EFFECTS OF HERBICIDAL SPRAYS ON NITRATE ACCUMULATION IN CERTAIN WEED SPECIES

By

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AN ABSTRACT

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Nitrate poisoning of livestock feeding on plants containing high concentrations of nitrate has been recognized since 1888. In recent years there have been a number of reports of livestock becoming poisoned after grazing on herbicidally treated vegetation. This poisoning was believed to have been caused by the ingestion of toxic quantities of nitrate which had been accumulated by the plants as a result of chemical treatment.

A study of the problem of nitrate accumulation was initiated during the summer of 1953 and continued through the summer of 1954. For this work, a group of weeds commonly found in cropped and pasture land in Michigan was selected. The following species were used:

1. *Amaranthus retroflexus* L.
2. *Ambrosia elatior* (L.) Descourtils
3. *Chenopodium album* L.
4. *Cirsium arvense* (L.) Scop.
5. *Eupatorium maculatum* L.
6. *Impatiens biflora* Walt.
7. *Poa pratensis* L.
8. *Polygonum Convolvulus* L.
9. *Polygonum Persicaria* L.
10. *Prunus virginiana* L.
11. *Sambucus canadensis* L.
12. *Solanum Dulcamara* L
13. *Spiraea alba* Du Roi
14. *Thalictrum dioicum* L.

These weeds were treated with the following herbicides:

1. Isopropyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D)
2. Butyl ether ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)
3. Sodium salt of 2-methyl-4-chlorophenoxyacetic acid (MCP)
4. Alkanolamine salt of dinitro-o-sec-butyl-phenol (DNBP)
5. Isopropyl-N-(3-chlorophenyl) carbamate (CIPC)
6. Diethanolamine salt of maleic hydrazide (MH).

The weeds were sprayed with sub-lethal dosages of these chemicals. This was done to prevent excessive deterioration or death of the plants during the period in which analyses were to be made. The herbicidal applications were timed so that the period of analysis coincided as closely as possible to the stage preceding reproduction.

Samples for analysis were obtained at 24 hour intervals for four days following treatment and an additional sampling was made two weeks after treatment. Samples were obtained in the early morning and immediately taken to the laboratory where extracts were prepared from the fresh materials. The nitrate content of the samples was determined by a colorimetric comparison with standard solutions of potassium nitrate, using a method involving the nitration of 3,4-xylenol.

Herbicidal treatments used in this study did not affect the nitrate content of the following species: Chenopodium album, Cirsium arvense, Prunus virginiana and Thalictrum dioicum.

The treatments resulted in significant increases in the nitrate content of five of the weeds. These weeds and treatments were:

1. The DNBP treatment of Amaranthus retroflexus
2. The 2,4-D, DNBP, CIPC and MH treatments of Eupatorium maculatum
3. All treatments of Impatiens biflora
4. The 2,4-D, 2,4,5-T and MCP treatments of Polygonum Convolvulus
5. The DNBP treatment of Polygonum Persicaria.

Ten of the fourteen weeds studied contained nitrate in sufficient quantities to cause nitrate poisoning of livestock even though no sprays had been applied.

In only two weeds, Eupatorium maculatum and Impatiens biflora, could the accumulation of toxic concentrations of nitrate be attributed solely to the effect of herbicidal treatment. None of the herbicides used appeared to have the same effect on nitrate accumulation in all of the weeds tested.

Chenopodium album and Amaranthus retroflexus were previously reported to accumulate nitrate following treatment with 2,4-D. In this study it was found that nitrate in Chenopodium was not affected by any of the treatments and Amaranthus accumulated nitrate following the DNBP treatment only.

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INTRODUCTION

Since the advent of the new and generally more efficient herbicides, a great deal of work has been done and a voluminous literature presented concerning their uses and possible mechanisms of action. While the more obvious effects of the chemicals have been described at length, the basic physiological mechanisms remain undefined.

The rapid incorporation of new herbicides into existing weed control practices, while a boon to agriculturalists, utility corporations, home owners and others, has not been without some criticism. This largely has been due to the failure of research agencies to conduct extensive experiments concerning the possible adverse effects in humans and in animals, from extended exposure to the chemicals and the products of the treated plants. There is, in addition to the problem of chronic toxicity, the possibility that application of herbicides may affect certain plants in such a way as to bring about the elaboration of a toxic substance by these plants or the accumulation of a toxic substance ordinarily present in non-toxic concentrations.

In recent years there have been numerous reports of death of livestock foraging on 2,4-D and 2,4,5-T treated members of the *Prunus* and *Sorghum* genera. Members of these two genera are known to accumulate hydrocyanic acid or glucosides which, upon hydrolysis, may produce this acid, sometimes in concentrations lethal to livestock. It has been postulated that the treated plants accumulated abnormal quantities of hydrocyanic acid which caused the livestock losses (12). Data from some experiments have indicated that treated plants do not accumulate large amounts of hydrocyanic acid (27,17).

In addition to the reports of poisoning by hydrocyanic acid, there have been a number of cases reported where livestock losses were attributed to nitrate poisoning (11). Livestock grazing on herbicide treated vegetation were believed to have ingested toxic quantities of nitrate which had been accumulated by the plants as a result of chemical treatment. That nitrate poisoning is not uncommon may be seen from the numerous cases reported over the past sixty years. However, the assumption that crop and weed plants may accumulate nitrate as a result of herbicidal treatment is a new development which requires careful experimental study.

*Willard (55), in discussing the indirect effects of herbicides, cites numerous reports of ordinarily unpalatable weeds becoming palatable to livestock following herbicidal treatment. Many of the weeds tested were found to contain toxic quantities of nitrate in both the treated and untreated state. The effect of herbicides on the palatability of these weeds could be a factor contributing to the nitrate poisoning of livestock.

A study of the problem of nitrate accumulation was initiated during the summer of 1953 and continued through the summer of 1954. For this work, a group of weeds commonly found in cropped and pasture land in Michigan was selected. This group included certain weeds known to accumulate high concentrations of nitrate as well as others suspected of having contributed in some manner to livestock losses. These weeds were treated with various herbicides and nitrate analyses were made at regular intervals following treatment in an attempt to determine the effects of the treatments upon the nitrate content.

REVIEW OF LITERATURE

High levels of nitrate in green weeds and crops as well as cured feeds may often have disastrous consequences. The first recognized case of nitrate poisoning of livestock was in 1888 and was reported, along with a number of other cases, in 1895 (49). The losses were traced to the feeding of both green and cured corn fodder and hay. The nitrate content, determined as potassium nitrate, amounted to as much as 25 percent of the dry weight and the salt crystals could be observed readily with the naked eye. In more recent years, nitrate poisoning from oat hay (4,54,31), hay containing pigweed (36), sorghum (36,39) and sugar beet tops (40) has been reported.

