THE EFFECT OF SELECTED PESTICIDES ON TURFGRASS GROWTH AND NITROGEN TRANSFORMATIONS IN SOIL

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ABSTRACT

THE EFFECT OF SELECTED PESTICIDES ON
TURFGRASS GROWTH AND NITROGEN TRANSFORMATIONS
IN SOIL

By James W. Timmerman

A study was made of the effects of selected pesticides on turfgrass growth, interactions with turf fertilizers, and nitrogen transformations in soil. In general, the two organophosphate insecticides B25141 and Diazinon and the fungicide Tersan-OM increased top growth, per cent nitrogen in the leaf tissue and nitrogen uptake during certain periods of growth in both the greenhouse and field experiments. Turf color was also improved by these pesticides in the field. Acti-dione thiram depressed growth in the greenhouse. All other pesticides had no effect. These effects were more noticeable in the first month after application, and were magnified when 10 times the recommended rate of the pesticides were used.

Soil mineral nitrogen (ammonium + nitrate) in pots receiving Milorganite as the nitrogen source was significantly higher than the no pesticide treatment when treated with B25141 and Cadminate at the recommended rate, and B25141, Diazinon, Dieldrin, and acti-dione thiram at 10 times the recommended rate in the greenhouse. This was not observed in the field.

In a laboratory incubation study, Tersan-OM applied at rates of possible accumulations over a period of time increased ammonification and nitrification in soil receiving Milorganite as the nitrogen source. Diazinon, B25141, Dieldrin, and Calo Clor at these rates resulted in inhibition of ammonification. All pesticides caused significant changes from the control soil in patterns of ammonification and nitrate accumulation from Milorganite.

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By

James W. Timmerman

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INTRODUCTION

The sport of golf today is enjoying it's greatest popularity. This is partly due to the impact of television, increasing population, and increasing leisure time of the people. Years ago, a golf course could expect around 5,000 to 7,000 rounds of play per year. Today, many courses have 20,000 to 50,000 rounds per year. This figure can be expected to rise as the population increases and lighted courses become prevalent.

As a result of this increase in play, conditions under which the turf is grown have been affected. One fact stands out clearly; growing conditions have so been altered that greater stress has been put on the actively growing turf. For example, increased irrigation and traffic lead to greater compaction of soil, causing a reduction in the oxygen supply available for plant growth. Furthermore, this added stress has been responsible for an increasing susceptibility of the grass plants to be injured from disease and soil insects and for weeds to invade turf areas. Compounding this situation is the problem that today's player demands the finest playing conditions that turf managers can produce. All this has led the turf manager of today to use increasing amounts of pesticides to control plant parasites and weeds.

Outside the area of golf courses, there has been an increased emphasis on quality home lawns with high maintenance requirements. Requirements have also risen for industrial grounds, parks, cemeteries, and school grounds. The trend here is toward applying more pesticides, either separately or contained in fertilizers, at increased levels.

Much research has been done in the area of how effective these pesticides are in controlling plant parasites and weeds. However, little research has been done on the possible effects these pesticides have on plant growth, other than controlling the target species.

Considerable research has been conducted with pesticides as they affect mineralization and nitrification of soil nitrogen. In the realm of turfgrass management, a number of different nitrogen fertilizers are used. Certain of these fertilizers require breakdown by the microbial population in the soil for release of available nitrogen. It is in this area that information is lacking on the possible effects pesticides may have on microbial activity and transformations of fertilizer and soil nitrogen.

It is the purpose of this investigation, first to study the effect of selected turf pesticides on turfgrass growth; second, to study possible interactions between these pesticides and turfgrass fertilizers; and third, to study what effect these pesticides have on nitrogen transformations in soil.

LITERATURE REVIEW

Turf fungicides and insecticides in current use have not been extensively studied as to their effects on turfgrass growth, other than controlling target species. Research on the fungicides in this respect is especially lacking. The following review of literature will focus on what is known of the effects pesticides have on plant growth and microbial activity.

Effects of Pesticides on Plant Growth - Beneficial

Ever since pesticides were first applied to plants and soil to control plant parasites and weeds, agricultural scientists have been interested in the effects these chemicals exert on plants, soils and non-target organisms, as well as on the target organisms.

If parasites or weeds are retarding plant growth and a pesticide application to the soil or plant reduces the concentration of the parasite, the normal effect is improvement in plant growth. Sometimes, however, a pesticide will improve plant yield in the apparent absence of the parasite or when a known parasite is not killed. This type of response has been called Increased Growth Response (35) and its cause ascribed to the killing of unrecognized parasites or to various side effects. Early workers believed this IGR was due to the increased availability of plant nutrients from the decomposition of the killed microbial cells, from the decomposition of soil humus, or from the pesticidal chemicals.

Fred (18) was of the opinion that, while moderate to large concentrations of a chemical might be toxic, small amounts could stimulate plant growth. Other investigators (48,63) believe that improved biological control by Trichoderma and other organisms are the factors responsible for IGR.

Several interesting biological control effects could be mentioned. Vaartaja (63) has shown that certain strongly antagonistic fungi and bacteria are relatively tolerant to some of the fungicides and fumigants. This fact was made use of by Bliss (4) who reported that treatment of infested soil with CS_2 did not kill Armillaria mellea (oak root fungus), but Trichoderma viride, which became dominant after treatment, parasitized and killed the fungus. Garret (21) stated that the CS_2 may not kill the root parasite, but may weaken it, and T. viride, stimulated by the treatment, finishes the job.

Muje et al (39) noted that certain chemicals added to the soil would stimulate growth of specific fungus species. Acrylic acid, sorbic acid, and furoic acid greatly stimulated growth of T. viride. In a greenhouse study (35) to determine if Phytophthora cinnamoni-infested soil treated with these chemicals would improve growth of avocado seedlings, they found the treatments stimulated growth of T. viride, improved growth of the avocado seedlings, but did not kill the P. cinnamoni.

In the area of physiological growth responses to pesticides, a number of effects have been investigated.

Ries et al (51) showed that peach and apple trees, growing in a soil sprayed with a mixture of simazine and amitrole-T had a higher leaf nitrogen content and longer terminal growth than trees grown in a weed-free environment. They suggested that the mixed herbicide, which is applied to the soil and not the plant, affected nitrogen uptake or nitrogen metabolism.

Freney (19) reported that simazine increased the yield of corn tops by 36%, and nitrogen uptake by 37%, but did not increase mineralization of soil nitrogen. These results suggest that simazine increased plant growth by a direct effect on plant metabolism and not through any interaction with the soil.

In other experiments by Ries and co-workers (49,50,62), simazine was shown to increase growth and nitrogen content of corn, and this effect was not due to a lack of weed competition nor to the additional available nitrogen in simazine. These responses to simazine occur in plants grown with nitrate, but not in plants grown with ammonium as the nitrogen source, and are greatest when nitrate and temperature are at sub-optimal levels. They also reported nitrate reductase activity in corn growing on sub-optimal levels of nitrate increased in a linear fashion with simazine concentration. From these observations, they presented the hypothesis that simazine enhanced nitrate utilization by increasing nitrate reductase activity.

Cooke (10) showed an increase in the soluble and total nitrogen content of legumes treated with monuron, although no increase in plant growth was found.

Roots of bluegrass treated with DCPA, amine methylarsonate, calcium propylarsonate and DMPA were shown by Singh and Campbell (54) to be significantly longer than on control plants.

Thompson (61) observed that bermudagrass turf sprayed with Daconil 2787 fungicide exhibited a darker green color than either the check or plots receiving kromad or velsicol 2/1 fungicide, although the plots receiving the latter two were not more diseased.

Eno and Everett (14) results showed that heptachlor, TDE, and DDT increased germination of Stringless Black Valentine beans.

Published results from greenhouse and laboratory work with atrazine suggest that its use for pre-emergent weed control may also result in an increase in the efficiency of water use for corn production. Smith and Buchholtz (55,56) reported a reduction in transpiration of several species, including 40% by corn and 65% by soybean plants six hours after addition of 20 ppm of atrazine to their nutrient solution. Transpiration was reduced in plants grown on atrazine-treated soil as well. Foliar applications were also effective.

Effects of Pesticides on Plant Growth - Detrimental

Occasionally, following the application of a pesticide which kills a known plant parasite or weed, plant growth is not improved and may

even be further retarded. Various factors may cause these unexpected results.

Almost any pesticide, if present in the soil in high enough concentrations will injure sensitive plants. Therefore, if insufficient time is allowed for a residual fumigant, fungicide, insecticide or herbicide to decompose, volatilize or leach out of the plant root area, the chemical itself may retard growth of sensitive crops. If the dosage is high, the temperature low, the soil excessively wet or high in colloids, longer intervals between pesticidal treatments will be necessary.

Repeated applications of the persistent chlorinated hydrocarbon insecticides may increase concentration to levels which are toxic to sensitive plants. Martin (34) noted that concentrations of residual DDT, toxaphene, chlordane, and other chlorinated hydrocarbons were high enough to injure some plants. Cucumbers, cantaloupes, watermelons, some varieties of beans, and potatoes were sensitive to one or more of the insecticides. Morrison et al (38) found that 137.5 pounds of DDT per acre caused severe stunting to bush and pole beans. Tomato transplants were slightly stunted and had small leaves.

Eno and Everett (14) showed that BHC reduced germination of Black Valentine beans 14.3% compared to the check. Root and top weights were reduced by chlordane, lindane, aldrin, dieldrin, TDE, and BHC.

Martin and Pratt (37) point out that inorganic end products, such as bromides and chlorides, released during the decomposition of pesticides may increase to sufficiently high levels to injure plants. Several crops are sensitive to bromine containing organic compounds or to the bromide ion. Included are beans, cabbage, celery, onions, peas, and potatoes. Often, citrus trees replanted in soil previously treated with high dosages of ethyiene dibromide to control citrus nematodes, grow much slower initially than trees planted in soil treated with other fumigants (36).

Sherman and Fujimoto (53) showed that increases in extractable or soluble Mn, Cu, of Zn following soil fumigation may be sufficient to cause plant injury.

Another little-understood temporary toxicity observed by Martin (34) may occasionally occur when soils are treated with a chemical which kills a large number of soil organisms. The toxicity may last from a few weeks to a year. Affected plants fail to absorb phosphorous even though the treatments may have increased the soluble or extractable soil phosphorous. The plants cease growing, but continue to adsorb Na, K, and B, which may reach toxic levels. With time and in a spotty manner, the toxic condition dissipates and the plants resume normal growth.

In the area of turfgrasses, certain effects on growth have been observed. Gaskin (23) reported that Kentucky bluegrass plants treated with crabgrass herbicides showed reduction of rhizome number and length and number of tillers. Zytron, dacthal, and trifluralin produced the greatest reduction at 1 1/2 times the standard rate of application.

Bandane and chlordane reduced rhizome development only at 1 1/2 times the standard rate. Crab-e-rad reduced the number of tillers and length of rhizomes.

Engel and Callahan (13) showed that bensulide, terbutol, chlordane, DMPA, and DCPA reduced top growth and that bensulide and terbutol seriously inhibited root development.

Singh and Campbell (54) noted that bluegrass turf treated with trifluralin and polychlorodicyclopentodiene resulted in considerably reduced turf density.

Jagschitz and Skogley (27) presented data that showed dacthal caused a reduction in turfgrass cover where nitrogen was not used. Red fescue was reduced more than bentgrass or bluegrass. Where nitrogen was applied no apparent reduction of turfgrass was caused by the addition of dacthal.

Effects of Pesticides on Microbial Numbers

Pesticides added to the soil can cause varied effects. First, there is the possibility of a direct, toxic action on a microorganism by affecting some aspect of its essential metabolic activity. Second, the pesticide may have a selective toxicity for certain groups of microorganisms, and third, pesticides may promote, either directly or indirectly, the growth of one or more types of soil organisms.

