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Environmental studies of Fusarium blight in Merion Kentucky bluegrass.

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ENVIRONMENTAL STUDIES OF FUSARIUM BLIGHT IN MERION KENTUCKY BLUEGRASS

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by

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INTRODUCTION AND LITERATURE REVIEW

Merion Kentucky bluegrass (*Poa pratensis* L. var.) is a widely used turf grass within the cool humid regions of the United States. With adequate management, Merion forms an excellent dense, dark green, tightly-knit turf which usually can withstand the rigors of northeastern climatic variability. Merion bluegrass is relatively resistant to leaf spot (*Helminthosporium* spp.) but is susceptible to stem rust (*Puccinia graminis* F. sp. agrostis, Eriks.), stripe smut (*Ustilago striiformis* (West) Niessl.) and mildew (*Erysiphe graminis* D.C.). (19) (71).

Couch (19), in 1959, first described the symptomology of Fusarium blight as a disease of fine turf grasses incited by *Fusarium roseum* (Lk), Snyder and Hansen. Among the grasses susceptible is Merion bluegrass. Since that time turf pathologists from other areas, in the cool, humid region, have reported similar findings. In further work, *Fusarium roseum* was described by Couch (21) to be pathogenic on bentgrass (Agrostis spp.) and creeping red fescue (*Festuca rubra*).

During the intervening years, from 1959 to the present time, the use of Merion bluegrass for aesthetic, recreational and utility purposes has increased markedly, partly because of local turf specialists' recommendations, and partly through advertising media and marketing techniques. In fact, its use is so widespread that Merion is not only planted in pure

stands but is also used as the dominant species where mixed stands are desirable because of economic requirements (57). Although Merion is relatively slow to germinate, 10 to 14 days, once it has become established it grows into a thick well-knit sward that may retard severe weed infestation. Merion has a rhizomatous growth habit which with clipping debris, and under sustained management, forms a thick blanket of thatch. This thatchy layer, although possibly beneficial in preventing high soil water losses from the soil, may also impede the percolation of nutrients and water into the soil; therefore, to maintain a healthy stand, dethatching is often necessary. Concomittant with the presence of this thatch, caused by sloughing off of roots and collected debris from clippings, is the possibility that this thatchy region may also be a reservoir for the build-up of all sorts of pathogenic inoculum.

The cost of fine turf maintenance on a nationwide basis has been estimated at about four and one-third billion dollars annually (59). About one-half of this total expenditure is spent within the cool humid growing regions, and of this total cost several hundred million dollars is spent on disease prevention and control. Although actual maintenance costs attributed to disease problems in any one grass genus or species is not known, it is quite likely that large outlays are involved in keeping a healthy Merion turf. It is a common practice among professional turf managers such as golf course

superintendents, landscapists, park and other grounds superintendents who are familiar with many of the disease problems associated with growing and maintaining fine turf to budget certain sums specifically for disease control annually. While the homeowner, on the other hand, attempts to keep a beautiful turf with a minimal outlay for grooming, fertilization, and weed-killers.

In order to control a disease, one must understand the whole epidemiology of the pathogen and its host. Couch (19, 20, 21) describes the symptoms of Fusarium disease aptly, and accurately. In overall view, affected turf grass stands first show scattered light green to grayish green patches 2-6 inches in diameter; the color of these patches changes in a 24-48 hour period to a dull reddish brown; then to a tan, and finally, to a light straw color. Initially, as described by Couch, the shapes of the affected areas are either elongate streaks, crescents, or circular patches (21).

Perhaps the most outstanding feature of this disease is the characteristic "frogeye" effect left when circular areas of turf have been blighted, with the exception of one to several, apparently unaffected grass plants remaining in the center. Under epiphytotic conditions the Fusarium mycelium moves out from the point of initial infection either in an ellipsoid or circular direction. Close macroscopic examination reveals whitish strands of mycelium covering the leaf blades. In its foliar pathogenic stage, Fusarium is said to

penetrate through wounds, natural openings or directly into the host (19, 20, 21). Leaf lesions which may occur at the cut ends, or randomly over the grass blade, are typically white-centered, surrounded by light to dark brown colored tissue. Some individual lesions may cover the entire width of a leaf in which case the leaf tips, or leaf portions above the lesions, assume a yellow coloring. Once germination takes place and successful entry has been made, the fungus organism moves inter and intracellularly upwards into the leaf tips and downward into the crown bud region. If environmental conditions necessary for the organism change, and the crown bud region has not been affected, chances for resumption of plant growth may be good. If, on the other hand, a large inoculum is present and environmental conditions in the rhizosphere and atmosphere are adequate for fungal proliferation, Fusarium roseum will usually cause death of the grass plants via crown rot and foliar cortical decay.

According to present ecological thought, each microorganism possesses an "ecological niche," for the occupation of which it is given a peculiar competitive advantage by its intrinsic genetic constitution. Ever since Sanford's classical paper of 1926 (67), in which he suggested that the control of potato scab by green manuring was biologically possible, because of the increase in the population of certain saprophtic bacteria that multiplied upon the organic material, eco-environmental studies have been carried on with ever-increasing fervor (8, 10, 12, 15, 24, 26, 27, 30, 31, 32, 33, 36, 38, 41,

44, 47, 55, 75). Of the thousands of investigators who have been involved in the study of the disease state in plants, since 1926, too few have been concerned specifically in developing a closer understanding of a specific "ecological niche," or of the underlying role of an associative environmental regime. It is with the latter association that this paper is concerned, i.e. what are the relations macro- and micro- that lead to the disease state and eventually bring widespread kill of Merion bluegrass. Fusarium spp. have been described by many investigators to be ubiquitous in nature, but not always does the mere presence of this organism lead to disease much less to an epiphytotic state (5, 10, 16, 33, 41).

Investigations by plant pathologists over many years have shown that soil inhabiting organisms have specific requirements for growth. Some of the ones most often mentioned are: C:N ratios, O_2, CO_2, free water, humidity, micronutrients, enzymatic capability, and sources of energy. However, even with all nutritive demands met, a pathogen may still not pass over into the disease state and may remain an innocuous saprophyte. Fusarium roseum, the incitant of Fusarium blight in Merion turf grass, seems to have its "ecological niche." What is it then that changes it from avirulent to virulent? If it is in a bio-competitive system, what part of that system favors proliferation and pathogenicity? Since some pathologists have dealt with the nutritional phase (13, 20, 27, 31, 49, 54, 58, 68, 72, 74, 77) requirements of Fusarium, it was of interest to this investigator to approach the

problem of Fusarium blight from an environmental standpoint.

A fungus growing and multiplying in a pure culture is quite unlike a fungus co-existing in the soil with other organisms; in the presence of a living host, in organic debris, on the organic-mineral gel surfaces of the colloidal aggregates of soil is another. Within a living host, perhaps, an organism may not meet competition, and only be subject to exo-environmental stresses. In the soil, either in organic debris or in a colloidal ecosystem bounded by stringent parameters of a physico-chemical environment, the organism may have severe competition from its co-inhabitants which it may, or may not, overcome in order to meet minimal nutritive requirements. Whatever the system is, it is most likely not an isolated one but rather part of a chain in the ecosystem.

Alexander, Mitchell and others (1, 15, 24, 25, 32, 37, 48, 51) have shown, in their work with certain field crops, that it might be possible to control a pathogenic organism by influencing populations of microflora lytic or competitive to the pathogen responsible for the disease state (1, 15, 24, 25, 32, 37, 45, 51,54, 55, 56). Their work evolved from a hypothesis that if a certain substrate, in a relatively pure form, was added to a soil then organisms that possessed the enzyme capability to utilize this material might build up their populations to either the exclusion or detriment of unwanted pathogenic organisms. Ground lobster shell was selected by them as a soil amendment for several reasons:

first, lobster shell was rich in chitin; secondly, the Fusaria spp. contains chitin as a significant structural component of the cell wall. Therefore, with the addition of lobster shell it was postulated that chitinovorous organisms would be stimulated, and once large populations were built up, these organisms would utilize any chitinaceous substrate present in the soil, and lyse those organisms that possessed it. Apparently, the investigators were somewhat successful, and based on their work this investigator studied the effect of ground lobster shell on Fusarium roseum, incitant of Fusarium blight (54, 55, 56).

In addition to the lobster shell study, the influence on the soil microflora of other soil amendments such as Diazinon, an organophosphate insecticide; fungicides, sucrose, and the influence of soil moisture systems were investigated.

Investigatory research was carried out in the laboratory, in the greenhouse, and in the field so that all aspects of the environmental regime could be adequately analyzed in the disease syndrome.

PATHOGENICITY STUDIES OF *FUSARIUM ROSEUM* f. sp. (Lk.), SNYDER AND HANSON ON MERION KENTUCKY BLUEGRASS

MATERIALS AND METHODS

Pathogenicity tests were conducted on Merion Kentucky bluegrass (*Poa pratensis* L. var.). Seed was obtained from stock supplies of a local seedsman, that had a certified purity of 90% with a germination potential of 84%. The seeds were surface-sterilized by immersion in a 1:10 dilution of 5.25% sodium hypochlorite (clorox) for 10 minutes and subsequently washed three times in distilled deionized water, air-dried and dusted with Ceresan M (7.7% N - (ethylmecuri) - p - (toluenesulfonanilide) (19, 20).

A sandy loam soil of pH 6.3 was selected as the growth medium to provide suitable aeration and drainage. Nine, four inch pre-sterilized clay pots were filled to one-half inch of the rim. The soil was brought to field capacity and steam sterilized for one hour. After the sterilization cooling period, 2 lbs. equivalent of N/1000 square feet in a granular 10-10-10 form was added.

After seeding, the containers were stored under an automatic misting system until the plants were established. Post-irrigation was performed by alloting one acre inch of distilled deionized water per pot, divided into two weekly applications.

The cultures of Fusarium roseum f. sp. (Lk.) Snyder and Hanson, were selected from several local Merion turf grass sites in which Fusarium blight had reportedly occurred. Foliar samples of blighted turf were selected on the basis of symptoms described by Couch (19, 20, 21). Leaf samples were immersed in a 1:10 dilution of 5.25% sodium hypochlorite for 10 minutes and washed 3 times in distilled deionized water. Randomly selected diseased samples were cut up, aseptically, into 3 - 5 mm. sections and sprinkled onto warm potato dextrose agar (Difco), adjusted to pH 5.6, in Petri dishes and placed in an incubation chamber at 28°C for 96 hours. After germination and growth, examination of mycelia and conidia was made for taxonomical and physiological characteristics. Additionally, a culture slant of a known pathogenic form of Fusarium roseum f. sp. (Lk.), Snyder and Hanson, obtained from Dr. Huston B. Couch, Head, Department of Plant Pathology, Virginia Polytechnic Institute, Blacksburg, Virginia, was plated out onto potato dextrose agar adjusted to pH 5.6; incubated at 28°C and compared for similar physiological responses and taxonomic features as those isolated from local turf grass sites.

The inoculum was prepared by growing pure cultures of the organism on 1% corn-meal sand mix (63), that had been autoclaved for 15 minutes at 120°C. The inoculum preparations were placed in an incubation chamber and checked periodically at 72-96 and 120 hours; at 120 hours the fungus had attained

sufficient growth for further work. The plates were then air-dried. Uniform amounts of inoculum were scattered over the foliage of the grass plants and maintained at air temperatures of about 21-23°C.

Prior to inoculation, three pots of Merion were wounded by cutting with ordinary grass shears; three pots of grass were cut by using a sterile razor and three pots were left uncut. After infection had taken place in each of the three treatments, the organism was re-isolated in the manner previously described, followed by a re-inoculation and re-isolation of the Fusarium.

RESULTS

Fusarium roseum f. p. (Lk.), Snyder and Hanson, isolates from selected sites proved to be extremely pathogenic to Merion Kentucky bluegrass tested. Lesions had occurred randomly at the cut ends more often than were found on the grass blade proper. According to results obtained by Couch (21), the lesions first appear as irregularly-shaped, dark green blotches of varying sizes from 2mm up to 10mm; these rapidly fade to a light green, then assume a reddish-brown cast, and finally in time become tan to straw color. Moreover, additional examination showed that rapid proliferation and extensive mycelium development took place in leaf regions where 3 or more lesions occurred within close proximity. Microscopic examination of the diseased blades showed, initially, that penetration of the infection peg appeared

very rapid through the intercellular spaces. Intracellular penetration occurred rapidly in younger paranchymatous cells, whereas, direct penetration by mycelia into surrounding older epidermal cells was less rapid. The fungus grew in both an acropetal and in a basipetal direction equally well. Young succulent blades were more readily susceptible to infection than the older blades. Since the number of successful penetrations and lesions were usually found more often on younger blades than on the older blades, it would appear that pectolytic enzymatic action is initially responsible for the dissolution of the younger tissues. Couch and Bedford (21) observed that a great number of successful penetrations, and a higher degree of mycelium development, occurred at the cut ends of leaves than on uncut blades; their results were in agreement with this investigator's findings. Although numbers of microconidia, macroconidia and chlamydospores were not recorded, it appeared under microscopic examination, that while chlamydospores were formed, there was a preponderance of micro and macrocondia. Size, shape and general taxonomic characteristics and tolerances of the organism in vivo were in agreement with observations by Snyder and Toussoun (73).

A higher observable percentage of inoculum take was found in the pots of grass which were cut by using a sharp razor. Not only was the take higher, but infection signs appeared only 30 hours after inoculation, compared to 44 hours and 50 hours in plants cut with grass shears and uncut plants,

respectively. Apparently, the razor-cut blades exuded and supplied more sustenance for a longer time, than the duller tearing action of shears. This treatment was the most indicative and significant.

Following the initial pathogenicity results, foliar samples from the diseased plants were surface sterilized, in the manner previously described, and an additional inoculation and infection series in accord with Koch's postulates proved the pathogenisis of Fusarium to Merion bluegrass, in the test performed. Pure cultures were derived and stored in potato dextrose agar slants and Petri dishes for following experimentation.

AN INVESTIGATION OF THE INFLUENCE OF CERTAIN SOIL AMENDMENTS ON A NEWLY ESTABLISHED TURF INOCULATED WITH FUSARIUM ROSEUM

MATERIALS AND METHODS

A fine sandy loam of pH 6.5 brought to field capacity was steam-sterilized for one hour in 1' x 1' x 6" wooden boxes. All the soil in the boxes received an application of 2 lbs. N/1000 square feet in the form of a 10-10-10 granular fertilizer after sterilization. Each box was divided into four equal six-inch compartments by corrugated 1/32" aluminum sheeting. Merion Kentucky bluegrass seed was surface sterilized and treated in a manner previously described.

Lobster shell was contributed by the Maritime Packers, Division of National Sea Products, Ltd., Pictou, Nova Scotia. The frozen, partially ground, lobster shell was air-dried in sunlight for 72 hours, ground on a Hammer mill grinder until the ground shell was able to pass readily through a 60 mesh sieve. The finely ground lobster shell was added at the rates of 600 lbs./acre and 1200 lbs./acre and evenly distributed into the top inch of soil prior to seeding.

The Geigy Chemical Corporation contributed liquid Diazinon AG 500, an emulsifiable insecticide solution containing as active ingredients: 48% 0-0-diethyl-0-(2 isopropyl-4 methyl-6 pyrimidinyl) phosphorothionate, and xylene 36%, plus, inert ingredients of 16%, Diazinon was applied at the

rate of 4 oz. in 3 gallons of water per 1000 square feet, and, at the 8 oz. rate in 3 gallons of water, the first treatment was added to the plots prior to seeding.

Sucrose was applied at a 700 lb./acre and 1400 lb./acre basis and evenly distributed into the upper inch of soil in randomly selected plots prior to seeding.

Individual Treatments	Rate
Sucrose	700 lbs/acre = xS
Sucrose	1400 lbs/acre = 2xS
Lobster shell	600 lbs/acre = xL
Lobster shell	1200 lbs/acre = 2xL
Diazinon	4 oz/1000 sq. ft. = xD
Diazinon	8 oz/1000 sq. ft. = 2xD

Combination Treatments	
Sugar - Diazinon - Lobster Shell	x - x - x
" " " "	x - 2x - x
" " " "	x - x - 2x
" " " "	2x - x - x
" " " "	2x - 2x - x
" " " "	2x - 2x - 2x
Sugar and Lobster shell	x - x
" " "	x - 2x
" " "	2x - x
" " "	2x - 2x

Sugar and Diazinon	x - x
" "	x - 2x
" "	2x - x
" "	2x - 2x
Diazinon and Lobster shell	x - x
" " " "	2x - x
" " " "	x - 2x
" " " "	2x - 2x

The plots received 3 applications of treatments, one just prior to seeding; the second 30 days after the grass was established, and 60 days following this treatment. The materials were evenly distributed and each plot selection within the boxes was completely randomized with untreated controls for each.

The grass plots were maintained at a 1 1/2 inch height of cut. All the plots received a measured 1 acre inch of water per week, applied in two, equal, weekly applications. Because of the large number of plots, the only practical means of maintaining a 1 1/2" cut was with well-kept, almost razor sharp, grass shears. A template of 1 1/2 x 1 1/2 inch thick hard pine was placed over the rim of each box and used as a guide for height of cut.

