with percent total N response to applied Mo in the greenhouse studies.

4. Anion exchangeable soil Mo was correlated with plant Mo concentration in field clover samples (r = 0.861).

5. Anion exchangeable soil Mo was correlated with soil pH (r = 0.803).

These afore mentioned plant and soil relationships with the anion exchangeable soil molybdenum suggest that the anion exchange resin method offers promise as an acceptable procedure in predicting Mo responsive soils. It appeared that soils which contained less than between 1.2 to 1.4 $\mu g/100$ g of anion exchangeable soil molybdenum responded to applied molybdate in the greenhouse experiments.

The studies also revealed that the molybdenum concentrations and total nitrogen content of clover were markedly increased whereas copper concentrations slightly decreased following the application of either molybdenum or lime to the soil. Also the greatest percent responses in clover yield and total nitrogen to applied molybdenum occurred in soils where less molybdenum was extracted with anion exchange resin.

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APPENDIX

Appendix Table 1. Information and chemical analysis of soils.

Sample No.	pH (1:2)	Exch. Mo (μg/100g)	Total Mo (μg/g)	TN (%)	OM (%)	Phos. (ppm)	Me/100g K	Ca	Mg	CEC
1a	4.95	0.50	0.44	0.337	8.22	2.8	1.03	4.4	2.3	25.0
1b	6.15	1.60	0.50							
1c	6.90	1.76	0.42							
2a	5.05	0.54	0.42	0.243	5.19	1.4	0.86	3.8	2.7	20.1
2b	6.75	1.36	0.50							
3a	5.15	0.64	0.44	0.268	6.87	1.0	0.37	2.8	1.3	19.5
3b	5.10	1.76	0.46							
3c	6.80	2.00	0.44							
4a	5.15	0.36	0.40	0.450	15.20	1.4	0.52	2.2	1.0	33.4
4b	5.25	1.20	0.46	0.364	11.36	1.4	0.50	4.4	1.1	27.4
5a	5.15	0.90	0.46							
5b	7.00	1.36	0.38							
6a	5.15	0.36	0.40	0.260	6.87	1.4	0.78	6.8	3.0	38.7
6b	7.10	2.10	0.42							
7	5.20	0.70	0.44	0.389	8.22	1.0	0.42	8.4	4.1	35.5
8a	5.20	0.80	0.53	0.489	13.15	1.0	0.64	2.0	0.7	30.3
8b	5.50	1.00	0.50							
9a	5.20	0.40	0.50	0.273	4.71	1.0	0.62	6.2	1.8	27.5
9b	5.50	0.70	0.63	0.313	6.33	4.1	0.56	11.7	2.0	29.1
10a	5.20	0.80	0.36							
10b	5.40	1.36	0.46	0.272	6.17	1.4	0.56	15.8	3.7	34.3
11a	5.25	0.80	0.46	0.289	7.03	1.4	0.64	4.1	1.8	20.6
12a	5.25	0.70	0.34	0.152	4.60	1.4	0.52	4.8	2.3	26.5
12b	5.25	2.00	0.30							
13a	5.25	0.54	0.95	0.301	6.98	4.8	0.24	5.0	2.5	24.5
13b	5.50	0.90	0.75	0.313	7.14	2.8	0.28	7.7	2.9	23.4
14a	5.30	0.75	0.30							
14b	5.30	0.85	0.26							

Appendix Table 1. (Continued)