One of the earliest and most striking cases of increase in bacterial numbers in the field, due to the utilization of an herbicide as a substrate for growth, is that of calcium cyanamide. Allison (1) showed that over a period of one or two weeks after application the total bacterial number can increase over thirty times and this is correlated with a general increase in soil respiration.

In a very extensive review of the phenoxy herbicides, Audus (3) points out that in some cases adverse and beneficial effects were noted, but on the whole the great majority of observations show that with normal practical rates of application there are no adverse effects of the phenoxy herbicides on the total number of microorganisms in the soil.

The effects of the halogenated aliphatic acids on the total soil populations are as diverse as those of the phenoxy herbicides. Kratochvil (30) reports a transitory reduction induced by herbicidal TCA, while Hale (25) notes that Dalapon has a slight stimulating effect on the general soil population.

The carbamates show the same diversity of effect. Reduction in the total numbers, as measured by soil respiration, has been reported with propham by Kratochvil (30) but could not be detected by Newman (42). EPTC temporarily depresses soil respiration (9); while, on the other hand, chloropham apparently increases it (60).

Audus (3) states that the nitrophenol herbicides are quite toxic to all soil microorganisms, although marked inhibitory effects are not always observed at normal rates of application.

Chandra et al (9) showed that simazine temporarily increased the carbon dioxide evolution 12 to 14% in two soil types.

Observations by Dubey (11) show that prometryne at 100 ppm had practically no effect on microbial population. Ametryne at 100 ppm reduced the actinomycete population in heavy soils, while diuron and picloram at 100 ppm reduced the actinomycetes in all types of soils.

In early studies on insecticides by Smith and Wenzel (57), DDT was shown to have no detrimental effects on soil microbes when applied up to 400 pounds per acre.

Wilson and Choudri (69), in laboratory studies, showed that BHC and DDT 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 (5) found BHC isomers added to clay adobe soil at 1000 ppm varied in their effects on the number of bacteria and molds which developed during incubation. They also made field application of gamma BHC at 20 pounds per acre on several loams and found no effects. These same investigators (6) also report that parathion, dieldrin, toxaphene and EPN applied at 10 pounds per acre on several loams had no immediate harmful effects on soil microorganisms.

Fletcher and Bollen (16) determined that aldrin at 200 to 1000 ppm in several types of soil had a stimulating influence upon the total number of soil organisms.

Richardson (48) showed that the fungicides thiram, nabam and ferbam increased the numbers of bacteria in the soil. Thiram and ferbam reduced the number of fungi; whereas, nabam killed nearly all fungi in the soil. The actinomycetes were unaffected by all the chemicals.

Effects of Pesticides on Ammonification and Nitrification

Ammonification is a very essential part of the nitrogen cycle. It represents the first stage in the decomposition of complex nitrogenous compounds of plant and animal origin and is carried out by the decay organisms in the soil. Whenever these microorganisms decompose organic matter, they set free more nitrogen than they are able to assimilate into their own protoplasm when excess nitrogen is present. Under aerobic conditions, the excess nitrogen appears in the soil as ammonium.

Several investigators (17,28) have been able to demonstrate no adverse effects from 2,4-D on ammonification.

Jones (29) observed that DDT applied to the soil at less than 0.1% did not injure any ammonifiers. Pochon (45) concluded that simizine and other triazine herbicides are similarly without action on ammonifying bacteria.

Allison (1) points out that cyanamide can itself be ammonified and thus contributes to total ammonium in the soil.

Nitrification is the next stage in the nitrogen cycle, and is one of the most important phenomena occurring in soils. More attention has been given to nitrification because it is easier to observe in both the field and laboratory than is ammonification. It includes two steps. First, ammonium is transformed to nitrite, which is an unstable compound; and second, nitrite is oxidized to nitrate. Nitrosomonas and Nitrobacter are the dominant microorganisms among the nitrifying population, which includes heterotrophic bacteria, actinomycetes and fungi.

The effects of arsenic compounds on the activity of these organisms in soils enriched by perfusion of ammonium chloride solutions have been followed by Quastel and Scholefield (46). They found that M/400 sodium arsenate had no effects on the activity, but that the same concentration of sodium arsenite halved the rate of ammonium oxidation and completely inhibited nitrite oxidation. This constituted a direct effect on the enzymatic processes themselves, since the soil columns used contained saturating populations of nitrifying organisms.

The phenoxy herbicides at normal field rates of application have either no effect or only a transitory one in the soil, although Flieg (17) showed that the activity of nitrifying organisms in culture can be considerably checked by such concentrations of 2,4-D. The addition of soil removes this inhibition, a phenomenon which may be related to the adsorption of 2,4-D by the soil colloids or to its degradation by other soil organisms.

From the majority of reports, it would appear that the chlorinated aliphatic acid herbicides are quite toxic to the nitrifying bacteria (44). The effect is temporary, however, and recovery can be complete in three weeks to a month (71).

A number of investigators (25,47,60) have determined that the carbamates and acetamide herbicides and their homologues all inhibit nitrification in soils. However, with normal rates of application recovery takes place after a month. This again suggests that the nitrifiers acquire some kind of tolerance to them.

Reports for monuron are conflicting. Quastel and Scholefield (47) claim it is a powerful inhibitor of nitrification; whereas, Hale (25) could not confirm these findings.

Dubey (11) found that prometyrne did not affect nitrification. Ametyrne at 100 ppm inhibited the Nitrobacter bacteria. This effect lasted longer in heavy soils. Diuron showed no effect on nitrification in fertile loam soil, but in light and heavy-textured soils it caused increased inhibition of nitrification with increases in its level. Picloram inhibited nitrification in all soils.

Farmer et al (15) carried out studies on nitrification with a perfusion unit in the presence of varying concentrations of simazine. Inhibition of nitrification was noted at concentrations of 6 ppm and above. A similar effect was noted with pure culture studies of Nitrobacter, but not with Nitrosomonas.

Several investigators (15,69) have shown no harmful effects to the nitrifiers from DDT.

Gray (24) observed that BHC and its isomers were toxic to the nitrifiers at a concentration of 0.01%.

Aldrin, dieldrin, and chlordane as reported by Brown (8), were shown to cause a significant retardation of nitrification in soil at the rate of 0.05% and 0.5%. Heptachlor and lindane decreased nitrification at 0.5% only, while DDT had little effect on nitrification.

Nishihara (43) found that diazinon, dithane, BHC, vapam and maneb are inhibitors of nitrification. He also points out that the effectiveness of these chemicals is increased under soil conditions which suppress the activity of the nitrifiers. Their effectiveness varied with the kind of soil.

Garretson (22) was able to show that aldrin, lindane and TDE at final medium concentration of 1 ug/ml were toxic to Nitrobacter agilis, with lindane being least toxic of the three. Malathion and parathion, two organic phosphate compounds, differed widely in their toxicity for Nitrobacter. Malathion was the least toxic, causing only delayed nitrification at 1000 ug/ml. Parathion, as toxic as aldrin, gave complete inhibition at 10 ug/ml or greater. Baygon, a methyl carbamate delayed nitrification by Nitrobacter at concentrations of 10 ug/ml. Lindane, malathion and baygon were also tested against Nitrosomonas europaea. All three compounds were at least 100 times more toxic for the Nitrosomonas culture than for the Nitrobacter.

METHODS AND MATERIALS

Greenhouse Experiment 1

In this study, the growth response of grass and nitrogen transformations in the soil were surveyed as affected by nine pesticides at two rates. Pennlawn creeping red fescue (Festuca rubra) was seeded in each of eighty 6-inch diameter pots. Each pot was lined with a polyethelene liner and 3,800 grams of an air-dried Hodunk sandy loam added. Soil test analysis showed this soil to be high in phosphorous and medium-high in potassium for turfgrasses, and to have a pH of 6.6. The cation exchange capacity was 5.65 me/100 g soil.

Milorganite, described in Table 2, was applied at the rate of 2/3 pound nitrogen per 1000 square feet at seeding time and on the fifteenth of each month until the experiment was completed. No additional phosphorous or potassium was added. The plants were grown in the greenhouse on tables with supplemental light to provide a photo period of 12 hours or longer until daylength reached this duration. Position of the pots on the tables was changed periodically to reduce border effects. Watering was done every third day or as needed to bring the soil up to field capacity.

Analysis and rate of application of the pesticides used are given in Table 1. Each pesticide was applied at the recommended rate and 10 times the recommended rate of application. Treatments were started March 14, 1967. The insecticides B25141, Diazinon, dieldrin, and

Table 1. Pesticide chemicals used in greenhouse, field, and laboratory experiments.

Material	Formula	A. I.*	Rate	Source
B25141	0,0-diethyl 0-(p-methylsulfinyl)phenyl)	64.2%	16 lbs/acre	Chemagro Corporation
Diazinon	0,0-diethyl 0-(2-isopropyl - 4 methyl-6-pyrimidinyl) phosphorothioate	48.0%	16 lbs/acre	Geigy Chemical Co.
Dieldrin	1,2,3,4,10,10-hexachloro-exo- 6,7,-epoxy-1,4,4a,5,6,7,8,8a,-octahydro-1,4,-endo-exo-5,8,dimethanonaphthalene	75.2%	3 lbs/acre	Shell Chemical Co.
Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro -.4,7-methanoindene	72.0%	10 lbs/acre	Velsicol Chemical Co.
Acti-dione Thiram	Thiram-(tetramethylthiuramisulfide) Cycloheximide-(3-(2-#,5, dimethyl - 2 oxocyclohexyl) - 2 - hydroxyethyl-glutaramide	75.0%	4 oz/1000 sq.ft.	Tuco Products Co.
Cadminate	Cadminate succinate Total cadmium	60.0% 29.0%	½ oz/1000 sq.ft.	Mallinckrodt Chemical Works
Calo Clor	Mercurous chloride Mercuric Chloride	60.0%	3 oz/1000 sq.ft.	Mallinckrodt Chemical Works
Dyrene	2,4-dichloro 6-0-chloroanilino - s - triazine	5.0%	4 oz/1000 sq.ft.	Chemagro Corporation
Tersan-OM	Thiram Hydroxymercurichlorophenl	45.0% 10.0%	4 oz/1000 sq.ft.	E.I.duPont Chemical Co.

* Active ingredient

chlordane were applied once a month and the fungicides Calo Clor, Cadminate, Tersan-OM, Dyrene and acti-dione thiram were applied every 14 days. All treatments were replicated four times.

The plants were clipped to a 1 1/2-inch height every 10 days. The clippings were collected and oven-dried at 35°C. Dry clipping weights were recorded on a monthly basis for three months. On June 14, 1967, soil samples were taken from each pot and the soil analyzed for total mineral nitrogen (ammonium + nitrate) by a micro-Kjeldahl procedure (7).

Greenhouse Experiment 2

In this experiment, the effects of four pesticides superimposed on nitrogen treatments of 0, 1/6, and 2/3 pounds of nitrogen per 1000 square feet per month for three nitrogen carriers were studied. The nitrogen analysis of these carriers is given in Table 2. Pennlawn creeping red fescue (*Festuca rubra*) was seeded in 140 six-inch pots. The preparation of the pots and the soil, the date and rate of seeding and other cultural practices used were the same as in experiment 1.

Table 2. Materials compared as sources of nitrogen.

Material	Formula	% N	Source
Ammonium nitrate	NH_4NO_3	33.5	Spencer Chem. Co.
Milorganite	Activated sewage sludge	7.0	Milwaukee Sewage Commission
Urea-formaldhyde	Mixture of methylene ureas	38.5	E. I. duPont Uramite

Analysis of the pesticides used and rates of application are given in Table 1. The insecticides B25141 and Diazinon were applied once a month and the fungicides Calo Clor and Cadminate were applied every 14 days. Treatments were started March 14, 1967, with four replications.

The plants were clipped to a 1 1/2 inch height every 10 days; clippings were oven-dried and weights recorded on a monthly basis for 5 months.