Fertilizer was added at the rate of 2 lbs. of N/1000 square feet in late May and late June using a 10-10-10 analysis granular fertilizer. Total N for the boxes was 6 lbs./1000 square feet.

Fusarium inoculum was prepared on a 1% corn-meal sand mix (65). After initial plant growth had attained a 2-inch height, the grass was clipped to the desired 1 1/2 inches. One square inch of air-dried corn-meal sand containing spores and fragmented mycelia was evenly distributed over each six-inch square plot.

Three 15 gram soil samples were removed from each plot by means of a 1/2 inch soil sampling tube. Samples were placed in marked wax treated cardboard cylinders. Plants and plant debris were separated from the soil immediately after removal of the core; these were placed in marked plastic bags and stored at 34°F for future examination. Soil sample replicates from each plot were thoroughly mixed, air dried, sifted through a 9-mesh sieve. The soil dilution method used was a variation after the technique of Johnson, Curl, and Freboug (46). After sieving, a 25 gram sample of dry soil was placed in a 250 ml. graduated cylinder. Sterilized, distilled, deionized water was added to bring the total volume to 250 ml. The cylinder was agitated by hand, and while the mixture was still in suspension, poured rapidly into a 500 ml. Erlenmeyer flask and agitated on a floating type agitator for 20 minutes.

While soil was still suspended, samples were drawn into a sterile 10 ml. pipette. The pipetted sample was transferred into a 90 ml. flamed sterile water blank. Successive 10 ml. transfers were made from the 1 in 10 dilution into successive

sterile water blanks to obtain 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} serials. Prior to each pipetting, suspensions were thoroughly shaken by hand. Concentrations of 10^{-4} were selected as best suited for fungal counts and 10^{-6} for bacterial and actinomycete population counts. 1.0 ml. pipette suspensions for fungal counts were transferred into plastic Petri dishes that had about 15 ml. of cool potato dextrose agar adjusted to pH 5.6, and the plates swirled to obtain a homogeneous mixture. A similar type of transfer was performed from 10^{-6} suspensions; however, 15 ml. of cooled bacterial agar adjusted to pH 7.0 was added to these aliquots. Plates were incubated at 28°C; examined at 24-hour intervals, after the first 48 hours. Fungal growth appeared optimum after 5 days, whereas bacterial growth required 7 days. A Quebec colony counter was used for bacterial, actinomycete, and fungal population determinations. Counts were recorded for future assimilation into a graphic and statistical analysis. All plate transfers were replicated 3 times.

Microscopic examination of plants was made on those plant blades in which lesions were found. The leaf samples were cleared and stained in a warm lacto-phenol-cotton blue solution, Couch and Bedford (21). Other leaf samples were surface sterilized, sectioned and placed in potato dextrose agar as previously described.

Visual examination of each grass plot was performed and comments regarding changes in growth habit, color and disease signs or symptoms were recorded.

RESULTS

Isolations of fungi, bacteria and actinomycetes, were made following 30 days after each application of treatments by the soil-dilution method, previously described. Counts were tabulated from the averages of 3 plates per dilution per treatment. The following trends were noted:

Fungi:

1.) Wherever a readily available organic C-source was added the numbers of fungi increased over the controls (Figure 1). At 30 days, 6 x 10^4 populations of predominantly Fusarium roseum colonies were found, as compared to 12 x 10^4 for the control; however, after the third application of sugar, at 60 days, subsequent 90-day counts showed 24 x 10^4 versus a control fungal count of 16 x 10^4. Both the control and the treated plots showed decreased counts at the 60-day level. Observable also, was that when actinomycete populations were high, fungal populations were found in low numbers. On the other hand, high fungal counts apparently had a competitive effect on actinomycete development.

2.) Applications of Diazinon and lobster shell had a suppressant effect (Figures 2, 3) which apparently displaces the C effect in unamended soils. Diazinon, applied at the manufacturer's recommended rate after 30 days had 8 x 10^4 fungi, whereas, the unamended plots had 12 x 10^4 fungal counts.

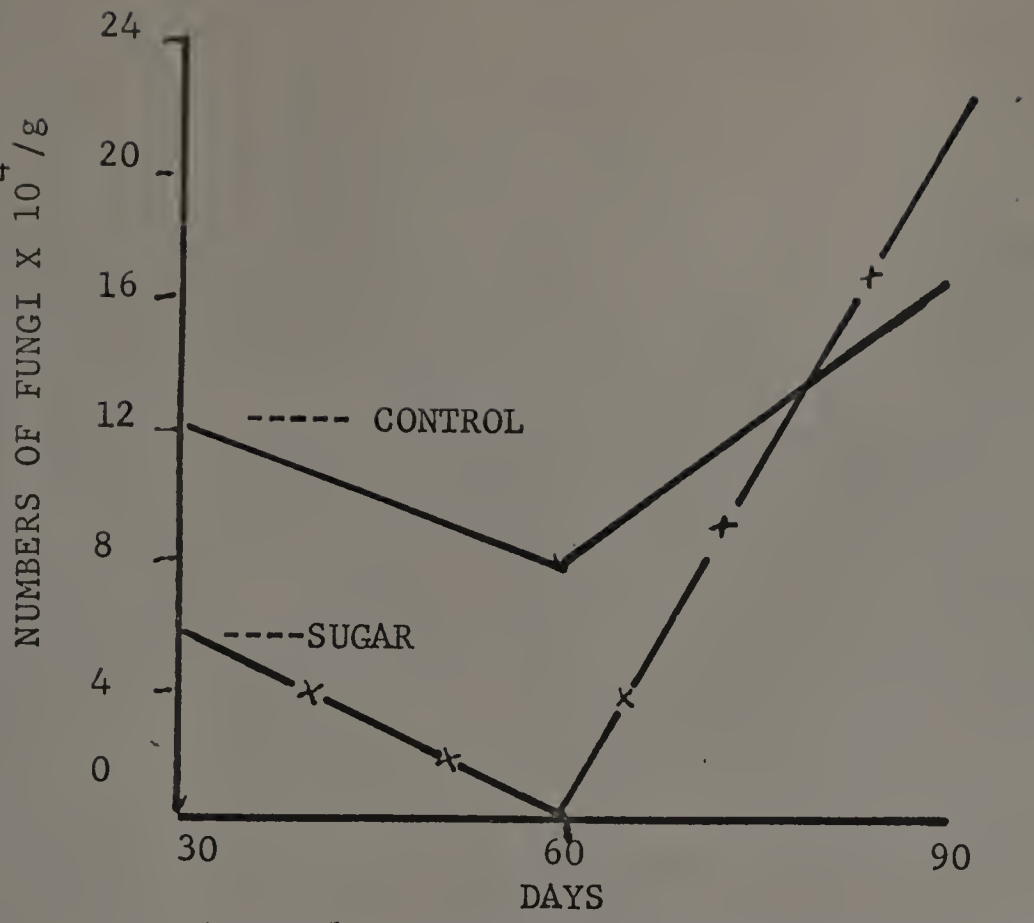


Figure 1; Influence of sugar 1200 lb/acre on fungal populations in newly established turf.

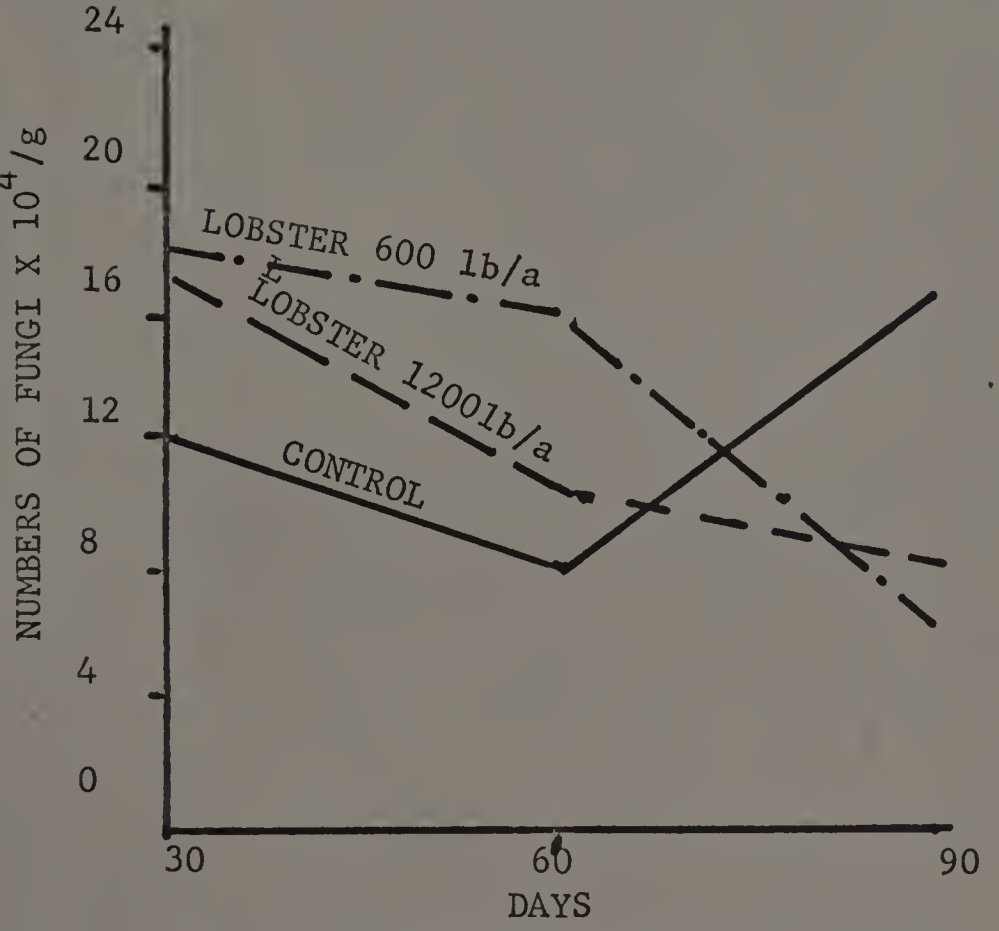


Figure 2: Influence of lobster shell on fungal populations in newly established turf

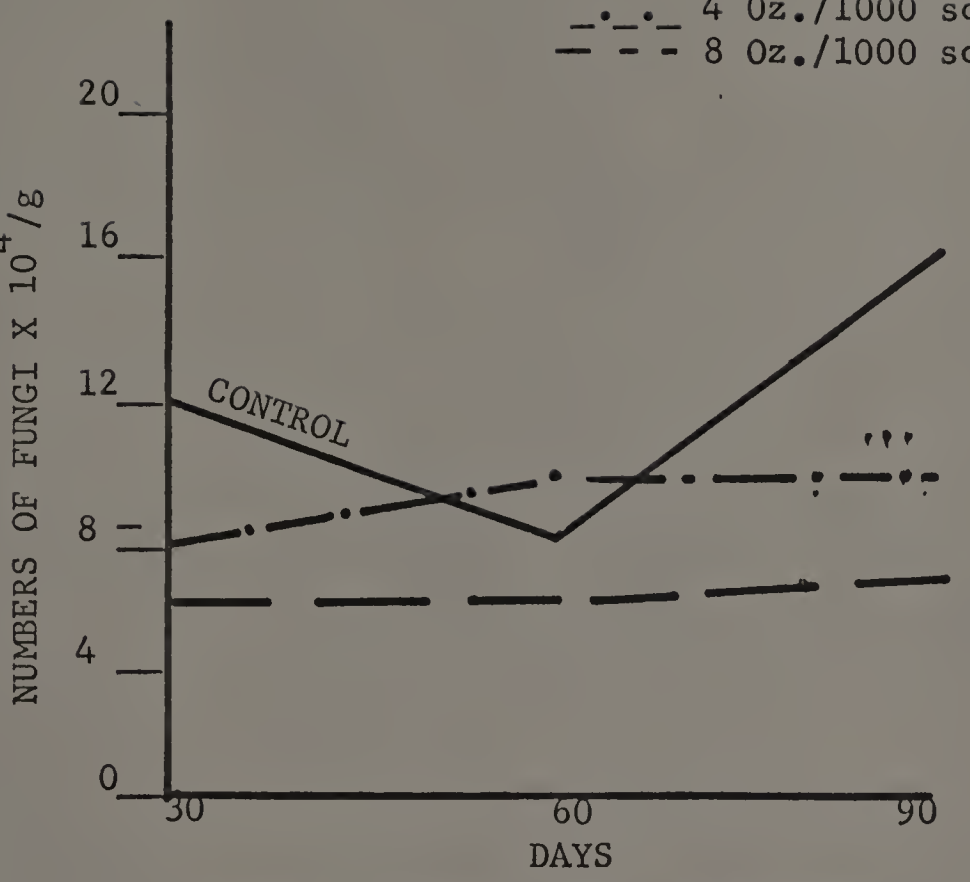


Figure 3: The influence of Diazinon at two rates on fungal populations in newly established turf.

At the 60-day sampling period the control level was at 8×10^4 versus a 10×10^4 counts for the Diazinon treated replicates. However, after 90 days the controls had increased to 17×10^4 while the Diazinon treated plots did not increase at all. At double the manufacturer's recommended rate there was a more definite continued suppression of fungi showing only a very slight increase at the 90-day counting period from 5×10^4 and 6×10^4. Ground lobster shell at 600 lbs./acre at the 30-day sampling time gave fungal population counts of 18×10^4 as opposed to 12×10^4 for the unamended controls. At 60 days the treated plot count changed to 16×10^4 and at 90 days markedly decreased to 6×10^4. Unamended controls at the 60-day period gave counts of 8×10^4 but increased considerably to 17×10^4 colonies. Lobster shell at 1200 lb./acre at 30 days gave 17×10^4 fungi, at 60 days 11×10^4, and at 90 days 8×10^4, a steady decline. Where carbon amendments were added their effect was primary while the Diazinon and lobster shell restrict these only slightly.

Actinomycetes:

Diazinon treated plots, at twice the manufacturer's recommended rates, showed an abrupt change of actinomycete colony counts from a high of 7×10^6 at 30 days to a low of 2×10^6 at the 60-day sampling time, with but a slight upward turn to 3×10^6 at the 90-day level (Figures 4, 5, 6). Comparing the control values of 6×10^6 at 30 and 60 days, and 11×10^6 at 90 days, this represents a decrease at all

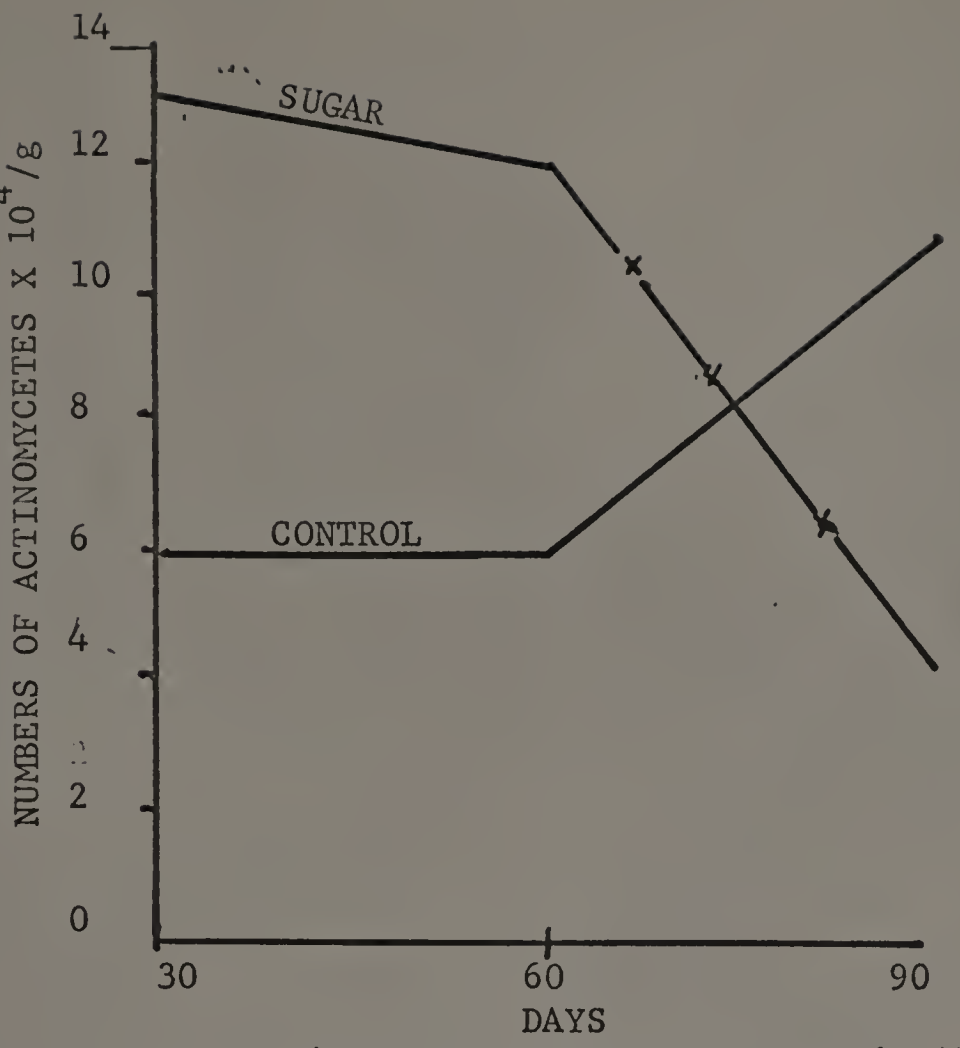


Figure 4: Influence of sugar at 6001b/a on Actinomycete populations in a newly established turf.

