EFFECTS OF FUMIGANT CHEMICALS ON MICROBIAL ACTIVITY AND NITROGEN TRANSFORMATION AND ON CROP RESPONSE IN ORGANIC SOIL

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY

James Irvin Kirkwood

1962

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EFFECTS OF FUMIGANT CHEMICALS ON MICROBIAL ACTIVITY AND NITROGEN TRANSFORMATION AND ON CROP RESPONSE IN ORGANIC SOIL

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Ph.D degree in Soils

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Major professor

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ABSTRACT

EFFECTS OF FUMIGANT CHEMICALS ON MICROBIAL ACTIVITY AND NITROGEN TRANSFORMATION AND ON CROP RESPONSE IN ORGANIC SOIL

by James Irvin Kirkwood

Field studies were conducted in 1959, 1960 and 1961 to investigate the effects of fall fumigation on seasonal accumulations of ammonium and nitrate and on yields of crops in nematode-free organic soil. Telone[1] (dichloropropene mixture) was used at rates of 32 to 48 gallons per acre.

Mineralization of organic nitrogen was enhanced, nitrification retarded in fumigated soil. Early applications of ammonium sulfate extended the delay in nitrification into late June. Where nitrate fertilizers were used, rapid nitrification in fumigated soil began in late May.

Wide ammonium:nitrate ratios, ranging up to 6:1 in late May, were associated with marked chlorosis and retarded early growth of celery. Nitrate sidedressings corrected early injury in 1959 and 1960. Under conditions of limiting manganese and phosphorus nutrition in 1961, there was no responde to nitrate. Foliar analysis indicated that increased availability of iron in fumigated soil had interfered with uptake or translocation of phosphorus. Yields of celery declined as iron: phosphorus ratios increased from .01 to .02. Manganese remained below 12 ppm.

In the same experiment, yields of sweet corn and lettuce were increased due to increased availability of manganese in fumigated soil. The manganese response of these two crops was identified by visual

symptoms and foliar analysis.

Laboratory incubation studies were conducted in 1961 to compare the effects of several fumigant chemicals on microbial numbers and activities in organic soil. Moisture conditions expected to prevail in the fall and spring months were simulated. Exposure and aeration periods recommended by the manufacturer were used in treating samples of organic soil with 1/2, 1, and 2 times the recommended application rates of Telone[1], Vidden-D[1], and Fumazone[1]. Untreated soil and a reference fumigant, chloropicrin, were included. N-Serve[1], a nitrification inhibitor, was included as a nonfumigant chemical which would specifically inhibit nitrifiers without materially influencing heterotrophic activities.

The ventilation method of incubation was used. Multiple subsamples of soil were incubated in duplicate respiration jars for each treatment. Three different temperature regimes were used.

The rate of CO_2 evolution was estimated titrimetrically every third day. At periodic intervals subsamples were removed from each respirometer jar for the determination of ammonium and nitrate and for the estimation of microbial numbers. Ammonium and nitrate were determined by microdiffusion. Enumeration of the soil microbial population was accomplished by dilution plate count.

Partial sterilization effects on both heterotrophic and autotrophic components of the soil microbial population were expressed by all fumigant chemicals. These results confirmed observations made in field studies.

However, the intensity and duration of effects of all fumigants

were markedly different under different sequences of soil temperature imposed 3 weeks after initial exposure. Nitrification was inhibited by chloropicrin for 14 to 16 weeks after treatment at 10° and 20°, but for only 9 to 10 weeks at 30° C. Inhibition by the other fumigants at 7 weeks was observed only at 10° or 20° C. Partial sterilization effects on the heterotrophic population, however, were expressed 3 to 4 months after treatment at the two lower temperatures. At 30°, heterotrophic numbers and activities were affected 7 weeks after treatment only by chloropicrin. The chloropicrin effects had largely disappeared 10 weeks after treatment.

The non-fumigant chemical, N-Serve, applied as a fertilizer additive on $(NH_4)_2SO_4$ reduced levels of nitrate and total mineral nitrogen under all three temperature regimes, without appreciable accumulation of ammonium after four weeks. This appeared to be due to chemical complexing of ammonium added with the chemical.

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1 Products of the Dow Chemical Company, Midland, Michigan.

EFFECTS OF FUMIGANT CHEMICALS ON MICROBIAL ACTIVITY AND NITROGEN TRANSFORMATION AND ON CROP RESPONSE IN ORGANIC SOIL

by

James Irvin Kirkwood

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science

1962

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Dedication

This work is dedicated to my wife, Jessie,
and to my children, Alledi and Paul
for earnestly and patiently waiting.

ACKNOWLEDGMENT

The writer is indebted and grateful to Dr. A. R. Wolcott for patient assistance in completion of this study and in preparing the manuscript. He is also grateful to the National Science Foundation, the John Hay Whitney Foundation, Dow Chemical Company, and to Dr. R. L. Cook for financial support which enabled the writer to prepare for, initiate, and complete the study.

Appreciation goes to Dr. L. N. Shepherd, Dr. R. E. Lucas, and Dr. C. L. SanClemente, and all the others who contributed to the completion of this work.

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INTRODUCTION

Numerous chemicals have a marked suppressive effect on microbial numbers and activities in the soil. Recognition of this fact led to the extensive use of carbon disulphide as a soil fumigant in Europe near the close of the last century. After World War I, the merits of chloropicrin as a fumigant were established, thereby increasing the interest in the use of volatile chemicals as soil fumigants.

The primary objective of soil treatment is to kill pathogenic organisms. The chemicals being used, however, are frequently non-specific. As a result, non-spore forming, nitrogen-fixing and nitrate-forming bacteria, as well as parasitic organisms are destroyed. Nitrification is strongly inhibited. The spore forming ammonifiers increase due to this partial sterilization. Ammonification, unaccompanied by nitrification, results in net accumulation of ammonium. Accumulation of ammonium may continue for weeks, especially in soils high in organic matter.

The intensity and duration of the suppression of nitrification vary with the chemical and the numerous mechanical, soil and environmental factors which influence the effectiveness of the chemicals as fungicides or nematocides.

Frequently crop injury results from soil fumigation due to the alteration of nitrogen nutrition arising from impaired nitrification. Whether or not the ammonium nitrogen which accumulates in the soil after fumigation will be taken up by plants and assimilated efficiently in growth will depend on the crop, soil pH and the supply of other cations, daylength, light intensity and the level of carbohydrates in the plant, as well as the form and placement of supplemental nitrogen fertilizer. The absorption by plants of nitrogen primarily in the ammonium form is

accompanied by a tendency toward rejection or immobilization of other cations, such as potassium, calcium, magnesium, and iron, and enhanced absorption of anions, notably chloride and sulfate. This may give rise to injurious physiological derangements in some plants. In the alkaline range, direct toxicity to the roots of a large number of plants may result from the presence of ammonia (NH_3) in the soil solution.

In laboratory studies, the large quantities of ammonium which accumulate in organic soils following treatment with various fumigant chemicals are converted rapidly to nitrate as the inhibitory effect of the fumigant on nitrification wears off. These results from laboratory experiments cannot be projected directly to field conditions to explain why crop injury is sometimes associated with one chemical and not with another, or why injury from the same chemical may occur in one location or season and not in another. The field research reported here was undertaken with a view to relating seasonal patterns of ammonification and nitrification in organic soil to fumigation treatment and climatic factors on the one hand and to crop response on the other. Laboratory studies were also conducted to determine the influence of temperature, as one climatic factor, on microbial response to fumigation treatment of organic soil.

LITERATURE REVIEW

Effects of Agricultural Chemicals on Microbial Numbers and Microbial Activities

Some of the earlier investigators advanced many speculative theories in the search for an explanation of the increases in plant growth following soil treatments with heat or fumigants. These have been well reviewed by DuBuisson (16) and Kopeloff and Coleman (35). DuBuisson indicates that the first record of an "antiseptic" soil treatment seems to be that of a German, C. Oberlin, around 1905. After using carbon disulfide as an insecticide in some of his vineyards that were attacked by Phylloxera, he noted a marked increase in the growth of vines.

Newhall (48) indicates, however, that we are indebted to earlier workers in the late 1800's for a number of well established facts. Among these are that treating soils with heat or "antiseptics" results in the following:

(a) Non-spore-forming, nitrogen-fixing, nitrite-forming and nitrate forming bacteria as well as parasitic organisms are destroyed, and nitrification is thereby inhibited. The spore-forming ammonifiers which survive increase in number and ammonification goes on almost uninterrupted for weeks, especially in soils high in organic matter.

(b) Soluble salts are often liberated, in some cases chlorides and sulfates of ammonia, and sometimes soluble manganese.

Effects of partial sterilization of the soil by heat or fumigants remained an enigma for several years. The first major breakthrough came

with the publication of a series of three papers by Waksman and Starkey (78). Conclusions reached by them were:

(a) Partial sterilization of soil by steaming brings about a chemical change in the organic matter of the soil, making it more available as a source of energy for microorganisms.

(b) A large proportion of the soil fungi are killed as a result of partial sterilization.

(c) The rapid increase in numbers of bacteria in the soil is at the expense of the organic matter made available.

(d) The actual amount of ammonia formed in partially sterilized soil is determined, not by the numbers of bacteria and fungi developing in the soil, but by the abundance of organic matter.

(e) The protozoa are suppressedin partially sterilized soil, but become active again long before bacterial numbers decline markedly.

(f) The more rapid the rise in bacterial numbers and the greater the maximum number reached, the sooner will the decline set in. This is true, also, of fungi. These fluctuations in numbers are related to the supply of readily available organic substrates released by the steam treatment and their subsequent exhaustion.

In their work using low and high dosages of chloropicrin, Stark, Smith, and Howard (65) concluded that since the total amount of nitrogen made available for plant growth was not increased except at high dosages then the increased plant growth obtained from low dosages could

not be accounted for solely by the hypothesis that more nitrogen was made available for plant growth.

Because of the high cost of chloropicrin and its lachrymatory, phytocidal and corrosive properties, other safer and cheaper soil fumigants were needed. The practice of soil fumigation was stimulated with the discovery of the nematocidal properties of D-D mixture (50-50 mixture of 1,3 dichloropropene and 1,2 dichloropropane) by Carter (10). This led to research with this and other fumigants using chloropicrin as the standard.

Tam and Clark (69), working with pineapple plants, showed that increased growth and nitrogen composition were associated with soil fumigated with chloropicrin. This was related to restriction of the plants to predominantly ammonium nutrition as compared to predominantly nitrate nutrition in unfumigated soil.

Tam (68), Thiegs (71), and Winfree and Cox (82) observed the same pattern of ammonium accumulation, followed by delayed but rapid conversion to nitrates about two months after treatment of soils with chemical fumigants.

Concomitant development of the insecticidal and herbicidal program created increased interest in the effect of chemicals such as D.D.T. (dichlorodiphenyl-trichloroethane) and BHC (benzene hexachloride) on specialized activities of microorganisms in soil.

Wilson and Choudri(81), in laboratory studies, showed that the BHC in amounts considerably exceeding practical field applications had no significant effect on development of bacteria and molds, nor on physiological activities important to soil fertility.

Bollen et al. (6) in other studies using various insecticides in

the field concluded that the observed stimulations and inhibitions of microbial activities were not intensive enough to materially influence fertility of the soil.

Kidson and Stanton (32) and Kidson (31) observed a long but not very pronounced "partial sterlization effect" when soil was treated with D.D. or chloropicrin. Smith et al (64) checked the effect of 2,4-D (2,4-Dichloro-phenoxyacetic acid) on the soil. Low concentrations had no visible effect but concentrations of 100 ppm or more displayed a weak sterilization effect without an appreciable increase in ammonia production. The nitrifying organisms proved to be very sensitive to 2,4-D. The results obtained by Koike and Gainey (33) are slightly different;2,4-D was found to exhibit a marked bactericidal action even in concentrations applied for weed control, and to cause afterward a temporary increase in bacterial numbers and in ammonia content in the soil. The nitrification process was suppressed for a period of two to four months.

Smith and Wenzel (63),however, found a marked suppression of the microflora and especially of the nitrifying bacteria with BHC. Chlordane and DDT were less harmful for microbes when applied in practical dosage.

Smith and Bell (62) reported some observations on light sandy soils in Florida. D.D., chloropicrin, D.D.T., and 2, 4-D all exhibited for a short period a partial sterilization effect. D.D.T. and 2,4-D had the strongest effect, lasting for 70 days.

Koike (34) conducted laboratory experiments to determine the effects of eight fumigants on the nitrification of $(NH_4)_2 SO_4$ and NH_4OH. Results indicated that under the conditions of the experiment the chemicals markedly inhibited nitrification from four to eight weeks.

Wolcott et al (83), using Telone as a fumigant applied at recommended nematocidal rates, found nitrification to be delayed in the laboratory about eight weeks at soil temperatures above 60°F. and for longer periods at lower temperatures. In the field, following fall fumigation, they found that nitrification was delayed 6 to 8 weeks after the soil warmed to 60°F. in the spring.

Sabey et al (58) initiated a study to determine the influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. In soils incubated at field capacity, the maximum nitrification rates increased from immeasurably low values at 0°C to as great as 900 ppm per week in some soils at 25°C. Delay periods decreased from about 32 weeks to less than one day, as temperatures increased from 0 to 25° C. Increase in initial population caused decreases in delay but did not appreciably affect the maximum rate above 10° C.

There are numerous contradictions among published reports dealing with the microbiological effects of these fungicides, insecticides, and herbicides. This is to be expected, considering the varied circumstances under which the work was carried out.

Effects of Agricultural Chemicals on Plant Nutrition and Metabolism

Nitrogen Nutrition

Tam (68) found that pineapple plants restricted to an ammonium nutrition due to D-D soil treatment were high in nitrogen, dark green, broad-leaved, soft, and succulent, as compared to slower growing, yellowish plants on nitrate nutrition.

Wolcott et al (83) in work with Telone (a mixture of dichloropropenes) reported that celery seedlings appeared to be unable to utilize ammonium

effectively at pH 6.3 and required nitrogen in the nitrate form during their early growth. Excessive nitrate during subsequent growth and particularly during the period immediately preceding harvest was detrimental to yield.

In his work with tomato plants Thiegs (71) found that the presence of nitrates seemed to decrease ammonium toxicity. As a result, he proposed that nitrate applied as fertilizer might counteract the detrimental effect of too much ammonium on ammonia sensitive plants. However, Winfree and Cox (82) felt that although it may be true that nitrate may counteract detrimental effects of too much ammonium, their data supported the conclusion that the benefit from applied nitrate came from substituting the more available nitrate for ammonium nitrogen.

Considerable research has been done with tobacco and potatoes and the interrelations of their nutrition and metabolism to soil fumigation treatment. The growth and development of tobacco is known to be adversely effected when the major portion of the available nitrogen remains in the ammonium form. The works of Evans and Weeks (17), Gilmore (21), and McEvoy (46) have indicated that the yield and percentage of dry matter in tobacco is lower when grown in media in which all the nitrogen is in the ammonium form than when grown in media containing nitrate nitrogen. Plants from ammonium cultures had a high percentage of total nitrogen, amide nitrogen, nicotine and pigments and a lower percentage of sugars and organic acids than did plants from the all nitrate cultures.

McCants et al (45) have presented data which showed that certain of the soil fumigants currently used for nematode control can have a significant influence upon the response of tobacco to the applications of nitrogen in the ammonium or the nitrate form. There was a greater yield response to nitrate from the fumigant treatments which had the most suppressing effect on nitrification. However, where nitrification was

inhibited and ammonium applied, there was a high ammonium and halogen content of leaves. This was associated with leaf abnormalities and stunting of the plants. Grogan and Zink (22) have shown that free ammonium hydroxide or free ammonia (NH_3) may cause severe injury to roots and tops of lettuce plants in California fields. Application of organic nitrogenous fertilizers to cold waterlogged soil, or the use of aqua or anhydrous ammonia, produced the injury. Ammonium sulfate, ammonium nitrate, or calcium nitrate caused relatively little damage.

Hoff and Newhall (26) reported that a severe root rot of head lettuce on the acid mucklands of New York could be reproduced in sand culture and steamed muck by an excess of ammonium. Lorenz et al (40) reported root injury and reduction of yields in spinach, radish, peas, cabbage, lettuce and onions following the application of aqua ammonia or of anhydrous ammonia. Ammonium sulfate, in contrast, increased yields in comparison with the unfertilized check plots.

Nightingale (49) (50) has given an excellent review of papers on nitrogen metabolism. Most of them indicate that neither nitrite nor ammonium nitrogen can accumulate in plants without causing damage. Nitrate nitrogen can be stored in considerable quantities by most plants without injury.

Through the use of $N^{15}H_3$, Vickery et al (77) showed that nitrogen of $N^{15}H_3$ is incorporated by tobacco plants into amides, amino acids, and proteins. The results of MacVickar and Burris (42) showed that glutamic and aspartic acids become highly labeled with N^{15} from $N^{15}H_3$. Their results suggests that glutamate and aspartate are the primary products of the assimilation of ammonium by plants. Rautenan (56) confirmed this when he showed that glutamate, aspartate, their amides, and

alanine are the major initial products of ammonium uptake in plants.

Grogan and Zink (22) have expostulated that if assimilation or detoxification of ammonium or nitrite keeps pace with absorption so that no accumulation occurs in the plant, injury is prevented. However, if ammonium accumulates because of slower assimilation at low pH values or because of too rapid absorption at high pH levels, injury to the plant may result. Lack of carbohydrates, which are necessary for the detoxification of ammonium by conversion to amines, may also result in damage from excessive ammonium uptake.

Response of plants to nitrate and ammonium varies with a number of environmental factors. Webster (80) has indicated, for example, that pH of the nutrient medium exerts a considerable influence on the relative utilization of nitrate and ammonium.

Arrington and Shive (2) found that a low pH favors nitrate uptake, while a high pH favors the uptake of ammonium.

Tiedjens and Robbins (73) reported that both absorption and assimilation of nitrate nitrogen was most rapid from acid media, whereas ammonium nitrogen is assimilated most rapidly from alkaline media.

Other conditions of the soil such as aeration, temperature, and soil moisture have their effect upon nitrification also.

Relationships Among Other Plant Nutrients as Influenced by the Form of Nitrogen Assimilated

As has been stated, the ability of plants to take up or assimilate either NH_4^+ or NO_3^- depends upon other nutritional factors which are strongly controlled by pH. The availability of many other nutrients is controlled by pH, and when the cation-anion balance is altered the metabolic patterns of the plant are likely to be altered.

The absorption by plants of nitrogen primarily in the ammonium form is accompanied by a tendency toward rejection or immobilization of the cations (K, Ca, Mg, and Fe) and enhanced adsorption of anions (Cl^-,$SO_4^=$). Bear (4) observed that the cation-anion equivalent ratio in plants can be represented as $\frac{Ca+Mg+K+Na}{N+Cl+S+P}$ = a constant. From the equation it follows that increased absorption of any one cation results in reduced absorption of some other cation or cations or increased uptake of one or more anions. Increased absorption of an anion results in reverse effects. These relationships may give rise to injurious physiological derangements in some plants.

Wallace et al (79) have shown evidence that increased chloride absorption results in decreased nitrogen in plants. The exact function of chloride in plant nutrition is undefined, although evidence has been forwarded that it interferes with carbohydrate metabolism, modifies chlorophyll content and affects relations between cation and anion absorption. Baslavskaja (3) has shown that large doses of chloride lower the content of chlorophyll in plants. The most apparent effect observed by Slatz et al (60) was the large increase in chloride content of plants associated with increasing chloride level in the nutrient medium. This increase did not cause a corresponding decrease in the content of phosphorus or sulfur.

Buchner (8) showed, in water culture experiments on various crops, that a shift in carbohydrate fractions associated with chloride ions was dependent on the kind of nitrogen supplied. Content of reducing sugars and sucrose declined much more as a result of chloride supply with ammonium than with nitrate nutrition. Absolute proportions of starch and total carbohydrate content were less influenced.

Harvard et al (25) made a study of the role of chloride and sulfate anions in the nutrition of Irish potatoes when different forms of nitrogen were used. Increasing Cl^- and decreasing $SO_4^=$ concentrations in the presence of NH_4^+ resulted in marked reduction in the yield of fibrous roots. Addition of Cl^- generally resulted in decreases in the percentage of dry matter in the tops. This effect was more pronounced in the presence of NH_4^+. The chloride content of the leaves was markedly increased in the presence of NH_4^+.

Vladmirov (76) reported that chloride and nitrate in culture solutions stimulated production of organic acids in tobacco, while sulfates and ammonium nitrogen retarded their accumulation.

Corbett and Gausman (12) concluded that chloride may affect potato tuber quality by affecting the uptake of phosphorus.

Kretschmer et al (36), in their work on chloride versus sulfate ions in nutrient-ion absorption, found that the most consistent response phenomenon involved an inverse relationship between nitrogen and chloride contents in plants.

Teater (70) using ammonium chloride as nitrogen fertilizer studied the effects of the chloride ion on yields and uptake of nutrients by crops. In general summary, he found that there appeared to be no great problem associated with the use of ammonium chloride for grain crops. Even though chloride accumulated in plants, there was little evidence that its interference with absorption of other essential anions was of any importance.

Another element that is affected by fumigation is manganese. Over three decades ago, Gilbert et al (20) found conclusive evidence that, through heavy liming, soil conditions are created in which many plants

develop chlorosis. This chlorosis was prevented by application of manganese sulfate.

Alexander (1) points out that manganese occurs in the soil in the divalent and tetravalent forms. The exchangeable divalent cation is water soluble, while the tetravalent cation is essentially insoluble, occurring as MnO_2. The ion that predominates is a function of pH. However, since there are manganese oxidizing microorganisms in the soil, they can also produce the insoluble form of manganese.

Lingle and Wright (38) studied the growth and manganese content of onions as influenced by source and rate of applied nitrogen, lime, and soil fumigation. The growth of onions on very acid coastal California soils was inversely related to the manganese content of the tissue. The manganese content was directly related to the soil pH as influenced by the rate and source of applied nitrogen, lime, and soil fumigants. It was also found that heavy nitrogen applications increased the manganese content independently of the effect of nitrogen on soil pH. Soil affected manganese uptake differently depending on lime application.

Sherman and Harmer (61) were the first to recognize the need for manganese on organic soils in Michigan. McCall and Davis (44) used manganese carriers effectively to increase the yield of onions on organic soils by foliar spraying and dusting, and by soil application.

Lucas and Davis (41) have indicated that the availability of manganese is influenced more by soil reaction than any other plant nutrient. Total manganese content in organic soils is of little value in predicting the need for manganese fertilizers. Availability decreases above pH 5.5. Shickluna and Davis (50) showed that the manganese content of onion tops dropped from 1125 ppm to 44 ppm when the pH of an organic soil was

raised by liming from 4.1 to 5.6. Another peat showed a drop of manganese in onion tops from 875 ppm to 25 ppm when the soil pH was raised from 4.9 to 7.0. Manganese toxicity is often credited with causing poor growth in very acid soils, especially if fumigated or sulfured.

Soluble Salts

Steam treatment of soil may release enough adsorbed salts to produce plant injury. The soluble salts may be, in some cases, chlorides and sulfates of ammonium, and sometimes soluble manganese. (48) A study was made by Markle and Dunkle (43) on the use of the soluble salt content of greenhouse soils as a diagnostic aid. Some observations and conclusions were:

(a) There is a close relationship between the total soluble matter and the electrical conductance of aqueous soil extracts.

(b) There is a close relationship between the inorganic soluble matter and the electrical conductivity of aqueous soil extracts.

(c) The electrical conductivity of the extract is a reliable measure of the soluble matter content.

Specific conductance has been used as a criterion for determining the range in soluble salt content over which plants will grow satisfactorily.

In correlating specific conductance with growth, a range between 61 and 106 x 10^{-5} mhos would appear favorable for celery plants, the critical electrical conductivity for a 1:5 (V/V) soil: water extract being about 110 x 10^{-5} mhos (15).

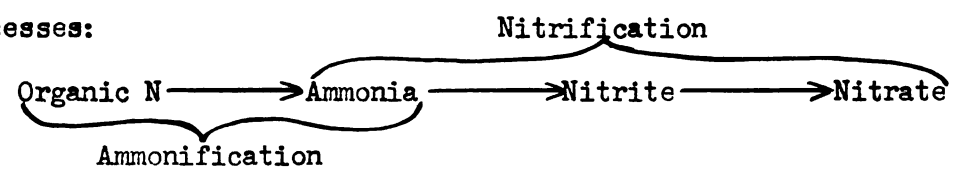
Methods for Studying Soil Microbial Population and Activities

Nitrogen Transformations

Harmsen and Van Schreven (24) have given a short review of the different methods used for determining the rate of nitrogen transformation in the soil. They divide the methods into three groups, field trials, pot experiments, and different procedures for incubation of soil samples under laboratory conditions. Field trials, being the most empirical, provide reliable results, but they are laborious, time- and space-consuming and subject to many external influences such as the effects of weather, season, crop grown, etc. Pot experiments are similar to field trials but the external conditions can be controlled and standardized better.

Incubation experiments have numerous limitations, also. By using both field and incubation data, however, the experimenter can develop a practically useful prediction of the pattern of nitrogen mineralization and transformation to be expected under a given set of field conditions.

It is generally accepted that organic nitrogen must be mineralized before it is available for plant uptake. In normal soil, nitrate is the end product of mineralization. The transformation of organic nitrogen to nitrate takes place in three steps by two general types of processes:



Soil microorganisms involved in the decomposition of organic matter set free more nitrogen than they are able to assimilate into their own protoplasm. Under aerobic conditions the excess nitrogen appears in the

soil as ammonium. In the nitrification process nitrate is formed from the ammonium.