Sample No.	pH (1:2)	Exch. Mo (μg/100g)	Total Mo (μg/g)	TN (%)	OM (%)	Phos. (ppm)	Me/100g			
							K	Ca	Mg	CEC
15	5.35	0.96	0.81	0.218	4.87	1.4	0.39	6.6	2.3	21.6
16a	5.40	0.96	0.75	0.214	5.84	2.1	0.50	7.8	5.9	23.9
16b	5.40	1.04	0.72							
17a	5.45	20.32	3.16	0.302	9.09	2.1	1.49	7.8	2.6	28.5
17b	5.85	31.86	10.30	0.235	6.82	3.8	0.90	5.0	1.8	22.0
18	5.50	0.80	0.77	0.260	7.41	4.1	1.47	9.6	4.1	29.7
19	5.50	0.50	0.40	0.213	4.81	5.5	0.88	12.5	5.0	25.9
20a	5.50	1.00	0.49	0.330	8.01	2.1	0.60	7.8	3.4	24.6
20b	5.80	1.36	0.55							
21	5.55	1.4	0.70	0.239	5.36	2.8	0.85	19.8	9.1	36.3
22a	5.55	0.90	0.36	0.197	5.30	2.8	0.66	6.6	4.6	23.6
22b	5.80	1.16	0.42							
23a	5.60	0.76	0.42	0.175	6.87	2.1	0.82	5.6	1.5	29.8
24	5.65	0.90	0.54	0.189	3.52	4.8	1.18	12.8	5.0	23.2
25a	5.65	0.90	0.28	0.274	4.98	1.0	0.43	12.4	6.4	30.9
25b	5.65	1.10	0.26							
26	5.75	1.54	0.61	0.217	6.98	2.8	0.84	8.7	2.4	23.3
27	5.80	0.84	0.28	0.119	2.81	4.8	0.28	5.3	3.3	11.3
28	5.80	70.40	9.60	0.164	3.52	2.1	0.26	7.1	4.6	16.4
29	5.80	1.10	0.50	0.341	10.0	9.0	0.24	15.8	7.1	26.2
30a	5.80	1.20	0.36	0.189	5.14	3.5	0.70	5.6	2.7	19.6
30b	5.75	1.26	0.38							
31	5.95	0.84	0.54							
32	6.15	2.00	0.42	0.246	9.31	12.4	0.86	22.8	15.6	49.6

Note: Refer Table 2 for details of sampling sites.

Appendix Table 2. Subterranean clover yield (dry matter/pot) and plant chemical analysis.

Sample	Mo	Cu	N	Yield (g/pot) Treatment		N (%) Treatment		TN (g/pot) Treatment		Cu (μg/g) Treatment		Mo (μg/g) Treatment	
No.	(μg/g)	(μg/g)	(%)	Check	Mo	Check	Mo	Check	Mo	Check	Mo	Check	Mo
1a	0.24	11.8	2.61	4.244	5.151	2.73	2.80	0.1159	0.1442	9.4	7.5	0.11	0.16
1b	1.23	8.3	3.63										
1c				5.995	6.815	2.84	3.31	0.1703	0.2256	6.7	6.7	0.11	0.29
2a	0.29	8.3	2.63	5.365	6.161	2.45	2.62	0.1314	0.1614	8.2	8.2	0.38	0.45
2b				6.202	6.485	3.27	3.30	0.2028	0.2140	8.2	7.9	0.20	0.68
3a	0.33	9.0	2.50	4.444	5.136	2.87	3.06	0.1275	0.1572	10.6	10.2	0.08	0.52
3b	1.00	7.0	3.36										
3c				6.208	6.702	3.09	2.97	0.1918	0.1990	8.2	9.5	0.30	0.60
4a	0.10	15.3	2.50	3.825	4.342	2.56	2.60	0.0979	0.1129	6.2	6.2	0.11	0.18
4b	0.40	7.6	3.19	4.877	5.941	2.10	2.57	0.1024	0.1527	8.2	7.9	0.07	0.72
5a	0.15	10.5	3.11										
5b				6.313	7.169	3.23	3.61	0.2039	0.2588	9.4	9.4	0.39	0.50
6a	0.07	10.8	2.48	5.760	6.080	2.78	2.78	0.1601	0.1690	10.0	10.0	0.05	0.27
6b				6.812	7.265	2.90	3.06	0.1975	0.2223	8.8	8.6	0.33	0.65
7	0.18	11.8	2.90	5.734	6.453	3.04	3.04	0.1743	0.1962	9.4	8.2	0.25	0.49
8a	0.16	16.0	2.77	3.630	3.954	2.79	2.82	0.1013	0.1105	9.2	8.2	0.03	0.16
8b	0.17	7.6	3.04										
9a	0.00	8.3	2.69	5.121	5.859	2.57	2.62	0.1316	0.1535	9.2	8.6	0.11	0.36
9b	0.12	8.3	2.81	5.038	5.654	3.24	3.27	0.1632	0.1849	9.2	6.7	0.16	0.31
10a	0.28	9.4	2.85										
10b	0.56	9.2	3.33	6.059	6.428	3.14	3.15	0.1902	0.2025	9.4	9.2	0.06	0.15
11a	0.14	11.3	2.65	6.300	6.451	3.01	3.17	0.1896	0.2035	11.0	8.5	0.28	0.41
11b	0.17	10.0	2.94										
12a	0.21	6.0	2.65	5.594	5.804	2.35	2.92	0.1315	0.1695	9.2	6.2	0.06	0.08
12b	0.80	6.0	3.50										
13a	0.19	25.3	2.73	5.692	6.268	3.00	3.12	0.1708	0.1956	11.9	11.9	0.35	0.41
13b	0.21	18.8	2.97	5.748	6.356	2.65	2.96	0.1523	0.1881	12.5	12.5	0.11	0.21
14a	0.14	21.0	2.78										
14b	0.25	10.0	2.92										
15	0.20	8.3	2.72	5.645	6.162	2.51	2.85	0.1417	0.1756	10.9	9.8	0.28	0.34