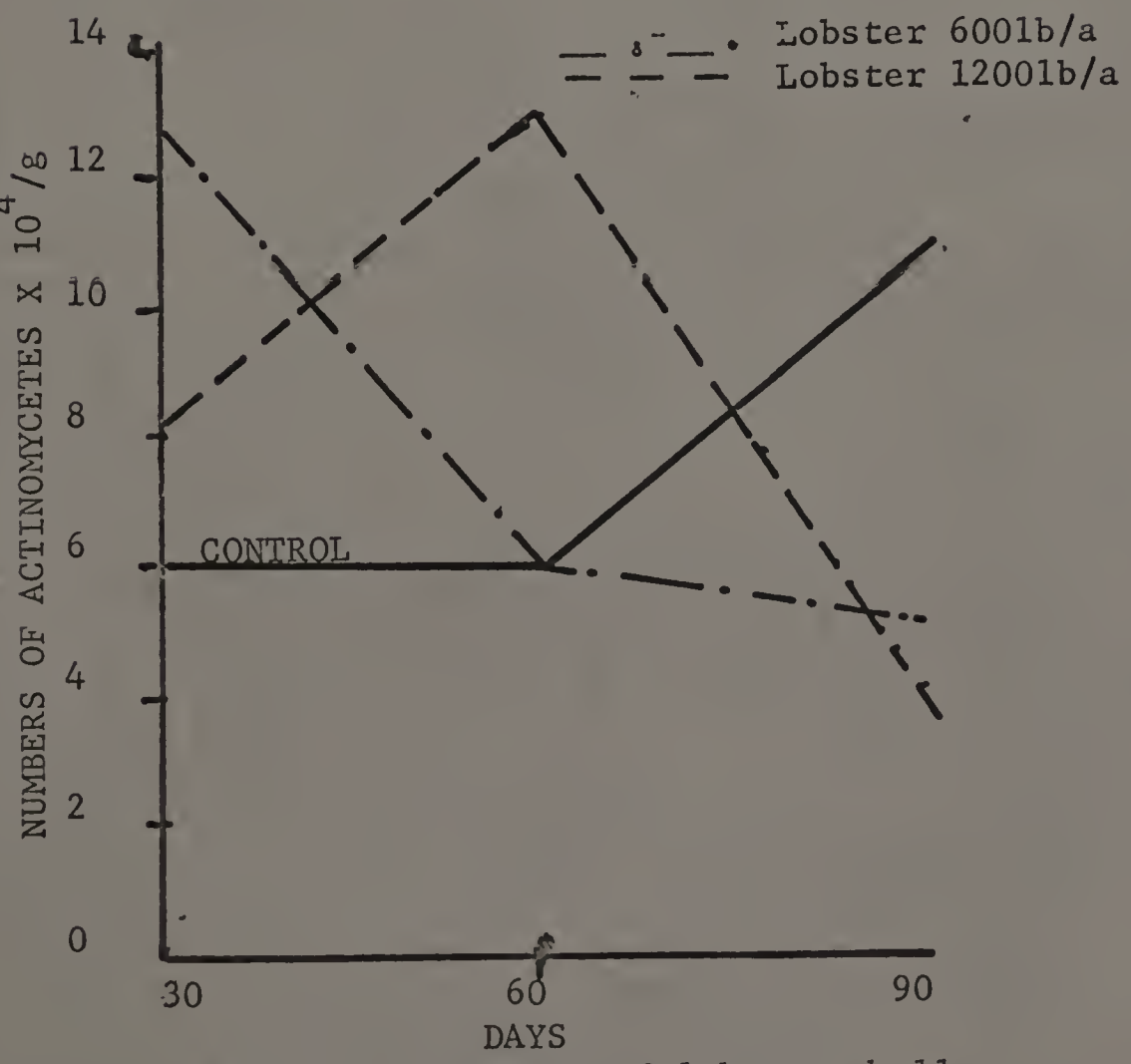


Figure 5: Influence of lobster shell on Actinomycete populations in a newly established turf.

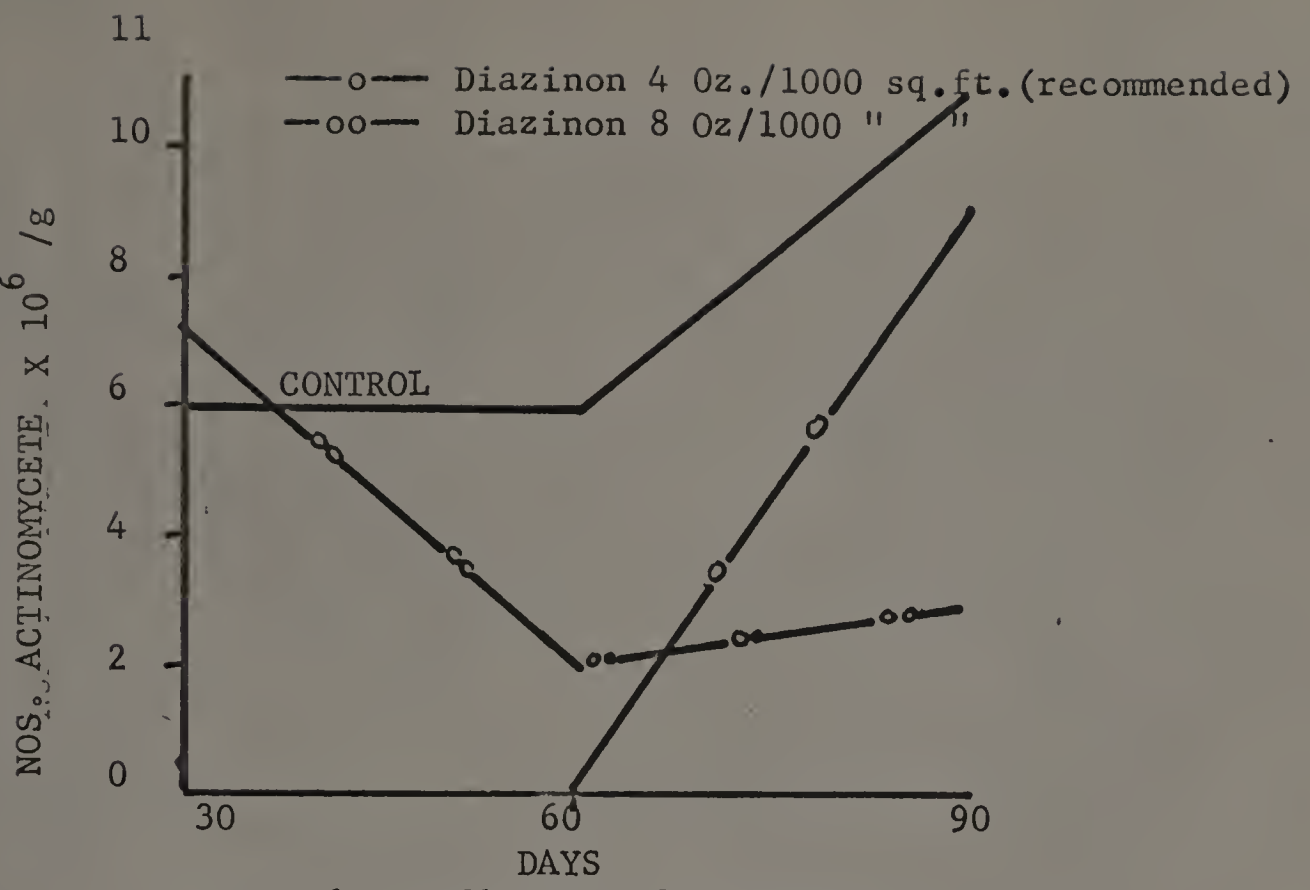


Figure 6: Influence of Diazinon on Actinomycete populations in newly established turf.

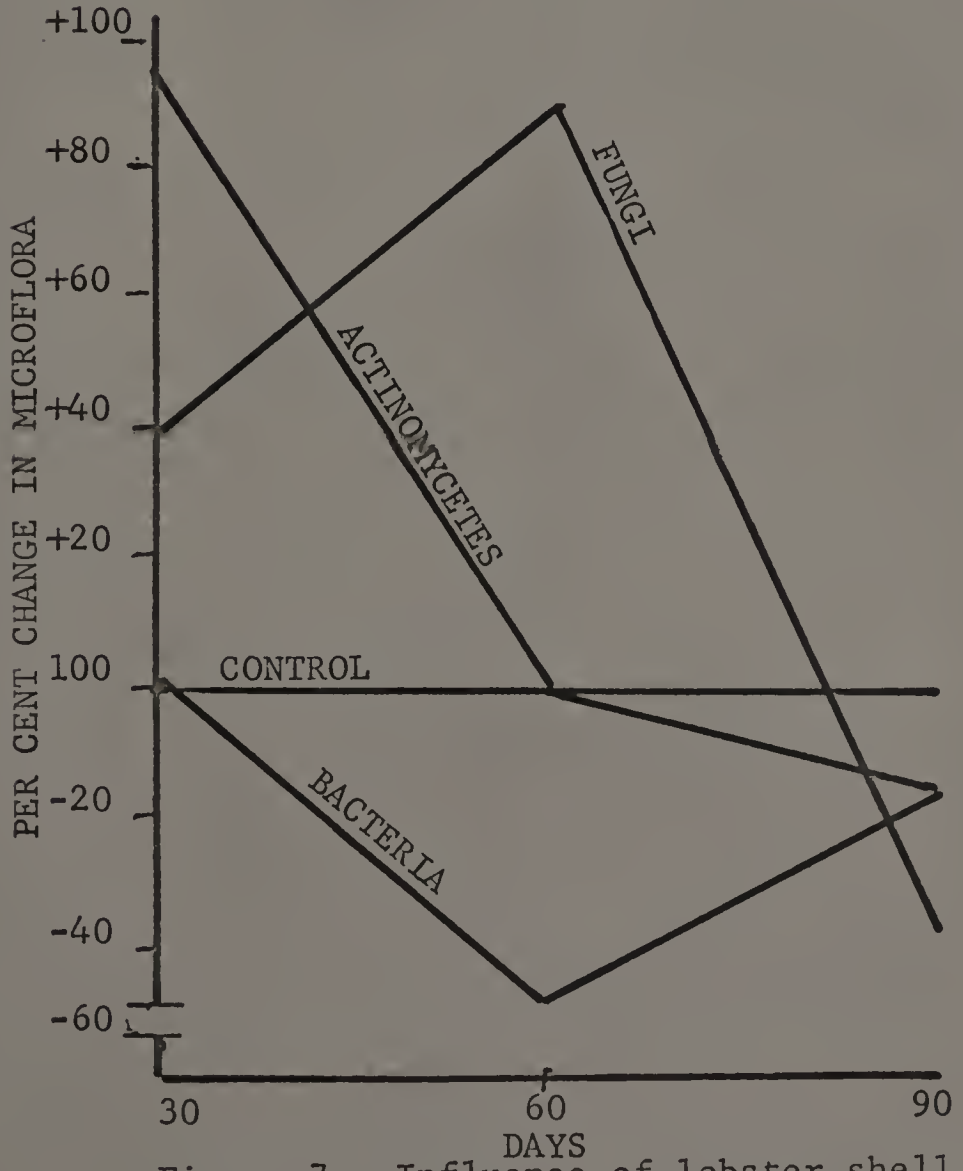


Figure 7: Influence of lobster shell 6001b/a on microfloral populations in new turf.

sampling times. More pronounced, perhaps, is the effect of Diazinon on actinomycetes at the manufacturer's recommended rate. Sampling in these plots 30 days after the initial treatment showed no actinomycete colonies present; this situation persisted after the second treatment at 30 days, and the subsequent 60-day sampling. However, a rapid regeneration and proliferation took place between the 60- and 90-day period, for counts at 90 days gave 9×10^6 for the treated plot, and 11×10^6 for the unamended controls.

The effect of ground lobster shell amendments on actinomycetes showed, initially, 30 days after the first treatments, colony counts of 13×10^6 at 600 lbs./ acre, and 8×10^6 actinomycete colonies per gram of soil at 1200 lb./ acre lobster shell. The control plots maintained rather stable population counts of 6×10^6 for both the 30- and 60-day sampling period. However, at the 60-day examination period the 1200 lb./acre lobster shell treatments assumed a higher level count at 13×10^6 colonies, while the 600 lb./acre treated plots equaled the unamended controls at 6×10^6 counts. The third application of lobster shell was made after the 60-day sampling, and its cumulative effect was shown at the 90-day observational level. Both the 600 lb./acre and 1200.lb./ acre treated plots fell below an increasing control reading. The untreated control had average counts of 11×10^6 as compared to 5×10^6 population counts for the 600 lb./acre treated plots and 4×10^6 actinomycete colonies for the 1200 lb./acre

lobster shell treated area.

Bacteria:

Results of population studies in the sugar treated plots reveal a heterogenous mixture of types of bacteria found, but there was a general suppression of numbers as compared to the control untreated plots (Figures 8, 9, 10, 11). For example, bacterial counts at 30, 60, 90 days in the treated plots were 130 x 10^6, 80 x 10^6, and 185 x 10^6 respectively, per gram of soil. While the control plot averages for 30, 60 and 90 days were 180 x 10^6; 150 x 10^6 and 205 x 10^6, in that order.

Plot treatments with ground lobster shell, at 1200 lbs./acre, showed a decline in recordable results 30 days after the initial application. Mean counts for this treatment, at 30 days, gave 60 x 10^6 bacteria per gram of soil in contrast to 180 x 10^6 bacteria per gram of soil for the control. Before the third application, at 60 days, soil dilution plate counts were 130 x 10^6 bacteria per gram of sampled soil versus 150 x 10^6 bacteria per gram of soil in the unamended treatments. At 90 days, 30 days after the third application of lobster shell at 1200 lbs./acre, 90 x 10^6 bacteria per gram of soil was found while control samples had increased to 210 x 10^6 bacteria per gram of sampled soil. Randomized treated lobster shell plots at the 600 lb./acre rate gave a wide range in the numbers of the various populations present during initial counts. At 30 days, 190 x 10^6

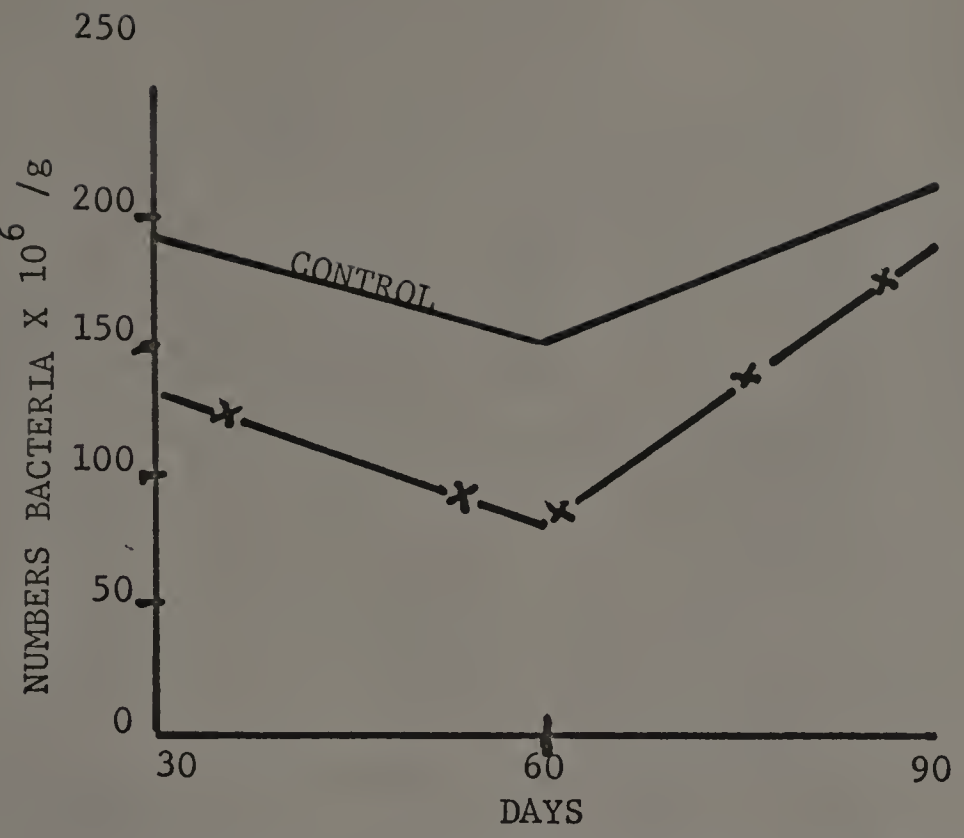


Figure 8: Influence of sugar at 600 lb/a on bacterial populations in a newly established turf