Harmsen and Van Schreven observed that the majority of earlier investigators determined the NO_3^- - N or the total mineral nitrogen (NO_3^- - N + NH_4^+ - N + NO_2^- - N) only in the field. They deduced from these periodically collected data their conclusions about the changes in the mineral nitrogen content as influenced by season, crops, climate, moisture, temperature, structure of the soil, and its total nitrogen and humus content.

With the introduction of incubation methods, the release of mineral nitrogen during incubation under standardized conditions could be followed: Alexander (1) noted that early microbiologists chose to limit their analyses to ammonium, the first inorganic product. The usually rapid conversion of ammonium to nitrate in soil invalidated the determination of ammonium alone as a measure of mineralization. Nitrate production has also been used as a mineralization criterion. This usually provides a valid measure of mineralization since ammonium and nitrite accumulate only under abnormal conditions. In some situations, however, ammonium and nitrite may accumulate, or denitrification losses may occur, so that the rate of accumulation of nitrate does not always reflect the rate of mineralization. Furthermore Jansson (29) has shown by N^{15} tracer studies that extensive cyclical re-utilization (immobilization) of mineralized nitrogen occurs. Usually it is not feasible to account for nitrogen recycled or that lost by denitrification. The most acceptable measure of mineralization is the total of all mineral products, - ammonium, nitrite and nitrate. The rate of mineral nitrogen accumulation is not an absolute measure of the rate of release of nitro-

gen from humus, proteins, nucleic acids or related materials. It is a measure only of net mineralization.

Respiratory Measurements

The rate of nitrogen mineralization in the soil can be estimated by measurements of CO_2 evolved by a sample during incubation. However, this is only an indirect measurement and may be entirely misleading if organic materials low in nitrogen are being decomposed, with extensive concomitant immobilization of nitrogen. Its more usual application is as a measure of microbial activity.

Starkey (66) in evaluating the usefulness of the CO_2 measurement stated that under aerobic conditions, with a mixed microbial population, it is the principal carbonaceous end product of decomposition. Determinations of CO_2 liberated from soils may be interpreted as indicating fairly accurately the speeds of decomposition of organic materials in soils. He felt this method to be much more accurate in estimating some of the influences of plant development on soil organisms than are plate counts.

Russell (57) stated that the number of microorganisms in a soil gives no direct measure of the activity of the microbial population. The activity of the total population is not a concept that can be given a quantitative definition. For many purposes,however, it is a useful concept. Total activity can be estimated by the amounts of either CO_2 or heat evolved by the population. In general, CO_2 evolution does increase as the number of microorganisms increases.

Gainey (18) studied the parallel formation of CO_2, NH_4^+, and NO_3^- in soil, air being drawn through the soil. There was a similarity between the curves for CO_2 and NH_4^+ release from soils incubated under

similar conditions. Insufficient moisture retarded both CO_2 and ammonium formation. Insufficient aeration also caused a depression or marked delay in CO_2 and NH_4^+ production. Where water content was varied, rate of nitrate accumulation was directly proportional to moisture content, the maximum rate not being reached until the maximum moisture that would be retained by the soil was reached. Where aeration was varied, insufficient aeration retarded the initial accumulation of nitrate, but after nitrification became active in all samples the rate of accumulation was inversely proportional to aeration.

Corbet (13) derived the following formula to describe, mathematically, the evolution of CO_2 from a soil sample, under laboratory conditions and at constant temperature:

$$y=Ft^m$$

where:

F=yield of CO_2 in unit time at the beginning of the experiment

y=total CO_2 evolved in time t.

m=constant less than 1, representing the progressive retardation in rate of gas evolution due to the restrictive experimental conditions of incubation.

This expression was shown to fit the pattern of CO_2 evolution from pure cultures of actinomycete species during the decline phase of the generalized bacterial growth curve. Since the same expression could be applied to the data from incubated soil samples, it was deduced that F represents the activity of the climax population present in the soil at the time of sampling in the field.

Vandecaveye (74) (75) in studies of microbial activities in soils concluded that, since increased rates of CO_2 production did not coincide

with increased microbial numbers, - the maximum CO_2 production preceding maximum number of microbes by 2-3 weeks, - then CO_2 production is by no means an accurate index of microbial numbers.

Lees and Porteous (37) stated that, in practice, the direct measurement of carbon retention in a medium naturally so highly organic as soil is difficult if not impossible. If assimilation is to be measured in a soil, it must be measured indirectly. The method they used involved the titrimetric estimation of CO_2 released from a known weight of an organic compound percolated through a soil. The decomposition of the perfused compound was measured by the surplus of CO_2 released over that released by the soil alone where no carbonaceous substrate was added to the perfusate. The difference between carbon added and carbon recovered as CO_2 represented carbon retained, or immobilized, in the soil.

Norman and Newman (52) collected CO_2 by diffusion into alkali in test tubes inserted with the soil samples in sealed jars. Replicate soil samples were sacrificed periodically to follow chemical and physical changes in the soil itself and changes in microbial numbers. The authors concluded that no single measurement could be used to adequately characterize microbial activities in soils. However, the pattern of CO_2 evolution over a period of time does provide a composite picture of the behavior of the organisms present acting on the substrates available under the environmental conditions that prevail. Delay periods and the cumulative total CO_2 evolved over a period of time can be used to make inferences about the size of the initial population and the biochemical capabilities of the population which develops during incubation.

Damirgi (14) used the "simultaneous absorption" method described by the previous authors for studying microbial populations and activity

in soils of a Prairie-Forest biosequence. Only small differences in CO_2 evolution were encountered between soils when incubated alone. This indicated a basic similarity in microbial activity in soils. No correlation appeared to exist between initial microbial numbers and total CO_2 evolved over 24 days from unamended soils.

Stotzky and Norman (67) studied the influence of several environmental factors on glucose decomposition in soil. Carbon dioxide evolution, substrate disappearance, formation of intermediates, and changes in soil pH were followed by periodic assay over intervals up to 48 days. Although good correlation was obtained between these parameters, microbial activity was not directly correlated with the numbers of bacteria and fungi estimated by dilution plating. High respiratory quotients were obtained during the early part of the incubation. Maximum numbers of bacteria and fungi developed after the rate of CO_2 evolution had decreased. A sequential devolpment of microbial populations differing qualitatively was indicated, with the early populations being characterized by a dominance of anaerobic types.

Quantitative Estimation of Microbial Numbers in Soil

According to Thornton (72), no fully satisfactory counting methods have been devised for the quantitative determination of microorganisms in soil. Colony counts from platings of diluted soil suspension have been the standard method for bacteria, actinomycetes, and fungi. Thornton indicates that one of the defects of this method is that no one medium is suitable for all the nutrionally varied organisms present. Work by Lochhead and Chase (39) showed that, just for bacteria, a quite complex medium was required that included unknown growth substances from yeast

extract and soil extract. On the other hand if the medium used for plating was too rich, competition between organisms on the plate reduced the colony count. In the case of fungi, there is additional uncertainty with regard to plate counts, because there is no way of knowing whether a colony is derived from a spore or from a fragment or from a clump of mycelium. Actinomycete colonies are usually counted along with bacterial colonies because a positive and complete separation of bacteria and actinomycetes is impossible on the basis only of colony characteristics.

Russell (57) offers as a major defect in the method that it gives only limited information about the reactions the various bacteria actually carry out in the soil, since bacteria can grow on organic substances in pure culture that they would be unable to use in the competitive environment of the soil.

In spite of the fact that plate counts inherently create uncertainty as to the absolute numbers of organisms in soil, they do have definite value (72). They are quite useful in comparing estimates made by the same technique from a number of soil samples. Hence, it is possible to demonstrate differences in number of organisms in soils variously treated.

Johnson et al (30) have given details of this method and various modifications that can be used.

MATERIALS AND METHODS

Field Experiments

Description

Field studies in 1959 and 1960 were directed towards evaluating the effects of fumigation with Telone (1, 3 dichloropropene) on seasonal patterns of nitrogen transformation in cropped and uncropped muck, and on celery yields. To eliminate nematodes as a factor in celery response, areas were selected on Houghton muck at the Michigan State University Muck Experimental Farm, Bath, Michigan, where previous crop performance had shown no evidence of nematode infestation. Houghton muck is an organic soil containing 85 percent organic matter. It was developed from organic deposits more than 42 inches deep. The pH averages 6.0 to 6.3 with a range of 5.0 - 7.0: Depending upon the crop, major and minor nutrients must be added. The water table at the muck experimental farm is controlled by a combination drainage and water level control pumping system.

In 1959, a split-plot design in four replications was used involving two levels of fumigation (none and 32 gpa Telone) as main plots, and no nitrogen or nitrogen applied as ammonium sulfate, ammonium nitrate or calcium nitrate as sub-plots. The fumigation treatment was made by injection, using a tractor mounted applicator, October 24, 1958. The entire experimental area was then aerated two weeks later by deep cultivation and again by plowing prior to planting celery on May 7, 1959. A basic fertilizer application of 1500 pounds per acre of 0-10-30 was plowed down. Nitrogen treatments were made in a split application of 50 pounds nitrogen at planting time and 50 pounds sidedressed on July 7. A sub-plot not planted to celery and receiving no nitrogen was included.

A split-split-plot design in 4 replications was used in 1960. Main plots were unfumigated or fumigated with 32 gpa of Telone on October 26, 1959. The entire experimental area was immediately cultipacked. One-half of each main plot was aerated by plowing just before transplanting celery, Utah 5270, May 5, 1960. All plots were subjected to three very shallow sweep cultivations during the growing season. Weeds which escaped cultivation were pulled by hand.

All plots in 1960 received 1500 pounds per acre of 0-10-30 before planting. Sub-sub-plots received 50-pound sidedressings of nitrogen at planting and on June 24. Nitrogen was applied as calcium nitrate, ammonium nitrate, or ammonium sulfate. A fourth sub-sub-plot received no nitrogen and a fifth sub-sub-plot was uncropped with no applied nitrogen.

Both in 1959 and 1960, ammonium and nitrate nitrogen were determined on field fresh soil samples taken periodically through the growing season, by methods to be described later. In 1960, soluble salt determinations were made on June 20. Estimates of dry matter and chloride content in celery also were made at this time. Final yields of celery were taken both years.

Field studies in 1961 were directed towards evaluating the effects of recommended and excessive rates of Telone fumigation on seasonal patterns of nitrogen transformation in cropped and uncropped muck and on yields of corn, celery, and lettuce. Soil population studies by J. A. Knierim, Nematologist, Entomology Department, Michigan State University, showed no evidence of parasitic nematodes in the selected plot area, which was located again on Houghton muck at the Michigan State University Muck Experimental Farm.

A split-split-plot design of four replications was used in 1961. Main plots were unfumigated or fumigated on October 4, 1960, with 32 gallons per acre and 48 gallons per acre of Telone. The entire experimental area was cultipacked as in 1959. The entire area was aerated by plowing on April 14, 1961.

Sub-plots were either uncropped or planted to lettuce, celery or sweet corn. Lettuce was planted with 800 pounds o-10-20 plus 1/4% boron + 1/4% copper + 2% manganese banded 2 inches below the seed and 1/2 inch to the side. The variety used was Great Lakes 659. Two celery varieties, Utah 5270 and Spartan 162, were planted on two rows of each celery sub-plot on May 11 and May 17 respectively. Basic fertilizer used was 500 pounds of 0-10-20 before planting.

Cropped and uncropped plots were further subdivided for broadcast nitrogen treatments. Sub-plots of lettuce and uncropped sub-plots received 50 pounds of nitrogen as a broadcast application on April 27, 1961. The nitrogen was applied to lettuce plots as calcium nitrate, ammonium sulfate, and ammonium sulfate treated with 1.6% N-Serve.[1] The uncropped plots received nitrogen as ammonium sulfate and treated ammonium sulfate. Nitrogen was sidedressed on celery and sweet corn sub-sub-plots on May 26 at the rate of 50 pounds per acre as ammonium sulfate and calcium nitrate. A treatment involving no supplemental nitrogen was included on all three crops.

Soil samples were taken periodically from all replicates of all treatment combinations beginning April 5 and continuing through September

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[1] N-Serve is the trade name for 2-chloro-6 (trichloromethyl) pyridine, a nitrification inhibitor manufactured by the Dow Chemical Company, Midland, Michigan.

13. Tissue samples were taken periodically through the growing season for all crops.

Harvest and final yield measurements were made for lettuce on July 13, for celery on August 14 and for sweet corn on August 25.

Sampling of Soils and Plants

As indicated in the previous section soil samples were taken periodically during the field experiments. Twenty soil cores to an eight inch depth were composited per plot. These were passed through a four-mesh screen, thoroughly mixed and sub-samples taken. Soil samples in 1959 and the April and May samplings in 1961 were frozen immediately and stored in a deep freezer for later analysis. Aliquots of all later samplings in 1961 and all samples taken in 1960 were extracted immediately for determination of ammonium and nitrate nitrogen as described in a later section.

The tissue samples taken of celery in June, 1960 consisted of five whole plants per plot; in 1961, ten petioles per plot were taken periodically through the growing season.

Prior to thinning, ten whole lettuce plants were taken per plot for tissue analysis in 1961; in later samplings ten to twenty leaves were taken. In the case of corn, tissue samples in 1961 consisted of ten basal midribs per plot. These tissue samples were dried at a temperature of 70-80° C. and stored in paper sacks. For the present study, samples from selected sampling dates were analyzed in the Department of Horticulture for N, P, K, Ca, Mg, Mn, Fe, Cu, Zn, Mo, B, Na and Al.[2] Nitrogen was determined by Kjeldahl procedure and potas-

[2] Under the supervision of A. L. Kenworthy and S. J. Gamble.

sium by flame photometer. The remaining elements were determined spectrographically, using a photoelectric spectrometer.

Laboratory Incubation Experiment

Most of the fumigant chemicals used extensively in commercial agriculture have been compared in incubation studies with regard to their immediate and residual effects on microbial numbers and activities. None of the reported studies, however, have taken into account the sequence of soil and climatic conditions which intervenes between fall fumigation and the planting of early spring crops.

The objective of the laboratory incubation studies reported here was to compare the effects of several fumigant chemicals on microbial numbers and activities in organic soil under moisture and temperature conditions which might be expected to prevail for significant periods of time in the field during the fall and spring months.

Preparation of Soils for Incubation

Virgin, uncropped, unfertilized Houghton muck was taken June 21, 1961, one day after two days of rain totalling 0.85 inches. At this time, the soil contained 263 percent moisture. Although wetter than normal for the growing season, it was not saturated.

Prior to incubation, the field moist soil was fumigated in 5-pound lots (oven dry basis) with 1/2, 1 and 2 times the recommended application rates of Telone, Vidden-D and Fumazone. In addition to these treatments, a check, and a reference chemical, chloropicrin, were used. The nitrification inhibitor, N-Serve, was included as a twelfth treatment. After addition of the volatile chemicals, the 5-pound lots of soil were sealed in plastic bags for an exposure period recommended by the manu-

facturer. Following exposure, water saturated air was forced through the samples for the minimum aeration period recommended by the manufacturer as necessary before shallowrooted crops may be planted.

Chemical identification of the materials used and their rates of application, exposure times, and aeration periods are shown in Table 1. Soils were treated at such times as to allow for completion of the indicated exposures and aeration at the beginning of incubation, on July 12, 1961. All of the lots of soil were amended with 100 ppm nitrogen (50 pounds N per acre) as ammonium sulfate just before dispensing in subsamples for incubation.

Table 1. - Soil treatments imposed on field moist Houghton muck prior to incubation.

Material	Rates Per Acre	Rates Per 5 lbs.	Exposure Time at 65°F.	Aeration Period at 65°F.
1 Check	-	-	2 weeks	1 week
2 Picfume	70 gal.	2.65 ml.	2 days	2 weeks
3 Telone	16 gal.	0.60 ml.	2 weeks	1 week
Telone	32 gal.	1.21 ml.	2 weeks	1 week
Telone	64 gal.	2.42 ml.	2 weeks	1 week
4 Vidden-D	20 gal.	0.76 ml.	2 weeks	1 week
Vidden-D	40 gal.	1.51 ml.	2 weeks	1 week
Vidden-D	80 gal.	3.02 ml.	2 weeks	1 week
5 Fumazone M-777	300 lbs.	1.37 g.	2 weeks	1 week
Fumazone M-777	600 lbs.	2.74 g.	2 weeks	1 week
Fumazone M-777	1200 lbs.	5.48 g.	2 weeks	1 week
6 N-Serve	4 lbs.	18.16 mg.	(added at beginning of incubation)	

1 - All soils, including the check, were held at field moisture content in sealed plastic bags through the exposure periods shown, after which they were aerated by forced passage of water-saturated air.

2 - Picfume contains 99 per cent active chloropicrin.

3 - Telone contains 90% active dichloropropenes plus 10% related hydrocarbons.

4 - Vidden-D is a mixture of dichloropropenes and 1,2 dichloropropane. Rates used supply the active dichloropropenes in the same quantities as the corresponding rates of Telone.

5 - M-777 is a granular formulation containing 10 percent active 1,2-dibromo-3-chloropropane on 30-60 mesh attaclay. Rates used correspond to 5, 10 and 20 gpa. of Fumazone 70E, the liquid formulation containing 8.6 lbs. active ingredient per gallon.

6 - Active 2-chloro-6-(trichloromethyl) pyridine supplied at a rate equal to 8% of the N added as $(NH_4)_2 SO_4$ to all soils (50 lbs. N per acre).

Incubation Procedure

In this experiment, the "ventilation" method of incubation was used. Figure 1 shows a single respiration unit and Figure 2 shows the arrangement of the apparatus in one of the constant temperature chambers. Ten-gram samples (oven dry basis) of moist soil, after exposure to chemicals and after aeration, were dispensed in 60 ml. plastic cups. Groups of 15 were placed in large respirometer jars. Duplicate jars were set up for each treatment.

Incubation was carried out at 10°, 20°, and 30° C., beginning July 12, 1961. Due to mechanical failure, the temperature in the 10° room was raised to 23°C. on October 3, and in the 20° room, to 24°C. on October 14.

The rate of CO_2 evolution was estimated every third day. At periodic intervals, subsamples of soil were removed from each respirometer jar for the determination of ammonium and nitrate and for the estimation of microbial numbers.

Collection of Carbon Dioxide

Carbon dioxide evolved from the incubating soil was swept out of the respirometer jars by a current of moist, carbon dioxide-free air. The incoming air was cleaned by passing through a series of absorbents consisting of a carboy of 4N sodium hydroxide to remove CO_2, followed by a carboy of concentrated sulfuric acid to remove ammonia. An absorption tower of granular zinc removed sulfuric acid from the air stream. From this point the air moved into each constant temperature room. The air was remoistened by passing through a column of water which served also as a manostat. It was distributed through manifolds into the appropriate jars and subsequently bubbled through tubes containing sodium

Figure 1. - A single respiration unit showing: A, CO_2 - free-air inlet tube; B, respirometer jar containing soil samples in plastic cups; C, tube containing KI for removal of halogen impurities from the air flowing through the respirometer jar; E, tube containing Ag_2SO_4 to indicate when to renew KI; D, tube containing NaOH for collection of CO_2.

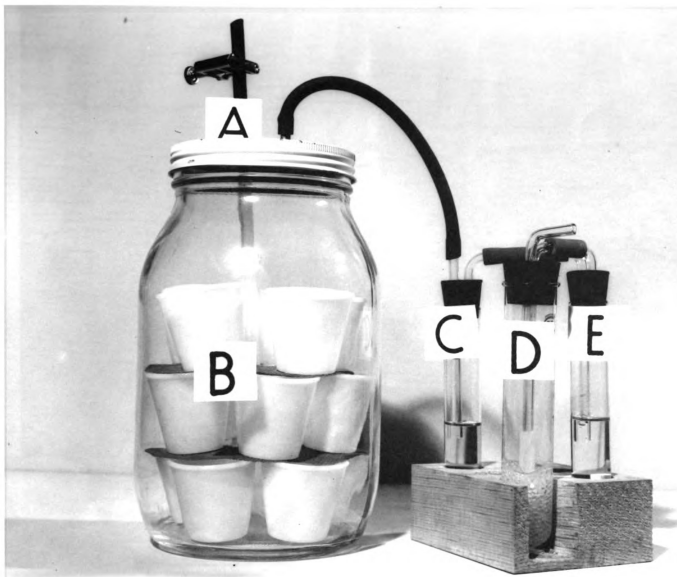


Figure 1.

Figure 2. - View of operator adjusting the incubation apparatus in one of the constant temperature rooms.

hydroxide to collect the evolved carbon dioxide. Prior to passing into the sodium hydroxide, halogens were removed by passing through a tube of potassium iodide solution. A tube of silver sulfate served as an indicator to show when the potassium iodide had been spent.

There were 24 respirometer jars in each room, each treatment being replicated twice at each temperature. Carbon dioxide evolution was determined for a 24-hour period every third day, using one replication only. The other replication in each room was aerated for two hours prior to switching the air current to the replication used for collecting carbon dioxide.

Carbon dioxide in the incoming air stream was monitored by passing through a blank tube of sodium hydroxide. The titration value for this blank was used directly to calculate the initial normality of the sodium hydroxide in the tubes used for collecting carbon dioxide from the corresponding respirometer jars. Unused sodium hydroxide was titrated against standard sulfuric acid in the presence of phenolpthalein and excess barium chloride. Carbon dioxide absorbed was calculated by difference and is reported as mgm. carbon per 100 gm. soil (oven dry basis) per day.

Laboratory Assays

Determination of Ammonium and Nitrate

The microdiffusion method described by Bremner and Shaw (7) was used for determination of ammonium and nitrate in both field and laboratory studies. The method is a modification of the procedure developed by Conway (11). A 1:10 extracting ratio (moist soil basis) was used, with a 30-minute extracting period (on a shaker). The extracting solution was 1 N potassium sulfate in 0.1 N sulfuric acid, having a pH between 1 and 2.

Aliquots of the extract were placed in plastic microdiffusion units. Ammonia was distilled off at room temperature in the presence of MgO and collected in boric acid in the center well of the microdiffusion unit. In separate units, nitrate was reduced to ammonia by titanous sulfate in the presence of magnesium oxide. In both units ammonia collected in the boric acid was determined by direct titration with .005 N sulfuric acid. Ammonium in the extracts was calculated directly, nitrate by difference. Ammonium and nitrate in the soil were calculated to ppm., oven dry basis.

Determination of Chlorine in Celery Tissue

The method described by Piper (54) was used for dry-ashing of the plant material for the determination of chlorine in celery tissue in 1960. Chlorine is readily lost during the ordinary ashing of many plant materials. For retaining it in the ash the sample must be ignited in the presence of an alkali. Chlorine-free lime was used in this case.

After ashing, the residue was dissolved, filtered, and brought to volume as described by Husband and Godden (27).

A microdiffusion procedure described by Conway (11) was used to determine the chlorine in the final extracting solution. The Öbrink Modified Conway Unit as developed by Öbrink (53) was employed in the determination.

The essentials of the method are as follows:

Chloride in the extracting solution is oxidized to chlorine by an oxidizing agent such as potassium permanganate. The chlorine volatilizes from the acid extract and is absorbed by a solution of potassium iodide in the center well of the microdiffusion unit. The iodide is oxidized and free iodine produced. The iodine is then titrated using sodium

thiosulfate with starch as the indicator.

Electrical Conductance Measurements

Electrical conductance of the soil solution in samples taken June 20, 1960 was measured in 1:2 (weight/volume) suspensions, using a Wheatstone bridge (Solu-Bridge). The general procedure followed is outlined by Jackson (28). Measured conductances were calculated to field moisture basis as recommended by Geraldson (19).

Dilution Plate Counts of Bacteria and Fungi

The soil dilution and plate count methods outlined by Johnson et al (30) were used for enumerating the soil microbial population. Thornton's standardized medium was used for estimating numbers of bacteria plus Streptomyces spp. and Martin's peptone dextrose medium was used for estimating fungal numbers.

Dilutions of 1/10, 1/T, 1/10T, 1/100T, and 1/M were made. For bacteria one milliliter of the 1/10T, 1/100T, and 1/M dilution was placed aseptically in each of two disposable petri dishes. For fungi, one milliliter of the 1/T, 1/10T, and 1/100T was used. Twelve to 15 milliliters of the appropriate agar medium, cooled to just above solidifying, were added to each dish. The dishes were rotated by hand in a broad swirling motion. The plates were incubated in the dark at room temperature (23°C.) for 4-6 days for fungi and 5-7 days for bacteria.

A magnifying colony viewer was used for counting. Data was recorded only for those dilution plates containing 20 to 200 colonies per plate. In the case of bacteria, usually these were the 1/100T and 1/M dilution plates. For fungi, the usual dilution plates used for counting were those of the 1/T and 1/10T dilutions.

RESULTS OF FIELD STUDIES

Fumigation Effects on Soil Nitrogen Transformations

Uncropped Soil

Fall fumigation with Telone markedly interfered with nitrification in the field through the middle of June. The seasonal pattern of interference can best be seen in data for uncropped plots where crop uptake was not a factor (Figs. 3 and 4). The difference in levels of ammonium and nitrate in fumigated and unfumigated soil was less in a cool season, 1960, than in the other two years. Soil temperatures during 1960 remained in the 50's and low 60's through May.