Appendix Table 2. (Continued)

Sample No.	Mo (μg/g)	Cu (μg/g)	N (%)	Yield (g/pot) Treatment Check	Mo	N (%) Treatment Check	Mo	TN (g/pot) Treatment Check	Mo	Cu (μg/g) Treatment Check	Mo	Mo (μg/g) Treatment Check	Mo
16a	0.54	7.5	3.47	7.888	8.623	3.14	3.18	0.2477	0.2742	11.9	11.2	0.27	0.50
17a				5.907	6.245	2.77	2.83	0.1636	0.1767	9.4	8.2	1.54	2.10
17b				4.627	5.438	2.50	2.54	0.1157	0.1381	9.0	8.2	22.80	23.40
18	0.23	9.4	2.82	7.790	7.912	2.94	2.96	0.2290	0.2342	11.2	10.2	0.21	0.29
19	0.22	10.0	2.75	6.907	8.325	3.14	3.20	0.2169	0.2664	11.3	10.9	0.21	0.30
20a				7.180	6.924	2.96	3.16	0.2125	0.2188	9.2	8.2	0.13	0.22
21	0.60	7.0	3.13	7.383	6.915	2.99	3.19	0.2207	0.2206	13.5	13.0	0.06	0.26
22a	0.41	10.0	3.05	4.816	5.262	2.37	2.61	0.1141	0.1373	10.2	9.8	0.02	0.05
22b	0.69	9.4	3.16										
23a	0.13	12.9	2.62	6.108	6.920	3.06	3.22	0.1869	0.2228	9.4	9.2	0.05	0.36
23b	0.67	4.7	3.04										
24	0.14	5.3	2.89	5.168	5.320	2.78	2.80	0.1437	0.1490	13.3	10.5	0.06	0.20
25a	0.17	5.7	2.94	4.242	4.380	2.71	2.57	0.1150	0.1126	9.4	8.6	0.07	0.09
26	0.60	13.0	3.21	7.073	6.983	2.64	2.73	0.1867	0.1906	9.4	9.2	0.04	0.20
27	0.12	12.9	2.72	4.472	4.793	2.45	3.26	0.1096	0.1563	7.5	5.9	0.38	0.80
28				4.120	4.385	2.48	2.91	0.1022	0.1276	8.3	6.8	11.10	13.59
29	0.54	11.3	3.33	6.714	6.095	3.11	3.35	0.2088	0.2042	10.2	10.2	0.36	0.43
30a	0.39	7.0	3.11	5.382	6.362	2.90	3.08	0.1561	0.1959	8.2	8.2	0.25	0.38
30b	0.44	4.1	3.39										
31	0.17	9.4	2.89										
32	1.14	10.5	3.61	3.263	4.360	2.87	3.02	0.0936	0.1317	11.2	8.6	0.42	0.72

Note: Refer Table 2 for details of sampling sites.

Appendix Table 3. ANOVA for treatment and soil effect on yield.

Source	SS	df	MS	F
Treatment	12.539	1	12.539	54.50**
Soil	268.959	37	7.269	31.59**
Treatment × Soil	9.702	37	0.262	1.13 NS
Error	34.969	152	0.230	
Total	326.167	227		

$F^{*}_{0.05(1,152)} = 3.91$ $LSD_{(0.05)} = 0.1245$g/908g soil

$F^{**}_{0.01(1,152)} = 6.81$ $LSD_{(0.01)} = 0.1636$g/908g soil

$F^{*}_{0.05(37,152)} = 1.47$

$F^{**}_{0.01(37,152)} = 1.72$

Appendix Table 4. ANOVA for treatment effect on total nitrogen.

Source	SS	df	MS	F
Treatment	1026371.60	1	1026371.60	5.69*
Error	13351039.00	74	180419.44	
Total	14377410.60	75		

$F^{*}_{0.05(1,74)} = 3.97$ $LSD_{(0.05)} = 0.0194$g/908g soil

$F^{**}_{0.01(1,74)} = 7.00$ $LSD_{(0.05)} = 0.0257$g/908g soil