Field Experiment

The experiment was located on a five-year-old stand of Cohansey bentgrass (Agrostis sp.) turf at the Soils Research Farm of Michigan State University. The soil was a specially prepared soil mixture consisting of 2 parts coarse concrete sand, 1 part Hodunk sandy loam soil, and 1 part peat by volume. Individual plot size was 3 by 6 ft. and each plot was separated by a 6-inch strip of aluminum grass guard placed vertically between plots.

In the year of the test, 1967, the plots were fertilized once each in April and May, with a 10-10-10 grade fertilizer at the rate of 1 pound nitrogen per 1000 sq. ft. No additional fertilizer was applied until treatments were started.

Four pesticides, Diazinon, B25141, acti-dione thiram and Tersan-OM were used at rates outlined in Table 1. A no pesticide treatment was included in the study. Treatments were begun July 2, 1967, and applied every 10 days thereafter until completion of the experiment. The pesticides were applied with an Ortho Spray-ette hand sprayer, having a fan-type nozzle, and the pressure used was 40 psi. When spraying, a 3 by 6 by 3 ft. plywood box (no bottom) was placed over the plot. On August 15, the entire area was sprayed with cadminate fungicide to control a slight infestation of Dollar Spot disease.

Superimposed on these treatments were applications of 0 and 1 pound of nitrogen per 1000 square feet applied July 1, August 1, and September 1. Nitrogen carriers were ammonium nitrate and Milorganite described in Table 2. In addition, a treatment of Tersan-OM applied every 10 days at 10 times the recommended rate of Table 1 with no nitrogen was studied.

The area was irrigated when necessary, so as to keep the soil moisture as close as possible to field capacity and also following each nitrogen application.

The plots were mowed every 5 days to a 1/2-inch height with a Jacobsen greens mower. Clippings were collected from a 7.6 sq. ft. area of each plot at each mowing, oven-dried at 35° C and weights recorded on a monthly basis for three months.

Color quality ratings were taken for each plot at the end of each month. A 1-10 scale, with 1 being darkest green color and 10 being lightest green color was used for rating.

Density counts were obtained from two 3-inch plugs randomly selected from each plot. Counts were taken on October 5, 1967.

Soil samples were taken from each plot on October 2, 1967, and analyzed for ammonium nitrogen and nitrate nitrogen by a micro-Kjeldahl procedure (7).

The plots receiving Tersan-OM, Diazinon, and acti-dione thiram and the control with ammonium nitrate as the nitrogen source were tested for effects on microbial populations. Sampling dates were: for Diazinon, September 11; for Tersan-OM, September 27; and for acti-dione thiram, October 12, 1967. An estimate of the population of fungi, actinomycetes, and bacteria in treated and untreated soil was obtained by use of soil dilution plate counts. The actinomycetes population was estimated after 5 days growth on chitin agar (31), the fungal population after 5 days on OAES agar (66), and the aerobic bacteria after 5 days on soil extract agar (32). In addition, three other media were used for estimation of aerobic bacteria. These were variations of media developed by Valera and Alexander (64) and Woldendorf (70). Their composition is listed below:

<u>Media</u>:

#121 - Glucose - nitrate agar plus growth factors

#103 - Glucose - nitrate agar without growth factors

#120 - Glutamate - nitrate agar without growth factors

Aneorobic bacteria were estimated on media 103, 121, and 120 incubated in 98% N_2 and 95% CO_2 atmospheres for 7 days. Bacteria suppressed by the N_2 and CO_2, but still viable, were estimated by counting the additional colonies which had appeared 3 days after removal from anaerobic conditions. All incubations were conducted at 25° C.

Laboratory Incubation Experiment

The objective of this experiment was to study the effects of some of the insecticides and fungicides used in the greenhouse and field studies on microbial activity and nitrogen transformations in soil. Milorganite fertilizer was used as the nitrogen source.

Prior to incubation, 200 gram lots of an air-dried Hodunk sandy loam were mixed with the insecticides B25141, Diazinon, and Dieldrin and the fungicides Calo Clor, acti-dione thiram, and Tersan-OM. At this time, Milorganite at 350 ppm (2 pounds nitrogen per 1000 sq. ft.) was also mixed with the soil. In addition to these treatments, a control receiving Milorganite but no pesticide and a check receiving no nitrogen and no pesticide were studied. Two blanks with no soil and no pesticide were included. Table 3 gives the rates of pesticides used per 200 grams of soil and a 5-fold rate.

Table 3. Soil treatments imposed on air-dried Hodunk sandy loam prior to incubation.

Pesticides	ppm Added per 200 grams of soil	5-Fold rate--ppm per 200 grams of soil
B25141	320*	1600
Diazinon	320	1600
Dieldrin	60	300
Acti-dione thiram	560	2800
Calo Clor	450	2250
Tersan-OM	560	2800

* Active ingredients added.

Incubation Procedure

In this experiment, a static method of incubation was used as described by Stotsky (59). Ten gram samples of the soil from the treated and untreated lots were placed in 60 ml plastic cups containing 30 grams of a 30-60 mesh white silica sand. The sand and the soil were thoroughly mixed. Six ml of distilled water was then added to each cup. Groups of 16 samples for each pesticide treatment were placed in one-gallon respirometer jars to permit continuous collection of CO_2 and periodic sacrifice of subsamples for determination of ammonium and nitrate nitrogen levels in the soil.

Incubation was carried out in a growth chamber, with a constant temperature of 30° C beginning January 27, 1968. Two subsamples of soil were removed from each respirometer jar for determination of ammonium-N and nitrate-N by a micro-Kjeldahl procedure (7) every fourth day beginning with January 27, 1968 until the experiment was completed.

Collection of Carbon Dioxide

A small vial containing 5 ml of 1N NaOH solution was placed in the bottom of each jar to collect the evolved carbon dioxide. The carbon dioxide was determined every day for the first two days and then every other day until the experiment was completed. The unused NaOH was titrated against 0.5N HCl in the presence of phenolpthalien and excess barium chloride. Carbon dioxide was calculated by difference (59) and is reported as mg. carbon per 100 grams soil (oven-dried basis) per day.

All data from the above experiments were analyzed statistically by analysis of variance (58). In some cases, means were compared by the Duncan's Multiple Range Test (12).

RESULTS AND DISCUSSION

Greenhouse Experiment 1

The treatments employed in this study were applied at two rates; the recommended rate and 10 times the recommended rate of application. For the insecticides the recommended rate is that required to control sod webworms and chinch bugs in turf, and for the fungicides the recommended rate was the manufacturer's recommendation for disease control.

In some instances, larger than normal rates are recommended for the control of a plant parasite. For example, some fungicides are used at 2 times the recommended rate to control a severe infestation of disease. They may be applied every 7-10 days in critical periods. Some insecticides are used at 3 times the recommended rate for chinch bugs in order to control nematodes. For these reasons, 10 times the recommended rate of application was used in this experiment. This rate may reflect the effects of large applications or accumulations of a pesticide over a period of time.

All data will be compared using the Least Significant Difference or Duncan's Multiple Range Test at the 5% level.

The results in Table 4 show that effects on top growth did occur with pesticide treatments. Analyses of variance for data is given in Table 15 of the Appendix. Insecticides will be compared first. For the sake of brevity, the notation "x" and "10x" will denote the recommended and 10 times the recommended rates of applications, respectively. During

Table 4. Effect of pesticides on top growth of Pennlawn creeping red fescue and soil mineral nitrogen in Hodunk sandy loam in the greenhouse.

Pesticide	Rate per[1] 1000 sq.ft.	Dry clipping weights - g/pot Mar. 27-Apr. 14	Apr. 14-May 14	May 14-June 14	Total	ppm Mineral N ($NH_4 + NO_3$) in soil June 14
None	--	.51	.77	.72	2.00	7.58
LSD .05[2]	--	.14	.15	.14	.29	3.64
			Insecticides[3]			
B25141	x	.60 ab	.95 b*	.96 b*	2.50 b*	12.45 bc*
Diazinon	x	.54 b	.98 b*	.86 bc*	2.38 bc	8.53 cd
Dieldrin	x	.50 b	.84 b	.72 cd	2.06 c	8.43 cd
Chlordane	x	.48 b	.86 b	.68 d	2.01 c	6.73 d
B25141	10x	.73 a*	1.21 a*	.25 e*[4]	2.18 bc	22.60 a*
Diazinon	10x	.63 ab	1.19 a*	1.13 a*	2.94 a*	15.38 b*
Dieldrin	10x	.64 ab	.81 b	.72 cd	2.18 bc	13.40 b*
Chlordane	10x	.63 ab	.81 b	.78 cd	2.22 bc	7.43 d

Fungicides[3]						
Calo clor	x	.51 b	.93 b*	.77 c	2.20 c	10.45 b
Cadminate	x	.53 b	.70 c	.68 cd	1.91 cd	11.35 b*
Tersan-OM	x	.51 b	.94 b*	.79 c	2.23 c	7.83 b
Dyrene	x	.43 b	.63 c	.69 cd	1.75 d	7.05 b
Acti-dione thiram	x	.42 b	.71 c	.57 cd*	1.70 d*	8.68 b
Calo clor	10x	.60 b	1.07 b*	.96 b*	2.62 b*	9.15 b
Cadminate	10x	.55 b	.63 c	.61 cd	1.79 d	10.48 b
Tersan-OM	10x	.86 a*	1.46 a*	1.15 a*	3.46 a*	8.90 b
Dyrene	10x	.40 b	.57 c*	.60 cd	1.58 d*	10.35 b
Acti-dione thiram	10x	.48 b	.34 d*	.35 e*	1.16 e*	18.33 a*

* Significantly different from no pesticide treatment at the 5% level.

[1]x = recommended rate from Table 1; 10x = 10 times recommended rate.

[2]For comparing pesticide treated soil with untreated control.

[3]a,b,c,d, and e--Duncan's Multiple Ranges of equivalence within columns. Values accompanied by same letter are not significantly different at 5%.

[4]Average of 3 pots; fourth died from treatment.

the first two weeks of the experiment (March 27-April 14), top growth was not significantly affected by any of the insecticide treatments at the recommended rate of application. At the 10x rate, B25141 was the only insecticide to significantly increase top growth compared to the control. In the last two months and for the entire 2 1/2 months of the experiment, B25141 at the x rate and diazinon at both rates, significantly increased growth over the control. B25141 at the 10x rate was significant over the control in the month of April 14-May 14, but in the last month, it became toxic and plant growth was greatly reduced. Visual observation showed that, while growth was still stimulated in the last month, the blade tips became yellow and the plants eventually died. Dieldrin and chlordane, at both rates of application, did not differ significantly from the control.

When comparing means for insecticides against each other (Duncan's Multiple Range Test), it is seen that at the x rate B25141 significantly increased top growth over Dieldrin and chlordane during the entire growing period. At the 10x rate, clipping weights from Diazinon-treated pots were significantly greater than for Dieldrin or chlordane. B25141 probably would have been significant over Dieldrin and chlordane had the plants not died. These results indicate that the organophosphate insecticides, Diazinon and B25141, significantly influenced top growth while the chlorinated hydrocarbons had no effect.

Among the fungicide treatments, the recommended rate of application of Calo Clor and Tersan-OM significantly increased top growth for the month of April 14-May 14, but not for the entire 2 1/2 months of the experiment. In addition, acti-dione thiram significantly decreased top growth over the 2 1/2 month period compared to the no pesticide treatment.

At the 10x rate, it is seen that Tersan-OM greatly influenced growth for each harvest period. Calo Clor gave a significant increase over the control, but not to the extent that Tersan-OM did. Dyrene and acti-dione thiram significantly reduced growth, while Cadminate had no effect.

When comparing means for fungicides against each other, it is seen that Calo Clor and Tersan-OM significantly increased growth over Dyrene and acti-dione thiram at the x rate for the 2 1/2 months of growth. This was also evident at the 10x rate, especially with the Tersan-OM treatment as it became significant over Calo Clor. Furthermore, Dyrene was significant over acti-dione thiram.