Recovery of nitrifying capacity after about June 20 was rapid following the strongly retarding effect of fumigant in 1959 and 1961. This recovery may have commenced about one week earlier at the lower rate of fumigant used in 1961.

Cropped Soil

Although nitrification was retarded in fall fumigated soil, it was not completely suppressed. A slow accumulation of nitrate was apparent by early May. This may be observed in data for cropped plots in Fig. 5. The greatly accelerated nitrification rates observed in uncropped plots after June 20 were not so apparent here since the celery was removing nitrate rapidly. However, the rapid rate of conversion to nitrate may be inferred from the fact that nitrate levels in fumigated soil were equal to or greater than in unfumigated soil during late July, a period of maximum growth and nitrogen requirement by celery.

During the period of retarded nitrification, ammonium accumulated in fumigated soil, reaching seasonally maximum levels in late May or early June. Where 50 pounds per acre of ammonium nitrogen (100 ppm)

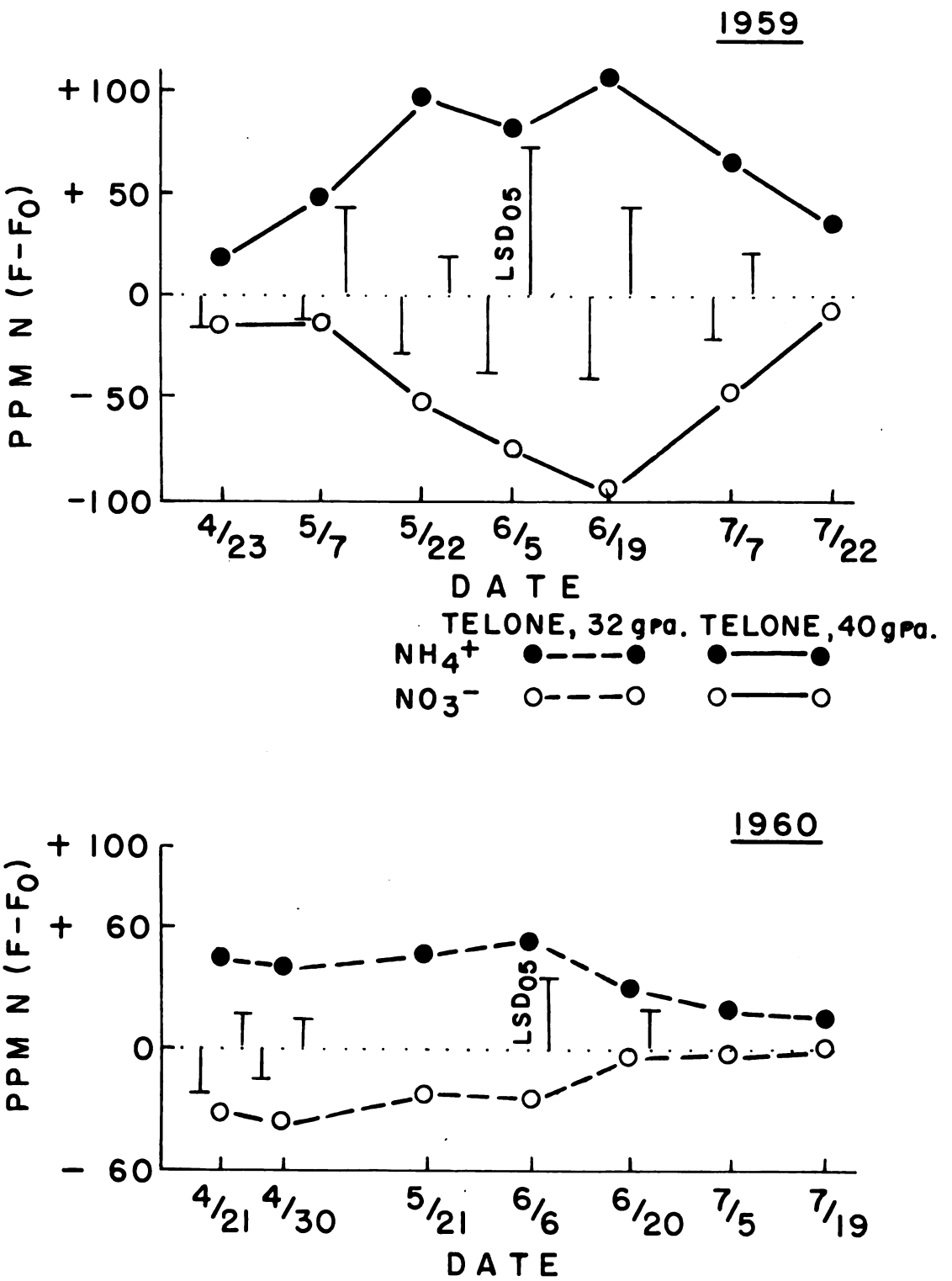
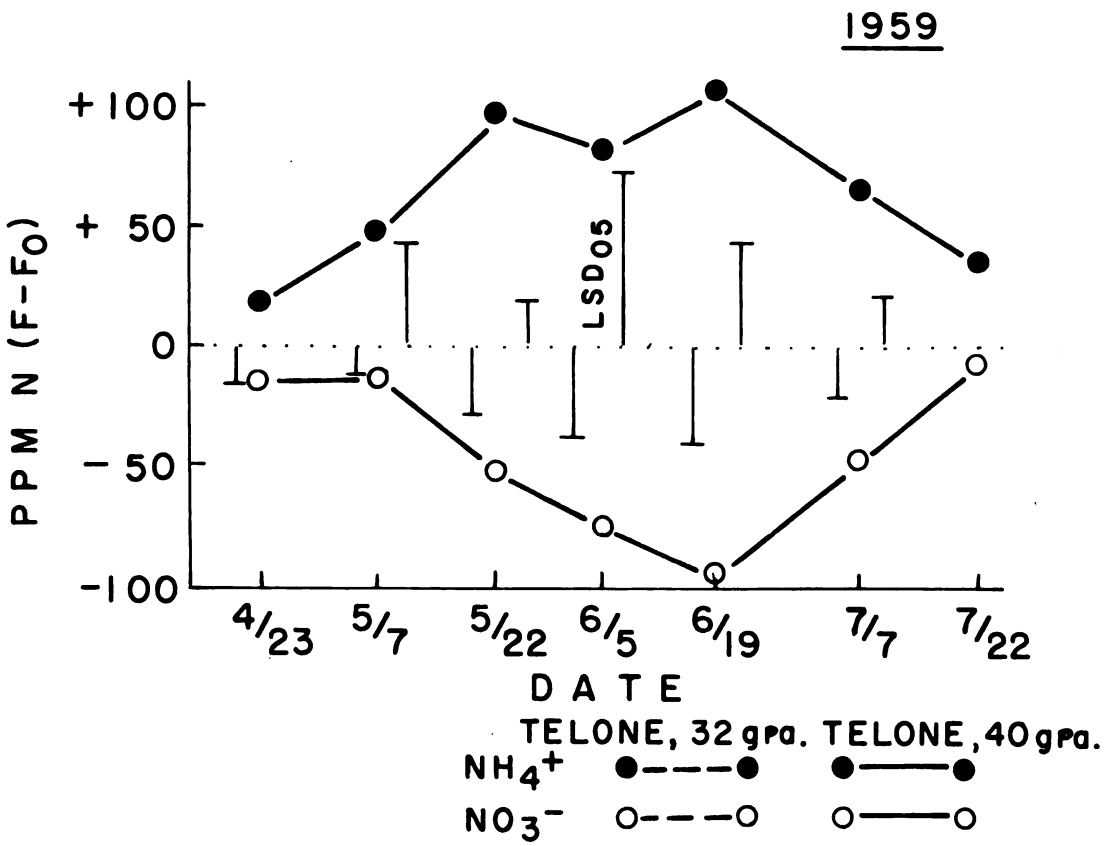


Figure 3. - Effects of fall fumigation with Telone on accumulation of ammonium and nitrate nitrogen in uncropped Houghton muck in 1959 and 1960. ($F - F_o$ = fumigated minus unfumigated.)



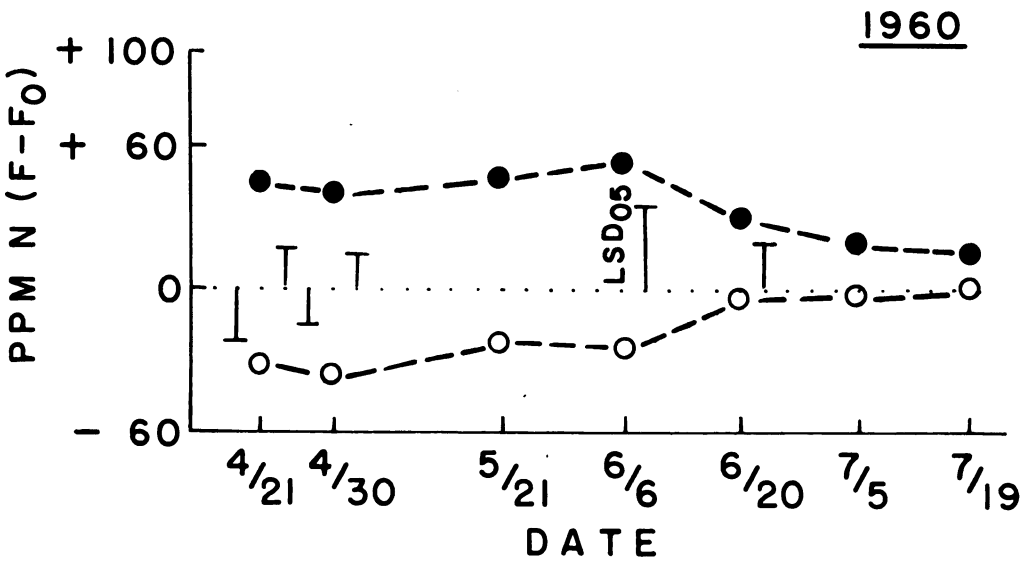


Figure 3. - Effects of fall fumigation with Telone on accumulation of ammonium and nitrate nitrogen in uncropped Houghton muck in 1959 and 1960. ($F - F_o$ = fumigated minus unfumigated.)

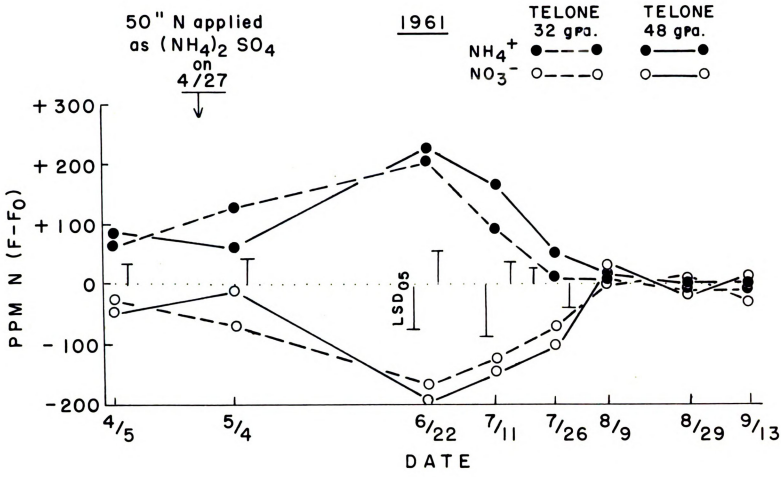


Figure 4. - Effects of fall fumigation with Telone on accumulation of ammonium and nitrate nitrogen in uncropped Houghton muck in 1961. ($F - F_o$ = fumigated minus unfumigated.)

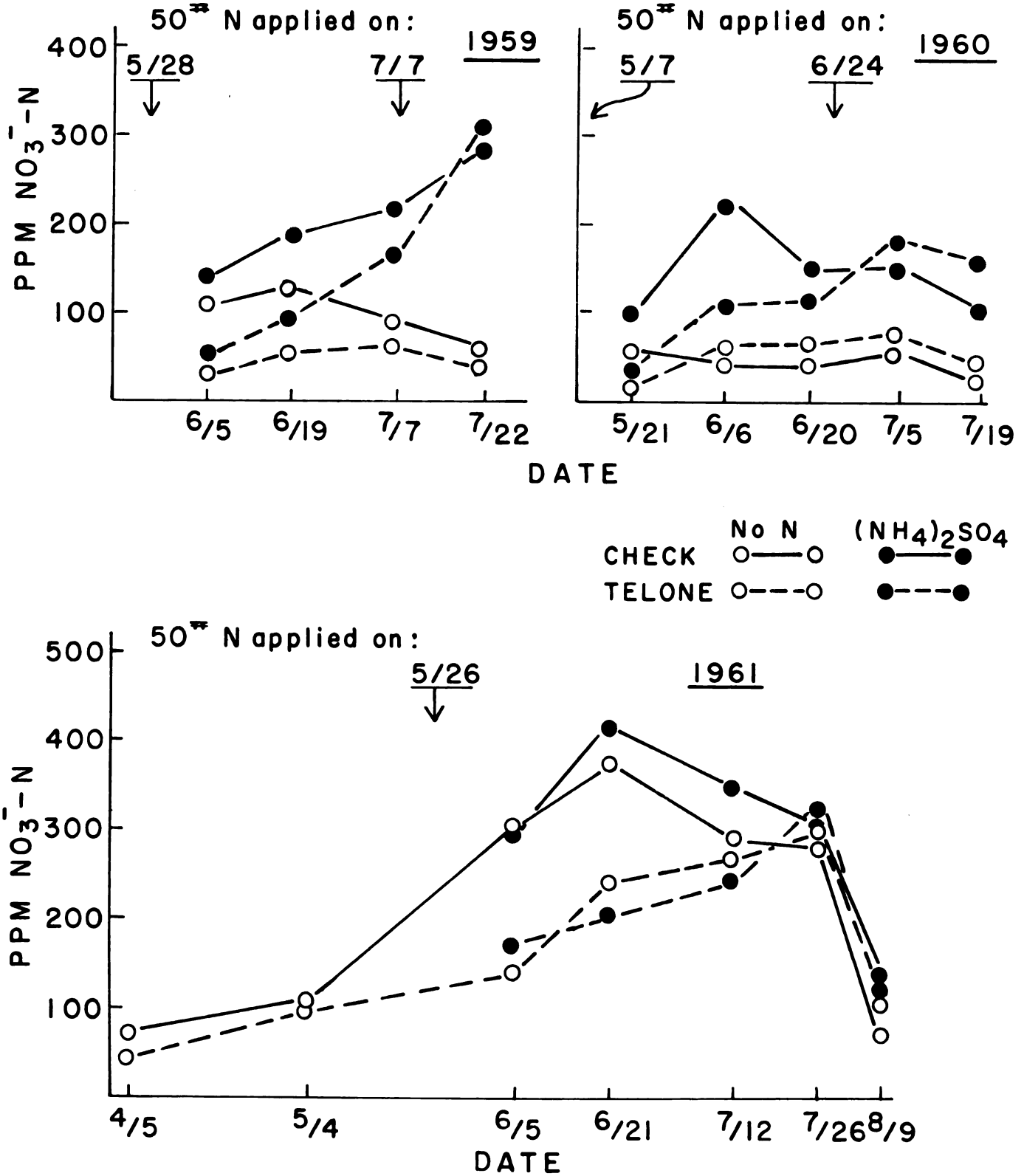


Figure 5. - Levels of nitrate nitrogen in fall fumigated and unfumigated Houghton muck planted to celery with and without supplemental ammonium fertilization.

had been side dressed previously, 250 to 300 ppm of ammonium nitrogen was found in the soil at this time. In 1959 and 1960, the ratio of ammonium to nitrate was 6 to 1 in fumigated soil which had received ammonium sulfate, as compared with ratios less than 1 in unfumigated soil without ammonium fertilizer. The maximum ratio found in 1961 was 2 to 1. (Fig. 6)

In addition to the retarding effect on nitrification, there was evidence that the fumigant exerted a partial sterilization effect which gave rise to heightened microbial activity and a greater release of inorganic nitrogen from soil organic matter (Fig. 7). The sum of ammonium and nitrate recovered was frequently significantly higher in fumigated than unfumigated soil. This was particularly true during the early part of the season, although significant increases for fumigation were also observed during later periods of high temperatures.

Interactions of Fumigation and Nitrogen Carriers

The effect of fall fumigation with Telone on nitrogen transformations in the soil was markedly influenced by the fertilizer form of supplemental nitrogen used. The effects of fumigation on soil nitrate levels, expressed as the difference between fumigated and unfumigated soil (F-Fo), is plotted in Fig. 8 for plots which received no supplemental nitrogen and those sidedressed with ammonium sulfate or calcium nitrate. The suppression of nitrate accumulation by Telone was strikingly and significantly enhanced by ammonium sulfate in the first two samplings in 1960. A similar tendency towards a synergistic suppression of nitrate levels by fumigation and ammonium fertilizer is expressed in 1959 and 1961, although statistical significance was

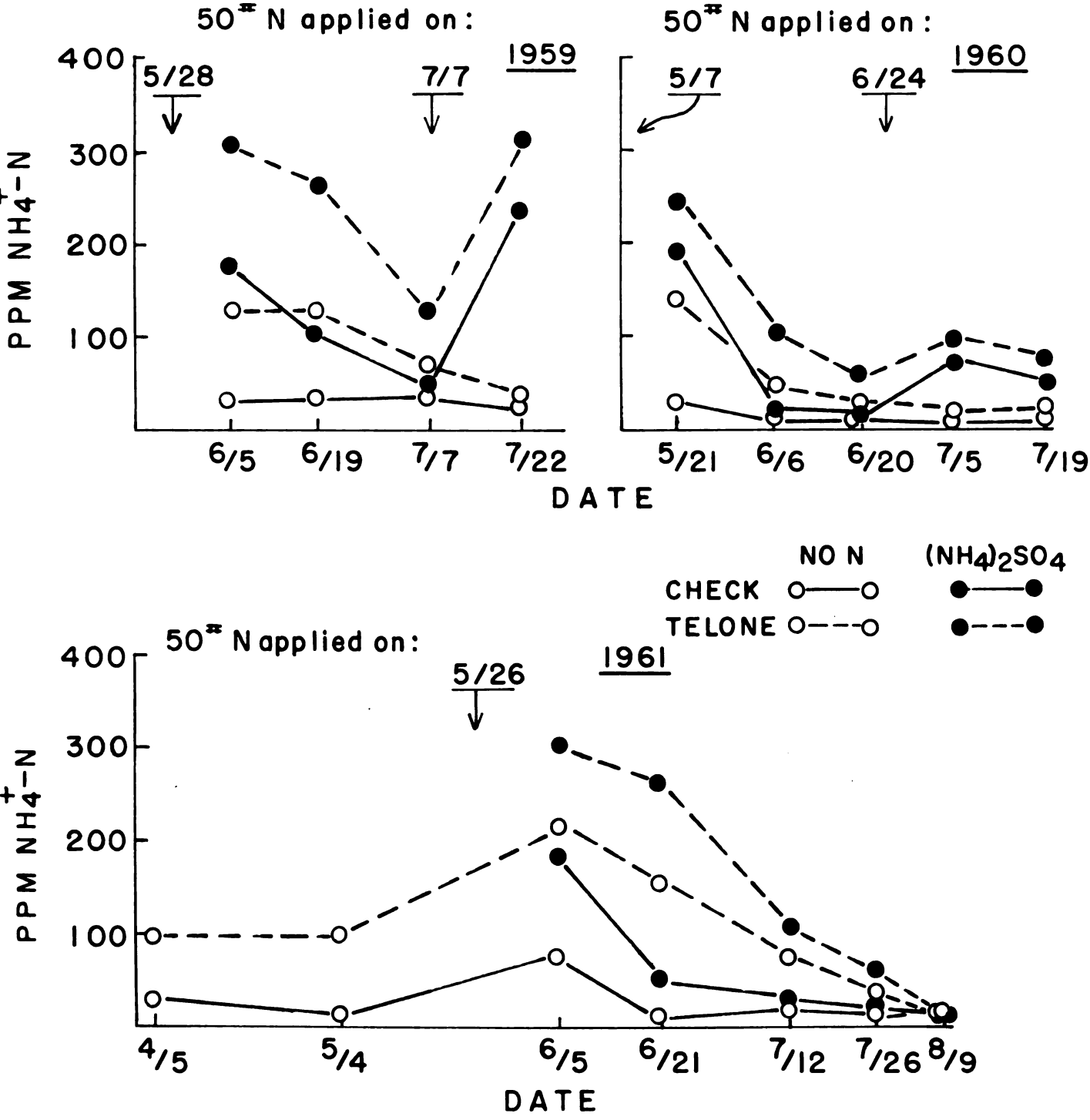


Figure 6. - Levels of ammonium nitrogen in fall fumigated and unfumigated Houghton muck planted to celery with and without supplemental ammonium fertilization. (1959, '60 and '61).

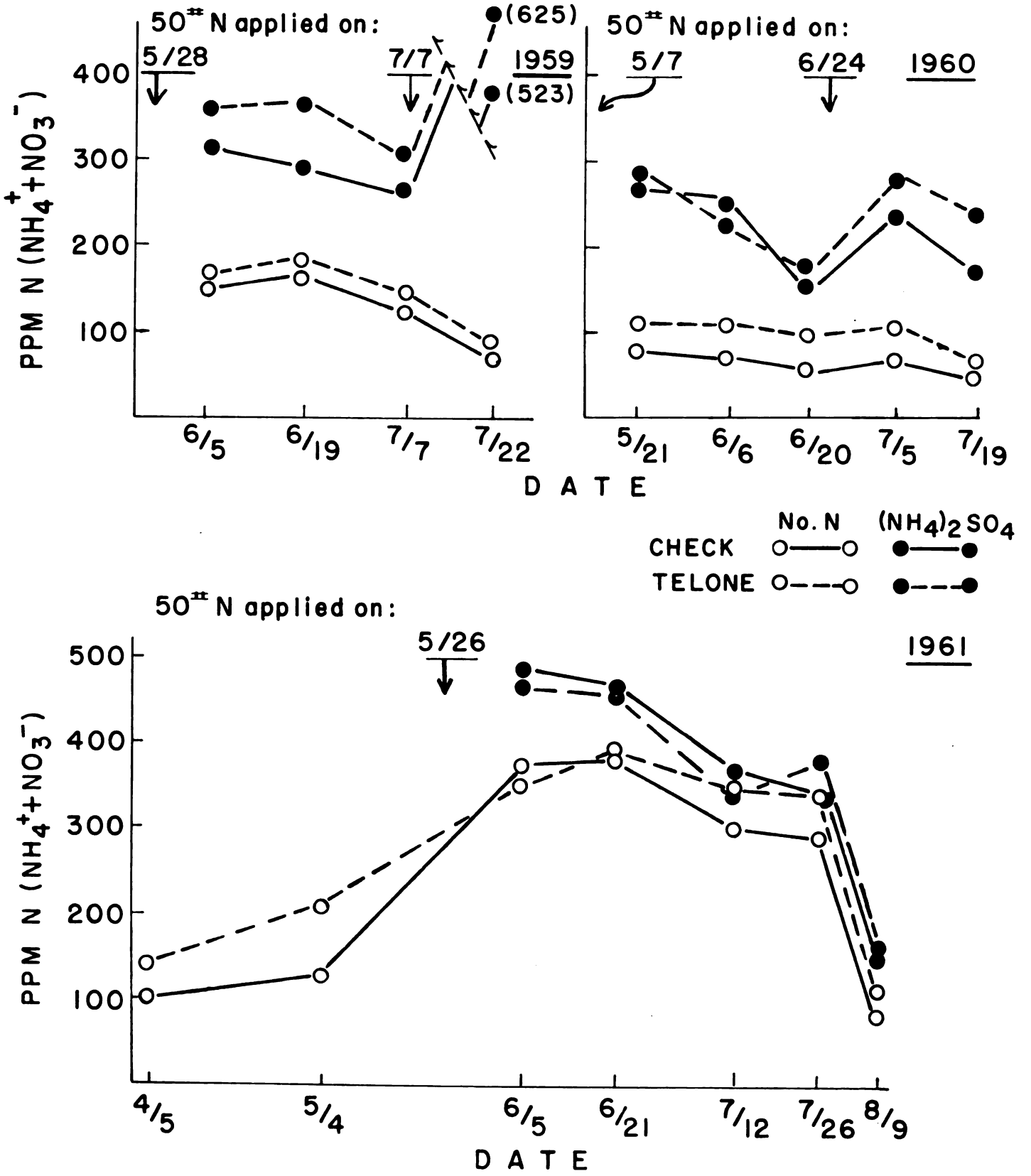


Figure 7. - Levels of total mineral nitrogen in fall fumigated and unfumigated Houghton muck planted to celery with and without supplemental ammonium fertilization.