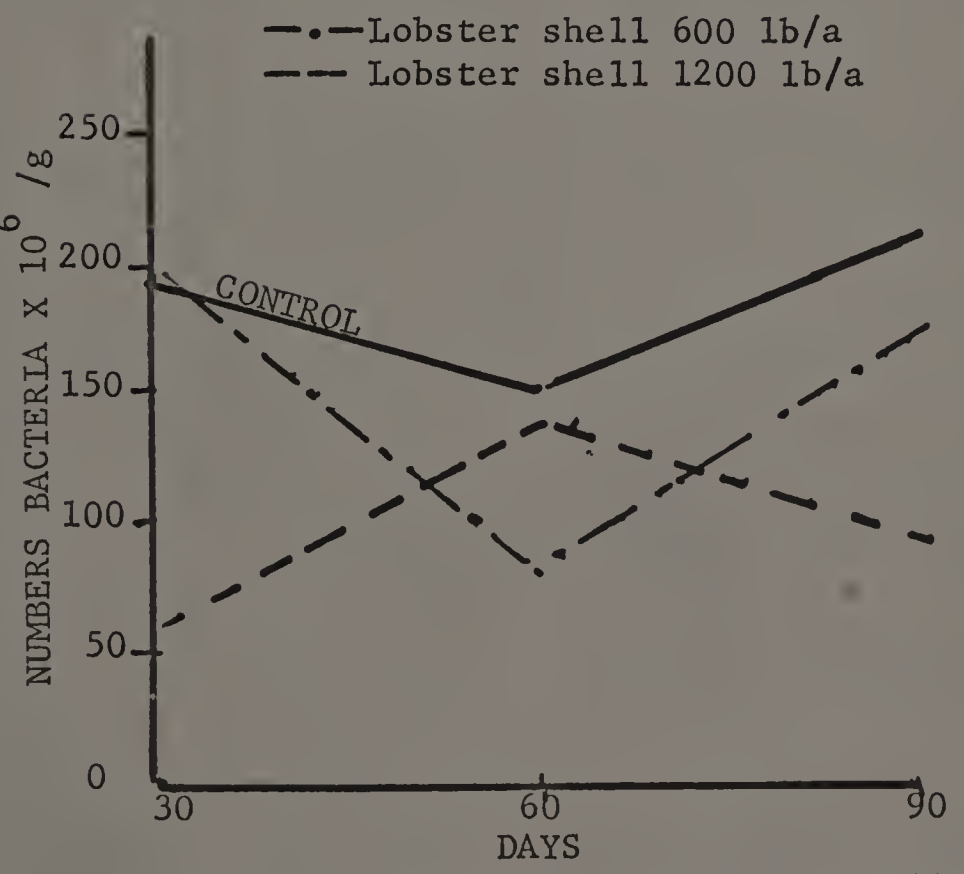


Figure 9: Influence of lobster shell on bacterial populations in a newly established turf.

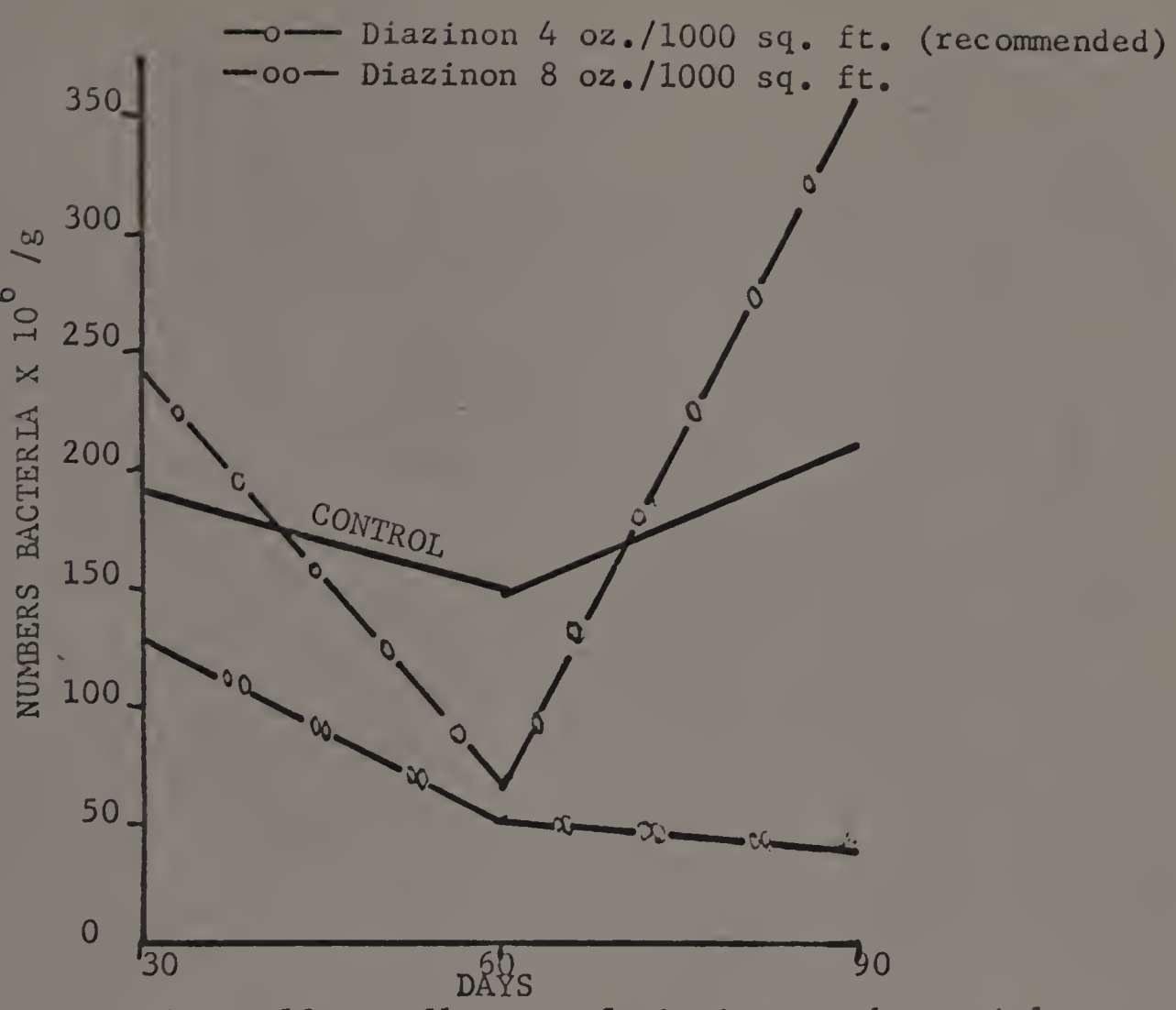


Figure 10: Influence of Diazinon on bacterial populations in newly established turf.

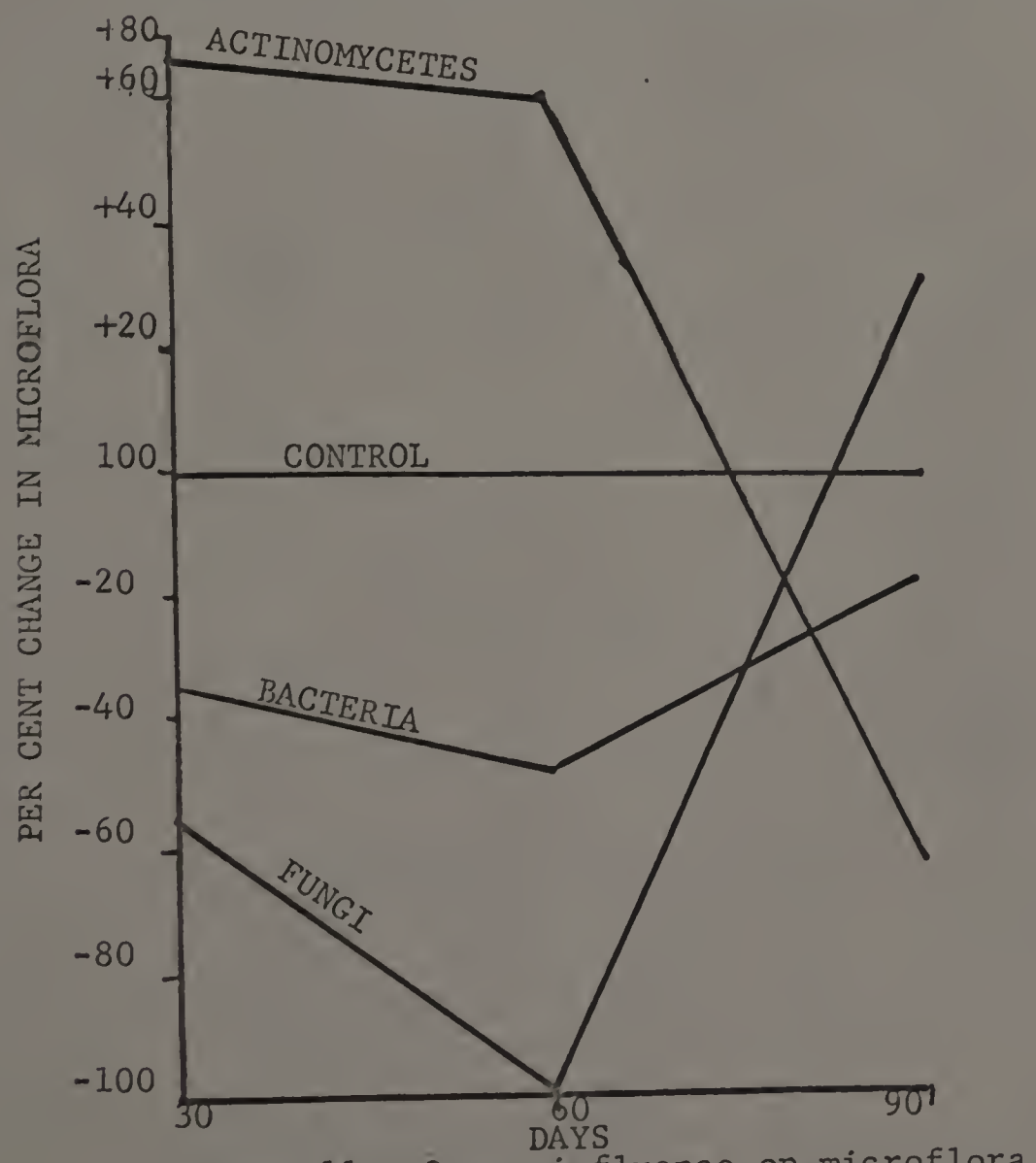


Figure 11: Sugar influence on microflora

colonies of bacteria per gram of soil were isolated while the control readings were 180 x 10^6 bacteria per gram. After 60 days, prior to the third treatment, control plot dilution plate counts were 150 x 10^6 bacteria, whereas a depressed count reading of 80 x 10^6 bacteria per gram of soil was recorded. At the 90-day testing period soil sampling and dilution plates gave relative increases for both the control and lobster shell at 600 lbs./ acre. Readings for the control were 210 x 10^6 as opposed to bacterial population counts of 120 x 10^6 per gram of soil.

Insecticide treated plots at the manufacturer's recommended rate and at two times this rate were tested for bacterial response. Dilution-plates were made and average counts recorded in a fashion similar to all others previously described. Thirty days following the first treatment of Diazinon at 4 oz./1000 square feet, bacteria counts were 240 x 10^6 per gram of soil while the control plot counts were 180 x 10^6 per gram (Figure 10). A second random sample, prior to the third application of Diazinon, gave a very much depressed population count of 65 x 10^6 as compared with a control counting of 150 x 10^6 colonies. A dramatic increase in bacterial counts took place in the insecticide plots 90 days after the first sampling. These counts showed 350 x 10^6 bacteria per gram although the control also increased somewhat, to 205 x 10^6. The increase in the treated plots, however, was due mainly to the proliferation of a selected bacterial

population of the genus Pseudomonad.

Plots treated with Diazinon at the 8 oz./1000 square feet rate, 30 days after the first application, gave counts of 125 x 10^6 in comparison to 180 x 10^6 bacteria per gram of soil for the control (Figure 10). At the 60-day random sampling period, bacterial counts showed a suppressed average reading of 50 x 10^6, while the unamended control, although slightly depressed, was 150 x 10^6 bacteria per gram of soil. The last reading, 30 days following the final Diazinon treatment, 90 days after the first sampling, showed another decline in bacterial populations to 35 x 10^6 bacteria per gram of soil. The control plots in the same period increased to 205 x 10^6 bacteria per gram of populations.

Changes and comparisons within the resultant ecosystem were recorded. The control plots for each series of treatments represented the 100 percent level. Decreases or increases in numbers were then related to this level and allocated a value accordingly.

Sugar:

The effects of the application of sugar on the microfloral population at 600 lbs./acre are shown in Figure 11. Actinomycete populations, one month after the first amendment, showed an increase of 120 percent over the control. Bacterial counts per gram of soil show a reduction of 65 percent. Fungal colony counts per gram of soil were also depressed by 46 percent. At 60 days, 30 days following the second sugar

addition, actinomycete populations had decreased to a level 100 percent above the control counts. At this time bacterial counts had been further depressed to 52 percent. Fungal populations per gram of soil, meanwhile, had declined to the point of disappearance. Ninety-day random sampling average counts, however, showed a reversal in trends. Fungi to 30 percent above the control level bacteria reduced to 18 percent less than the control, and actinomycete populations counts drastically lowered to 64 percent below the control.

Lobster Shell:

The ground lobster shell effect at 1200 lbs./acre is illustrated in Figure 7a. Actinomycete populations at the 30-day recording, and sampling time, gave a 32 percent increase over the control; fungi, a 30 percent increase, and bacteria were 70 percent less than the control. After 60 days, 30 days following the second amendment of lobster at 1200 lbs./acre, showed actinomycete counts to have risen to 106 percent above the contrasting control value. Fungal counts moved downward somewhat, but still remained above the unamended control by 12 percent. Bacterial population average sample counts showed an increase but were still below the control by 10 percent. At the 90-day sampling period all counts of fungi, bacteria and actinomycetes were depressed; fungi by 56 percent; bacteria by 50 percent; and actinomycetes by 64 percent.

Treated plots at 600 lbs./acre of ground lobster shell in three applications, showed a differentiation in the ecological adjustment as compared to the higher rate of lobster

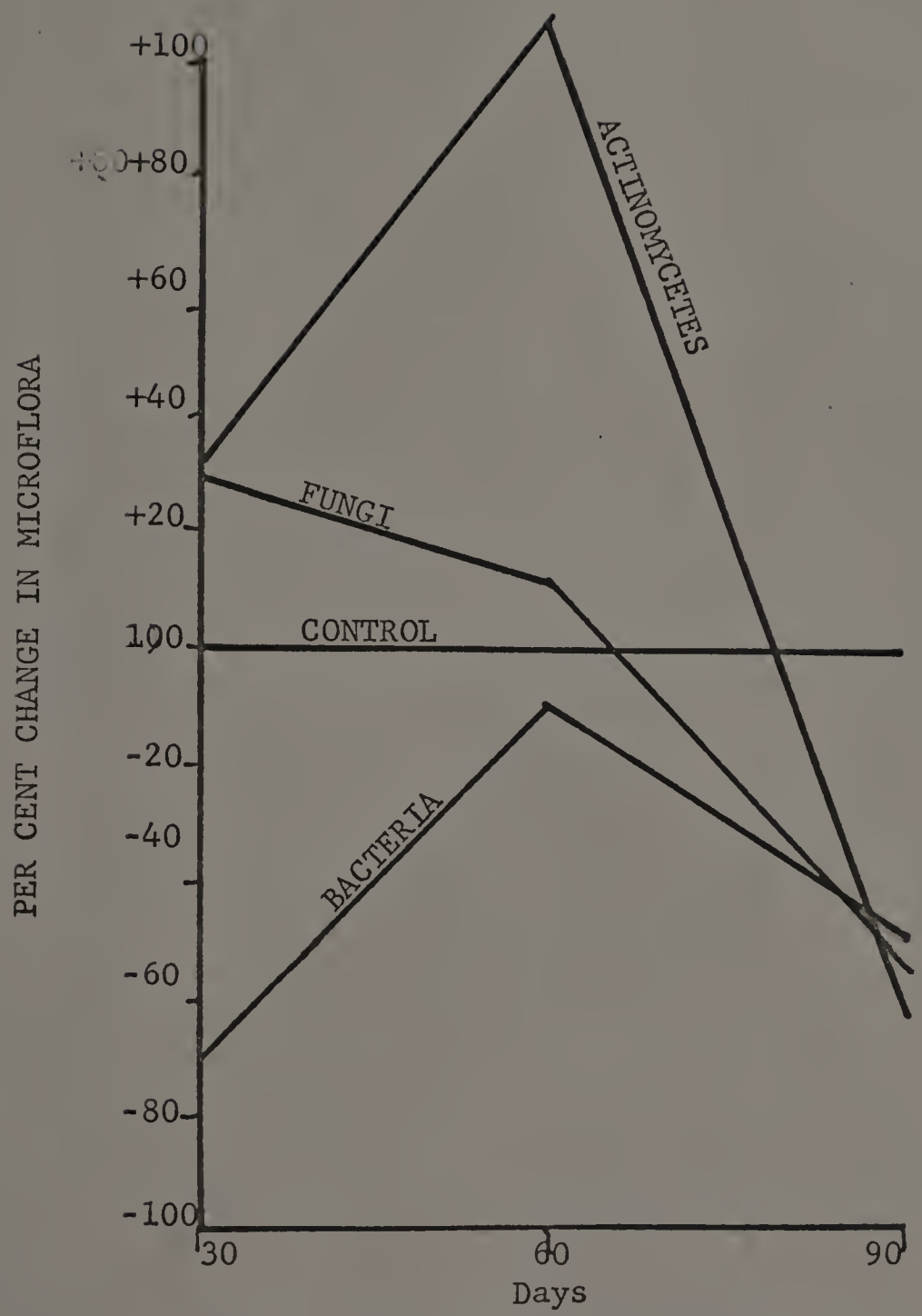


Figure 7a: Influence of lobster shell at 1200 lb/A on microfloral populations in newly established turf.

shell application. At 30 days, following the first application (Figure), actinomycete counts were 205 percent above the control. Bacteria equalled the control level, i.e. were unaffected, while fungal counts were 40 percent above the control. The 60-day sampling period showed fungal populations had assumed dominance at 200 percent above the control; actinomycete counts had dropped to a low level. Bacteria were suppressed, and 48 percent below the control. Thirty days following the third application, at the 90-day sampling period, all microfloral counts were below the level of the unamended controls; fungi by 36 percent, actinomycete and bacteria by 26 percent. Bacterial counts, however, did increase by 22 percent from previous dilutions, but were still below those of unamended control plots.