The effect of these pesticides on the mineral nitrogen (NH_4, NO_2, and NO_3) in the soil was determined at the conclusion of the experiment. Milorganite was used as the nitrogen source. Results in Table 4 reveal that for the insecticide B25141 at both rates of application, and Diazinon and Dieldrin at the 10x rate, significantly increased mineral nitrogen over the untreated control. B25141 was, at the 10x rate, the most effective treatment--nearly tripling the mineral nitrogen level.

Soils treated with Diazinon, B25141, and Dieldrin at the 10x rate significantly increased mineral nitrogen over chlordane-treated soils, according to Duncan's Multiple Range Test. At the x rate, only the B25141-treated soil contained significantly more mineral nitrogen compared to chlordane.

For the fungicides, only Cadminate at the x rate and acti-dione thiram at the 10x rate were effective in increasing mineral nitrogen over the control. Acti-dione thiram at the 10x rate gave increased nitrogen over all other fungicide treatments. This may have been due to decreased top growth of grass under the high rate of acti-dione thiram, allowing a buildup of mineral nitrogen.

Greenhouse Experiment 2

In our modern era of turf management, there are a variety of nitrogen sources being used at varying rates. Also, in some instances, such as turf maintenance programs on golf courses, many insecticides and fungicides are used in connection with these nitrogen fertilizers. It was, therefore, the purpose of this experiment to study the effect of applying certain pesticides on growth of Pennlawn red fescue when treated with three different forms of nitrogen carriers. These sources were ammonium nitrate, a readily available form of nitrogen; activated sewage sludge, a natural organic form of nitrogen; and ureaform, a slow release synthetic organic carrier.

The results given in Table 5 show the effect of fertilizers and pesticides on top growth over a 5-month period in the greenhouse. Analyses of variance for data is given in Table 16 of the Appendix. Where no fertilizer was applied, the insecticide B25141 was the only pesticide treatment to significantly increase growth over the no pesticide control. Compare means for pesticides horizontally using the LSD (5% level) of 0.59 for a given nitrogen treatment.

When ammonium nitrate was the nitrogen source used, no pesticide treatment significantly increased growth over the untreated control for either nitrogen rate. However, it can be seen that while clipping weights for the B25141 treatment were not significantly greater than the control, it was significant over the effects of the Cadminate treatment at the low rate of nitrogen; and that B25141, Diazinon, and Calo Clor were significant over Cadminate at the high rate of nitrogen.

Where Milorganite and ureaform were the nitrogen sources used, B25141 again significantly increased growth over the untreated control, and over the Cadminate treatment for both rates of nitrogen and both carriers; but, Cadminate was not significantly different from the control in either instance. In addition, the Diazinon treatment was significant over the control under Milorganite applied at the lower rate and ureaform applied at the higher rate. Diazinon and Calo Clor significantly increased growth over Cadminate at both rates of ureaform, but not for Milorganite.

The overall average effect of pesticides, regardless of nitrogen source, is given in the last row of Table 5. It shows that B25141, Diazinon, and Calo Clor stimulated growth significantly, while Cadminate had a depressing effect.

When comparing the effectiveness of the nitrogen carriers under the different pesticide treatments, certain differences are noted (Pesticide means compared by Duncan's Multiple Range test within vertical columns). For the no pesticide and Diazinon treatments, and for the average effect of nitrogen fertilizers regardless of pesticide, the order of effectiveness is the same; i.e. ammonium nitrate at the high rate is significant over all other fertilizer treatments: Milorganite and ureaform at the high rate and ammonium nitrate at the low rate; Milorganite and ureaform at the low rate; and the no nitrogen treatment.

Table 5. Effect of pesticides and nitrogen carriers and rate on top growth of Pennlawn creeping red fescue, grown in Hodunk sandy loam in the greenhouse. Average total weight for 5 months growth.

Nitrogen source	Rate-lbs. N per 1000 sq.ft.	Dry clipping weights g/pot: No Pesticide	Insecticides: B25141	Insecticides: Diazinon	Fungicides: Cadminate	Fungicides: Calo Clor	LSD .05	Average for Fertilizer
None	--	2.85 d[1]	3.92 d*	3.06 d	2.86 e	3.32 e	.59	3.22 d
Ammonium + nitrate	1/6	4.84 b	5.14 c	4.99 b	4.53 b	4.88 c	.59	4.87 b
"	2/3	7.70 a	7.84 a	8.18 a	7.20 a	8.23 a	.59	7.85 a
Milorganite	1/6	3.50 c	4.52 c*	4.11 c	3.59 cd	3.95 d	.59	3.93 c
"	2/3	5.05 b	5.98 b*	5.08 b	4.97 b	5.51 b	.59	5.31 b
Ureaform	1/6	3.74 c	4.54 c*	4.25 c	3.16 d	3.93 d	.59	3.93 c
"	2/3	4.45 b	6.02 b*	5.13 b*	3.89 c	5.01 bc	.59	4.91 b
Average for pesticides		4.59	5.43*	4.96*	4.32*	5.01*	.22	

* Significantly different from no pesticide treatment at 5%.

[1] a,b,c, and d--Duncan's Multiple Ranges of equivalence, values within columns accompanied by same letter are not significant at 5%.

Table 5. Effect of pesticides and nitrogen carriers and rate on top growth of Toronto creeping bent grass, grown in Hubbard sandy loam in the greenhouse. Average total weight for [illegible]

Dry clipping weights g/pot

Nitrogen carriers	Rate-lbs. N per 1000 sq.ft.	Fertilizer	Insecticides [illegible]	Insecticides Dieldrin	Fungicides Cadminate	Fungicides [illegible]	[illegible]	Average [illegible]
None		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Average for pesticides		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

* Significantly different from no pesticide treatment at [illegible]

[illegible]

For the remaining pesticide treatments, B25141, Cadminate, and Calo Clor, ammonium nitrate at the high rate is again significantly greater than all other fertilizer treatments. Differences occur with the other carriers and rates. Where B25141 is used under the Milorganite and ureaform at the high rate, top growth is stimulated to the point where it is significant over ammonium nitrate at the low rate. Further, the Milorganite and ureaform treatments at the low rate of nitrogen compare favorably with ammonium nitrate at the low rate.

For the Cadminate treatment, the only difference seen is that the top growth from ureaform at the high rate is depressed to a point where it is significantly lower than top growth from ammonium nitrate at the low rate.

Where Calo Clor is used, the only difference compared to the no pesticide treatment noted is that top growth under the Milorganite applied at the high rate isstimulated to the point where it is significant over ammonium nitrate at the low rate.

Field Experiment

The field experiment was conducted in the summer of 1967. Treatments employed were based on results from the two preceding greenhouse experiments. The nitrogen carriers used were ammonium nitrate and activated sewage sludge. A no nitrogen level was also used as a comparison against the nitrogen carriers.

The insecticides used were B25141 and Diazinon, and the fungicides were Tersan-OM and acti-dione thiram. In addition, Tersan-OM was applied at 10 times the recommended rate of application, under the no nitrogen level. All pesticide treatments were applied 9 times over a 3-month period. For the insecticides (B25141 and Diazinon) and Tersan-OM at the 10x rate of application, this rate exceeds the normal usage for turf, although the insecticides are applied at 5 times the recommended rate for nematode control. Normally, these insecticides are used only on turf once or twice during a growing season. The more frequent applications were used to determine what effects on plant growth and soil nitrogen transformations may result from more numerous applications or from a build-up in the soil over a period of time under field conditions.

Analysis of variance for all field data is given in Tables 17, 18, 19, and 20 of the Appendix.

Clipping Weights

The results in Table 6 show that in the month of July the insecticides

Table 6. Effect of pesticides and nitrogen carriers on top growth of Cohansey bentgrass under field conditions.

Pesticide and	Dry clipping weights g/1000 sq. ft.			
nitrogen treatment	July	August	September	Total
Ammonium nitrate[1]:				
Diazinon	8037 a*	8604 ab	7797 a	24437 a
B25141	7308 ab	9188 ab	7199 a	23695 ab
Acti-dione thiram	6816 bc	9162 ab	7352 a	22417 bc
Tersan-OM	6850 bc	9494 a	7805 a	24149 ab
Check	6086 cd	8351 b	7308 a	21745 c
Milorganite[2]:				
Diazinon	6130 cd	6728 c	4341 b	17199 d
B25141	5912 d	7086 c	4442 b	17439 d
Acti-dione thiram	5096 ef	7055 c	4394 b	16545 d
Tersan-OM	4869 fg	7212 c	5052 b	17134 d
Check	5057 ef	6597 c	4410 b	16065 d

No nitrogen:				
Diazinon	3966 h	3805 d	2600 c	10371 ef
B25141	4267 fgh	3966 d	2592 c	10825 e
Acti-dione thiram	3582 h	3665 d	2635 c	9882 ef
Tersan-OM	4018 gh	4101 d	2880 c	10999 e
Check	3599 h	3303 d	2138 c	9040 f
Tersan-OM 10x[3]	6099 cd	6675 c	4599 b	17373 d
Average for pesticides:				
Diazinon	6044 a	6379 ab	4913 ab	17336 a
B25141	5829 a	6747 a	4744 b	17320 a
Acti-dione thiram	4860 b	6627 a	4793 b	16281 b
Tersan-OM	5246 a	6936 a	5246 a	17427 a
Check	4914 b	6083 b	4619 b	15616 b

* a,b,c,d,e,f,g, and h--Duncan's multiple ranges of equivalence within columns. Values accompanied by same letter are not significantly different at 5%.

[1]Ammonium nitrate applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[2]Milorganite applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[3]Ten times recommended rate, as given in Table 1.

B25141 and Diazinon significantly increased clipping weights over the check when ammonium nitrate and Milorganite were used as the nitrogen sources. This trend continued in August and September for Diazinon. B25141-treated plots gave greater yields in August, but in September they were about equal with the check, indicating that toxicity may have occurred. For the total seasonal clipping weights, these two insecticides again were significant over the check for the ammonium nitrate treated plots. The significance was not evident under Milorganite, but the values tended to be higher. This trend of higher clipping weights over the check for B25141 and Diazinon was also evident in all three months under the no nitrogen treatment and resulted in a significant increase for the total clipping weights for the B25141 treatment.

Among the fungicide treatments, Tersan-OM significantly increased top growth over the check in August and for the three-month period of growth under the ammonium nitrate treatment. Values were also higher in July and September. This trend for higher clipping weights from the application of Tersan-OM was also observed for the Milorganite and no nitrogen treatment, but was significant only for the total clipping weight under the no nitrogen treatment. Acti-dione thiram showed a slight tendency to increase top growth for all nitrogen treatments over the season, but no significant differences were noted.

A very definite increase in growth was observed from Tersan-OM applied at 10 times the recommended rate under the no nitrogen treatment.

Growth for each month compared favorably with plots receiving Milorganite.

The last section of Table 6 shows the average effect of pesticides, regardless of nitrogen treatments. B25141, Diazinon and Tersan-OM stimulated growth significantly over the check, particularly in July and for the total clipping weight.

Per Cent Nitrogen in Leaf Tissue

The data presented in Table 7 show that when ammonium nitrate was the nitrogen source, applications of the insecticides B25141 and Diazinon resulted in a significantly greater percentage of nitrogen in the leaf tissue than the check in July. B25141 continued to be significant in August but not in September.

When Milorganite was the nitrogen source used, B25141, Diazinon, and Tersan-OM increased the per cent of nitrogen in the leaf tissue over the check in July. In August and September, however, this trend was not significant.

When no nitrogen was applied, no significant increase in per cent nitrogen in the leaf tissue was observed for the months of July and September, but in August B25141 and Diazinon caused a significant increase. The acti-dione thiram treatment resulted in a significant decrease in per cent nitrogen in the leaf tissue in August. This tendency was also observable in August and September under the two nitrogen carriers, although not significant.

Table 7. Effect of pesticides and nitrogen carriers on per cent nitrogen in leaf tissue of Cohansey bentgrass under field conditions.