Poisoning from ingestion of high nitrate plants is due to the reduction of nitrate to nitrite by microorganisms in the intestinal tracts of animals. The nitrite is taken into the blood stream where it combines with hemoglobin to form methemoglobin. The latter is incapable of giving up its oxygen to the tissues and thus causes asphyxia (4). Bradley _et al._ (4), have determined the minimum lethal dose to be 25 grams of potassium nitrate per one hundred pounds of animal weight. At this level, a five-hundred-pound animal would have to eat but five and one-half pounds of oat hay containing 5 percent nitrate to become fatally poisoned.

The absorption and elaboration of nitrogenous nutrients in plants is dependent upon such external factors as the pH and nitrate concentration of the growth medium, and the relative availability of various mineral solutes. Such factors as light, temperature, moisture and oxygen supply are also important. Internal factors of equal importance are the availability of carbohydrates and the stage of growth of the plants.

In considering the possible effects of herbicides on the nitrate content of plants, it may be desirable to consider first some of the factors affecting the absorption and assimilation of nitrates. The order in which these are treated should not be taken as the order of importance as it is impractical if not impossible to assume that any one is of more importance than another.

Availability of Nitrate to the Plant

That there is a correlation between the nitrate content of plants and the amount of available nitrate in the soil has been recognized by agronomists for a number of years. Some of the more improved methods of fertilizer application are based on the fact that a low plant nitrate level usually indicates a low level of soil nitrate. Bradley _et al._ (4) investigated the causes of high nitrate level in crop plants and found that soils with an abundanse of nitrogen produced crops with a high level of nitrate. While toxic concentrations of nitrate in corn appeared to be exceptional, where nitrate was available to the plants it was absorbed and represented a considerable fraction of the dry weight (56). Corn plants growing on very fertile plots of soil previously used as animal enclosures, were found to have accumulated nitrate to such an extent that it constituted 18.8 percent of the dry weight of the plants (49).

Moisture Supply

Most of the severe losses of livestock as a result of nitrate poisoning have occurred in the drier sections of the country. Studies conducted with plants having abundant moisture indicate that these plants could very well accumulate toxic levels of nitrate if grown under drought conditions (56). Grasses, in contrast to many other plants, were found to contain only traces of nitrate, which was thought to result from the removal of excess nitrate from the plant by guttation. Maintaining droughty soil conditions, such as keeping the soil moisture as low as 15 percent, resulted in a much higher concentration of nitrate in the plants. Whitehead and Olson (54) found that as the soil moisture increased there was a corresponding decrease in nitrate until the soil moisture reached a level of 25 percent. No differences were found above this level.

Temperature

The effects of temperature are for the most part indirect. The nitrifying ability of soil microorganisms is affected by the temperature. Jones and Greaves (23) found that of the nine soils tested for nitrifying ability, maximum nitrification occurred in six of the soils at 20°, and at 30° in the remaining three. Active absorption of ions is accelerated by a rapid rate of respiration in the roots. Temperatures which retard root respiration therefore inhibit the uptake of nitrate and other ions.

The rates of photosynthesis and respiration, which are enzymatically regulated processes, are affected by temperature. This has a direct bearing on the availability of carbohydrates which has been shown to affect nitrate metabolism in plants.

pH of the Nutrient Medium

Soil pH is known to affect the number of soil microorganisms and the rate of nitrification. In addition, the soil pH affects the availability of various ions directly or indirectly involved in nitrate uptake and assimilation. Davidson and Shive (7) observed that peach trees absorbed and utilized ammonium nitrogen more readily than nitrate nitrogen at pH 6 while the reverse was true when the medium was maintained at pH 4.

Oxygen Supply

The level of available oxygen in a nutrient substrate was found by Gilbert and Shive (14) to be a factor in the rate of nitrate absorption and assimilation. They, and others (57), found that the greater the oxygen tension the greater was the nitrate absorption by roots. Shive (41) demonstrated that nitrate assimilation could be inhibited by oxygen and that nitrate utilization was inversely proportional to the oxygen level. It was found that nitrogen deficiency occurred in plants cultivated in a nitrate solution when the oxygen level was as high as 8 to 16 parts per million in the solution (13). Nance (30) observed that oxygen did not inhibit active nitrate absorption but that the assimilation of previously absorbed nitrate was inhibited.

Effects of Light

Light may affect the assimilation of nitrate in a number of ways. Light is required for the synthesis of carbon compounds necessary for the formation of organic nitrogen and for the synthesis of carbohydrates which, when oxidized, probably provide the energy for nitrate reduction in the dark. There is, in addition, considerable evidence that light plays a more direct role in nitrate reduction. Plants kept in continuous light have been found to contain less nitrate than other plants (54) while shading resulted in an increased nitrate content (47).

Burstrom (5) found that wheat leaves did not assimilate nitrate in the dark but did in the light and that the rate of assimilation increased with an increase in light intensity. Excised wheat roots were found to be capable of reducing nitrate in the dark at the expense of respired carbohydrates. Nitrate reduction in wheat leaves appeared to be induced by light and was independent of respiration.

Mendel and Visser (28), using N^{15} and green leaf disks, carried out experiments similar to those of Burstrom and, in addition, investigated the effects of respiration inhibitors on nitrate reduction in light and in darkness. Respiration inhibitors completely inhibited nitrate reduction in darkness but had no effect on nitrate reduction in the light. Van Niel et al. (48) obtained experimental evidence which supports the contention that photochemical reduction of nitrate represents a process in which nitrate acts directly as an alternate hydrogen acceptor in photosynthesis.

Molybdenum Supply

Certain experiments indicate that molybdenum functions as a biological catalyst in symbiotic-nitrogen fixation and in the reduction of nitrate in plants. Plants starved for molybdenum have been found to accumulate considerable nitrate in the petiolar tissue (20). When nitrate is the source of nitrogen, the molybdenum requirement is greater than when ammonia serves as the source (54,29). Plants deficient in molybdenum were found to have a depressed content of sugars, chlorophyll and organic nitrogen and a greatly increased content of nitrate (1,29). Nitrate reductase activity has been shown by a number of workers to be decreased in molybdenum deficient plants. Nicholas et al. (32) have shown that the nitrate reductase activity of some microorganisms deficient in molybdenum was reduced to one-tenth to one-thirtieth of that of the controls. Plants provided with ammonia as the sole source of nitrogen may still show signs of molybdenum deficiency indicating that this element may play some role in plant metabolism other than in nitrate reduction. Since the energy required for nitrate reduction in darkness in plants must be supplied by dehydrogenation reactions in respiration, it is possible that molybdenum, as a part of one or more dehydrogenases, indirectly affects nitrate reduction by enzymatically catalyzing certain dehydrogenation reactions (9).