Diazinon plots which received the manufacturer's recommended rates, at 30 days following the initial application (Figure 12), were compared to the unamended control plots. Fungal counts were 32 percent below the control; bacteria 28 percent above the control, but actinomycetes did not appear to be present at all. Sixty days following the initial application and 30 days after the second treatment, fungal numbers had risen to 20 percent above the control; bacteria had dropped to 60 percent below the control, and only negligible numbers of actinomycetes had appeared. Ninety days after the initial application and 30 days after the third treatment, actinomycetes had risen to 16 percent above the

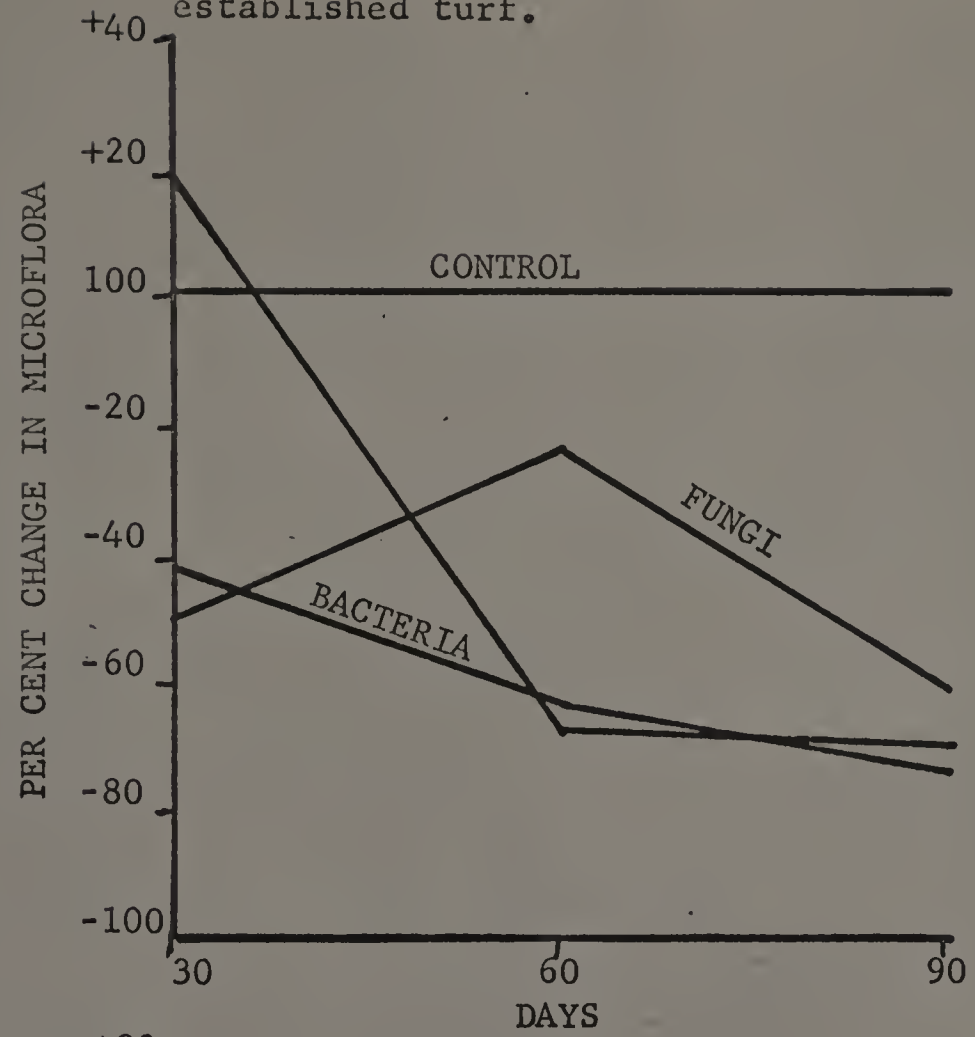
control, and a selective, apparently dominant, bacterial population had increased to counts 68 percent above the number in the control plots.

At twice the normal Diazinon application 30 days following the first treatment, actinomycetes were 18 percent above the control; fungi and bacteria were 50 percent and 44 percent below their respective controls (Figure 13). Fungi were 26 percent; bacteria 64 percent below their respective controls, and actinomycetes were 68 percent over the control at the 60-day sampling period. After 90 days fungi were depressed to 64 percent; actinomycetes to 72 percent and bacteria to 70 percent below their respective controls, indicating the general depressant effect of this organophosphate at a narrow range of increased application (from 4 to 8 oz. per 1000 square feet).

Tabulations of microfloral counts and the effect of treatments, in time, were submitted to the CDC 3600 computer, University of Massachusetts, Amherst, and programmed for an analysis of variance factorial design, BM02V, version May 20, 1964, Health Sciences Computing Facility, U.C.L.A. Results in Table I. Count numbers were transformed to log base E.

None of the treatments had any significant effect, in time . Microfloral populations, although affected, following each treatment, were apparently able to adapt to the soil amendments added without any permanent change in the ecosystem. As shown in Table I, the cumulative effect of time and the counts of microflora were highly significant.

Figure 12: Influence of Diazinon at 8 0z./1000 sq. ft. on microfloral populations in newly established turf.



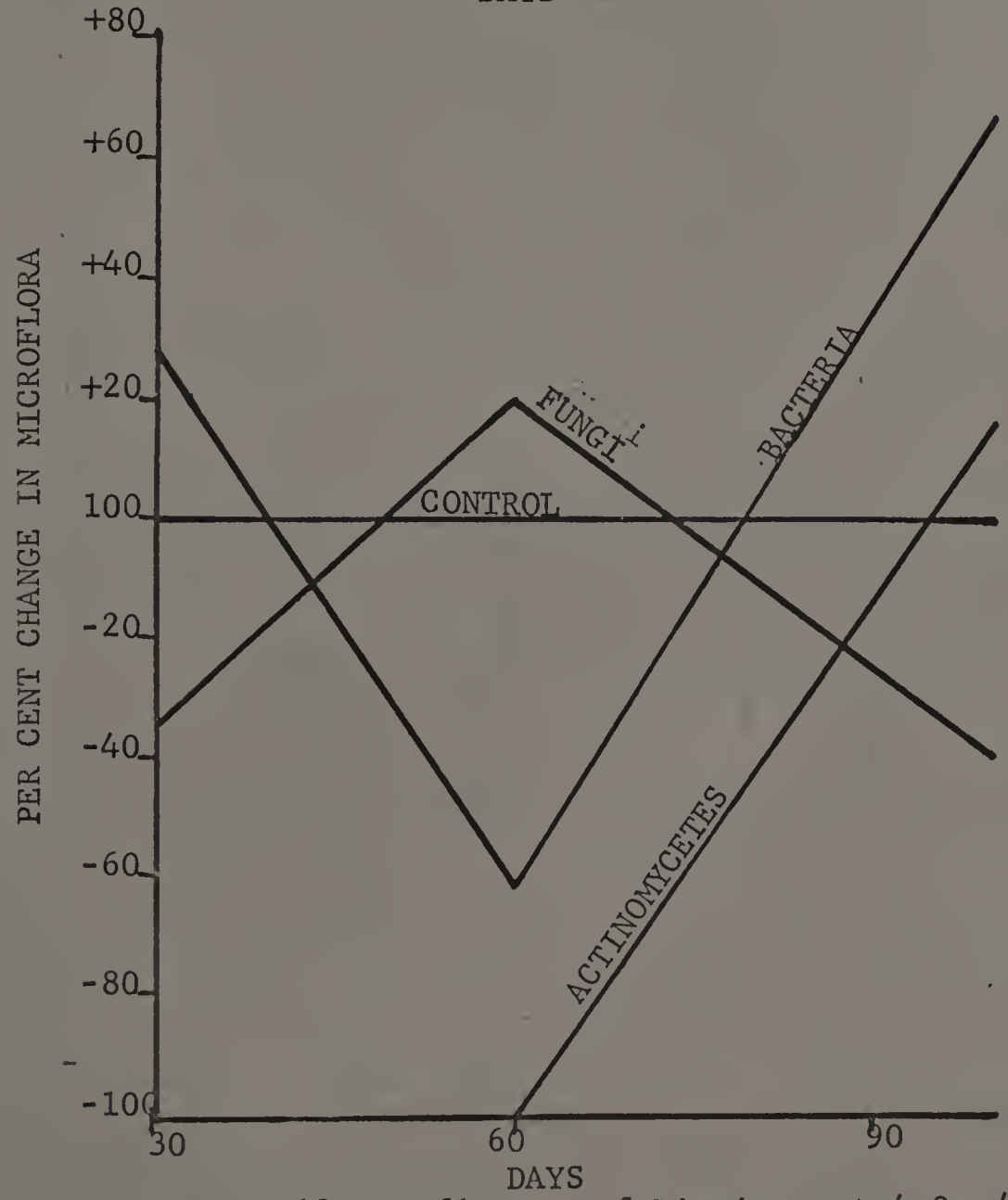


Figure 13: Influence of Diazinon at 4 0z./1000 sq. ft. on microfloralin newly established turf

TABLE I

ANALYSIS OF VARIANCE FOR FACTORIAL DESIGN[1]

Source	DF.	Sum Squares	Mean Square	Tabulated F.05	F/.05
Days	2	311.846	155.923	10.15	
Counts	2	10569.624	5284.812	34.24	
Treatments	29	975.876	33.651	2.18	
Days x Counts	4	145.885	36.471	2.31	
Days x Treatments	58	1501.685	25.891	1.68	
Counts x Treatments	58	1836.375	31.662	2.05	
Days x Counts x Treatments	116	2366.706	20.403	1.32	
Within Replicates	540	8335.241	15.436		3.00
Total	809	26043.238			

[1]Data submitted to CDC 3600 computer, University of Massachusetts, Amherst, Massachusetts.

STUDY OF THE EFFECTS OF SOIL AMENDMENTS ON SOIL MICROFLORA ON A WELL-ESTABLISHED MERION TURF SITE, AND TO STUDY THE OCCURRENCE AND INCIDENCE OF FUSARIUM BLIGHT

MATERIALS AND METHODS

The University of Massachusetts Alumni football field was selected as the site for this experiment. Alumni field was sodded to Merion Kentucky bluegrass in the late spring and early summer of 1965. The turf was grown on selected acreage in South Deerfield, Massachusetts, for three years before its transference to this site. The stadium was opened officially and used by the football team for home games in the fall of 1965.

The turf was cut at a 1 1/2 inch height of cut and clippings removed; a liming and fertilization program was also initiated. Artificial irrigation was applied to supplement natural rainfall, to insure the turf received at least one acre inch of water per week. A problem of water percolation and drainage on the field was present, and this was especially evident immediately following irrigation, or after a heavy downpour. The inadequate drainage was noted for a possible influence on the development or inhibition of disease in the future.

On May 15, 1966 a randomly selected location was chosen at the southwest end of the field, beginning at the goal line, and running northwesterly for 40 feet toward midfield, and 35

feet in a westerly direction, toward the center of the field. The area was divided into 5' x 5' plots. Treatments were assigned numbers from 0 to 12. Plots for complete treatment were randomly selected and numbers assigned. Non-amended controls were included in the random selection.

The lobster shell used in this experiment was dried and ground in the same manner as described previously, and subsequently evenly distributed over the plots. Diazinon AG 500 was mixed according to the manufacturer's recommended procedure and applied, using a portable hand pressure pump emitting a fan-shaped spray. One-half of individual treatments were applied in one direction, and the other half applied at right angles to the first. Only one application of the following treatments were made:

Diazinon 2 oz/1000 sq. ft. = 1/2 Dx

Diazinon 4 oz/1000 sq. ft. = Dx

Diazinon 8 oz/1000 sq. ft. = D2x

Diazinon 16 oz/1000 sq. ft. = D4x

Ground lobster shell 300 lbs/acre = 1/2 Lx

Ground lobster shell 600 lbs/acre = Lx

Ground lobster shell 1200 lbs/acre = L2x

Ground lobster shell 2400 lbs/acre = L4x

Mixed Treatments:

1/2L - 1/2D at 1/2X

1/2L - 1/2D at X

1/2 L - 1/2D at 2X

1/2 L - 1/2D at 4X

Unamended Controls

Soil samples were removed on June 24, July 25 and August 25, using a 1/2-inch metal sampling tool inserted to a depth of 6 inches. Five soil samples were removed from each replicate, shaken directly into plastic bags and tagged according to number and date. The composite sample was mixed thoroughly and a 25 gram air-dried portion of the composite was used for determining microflora numbers by the soil dilution-plate method described previously.

A 1.0 ml. serial dilution sample of 10^{-4} was pipetted into Petri dishes containing 15 ml. of cooled potato dextrose agar (Difco) adjusted to pH 7.0 for bacterial and actinomycete counts. All plate samples were replicated 3 times and the averages of these counts recorded. Counting was performed with a Quebec colony counter.

Grass plants removed during the soil sampling were examined macro- and microscopically for signs of "Fusarium blight" disease or for any other turf grass disease prevalent. Visual observations of color, texture and general conditions of the plots were made. Observations of disease occurrence were also noted in other non-treated portions of the football field.

All data of counts, treatments and sampling days were subjected to an analysis of variance factorial design. Tabulations were programmed using a BMO2V, version of May 20, 1964 Health Sciences Computing Facility, U.C.L.A., California. Computations were submitted to the CDC 3600 computer, Uni-

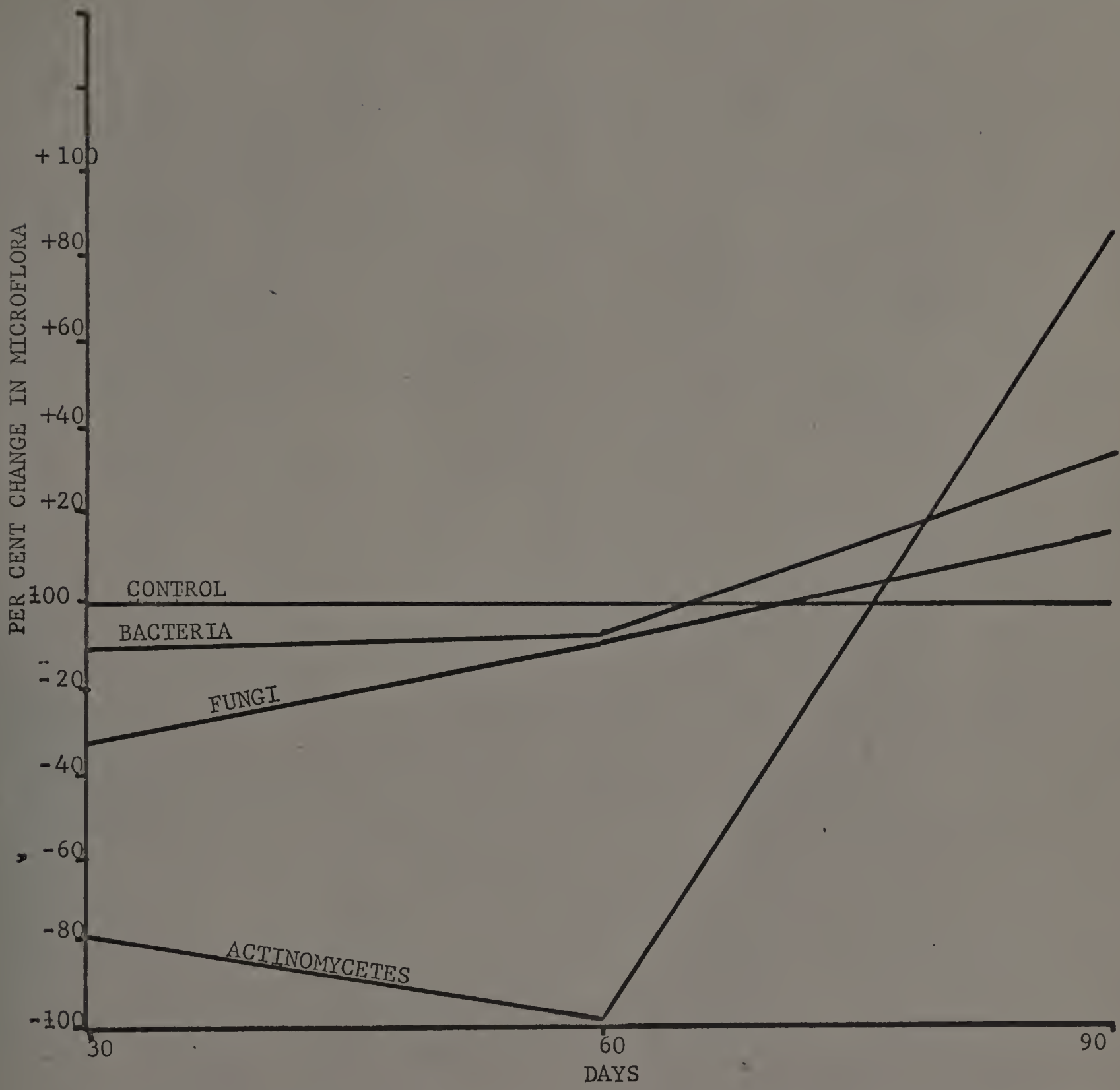


Figure 18: The effect of lobster shell at 300 lbs./A on soil microflora of an established turf.

versity of Massachusetts. Microfloral counts were submitted as a log transformation base E.