Pesticide and nitrogen treatment	Per cent nitrogen in leaf tissue		
	July	August	September
Ammonium nitrate[1]:			
Diazinon	5.00 ab*	4.25 ab	4.37 a
B25141	5.20 a	4.43 a	4.15 a
Acti-dione thiram	4.79 bc	4.05 b	4.09 a
Tersan-OM	4.78 bc	4.23 ab	4.34 a
Check	4.63 cd	4.12 b	4.27 a
Milorganite[2]:			
Diazinon	4.49 cde	3.64 c	3.42 bc
B25141	4.58 cd	3.65 c	3.64 b
Acti-dione thiram	4.24 efg	3.43 cd	3.30 c
Tersan-OM	4.34 de	3.59 c	3.44 bc
Check	4.09 fg	3.43 cd	3.41 bc

No nitrogen:			
Diazinon	4.03 fgh	3.16 d	2.92 def
B25141	4.02 gh	3.22 d	2.98 de
Acti-dione thiram	3.75 h	2.58 f	2.68 f
Tersan-OM	3.84 gh	2.82 e	2.74 ef
Check	3.80 gh	2.93 e	2.81 ef
Tersan-OM 10x[3]	4.48 cde	3.35 d	3.19 cd
Average for pesticides:			
Diazinon	4.51 a	3.69 a	3.57 a
B25141	4.60 a	3.77 a	3.59 a
Acti-dione thiram	4.26 b	3.41 c	3.36 b
Tersan-OM	4.32 b	3.54 b	3.51 a
Check	4.17 b	3.49 bc	3.50 ab

* a,b,c,d,e,f, and g--Duncan's multiple ranges of equivalence within columns. Values accompanied by same letter are not significantly different at 5%.

[1]Ammonium nitrate applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[2]Milorganite applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[3]Ten times recommended rate, as given in Table 1.

Again, as with the clipping weights, Tersan-OM at the 10x rate under no nitrogen compared quite favorably with Milorganite-treated plots.

The averages for the pesticides, regardless of nitrogen carrier or level, show that the two insecticides B25141 and Diazinon were significant over the check in July and August, but not in September.

Nitrogen Uptake

In Table 8, clipping weights and nitrogen composition data were used to determine total nitrogen uptake. The results show that on plots receiving ammonium nitrate, Diazinon caused a significant increase in nitrogen uptake over the check in July but not August or September, although values tended to be higher in these two months. B25141 was significant over the check in July and August, but not in September. Tersan-OM gave a significant increase in nitrogen uptake in August, but not in July or September; however, again values tended to be higher in these two months than for the check. All three materials were significant over the check for total seasonal nitrogen uptake.

Diazinon, B25141, and Tersan-OM tended to increase nitrogen uptake for the plots receiving Milorganite and no nitrogen. However, the only significant difference noted over the check was for Diazinon and B25141 in July under Milorganite and for B25141 over the 3-month period of growth for both nitrogen treatments.

Table 8. Effect of pesticides and nitrogen carriers on nitrogen uptake of Cohansey bentgrass under field conditions.

Pesticide and	Grams nitrogen uptake per 1000 sq. ft.			
nitrogen treatment	July	August	September	Total
Ammonium nitrate[1]:				
Diazinon	401 a*	366 ab	341 a	1108 a
B25141	380 a	407 a	299 b	1085 a
Acti-dione thiram	283 bc	372 ab	300 b	955 b
Tersan-OM	327 b	401 a	339 a	1067 a
Check	282 bc	344 b	312 ab	939 b
Milorganite[2]:				
Diazinon	276 c	245 c	148 c	668 cd
B25141	270 c	259 c	163 c	692 c
Acti-dione thiram	216 d	241 c	145 c	602 d
Tersan-OM	212 de	259 c	174 c	645 cd
Check	206 de	226 c	151 c	583 d

No nitrogen:				
Diazinon	160 f	121 d	76 d	357 ef
B25141	171 ef	128 d	77 d	376 e
Acti-dione thiram	134 f	100 d	70 d	304 ef
Tersan-OM	154 f	116 d	79 d	349 ef
Check	138 f	97 d	60 d	295 f
Tersan-OM 10x[3]	273 c	223 c	147 c	643 cd
Average for pesticides:				
Diazinon	279 a	244 ab	188 ab	711 a
B 25141	274 a	264 a	180 ab	717 a
Acti-dione thiram	211 b	238 b	172 b	621 b
Tersan-OM	231 b	258 a	197 a	687 a
Check	209 b	223 b	174 b	606 b

* a,b,c,d,e, and f--Duncan's multiple ranges of equivalence.within columns. Values accompanied by same letter are not significantly different at 5%.

[1]Ammonium nitrate applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[2]Milorganite applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[3]Ten times recommended rate, as given in Table 1.

Acti-dione thiram had no effect under any nitrogen treatment. Tersan-OM at the 10x rate under no nitrogen again performed as well as plots receiving Milorganite.

The averages for all pesticides showed Diazinon, B25141, and Tersan-OM caused increases in nitrogen uptake throughout the experiment.

Turf Color Rating and Density Counts

For plots receiving ammonium nitrate, the data in Table 9 show no significant difference in color rating was observed for any pesticide treatment in July, but Diazinon and Tersan-OM plots rated significantly better than the check in August and September.

Tersan-OM-treated plots also rated significantly better than the check in August when Milorganite was the nitrogen source. No other pesticide affected turf color ratings.

Where no nitrogen was applied, plots receiving Tersan-OM showed significantly better color than the check in all three months. In addition, B25141-treated plots were significantly better in September. A marked effect on color was observed for Tersan-OM applied at 10 times the recommended rate. In July and August, these plots rated with those receiving ammonium nitrate. In September, they were as good as plots receiving Milorganite.

The average for the pesticides regardless of nitrogen source shows that again Tersan-OM rated significantly better than the check for all

Table 9. Effect of pesticides and nitrogen carriers on relative turf color rating and density counts of Cohansey bentgrass under field conditions.

Pesticide and nitrogen treatment	Average turf color rating[4] July	August	September	Density counts Shoots per sq dm
Ammonium nitrate[1]:				
Diazinon	2.0 ab*	1.8 a	1.5 a	1077 a
B25141	2.5 b	2.0 ab	1.8 ab	1108 a
Acti-dione thiram	2.3 b	2.5 abc	2.3 abc	1080 a
Tersan-OM	1.7 ab	1.7 a	1.5 a	1071 a
Check	2.7 bc	2.8 bc	2.5 bcd	1058 a
Milorganite[2]:				
Diazinon	2.5 b	3.0 cd	3.2 cde	1052 a
B25141	2.8 b	3.2 cde	3.2 cde	1077 a
Acti-dione thiram	2.5 b	2.8 bcd	3.7 e	1056 a
Tersan-OM	2.5 b	2.5 abc	3.3 de	1069 a
Check	2.8 b	3.5 de	3.8 e	1039 a

Table 9. Effect of pesticides and nitrogen carriers on relative turf color rating and density counts of Cohansey bentgrass under field conditions.

Pesticide and nitrogen treatment	Average turf color rating[4]			Density counts
	July	August	September	Shoots per sq. dm.
Ammonium nitrate[1]:				
Diazinon	2.0 ab*	1.8 a	1.5 a	1077 a
B25141	2.5 b	2.0 ab	1.8 ab	1108 a
Acti-dione thiram	2.3 b	2.5 abc	2.3 abc	1089 a
Tersan-OM	1.7 ab	1.7 a	1.5 a	1071 a
Check	2.7 bc	2.8 bc	2.5 bcd	1058 a
Milorganite[2]:				
Diazinon	2.5 b	3.0 cd	3.2 cde	1052 a
B25141	2.8 b	3.3 cde	3.2 cde	1077 a
Acti-dione thiram	2.5 b	2.8 bcd	3.7 e	1066 a
Tersan-OM	2.5 b	2.5 abc	3.3 de	1069 a
Check	2.8 b	3.5 de	3.8 e	1039 a

No nitrogen:				
Diazinon	5.5 e	5.5 f	6.2 fg	1007 a
B25141	5.0 e	5.2 f	5.5 f	996 a
Acti-dione thiram	5.0 e	5.8 f	6.3 fg	1018 a
Tersan-OM	4.0 d	4.2 e	5.5 f	998 a
Check	5.3 e	5.8 f	6.7 g	973 a
Tersan-OM 10x[3]	1.8 ab	2.0 ab	3.5 e	1036 a
Average for pesticides:				
Diazinon	3.3 b	3.4 b	3.6 a	
B25141	3.7 b	3.5 b	3.5 a	
Acti-dione thiram	3.3 b	3.7 bc	4.1 b	
Tersan-OM	2.2 a	2.8 a	3.4 a	
Check	3.6 b	4.1 c	4.3 b	

* a,b,c,d,e, and f--Duncan's multiple ranges of equivalence within columns. Values accompanied by same letter are not significantly different at 5%.

[1]Ammonium nitrate applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[2]Milorganite applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[3]Ten times the recommended rate, as given in Table 1.

[4]Color rating base on 1-10 scale; 1 = darkest green color, 10 = lightest green color.

three months, and that Diazinon and B25141 rated significantly better in August and September.

No significant effect on density counts taken October 1 was observed from any pesticide; however, plots receiving nitrogen tended to have higher counts than those receiving no nitrogen.

Mineral Soil Nitrogen

Mineral nitrogen (ammonium + nitrate) was determined on soil samples taken October 5. The results in Table 10 show that no significant differences in the level of ammonium nitrogen in the soil resulted from the use of these pesticides under either nitrogen carrier or where no nitrogen was applied.

For the plots receiving ammonium nitrate, the insecticide B25141 significantly increased the level of nitrate nitrogen over the check. All other pesticides tended to give higher values than the check, although no significance was noted.

Where Milorganite was applied and for the plots receiving no nitrogen, no significant difference in the level of nitrate nitrogen in the soil was observed from any pesticide treatment.

For the total mineral nitrogen (ammonium + nitrate) in the soil, again no significant differences were noted from the use of these pesticides for either nitrogen carrier or where no nitrogen was used. The averages for the pesticides, regardless of the nitrogen source or level, show no significant effect on ammonium and nitrate levels or total mineral nitrogen in the soil.

Table 10. Effect of pesticide and nitrogen carriers on mineral nitrogen content of field soil on October 5.

Pesticide and nitrogen treatment	ppm Ammonium nitrogen	ppm Nitrate nitrogen	ppm Ammonium + nitrate nitrogen
Ammonium nitrate[1]:			
Diazinon	5.0 ab*	6.6 abc	11.6 abc
B25141	4.9 ab	8.4 a	13.3 a
Acti-dione thiram	4.9 ab	7.9 ab	12.8 ab
Tersan-OM	5.6 a	6.3 abcd	11.9 abc
Check	5.6 a	5.8 bcd	11.4 abc
Milorganite[2]:			
Diazinon	5.1 ab	6.1 abcd	11.2 abc
B25141	5.4 a	5.6 bcd	11.0 abc
Acti-dione thiram	4.5 ab	4.8 cd	9.3 cde
Tersan-OM	4.6 ab	5.6 bcd	10.3 abcde
Check	5.3 a	5.2 cd	10.5 abcd

No nitrogen:			
Diazinon	4.4 ab	5.1 cd	9.6 bcde
B25141	4.3 ab	4.9 cd	9.2 de
Acti-dione thiram	3.7 ab	4.1 d	7.8 de
Tersan-OM	3.4 b	4.0 d	7.4 e
Check	4.4 ab	4.1 d	8.5 cde
Tersan-OM 10x[3]	4.7 ab	6.2 abcd	10.9 abcd
Average for pesticides:			
Diazinon	4.8 a	6.0 a	10.8 a
B25141	4.8 a	6.3 a	11.1 a
Acti-dione thiram	4.3 a	5.6 a	9.9 a
Tersan-OM	4.5 a	5.2 a	9.7 a
Check	5.1 a	5.2 a	10.3 a

* a,b,c,d, and e--Duncan's multiple ranges of equivalence within columns. Values accompanied by same letter are not significantly different at 5%.

[1]Ammonium nitrate applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[2]Milorganite applied at the rate of 1 lb. nitrogen per 1000 sq. ft. per month.