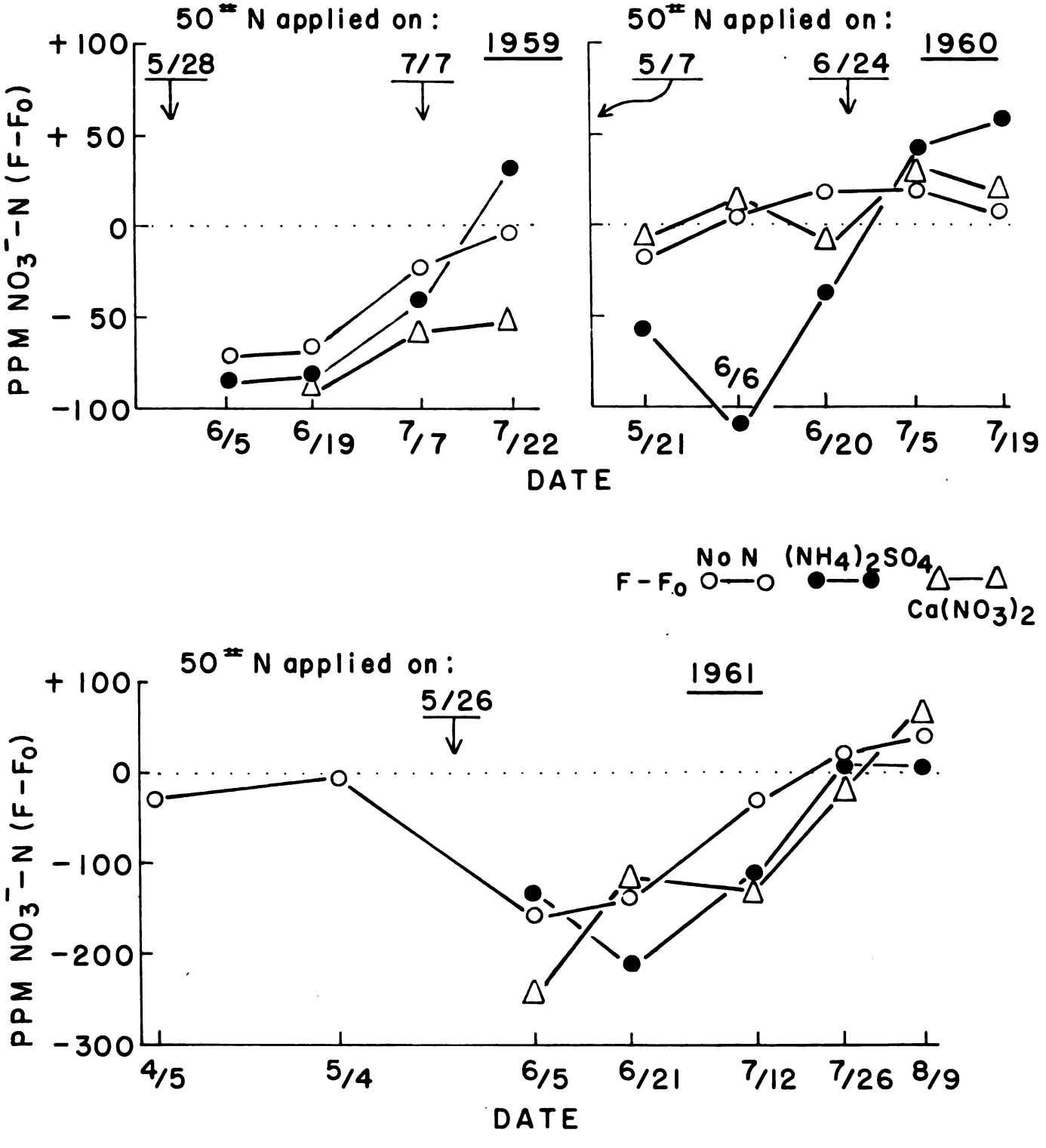


Figure 8. - Effects of fall fumigation with Telone on the accumulation of nitrate as related to source of applied nitrogen. Houghton muck, planted to celery, 1959, '60, '61.
($F - F_o$ = fumigated minus unfumigated.)

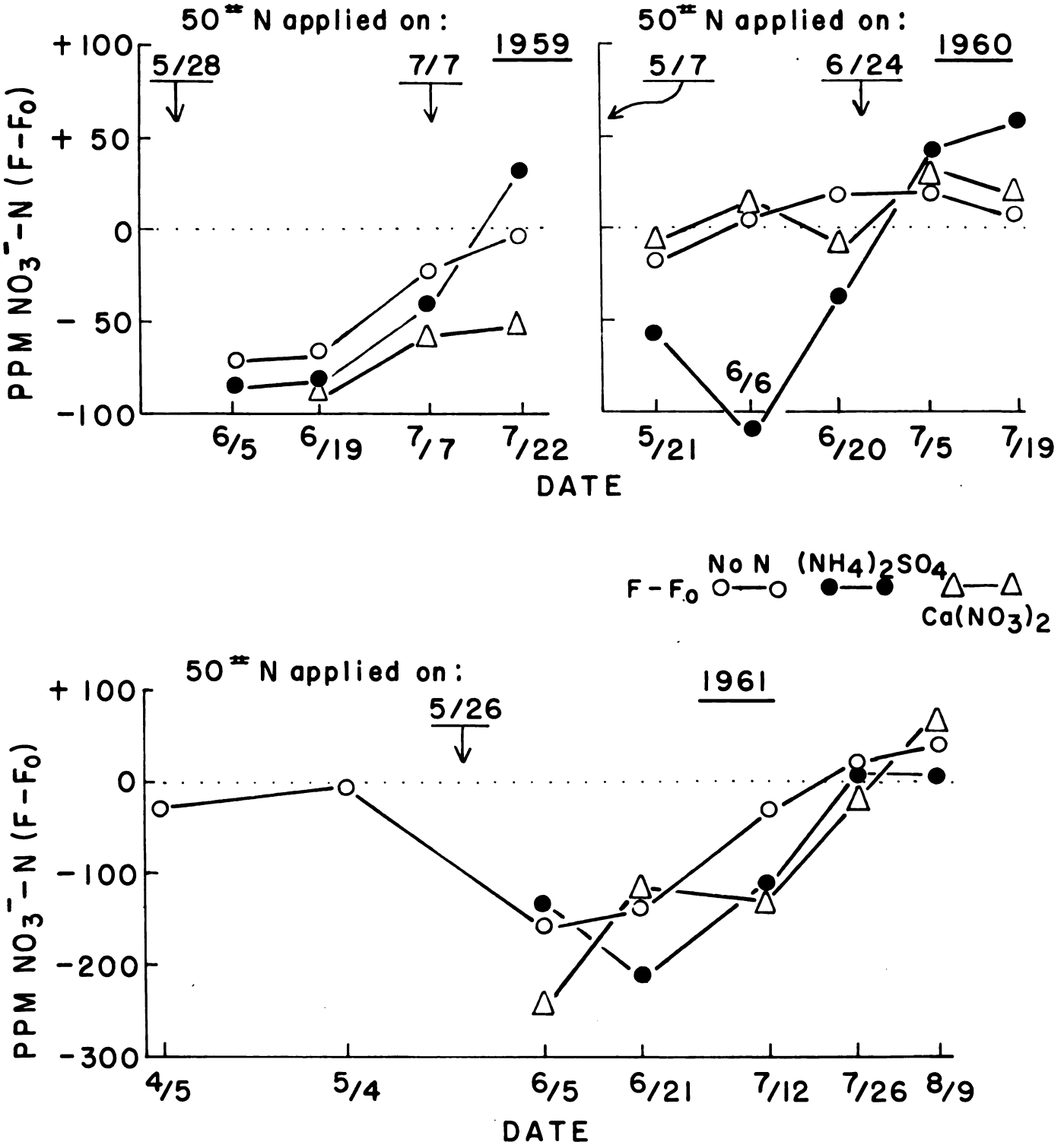


Figure 8. - Effects of fall fumigation with Telone on the accumulation of nitrate as related to source of applied nitrogen. Houghton muck, planted to celery, 1959, '60, '61.
($F - F_o$ = fumigated minus unfumigated.)

not attained. After dissipation of the retarding effect of the fumigant, nitrate accumulation in 1959 and 1960 rose to significantly higher levels in fumigated ammonium sulfate plots than in those which received calcium nitrate or no nitrogen. This happened during a period when nitrate was being removed rapidly by the crop..

The fumigation effect on nitrate levels in plots which received calcium nitrate was erratic. This appeared to be due to differences in rate of removal of nitrate by the celery in the different seasons and at different times during each growing season. However, there was evidence in 1960 and 1961 that periods of rapid nitrate accumulation in fumigated soil receiving calcium nitrate preceded full recovery of nitrifying capacity in those plots which received no nitrogen or received nitrogen as ammonium sulfate.

Since nitrate levels were depressed by fumigation to a greater extent when combined with an ammonium fertilizer source, it would be expected that fumigation would also give rise to greater accumulations of ammonium where this form of nitrogen was used. Such was actually the case, as may be seen in Fig. 9. Differences in fumigation effect between ammonium sulfate, on the one hand, and no nitrogen or calcium nitrate, on the other, were statistically significant during June of 1959 and 1960. The combination of fumigation and ammonium sulfate significantly enhanced net mineralization of soil organic nitrogen through most of the season 1959 and in June of 1960 (Fig. 10). This was not true in 1961.

It is important to note in Fig. 9 that, during 1959 and 1960, the increase in ammonium levels due to fumigation was never as great with calcium nitrate as it was where ammonium sulfate was used. In 1960, the earlier decline in stimulus to ammonification in calcium

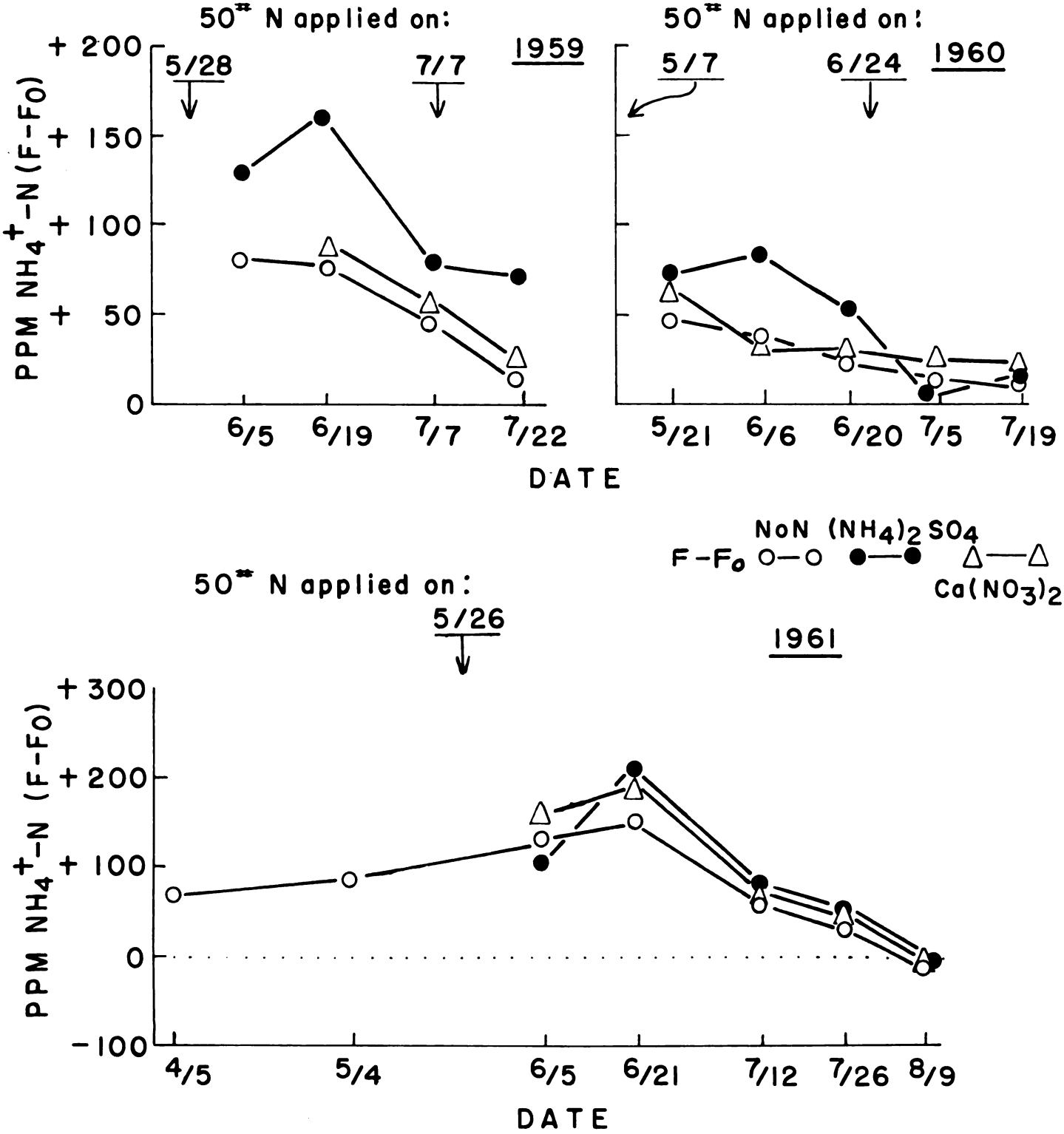


Figure 9. - Effects of fall fumigation with Telone on the accumulation of ammonium as related to source of applied nitrogen. Houghton muck planted to celery, 1959, '60, '61.
(F - F = fumigated minus unfumigated.)
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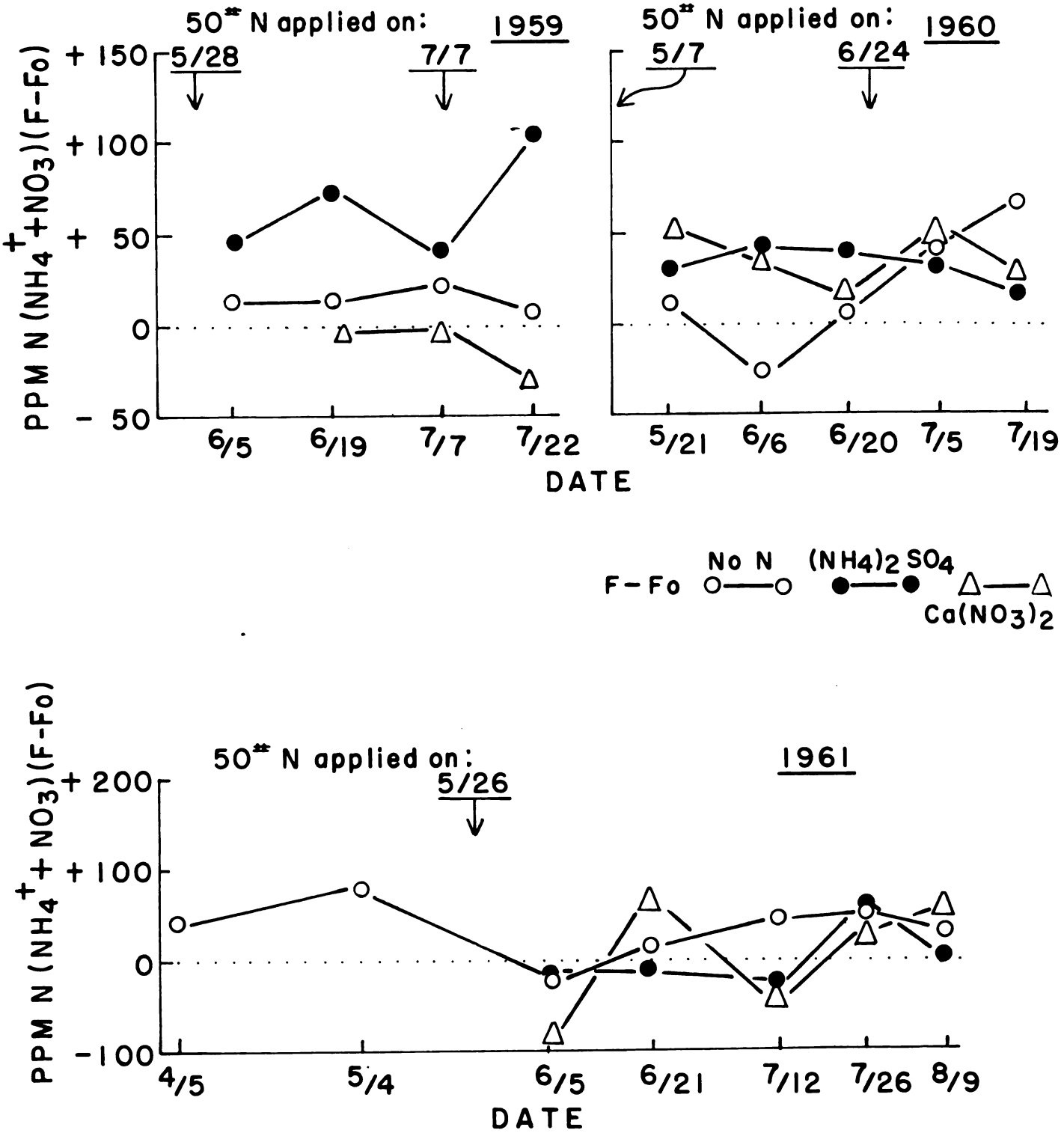

Figure 10. - Effects of fall fumigation with Telone on the level of mineral nitrogen (NH_4^+ plus NO_3^-) as related to source of applied nitrogen. Houghton muck, planted to celery, 1959, '60, '61. ($F - F_o$ = fumigated minus unfumigated.)

nitrate plots is distinctly paralleled by an earlier recovery of nitrifying capacity in the fumigated plots (cf. Fig. 8 and 9).

Effects of the nitrate fertilizer on the fumigation stimulus to mineralization were erratic and generally nonsignificant (Fig. 10). However, a statistically significant synergistic relationship was expressed in the May 21 and June 6 samplings in 1960.

Interactions of Fumigation, Nitrogen Carriers and Tillage

In 1960, one half of the plots in the field fumigation experiment were not disturbed after they were heavily compacted immediately after injection of the Telone in the fall of 1959. The other half of the plots were plowed just before planting celery in the spring. Plowed and unplowed plots, therefore, represented a differential compaction and aeration status which was maintained through the growing season.

Numerous significant first and second order interactions between fumigation, aeration treatment and nitrogen carriers in their effects on the accumulation of ammonium and nitrate were encountered (Tables 2 and 3). The LSD noted for the FxN within A comparison is appropriate for comparing two differences (F-Fo) in the data plotted in Fig. 11.

Where no supplemental nitrogen was applied, there was no effect of the plowing on the extent to which ammonium accumulated after fumigation (Fig. 11-A). The accumulation of nitrate was retarded to a greater extent by the fumigant in unplowed soil (Fig. 11-B).

By contrast, in the May 21 sampling, the accumulation of ammonium was dramatically enhanced in unplowed soil by calcium nitrate (Fig. 11-A) and by ammonium nitrate (Fig. 11-C). Concurrently, a marked disappearance of the nitrate added on May 7 was noted in the fumigated, unplowed plots. This appears in Figs. 11-B and 11-D as a

Table 2. - Nitrate nitrogen in Houghton muck planted to celery as related to aeration, fumigation and nitrogen carriers in 1960

Aeration treatment[1]	Fumigation treatment[2]	Nitrogen carriers[3]	PPM NO_3 - N on May 21	June 6	June 20	July 5	July 19
A	F	$(NH_4)_2SO_4$	54	123	104	182	174
		NH_4NO_3	159	164	118	236	154
		$Ca(NO_3)_2$	228	172	108	195	160
		NO N	38	64	74	96	52
A	F_o	$(NH_4)_2SO_4$	98	220	125	138	90
		NH_4NO_3	138	223	114	208	140
		$Ca(NO_3)_2$	134	232	131	148	187
		NO N	48	55	38	82	44
A_o	F	$(NH_4)_2SO_4$	27	109	121	190	146
		NH_4NO_3	82	208	153	304	160
		$Ca(NO_3)_2$	120	230	146	247	218
		NO N	29	59	58	66	42
A_o	F_o	$(NH_4)_2SO_4$	92	240	151	170	120
		NH_4NO_3	140	225	84	216	84
		$Ca(NO_3)_2$	230	154	146	232	154
		NO N	54	59	56	41	40
LSD .05 (A within FN)			90	NS	NS	NS	NS
LSD .05 (F within AN)			90	89	63	NS	56
LSD .05 (N within AF)			94	79	53	103	56
LSD .05 (F N within A)			93	NS	60	NS	56

1 - All plots compacted after fumigation on October 26, 1959.
A_o = Soil undisturbed except for planting operations in 1960.
A = Plowed May 2, 1960, prior to planting celery on May 5.

2 - F_o = no fumigant. F = 32 gpa Telone.

3 - 50 pounds per acre N (100 ppm) applied May 7, 1960, and again on June 24.

Table 3. - Ammonium nitrogen in Houghton muck planted to celery as related to aeration, fumigation and nitrogen carriers in 1960.

Aeration treatment[1]	Fumigation treatment[2]	Nitrogen carriers[3]	PPM NH_4 - N on May 21	June 6	June 20	July 5	July 19
A	F	$(NH_4)_2SO_4$	290	131	56	80	100
		NH_4NO_3	188	83	34	61	40
		$Ca(NO_3)_2$	93	49	38	33	20
		NO N	76	52	38	26	19
A	F_o	$(NH_4)_2SO_4$	193	26	5	43	47
		NH_4NO_3	95	33	9	39	28
		$Ca(NO_3)_2$	112	22	7	12	14
		NO N	43	12	14	13	15
A_o	F	$(NH_4)_2SO_4$	221	82	54	120	59
		NH_4NO_3	227	86	46	94	48
		$Ca(NO_3)_2$	158	48	42	44	23
		NO N	76	42	24	25	26
A_o	F_o	$(NH_4)_2SO_4$	187	17	5	136	83
		NH_4NO_3	53	10	22	85	22
		$Ca(NO_3)_2$	14	6	15	21	10
		NO N	16	8	7	11	13
LSD .05 (A within FN)			NS	NS	NS	45	38
LSD .05 (F within AN)			94	37	22	NS	31
LSD .05 (N within AF)			95	37	22	45	29
LSD .05 (FXN within A)			96	38	22	38	31

1 - All plots compacted after fumigation on October 26, 1959.
A_o = Soil undisturbed except for planting operations in 1960.
A = Plowed May 2, 1960, prior to planting celery on May 5.

2 - F_o = no fumigant. F = 32 gpa Telone.

3 - 50 pounds per acre N (100 ppm) applied May 7, 1960, and again on June 24.

markedly enhanced suppression of nitrate on May 21 associated with the two nitrate carriers in unplowed soil. Apparently the nature of the recovery population in fumigated soil was such as to effect direct reduction of nitrate to ammonium in the poorly aerated environment represented by compacted, unplowed soil at high moisture content. Nitrate had completely disappeared on May 21 in all fumigated, unplowed plots in one replication which happened to fall on a slight depressional area which had not drained as quickly as the rest of the plots.

In the 17 days immediately following this marked disappearance of nitrate, it appeared that essentially complete recovery of nitrifying capacity had been achieved in unplowed plots which had received calcium nitrate or ammonium nitrate. Nitrate accumulated at rates of 45 to 50 ppm per week. This compared with rates of 50 to 60 ppm per week in unfumigated plots which had received ammonium sulfate and in which maximum rates were expressed during this period. These rates exceeded those in unplowed, unfumigated plots and appear in Figs. 11-B and 11-D as marked differential accumulations of nitrate on June 6. This dramatic recovery of nitrifying capacity was reflected also in the very rapid differential disappearance of ammonium in unplowed, fumigated plots when nitrate was added (cf. Figs. 11-A and 11-D).

During this same period, differentially enhanced crop removal or microbial immobilization in fumigated, aerated plots resulted in a marked differential disappearance of nitrate, similar to that expressed in all plots which received ammonium sulfate. This differential nitrate disappearance, as plotted in Figs. 11-B and 11-D, leads to an erroneous inference regarding the onset of rapid nitrification in plowed soil following application of the two nitrate carriers. Large

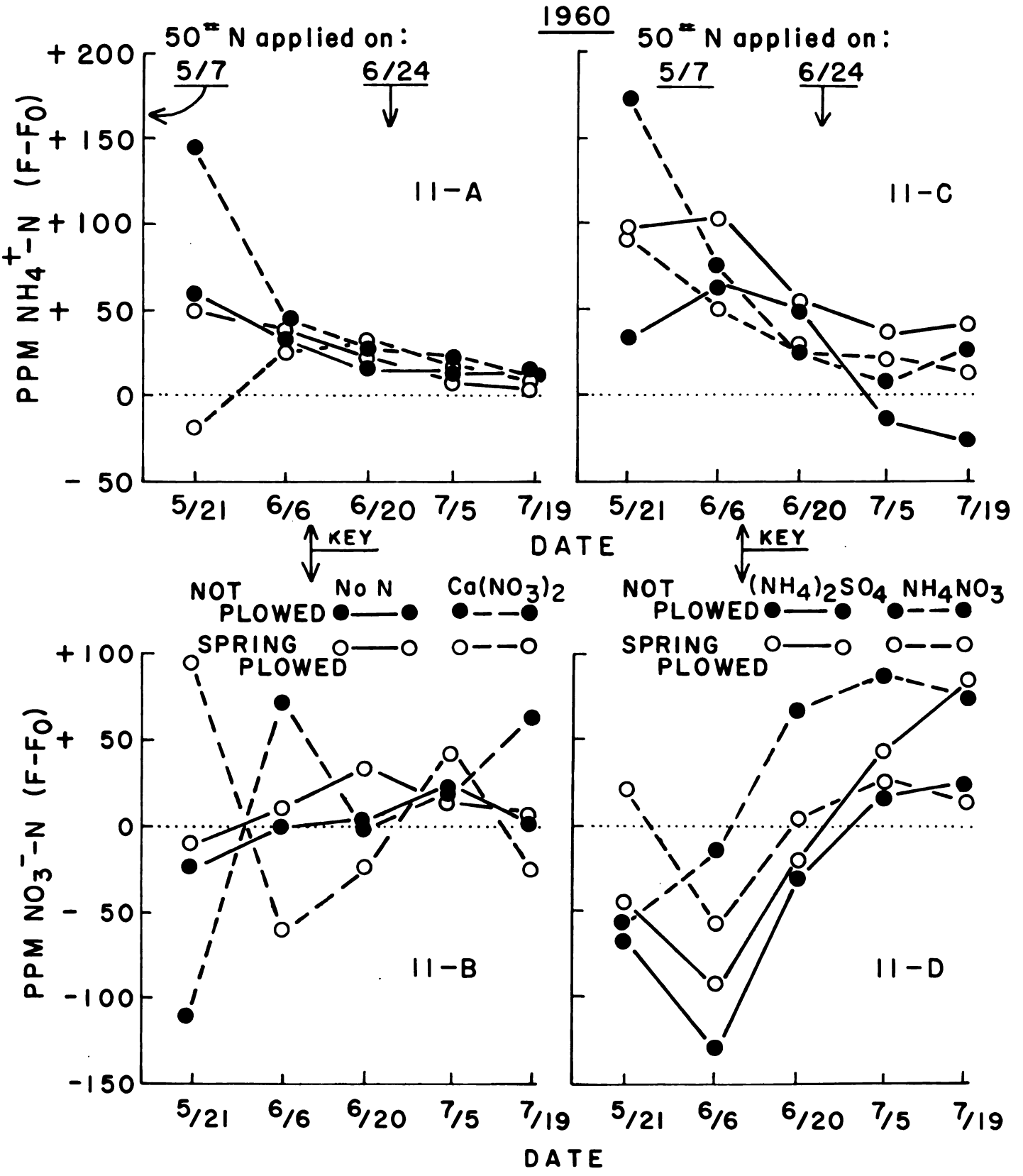

Figure 11. - Effects of Telone fumigation on accumulation of ammonium nitrogen and nitrate nitrogen in Houghton muck as influenced by source of added nitrogen and aeration treatment. ($F - F_o$ = fumigated minus unfumigated).

accumulations of nitrate were observed already on May 21 in fumigated, aerated plots which had received these two materials (Table 2). Thus, it may be inferred that plowing and the use of nitrate containing fertilizer maximally reduced the period of retarded nitrification resulting from fumigation. Rapid nitrification apparently commenced in plowed and fumigated plots receiving fertilizer nitrate even earlier than when these materials were applied on plowed plots that had not been fumigated (Table 2).