Very minute amounts of molybdenum are required for nitrate reduction while much greater quantities are required for symbiotic-nitrogen fixation. It has been reported that nodules from alfalfa contain five to fifteen times as great a concentration of molybdenum as roots of the same plant (22).

There is some evidence for considering the possibility of a role of nitrate reductase in nitrogen fixation as well as in nitrate assimilation (10).

Other Ions

A number of nutrient ions directly or indirectly affect the absorption and utilization of nitrate in plants. Potassium ions are necessary for nitrate reduction. A deficiency of potassium results in an accumulation of both nitrate and carbohydrates. When potassium is supplied to plants deficient in this ion there is a sudden increase in nitrate reduction and protein formation accompanied, in some cases, by the temporary appearance of nitrite (34).

Phosphate ions not only affect the absorption of nitrate but also the utilization of nitrate already absorbed. Helder (19) found that the growth rate of five to six week old corn plants when placed in a nutrient solution deficient in phosphorus was inhibited and the uptake of nitrate ceased. This was in spite of the fact that the plants were at the stage of growth in which nitrate absorption was still high. Tanada (47) found, on the contrary, that a deficiency of phosphorus resulted in abnormally large increases in nitrate content. Phosphorus and nitrate appear to be mutually antagonistic with respect to uptake by plants. A high concentration of either in the plant inhibits the absorption of the other.

Oats in manganese deficient cultures have been observed to contain an extremely high percentage of nitrate (54). This ion enhances the reduction and assimilation of nitrate in both roots and leaves of wheat (30,6). Jones _et al_. (24) found that soy beans growing in a nitrate solution without manganese turned yellow and exhibited symptoms of nitrogen deficiency. The addition of manganese ions resulted in complete recovery. They concluded that manganese was essential for the normal reduction of nitrate and the formation of amino compounds.

An absence of ions containing sulfur has been shown to inhibit the fixation of nitrogen by nitrogen-fixing bacteria (2). This is an indirect effect brought about by the influence of sulfur on organic nitrogen metabolism. Sulfur may affect the reduction of nitrate in a similar way.

Carbohydrate Supply

A readily available supply of carbohydrates is associated with the reduction of nitrate in plants. When the carbohydrate supply is limited, nitrate reduction proceeds very slowly if at all. Plants having a high content of carbohydrates rapidly reduce nitrate and synthesize new protein, indicating that some relationship exists between carbohydrate respiration and nitrate reduction. A number of workers have shown that there is a relationship between the rate of nitrate reduction and the evolution of respiratory carbondioxide (14,5,18). Goksoyr (15) determined the rate of nitrate assimilation from the amount of carbon dioxide given off and found that the quotient of extra carbon dioxide/ nitrate reduced to be 2.85. The empirical quotient of carbon dioxide evolved/ nitrate reduced was found to be 2.84. These workers have shown that carbon dioxide evolution was increased during the periods of nitrate reduction. The oxygen made available by the reduction was utilized in the oxidation of carbohydrates which supplied the energy necessary for the reduction.

Soybean and oat plants are known to accumulat carbohydrates in the absence of nitrate (33). The addition of either nitrate or ammonium ions was found to reduce the carbohydrate content and in particular the soluble sugars. Vladimerov (53) found that the addition of nitrate to nitrogen deficient plants resulted in an increase in citric acid while the addition of ammonium nitrogen reduced the citric acid content. From this he concluded that nitrate, which is in a highly oxidized state, created conditions conducive to the intensification of the oxidation process leading to the formation and storage of acids.

Stage of Development

The ability of cultivated and weedy plants to draw nutrients from the soil is dependent, to a large extent, upon the stage of growth. Singh and Singh (42) observed that the maximum value of absorption was reached in weeds at the stage when the plants were about to enter their reproductive period. After this stage there was a gradual decrease in absorption. They also noted that the order of concentration of various elements was the same for all of the weeds of a related group. The largest group being that in which nitrogen is the nutrient first absorbed in large amounts.

The stage of development may also affect the form of nutrient ions absorbed. According to Stahl and Shive (44,45), some plants, during the early stages of growth, absorbed nearly all their nitrogen in the form of ammonium ions. As the plants increased in age the amount of ammonium ions absorbed decreased while the absorption of nitrate ions increased.

Inherent Ability to Accumulate Nitrate

Different species of plants vary in their ability to absorb and store nitrate and other nutrients. Where nitrate is the chief source of nitrogen, fairly high concentrations must often be maintained for normal plant growth (36). Jacques and Osterhout (21), for example, found that the sap of certain algae may contain a nitrate concentration of from 500 to 2,000 times that of sea water.

The nitrate content in the expressed saps of different plants growing in the same association, was found by Wilson (56) to differ greatly. In one association, the sap of soybeans was found to contain 1,000 parts per million of nitrate while that of purslane contained 5,882 parts per million. In another association oats were found to contain 500 parts per million of nitrate and _Amaranthus_ was found to contain 4,140 parts per million. Most weed plants were found to contain a much higher content of nitrate and other nutrients than the common crop plants growing in the same association (42).

Production of Nitrate within the Plant

Evidence that nitrate may be synthesized by plants is rather meager. Vickery *et al.* (50,51) have demonstrated that the nitrate levels of excised leaves of tobacco and rhubarb were increased when these leaves were cultured in darkness in water or in solutions containing ammonium sulfate. The nitrate was assumed to have been formed by the oxidation of the ammonium ions. These workers have also shown that nitrate-free *Narcissus* bulbs developed traces of nitrate when cultivated in either distilled water or in complete nutrient solutions containing ammonium sulfate (52). Nitrate synthesis by segments of *Narcissus* leaves has also been reported (37).

Herbicidal Treatment

Stahler and Whitehead (46) report a case in which several hundred acres of sugar beets on seven farms were accidently treated with 2,4-D. Samples of beet leaves from the seven farms, together with several samples from untreated fields of adjacent farms, were analyzed for nitrate. The average nitrate level in the untreated beet leaves, calculated in terms of potassium nitrate, was found to be 0.22 percent of the dry weight. The nitrate levels in the treated leaves ranged from 1.81 percent to 8.77 percent, all of which were considered above the minimum lethal concentration. The author has also observed that 2,4-D has a profound effect on the nitrate content of sugar beet leaves. These results indicate that 2,4-D does affect the nitrate level of beet leaves to the extent that considerable losses could be expected if the leaves were fed to livestock.