[3]Ten times recommended rate, as given in Table 1.

Microbial Counts

The soil samples for microbial studies were taken over a 3-week period starting in the last week of September. The variation in counts for the control plots is probably due to differences in sampling dates. Time and facilities did not allow counts to be taken for all pesticide or nitrogen treatments. The plots receiving Diazinon, acti-dione thiram and Tersan-OM using ammonium nitrate as the nitrogen source were the only plots tested.

Data in Table 11 show that Diazinon significantly reduced the number of bacteria grown on media 116 (soil extract agar). This effect was not noticeable on the other media. Tersan-OM had no effect on bacteria counts for any of the media. Acti-dione thiram significantly increased bacteria able to grow in a CO_2 atmosphere when media 103 was used. This effect was not observed with media 120. While no other significant differences were noted for acti-dione thiram, there was a tendency for bacteria counts to be higher than the control for all media and incubation conditions where it was applied.

No significant difference occurred in fungal or actinomycetes numbers from the application of any pesticide.

Table 11. Effect of pesticides on relative numbers of microbial groups in field treated soil, September-October 1967.

Incubation atmosphere	Medium[1]	Microbial group	Geometric mean numbers per g dry soil x 10^4						Significance
			Control	Diazinon	Control	Tersan-OM	Control	Acti-dione thiram	
Air	116	Bacteria	713.9 a*	596.2 b	889.2	926.8	837.9	1048.0	
Air	117	Actinomycetes	148.9	149.4	161.4	145.2	144.6	167.0	N.S.
Air	119	Fungi	2.4	2.9	1.6	1.6	1.8	2.7	N.S.
Air	121	Bacteria	532.8	531.9	751.5	760.0	659.3	733.3	N.S.
Air	103	"	571.2	630.6	670.8	749.4	605.0	679.8	N.S.
Air	120	"	923.9	902.0	1047.0	992.8	952.6	1042.0	N.S.
N_2	103	"	12.3	13.4	15.4	15.8	16.6	17.5	N.S.
" plus air[2]	"	"	245.1	254.8	372.0	302.0	283.2	288.0	N.S.
N_2	120	"	41.9	37.2	42.8	49.9	43.2	52.1	N.S.
" plus air	"	"	338.7	351.8	560.4	484.9	343.5	432.7	N.S.
CO_2	103	"	19.4	34.9	10.1	12.1	5.8 b	18.9 a	
" plus air	"	"	106.7	105.7	88.7	120.1	73.4 b	129.6 a	
CO_2	120	"	33.6	30.4	24.2	29.7	32.5	34.4	N.S.
" plus air	"	"	286.3	325.0	222.3	234.5	225.3	233.8	N.S.

* a and b--Duncan's Multiple Ranges of equivalence, values within rows accompanied by same letter are not significant at 5% level.

[1]Medium 116, soil extract agar (32), medium 117, chitin agar (31); medium 119, OAES agar (66); medium 121, 103, and 120--see page of Methods and Material chapter.

[2]Plates exposed 3 days in air after 5 days in N_2 or CO_2 atmosphere. Only new colonies which developed in air were counted.

Discussion of Greenhouse and Field Results

The results of the greenhouse and field experiments indicate that pesticides may exhibit stimulatory, inhibitory, or no influence on plant growth. Under higher than recommended rates, it was seen that effects tended to be magnified.

If a pesticide is successful in killing a plant parasite, plant growth should be improved. However, in both the greenhouse and field experiments no apparent effects from disease or insects were noted on control plants receiving nitrogen in any form. A slight infestation of dollar spot did occur on the no nitrogen plots of the field experiment. This was cleared up with an application of Cadminate, which was applied to all plots. No changes in patterns of growth were observed after this application. This does not, however, rule out the possibility that unrecognized parasites could have been controlled.

If no apparent disease or insects were controlled, these results suggest that the stimulus to growth may be physiological or microbiological in nature. A number of explanations may exist for these influences and will be discussed here and in the laboratory incubation study that follows.

The results of the field experiment substantiate, to a certain degree, the results of the two greenhouse experiments. It is seen that, in most cases, the two organophosphate insecticides, B25141 and Diazinon increased growth over the no pesticide treatment in both the greenhouse and field experiments, but differences occurred with nitrogen carriers. In the greenhouse, when Milorganite was used, the two insecticides increased growth. This was not evident when ammonium nitrate was used. In the field, they increased growth under both nitrogen carriers. The difference in turf species or environmental or soil conditions between the greenhouse and field experiments may be responsible.

The increased growth response due to applying Tersan-OM was also evident in both the greenhouse and field, especially at the higher rate of application. For acti-dione thiram, the apparent decrease in growth in the greenhouse was not observed in the field. Again, specie tolerance or environmental factors may play some role in this result.

The field results point out that the percentage nitrogen in the leaf tissue and nitrogen uptake were stimulated by Diazinon, B25141 and Tersan-OM. This effect was more noticeable in July for the two insecticides Diazinon and B25141, and more noticeable in August for Tersan-OM.

Increases in growth and nitrogen uptake caused by pesticides have been observed by other investigators. Freney (19) and Ries et al (51) showed increases in growth and nitrogen uptake in fruit trees and corn treated with simazine. The work of Ries and co-workers (50,62) demonstrated that small amounts of simazine stimulate nitrate reductase activity in corn and they have presented the hypothesis that simazine enhances nitrate utilization by increasing nitrate reductase activity.

Roberts et al (52) observed that the action of DDT and nonherbicidal concentrations of 2,4-D on plant growth closely resemble that of some plant hormones.

These observations indicate that pesticides can have an effect on the physiology of the turf plants which enhances growth and nitrogen uptake.

Musser and Duich (40) have raised a question as to whether serious consideration should be given to an increase in growth and nitrogen uptake unless color quality of the turf is improved. To many turf managers, the quality of color or how green the grass is has become a leading criterion for rating turfgrasses. In this respect, it was seen that Diazinon and Tersan-OM were effective in producing significantly better color in the field during certain periods of growth for all nitrogen treatments.

The increases in the total mineral nitrogen in the soil found for Diazinon, B25141, and acti-dione thiram in the greenhouse were not detectable under field conditions. Possibly, the soil and management conditions could explain these differences. In the greenhouse, the turf was grown in plastic-lined pots so no leaching took place. Also, the soil used was considerably less sandy than out in the field. The field soil was a specially prepared soil mixture that was approximately 50% coarse sand. This required more frequent applications of water than for the greenhouse soil. Any appreciable increases in nitrate nitrogen would have been subjected to leaching. If the soil had been had been sampled to a deeper depth or sampled more frequently during the experiment, these increases in soil nitrogen may have been detectable.

The increase in total mineral nitrogen in the soil observed for acti-dione thiram in greenhouse experiment 1, may have been due to the decreased growth of the turf resulting in less nitrogen uptake. This was not the case with B25141 and Diazinon, as total mineral nitrogen and growth were increased, suggesting that these two insecticides enhance mineralization and nitrification.

Another possible explanation for increases in mineral nitrogen is that these pesticides may cause destruction of certain predators whose cell material becomes an added food supply to the surviving microbial population and upon decomposition increases the amount of nitrogen in the soil.

Clipping weights, nitrogen composition, and uptake were all increased by nitrogen application. The greater availability of nitrogen in ammonium nitrate compared to Milorganite and ureaform was particularly apparent.

This increase in growth from ammonium nitrate over Milorganite and ureaform has been observed by other investigators (26,40). This is due to a greater amount of nitrogen available to the plant in the first few weeks after application. Lunt (33) points out that, usually, about one-third to one-half of the nitrogen in Milorganite is mineralized by soil organisms in four weeks time when conditions are favorable. The remaining fraction is very slowly available. He further notes that about

25% of the nitrogen in ureaform is cold water soluble, with the remaining cold water insoluble fraction able to mineralize at a rate of about 7 to 11 per cent per month depending on soil conditions influencing microbial activity. A possible explanation for the increase in growth over the control when pesticides are applied to turf receiving Milorganite or ureaform, is that the pesticide could influence microbial activity to a point where more rapid mineralization of the fertilizers occurs, thus releasing more nitrogen for plant growth. Waksman (65) has suggested partial sterilization effects could be responsible for a phenomenon of this type. He points out that partial sterilization of a soil brings about a chemical change in the organic matter of the soil, making it more available as a source of energy for microorganisms, resulting in higher nitrogen levels in soils.

Laboratory Incubation Experiment

The laboratory experiment was designed to investigate effects of some of the pesticides used in the greenhouse and field experiments on microbial activites in Hodunk sandy loam. Of specific concern, was the microbial activity as it affected nitrogen transformations in the soil using Milorganite as the nitrogen source. This carrier requires microbial breakdown for release of its nitrogen, and is used extensively in the turfgrass field.

Nitrogen Transformations

The data for nitrogen transformations are given in Table 12, 13, and 14. The tables are designed so that quantitative changes in ammonium, nitrate, and ammonium plus nitrate, respectively are shown for each 4-day incubation period.

During the 28 days of incubation marked changes in patterns of ammonium accumulation were caused by the added pesticides. The results in Table 12 show that Tersan-OM at both rates of application greatly stimulated ammonification over the 28 days of incubation. This is illustrated in Fig. 1.

Calo Clor and B25141 at both rates and Dieldrin at the high rate strongly inhibited ammonification. In addition, Dieldrin at the low rate caused significantly less ammonification than the control, but not to the extent of the high rate.

Table 12. Effect of pesticides and time of incubation on the change in soil ammonium levels between each sampling date in Hodunk sandy loam with Milorganite applied as the nitrogen source.

Treatment	Pesticide[1] rate	Change in soil ammonium levels - ppm ammonium nitrogen							
		Time of incubation, days							Total
		4	8	12	16	20	24	28	change
Check (no N)[2]		-1.0	5.5	-2.5	-6.5	2.5	-8.0	-1.5	-11.5
Control[3]		11.5	42.5	25.5	-9.5	75.5	-2.0	18.5	162.0
B25141	x	3.5*	10.0*	-1.0*	6.0*	-0.5*	-6.5	8.0	19.5*
"	5x	4.5*	1.5*	0.0*	1.0*	2.5*	6.0	-4.0	11.5
Diazinon	x	13.0*	48.0	64.5*	17.0*	1.5*	-20.5	57.0	180.5
"	5x	3.0*	2.5*	-3.0*	19.5*	83.5	-35.0	0.5	71.5*
Dieldrin	x	3.5*	10.0*	6.5*	-7.5	11.5*	45.5*	33.5	103.0*
"	5x	4.5*	-1.0*	3.5*	-6.0	-1.0*	1.0	0.5	3.5*
Acti-dione thiram	x	12.5	1.5*	4.0*	70.5*	24.5*	49.0*	19.5	181.5
"	5x	6.5	35.5	39.5	0.0	50.0*	17.0	26.0	174.5
Tersan-OM	x	53.0*	85.5*	89.0*	-4.5	30.0*	83.5*	48.5	385.0*
"	5x	94.0*	32.0	43.0	54.5*	48.0*	31.5	38.0	341.0*
Calo Clor	x	1.0*	4.0*	9.0	-12.0	2.5*	1.5	4.0	10.0*
"	5x	0.0*	2.5*	4.0*	-4.0	1.5*	-0.5	-2.5	1.0*
LSD .05		6.6	17.3	17.4	21.1	23.4	45.1	39.8	32.2

* Significantly different from control at the 5% level.

[1]x = rate per 200 grams soil, 5x = 5-fold rate per 200 grams soil as given in Table 3 of the Methods and Materials chapter.

[2]No pesticide and no Milorganite fertilizer added.

[3]No pesticide added, but 350 ppm nitrogen added as Milorganite.