Totals of mineral nitrogen (NH_4^+ plus NO_3^-) are shown in Table 4. The effects of nitrogen carriers and plowing on the basic mineralization response to fumigation are depicted in Fig. 12. In unplowed soil, fumigation tended to depress the level of mineral nitrogen early, leaving it unchanged at the end of the season, in plots which received ammonium sulfate (Fig. 12-B); in plots fertilized with ammonium nitrate, fumigation greatly enhanced net mineralization all through the season. Plowing resulted in a marked reduction of the fumigation - enhanced mineralization in plots which received ammonium sulfate. Results with calcium nitrate (Fig. 12-A) were erratic, but the relative effect of plowing on June 6 and July 19 was the same as that observed where ammonium nitrate was used.

There was a tendency during June for behavior in plots not supplemented with nitrogen (Fig. 12-A) to parallel that in ammonium sulfate plots (Fig. 12-B).

Summary of Fumigation Effects

The interactions described are complex. They suggest that the nature of the recovery population following partial sterilization of soil is strongly influenced by environmental conditions imposed during

Table 4. - Total mineral nitrogen in Houghton muck planted to celery as related to aeration fumigation and nitrogen carriers in 1960.

Aeration treatment[1]	Fumigation treatment[2]	Nitrogen carriers[3]	PPM NH_4^+ - N + NO_3^- - N on May 21	June 6	June 20	July 5	July 19
A	F	$(NH_4)_2SO_4$	344	254	168	261	274
		NH_4NO_3	348	248	152	297	194
		$Ca(NO_3)_2$	320	220	146	228	180
		NO N	113	116	112	122	71
A	F_o	$(NH_4)_2SO_4$	291	246	130	181	137
		NH_4NO_3	233	256	122	247	169
		$Ca(NO_3)_2$	246	254	138	160	201
		NO N	91	67	52	95	57
A_o	F	$(NH_4)_2SO_4$	248	192	175	311	205
		NH_4NO_3	309	294	224	398	208
		$Ca(NO_3)_2$	278	278	188	291	241
		NO N	104	101	83	91	68
A_o	F_o	$(NH_4)_2SO_4$	279	251	181	306	202
		NH_4NO_3	193	235	105	301	106
		$Ca(NO_3)_2$	244	161	161	253	164
		NO N	70	67	63	52	52
LSD .05 (A within FN)			NS	NS	62	NS	NS
LSD .05 (F within AN)			76	NS	58	NS	72
LSD .05 (N within AF)			76	95	47	126	72
LSD .05 (FXN within A)			76	96	52	NS	72

1 - All plots compacted after fumigation on October 26, 1959.
A_o = Soil undisturbed except for planting operations in 1960.
A^o = Plowed May 2, 1960, prior to planting celery on May 5.

2 - F_o = no fumigant. F = 32 gpa Telone.

3 - 50 pounds per acre N (100 ppm) applied May 7, 1960, and again on June 24.

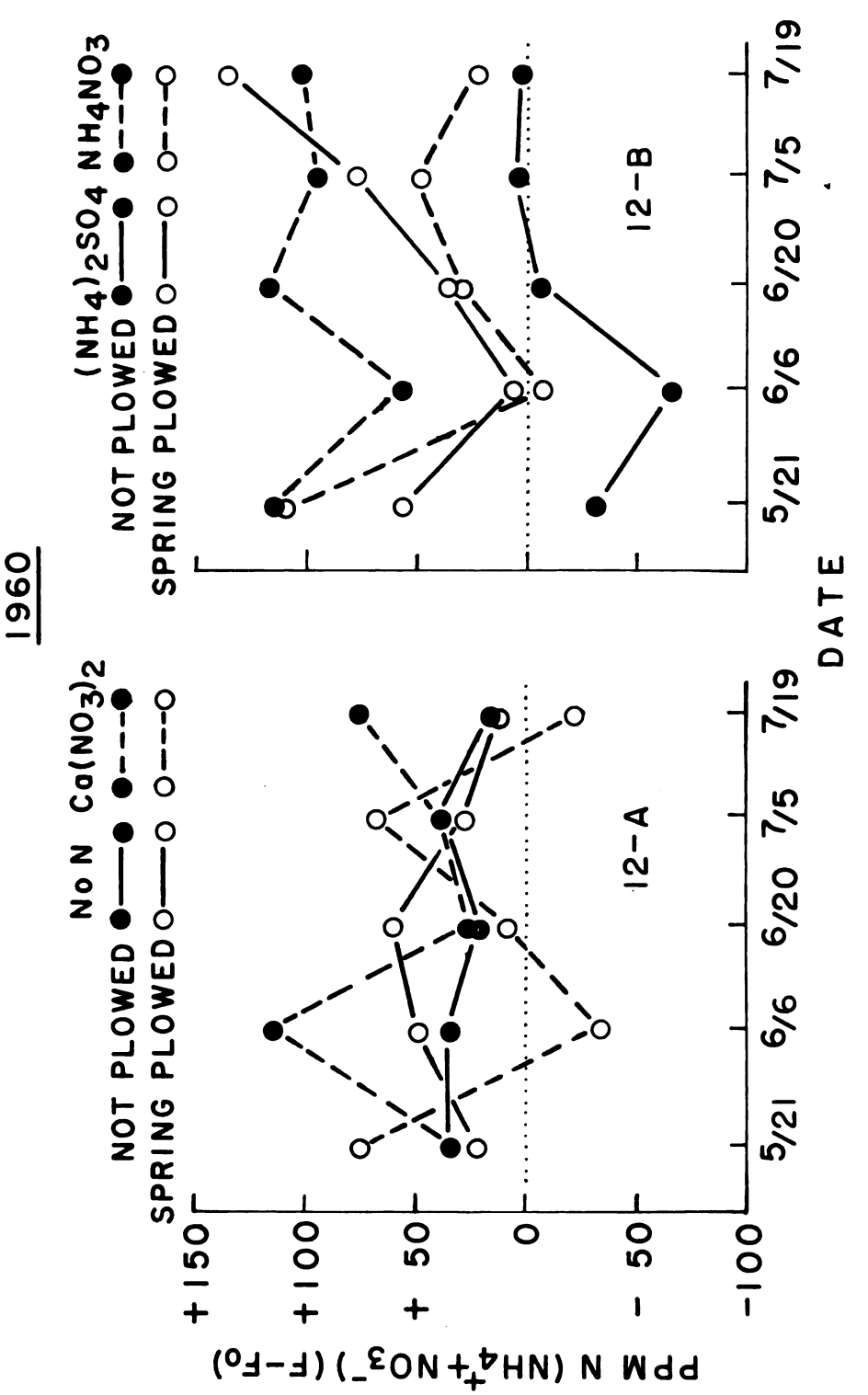

Figure 12. - Effects of Telone fumigation on accumulation of total mineral nitrogen in Houghton muck as influenced by source of added nitrogen and aeration treatment. ($F - F_o$ = fumigated minus unfumigated.)

the recovery period. Aeration and the form of fertilizer applied are potent factors in this environment. The synergistic effect of ammonium fertilizer on the retardation of nitrification by Telone may be related in some manner to the known sensitivity of Nitrobacter to ammonia, - a sensitivity, however, which is usually expressed only at alkaline pH. The marked benefit to the nitrification process from application of nitrate fertilizer appears to have no basis in what is known of the physiology of the nitrifiers.

Practical conclusions to be drawn from the three years' observations may be summarized as follows:

1. Fall fumigation with Telone at recommended rates retarded nitrification in organic soil through the middle of June.
2. Nitrification was not completely suppressed. Nitrate accumulated more slowly than in unfumigated soil until about June 20, after which the rate increased dramatically and exceeded that in unfumigated soil.
3. The use of fertilizer containing only ammonium nitrogen enhanced the inhibitory effect of the fumigant and prolonged the delay period one to two weeks. Impaired soil aeration enhanced these affects of ammonium nitrogen.
4. The use of fertilizer nitrogen in the nitrate form greatly reduced the period of restricted nitrification. Near normal accumulation of nitrate was observed by the first of June in compacted soil, and probably one or two weeks earlier in plowed soil. Ammonium nitrate was about as effective as calcium nitrate supplying twice as much nitrate.
5. During the period of retarded nitrification, ammonium

accumulated, reaching peak levels of 200 to 350 ppm. late in May where ammonium fertilizers had been used. Ammonium to nitrate ratios at this time were about 6 to 1, in contrast to ratios less than unity in unfumigated soil.

6. Rates of application ranging from the recommended 32 gallons per acre up to 48 did not materially intensify the observed affects of the fumigant. Recovery of nitrifying capacity may have been delayed about a week at the higher rates.

Field Studies With N-Serve

The nitrification inhibitor, N-Serve, was used in 1961 in unfumigated soil and soil fumigated with 32 and 48 gpa of Telone. These plots were not cropped.

Quantities of nitrate found in the soil through the season are tabulated in Table 5. The fumigation treatment depressed nitrate levels through July, although the effect was statistically significant only in June and July. There were no significant differences between the two rates of Telone used. Marked decreases in nitrate during August were due to removal by leaching. Six inches of rainfall were recorded between July 26 and August 29.

Fifty pounds per acre (100 ppm) of nitrogen was applied as ammonium sulfate on April 27, with and without the addition of 1.6 percent N-Serve. A significant suppression of nitrate by the N-Serve was observed only on June 22. This effect was expressed at all three fumigation levels and was strictly additive, there was no evidence of

Table 5. - The effects of Telone and nitrogen treatments with ammonium sulfate and ammonium sulfate + 1.6% N-Serve, on nitrate levels in uncropped Houghton muck. 1961.

Treatment [1]	4/5	5/4	6/22	7/11	7/26	8/9	8/29	9/13
F_o	68	128	376	427	342	191	152	251
F_1	42	52	206	304	275	191	162	217
F_2	23	119	175	281	238	224	134	267
LSD .05	NS	NS	78	85	40	NS	NS	NS
N_3		92	276	326	302	192	149	262
N_4		108	229	348	268	212	150	228
LSD .05		NS	32	NS	NS	NS	NS	NS
F_o N_3		125	389	368	354	191	164	260
N_4		130	363	485	331	190	139	241
F_1 N_3		40	247	306	304	171	179	219
N_4		65	166	302	246	212	146	215
F_2 N_3		110	191	304	249	213	103	306
N_4		128	159	258	226	235	165	227
LSD .05 F wi N		NS	80	115	66	NS	NS	NS
LSD .05 N wi F		NS	55	NS	NS	NS	NS	NS

1 - F_o = no fumigant. F_1 = 32 gpa Telone. F_2 = 48 gpa Telone.

N_3 = 50 pounds per acre N (100 ppm) applied April 27, as ammonium sulfate.

N_4 = 50 pounds per acre N (100 ppm) applied April 27, as ammonium sulfate + 1.6% N-Serve.

Table 6. - The effects of Telone and nitrogen treatments with ammonium sulfate and ammonium sulfate + 1.6% N-Serve, on ammonium levels in uncropped Houghton muck, 1961.

Treatment[1]	4/5	5/4	6/22	7/11	7/26	8/9	8/29	9/13
F_o	34	252	35	24	31	10	43	31
F_1	100	376	238	112	46	17	35	24
F_2	120	311	260	186	83	18	43	33
LSD .05	35	42	58	34	24	NS	NS	NS
N_3		320	152	74	43	15	40	30
N_4		306	203	141	63	16	41	29
LSD .05		NS	20	39	NS	NS	NS	NS
F_o N_3		266	10	14	17	11	48	28
N_4		238	60	36	44	9	39	33
F_1 N_3		392	202	78	39	16	35	28
N_4		360	275	147	54	18	36	20
F_2 N_3		303	244	132	74	17	37	32
N_4		320	276	240	91	20	48	34
LSD .05 F wi N		54	61	51	19	NS	NS	NS
LSD .05 N wi F		NS	35	68	NS	NS	NS	NS

1 - F_o = no fumigant. F_1 = 32 gpa Telone. F_2 = 48 gpa Telone.

N_3 = 50 pounds per acre N (100 ppm) applied April 27, as ammonium sulfate.

N_4 = 50 pounds per acre N (100 ppm) applied April 27, as ammonium sulfate + 1.6% N-Serve.

Table 7. - The effects of Telone and nitrogen treatments with ammonium sulfate and ammonium sulfate + 1.6% N-Serve on levels of total mineral nitrogen in uncropped Houghton muck, 1961.

Treatment[1]	4/5	5/4	6/22	7/11	7/26	8/9	8/29	9/13
F_o	102	380	411	451	373	201	195	282
F_1	142	428	444	416	321	208	197	241
F_2	143	430	435	467	321	242	177	300
LSD .05	NS	NS	NS	NS	42	NS	NS	NS
N_3		412	428	400	345	207	189	292
N_4		414	432	489	331	228	191	257
LSD .05		NS	NS	NS	NS	NS	NS	NS
F_o N_3		391	399	382	371	202	212	288
F_o N_4		368	423	521	375	199	178	274
F_1 N_3		432	449	384	343	187	214	247
F_1 N_4		425	441	449	300	230	182	235
F_2 N_3		413	435	436	323	230	140	338
F_2 N_4		448	435	498	317	255	213	261
LSD .05 F wi N		NS	NS	NS	NS	75	NS	NS
LSD .05 N wi F		NS	NS	NS	NS	NS	NS	NS

1 - F_o = no fumigant. F_1 = 32 gpa Telone. F_2 = 48 gpa Telone.

N_3 = 50 pounds per acre N (100 ppm) applied April 27, as ammonium sulfate.

N_4 = 50 pounds per acre N (100 ppm) applied April 27, as ammonium sulfate + 1.6% N-Serve.

interaction.

Although the N-Serve retarded nitrate accumulation only through June, the disappearance of ammonium was retarded through July, and increasingly so with increasing level of fumigation (Table 6). There was no evidence that net mineralization was influenced by the chemical additive (Table 7).

Effects of Fumigation on Celery in 1959 and 1960

Early Growth

In 1959, early growth of celery was severely retarded on plots fumigated with Telone the previous fall. Plants were small and chlorotic. This early injury in 1960 was not so general. It was most pronounced on portions of two replications which covered a slight depression which remained wetter than the rest of the experimental area. Growth was most retarded by fumigation, both in 1959 and in 1960, in plots which received supplemental nitrogen in the form of ammonium sulfate (Fig. 13). Calcium nitrate very effectively corrected the fumigation injury and promoted vigorous early growth (Fig. 14).

These differences were apparent five weeks after transplanting. By seven weeks after transplanting, visible differences were beginning to disappear.

Figure 13. - Growth of celery six weeks after transplanting on fumigated (F) and unfumigated (F_o) Houghton muck with 50 pounds of nitrogen as ammonium sulfate (N_1) sidedressed at planting. 1960.

Figure 14. - Growth of celery six weeks after transplanting on fumigated (F) Houghton muck with 50 pounds of nitrogen as calcium nitrate (N_3) and as ammonium sulfate (N_1) applied at planting time. 1960.

Table 8. - Dry matter yield of celery 7 weeks after transplanting in Houghton muck as related to fumigation, aeration, and nitrogen source. 1960.

Nitrogen Sources	Non-aerated		LSD .05	Aerated		LSD .05
	Non-fum.	Fum.	F wi AN	Non-fum.	Fum.	F wi AXN
	#/A	#/A		#/A	#/A	
$(NH_4)_2 SO_4$	908	925	NS	908	1048	NS
$NH_4 NO_3$	1152	1013	NS	1117	908	NS
$Ca(NO_3)_2$	1048	978	NS	995	960	NS
None	803	925	NS	768	681	NS
LSD .05 N wi AF	248	NS		248	248	
LSD .05 A wi FN			NS			

Dry matter determinations were made seven weeks after planting in 1960. On the average there was a significant growth response to all nitrogen sources. In Table 8 it may be seen that the largest dry matter production occurred with the two nitrate carriers, except in aerated soil with fumigation.

Table 9. - Percent dry matter in celery 7 weeks after transplanting as related to fumigation, aeration and source of nitrogen. 1960.

Nitrogen Sources	Non-aerated		LSD .05	Aerated		LSD .05
	Non-fum.	Fum.	F wi AXN	Non-fum.	Fum.	F wi AN
	%	%		%	%	
$(NH_4)_2 SO_4$	13.1	13.2	NS	12.7	13.9	NS
$NH_4 NO_3$	11.7	12.0	NS	11.0	14.2	NS
$Ca(NO_3)_2$	11.8	13.0	NS	12.3	13.9	NS
None	15.6	13.9	NS	16.1	15.9	NS
LSD .05 N wi AF	3.6	NS		3.6	NS	
LSD .05 A wi N			NS			

Percent dry matter was inversely related to dry matter yield (cf. Tables 8 and 9). Increasing dry matter yields were associated with increasing succulence of the celery plants.

Table 10. - Percent chloride in celery 7 weeks after transplanting as related to fumigation, aeration and source of nitrogen. 1960.

Nitrogen Sources	Non-aerated		LSD .05	Aerated		LSD .05
	Non-fum.	Fum.	F wi AN	Non-fum.	Fum.	F wi AN
	%	%		%	%	
$(NH_4)_2 SO_4$	4.44	4.95	NS	5.06	4.72	NS
NH_4NO_3	4.93	4.60	NS	5.77	6.19	NS
$Ca(NO_3)_2$	4.68	4.78	NS	4.78	4.45	NS
None	4.86	5.96	.83	4.91	5.57	NS
LSD .05 N wi AF	NS	.83		.83	.83	
LSD .05 A wi FN			.83			

Chloride content of the tissue was determined in celery plants harvested seven weeks after transplanting (Table 10). Chloride tended to be high (relative to mean) in celery grown in fumigated soil which received no supplemental nitrogen. The highest chloride content was found in celery grown in aerated soil which had received nitrogen as ammonium nitrate. Fumigation tended to enhance this aeration effect with ammonium nitrate.

While the chloride values appear to be high, they are within the range of reported values for celery (5). They do not appear to have been excessive, since there was no apparent relation to dry matter yields on this date.

Table 11. - Electrical conductance of the soil solution in Houghton muck 6 weeks after transplanting celery as related to fumigation, aeration and source of nitrogen. 1960. [1]

Nitrogen Sources	Non-aerated		LSD .05	Aerated		LSD .05
	Non-fum.	Fum.	F wi AN	Non-fum.	Fum.	F wi AN
	mnos.x 10^5	mhos.x 10^5		mhos.x 10^5	mhos.x 10^5	
$(NH_4)_2SO_4$	278	240	NS	194	201	NS
NH_4NO_3	199	226	NS	172	178	NS
$Ca(NO_3)_2$	199	258	47	198	166	NS
None	170	172	NS	159	164	NS
LSD .05 N wi AF	47	47		NS	NS	
LSD .05 A wi FN			47			

1 - Electrical conductance calculated to field moisture basis, according to Geraldson, 1957, Proc. Florida Hort. Soc. 70:121-126.

Electrical conductance in the soil solution was measured in soil samples taken six weeks after celery transplanting (Table 11). Significantly high conductances were obtained only in non-aerated soils to which supplemental nitrogen was added. Fumigation enhanced this effect where the two nitrate sources were used. In the non-aerated soils, treatments with high electrical conductivities were also those in which extremely high levels of ammonium and low levels of nitrate were found in earlier samplings.

All conductance values were well below the 600 mhos. considered critical by Geraldson (19). Nevertheless in non-aerated soils, conductivities were inversely related to dry matter yields taken one week later. It is possible that injuriously high concentrations of soluble salts may have occurred earlier in the season.

It is of note that, while the highest conductances occurred in non-aerated soils, the highest chloride content occurred in celery

grown on aerated soils.

Final Yields

Yield differences at harvest time were small, but significant interaction effects were noted (Table 12).

Table 12. - Final yields of celery grown on Houghton muck in 1960 as related to fumigation, aeration and source of nitrogen.

Nitrogen Sources	Non-aerated		LSD .05	Aerated		LSD .05
	Non-fum.	Fum.	F wi AN	Non-fum.	Fum.	F wi AN
	cwt	cwt		cwt	cwt	
$(NH_4)_2SO_4$	570	599	NS	619	561	30
NH_4NO_3	580	558	NS	599	569	30
$Ca(NO_3)_2$	583	594	NS	581	556	NS
None	482	523	30	535	561	NS
LSD .05 N wi AF	71	71		71	NS	
LSD .05 A wi FN			NS			

Fumigation increased celery yields in non-aerated soils, particularly where no supplemental nitrogen was used. In aerated soil, fumigation depressed yields where supplemental nitrogen was applied, particularly when an ammonium carrier was used. This depression of final yield by fumigation in aerated soil treated with ammonium sulfate and ammonium nitrate was associated with rapid late season release of nitrate and high levels of nitrate during the two or three weeks preceding harvest.

Summary of Effects on Celery in 1959 and 1960.

Early injury to celery, resulting from fall fumigation with Telone, appeared to be due to reduced availability of nitrate, since the plants responded dramatically to the addition of nitrate fertilizer. However, the possibility of ammonium toxicity cannot be discounted. Ammonium sulfate enhanced the fumigation injury. The large concentrations of

ammonium encountered (200 to 350 ppm), and the high ratios of ammonium to nitrate (6:1) are certainly suspiciously high. The soil studies reported in an earlier section showed that soil ammonium disappeared much more rapidly where nitrate fertilizers were used. The beneficial effect of nitrate fertilizer on the celery may have been due to this more rapid disappearance of ammonium.

While the early injury was dramatic, effects on final yield were largely overcome by rapid later growth associated with the accelerated nitrification which occurred after the inhibitory effect of the fumigant had been dissipated. Soil tests and the visible improvement in celery growth both indicated that this occurred about the middle of June.

The delayed but rapid release of nitrate during the later stages of growth of celery in fumigated soil where ammonium fertilizer was used appeared to be unfavorable for maximum yields of celery. This alteration of the seasonal pattern of nitrate release may represent the most significant factor influencing the response of different crops to fumigation.

FIELD STUDIES WITH CELERY, SWEET CORN AND LETTUCE IN 1961

Previous experience with sweet corn on Houghton muck has shown that sweet corn responds readily to ammonium fertilizer. Grogan and Zink (22) in California and Hoff and Newhall (26) in New York have reported ammonium toxicity to lettuce. Previous experiments on Houghton muck, including those reported in the previous section have provided evidence suggesting that celery cannot utilize ammonium in early growth, since it responds to nitrate in the presence of large supplies of ammonium.

For these reasons, field studies in 1961 were directed towards evaluating the effects of Telone fumigation and nitrogen carriers on yields and nutrient status of celery, sweet corn and lettuce.

The yield responses of these three crops are summarized in Tables 13 and 14. Yields of lettuce, sweet corn and one variety of celery were increased by fumigation, yields of Utah 5270 celery were reduced. Only lettuce responded to applied fertilizer nitrogen. The maximum response was to ammonium sulfate, but this response was significantly reduced when the ammonium sulfate was treated with the nitrification inhibitor, "N-Serve."

Clues to the behavior of celery in 1961 were supplied by the performance of sweet corn and lettuce. Visual observations and nutrient status of these two crops will be discussed first.

Sweet Corn

The response of sweet corn to fumigation appeared to be primarily due to increased availability of manganese. By June 30, there was a strikingly beneficial effect of fumigation at both levels of treatment,

Table 13. - Yields of celery varieties, lettuce and sweet corn on Houghton muck as related to fumigation. 1961.