Lambs quarters, pigweed and smart weed when treated with 2,4-D were found by Jones (25) to be very high in nitrate while the controls contained very little. This was thought to be due to the 2,4-D checking the assimilation of nitrates into protein. These weeds, as well as sugar beet leaves, have been found by various other workers to accumulate nitrates in toxic concentrations even when not treated with 2,4-D.

Fertig (11) studied the effect of 2,4-D and MCP on the accumulation of nitrate in lambs quarters, ragweed, pigweed and curled dock but was unable to obtain conclusive results.

It is apparent that there are many factors, in addition to herbicidal treatment, capable of influencing the level of nitrate in plants. Some of these factors may possibly be modified by the application of sprays to the plants. Applications of 2,4-D and related compounds are known to have a considerable effect on the metabolism and growth process of plants. This effect could bring about changes in the rates of photosynthesis, root growth, nutrient absorption and transpiration. Because of the numerous factors influencing the uptake and assimilation of nitrates, considerable variations in the nitrate levels should be expected, not only in plants growing under normal conditions, but also in those herbicidally treated plants.

MATERIALS AND PROCEDURES

The species of weeds used in this work were selected on the basis of availability, occurrence in pastured areas, and reports concerning the ability of certain of the weeds to accumulate nitrate. All of the species were found growing on or bordering the muck farm used for weed control investigations. The following species were selected:

1. *Amaranthus retroflexus* L.
2. *Ambrosia elatior* (L.) Descourtils
3. *Chenopodium album* L.
4. *Cirsium arvense* (L.) Scop.
5. *Eupatorium maculatum* L.
6. *Impatiens biflora* Walt.
7. *Poa pratensis* L.
8. *Polygonum Convolvulus* L.
9. *Polygonum Persicaria* L.
10. *Prunus virginiana* L.
11. *Sambucus canadensis* L.
12. *Solanum Dulcamara* L.
13. *Spiraea alba* Du Roi
14. *Thalictrum dioicum* L.

Some of the more commonly used herbicides were selected for this work. Included in these were herbicides which were believed to offer differences in physiological activity. The herbicides selected were:

1. Isopropyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D)
2. Butyl ether ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)
3. Sodium salt of 2-methyl-4-chlorophenoxyacetic acid (MCP)
4. Alkanolamine salt of dinitro-o-sec-butyl-phenol (DNBP)
5. Isopropyl-N-(3-chlorophenyl) carbamate (CIPC)
6. Diethanolamine salt of maleic hydrazide (MH)

The weeds were treated with sub-lethal dosages of herbicide applications. This was done to prevent excessive deterioration or death of the plants during the period in which analyses were to be made. With a few exceptions the spray mixtures were applied in a volume of 80 gallons of water per acre. The exceptions were treated with a volume of 40 gallons per acre. It was known that the period of greatest accumulation of nitrate was during the stage of growth immediately preceding the reproduction stage. For that reason all herbicidal applications were timed so that the period of analysis coincided as closely as possible to the stage preceding reproduction.

Samples for analysis were obtained at 24 hour intervals, for four days following treatment. An additional analysis was made two weeks after treatment. By sampling in this manner, both rapid and slow accumulations of nitrate could be detected. Sampling was done early in the morning to eliminate, as much as possible, the effect of sunlight on the nitrate accumulated during the dark hours.

The samples were taken immediately to the laboratory where extracts were prepared from duplicate samples of the fresh material. The extracts were then analyzed for nitrate content using a colorimetric method which involved the nitration of 3,4 xylenol.[1,2] This method was reported to have been successfully

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[1] Analytical method supplied by Dr. G.H. Ellis, Head of Chemical Division of United Co-operatives, Inc., Ithaca New York.

[2] See appendix for analytical method and standard curve.

used at the U. S. Plant Soils and Nutrition Laboratory, Ithaca, New York. Preliminary work indicated that the method gave consistant and reproducible results and that it was much less time consuming than the Kjeldahl method of nitrate determination. A standard curve for use in comparing the results of the nitrate determinations was obtained by analyzing solutions containing known quantities of analytical grade potassium nitrate. All of the potassium nitrate values in the tables represent the average of two determinations.

Prior to the summer in which the bulk of this work was performed, a preliminary survey was made on several weed species. These analyses were made two weeks following treatment. The results are presented in Table 1.

EXPERIMENTAL RESULTS

In the preliminary study, ten weeds were treated with the various chemicals and the effect on the nitrate accumulation determined. The results (Table 1) indicated that nitrate accumulation, if it occurred, did not persist for the two-week period following application of the chemicals.

Based on the data obtained, the weeds sprayed in the later experiments were divided into four groups. These groups are as follows:

1. Weeds which showed no significant differences between either chemical treatments or days
   - *Chenopodium album*
   - *Cirsium arvense*
   - *Prunus virginiana*
   - *Thalictrum dioicum*

2. Weeds in which there were differences only between chemical treatments
   - *Eupatorium maculatum*
   - *Impatiens biflora*
   - *Sambucus canadensis*
   - *Solanum Dulcamara*

3. Weeds in which there were significant differences between chemical treatments and between days on which analyses were made
   - *Amaranthus retroflexus*
   - *Ambrosia elatior*
   - *Polygonum Convolvulus*
   - *Polygonum Persicaria*

4. Weeds which showed significant differences only between the days on which analyses were made.
   - *Poa pratensis*
   - *Spiraea alba*

TABLE 1

NITRATE CONTENT OF WEEDS TWO WEEKS AFTER APPLICATION OF HERBICIDAL SPRAYS

Weed Species	Mg. of KNO_3 per Gram of Dry Weight						
	Chemical and pounds per acre						
	2,4-D 1/4 lb	2,4,5-T 1/4 lb.	MCP 1/4 lb	DNBP 1/2 lb	CIPC 1 lb.	MH 1 lb.	Control
Ambrosia elatior	9.30	10.37	9.30	10.37	9.15	9.15	9.60
Bidens vulgata	.59	.74	.44	.51	.51	.59	.66
Chenopodium album	12.40	12.40	12.40	11.67	12.10	11.76	12.10
Eupatorium maculatum	1.39	1.57	1.57	2.11	1.93	1.84	1.84
Impatiens biflora	3.72	5.99	6.26	6.74	6.50	6.74	7.78
Poa pratensis	1.86	1.42	1.35	.97	1.05	1.20	2.09
Polygonum Persicaria	3.31	4.13	3.65	1.98	3.81	3.65	3.47
Prunus virginiana				15.08	14.64	18.84	17.66
Spiraea *alba*	1.29	1.35	1.23	1.23	1.46	1.23	1.40
Thalictrum dioicum	3.14	3.27	1.71	1.10	1.84	1.71	3.27

Group One

The nitrate levels of _Chenopodium album_ (Table 2) and _Cirsium arvense_ (Table 3) were found to be quite high, while the nitrate levels of _Prunus virginiana_ (Table 4) and _Thalictrum dioicum_ (Table 5) were relatively low. Statistical analyses showed that variations between treatments and between days were not large enough to be significantly different.