RESULTS

Thirty days following the application of the various soil amendments, serial dilution plates were made and microflora counts tabulated. Major representative groupings of bacteria, fungi and actinomycetes were recorded.

Fungi:

Ground lobster shell treated plots, at the 300 lb/acre rate, counted and averaged on 3 plate replicates showed fungal colony counts at 2×10^4/g. The unamended control plots gave average counts of 3×10^4/g at the same sampling period. Thirty days later, sampling counts showed lobster shell at 300 lbs./acre were 9×10^4 as compared to control plot counts of 10×10^4. Soil dilution plates at 90 days gave treated plot counts of 7×10^4, while control plots at this time were 6×10^4 (Figure 18).

Lobster shell at 600 lbs./acre showed no difference from the control plots at 30 days, both having 3×10^4 fungal counts. However, thirty days later there was some slight divergence, 600 lbs./acre lobster treated plots were 7×10^4, while the control untreated plots were 10×10^4. However, after 90 days, lobster shell plots had increased almost linearly to a count of 10×10^4. The control plots, on the other hand, had declined to 6×10^4 (Figure 19).

Plots amended with ground lobster shell at 1200 lbs./acre

rate showed counts of 4×10^4/g after 30 days, while control plots were slightly less at 3×10^4. By 60 days the lobster treated plots increased to 25×10^4, whereas the control plots, although showing an increase, were below this at 10×10^4. At the 90-day sampling level lobster shell plot counts had fallen somewhat to 13×10^4 as compared to 6×10^4 for the controls (Figure 20).

At 2400 lbs./acre lobster treatment, fungal counts after 30 days were 6×10^4 for the controls. Thirty days later fungal colony counts for the treated plots were 7×10^4 as opposed to control fungal counts of 10×10^4. Unamended control plots at the 90-day sampling period were 6×10^4 per gram of soil.

Diazinon AG 500 treated plots at 2 oz./square feet, 30 days after application, equalled the unamended control plot counts at 3×10^4 fungal colonies per gram of soil. This pattern changed, however, at the 60-day sampling. At this point the unamended control was 10×10^4 while the Diazinon treated plots averaged 4×10^4 fungi per gram of soil. On the last sampling at 90 days the control fell to 6×10^4, but the pesticide treated plots declined as well to 2×10^4 (Figure 14).

Diazinon at twice the manufacturer's recommended rates showed depressed fungal counts at the 30-day sampling period following application. Treated plot average fungal counts were 1×10^4. Untreated control plots read 3×10^4 at this

time. Sixty days after application in the Diazinon treated plots, at twice the rate, fungi populations were 2×10^4, representing only a slight increase. Comparable samplings on unamended plots showed a peak fungal population of 10×10^4 (Figure 14).

At four times the recommended rate of Diazinon application, counts were no different from those of the controls, 2×10^4/g. Thirty days later, at the second soil dilution sampling, controls were 10×10^4 while the treated plots were up to 5×10^4 fungal colonies per gram of soil. On the last sampling date, the 90-day period, there was no change in the treated plots and the controls, 6×10^4 fungi, per gram of soil (Figure 14).

Bacteria:

Ground lobster shell at 300 lbs./acre gave bacterial populations of 60×10^6, similar to that of the control. Sixty days after the initial treatment of lobster shell at the half rate showed bacterial colony counts were 150×10^6 and thirty days later 190×10^6, per gram of soil (Figure 17).

Lobster shell applied at 1200 lbs./acre at the 30-day sampling gave bacterial population counts of 80×10^6, at 60 days 112×10^6, and at 90 days 226×10^6 bacteria per gram of soil. Control plots during the 30, 60, and 90-day period were 60×10^6, 120×10^6, and 160×10^6 respectively (Figure 17).

Completely randomized plots treated with three different rates of Diazinon, in one application, yielded the following

results: Diazinon at 2 oz./1000 square feet, equal to one-half the manufacturer's recommended rate showed at the first sampling period a bacterial population of 74 x 10^6, compared to unamended control plots of 58 x 10^6. After 60 days at one-half the recommended rate of Diazinon, the plots had a depressed value of 64 x 10^6 while the control plots inerred linearly to 120 x 10^6. At the last sampling time there was no apparent difference between the one-half Diazinon rate treated plots compared to the unamended controls. Treat plot values were 122 x 10^6 bacteria per gram of soil while the bacterial count of untreated plots after 90 days was 160 x 10^6 bacteria per gram of soil (Figure 15).

Plots which received the 4 oz./1000 square feet Diazinon rate gave bacterial plate counts of 64 x 10^6 per gram of soil, and at this same sampling time control plating yielded 58 x 10^6 bacteria per gram. Both treated plots and untreated plots, at the end of the 60-day period, had almost similar population counts. Treated, 116 x 10^6 bacteria; untreated, 120 x 10^6 bacteria per gram of soil. A different picture was found at 120 days. The Diazinon treated plot counts had fallen from their peak counts to 74 x 10^6, whereas control plots continued in an upward manner to 160 x 10^6 bacteria per gram of soil. (Figure 15).

Dilution plate counts for twice the recommended Diazinon rate, i.e. 8 oz./1000 square feet sampled at 30 days, showed 58 x 10^6 and a control count of 46 x 10^6 bacteria per gram of

soil. However, at the 60-day sampling time the treated plots shifted to a much higher level of 182 x 10^6 bacteria per gram. In the same period, control plots gave counts of 120 x 10^6. At the 120-day soil dilution plate examination, treated plots gave a bacterial count of 144 x 10^6, and the unamended controls were 160 x 10^6 bacteria per gram of soil (Figure 15).

At four times the manufacturer's recommended rate of Diazinon application 16 oz./1000 square feet plate counts followed the general trend seen at twice the rate. Thirty-day soil dilution plate counts showed 52 x 10^6 bacteria per gram. Sixty days, 178 x 10^6 and 90 days at a level slightly higher than the control plots at 161 x 10^6 bacteria per gram.

Actinomycetes:

The effect of finely-ground lobster shell as a soil amendment at 300 lbs./acre showed colony counts for the amended and unamended plots were both 1 x 10^6. At 60 days amended plot counts were to 10 x 10^6, but controls markedly increased to 15 x 10^6. On the 90-day sampling period of amended soil plots, no actinomycetes were found. During the same period the control plots had decreasing actinomycetes counts, of 9 x 10^6.

Lobster shell applied to the randomly selected plots at 600 lbs./acre appeared to have an initial stimulation effect on actinomycete development, and in time a degeneration had taken place. For example, at 30 days counts were 4 x 10^6; at 60 days, 1 x 10^6, and finally at 90 days no actinomycetes

were discernible.

Actinomycete population plate counts from plots treated with 2 oz. of Diazinon AG 500 showed only 3 x 10^6 per gram at 30 days. Control samples were 1 x 10^6. After an additional 30-day period samples showed, at that time, no actinomycetes in the treated random plots. Control plots, however, escalated to 15 x 10^6 actinomycetes per gram of soil. After 120 days following the Diazinon application the control plot counts were 9 x 10^6 actinomycetes per gram while the treatment plots still had no recordable actinomycete counts (Figure 16).

Diazinon at the recommended rate of 4 oz./1000 square feet was 4 x 10^6 actinomycetes, while the unamended plots were 1 x 10^6 at 30 days. After 30 additional days, Diazinon plots were 1 x 10^6 actinomycetes per gram, whereas the control plots were 15 x 10^6. Thirty days later, 120 days after initial application, in the regular rate of Diazinon application, no actinomycetes were found. On the other hand, investigation of control soil samples revealed 9 x 10^6 actinomycetes per gram of soil.

Plots treated at 8 oz./1000 square feet of Diazinon did not evince much change and countings of plates at 30 days showed 1 x 10^6 actinomycetes, and the 60 and 90-day counts yielded no actinomycetes in the sample tested. Controls, meanwhile, at 30, 60 and 90 days were 1 x 10^6; 15 x 10^6 and 9 x 10^6 actinomycetes per sampling, respectively (Figure 16).

When plots of Diazinon at 16 oz./1000 square feet were sampled at 30 days, no actinomycetes were found. In fact,

not until the 90-day sampling time were any seen, and these samplings showed actinomycete populations at 1 x 10^6 per gram of soil. Controls in the same period of 30, 60 and 90 days were much higher at 1 x 10^6, 15 x 10^6 and 9 x 10^6 actinomycetes per gram of soil in the samples.

Mixed Applications of Ground Lobster Shell and Diazinon Applied at Varying Rates

Fungi:

Ground lobster shell at the rate of 600 lbs./acre plus 2 oz. of Diazinon per 1000 square feet treated plots gave, initially, rather high fungal populations of 8×10^4 per gram of soil thirty days after treatment. After 60 days plots treated at this rate showed counts of 16×10^6, and at 90 days 8×10^6 fungi per gram of soil (Figure 26).

Lobster shell at 600 lbs./acre plus 4 oz. of Diazinon per 1000 square feet 30 days after application gave soil dilution plate count averages of 8×10^4 fungi per gram of soil. Further sampling at 60 days showed a high count of 17×10^4, while the 90-day sampling yielded 10×10^6 fungi per gram (Figure 26).

Treated randomized plots of lobster shell at 600 lbs./acre and Diazinon at 8 oz./1000 square feet 30 days after application showed counts of 6×10^6 fungi per gram of soil. Sixty days after application samples showed counts of 12×10^4 fungi per gram, and 9×10^6 fungal propagates were found at the final, 90-day sampling.

The treatment of plots with Diazinon at 16 oz./1000 square feet plus lobster shell at 600 lbs./acre, 30 days after application, gave counts of 3×10^6. At 60 days fungal population counts were 5×10^6 per gram of soil. Thirty days later these numbers remained unchanged (Figure 26).

Bacteria:

Results, in which ground lobster shell at 600 lbs./acre was kept at a constant rate, and the rates of Diazinon AG 500 were varied, are reported below (Figure 26).

Plots receiving finely-ground lobster shell and Diazinon at 2 oz./1000 square feet were sampled at 30, 60 and 90 days. At 30 days these yielded average counts of 188 x 10^6 bacteria per gram of soil (Figure 27). At 60 days the colony counts increased to 150 x 10^6, and at 90 days 188 x 10^6 bacteria per gram of soil. At 30 days plots receiving Diazinon at the 4 oz./1000 square foot rate showed bacterial populations of 90 x 10^6; at 60 days yields were 144 x 10^6 bacteria per gram. The last sampling at the 90-day level showed an upward trend to a 160 x 10^6 bacterial count.

Plot samples in which the Diazinon application rate was 8 oz./1000 square feet plus lobster shell showed bacterial counts of 80 x 10^6 per gram of soil after 30 days. Thirty days later, counts rose to 114 x 10^6 per gram, the populations dominated by a few genera. At 90 days the plots receiving 8 oz./1000 square feet of Diazinon yielded very high counts of 226 x 10^6 per gram of soil, with the predominant populations found to be of the genera Pseudohomas (gram negative polar flagellated rods). Confirmation of these observations were found when the plots receiving four times the Diazinon recommended rate (16 oz./1000 square feet) were plated. In these plots, after 30 days, bacterial counts

averaged 84 x 10^6 and at the 60-day counting period the counts showed a bacterial population of 246 x 10^6, predominantly Pseudomonads. The final sampling at 90 days showed a slight decrease in numbers to 238 x 10^6 bacteria, but again the major portion of the population was Pseudomonads.

Counts of unamended control plots showed bacterial colony counts of 58 x 10^6 after 30 days. At the 60 and 90-day sampling times a steady increase of a diverse population was seen. The counts at 60 days were 118 x 10^6, and at 90 days 160 x 10^6 bacteria per gram. Significantly, the control plates examined displayed a wide array of bacterial genera unlike the treated plots which were dominated by Pseudomonas spp.

Actinomycetes:

Ground lobster shell applied to the plots checked was held to a constant rate of 600 lbs./acre: the variant being Diazinon AG 500. Treatments were made at time zero, and samples were taken 30 days after for counting (Figure 28).

At 30 days counts of plots treated with lobster shell plus Diazinon AG 500 were 1 x 10^6, the same count as the controls. The population peak for this treatment was recorded at 60 days at a count of 10 x 10^6 actinomycetes colonies per gram of soil. However, after 90 days no actinomycetes were found within the plots sampled.

Diazinon at the recommended rate of 4 oz./1000 square feet plus lobster shell, 30 days after treatment, gave yields of 4 x 10^6 actinomycetes. These plot counts decreased

rapidly to 1 x 10^6 colonies at 60 days and zero at 90 days. Increasing the Diazinon application to 8 oz./1000 square feet showed after 30 days actinomycete counts of 7 x 10^6, and at 60 days a peak of 15 x 10^6 per gram of soil, but at the 90-day sampling time no actinomycete colonies were found. Doubling the manufacturer's recommended rate while maintaining lobster shell at a constant rate had similar effects. At the 30-day sampling time actinomycete colonies counts per gram of soil were 8 x 10^6; at 60 days they reached a maximum of 20 x 10^6, but at 90 days no actinomycete colonies were observed in the soil dilution plates.

Control plots where no amendments were added to the soil gave population counts of 1 x 10^6 actinomycetes after 30 days; a high of 15 x 10^6 colonies per gram of soil after 60 days. Unlike all other treated plots, however, the control plots maintained actinomycete representation at a level of 9 x 10^6 per gram of soil.

When finely-ground lobster shell was added at the low rate of 300 lbs./acre, soil microfloral populations were suppressed for 60 days while the unamended controls retained a stable distribution of populations. In time, the populations of lobster-shell treated plots increased. Actinomycetes increased 88 percent above the controls, and fungi normally biotically inhibited when actinomycetes predominate, were only 16 percent above the control (Figure 18). Bacterial populations, meanwhile, steadily increased and were apparently intermediate in responsiveness to the natural additive

at 34 percent above control.

Increasing the amount of ground lobster shell to 600 lbs./ acre had a slightly greater stimulating effect on the soil inhabitants, but especially on the actinomycete fraction, in soils sampled (Figure 19). Actinomycetes were far below numbers of fungi, and the control counts responded almost in an arithmetic linear fashion to the addition of organic carbon. At the end of the testing, 120 days after application, counts showed actinomycetes to be 50 percent above the controls. In this treatment series, fungi were evidently able to overcome whatever suppressant effect actinomycetes might have had by 120 days, since fungal counts were 40 percent above the controls. Significantly, during the 120-day period of experimentation, moisture was at a maximum: artificial irrigation supplied at least one acre inch of water per week, but drainage was apparently impeded to such an extent, that free water existed for up to 24 hours on the surface after water was applied. Under these conditions, the soil remained at field capacity for long periods between water amendments.