Fumigation Treatment	Celery		Lettuce	Sweet Corn
	Utah 5270	Spartan 162		
	cwts.	cwts.	cwts.	cwts.
None	636	479	325	130
32 gpa Telone	555	486	461	147
48 gpa Telone	521	515	446	158
LSD .05	35	NS	86	15

Table 14. - Yields of celery varieties, lettuce and sweet corn on Houghton muck as related to nitrogen carriers. 1961.

Nitrogen Treatment[1]	Celery		Lettuce	Sweet Corn
	Utah 5270	Spartan 162		
	cwts.	cwts.	cwts.	cwts.
None	581	486	355	145.6
$Ca(NO_3)_2$	548	503	414	141.3
$(NH_4)_2SO_4$	582	491	474	146.9
$(NH_4)_2SO_4$ + N-Serve[2]	-	-	401	-
LSD .05	NS	NS	68	NS

1 - 50 lbs. N sidedressed, April 27 on lettuce, May 26 on celery and sweet corn. Basic fertilizer at planting: Lettuce, 800 lbs. 5-10-20 (plus 1/4% B, 1/4% Cu, 1% Mn); celery, 1000 lbs. of 0-10-30/A; corn, 500 lbs. 0-10-20.

2 - N-Serve (2-chloro-6-(trichloromethyl) pyridine) added to ammonium sulfate at the rate of 1.6% by weight.

which became more and more pronounced as the season progressed (Fig. 15). Close-ups of leaves in Fig. 16 reveal the characteristic interveinal streaking of manganese deficiency in leaves from unfumigated plots.

In unfumigated plots, ammonium sulfate delayed the appearance of severe manganese deficiency symptoms. This effect was pronounced at the end of June but soon became negligible. Final yields were not significantly greater than where no nitrogen was used.

Foliar analyses of composite midrib samples taken opposite the ear shoot on July 14 are tabulated in Table 15. The manganese content of midribs from unfumigated plots were in a critically deficient range; at the 48 gpa level of fumigation it was approaching a normal level.[3] Although the observed increases in manganese content were not significant statistically, they were associated with consistent decreases in the content of the major and secondary nutrients and consistent increases in yield. The low manganese contents were associated with extremely high levels of iron, the iron to manganese ratio for no fumigation being 20:1; for the high rate of fumigation it was close to unity.

Lettuce

Composite samples of midribs from the largest, vigorous outer leaves of lettuce were taken on July 20, when the lettuce was beginning to ball up (Table 16). Manganese contents were again in a critically

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[3] Values below 25 ppm are associated with deficiency symptoms in most crops. Levels of 50 to 300 ppm are normal. Lucas, R.E. The need for micronutrients in plant nutrition. Michigan Fertilizer Conference Proceedings. 1961.

Figure 15. - Response of sweet corn to fumigation under conditions of critically limiting manganese nutrition. Houghton muck, 1961.

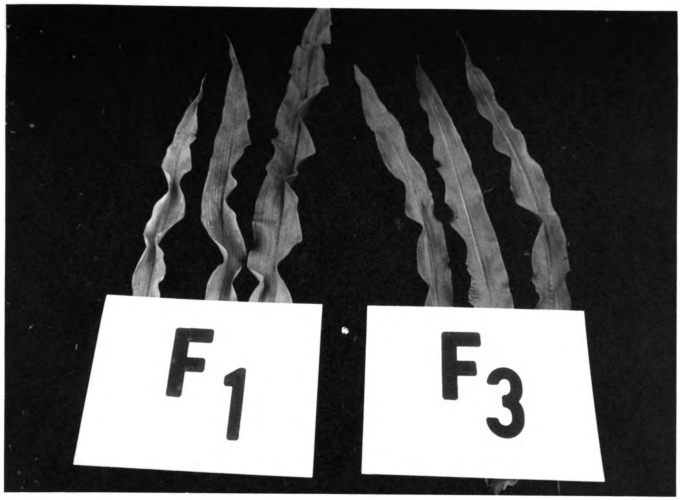


Figure 16. - Manganese deficiency symptoms in leaves of sweet corn grown on unfumigated (F_1) Houghton muck in 1961. Normal leaves were from plots treated in the fall with 48 gpa of Telone (F_3).

Table 15. - Yields of sweet corn and foliar nutrient contents as related to fumigation. Houghton muck, 1961.

Fumigation Treatment	Corn Yields 8/25	Analysis of midrib samples taken July 14[1]						
		Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Manganese	Iron
	cwt.	percent	percent	percent	percent	percent	percent	percent
None	130	2.86	.193	6.82	0.61	.387	.0005	.010
32 gpa Telone	147	2.47	.159	6.23	0.54	.303	.0007	.010
48 gpa Telone	158	2.32	.159	5.86*	0.48	.280	.0050	.006
LSD .05	15	.17	NS	NS	.08	.04	NS	NS

*Sig. @ 10%

1 - Average content of other nutrients not significantly related to fumigation: sodium, .021; copper, .001; boron, .001; zinc, .002; molybdenum, .003; aluminum, .021.

Table 16. - Yields of lettuce and foliar nutrient contents as related to fumigation. Houghton muck, 1961.

Fumigation Treatment	Lettuce Yields 7/13	Analysis of midrib samples taken June 20[1]								
		Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Manganese	Iron	Sodium	Aluminum
	cwts.	percent	percent	percent	percent	percent	percent	percent	percent	percent
None	325	4.36	.474	7.46	1.01	.355	.001	.014	.033	.007
32 gpa Telone	461	4.31	.458	7.34	.82	.312	.001	.012	.024	.005
48 gpa Telone	446	4.02	.404	6.78	.69	.260	.001	.009	.022	.004
LSD .05	86	.08	NS	NS	.17	.07	NS	NS	.007	.002

1 - Average content of other nutrients not significantly related to fumigation: copper, .0004; boron, .002; zinc, .003; molybdenum, .0003.

deficient range. Even though there were no changes in manganese content, it appears extremely likely that the major factor in the response of lettuce to fumigation was the increasing availability of manganese. Decreasing contents of the major and secondary nutrients, as well as of iron, sodium and aluminum, were associated with increasing yields. Although the content of iron was high, it is well within the reported ranges for this crop (5).

While the basic response to fumigation appeared to be related rather simply to manganese availability, the yield response to forms of fertilizer nitrogen noted in Table 14 involved a rather complex sequence of developmental responses. On June 5, root browning reportedly characteristic of ammonium toxicity (22 and 26), was wide spread, even in plots where negligible accumulations of ammonium were found. The severity of root discoloration increased with fumigation level, and was most marked where ammonium sulfate and ammonium sulfate plus N-Serve had been used. Ammonium levels in excess of 300 ppm were found in some of these plots. Calcium nitrate plots were least affected. However, the levels of root injury observed were not reflected by visible differences in top growth.

On June 20, growth was remarkably uniform on all plots, except that fumigated plots tended to be darker green and slightly slower in forming heads, particularly where ammonium sulfate, - with or without N-Serve, - was used. On the fumigated areas, calcium nitrate plots were distinctly lighter green in color than those receiving ammonium sulfate. The plots which received ammonium sulfate plus N-Serve were still darker green and not fully ready for harvest when yields were taken on July 13.

Final yields were not related to the root browning observed in early June, since maximum yields were obtained with ammonium sulfate in fumigated plots, - where the root discoloration had been most severe (Table 17-B). Preferential assimilation of ammonium rather than nitrate nitrogen was ruled out because of the unfavorable response to the nitrification inhibitor, N-Serve, which effectively maintained higher concentrations of ammonium in the soil through harvest. An inadequate availability of nitrate nitrogen might have been involved with this treatment, but not where calcium nitrate was used, since 100 to 200 ppm of nitrate nitrogen was maintained in the soil through harvest where the fertilizer nitrate was applied. Also tissue nitrogen was inversely related to the yield differences associated with nitrogen sources, indicating that growth was being limited by some other factor.

This limiting factor again appears to have been manganese. The tissue manganese values in Table 17-A do not include any statistically significant differences. Significant differences among yields were found only among the averages for fumigation and for nitrogen sources (cf. Tables 13 and 14). However, the close correspondence between manganese levels and yields indicates that the response to ammonium sulfate was a response to manganese expressed additively on the manganese response associated with fumigation.

The significantly reduced response to ammonium sulfate when combined with N-Serve suggests that the nitrification inhibitor counteracted the solubilizing action of the sulfate on soil manganese. The solubilizing action of ammonium sulfate arises from its acidifying effect, half of which is expressed only as the ammonium is nitrified. It appears that the seasonal distribution of manganese availability closely paralleled seasonal patterns of nitrification as influenced

Table 17. - Manganese contents and yields of lettuce as related to fumigation and nitrogen sources. Houghton muck, 1961.

Fumigation Treatment	Nitrogen source				Ave. for fumigation
	No N	$Ca(NO_3)_2$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$ plus N-Serve	
	A. PPM manganese in leaf midribs on June 20				
Unfumigated	4	12	19	8	11
32 gpa Telone	19	4	16	4	11
48 gpa Telone	12	12	8	8	10
Ave. for N source	12	9	14	7	-
	B. Final yield, - Cwts.				
Unfumigated	234	270	433	364	325
32 gpa Telone	412	493	511	430	461
48 gpa Telone	419	478	479	409	446
Ave. for N. source	355	414	474	401	-

by fumigation and fertilizer sources of nitrogen. An optimal seasonal release of manganese was associated with fumigated ammonium sulfate plots. Where ammonium sulfate was treated with N-Serve, recovery of nitrifying capacity was additionally retarded. The associated delay in manganese release accounts for the delayed development and darker green color of lettuce on these plots on July 13 when lettuce on other plots had reached market size. If these plots had been left unharvested another week or ten days, it is likely that final yields would have equalled those where ammonium sulfate without N-Serve was used.

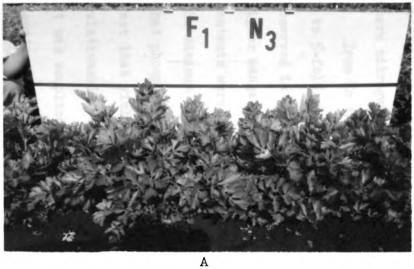
The slow, but continuing release of manganese associated with retarded nitrification in fumigated soil accounts for the fact that lettuce yields four times greater than the state average of 100 to 140 cwt. per acre were produced, even though manganese in the plants was at critically deficient levels.

Celery

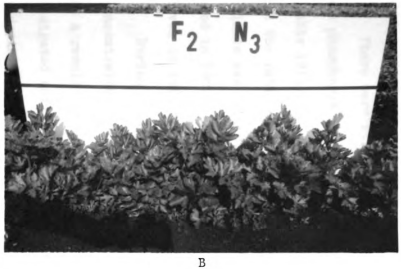
Of the two celery varieties used in 1961, Utah 5270 was the only one injured by fumigation. This was the same hybrid used in 1960. Early injury was as striking as it had been on poorly drained replications in 1960. The degree of injury increased with increasing rate of Telone application (Figs. 17-A, B, C). However, in contrast to 1960, there was no intensification of injury with ammonium sulfate and no beneficial effect of calcium nitrate. In fact, the latter material tended to intensify the injury (Fig. 17-D). Differences in final yields associated with these nitrogen sources were not significant. The fumigation injury was reflected in significant reductions in yield.

The content of N, K, Mg, Mn, Fe and B in celery petioles on July 1 tended to increase as yield potentials associated with fumigation

Figure 17. - Utah 5270 celery two months after planting in unfumigated soil [F_1] and following fall fumigation with 32 gpa [F_2] and 48 gpa [F_3] of Telone. Injury increased with fumigation level where $(NH_4)_2SO_4$ [N_3] was used. Failure to respond to $Ca(NO_3)_2$ [N_2] was due to limiting Mn and P, and an interaction with increased availability of Fe in fumigated plots.



A



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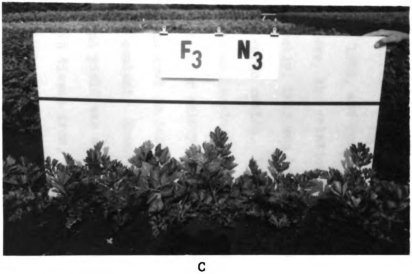
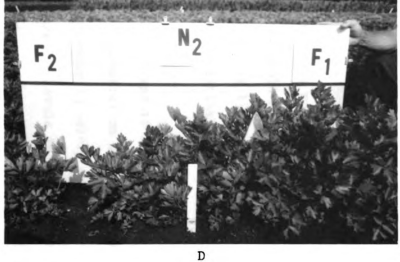


C



D

Figure 17.

levels decreased (Table 18). Phosphorus content decreased.

There were striking differences in the status of iron, manganese and phosphorus in celery tissue grown in 1961, as compared with 1960 (Table 19). The content of iron and manganese was much lower in 1961, but the ratio of iron to manganese was several-fold higher. Manganese sulfate was included in all insecticidal sprays in 1960 but was not used in 1961. As a result, manganese was at a critically deficient level in celery tissue in 1961.

During both years, the effect of fumigation was to increase iron concentrations in the plant and reduce the concentration of phosphorus. As a result, iron to phosphorus ratios were wider in celery grown on fumigated soil. At the critically low manganese levels in 1961, these widening Fe:P ratios were closely related to declining yields (Fig. 18). This apparent immobilization of phosphorus by iron in fumigated plots was most injurious to yields where calcium nitrate was used and least where nitrogen was supplied as ammonium sulfate.

Iron in the tissue was much higher in 1960, and Fe:P ratios were 2 to 5-fold greater (Table 19). Nevertheless, the relation to yield observed in 1961 was not obtained. Apparently, this was because of the higher manganese levels and much narrower Fe:Mn ratios. Phosphorus contents were also higher in 1960. Phosphorus levels in 1961 approached the minimum reported value of 4300 ppm cited for celery by Beeson (5). That phosphorus was in a deficient range in 1961 is indicated by the fact that yields declined with phosphorus content. However, the relationship between phosphorus content and yield was not a simple one. It was complicated by the interactions with tissue iron, fumigation treatments and nitrogen carriers which are depicted in Fig. 18.

Table 18. - Yields of celery and foliar nutrient contents as related to fumigation. Houghton muck, 1961.

Fumigation Treatment	Celery Yields 8/14	Analysis of petiole samples taken July 1[1]							
		Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Manganese	Iron	Boron
	cwts.	percent	percent	percent	percent	percent	percent	percent	percent
None	636	2.40	.488	7.24	1.86	.260	.0007	.005	.002
32 gpa Telone	555	2.60	.458	7.38	1.91 } *	.277	.0005	.007	.003
48 gpa Telone	521	2.62	.435*	7.63	1.82 }	.277	.0009	.008	.003
LSD .05	35	.11	NS	NS	NS	NS	NS	.002	.0001
			(*Sig. @10%)		(*Sig. @10%)				

1 - Average content of other nutrients not significantly related to fumigation: copper, .0005; zinc, .003; molybdenum, .0006; aluminum, .006.

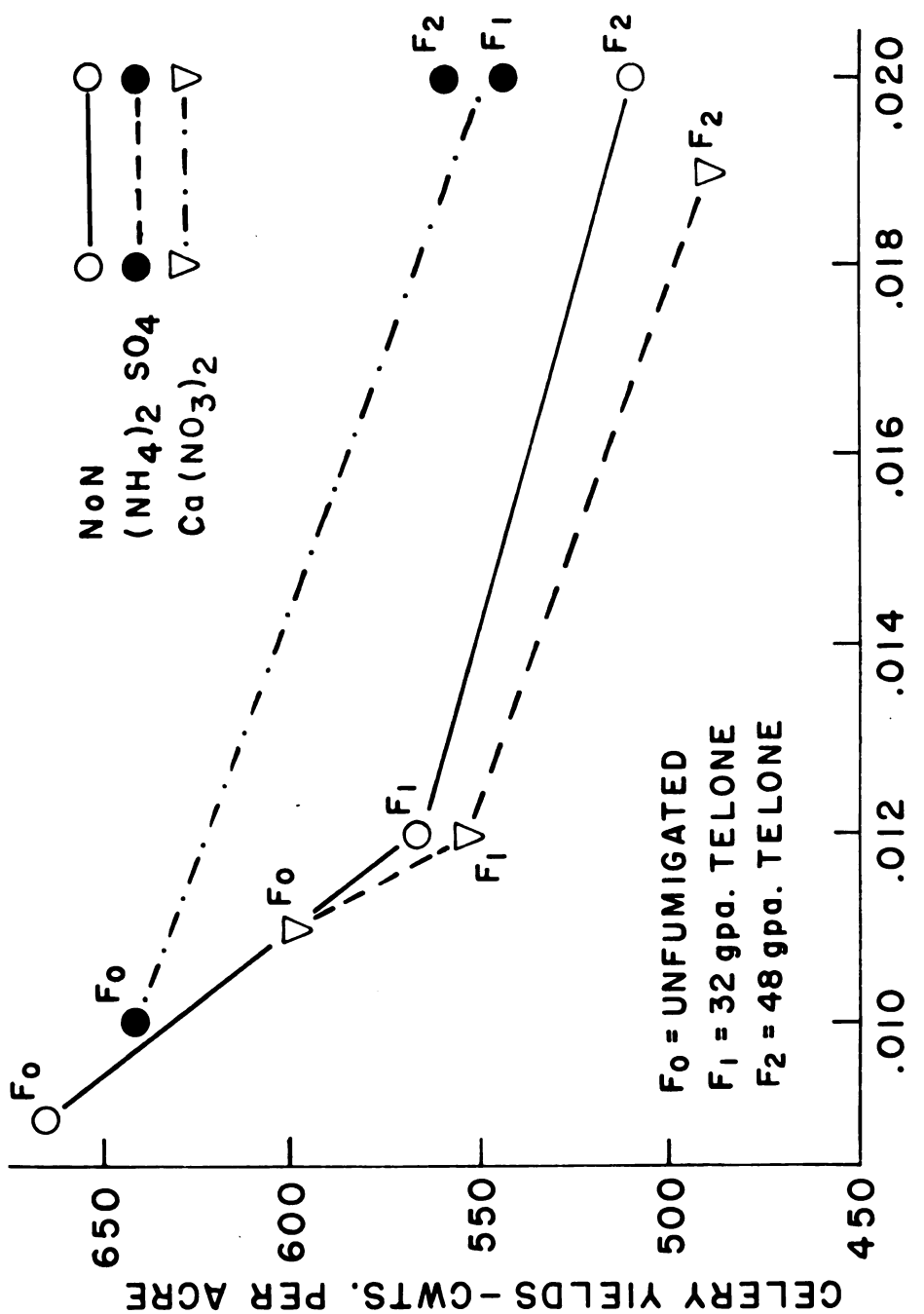


Figure 18. - Celery yields as related to the ratio of iron to phosphorus in leaf petioles six weeks before harvest on Houghton muck with variable fumigation and nitrogen treatment. 1961.

Table 19. - Status of iron, manganese and phosphorus in celery petioles in 1960 and 1961 as related to fumigation and final yields on Houghton muck.

Fumigation Treatment	Nutrient content of petioles [1] Fe	Mn	P	Fe/Mn	Fe/P	Celery Yield
	PPM	PPM	PPM			Cwt.
			A. 1960			
Unfumigated	280	176	6990	2	.039	569
32 gpa Telone	324	176	6390	2	.051	565
LSD 05	31	NS	400			NS
LSD 10	26	NS	340			NS
			B. 1961			
Unfumigated	50	7	4880	7	.010	636
32 gpa Telone	70	5	4580	14	.015	555
48 gpa Telone	80	9	4350	9	.018	521
LSD 05	22	NS	NS			35
LSD 10	11	NS	340			34

1 - The most vigorous petiole on each plant taken from the second or third whorl from the outside on June 24, 1960, and July 1, 1961. Twelve petioles per plot and 4 replicate plots were composited. Values for 1960 are means of 8 composite samples, for 1961 they are means of 3 composites.

Summary of 1961 Field Studies with Crops

The results of studies in 1959 and 1960 had suggested that injurious effects of fumigation on celery in organic soil could be explained in terms of the nitrate vs. ammonium nutrition of the crop as related to effects of the fumigant on the seasonal distribution of these two forms of nitrogen. The 1961 results demonstrate that, in special situations, direct effects of fumigation on the availability of reducible trace nutrients such as iron and manganese may be of overriding importance.

The stimulus to heterotrophic activity, commonly referred to as the "partial sterilization effect" of fumigation, gives rise to strongly reductive soil conditions. The foliar analyses reported here for celery for both 1960 and 1961 indicated that the availability of iron, as well as of manganese, was enhanced by this reductive influence of fumigation. While this effect on manganese was apparent with all three crops, the effect on iron was expressed only by the one variety of celery which was injured by fumigation. Marked genetic differences in response are indicated. These may involve physiological differences within the plant, as well as differences in rhizosphere environment imposed by the nature of root exudates associated with species or variety of plant.

Under conditions of restricted minor element nutrition, differences in the solubilizing action of various fertilizer nitrogen sources become more significant than differences in assimilability of NH_4^+ or NO_3^- by specific crop plants. This solubilizing action arises from the acidifying effect of nitrogen fertilizers. Ammonium salts and nitrate salts have an immediate acidifying effect on the soil solution

associated with the absorption of the cationic component and displacement of H+ from the exchange complex. With nitrate salts, this acidifying effect is largely dissipated as the nitrate is removed by leaching or crop removal. With ammonium salts, however, there is a progressive intensification of H+ concentration as the NH_4^+ is nitrified to NO_2^- and NO_3^-. In the case of $(NH_4)_2SO_4$, the direct acidity associated with the SO_4^{--} ion and the "physiological acidity" associated with NH_4^+ result in a net residual acidity equivalent to 110 pounds of $CaCO_3$ per unit (20 pounds) of N.[4]

Interaction between the reductive effect of partial sterilization and the direct and physiological acidification associated with nitrogen carriers gave rise to complex patterns of response in all three crops grown in 1961.

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[4] Dictionary of Plant Foods. Handbook published by Farm Chemicals, 317 N. Broad St., Philadelphia, Pa. 1958.

INCUBATION STUDY

The incubation experiment was undertaken to investigate effects of temperature and rates of application of three fumigant chemicals on soil microbial activities in Houghton muck. Specific concern was in microbial activities related to nitrogen transformations in the soil, and in effects expressed after the soil had gone through periods of exposure and aeration, as recommended in field practice. Chloropicrin at a single rate was used as a reference fumigant. N-Serve (2-chloro-6-(trichloromethyl) pyridine) was used at a single rate as a chemical additive which might affect nitrification specifically without affecting the activity of heterotrophic organisms.

It will not be fruitful to discuss here all of the effects which were expressed with statistical reliability. Many of the significant effects were reversed as incubation progressed or were expressed only for short periods. Numerous cyclic readjustments in balance of the soil population were indicated by the CO_2 evolution data. These patterns of readjustment were very likely determined by the conditions of incubation and would not have wide applicability under varying field conditions.

However, a number of relationships which appear to have general validity will be discussed in the following sections.

Nitrogen Transformations

The distribution of significant F ratios as they apply to differences in total mineral nitrogen, ammonium nitrogen and nitrate nitrogen associated with various treatment combinations are presented in the Appendix (Tables 21, 22 and 23). Means for chemicals are plotted in the histograms of Fig. 19. The values for Vidden-D, Telone

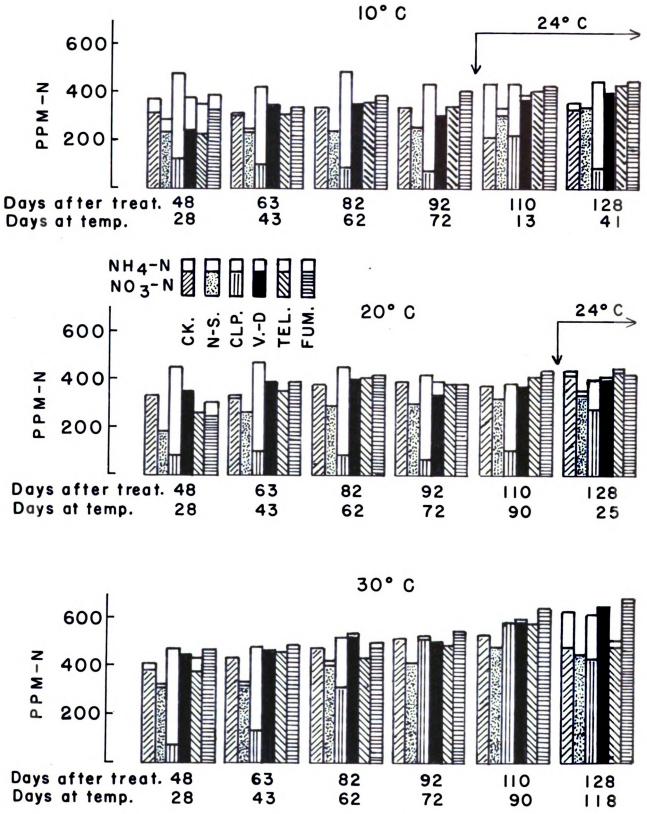


Figure 19. - Levels of ammonium and nitrate nitrogen in unfumigated organic soil and in soil previously treated with N-Serve, chloropicrin, Vidden-D, Telone and Fumazone, during incubation under three temperature regimes.

and Fumazone are those for the highest rate of application. Consistent effects of rate were expressed only with Fumazone.

The principal significant differences between the check and treated soils were associated with N-Serve and chloropicrin.