Group Two

Chemical treatments of _Eupatorium maculatum_ (Table 6), with the exception of MCP, resulted in significant increases in nitrate content over the control at some time during the two week period. There was an immediate increase (24 hours) in nitrate concentration followed by a decline over the two week period. At the fourteenth day, only the plants which received the DNBP treatment remained significantly higher in nitrate than the control. There was no definite pattern of nitrate increase or decrease after the first day. That is, some treatments caused a gradual increase in nitrate followed by a decrease while others brought about a decrease in nitrate followed by an increase. MCP produced significant decreases at the second, third and fourth day intervals.

One the first day following treatment of _Impatiens biflora_ (Table 7), significant increases were observed for all treatments except that of DNBP. In most cases the nitrate content

TABLE 2

NITRATE CONTENT OF *CHENOPODIUM ALBUM* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	38.62	36.02	41.57	39.79	41.57
2,4,5-T 1/4 lb.	42.81	42.19	42.19	42.81	39.14
MCP 1/4 lb.	40.95	41.57	40.95	42.81	45.24
DNBP 1/2 lb.	44.65	43.42	45.24	44.04	43.42
CIPC 1 lb.	42.86	44.04	45.24	42.81	30.96
MH 1 lb.	42.19	45.24	44.04	46.63	40.95
Control	45.24	42.22	40.99	42.19	44.62

Statistical analysis showed no significant differences between days or treatments.

TABLE 3

NITRATE CONTENT OF *CIRSIUM ARVENSE* (L.) SCOP. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	28.72	29.15	36.38	35.83	37.44
2,4,5-T 1/4 lb.	39.63	35.83	35.31	38.53	39.08
MCP 1/4 lb.	39.08	33.70	34.79	37.44	37.99
DNBP 1/2 lb.	37.99	36.90	36.35	35.83	36.35
CIPC 1 lb.	40.15	35.31	35.31	35.83	34.79
MH 1 lb.	38.53	35.83	35.31	33.35	32.32
Control	39.63	33.70	39.60	36.90	37.99

Statistical analysis showed no significant differences between days or treatments.

TABLE 4

NITRATE CONTENT OF *PRUNUS VIRGINIANA* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	10.88	9.92	10.38	10.10	10.10
2,4,5-T 1/4 lb.	7.84	8.85	9.47	9.23	8.97
MCP 1/4 lb.	9.22	9.60	10.23	9.72	9.72
DNBP 1/2 lb.	9.85	9.46	9.96	9.72	8.97
CIPC 1 lb.	9.48	10.10	9.61	10.88	9.98
MH 1 lb.	10.10	9.47	10.46	10.16	9.73
Control	9.35	10.23	9.84	9.96	8.97

Statistical analysis showed no significant differences between days or treatments.

TABLE 5

NITRATE CONTENT OF *THALICTRUM DIOICUM* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	6.60	4.21	6.21	5.06	
2,4,5-T 1/4 lb.	4.70	3.78	4.85	6.70	
MCP 1/4 lb.	4.57	3.86	4.99	5.47	
DNBP 1/2 lb.	4.92	5.39	5.23	5.06	
CIPC 1 lb.	7.67	5.33	4.47	5.84	
MH 1 lb.	3.34	3.60	3.66	3.40	
Control	3.27	2.54	4.78	6.60	

Statistical analysis showed no significant differences between days or treatments.

of sprayed plants remained relatively constant throughout the two-week period. The nitrate level of the control increased constantly, with the result that at the end of the two-week period only the MCP treated plants remained significantly higher in nitrate.

In no case did the spraying of <u>Sambucus canadensis</u> result in a significant increase in nitrate over the control (Table 8). The 2,4-D treatment resulted in a highly significantly lower nitrate content for at least four days following the application. At the end of the two-week period however, the nitrate level in this treatment was similar to that in the majority of other treatments. The nitrate level in the 2,4,5-T sprayed plants remained normal for the first four days but was considerably reduced at the end of two weeks.

Chemical treatments, with the exception of DNBP, resulted in a significantly lower content of nitrate in <u>Solanum Dulcamara</u> (Table 9). On the fourth day following application of the chemicals, the DNBP treated plants contained a significantly greater amount of nitrate than the control. However, analyses at the end of the two-week period showed that the nitrate content of these plants had fallen much below that of the control.

TABLE 6

NITRATE CONTENT OF *EUPATORIUM MACULATUM* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	13.06*	17.87**	17.56**	14.89	10.76
2,4,5-T 1/4 lb.	12.09	11.38	10.95	8.88**	5.37**
MCP 1/4 lb.	8.39	6.84	4.80*	7.13**	13.14
DNBP 1/2 lb.	23.68**	23.38**	16.58**	19.26*	23.02**
CIPC 1 lb.	14.87**	15.34**	22.35**	16.08	11.44
MH 1 lb.	18.05**	18.55**	14.89**	10.57*	15.66
Control	8.86	9.17	9.38	14.89	14.44

* Least significant difference between treatments at 5% = 3.84
** Least significant difference between treatments at 1% = 5.22
No significant difference between days

TABLE 7

NITRATE CONTENT OF IMPATIENS BIFLORA WALT. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	12.60*	11.30	12.27	12.28	12.60**
2,4,5-T 1/4 lb.	13.58**	13.96*	11.62*	13.96	14.93
MCP 1/4 lb.	22.98**	23.35**	16.73	23.36**	19.50*
DNBP 1/2 lb.	10.64	12.93	11.62*	11.95	12.93**
CIPC 1 lb.	12.60*	13.96*	16.02	12.27	12.28**
MH 1 lb.	13.96**	18.47**	15.31	14.28*	15.31
Control	9.67	11.30	14.28	11.62	16.37

* L.S.D. between treatments at 5% = 2.50
** L.S.D. between treatments at 1% = 3.40
No significant difference between days

TABLE 8

NITRATE CONTENT OF *SAMBUCUS CANADENSIS* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	14.30**	15.22**	13.67**	16.11**	24.20**
2,4,5-T 1/4 lb.	26.34	23.50	24.81	25.64	16.79**
MCP 1/4 lb.	24.20	23.85	25.51	25.94	24.81*
DNBP 1/2 lb.	22.00	26.29	25.94	26.34	25.51*
CIPC 1 lb.	21.74*	23.50	22.63	28.37	25.64
MH 1 lb.	25.90	26.34	24.20	25.25	26.29
Control	25.51	25.94	23.85	28.76	29.17

* L.S.D. between treatments at 5% = 3.60
** L.S.D. between treatments at 1% = 4.89
No significant difference between days

TABLE 9

NITRATE CONTENT OF *SOLANUM DULCAMARA* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	13.48**	13.08**	10.92**	14.62**	11.78**
2,4,5-T 1/4 lb.	11.43**	10.55**	10.36**	11.95**	13.46**
MCP 1/4 lb.	13.91**	11.07**	10.91**	11.78**	12.85**
DNBP 1/2 lb.	21.90	22.92	22.61	23.89	16.03**
CIPC 1 lb.	14.14**	12.49**	16.27**	16.77**	16.01**
MH 1 lb.	13.55**	16.01**	14.14**	16.25**	14.36**
Control	22.92	22.00	22.21	20.55	22.21

* L.S.D. between treatments at 5% = 2.32
** L.S.D. between treatments at 1% = 3.16
No significant difference between days

Group Three

The nitrate content of this group differed significantly between treatments as well as between the days following treatment.