There appeared to be a definite shift in microfloral activity when chitinaceous increments were increased. For example, at an application rate of 1200 lbs. of ground lobster shell per acre (Figure 20), fungal counts 30 days after initial application were 25 percent above the unamended controls. Thirty days later fungi were 60 percent above the control level. Under the evident predominance of fungi,

bacteria competing for nutrients started at a level 11 percent higher than fungal activity, but at the 60-day sampling time bacterial counts were 2 percent below the control. Another evident reaction to the 1200 lb. lobster shell application was the slow response by actinomycetes. Readings showed actinomycete counts to be 60 percent less than controls after 30 days. However, after 60 days actinomycetes had increased to 12 percent above the control samples concomittant with a decrease in bacteria and the peak of fungal counts. On the final sampling, 30 days following, actinomycetes were found to be 66 percent above the controls, and appeared to be successfully competitive with the fungi restricted to 12 percent less than control level. It would appear that in the short term fungi responded rapidly to the carbon amendment added, but were not able to utilize less easily hydrolyzed fractions of the lobster shell. This observation is borne out with population samplings taken in plots receiving 2400 lbs. space of lobster shell per acre, four times an average treatment (Figure 21). In these high level treatment plots, fungi were predominant and 50 percent above the levels of unamended controls. Bacteria, at this time, were 15 percent below the controls while actinomycete counts equalled control counts. Populations shifted radically after 39 additional days with bacterial numbers reaching 42 percent above the controls; actinomycetes 25 percent below, and lowest at 30 percent less than in the control

plots. One hundred and twenty days after the initial 2400 lbs./ acre lobster shell treatment, results showed an abrupt, but not unexpected, population shift. At that time actinomycetes increased explosively in numbers to 95 percent greater than the controls; bacterial numbers remained about stable, dropping slightly to 35 percent above the control numbers, a loss of 7 percent. Fungi increased during this interval to 50 percent above the controls but apparently not sufficiently to challenge actinomycete predominance.

The second portion of the experiment involved the investigation of the microfloral changes associated with the addition of Diazinon when it was applied in conjunction with lobster shell. According to Mitchell and Alexander (54, 55, 56), the application of ground lobster shell to a soil system selectively stimulated soil microflora capable of utilizing N-acetylglucosamine (chitin) as a carbon source, and rapidly increased actinomycete populations. Any groups, so selected, could hypothetically be a serious competitor for basic carbon and nitrogen with pathogenic fungi. Further, by selecting organisms capable of using chitin, and subsequently attaching fungal hyphae-containing chitin as a component, a controlled population would be achieved which would create a beneficial change. The addition of an organophosphate Diazinon appeared to affect the lobster shell (chitin) influence. Since fine turf management requires, on occasion, the application of materials designed for the control of root or foliage destroying insects, or grubs, and Diazinon AG 500 is typical of the oganophosphate designed and approved for this purpose, the effect of

adding the compound to a lobster shell amendment was of some interest.

Finely ground lobster shell at a constant rate was added to randomized plots; the same plots received applications of Diazinon AG 500 at varying rates in one treatment.

Results showed that under the influence of Diazinon at all rates there was practically an elimination of actinomycetes in spite of the lobster shell. Fungal populations, apparently, were generally unaffected at the 4 oz./1000 square foot and 8 oz./1000 square foot rates but responded to the 2 oz. and 16 oz. rate (Figures 22, 23, 24 and 25). At the 2 oz. rate a highly selective Pseudomonad predominated. At the 4 oz./1000 square foot treatment fungal numbers remained unchanged throughout the period of the experiment. Actinomycete numbers were initially influenced by what appeared to be the available lobster shell fraction, but in all cases this effect was not sufficient to counteract the inhibitory action of Diazinon. Moisture was not a problem since supplementary artificial irrigation was used at regular intervals. Bacteria were less affected, generally, than other microflora. However, there was a marked change from a diverse grouping of bacteria species at the first sampling, to one grouping. In general, the higher the rate of Diazinon applied, the more selective the bacterial population became. Several bacteria were isolated from the 16 oz. Diazinon treated plots that were inhibitory to the development of fungal colonies,

notably to Fusarium roseum and Trichoderma viride (see Figure 25).

Plots to which Diazinon alone was applied showed actinomycetes and fungi to be generally suppressed in time. A comparison of microfloral counts in which the unamended controls strikingly exhibit this effect is shown in Figures 22, 23, 24 and 25. The bacteria isolated from the Diazinon plots at the 8 oz. and 16 oz. rates were a selected group of Pseudomonad similar to those found where Diazinon was used in conjunction with lobster shell.

The field ecosystem, in general, appeared little influenced by the application of ground lobster shell. This appeared to result from (1) the nature of the chitinaceous material itself which, even though finely ground, was relatively insoluble and could not move into the root system, and (2) since this was an established turf, the close-knit roots, rhizomes and grass plants acted as a barrier to the downward movement of the lobster shell material. It would suggest that hydrolyzing lobster shell to render its active component, N-acetylglucosamine more immediately available would enhance its effect in stimulating chitenovorous populations. In a newly-established turf, such material could readily be tilled into the upper two inches of soil, insuring greater contact and availability.

Sampling during the 120-day period of examination yielded a variety of soil microflora. The dominant fungi

were: Alternaria spp, Rhizoctonia spp, Fusarium spp; particularly F. roseum, Curvularia spp, Cyclindrocarpon, Aspergillus spp and Penicillium. Soil dilution counts showed high numbers of F. roseum to be present, but during the entire program only insignificant numbers of foliar lesions attributable to this organism were found.

In plots that received ground chitinaceous material the grasses appeared to assume a darker green color than non-treated plots, perhaps through response to the release of soluble nitrogen. The color difference did nd persist beyond 2 or 3 weekly clippings.

Statistical results programmed for a factorial design, as previously described, are shown in Table II. Highly significant F values are shown for the counts of fungi, bacteria and actinomycetes and days, emphasizing that single applications of lobster shell, Diazinon, sugar and combinations of these might have had an immediate effect following application but that in time microfloral populations tended toward re-stabilization of their numbers.

TABLE II

Analysis of Variance for factorial design of treated plots in established turf site.[1]

Sources	Degree Freedom	Sum Squares	Squares	Tabulated F.05	F/.05
Days	2	71.65	35.83	2.57	
Counts	2	1581.38	790.69	56.91	
Treatments	12	242.92	20.24	1.45	
Counts x Treatments	24	376.08	15.67	1.13	
Counts x Days	24	358.20	14.92	1.07	
Days x Counts	4	271.06	67.77	4.88	
Within Replicates	48	666.90	13.89		4.05
Totals	116	3568.19			

[1]In one application

Part I. Environmental Investigations of Fusarium Blight in a Fusarium roseum endemic Merion Kentucky bluegrass site.

Part II. Observation and Investigation of the Effect of some Fungicides and Chitinaceous Materials on a Naturally Infected Merion Kentucky bluegrass site.

Materials and Methods

Part I. An endemic Fusarium roseum Merion Kentucky bluegrass site was reportedly located on a flood plain within a few miles of the University of Massachusetts, Amherst campus. Investigation revealed that within the past few years diseased areas had appeared, but never reached epiphytotic proportions. No concerted chemical control program had been initiated to alleviate the condition.

Dr. Joseph Troll, Turf Specialist, Department of Plant and Soil Science, University of Massachusetts, had identified the causal organism as Fusarium roseum, during previous disease eruptions. According to Dr. Troll's observations, the macro-symptoms of the disease, i.e. dead patches, streaks and/or frogeye effects appeared to be most prevalent close to a wild dogwood tree trunk; along the edge of a driveway, and in other areas that were well-drained. In previous growing periods, up to the present, the grass plants had received ample applications of lime and fertilizer. The area suffered an extended drought period but the grasses

did receive a sufficient supplemental water supply by sprinkler and soaker hose. Visual observation by the investigator had revealed that this turf was apparently in a healthy condition judged by color, close-knit habit of growth, lack of weeds and its general healthy appearance--an ideal home lawn.

Core samples were removed, randomly, by a 1/2" soil sampler. Samples removed showed an extensive root system; a rather heavy layer of thatch, but not disproportionate to the age of the sward. Initial investigatory soil-dilution plates made from these samples showed a predominant Fusarium roseum population. Also present were Curvularia spp, Alternaria spp, Helmenthosporium spp and Trichoderma viride. The latter organism was not observed in previous investigation in other plots. Soils removed from the site were examined to determine soil classification, pH and soil moisture equivalence. Soil and plant samples were removed from the site during the investigation to evaluate the effectiveness of the superimposed treatments within the plot design. Development of the disease was recorded and, any unusual climatic variability effects on the system was observed and recorded.

Part II. A completely randomized plot design was established. The plot location was selected to encompass an area in which symptoms of the disease had developed in prior growing season. Treatments consisted of: Difolatan at the manufacturers' recommended rate, and at twice the rate; Fore at the formulators' recommended rate; Niagara's

experimental fungicide, at the recommended rate. All fungicides tested had been reported as presumptively effective in the control of unspecified fungus diseases in fine turf grass. All fungicides were mixed according to manufacturer's directions, and applied with a hand pressurized sprayer. Applications every 14 days were uniformly applied with a fan-shaped nozzle spray, one-half the treatment applied in one direction, the other half at a right angle to the first. Lobster shell used was first dried, then ground in a Hammer Mill grinder. The final product was passed through a 60-mesh screen. Lobster shell was applied at 600 lbs./acre and 1200 lbs./acre rates every 14 days, and the area thoroughly watered.

Disease incidence on a percentage basis was recorded by this investigator and an independent observer. Correlated readings were incorporated into a master system of tabulation. Disease index percentages ranged from zero, or no disease apparent, to 100 percent, or completely diseased, or dead. Readings for the disease index were based on the appearance of the foliar portions of the grass plants. Fungicidal and lobster shell applications were to proceed until either a positive control was imminent or for a period no longer than 90 days.

Five 25 gram soil samples were removed from each treated plot in a random fashion. From these randomized soil samples, soil dilution plates were made for observation of changes in

microfloral populations. Fifteen ml of potato dextrose agar adjusted to pH 5.6 was the sole nutrient source in plastic Petri dishes. Random diseased foliar samples were removed from within the various treated and untreated plots intermittently to ascertain and identify the responsible pathogen. These leaf samples were immersed in a 1:10 dilution of commercial Clorox for 10 minutes; washed three times in distilled deionized water and macerated in a Waring blendor. A 1 ml sample was then transferred to 15 mls of potato dextrose agar (Difco). After 72 hours germination at room temperature plates were examined for the presence of Fusarium roseum.

Results: Part I.

Symptoms of disease appeared about June 9, 1966. The preceding month, May 1966, was relatively dry with a precipitation of 2.26 inches, most of this having occurred within the first 19 days of the month. Only 1.42 inches of recordable precipitation occurred from May 19 until June 9. Normally, according to local weather data, this was a period when two to three times that amount occurred. Temperature means for this period were 53.3°F for May and 65.9°F for the first 8 days in June preceding the disease state. The major concern of most turf owners, was that the northeast would, according to meteorologists, experience a third successive year of extreme drought and a fifth year of less than normal

rainfall. Local officials concerned about the drought's effect on the dwindling water resources prepared for a ban on the use of water for lawns and other esthetic purposes in the future. However, water was available at the outset, applied at rates of about 1 1/2-acre inches per week. The symptoms which first became noticeable were located in several areas, but principally in the peripheral area surrounding a young wild dogwood tree, quite likely in regions with surface feeder roots of the tree. Other Fusarium symptoms also were found and identified in an area which the subsurface was compacted, formerly part of an old driveway. This latter area was well drained with a considerable portion of the surface water draining off rapidly due to slope and the nature of the underlying material.

Circular patches of turf were destroyed in shaded areas as well as those portions that received full sunlight, contrary to reports of other investigators. (23). Occurrence of disease patches was perhaps more a result of the moisture problem in these areas rather than a light requirement per se. Merion bluegrass was not the only grass responsive to pathogenic virulence; creeping red fescue (Festuca rubra var.) was also infected and killed. Patches of diseased red fescue were observed along the foundation wall of the house in an area that received full sun for the greater portion of the day and in soils that dried rapidly and were silty in texture.

During the investigation, the disease progressed unchecked except in one period when a 1.9 inch rainfall occurred within one twelve-hour period. At that time, and for 7--9 days following, there were no new disease patches. In fact, some partially affected spots were rejuvenated and appeared to recover somewhat. Following this rain, an extended period of drought ensued and, apart from nocturnal dew and artificially applied water, only insignificant amounts of rain fell.

Periodically, disconnected areas of the lawn became infected, leaf samples taken from these areas, were cleared and stained in a warm lacto phenol cotton blue solution. Microscopic examination showed Fusarium to be present. Penetration by mycelia was both direct at the juncture of epidermal cells as well as through leaf tips wounded during mowing.

Macroscopic examination of both the plots and the rest of the lawn area showed, at the onset of disease, wettish areas of 3 inches to a foot in diameter to appear first. These patches had a gray-green appearance and after 24 hours became light brown or staw colored, and within 48 hours the area within these patches became reddish brown in color. Diseased patches observed from an oblique angle gave the grass "pock-marked" appearance under direct sunlight. The grasses within these "pock-marks" were limp, appearing to be subjected to wilt (Figure 33). The blades were slightly moist to the touch. A peculiar pungent odor permeated the

area in which the fungus was active.

The turf site was located in an area composed of approximately 60 percent sand, 37 percent silt and 3 percent clay, with a moisture equivalence of 34 percent. The sand fraction consisted of 25 percent very fine sands, 35 percent of fine sand, and the remainder equally divided into the upper size ranges. This soil once dried, was with difficulty, receptive to water penetration. The soil, once wetted, thoroughly, maintained a rather high degree of moisture due either to a drainage or high organic content in the thatch area. In the early summer of 1966 the use of water was banned for home use. During this period, when drought conditions existed, areas of the blight rapidly coalesced and engulfed most of the turf site. The first portions affected by the blight were the well-drained regions along the driveway, slopes near the road, the periphery of small trees and other areas which might dry rapidly (Figures 33, 34, 35, 36, 37, 38 and 39). The only areas at that time not affected were grasses located close to the foundation evergreen planting. These had continuously received a soaker hose water treatment to save valuable and delicate shrubs from kill. Within 3-4 days the entire randomized plot was blighted except for some patches which normally are apparently left uninfected in the center of blighted areas described by Couch (20, 21) as the "bullseye" or "frogeye" effect.

Weather observations were discontinued by the University of Massachusetts Experiment station on June 30, 1966, but

through the courtesy of Philip Ives, Amherst College, Biology Department, this investigator was able to maintain a degree of meteorological continuity. According to Ives' records, the last half of June, 1966 showed the average mean temperature to be 70.5°F with .59 inches of rainfall. The first half of July the average mean temperature was 74.6°F with 2.09 inches of precipitation; of this latter figure 1.75 inches occurred during a severe thunderstorm. Following the storm there was generally no new areas of blight, and those areas already infected, it appeared, were generally inhibited, characterized by a diminuition of fungal activity. This fact is borne out by a general decrease in the index shown on Figures 40, 41, 42, 43, 44 and 45 at the 28-day level. Total precipitation for July 1966 was 3.58 inches, only a trace of precipitation in the last week. Records purportedly show August the driest on record with only .79 inches of rain with an average mean temperature of 71.2°F; an average maximum of 83.8°F; and an average minimum of 58.5°F. In the early morning hours a rather heavy dew settled on the grass blades but the soil, although dry, did not reach the permanent wilting stage. The disease index during this period, the 42 and 56-day level increased significantly in most plots and treatments.

Wherever Fusarium blight was found in its virulent pathogenic stages Trichoderma viride was present, usually in numbers comparable to those of Fusarium. Foliar examination of stained samples, under the microscope, showed only Fusarium roseum

present within the plant, both inter and intracellularly. Trichoderma viride was not found in any foliar samples.

None of the soil amendments effectively retarded the development of disease within the plots investigated. There was some suppression of disease incidence after a heavy rainstorm in July. This condition did not persist for long, once dry conditions resumed. Concomittant with the lack of rainfall, and the stoppage of irrigation by a local water ordinance, there was a sharp increase in the disease state. The dry conditions apparently accounted for some of the stresses placed on the plants ecosystem and the normal growth habit of the grasses, and thus was perhaps contributory to a decline in plant vigor. In addition, once dry conditions had prevailed, the presence of Trichoderma became more pronounced, and possibly effectively restricted other microflora inhibitory to the proliferation of Fusarium roseum.

Dilution plates media showed during the investigation that Fusarium roseum grew satisfactorily on potato dextrose agar either when Fusarium was added first, and allowed to germinate, followed by the addition of Trichoderma, or if Trichoderma was seeded on the plates first, and allowed to germinate, followed by seeding of Fusarium cultures. Both organisms grew well in the same system without any apparent mutual inhibition. Centrifuged, cell free, filtrates of both Fusarium and Trichoderma cultures, tested separately and together and placed in penniciylinders on seeded plates, showed no antibiotic reaction. Thus, it would appear that Fusarium roseum

and Trichoderma viride exercise little influence on one another.

Results: Part II.

Prior to the application of the various fungicides and ground lobster treatments, readings were recorded at zero time on a percentage-per-plot disease index basis. Averages were arrived at for the replicates of the treatments and recorded graphically (Figures 40, 41, 42, 43, 44, 45 and 46).

Visual examination of the graphs and the percentages of disease incidence occuring shows no definite trend of either inhibition or control. There were intermittent periods of Fusarium blight control, but these periods also occurred simultaneously with the previously suggested influence of natural precipitation. Of course at zero time some disease had already taken place, but allowances were made for this state by selecting plots on a completely random basis without regard for disease percentage within a treatment.

In Figure 40 it would appear that Difolatan at both the recommended and twice the recommended rates offered some protection against spread of Fusarium blight. However, the areas in which this treatment had its random plots located were the last to be overrun during the virulent period of fungal attack, mainly because the plots were located in areas which had benefited from the water applications given to a bordering stand of evergreens. Moreover, several weeks after the experiments were concluded these plots showed severe damage not unlike the other treated regions.