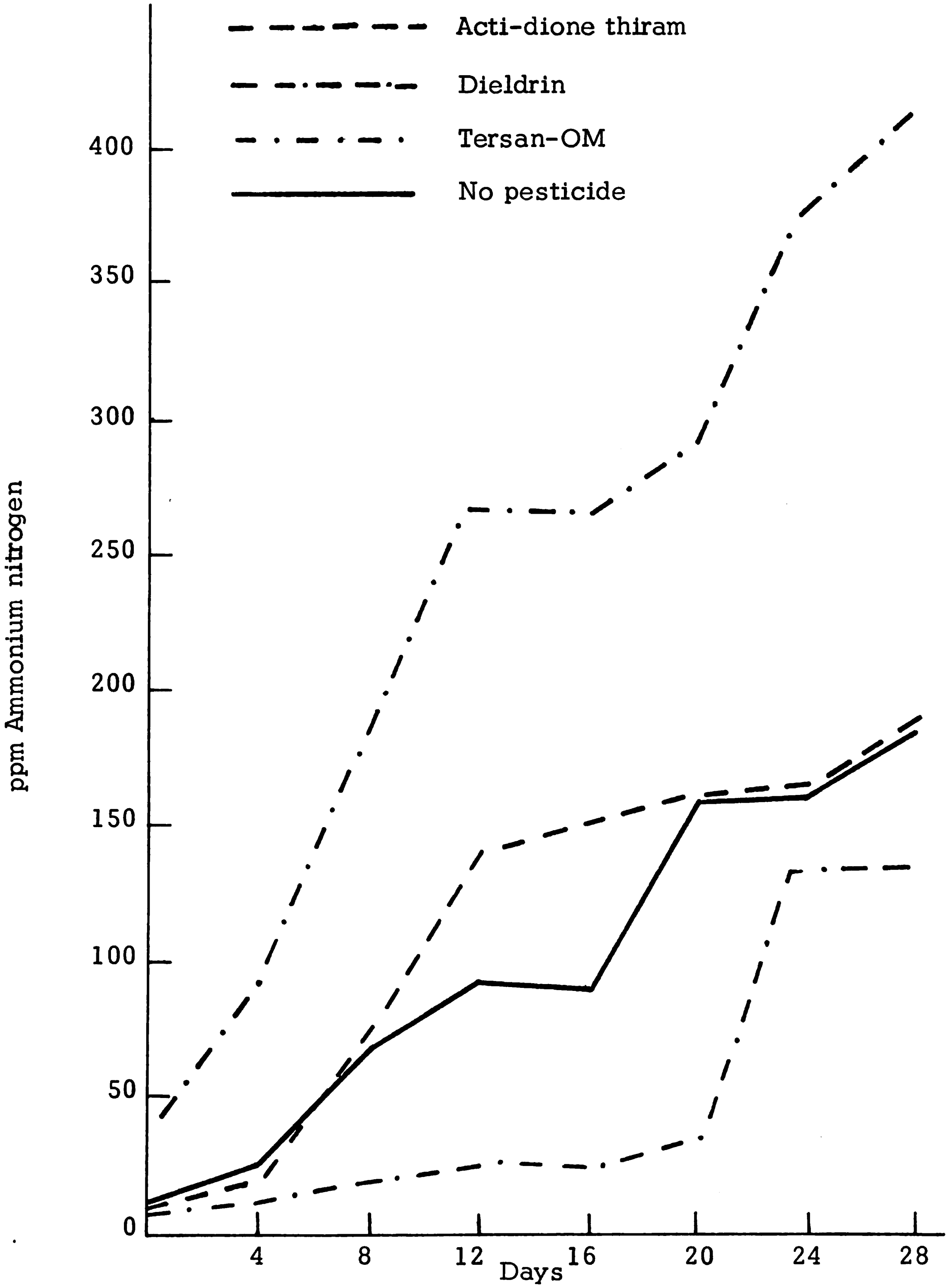

Fig. 1 Effect of pesticides on ammonification of Milorganite in Hodunk sandy loam.

While Diazinon at the low rate and acti-dione thiram at both rates did not significantly affect ammonification, patterns of accumulation of ammonium were significantly altered from the control. For example, the control soil showed a disappearance of 9.5 ppm ammonium between the twelfth and sixteenth day, and an increase of 75.5 ppm between the sixteenth and twentieth days. Whereas, Diazinon-treated soils showed an increase of 17 ppm ammonium between the twelfth and sixteenth day and only an increase of 1.5 ppm between the sixteenth and twentieth day.

Similar fluctuations in rate of release appear for the other pesticides that allowed ammonification.

Table 13 shows the effect of pesticides on soil nitrate levels. Here it is observed that the accumulation of nitrate in the control soil was inhibited by Milorganite itself. This would suggest that some other factor associated with Milorganite inhibited activity of nitrifying organisms or stimulated activity of microbial groups using nitrate assimilatively or in dentrification. It has been reported by Fuller (20) that high ammonium salt concentration under high soil pH inhibits nitrate production. However, the pH of the control soil was 6.3. This would indicate that something other than high pH inhibited the Nitrobacter activity. On the basis of these data, it was impossible to identify what effects B25141, Diazinon, Dieldrin, and Calo Clor at both rates and acti-dione thiram at the low rate may have had on any specific microbial processes leading to the appearance or disappearance of nitrate.

Table 13. Effect of pesticides and time of incubation on change in nitrate nitrogen levels between each sampling date in Hodunk sandy loam with Milorganite applied as the nitrogen source.

		Change in soil nitrate levels - ppm nitrate nitrogen							
	Pesticide[1]	Time of incubation, days							Total
Treatment	rate	4	8	12	16	20	24	28	changes
Check (no N)[2]		1.0	6.0	9.5	-1.5	4.0	2.0	2.5	23.0
Control[3]		0.5	-1.0	0.0	1.0	-0.5	0.0	0.5	0.5
B25141	x	-3.5	5.0	-6.0	2.0	0.0	4.0	-5.0	-3.5
"	5x	-2.0	1.0	1.0	0.5	0.0	-3.0	1.0	-1.5
Diazinon	x	-1.5	0.5	0.0	-3.5	0.0	-0.5	0.0	-5.0
"	5x	0.5	1.5	-3.0	1.0	-1.0	0.0	0.5	-0.5
Dieldrin	x	-0.5	0.5	1.0	-1.5	-1.5	-0.5	3.5	1.0
"	5x	-1.0	-1.0	0.5	-2.0	0.5	1.0	0.0	-2.0
Acti-dione thiram	x	3.0	-5.0	5.5	-5.5	-1.0	3.5	-2.5	-1.5
"	5x	13.0*	-10.5*	15.0*	-10.5	4.0	24.5*	6.0	41.5 *
Tersan-OM	x	-16.5*	1.0	9.0	-3.5	-6.5	4.0	49.5*	37.0*
"	5x	8.0	38.0*	6.0	-4.5	-14.0	36.5*	-38.5*	31.5*
Calo Clor	x	1.5	-0.5	12.0*	-10.5	-1.5	-1.0	1.0	1.0
"	5x	-1.0	0.5	0.5	0.5	-1.0	-0.5	1.5	0.5
LSD .05		9.2	8.2	9.9	16.4	19.0	6.7	15.4	12.8

* Significantly different from control at the 5% level.

[1] x = rate per 200 grams soil, 5x = 5-fold rate per 200 grams soil as given in Table 3 of the Methods and Material chapter.

[2] No pesticide and no Milorganite fertilizer added.

[3] No pesticide added, but 350 ppm nitrogen added as Milorganite.

The data of Table 13 do, however, reveal that Tersan-OM at both rates and acti-dione thiram at the high rate significantly increased nitrate accumulations. These pesticides were evidently able to overcome the apparent inhibition.

The effect of these pesticides on total nitrogen changes (ammonium + nitrate) as shown in Table 14, follows closely the patterns for ammonium. This is related to the apparent inhibition of nitrate production. However, due to the increases in nitrates caused by acti-dione thiram at the high rate, a significant increase in total nitrogen over the control was observed.

Carbon Dioxide Evolution

Data for the carbon dioxide evolution were not consistent. With certain pesticides, evidence of microbial activity was shown by increases in ammonium in the soil. This apparent microbial activity did not consistently result in increases in carbon dioxide evolution. These results indicate that the carbon dioxide evolved was in some way being held in the soil. Therefore, no basis for comparison of pesticides is valid.

Table 14. Effect of pesticides and time of incubation on the change in total soil nitrogen levels (ammonium + nitrate) between each sampling date in Hodunk sandy loam with Milorganite as the nitrogen source.

		Change in total mineral nitrogen (ammonium + nitrate) levels - ppm total nitrogen							
	Pesticide[1]	Time of incubation, days							Total
Treatment	rate	4	8	12	16	20	24	28	change
Check (no N)[2]		0.0	11.5	7.0	-8.0	6.5	-6.0	1.0	12.0
Control[3]		12.0	41.5	25.5	-8.5	75.0	-2.0	19.0	162.0
B25141	x	0.0	15.0*	-7.0*	8.0	-0.5*	-2.5	3.0	16.0*
"	5x	2.5	2.5*	1.0*	1.5	2.5*	3.0	-3.0	10.0*
Diazinon	x	11.5	48.5	64.5*	13.5	1.5*	-21.0	57.0	175.0
"	5x	3.5	4.0*	-6.0*	20.5	82.5	-35.0	1.0	70.0*
Dieldrin	x	3.0	10.5*	7.5	-9.0	10.0*	45.0*	37.0	104.0*
"	5x	3.5	-2.0*	4.0*	-8.0	-0.5*	2.0	2.0	1.0*
Acti-dione thiram	x	15.5	-3.5*	9.5	65.0*	23.5*	52.5*	17.0	180.0
"	5x	19.5	25.0	54.5*	-10.5*	54.0	41.5	32.0	216.0*
Tersan-OM	x	36.5*	86.5*	98.5*	-8.0	23.5*	87.5*	98.0*	422.0*
"	5x	102.0*	70.0*	49.0*	50.0*	34.0*	68.0*	-0.5*	372.0*
Calo Clor	x	2.5	3.5*	21.0	-22.5	1.0*	0.5*	5.0*	11.0
"	5x	-1.0*	3.0*	4.5*	-3.5	0.5*	-1.0	-1.0	1.0
LSD .05		12.5	20.2	19.9	26.9	23.7	45.3	39.5	35.7

* Significantly different from control at 5% level.

[1] x = rate per 200 grams soil, 5x = 5-fold rate per 200 grams soil as given in Table 3 of the Methods and Material chapter.

[2] No pesticide and no Milorganite fertilizer added.

[3] No pesticide added, but 350 ppm nitrogen added as Milorganite.

Discussion of Results of Incubation Experiment

Pesticides added to soil in the laboratory produced some stimulating and depressive effects on ammonification and nitrification. Two rates of application were used for each pesticide, as shown in Table 3 of the Methods and Materials chapter. The high rates are higher than recommended for field use. This was done to allow for effects of potential residues resulting from long periods of extended use and to force possible microbial effects. The rates are based on a one-inch depth of soil as the effective zone of pesticidal action.

The low rate, however, may represent amounts that reach the soil in the course of one growing season. For example, Tersan-OM applied at 4 oz. per 1000 sq. ft. is equivalent to 35 ppm, based on a one-inch depth of soil with a density of 1.55. It is not uncommon for fungicides to be applied every 7-10 days by turf managers as a disease preventative during a growing season giving a likely maximum of 16 applications per year. Therefore, 16 times the recommended rate (35 ppm) of Tersan-OM was used to study effects of possible accumulation in the soil over the course of one growing season. Actual amounts used will depend on turf and environmental conditions. This basis for application rates was used for the other fungicides as well. For the insecticides, 6 times the recommended rate was used.

The point can be raised as to what depth of soil pesticides can exert an influence on microbial activity. If the solubility of pesticides is low and they are adsorbed onto clay minerals, they may be concentrated in the top 1/4 to 1/2 inch of soil. This would result in even greater concentrations of pesticides in the soil than were used in this experiment, assuming no decomposition of the pesticide takes place. Further research on pesticide residues is certainly needed to determine what actually is the effective zone of soil pesticide influence.