At no time did the herbicidal treatment of Ambrosia elatior (Table 10) result in an increase in nitrate content above that of unsprayed plants. The 2,4-D treatment appeared to be most effective in reducing the nitrate level. Two weeks following the spraying, nitrate levels were reduced well below both that of the control and the levels observed 24 hours after treatment.

The results of treating Amaranthus retroflexus (Table 11) indicate that on the first day following the application of the herbicides, the nitrate content of all treatments, except that of CIPC, was significantly lower than that of the control. The nitrate level of all lots showed an increase on the second and third days. At the fourth day the nitrate content decreased as compared to the control and at two weeks the level of all treatments was considerably below that observed on the first day. Analyses on the fourteenth day showed the DNBP and MH sprayed plants to be significantly higher in nitrate than the control. All other differences between the treated and untreated plants on different days were due to the lower nitrate content in the sprayed plants.

Unlike most of the weeds worked with, there was not a consistant upward or downward trend in the nitrate content of Polygonum Convolvulus (Table 12). The nitrate levels varied considerably from day to day but the pattern was not consistent for the different treatments. The 2,4-D treated plants contained a significantly higher nitrate level than the control on the first, second, third and fourteenth days, following spraying. The 2,4,5-T treated lot contained a higher nitrate level than the untreated plants on the fourth and fourteenth days, while plants which were treated with MCP contained more nitrate on the second, third and fourth day intervals. With the exception of the 2,4,5-T lot, the increases in nitrate were not significantly higher than in the control plants.

The nitrate levels of Polygonum Persicaria (Table 13) varied considerably on the first day following treatment, but all compounds except 2,4-D brought about significant reductions. The nitrate content increased on the second, third and fourth days but the increases were not significantly different from the control plants. At the fourteenth day only 2,4-D had failed to cause a highly significant decrease in the nitrate content of this weed.

Group Four

Poa pratensis (Table 14) was found to have a very low nitrate content throughout the period in which analyses were made. There were significant differences in nitrate content between the 24 hour periods in which analyses were made. However, these were due to the downward trend in nitrate content which occurred in all Poa samples.

TABLE 10

NITRATE CONTENT OF *AMBROSIA ELATIOR* (L.) DESCOURTILS FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	20.29**	17.99**	22.00**	21.60**	13.86**
2,4,5-T 1/4 lb.	27.07*	24.22**	25.27**	25.55**	12.32**
MCP 1/4 lb.	20.96**	23.28**	24.22**	23.63**	13.49**
DNBP 1/2 lb.	26.98*	26.31*	27.07**	26.69**	10.38**
CIPC 1 lb.	23.94**	24.29*	27.88**	25.55**	11.09**
MH 1 lb.	24.96**	26.60*	28.63*	25.55**	13.42**
Control	28.63	29.06	31.26	32.16	18.01

* L.S.D. between treatments at 5% = 2.31
** L.S.D. between treatments at 1% = 3.15
L.S.D. between days at 5% = 1.96
L.S.D. between days at 1% = 2.66

TABLE 11

NITRATE CONTENT OF *AMARANTHUS RETROFLEXUS* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	35.37**	37.63*	37.57**	37.63**	19.24**
2,4,5-T 1/4 lb.	36.00**	34.37**	39.84*	37.57**	22.96
MCP 1/4 lb.	37.06*	38.69	40.41	37.63**	18.06**
DNBP 1/2 lb.	31.32**	34.01**	38.72**	37.57**	28.37**
CIPC 1 lb.	39.84	40.41	42.10	41.56	25.85
MH 1 lb.	35.37**	37.06**	42.86	39.84**	27.12*
Control	39.84	40.99	42.64	43.40	24.50

* L.S.D. between treatments at 5% = 2.60
** L.S.D. between treatments at 1% = 3.53
L.S.D. between days at 5% = 8.69
L.S.D. between days at 1% = 11.81

TABLE 12

NITRATE CONTENT OF *POLYGONUM CONVOLVULUS* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	19.39*	21.57**	17.87*	16.55	17.07**
2,4,5-T 1/4 lb.	17.60	19.91	12.12**	17.10*	17.07**
MCP 1/4 lb.	19.14	21.85**	20.68**	20.16**	16.06
DNBP 1/2 lb.	9.14**	13.74**	12.00**	13.12	12.75
CIPC 1 lb.	18.89	19.14	16.30	15.09	16.05
MH 1 lb.	14.64*	13.50**	12.00**	12.92	12.75
Control	17.07	18.64	15.11	14.86	14.22

* L.S.D. between treatments at 5% = 2.08
** L.S.D. between treatments at 1% = 2.83
L.S.D. between days at 5% = 2.44
L.S.D. between days at 1% = 3.32

TABLE 13

NITRATE CONTENT OF <u>POLYGONUM PERSICARIA</u> L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	32.34	37.63	38.04	38.58	37.09
2,4,5-T 1/4 lb.	29.43*	32.34	33.80	34.24*	30.88**
MCP 1/4 lb.	26.82**	35.19	37.63	36.55	25.77**
DNBP 1/2 lb.	24.38**	33.29	27.49**	34.65	32.34**
CIPC 1 lb.	28.00**	35.16	39.17	37.63	28.20**
MH 1 lb.	26.10**	30.88*	34.24	37.85	19.97**
Control	34.24	36.14	36.68	38.58	38.04

* L.S.D. between treatments at 5% = 4.05
** L.S.D. between treatments at 1% = 5.50
L.S.D. between days at 5% = 3.42
L.S.D. between days at 1% = 4.65

The results obtained from the treatment of Spiraea alba (Table 15) were similar to those obtained for Poa pratensis. The differences observed were due to the continuous decrease in nitrate content of both the treated and untreated plants.