The N-Serve was not added until the soils were placed in the constant temperature rooms 28 days prior to the first determination of mineral forms of nitrogen. Significantly lower levels of nitrate and total mineral nitrogen relative to the check were found on most sampling dates at all three temperatures. Only traces of ammonium or none at all were encountered, except in the first sampling at 10° C.

In the case of chloropicrin, total mineral nitrogen was significantly higher than in check soils through 72 days of incubation at 10°C., 62 days at 20° and only in the sampling taken after 28 days at 30°. Half or more of the mineral nitrogen was in the form of ammonium throughout the incubation at 10°C., through 90 days at 20°, and through 43 days at 30°.

Significant increases in total mineral nitrogen over the check were rarely encountered in soil treated with Vidden-D, Telone or Fumazone. Such increases were found after 28 days at 30°C., and occasionally during later stages of incubation at the two lower temperatures. Significant decreases relative to the check were found frequently at the low rate of Fumazone treatment. These will be discussed later. With these three materials, significant accumulations of NH_4^+ and/or depressions of nitrate relative to the check were encountered only in the first sampling at 10° and 20° C.

Differences in patterns of accumulation of nitrate and total mineral nitrogen associated with N-Serve, chloropicrin and Telone are

best observed in Figs. 20 and 21.

In the case of N-Serve, the accumulation of nitrate and total mineral nitrogen during incubation was less than the check at all three temperatures. The depression was approximately equivalent to the 100 ppm.of ammonium N added with the N-Serve at the beginning of controlled temperature incubation. This relative difference was maintained rather consistently throughout incubation. Only at 20° C. was there any evidence of a specific inhibition of nitrification during the first 28 days of incubation. After that, curves for treated and untreated soils tended to parallel each other at all temperatures. Where the temperature in the 10° room was raised to 24° C. after 77 days, a marked disappearance of NO_3^- in the check soil (Fig. 20) was accompanied by a marked increase in NH_4^+ and in total mineral nitrogen (Fig. 21). This did not occur in soil treated with N-Serve, and NO_3^- with this treatment was higher than the check in the last two samplings.

A sharp contrast is presented by the behavior of soils treated with chloropicrin: Nitrate levels (Fig. 20) were markedly depressed 48 days after treatment (28 days at controlled temperature) at all three temperatures. After 43 days at 30° C., rapid nitrification began in chloropicrin treated soil, giving rise to higher levels than in the checks after 90 days. At 10° and 20° C., there was no evidence of recovered nitrifying capacity until after 70 to 90 days.

A marked partial sterilization effect, giving rise to significantly increased net mineralization of nitrogen in chloropicrin treated soil was also noted (Fig. 21). Ammonium and nitrate levels with chloropicrin were similar after 28 days at all three temperatures

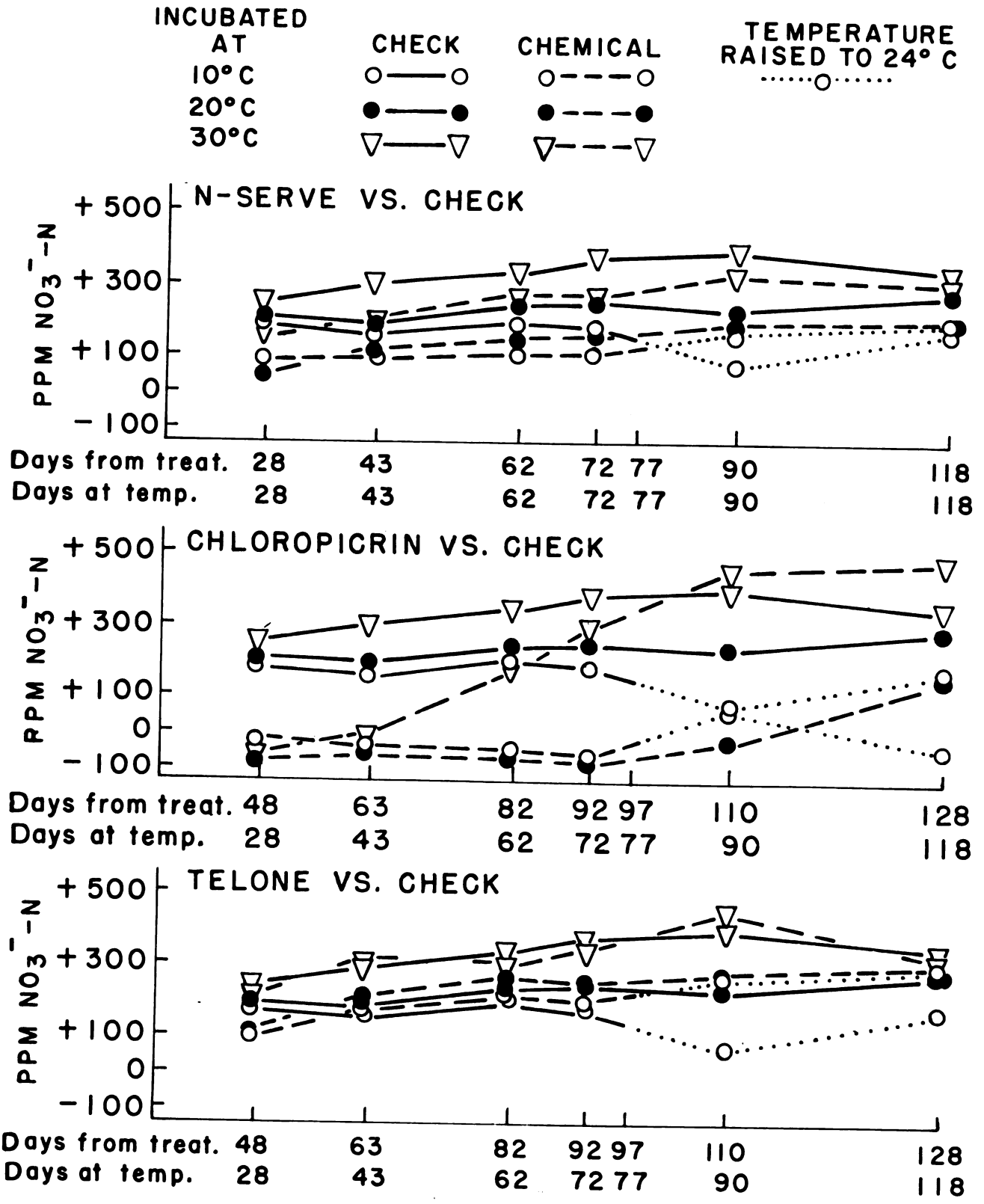


Figure 20. - Changes in soil nitrate following chemical treatment of Houghton muck. The soil contained initially 142 ppm NO_3^- - N and 3 ppm NH_4^+ - N. 100 ppm N was added as $(NH_4)_2SO_4$ when soils were placed in constant temperature rooms 48 days after adding chloropicrin and Telone. Prior to this time, soils were held at 18° to 20° C.

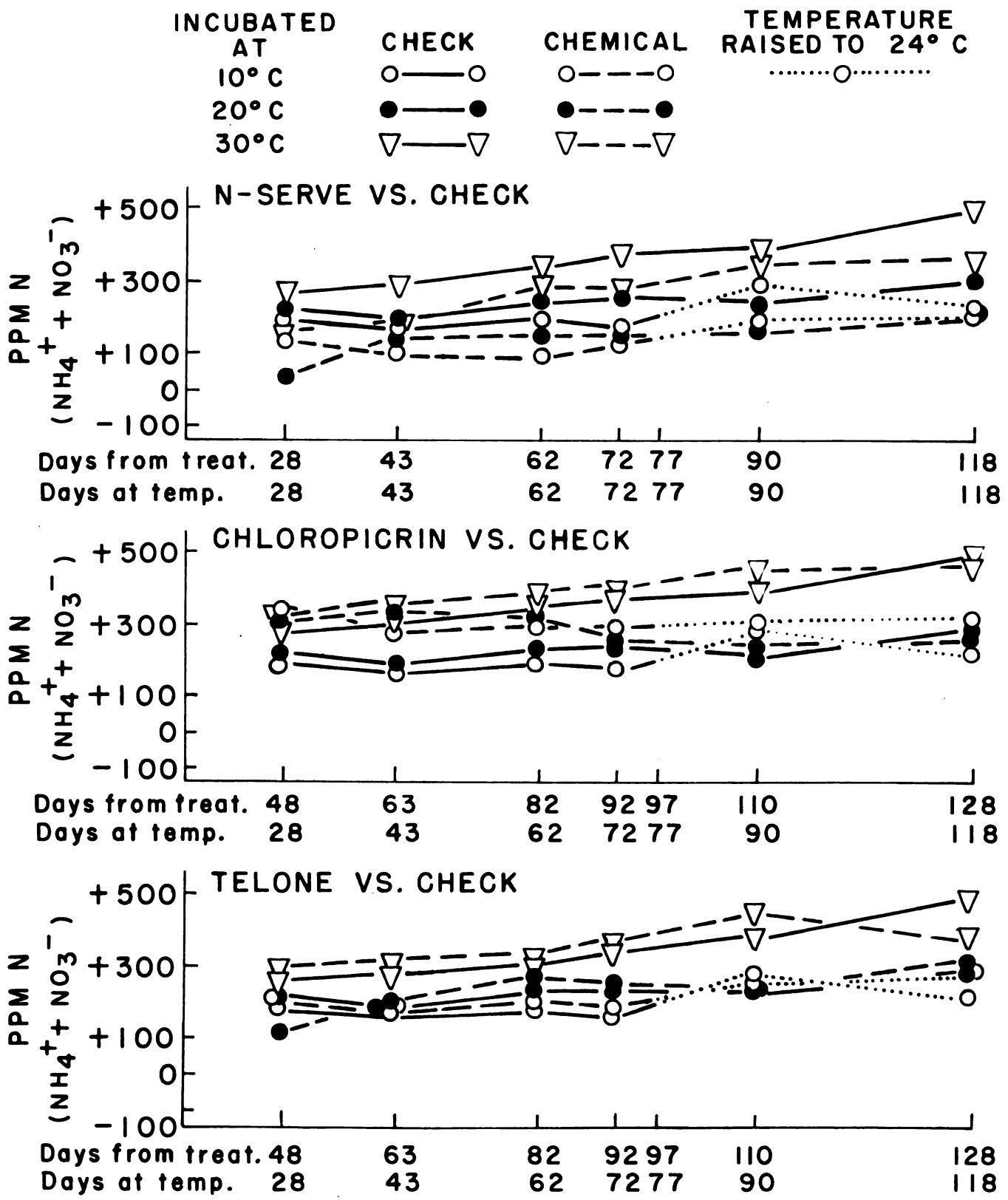


Figure 21. - Changes in total mineral nitrogen following chemical treatment of Houghton muck. Initially the soil contained 145 ppm mineral N. 100 ppm N was added as $(NH_4)_2SO_4$ when soils were placed in constant temperature rooms 48 days after adding chloropicrin and Telone. Prior to this time, soils were held at 18° to 20° C.

(cf. Fig. 19). This indicates that the major stimulus to resistant species in the recovery population had occurred during the earlier exposure and aeration periods at 18° to 20° C. At 10° C., the residual effect on total mineral nitrogen levels was maintained throughout the incubation period (Fig. 21). At 20° and 30° C., differences between treated and untreated soils had largely disappeared after 70 to 90 days at each temperature.

In the case of Telone at the highest rate of application (64 gpa), significantly reduced accumulations of nitrate were observed only in the first sampling at 10° and 20° C. (Fig. 20). In later samplings, nitrate levels tended to be higher in the Telone treatments than in the checks, becoming significantly higher in the 10° room after the temperature was raised to 24° C. Differences in total mineral nitrogen between check and Telone treated soils were not statistically significant at any time (Fig. 21). However, there was evidence that a stimulus to net mineralization, similar to that expressed with chloropicrin had occurred following addition of Telone. Total mineral nitrogen was maintained rather consistently at levels higher than the check through 70 to 90 days of incubation at each temperature.

Telone and Vidden-D were very similar in their action at all rates of treatment. Significant accumulations of ammonium occurred only in the first sampling at 10° C. and the quantities found increased with rate, reaching maxima of 100 to 150 ppm at the highest rate. At this same time, at 30° C., both total mineral nitrogen and nitrate nitrogen were significantly increased over the check at the higher rates of treatment only. A similar enchancement of nitrate and total mineral nitrogen at higher rates, sometimes associated with significant

depression at the low rate, was observed after 62 and 72 days at 10° C. with these two materials. Generally, however, significant or consistent effects of rate were not observed.

On the other hand, with Fumazone, significant effects of rate were expressed on net mineralization and nitrate accumulation on all sampling dates at one or more temperatures (Appendix Tables 21 and 23). Nitrate and total mineral nitrogen were consistently higher at the higher rates of treatment (7 and 14 gpa) than at the low rate (3½ gpa), at all temperatures (Fig. 22). Levels at the low rate were frequently reduced significantly below those in the check.

As was true with Telone and Vidden-D, accumulations of ammonium were found in the first sampling at 10° C. Sixty ppm were still present at 20° C. where the highest rate was used. There was a transient accumulation of NH_4^+ immediately following the temperature change in the 10° room. This was observed also where Telone and Vidden-D had been used, but not with N-Serve. In no case was the ammonium accumulation associated with this temperature change as dramatic as in the check soil. Ammonium accumulations near the end of the incubation at 30° C. appeared to be related to drying of the soil. Since the soil was initially high in moisture, drying associated with this high air temperature promoted a generally increasing level of microbial activity, as reflected by CO_2 evolution.

Microbial Numbers and Evolution of Carbon Dioxide

The generally increasing level of microbial activity associated with drying of soil during incubation at 30° C. is apparent in Fig. 23. It was much less apparent, or did not appear, in connection with soils

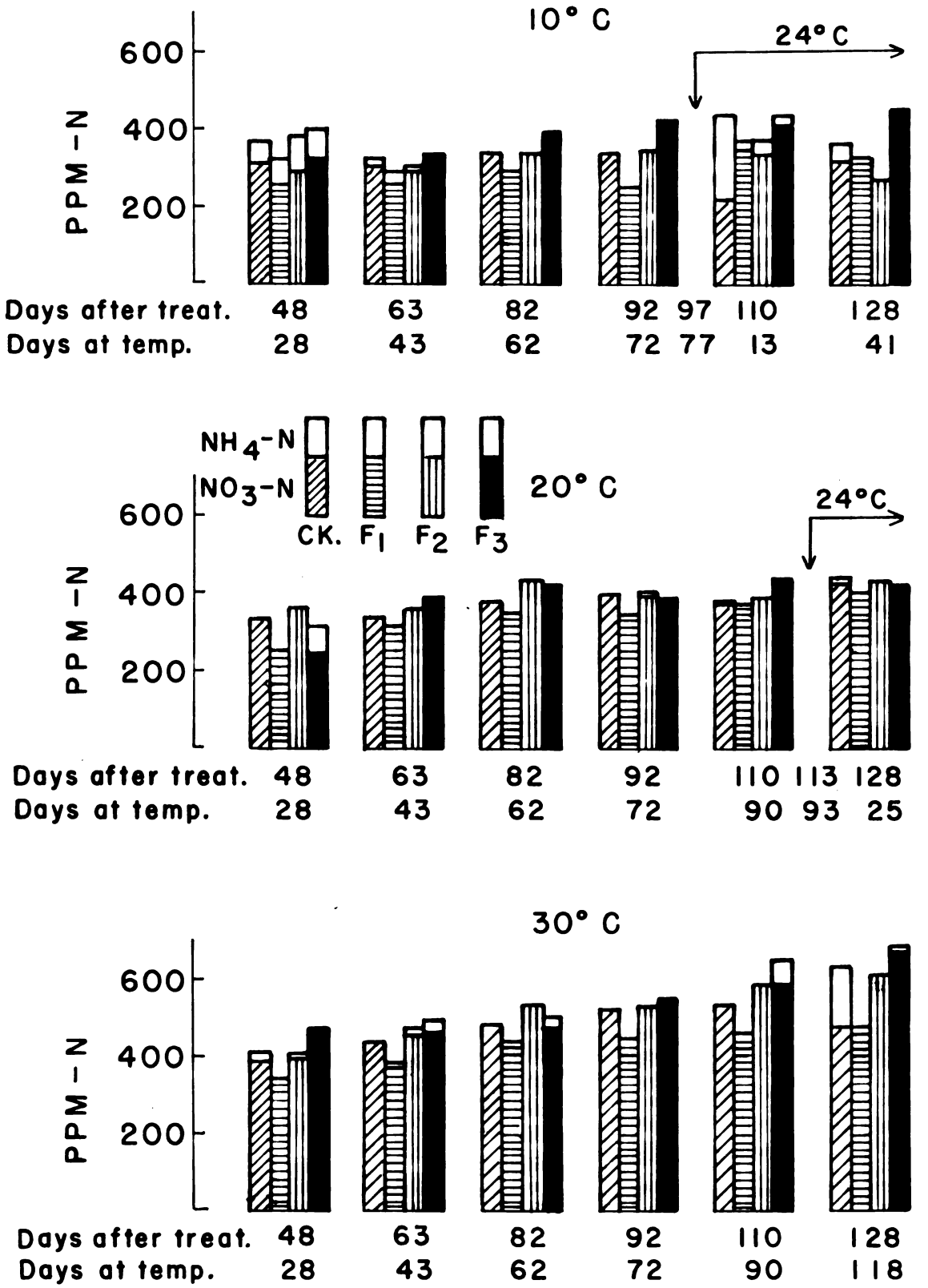
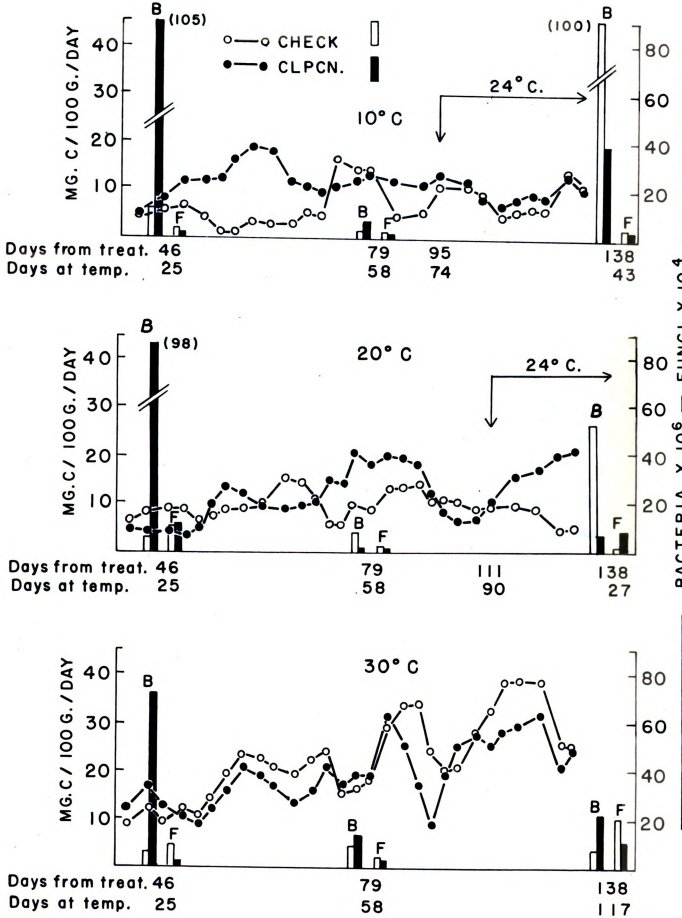


Figure 22. - Levels of ammonium and nitrate nitrogen in unfumigated muck and in muck previously treated with three levels of Fumazone, during incubation under three temperature regimes.
(F_1=3½ gpa; F_2=7 gpa; F_3=14 gpa)

Figure 23. - Effect of chloropicrin on the evolution of carbon dioxide and the numbers of bacteria (B) and fungi (F) in Houghton muck at three incubation temperatures.


B
(105)
CHECK
CLPCN.
(100)
B
24° C.
10° C
MG. C / 100 G. / DAY
40
30
20
10
80
60
40
20
F
B
F
Days from treat. 46 79 95 138
Days at temp. 25 58 74 43
B
(98)
20° C
24° C.
MG.C/ 100G./DAY
B
F
Days from treat. 46 79 111 138
Days at temp. 25 58 90 27
BACTERIA X 10⁶ — FUNGI X 10⁴
30° C
MG.C / 100 G. / DAY
Days from treat. 46 79 138
Days at temp. 25 58 117

incubated at 10° and 20° C.

Periodic readjustments in composition and size of the soil population were very likely responsible for the peaks and valleys in the rate curves for CO_2. At 30° C., these readjustments occurred at about the same time in treated and untreated soils. At 10° and 20° C., this coincidence was markedly disrupted with all chemical treatments. The degree of phase displacement and the maximum rates attained varied with the different chemicals and with rate of application.

At the high rates of treatment with Vidden-D, Telone and Fumazone, higher rates of CO_2 evolution than in the check soil were maintained over major portions of the incubation period at 10° and 20° C. In this respect their behavior was similar to that shown in Fig. 23 for chloropicrin at these lower temperatures. Total CO_2 evolution, accordingly, was higher for all of these treatments. With N-Serve and the low rates of Vidden-D, Telone and Fumazone, total CO_2 evolved was greater than the check only at 10° C.

Chloropicrin treated soil exhibited a markedly increased activity following the temperature change in the 20° room (Fig. 23). There was no similar response to the earlier change in the 10° room. In the case of N-Serve and the high rates of Vidden-D, Telone and Fumazone, these temperature changes were accompanied by enhanced CO_2 evolution in both the 10° and 20° rooms. At the low rates of the last three fumigants, these late temperature changes had no effect.

The relative differences described above indicate that the composition and general activity of the soil microbial population was altered in varying degrees by different chemicals and by rates of application of a given chemical. The altered populations responded

differently when temperatures were raised or lowered 3 weeks after fumigation. Even a minor temperature change from 20° to 24° imposed 4 months after fumigation produced differential responses in activity.

The sequential pattern of adjustment to the 10° temperature was markedly disturbed by all chemical treatments. Adjustments when the temperature was later increased to 24° C. were distinctly different for different chemicals, particularly at high rates of treatment. It appeared, however, that at 30° C. these residual effects on the soil population were rapidly dissipated. As a result, fluctuations in activity associated with normal population changes and with drying of soil followed similar patterns in treated and untreated soil.

Attempts to characterize qualitative changes in the composition of the population were not highly successful because of the great variability encountered in plate counts for bacteria and fungi. Significant differences in geometric means for various treatment comparisons were obtained principally in the first counts, made after 25 days of controlled temperature incubation (Appendix, Table 24).

The major differences at this time were associated with the chloropicrin treatment (Table 20). Bacterial numbers were dramatically increased at all three temperatures. Numbers of fungi were sharply reduced where the temperature had been lowered to 10° or raised to 30° from the pre-incubation temperature of 18° to 20° C. There were few significant differences for other treatments. Those that were obtained were consistent with the conclusion that chemicals other than chloropicrin had had a stimulating effect on bacterial numbers at 10° and 20°, similar to but less pronounced than chloropicrin. These other chemicals had had no effect on bacterial numbers at 30° C.,

Table 20. - Geometric mean numbers[1] of bacteria and fungi during incubation of Houghton muck as related to chemicals and incubation temperature.

Days at temp.	25			68			117		
Centigrade	10	20	30	10	20	30	10[3]	20[4]	30
Treatment	A. Bacteria x 10^6								
Check	11.94	6.26	5.46	2.05	9.25	9.12	100.57	53.70	7.74
N-Serve	19.56	10.74	3.96	3.54	3.98	4.53	4.88	7.08	3.74
Chloropicrin	104.60	98.40	74.01	5.92	1.61	13.21	37.24	6.79	20.89
Other fumigants[2]	20.49	11.80	5.35	4.29	9.17	8.56	14.68	14.11	7.46
	B. Fungi x 10^4								
Check	4.24	9.51	8.38	1.45	2.20	3.70	4.22	1.37	19.88
N-Serve	14.69	2.51	6.64	2.18	2.98	1.40	2.96	4.98	2.79
Chloropicrin	1.42	10.96	1.06	.94	.89	2.92	2.72	9.44	10.67
Other fumigants	6.79	10.78	8.63	2.32	2.26	4.87	3.88	7.94	8.20

1 - Antilogs of mean logarithms. Significant F ratios for various comparisons among mean logarithms are shown in Table 23 of the Appendix.

2 - Geometric means combining replications and rates of Vidden-D, Telone and Fumazone.

3 - Temperature in 10° room raised to 24° C. for last 40 days.

4 - Temperature in 20° room raised to 24° C. for last 24 days.

and essentially none on numbers of fungi at any temperature.

It is of interest that bacteria were more numerous in the first enumeration with all chemicals at 10° and 20° than at 30° C. After 68 days at each temperature, bacteria in these soils had declined to levels comparable to those at 30° C. In chloropicrin treated soil, bacteria at 20° and fungi at 10° and 20° were significantly fewer than in check soil or soil treated with other chemicals.

Following the temperature increases in the 10° and 20° rooms, bacterial numbers increased rapidly in the check soil. Smaller gains were observed also for all fumigated soils, although differences in numbers among chemicals were non-significant. Few differences in fungal count were statistically significant. However, it did appear that fungi were on the increase in check and chloropicrin treated soils at 30° C. This may have been a reflection of the drying soil conditions.