Certain effects of pesticides observed in this incubation study may explain some of the responses in the greenhouse and field experiments. It was seen that Tersan-OM, at both rates of application greatly stimulated ammonification. Lunt (33) has reported that from 1/3 to 1/2 of the nitrogen in Milorganite is mineralized in four weeks time when conditions are favorable. This is consistent with data in Table 12, where 162 ppm (nearly half of the 350 ppm nitrogen added to the control soil) was mineralized during the four weeks of incubation.

When Tersan-OM was applied, however, 380 ppm (over 100%) of nitrogen was released from Milorganite. The amount of nitrogen recovered above the 350ppm added may have resulted from the breakdown of organic matter already in the soil, stimulated nitrogen fixation, or variation in the per cent nitrogen in Milorganite. Regardless of the greater nitrogen recovery, the data do suggest that Tersan-OM may stimulate microorganisms that are able to decompose Milorganite rapidly. Increases in growth observed in greenhouse experiment 1 when Tersan-OM was applied at 10 times the recommended rate may have been due to the increased availability of nitrogen from Milorganite.

Another possible explanation for increases in ammonium levels from soil treated with Tersan-OM, is that partial sterilization may occur (65). Tolerant organisms may decompose killed microbial cell material, resulting in increased nitrogen availability. Increases in growth observed in the greenhouse and field experiments from B25141, Diazinon, and Calo Clor could not be correlated with responses in the incubation study. Rates used may have been toxic to ammonifying organisms. Furthermore, these responses may be physiological in nature and not related to microbial activity.

One point brought out in this experiment that is of particular interest is the apparent cyclic pattern of ammonification and nitrification of Milorganite (see Fig. 1). This suggests that high populations of specific microbes may be present in the soil at certain periods. As was seen in Tables 12 and 13, when ammonification and nitrification occured a number of the pesticides caused significant variation in the pattern of ammonium accumulation and nitrification. This would indicate that microbial activity is also being affected. Naumann (41) has shown peak ofmicrobial populations at 5-7, 11-12, and 21-22 days from a 1% application of Parathion to soils.

What effects on plant growth the cyclic patterns of ammonification and nitrification of Milorganite have cannot be determined by this experiment. Further investigations are certainly needed to establish if this effect will influence soil fertility and plant growth to any significant degree.

Disappearance of ammonium and nitrate is evident from Table 12 and Table 13, and in many cases is not accounted for by nitrification. Milorganite is high in carbonaceous material. It is possible that during its decomposition the high population of specific microbes suggested by the observed cyclic patterns may result in assimilation of some mineral nitrogen. As shown in Table 11, acti-dione thiram increased the populations of carbon dioxide tolerant and nitrate utilizing bacteria. This could account for some of the disappearance of nitrate, if these microorganisms were stimulated.

Denitrification losses can occur in well-aerated soils (67). It is possible that disappearance of nitrogen in this experiment was due to this process. Fixation by certain clay minerals, as reported by Allison et al (2) can result in apparent losses of nitrogen by rendering significant amounts of ammonium unavailable for nitrification.

SUMMARY AND CONCLUSION

The purpose of this investigation was to study the effect of selected pesticides on turfgrass growth, interaction with turf fertilizers, and nitrogen transformations in soil. The following conclusions were drawn from greenhouse, field, and laboratory incubation experiments.

Greenhouse Experiment 1:

1. The organophosphate insecticides B25141 and Diazinon increased top growth of Pennlawn red fescue significantly over the control at both the recommended and 10 times the recommended rate of application. Tersan-OM and Calo Clor fungicides greatly stimulated growth at 10 times the recommended rate. Acti-dione thiram at both rates and Dyrene at the high rate depressed growth.

2. B25141 at both rates, Diazinon, Dieldrin, and acti-dione thiram at 10 times the recommended rate, and Cadminate at the recommended rate resulted in higher levels of soil mineral nitrogen than the control at the conclusion of the experiment.

Greenhouse Experiment 2:

1. Pots treated with the insecticide B25141 gave significantly greater top growth of Pennlawn red fescue over a 5-month period when Milorganite or ureaform were used as the nitrogen source. This was also true when no nitrogen was applied. Diazinon-treated pots gave greater top growth

when ureaform was used at the higher rate. Pots receiving ammonium nitrate were not significantly affected by any pesticides.

Field Experiment:

1. Top growth of Cohansey bentgrass, receiving ammonium nitrate and Milorganite as the nitrogen source, was significantly increased over the no pesticide treatment when treated with B25141 and Diazinon. This stimulus to growth was especially noticeable in July.

2. Continued usage of Tersan-OM increased top growth significantly over the no pesticide treatment when ammonium nitrate and no nitrogen were used.

3. Diazinon and B25141-treated plots, receiving ammonium nitrate or Milorganite as the nitrogen source, had a significantly higher per cent nitrogen in the leaf tissue in July and in August when no nitrogen was applied.

4. Nitrogen uptake was significantly increased over the no pesticide treatment in plants receiving ammonium nitrate or Milorganite, when B25141, Diazinon, and Tersan-OM was applied, especially in July. When no nitrogen was used, the B25141 treatment resulted in a significant increase in nitrogen uptake over the 3-month period of growth.

5. Tersan-OM-treated plots receiving ammonium nitrate as the nitrogen source and no nitrogen had significantly better color than the no pesticide treatment in all three months of growth. Diazinon-treated

plots receiving ammonium nitrate rated better than the no pesticide treatment in August and September.

6. Applications of B25141 insecticide resulted in a significantly higher soil nitrate level than the no pesticide treatment at the end of the experiment, when ammonium nitrate was the nitrogen carrier used.

7. When ammonium nitrate was used, Diazinon treatments resulted in lower numbers of bacteria in the soil than the no pesticide treatment in September. Acti-dione thiram treatments resulted in increased CO_2 tolerant and nitrate utilizing bacteria in September.

8. For all evaluations, plots treated with Tersan-OM at 10 times the recommended rate receiving no nitrogen rated with plots receiving Milorganite.

Laboratory Incubation Experiment

1. Ammonification and accumulations of nitrate were significantly increased over the no pesticide treatment in soil receiving Milorganite as the nitrogen source when Tersan-OM was applied at rates that may accumulate in soils over a period of time.

2. At these rates, B25141, Diazinon, Dieldrin and Calo Clor resulted in inhibition of ammonification.

3. All pesticides caused significant changes in patterns of ammonification and nitrification of nitrogen released from Milorganite.

The results of this investigation indicate that pesticides may affect the physiology of turfgrasses, the ecological system, or the nitrogen relations in the soil With the increased use of pesticides on turf, turf managers should be aware of these possible effects. This study indicates a need for further research in this area and suggests that caution should be used when applying pesticides to turf.

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APPENDIX

Table 15. Analyses of variance for data of greenhouse experiment 1.

Source	d.f.	Mean sq.	F	Approximate significance
		Clipping weights March 27 - April 14		
A (pesticide)	8	0.062	4.671	0.0005
B (rate)	1	0.220	16.623	0.0005
A x B	8	0.024	1.799	0.098
Error	54	0.132		
		Clipping weights April 14 - May 14		
A	8	0.454	32.290	0.0005
B	1	0.056	3.994	0.051
A x B	8	0.130	0.276	0.0005
Error	54	0.014		
		Clipping weights May 14 - June 14		
A	8	0.242	18.548	0.0005
B	1	0.003	0.197	0.659
A x B	8	0.206	15.770	0.0005
Error	54	0.013		
		Total clipping weights		
A	8	1.693	29.162	0.0005
B	1	0.429	7.395	0.009
A x B	8	0.567	9.767	0.0005
Error	54	0.058		
		ppm Soil mineral nitrogen		
A	8	78.288	8.033	0.0005
B	1	264.883	29.9942	0.0005
A x B	8	37.592	3.831	0.001
Error	54	9.813		

Table 16. Analyses of variance for data of greenhouse experiment 2.

Source	d.f.	Mean sq.	F	Approximate significance
		Total clipping weight		
A (pesticide)	4	5.036	28.104	0.0005
B (nitrogen carrier)	6	45.231	252.402	0.0005
A x B	24	0.312	1.741	0.029
Error	105	0.179		

Table 17. Analyses of variance for clipping weight data of the field experiment.

Source	d.f.	Mean sq.	F	Approximate significance
		Clipping weights - July		
A (pesticide)	4	2582400.199	12.053	0.0005
B (nitrogen carrier)	2	32653196.183	152.404	0.0005
A x B	8	481240.806	2.246	0.054
C (replication)	2	827325.640	3.861	0.330
Error	28	214253.733		
		Clipping weights - August		
A	4	990740.233	4.328	0.008
B	2	102720518.141	448.772	0.0005
A x B	8	76828.425	0.336	0.945
C	2	989811.287	4.234	0.023
Error	28	228892.520		
		Clipping weights - September		
A	4	511952.188	2.764	0.047
B	2	92155654.082	497.456	0.0005
A x B	8	106943.988	0.577	0.788
C	2	9472.514	0.053	0.949
Error	28	185253.805		
		Clipping weights - Total		
A	4	5894592.391	6.490	0.001
B	2	640218930.516	704.873	0.0005
A x B	8	474302.262	0.522	0.830
C	2	21704.601	0.024	0.976
Error	28	908275.834		

Table 18. Analyses of variance for per cent nitrogen in leaf tissue data of the field experiment.

Source	d.f.	Mean sq.	F	Approximate significance
		Per cent nitrogen in leaf tissue - July		
A (pesticide)	4	0.279	10.815	0.0005
B (nitrogen carrier)	2	3.701	143.513	0.0005
A x B	8	0.016	0.612	0.760
C (replication)	2	0.013	0.501	0.611
Error	28	0.026		
		Per cent nitrogen in leaf tissue - August		
A	4	0.186	12.544	0.0005
B	2	5.747	387.466	0.0005
A x B	8	0.019	1.304	0.282
C	2	0.042	2.855	0.074
Error	28	0.014		
		Per cent nitrogen in leaf tissue - September		
A	4	0.076	3.673	0.016
B	2	7.561	367.239	0.0005
A x B	8	0.030	1.470	0.213
C	2	0.010	0.485	0.621
Error	28	0.021		

Table 19. Analyses of variance for turf color rating and density count data of the field experiment.

Source	d.f.	Mean sq.	F	Approximate significance
		Turf color rating - July		
A (pesticide)	4	1.361	4.744	0.005
B (nitrogen carrier)	2	30.717	107.062	0.005
A x B	8	0.369	1.288	0.290
C (replication)	2	2.317	8.075	0.002
Error	28	0.287		
		Turf color rating - August		
A	2	1.986	9.931	0.0005
B	2	39.267	196.333	0.0005
A x B	8	0.315	1.576	0.177
C	2	0.950	4.750	0.017
Error	28	0.200		
		Turf color rating - September		
A	4	1.425	6.401	0.001
B	2	64.550	289.957	0.0005
A x B	8	0.154	0.693	0.695
C	2	1.217	5.465	0.010
Error	28	0.223		
		Density counts		
A	4	2318.611	0.373	0.862
B	2	27482.067	4.416	0.022
A x B	8	161.594	0.026	1.000
C	2	130.400	0.021	0.979
Error	28	6223.995		

Table 20. Analyses of variance for soil mineral nitrogen data of the field experiment.

Source	d.f.	Mean sq.	F	Approximate significance
		ppm Ammonium nitrogen		
A (pesticide)	4	0.826	1.072	0.389
B (nitrogen carrier)	2	5.931	7.695	0.002
A x B	8	0.394	0.511	0.838
C (replication)	2	5.435	7.051	0.003
Error	28	0.771		
		ppm Nitrate nitrogen		
A	4	1.981	1.406	0.285
B	2	21.095	14.997	0.0005
A x B	8	1.743	1.237	0.315
C	2	1.558	1.106	0.345
Error	28	1.408		
		ppm Total nitrogen		
A	4	3.047	1.242	0.316
B	2	46.430	18.929	0.0005
A x B	8	1.744	0.711	0.680
C	2	10.238	4.147	0.026
Error	28	2.453		