TABLE 14

NITRATE CONTENT OF *POA PRATENSIS* L. FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	5.62	2.94	3.47	3.38	3.38
2,4,5-T 1/4 lb.	5.34	2.59	3.65	3.12	3.03
MCP 1/4 lb.	4.47	2.59	4.64	2.85	2.77
DNBP 1/2 lb.	4.45	2.50	3.83	3.29	2.94
CIPC 1 lb.	4.47	4.85	3.03	2.59	2.85
MH 1 lb.	2.59	4.28	2.33	2.68	1.55
Control	4.75	2.77	2.68	3.91	2.59

No significant difference between treatments
L.S.D. between days at 5% = .85
L.S.D. between days at 1% = 1.15

TABLE 15

NITRATE CONTENT OF *SPIRAEA ALBA* DU ROI FOLLOWING APPLICATION OF HERBICIDAL SPRAYS

Chemical and Pounds Applied per Acre	Mg. of KNO_3 per Gram of Dry Weight				
	Days after treatment				
	1	2	3	4	14
2,4-D 1/4 lb.	10.16	11.42	10.46	6.63	8.19
2,4,5-T 1/4 lb.	11.39	12.35	9.35	9.24	7.64
MCP 1/4 lb.	12.77	12.35	9.35	9.80	7.76
DNBP 1/2 lb.	12.25	11.56	8.58	9.01	7.18
CIPC 1 lb.	12.43	11.74	8.84	8.71	7.29
MH 1 lb.	12.60	9.60	8.11	7.80	7.30
Control	13.40	12.90	11.42	10.61	7.76

No significant difference between treatments
L.S.D. between days at 5% = 4.30
L.S.D. between days at 1% = 5.84

DISCUSSION

A number of research workers have shown that sub-lethal dosages of 2,4-D, and other herbicides, have remarkable effects on the metabolism of the treated plants (8,3,16,43,26). Not all species of plants are affected in the same manner nor to the same degree. This is evident from the varying degrees of tolerance exhibited by different species of plants to a particular chemical. The metabolic abnormalities, brought about by herbicidal treatment, may cause plants to accumulate nitrate in the same manner as is done when plants are placed under adverse growing conditions, such as drought.

Nitrate accumulation following treatment with 2,4-D has been reported in the case of *Chenopodium album* (25). This effect was not found in this study and may be accounted for by differences in environmental conditions under which the work was done. Bradley *et al*. (4) have shown that the type of soil determined to a great extent the amount of nitrate in plants, even when the nitrate level of the soil was high. Wide differences in soil types and soil moisture conditions may give rise to entirely conflicting results.

Herbicidal treatments used in this study did not affect the nitrate content of the following species: *Chenopodium album*, *Cirsium arvense*, *Prunus virginiana* and *Thalictrum dioicum*.

The herbicidal treatments on two species, _Eupatorium maculatum_ and _Impatiens biflora_, resulted in rapid increases in nitrate content. In the majority of weeds treated there was either a reduction or no change in nitrate content during the same period.

Some weeds, for example: _Ambrosia elatior_, _Amaranthus retroflexus_ and _Polygonum Persicaria_, were observed to accumulate nitrate on the second, third and fourth days following herbicidal treatment. In these weeds there was some reduction in nitrate content from the fourth to the fourteenth day following treatment. These increases and decreases in nitrate level occurred in both the treated plants and the controls, indicating that factors other than the herbicidal treatments were responsible for the changes in the nitrate levels. Variations of temperature, light, moisture and the progress of the plants from one stage of growth to another could account for the changes in the nitrate level of both treated plants and the control.

In no case did any particular herbicidal treatment result in consistantly higher or lower nitrate levels in all species. For example, it was observed that the 2,4-D treatment resulted in lower nitrate levels than the other treatments when applied to _Ambrosia elatior_, _Sambucus canadensis_ and _Polygonum Persicaria_. The reverse was true in the case of _Polygonum Convolvulus_.

Ten of the fourteen weeds were found to contain nitrate levels in excess of the one and one-half percent considered by Bradley *et al*. (4) to be the maximum concentration of nitrate in plants which livestock could safely consume. These ten weeds were: *Amaranthus retroflexus*, *Ambrosia elatior*, *Chenopodium album*, *Cirsium arvense*, *Eupatorium maculatum*, *Impatiens biflora*, *Polygonum Convolvulus*, *Polygonum Persicaria*, *Sambucus canadensis* and *Solanum Dulcamara*. In only two weeds could the toxic level of nitrate be attributed to the effects of herbicidal treatment. The application of 2,4-D, DNBP, CIPC and MH to *Eupatorium maculatum* resulted in a rapid accumulation of nitrate to the extent the the plant could, if consumed by livestock in sufficient quantities, cause nitrate poisoning. The application of MCP, CIPC and MH to *Impatiens biflora* resulted in similar increases in nitrate concentration.

Livestock poisoning by *Solanum Dulcamara* and *Prunus virginiana* has frequently been reported. The 2,4-D and 2,4,5-T treatments, which often have been employed in eradicatin these two species, did not result in increased levels of nitrate in these plants.

The data indicate that large day to day variations in nitrate content were common and were often of greater significance than the variations due to the chemical treatments.

SUMMARY AND CONCLUSIONS

1. Fourteen species of weedy plants were treated with six different herbicides and the effect of the treatments on the nitrate content was determined.

2. The herbicides were applied at sub-lethal dosages and analyses of the nitrate content were made on the first, second, third, fourth and fourteenth days following the application.

3. The nitrate content of the weeds was determined by a comparison with standard solutions of potassium nitrate using a method involving the nitration of 3,4 xylenol.

4. The nitrate levels of four of the treated weeds were unaffected by the herbicidal application. These weeds were: *Chenopodium album*, *Cirsium arvense*, *Prunus virginiana* and *Thalictrum dioicum*.

5. Herbicidal treatment of five of the weeds resulted in significant increases in nitrate content. These weeds and treatments were:

   a. The DNBP treatment of *Amaranthus retroflexus*
   b. The 2,4-D, DNBP, CIPC and MH treatments of *Eupatorium maculatum*
   c. All treatments of *Impatiens biflora*
   d. The 2,4-D, 2,4,5-T and MCP treatments of *Polygonum Convolvulus*
   e. The DNBP treatment of *Solanum Dulcamara*.

6. Treatments on *Polygonum Persicaria* caused significant reductions in nitrate content.

7. Ten of the fourteen weeds studied contained nitrate in sufficient quantities to cause nitrate poisoning of livestock if consumed in considerable quantities even though no sprays had been applied.

8. In only two of the weeds could the accumulation of toxic concentrations of nitrate be attributed solely to the effect of herbicidal treatment. These two weeds were *Eupatorium maculatum* and *Impatiens biflora*.