Summary of Incubation Results

Results of both field and laboratory studies indicate that, in organic soil, effects of chemical fumigation on patterns of nitrogen transformation are compounded of effects on both heterotrophic and autotrophic components of the soil microbial population. In the incubation study, partial sterilization effects on both components were expressed by all fumigant chemicals. Effects of Vidden-D, Telone and Fumazone were neither so drastic nor so prolonged as with chloropicrin. However, the intensity and duration of effects of all fumigants were markedly different under different sequences of soil temperature imposed 3 weeks after initial exposure.

At soil temperatures normal to the summer season (30° C., 86° F.), effects of the fumigants, including chloropicrin were rapidly dissipated. Heterotrophic populations in both treated and untreated soils were characterized by relatively low numbers of bacteria, high ammonifying capacity (rapid net mineralization of nitrogen), and by synchronous population readjustments reflected in essentially parallel fluctuations in rate of CO_2 evolution. Autotrophic nitrifiers had recovered full activity 7 weeks after initial exposure, except in chloropicrin treated soil, where an additional 3-week delay was observed.

At soil temperatures normal to late autumn and spring (10° and 20° C., 50° and 58° F.), a slight delay in nitrification beyond 7 weeks was observed with fumigants other than chloropicrin, notably at recommended and higher than recommended rates. With chloropicrin, nitrification at both temperatures was delayed for about 3 months after treatment. Heterotrophic bacteria were greatly increased in numbers 7 weeks after exposure to chloropicrin; increases with the

other three fumigants were much less, though statistically significant in some instances, approaching significance in others. Fungi were significantly suppressed by chloropicrin but little affected by the other three fumigants.

These changes in the proportion of major taxonomic groups gave only a very meagre indication of the extent to which heterotrophic populations were altered in their physiological composition. This is indicated by the very marked chronological displacement of sequential phases of increase and decline in rates of CO_2 evolution in treated and untreated soils. This was apparent at both 10° and 20° C., but it was most prominent at the lower temperature, and with chloropicrin and the higher rates of Vidden-D, Telone and Fumazone.

Heterotrophic activity at these lower temperatures was characterized by enhanced net mineralization in soils treated with chloropicrin and higher than recommended rates of the other fumigants. At lower than recommended rates, net mineralization was reduced. Carbon dioxide evolution was similarly affected by dosage of these three chemicals. These differences in efficiency of retention of carbon and nitrogen indicate that microbial populations which were able to survive intensive levels of fumigation were qualitatively different than those which survived treatment at lower dosage or with less toxic materials.

Since the nitrifiers are very sensitive to fumigants, it may be assumed that toxic quantities of chemical had been physically removed from the soil prior to recovery of nitrifying capacity. Differential responses in heterotrophic numbers and activities were, nonetheless, observed when temperatures were raised in the 10° and 20° rooms near

the end of the incubation period, - long after effects on nitrification had disappeared with most treatments.

These residual effects of fumigation treatment parallel those observed in the field where temperature sequences following fall fumigation were somewhat similar to those imposed on soils incubated in the 10° room. It was observed in the field that nitrification was not completely suppressed in the early spring months following fall fumigation, although it was retarded. Recovery of full nitrifying capacity was associated with rapid warming of the soil in June. Enhanced mineralization of nitrogen was observed in fumigated soil in the field during the spring and summer, paralleling the differential stimulus to microbial numbers or CO_2 evolution or net mineralization associated with different chemical treatments when temperatures were raised during the later stages of incubation in the 10° and 20° rooms.

Much larger accumulations of NH_4^+ were encountered in the field with fall fumigation with Telone than with the same material in the laboratory. A substantial portion of this was already present at the time of the earliest samplings in April. These early accumulations had very likely accrued during the late October exposure period and remained unchanged during the late fall and winter months when soil temperatures were near freezing or below. Such temperatures were not reproduced in the laboratory, - nor were the completely saturated moisture levels which develop normally at the field location during late winter and early spring.

Low nitrate levels in the early spring in the field were very likely due to leaching of nitrate which may have been present in the

fall. The slow accumulation of nitrate in fumigated soil during the spring months suggests that nitrification was limited by low numbers of nitrifiers rather than by any specific residual inhibition by the chemical. The early recovery of nitrifying activity in Telone treated soils in the laboratory supports this conclusion.

If this conclusion is correct, then the delays in rapid nitrification observed in fumigated soils in the field were due to factors which restricted growth and reproduction of the nitrifiers without hampering the activity of those present. Antagonism by heterotrophic components of the recovery population may have been involved. Inhibition by excessive NH_4^+ is suggested by the synergism observed between fumigant and $(NH_4)_2SO_4$ fertilizer. However, the very marked reduction of the delay period by nitrate fertilizers (both the ammonium and the calcium salt) suggest that nitrate itself may have stimulated the growth of the nitrifiers. It may be relevant to note that the incubation study was conducted with soil which contained initially 142 ppm of nitrate nitrogen.

In the case of the non-fumigant fertilizer additive N-Serve, specific inhibition of nitrification was observed 4 weeks after treatment only at 20°C. At this time, some stimulus to bacterial numbers was expressed at 10° and 20°, and CO_2 evolution remained at a higher level than in untreated soil through most of the incubation period at 10° C. Significantly reduced levels of nitrate and total mineral nitrogen at all three temperatures appeared to be due to immobilization of the ammonium nitrogen added with the chemical.

It has recently been reported that this nitrification inhibitor

is inactivated by sorption on organic matter.[5] The results reported here for an organic soil suggest that this sorption of N-Serve may be accompanied by a simultaneous irreversible complexing of NH_4^+.

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5 Goring, C.A.I. 1962. Control of nitrification by 2-chloro-6-(trichloromethyl) pyridine. Soil Sci. 93:211-218.

GENERAL DISCUSSION

The significant effect of fumigation on the nitrogen economy of soils and crops is to alter the seasonal distribution of ammonium and nitrate. This is a conclusion reached in earlier investigations in Michigan by Wolcott, Maciąk et al (83). It was substantiated by the field and laboratory studies reported here and is consistent with results reported by numerous investigators down through the years (48)(68)(69)(71)(82).

These effects on the seasonal pattern of nitrogen transformations are mediated by partial sterilization effects on both heterotrophic and autotrophic components of the soil microbial population (78)(31)(32). The results of the present incubation experiment demonstrate that these partial sterilization effects may be projected over long periods of time where soils are subjected to reduced temperatures after initial exposure and aeration to remove volatile fumigant chemicals. It appeared that these residual effects were generated by heterotrophic components of the recovery population, and were not due to retention of toxic quantities of the chemicals themselves in the soil.

In field studies, high concentrations of ammonium nitrogen (200 to 350 ppm) and wide $NH_4^+ : NO_3^-$ ratios (up to 6:1) were encountered in May in organic soil fumigated in the fall with 32 to 48 gpa of Telone (mixture of dichloropropenes). Such abnormally high proportions of ammonium to nitrate may seriously impair metabolism in some plants(23)(51)(55)(47)(49)(50)(83). Celery appears to be a crop which is sensitive to such imbalances. Early injury from fumigation was corrected in 1959 and 1960 by sidedressings of nitrate fertilizers.

However, the residually enhanced levels of heterotrophic activity in fall fumigated organic soils give rise also to reductive soil con-

ditions the following season. As a result, soil fumigation increases the availability of reducible nutrients, notably manganese (15)(38)(48).

Such increases in manganese availability were responsible for dramatic increases in yields of sweet corn and lettuce in fumigated soil in 1961.

With celery, increased availability of iron was reflected by significantly increased concentrations in tissue samples taken in 1960 and 1961.

Under conditions of limiting manganese and phosphorus nutrition in 1961, immobilization of phosphorus in the soil or in the conductive tissues of the plant by increased levels of available iron in fumigated soil resulted in significantly reduced yields of celery. Under these conditions, there was no response to early sidedressings of $Ca(NO_3)_2$.

The oxidation state and the solubility of iron and manganese are strongly influenced by soil reaction. Various fertilizer nitrogen sources differ in their direct and indirect effects on soil pH. For this reason, responses to different nitrogen carriers cannot be ascribed indiscriminantly to differences in availability or assimilability of the nitrogen. The early response of celery to nitrate fertilizers in 1959 and 1960 appeared, however, to be specifically a response to the nitrate form of nitrogen. No such response to nitrate was obtained in 1961 with celery, - nor with sweet corn or lettuce. The latter two crops responded to $(NH_4)_2SO_4$, but leaf symptoms and tissue analysis revealed that this was a response to manganese rather than nitrogen.

Although the early response of celery to nitrate in 1959 and 1960 appeared to have been a specific response to the nitrate form of

nitrogen, the possibility remains that it was also a reflection of the much earlier rapid disappearance of toxic concentrations of ammonium. There was evidence in each of the three years that fertilizer nitrate greatly accelerated the recovery of full nitrifying capacity in fumigated soil, whereas $(NH_4)_2SO_4$ extended the delay period.

The basis for stimulation of nitrification by nitrate is not readily apparent. The possibility presents itself that the Nitrosomonas group of nitrifiers may be able to use nitrate as a terminal electron acceptor in growth metabolism. In 1960, almost complete disappearance of applied nitrate immediately preceded full recovery of nitrifying capacity in fumigated plots which had been compacted in an area subject to impeded drainage. The nitrate lost in plots which had received $Ca(NO_3)_2$ or NH_4NO_3 appeared almost quantitatively as NH_4^+.

If reduction of nitrate by nitrifiers under poorly aerated conditions actually contributed to the observed transformation, the Nitrosomonas group, rather than Nitrobacter, appears more likely to have been implicated. Qualitative tests revealed no accumulations of nitrite in fumigated plots, - even when traces were occasionally found in unfumigated soil in the spring of the year. This would suggest that nitrification was blocked at the first step, leading from NH_4^+ to NO_2^-.

The fertilizer additive, N-Serve, appears to have no appreciable direct effect on the heterotrophic population. Its inhibitory effect on the autotrophic nitrifiers is reported to be greatly reduced in soils high in organic matter.[5] However, a significant effect on both NH_4^+ and NO_3^- levels in Houghton muck was observed in the field, and

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[5] See footnote, P. 106

this effect was additive when combined with fumigation. The retarded nitrification of $(NH_4)_2SO_4$ delayed the development of the residual acidity associated with this carrier sufficiently to reduce manganese availability and yields of lettuce in 1961, under conditions of critically deficient manganese nutrition.

Laboratory studies with N-Serve suggested a simultaneous complexing of the chemical and NH_4^+ by organic matter. Appreciable release of the complexed nitrogen was not observed during nearly four months of incubation at 30° C. If this observation is borne out by further work, it suggests the use of this chemical as a direct soil additive to retard subsidence of organic soils and to reduce accumulations of nitrate late in the season. Crops such as sugar beets are reduced in yield and quality on organic soils by reason of excessively high nitrogen nutrition during the fall.

SUMMARY

The effects of chemical fumigants and a non-fumigant nitrification inhibitor on nitrogen transformations in soil and on availability of nutrients to crops were studied in the field and in the laboratory. An organic soil known to be free of nematodes was used.

Marked effects on the seasonal distribution of NH_4^+ and NO_3^- were observed following fall application of a fumigant chemical in the field. Laboratory studies confirmed field observations which indicated that these effects were due to readjustments in the heterotrophic and autotrophic components of the recovery population rather than to retention of toxic concentrations of the fumigant in the soil through the winter.

The non-fumigant chemical, applied as a fertilizer additive on $(NH_4)_2SO_4$ in the laboratory, inhibited nitrification without appreciably influencing heterotrophic activities. Simultaneous irreversible complexing of the chemical and NH_4^+ by organic matter appeared to be responsible for the uniform reduction in total mineral nitrogen which was maintained for long periods after addition of this material to the soil.

Enhanced mineralization of organic nitrogen and retarded nitrification in fall fumigated soil resulted in large accumulations of NH_4^+, reaching maxima of 200 to 350 ppm in May or early June. Wide ratios of NH_4^+ to NO_3^-, ranging up to 6:1, appeared to be injurious to early growth of celery. Application of nitrate fertilizer corrected this early injury during two seasons. In the third season, under conditions of limiting manganese and phosphorus nutrition, there was no response to fertilizer nitrate. Foliar analysis indicated that increased availability of iron in fumigated soil had interferred with

uptake or translocation of phosphorus and significantly reduced the yields of celery. In the same season, and in the same experiment, yields of sweet corn and lettuce were significantly increased by the increased availability of manganese in fall fumigated soil.

These increases in availability of iron and manganese were attributed to the reductive conditions associated with enhanced heterotrophic activity in the recovery population.

In the case of lettuce, an additive response to $(NH_4)_2SO_4$ at all levels of fumigation was shown by foliar analysis to be due to an additive effect on manganese availability resulting from the acidifying action of this nitrogen carrier. Leaf symptoms in sweet corn showed a similar manganese response to $(NH_4)_2SO_4$ prior to silking, but additional increases beyond the fumigation response were not significant.

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APPENDIX

Table 21. - Distribution of significant F ratios among various comparisons of the effects of temperature and chemical treatments on total mineral nitrogen (NH_4^+ plus NO_3^-) in incubated Houghton muck.

Source of variation	F ratios significant at 5% (*) and 1% (**)					
	Days under controlled temperature					
	28	43	62	72	90	118
	A. Combining three temperatures					
Temperature	22.98*	113.17**	106.22**	27.32*	30.74*	318.34**
Check vs. individual treatments	11.37**	14.82**	9.49**	6.79**	3.10**	2.36*
Check vs. all fumigants	-	4.67*	-	-	-	-
Clpcrn. vs. other fumigants	42.92**	52.09**	19.13**	10.49**	-	-
Among 3 fumigants	3.42*	-	4.66*	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	9.97**	12.14**	9.83**	11.96**	6.85**	4.41*
Among 3 rates	5.38**	7.87**	10.23**	-	4.50*	-
Fumigants at low rates	6.32**	5.64**	3.32*	7.25**	-	3.44**
Fumigants at med. rates	-	-	-	4.86*	-	-
Fumigants at high rates	-	-	-	-	-	-
	B. At 10° C.					
Check vs. individual treatments	7.47**	11.82**	23.67**	8.46**	-	-
Check vs. all fumigants	-	5.86*	-	-	-	-
Clpcrn. vs. other fumigants	41.26**	62.80**	80.95**	22.88**	-	-
Among 3 fumigants	-	7.68**	-	-	-	-
Rates of Vidden-D	-	-	7.14**	6.43*	-	-
Rates of Telone	-	-	12.73**	-	-	-
Rates of Fumazone	4.38*	4.52*	18.35**	17.34**	-	-
Among 3 rates	-	5.35*	32.72**	11.07**	-	-
Fumigants at low rates	-	-	-	4.45*	-	-
Fumigants at med. rates	-	6.50*	-	-	-	3.97*
Fumigants at high rates	-	-	-	7.55**	-	-

C. At 20° C.

Check vs. individual treatments	3.32*	5.41**	2.96*	-	-	-
Check vs. all fumigants	-	-	-	-	-	-
Clpcrn vs. other fumigants	12.11**	20.48**	5.71*	-	-	-
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	-	-	-	-	-	-
Among 3 rates	-	-	-	-	-	-
Fumigants at low rates	-	-	-	-	-	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	-

D. At 30° C.

Check vs. individual treatments	21.48**	5.24**	-	-	-	8.98**
Check vs. all fumigants	-	-	-	-	-	-
Clpcrn. vs. other fumigants	12.79**	4.86*	-	-	-	-
Among 3 fumigants	6.09*	-	-	-	-	10.28**
Rates of Vidden-D	6.32*	-	-	-	-	-
Rates of Telone	7.55**	-	-	-	-	-
Rates of Fumazone	35.90**	9.48**	-	-	5.93*	15.31**
Among 3 rates	35.66**	7.86**	-	-	-	9.97**
Fumigants at low rates	11.58*	-	-	-	4.02*	7.70**
Fumigants at med. rates	7.21**	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	11.01**

Table 22. - Distribution of significant F ratios among various comparisons of the effects of temperature and chemical treatments on levels of ammonium nitrogen in incubated Houghton muck.

Source of variation	F ratios significant at 5% (*) and 1% (**)					
	Days under controlled temperature	28	43	62	72	90	118
	A. Combining three temperatures						
Temperature (T)		-	-	-	-	-	-
Check vs. individual treatments		106.64**	654.88**	-	20.42**	6.87**	6.28**
Check vs. all fumigants		18.10**	63.03**	-	-	-	-
Clpcrn. vs. other fumigants		1,134.62**	70,830.42**	49.18**	221.48**	73.32**	67.87**
Among 3 fumigants		-	-	-	-	-	-
Rates of Vidden-D		-	-	-	-	-	-
Rates of Telone		-	-	-	-	-	-
Rates of Fumazone		-	-	-	-	-	-
Among 3 rates		-	-	-	-	-	-
Fumigants at low rates		-	-	-	-	-	-
Fumigants at med. rates		-	-	-	-	-	-
Fumigants at high rates		-	-	-	-	-	-
	B. At 10° C.						
Check vs. individual treatments		19.81**	300**	1,694**	16,299**	-	1,695**
Check vs. all fumigants		7.75*	-	201.83**	1,821.05**	-	150.92**
Clpcrn. vs. other fumigants		186.88**	324.24**	18,258.50**	175,940**	11.84**	18,351.23**
Among 3 fumigants		-	-	-	-	-	-
Rates of Vidden-D		5.74*	-	-	-	-	-
Rates of Telone		-	-	-	-	-	-
Rates of Fumazone		-	-	-	-	-	-
Among 3 rates		4.21*	-	-	-	-	-
Fumigants at low rates		-	-	-	-	-	-
Fumigants at med. rates		-	-	-	-	-	-
Fumigants at high rates		-	-	-	-	-	-

C. At 20° C.

Check vs. individual treatments	342.8**	624**	205**	501**	139**	3.94*
Check vs. all fumigants	41.84**	52.86**	20.96**	45.67**	15.33**	-
Clpcrn. vs. other fumigants	3,693.52**	6,748.27**	2,210.54**	5,412.38**	1,493.61**	46.62**
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	-	-	-	-	-	-
Among 3 rates	-	-	-	-	-	-
Fumigants at low rates	-	-	-	-	-	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	-

D. at 30° C.

Check vs. individual treatments	811.94**	933**	3.49*	995**	-	-
Check vs. all fumigants	69.38**	11.32**	-	-	-	-
Clpcrn. vs. other fumigants	8,786.28**	1,006.62**	37.78**	100.81**	-	-
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	-	-	-	-	-	-
Among 3 rates	-	-	-	-	-	-
Fumigants at low rates	-	-	-	-	-	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	-

Table 23. - Distribution of significant F ratios among various comparisons of the effects of temperature and chemical treatments on levels of nitrate nitrogen in incubated Houghton muck.

Source of variation	Days under controlled temperature	F ratios significant at 5% (*) and 1% (**)					
		28	43	62	72	90	118
		A. Combining three temperatures					
Temperature (T)		11.77*	94.71**	141.72**	34.722**	59.94**	190.89**
Check vs. individual treatments		-	56.37**	19.53**	11.84**	3.38**	2.91**
Check vs. all fumigants		-	-	-	-	-	-
Clpcrn. vs. other fumigants		17.88**	544.35**	180.79**	99.26**	20.29**	15.81**
Among 3 fumigants		-	4.18*	-	-	-	-
Rates of Vidden-D		-	-	-	-	-	-
Rates of Telone		-	-	-	-	-	-
Rates of Fumazone		-	15.39**	5.01**	6.77**	-	-
Among 3 rates		-	13.77**	5.85**	-	-	-
Fumigants at low rates		-	6.47**	-	5.06**	-	-
Fumigants at med. rates		-	-	-	-	-	-
Fumigants at high rates		-	-	-	-	-	-
		B. At 10° C.					
Check vs. individual treatments		6.20**	17.01**	60.03**	23.16**	-	3.81*
Check vs. all fumigants		-	-	-	-	-	-
Clpcrn. vs. other fumigants		44.52**	153.55**	518.35**	189.64**	-	30.29**
Among 3 fumigants		-	5.06*	-	-	-	-
Rates of Vidden-D		-	-	8.63**	-	-	-
Rates of Telone		-	-	13.49**	-	-	-
Rates of Fumazone		-	4.88*	196.33**	18.70**	-	-
Among 3 rates		-	4.76*	36.59**	11.67**	-	-
Fumigants at low rates		-	-	-	4.81*	-	-
Fumigants at med. rates		-	4.49*	-	-	-	-
Fumigants at high rates		-	-	-	8.16*	-	-

C. At 20° C.

Check vs. individual treatments	5.21**	24.06**	16.38**	7.52**	13.25**	-
Check vs. all fumigants	-	-	-	-	-	-
Clpcrn. vs. other fumigants	34.78**	229.04**	158.88**	68,538**	125.56**	15.17**
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	-	-	-	-	4.08*	-
Rates of Fumazone	-	5.02*	-	-	-	-
Among 3 rates	-	-	-	-	-	-
Fumigants at low rates	-	-	-	-	4.82	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	-

D. At 30° C.

Check vs. individual treatments	32.51**	21.62**	-	-	-	-
Check vs. all fumigants	-	-	-	-	-	4.77*
Clpcrn. vs. other fumigants	303.65**	204.92**	14.17**	-	-	-
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	6.51*	-	-	-	-	-
Rates of Fumazone	10.73**	7.06**	-	-	6.09*	4.30*
Among 3 rates	9.69**	6.39*	-	-	4.01*	-
Fumigants at low rates	-	-	-	-	4.17*	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	6.36*	-	-	-	-	-

Table 24. - Distribution of significant F ratios among various comparisons of the effects of temperature and chemical treatments on geometric mean numbers of bacteria and fungi in incubated Houghton muck.

Source of variation	F ratios significant at 5% (*) and 1% (**)					
Days under controlled temperature	25		68		117	
Microbial group	Bact.	Fungi	Bact.	Fungi	Bact.	Fungi
A. Combining three temperatures						
Temperature (T)	-	-	-	-	-	-
Check vs. individual treatments	10.38**	-	-	-	-	-
Check vs. all fumigants	7.82**	-	-	-	4.91*	-
Clpcrn. vs. other fumigants	97.89**	-	-	5.16*	-	-
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	-	-	-	-	-	-
Among 3 rates	-	3.22**	-	-	-	-
Fumigants at low rates	-	-	-	-	-	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	-
B. At 10° C.						
Check vs. individual treatments.	-	2.91*	-	-	-	-
Check vs. all fumigants	-	-	-	-	-	-
Clpcrn. vs. other fumigants	6.86*	13.91**	-	-	-	-
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	3.86*	-	-	-	5.50*
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	-	-	-	-	-	-
Among 3 rates	-	-	-	-	-	-
Fumigants at low rates	-	-	-	-	-	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	4.43*

		C. At 20° C.				
Check vs. individual treatments	17.13**	-	-	-	-	-
Check vs. all fumigants	22.51**	-	-	-	-	-
Clpcrn. vs. other fumigants	140.03**	-	6.86*	-	-	-
Among 3 fumigants	4.47*	-	-	-	-	-
Rates of Vidden-D	6.15*	-	-	-	-	-
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	-	-	-	-	-	-
Among 3 rates	3.93*	-	-	-	-	-
Fumigants at low rates	5.38*	-	-	-	-	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	-
		D. At 30° C.				
Check vs. individual treatments	6.70**	-	-	-	-	-
Check vs. all fumigants	-	-	-	-	-	-
Clpcrn. vs. other fumigants	25.00**	-	-	6.27*	-	-
Among 3 fumigants	-	-	-	-	-	-
Rates of Vidden-D	-	-	-	-	-	-
Rates of Telone	-	-	-	-	-	-
Rates of Fumazone	-	-	-	-	-	-
Among 3 rates	-	-	-	-	-	-
Fumigants at low rates	-	-	-	-	-	-
Fumigants at med. rates	-	-	-	-	-	-
Fumigants at high rates	-	-	-	-	-	-

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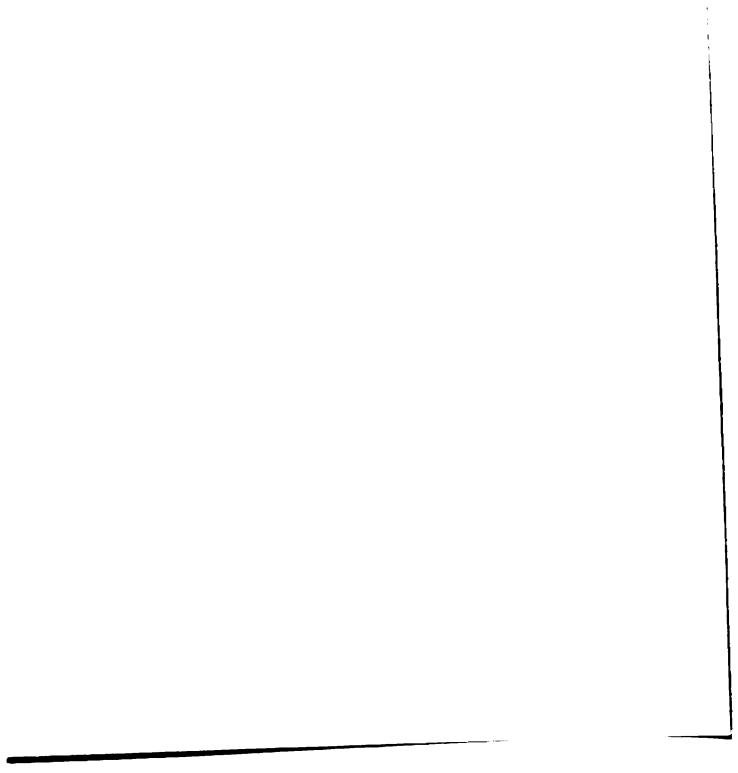

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