9. No herbicide appeared to have the same affect on all of the weeds tested.

10. Of the two weeds, *Chenopodium album* and *Amaranthus retroflexus*, previously reported to accumulate nitrate following treatment with 2,4-D (25), it was found that *Chenopodium* was not affected by any of the treatments and *Amaranthus* accumulated nitrate only as a result of the DNBP treatment.

11. During the course of this study it was observed that soil conditions often had a greater effect upon the plant nitrate content than did the herbicidal treatment.

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APPENDIX

Xylenol Method for Nitrate Determination

1. Reagents

   a. 0.1 % NaOH

   b. H_2SO_4 - 1 volume H_2O to 3 volumes of concentrated H_2SO_4

   c. H_2SO_4 - 5 volumes of H_2O to 1 volume of concentrated H_2SO_4

   d. 2 % acetone solution of 3,4 xylenol (4 hydroxy 1,2 dimethylbenzene)

   e. Phosphotungstic acid 40 %

   f. $Ag(NH_3)_2^+$ - Saturate concentrated, boiling NH_4OH with Ag_2SO_4. Concentrate 60 ml. to 30 ml. by boiling and make to 100 ml. with distilled H_2O

   g. CCl_4 - Distill over CaO and filter.

2. Procedure

   a. Extract sample in Waring Blendor, 1-10 grams in 100 ml. of H_2O, for 6 minutes. Filter through cotton milk filters, folded like conventional filter paper. Take aliquot, 20 ml., add 2 ml. H_2SO_4 (5 to 1) and 2 ml. phosphotungstic acid. Centrifuge again and take an aliquot for nitration.

b. Add 1 ml. of xylenol and add H_2SO_4 (1 to 3) to 3 times the volume of the aliquot, ie 5 ml. aliquot and 15 ml. H_2SO_4. Put on shaker for 15 minutes at from 35-55^oC. Caution: The reaction temperature is extremely important. Where total reactant volume is under 10-15 ml., it may be necessary to warm the tubes before the 15 minute interval has elapsed. If the temperature goes above 55^o , serious loss will be encountered due to side reactions.

c. After the 15 minute shaking, dilute with approximately 50 ml. H_2O. Cool and transfer to 125 ml. separatory funnels. Extract with two 15 ml. portions of CCl_4. Extract the CCl_4 phase with 50 ml. of 0.1% NaOH and discard the CCl_4 layer. Filter the aqueous solution and read in an Evelyn Colorimeter with a 440 mu filter. A standard curve is run by carrying through known quantities of KNO_3 from step b in the procedure with the blank used as 100 % transmission.

The xylenol method of determining the nitrate in plant materials was reported by Rauterberg and Benischke (38) in 1949. Ellis (footnote p. 19), in personal communication, reported making several modifications in the procedure.

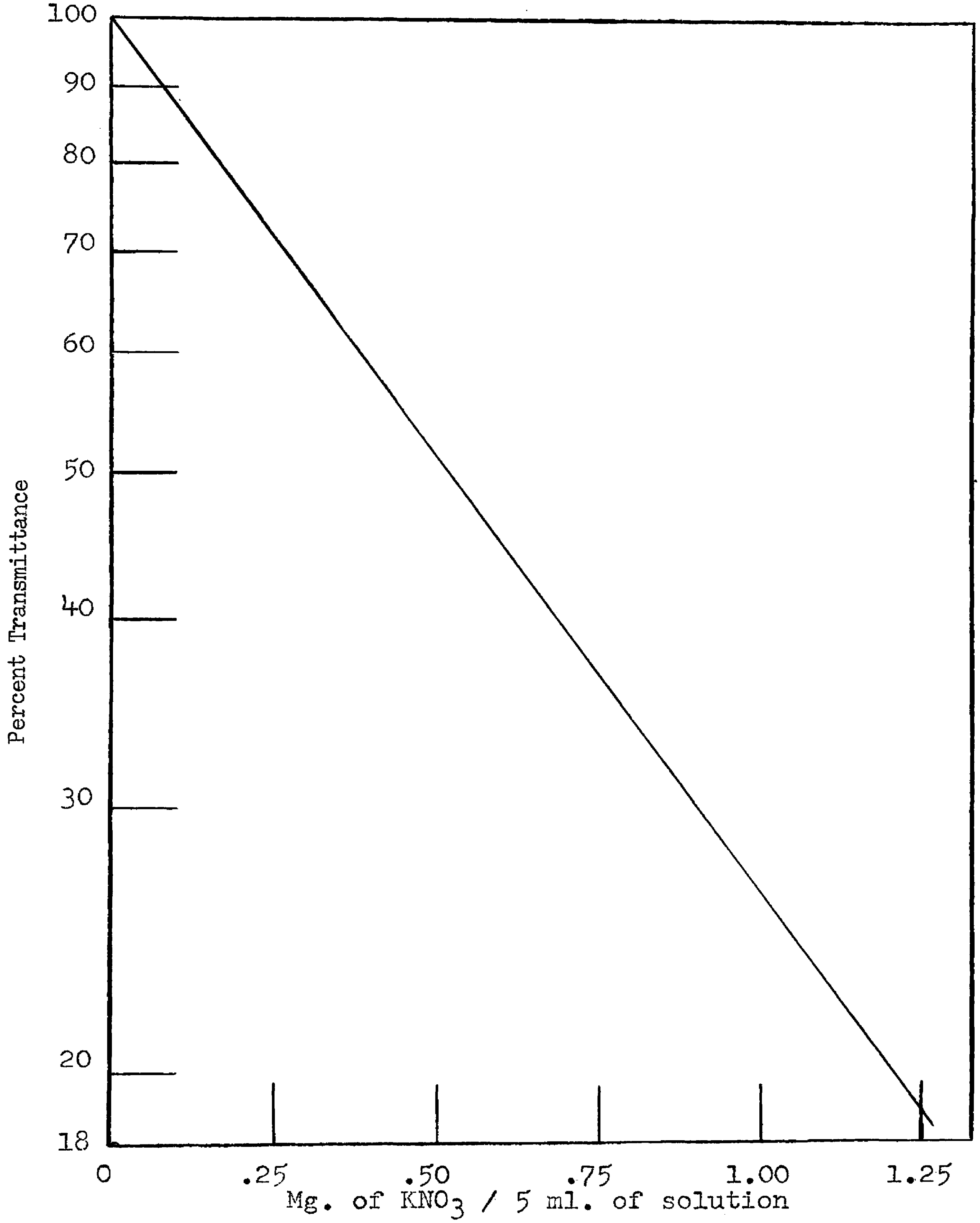

Fig. 1. Standard curve of transmittance of KNO_3 solutions.