Niagara's experimental fungicide showed a 25 percent increase in disease from zero time to the conclusion of the experiment for a total loss of 60 percent. (Figure 42). This was not satisfactory because over 50 percent loss of turf in an area will usually compel complete re-establishment of a turf site. Fore plot readings showed an overall increase of 43 percent from the inception of testing. There were, however, two readings for Fore at the 28-day level where a slight decrease was noted (Figure 41). This level, hypothetically, was due to precipitation since this was found to have occurred in most other treatments; but the sharp decline of the disease index from 30 percent at 56 days to 23 percent at 70 days is noteworthy. This recession is negated at the final 84-day reading which was much higher than before.

Ground lobster shell applied at 600, 1200 and 2400 lbs./acre in all cases, generally gave poor results. (Figures 44, 45 and 46). The disease index percentage showed either some amelioration after 28 days, or none. Even at the 1200 lbs./acre rate of lobster shell, although the percentage of disease showed 7 percent lower than the control, there is no great difference since the control plots had a 4 percent higher disease index at the outset of the testing.

At the initial sampling, zero time, fungi found represented a broad spectrum. In particular, Fusarium roseum, Alternaria spp., Helminthosporium spp., Curvularia spp., Aspergillus spp., and Trichoderma viride were among the most

common. Areas sampled that were infected, or had been subject to infection showed only the presence of Fusarium roseum and Trichoderma viride, in general. Bacteria in the uninfected region were also represented by a wide array of bacterial flora. Few actinomycetes were present in the uninfected area. In the infected reginns few bacteria were observed, and these were of the Pseudomonad type. No actinomycetes were observed in the infected areas.

A Study of the Relationship of Different Soil Moisture Equivalences When Inoculated with Fusarium and Trichoderma, Alone and Together

Materials and Methods

Soil was selected from several local sites: a silt loam, a clay loam and a sandy loam. These soils were then mechanically sieved in order to isolate fractions of sand, silt and clay for incorporation into artificial mixtures. Two other soils, one a sandy loam saved from previous greenhouse experiments, and the other a silt loam from a Fusarium blighted turf were used without any changes or amendments.

Composite sub-samples of sand, silt and clay were mixed in varying amounts to attain a relative degree of soil moisture equivalence. The centrifuge method for determining the moisture equivalent of soils was used, after the method of the American Society of Testing Materials design 425:39 (2). The moisture equivalent by the centrifuge method is the amount of moisture expressed as a percentage of the weight of oven-dried soil and its moisture retention when rewetted and subject to a force equal to 1000 times the force of gravity for one hour. Soil M.E. percentage was determined by the formula $\frac{A-b) - A1-b}{Me=A_1 - (C-6_1} \times 100$. Where A equals weight of crucible plus contents after centrifuging; A_1 equals weight of crucible plus contents after drying; C equals weight of the crucible; b equals weight of dry filter paper. An element of moisture equivalence accuracy was attained with the smaller sampling; increasing sample sizes tended to diminish accuracy but still

maintained a moisture relationship within the general scope needed for the experiment. Values of 20, 40, 60 and 80 percent were used as a guideline with some variance within each sample.

Mixtures used in a system of percentages by weight were: 79 percent sand, 12 percent silt and 9 percent clay for the 20 percent soil moisture equivalence ; 70 percent sand, 10 percent silt and 15 percent clay for the 40 percent equivalent; 41 percent sand, 21 percent silt and 38 percent clay for the 60 percent equivalence; 23 percent sand, 45 percent silt and 32 percent clay for the 80 percent equivalence. The sandy loam used as a check had a soil moisture equivalence of 48 percent. Soil from the endemic Fusarium disease area had a moisture equivalence of 34 percent.

Ninety-six 4 inch clay pots were washed and subsequently steam-sterilized for one hour. Soils which had been previously mixed and prepared were placed into each pot up to 1/2 inch of the rim. Pots containing the soil were brought up to field capacity, placed in flats, and steam-sterilized for one hour. After removal from steam cabinets; flats were covered with ventilated polethylene covers for 24-36 hours until the soils were cool. The covers offered some protection from extraneous contaminants being introduced during the cooling off period.

Merion Kentucky bluegrass (Poa pratensis L. var.) seed used was from the same source and stock supply used in prior investigations. The grass seed was surface sterilized in the

manner described in previous work. No fungicidal material was added to the seedcoat.

Prior to seeding, random sampling for pH determination was made within each moisture range. All soils received an application of 2 lbs. equivalent N/1000 square feet and raked into the upper inch of the soil with a flamed fork.

After seeding, pots were supplied with water gravimetrically in an automatic cyclic irrigation system. All containers were kept under the cyclic irrigation system until roots had permeated the soil masses and topgrowth had attained one inch. Plant containers were relocated and placed on a strict regime of one-acre inch of water, applied in a split application, twice a week. Deionized distilled water was used. Air temperatures of 74°F ± 2°F were maintained by an automatic thermostatic system.

Grasses were cut and maintained at a 1 1/2" height. Five-gram soil samples were removed, randomly, within the various soil moisture regimes and examined periodically for soil moisture changes by the centrifuge system described above.

Pure cultures of Trichoderma viride and Fusarium roseum isolates were prepared on potato dextrose agar adjusted to pH 5.6. Fusarium was later seeded onto a sterile 1 percent corn meal sand mix prepared according to Riker and Riker (63). Immediately following the shearing of the grass plants, Fusarium corn-meal sand inoculum, air-dried, was evenly distributed into the designated containers. A casein-Trichoderma

slurry was prepared in a Waring blendor from Petri plate isolates, and the inoculum was distributed equally into the assigned marked containers. Another series of each treatment received both Trichoderma and Fusarium inoculum distributed in the same manner.

Within each soil moisture regime 4 pots received Trichoderma alone; 4 pots received Fusarium alone; 4 pots received a combination of Fusarium and Trichoderma and 4 pots were left uninoculated as controls. Sixteen tagged pots were involved in each treatment. Treatments were repeated four times for each soil moisture level as well as for treatments for the endomic and sandy loam soil.

Grasses were observed for changes in color, texture and disease every 24 period following inoculations. A disease index based on the percent blighted plants per pot: zero for no disease to 100 percent total plant death was used. Every 72 hours random 15-gram soil samples with adhering plant material were examined for the presence of organisms inciting disease. Dilution plate-series, as previously described, were initiated to corroborate findings, and to ascertain if other organisms might have contaminated the system during the course of the experiment, and as a check on the viability of the previously artificially introduced organisms.

At the conclusion of the experiment an entirely new second series was prepared in the same manner described above.

Results:

Satisfactory soil moisture equivalences were maintained throughout the investigation: only some slight variations occurred as foliar growth, root systems and rhizomes developed.

Within the first 72-hour period the Trichoderma plus Fusarium pots at the 20 and 40 percent M.E. levels showed signs of infection, i.e. lesions were observed on a random basis within the pots. Twenty-four hours later all plants were sheared to maintain a 1 1/2" height of cut; 8 hours following this, at all M.E. level treatments where Fusarium was included, there were some lesions on both upper and lower portions of the leaf blade and sheath. Microscopic examination of cut-sections of the infected area showed Fusarium had penetrated the leaf surface at the juncture of the epidermal cells and through stomates. Stomate penetration was not common. Most penetrations occurred at the wounded tips or directly through the cuticular and epidermal portions. Lesions and signs of fungal infection or fungal proliferation did not persist at the higher moisture levels of 60-80 percent or in the sandy-loam mix. Nor did the disease state increase in intensity in those pots in which only Fusarium had been added, and most of these plants so inoculated appeared to recover after a clipping, 6 days after inoculum was added to the system. The clipping evidently aided removal of active Fusarium stages. Grass plants that had been inoculated with both Trichoderma and Fusarium at the 20 percent and 40 percent M.E., and in

the endemic soils, increased in disease symptoms at this time, and death to plants on a percentage basis significantly increased. Seven days after inoculation it was observed that the Fusarium-Trichoderma infection syndrome in the 20 percent M.E. systems had lost from 40 to 100 percent of the grass plants as a result of Fusarium blight. At the 40 percent M.E. in the Fusarium-Trichoderma inoculated containers a loss from 15 to 50 percent was recorded. At 60 percent M.E. in the Trichoderma-Fusarium combination system only 10 to 15 percent of the plants were killed. The 80 percent or saturated type moisture level the plant death percentage was only 1 to 5 percent. The sandy loam, approximately 48 percent M.E., had a lethal disease percentage of 2 to 10 percent. Readings taken from the endemic soil inoculated with Fusarium-Trichoderma combination showed that plant kill percentages were 50-75-80 and 100 percent in the four pots used. The soil moisture equivalence for the endemic soil was 34 percent.

Comparisions within the Fusarium-Trichoderma pots, on an individual treatment basis, assayed in culture, showed (Table III) only Trichoderma spp. present in soil particles adhering to rootlets with some Pseudomonad-like bacteria present. Fusarium treated grass plots showed only Fusarium present in the rhizosphere accompanied by a similar Pseudomonad-like bacterium. In the combined treated plots (Fusarium-Trichoderma), Fusarium roseum was identified as the incumbent pathogen, in

TABLE III

Percent Disease in Soil Moisture Systems Inoculated with *Fusarium* and *Trichoderma*, Alone and Together

Moisture Equivalence %	Fusarium Replicates				Trichoderma Replicates				Fusarium-Trichoderma Replicates			
	1	2	3	4	1	2	3	4	1	2	3	4
20	10.0	10.0	25.0	30.0	15.0	20.0	20.0	25.0	40.0	40.0	80.0	100.0
34[1]	30.0	30.0	30.0	40.0	10.0	15.0	20.0	25.0	50.0	75.0	80.0	100.0
40	20.0	5.0	5.0	10.0	2.0	5.0	5.0	5.0	15.0	20.0	30.0	50.0
48[2]	TR	TR	2.0	TR	TR	TR	1.0	TR	20.0	1.0	10.0	0.0
60	2.0	2.0	5.0	10.0	TR	TR	1.0	1.0	10.0	10.0	15.0	15.0
80	TR	1.0	1.0	2.0	TR	0.0	0.0	0.0	0.0	2.0	5.0	5.0
Control[3]	--	--	--	--	--	--	--	--	--	--	--	--

[1] Endemic soil

[2] Sandy loam

[3] No disease found at any level

regions where foliar cortical decay, crown bud invasion and defoliation had taken place, and the end result was the death of the plants. There were sufficient repeated manifestations of the disease syndrome, as described by Couch (21), to conclude, apparently, that Fusarium was the sole pathogen responsible for the death, and decay of the leaves and crown buds. On the other hand, those symptoms found in Trichoderma inoculated containers appeared to be more the result of a distress condition engendered by chlorosis in the leafy areas of the plant.

Soil samples over the 54-day period of the investigation showed some slight variations and some increase in the soil moisture equivalence, within treatments. Probably due to increasing quantities of sloughed-off root materials, the other organic debris or gels. The changes which, in general, were not over 2 to 4 percent did not appear to be generally significant to interfere with the final results of the overall disease situation.

Soil-dilution plate samples taken intermittently verified that there was apparently no cross contamination from one series of pots to another. Only those organisms that had been originally inoculated were found, although some bacteria, as previously noted, were present particularly--Pseudomonas spp.

Figures 46, 47, and 48 show the influence of soil moisture on disease incidence in pots inoculated with Trichoderma viride. The control pots did not show any signs

of disease or chlorosis. While those grass plants in the 20 percent M.E. system reached a disease index of 25 percent after 14 days and continued at that level for the next two readings, there was then some easing of the stress conditions after 40 days. In general, however, in the Trichoderma inoculated pots there was no general plant debilitation even in the 20 percent M.E. treated pots. It is noteworthy, however, that at all higher soil moisture systems the plants recovered much sooner following inoculation.

Figure 47 illustrates the influence of soil moisture on disease incidence in pots inoculated with Fusarium roseum and shows that the immediate effect of the introduced pathogen is not as rapid as that when Trichoderma is present; and it was some 28 days before peak infection was seen at the 20 percent moisture level. In addition, it is to be noted that the disease rating at 40 percent M.E. is much higher than in parallel Trichoderma infected pots. In the case of the Fusarium infected pots, lesions were found and the mycelia and spores of Fusarium were observed throughout the plants so infected. In this instance there was no recovery of the grass plants once infected, and in which tissue degradation had taken place. Moreover, once a plant had been killed, other close growing grass plants did not spread or grow into the void left by the dead plant. Higher soil moisture systems, i.e. 60 and 80 percent, were affected by the presence of the pathogen only slightly, and, in general, invasion of just the

aerial portions of the plants was found. The uninoculated controls showed no disease signs at all.

Figure 48 shows the influence of soil moisture in association with disease incidence when the plants were inoculated with a combination of Fusarium and Trichoderma. At 20 percent M.E. the disease index climbs rapidly and by 28 days an average of 65 percent of the plants had been lost as a result of Fusarium invasion. The disease index of the 40 percent M.E. pots was not as high as the 20 percent M.E., but an average of 30 percent of the plants had been killed by Fusarium.

Fungal population counts at various soil moisture levels in inoculated pots are recorded in Table IV. It is interesting to note that the populations of Fusarium are high at 20 percent M.E., somewhat akin to the figures recorded for the endemic soil sample which has a soil moisture equivalence of 34 percent. Also, the population counts of Fusarium in the Fusarium-Trichoderma pots were not unusually higher than when Fusarium was inoculated alone. It is apparent that Trichoderma counts are, however, very much higher than the Fusarium counts; especially at the lower soil moisture levels. When the soil moisture relationship is examined it will be seen that Trichoderma and Fusarium are inhibited at the higher moisture levels.

Random soil sampling at various times and intervals during the investigation showed pH value ranges at 20 percent

moisture equivalence 6.0-6.2; at 40 percent M.E. pH ranges of 6.0 to 6.3; 60 percent M.E. pH ranges of 6.1 to 6.3, and at 80 percent M.E. pH ranges were 6.1 to 6.6. Endemic soil pH ranged widely from 6.0 to 6.5. Sandy loam soil pH values fluctuated from 6.2 to 6.6 during the course of the experiment, and did not appear to be a factor in these experiments.

A statistical analysis, using a factorial design, was programmed through the CDC 3600 computer at the University of Massachusetts Computer Center; results are shown in Table V. Significant differences were found at the 95 percent confidence levels within those containers that had been inoculated with a combination inoculum of Fusarium-Trichoderma in the 20 percent M.E. system. Further, it is apparently evident that those pots that received inoculations of only Fusarium were more effective than those pots which received Trichoderma alone. The tendency toward the disease state was found to be more significant when soil moisture levels were decreased. The analysis showed the highly significant effect of both the soil moisture and the interaction of the dual inoculation.

At the completion of the above described experiment an entirely new soil moisture equivalence series was initiated. In this series all pots, soils, seeds, plants, inoculum were treated in the same manner as previously described. The results of the second repeated series corroborated results obtained from the first run series experiment.

TABLE IV

Fungal Population Counts at Various Soil Moisture Levels in Inoculated Pots

Days	Moisture Equivalence %	Nos. x 10^4/g Fusarium	Trichoderma	Fusarium-Trichoderma	
14	20	48	240	75	229
	40	33	198	40	173
	60	14	40	5	26
	80	12	8	0	1
	E *	96	232	63	260
	SL**	3	66	8	79
28	20	85	176	91	205
	40	42	210	80	162
	60	13	15	14	30
	80	16	2	4	12
	E *	71	191	56	297
	SL**	11	41	10	55
42	20	59	135	60	198
	40	27	152	35	88
	60	29	6	34	18
	80	15	15	4	22
	E *	110	288	102	29
	SL**	6	18	10	29

* Endemic Soil, 34% M.E.
** Sandy Loam, 48% M.E.

TABLE V

Analysis of Variance for Factorial Design for Soil Moisture Systems inoculated with *Fusarium*, *Trichoderma*, Alone and Together

Sources	Degree Freedom	Sum of Squares	Mean Squares	Tabulated F.05	F/.05
Fungi	2	2591.79	1295.90	8.29	
Moisture	5	13388.40	2677.69	17.29	
Fungi x Moisture	10	1189.61	118.96	0.76	
Within Replicates	54	8444.66	156.38		3.10
Total	71	25614.46			

DISCUSSION

The ecosystem of a given soil micro-environment is the resultant of a vast array of forces operative simultaneously. Moisture, pH, organic matter, gas exchange, soil texture and mineral constituents represent the gross determinants for the selection of microbial populations. Nonetheless, the work presented in this thesis has demonstrated that the intrusion of even small amounts of Diazinon, an organophosphate; chemical; or of lobster shell, an organic amendment, consisting largely of chitin, can induce broad changes in the spectrum of the soil microflora. Further, that changes in the relative proportions of these materials will be reflected by immediate and pronounced changes among the broad grouping of microorganisms of the soil.

In general, these changes may be catalogued as follows: Diazinon elicited little response from the fungi, while initially depressing actinomycete counts. It did exercise a sharply selective effect on the soil bacteria enhancing the appearance of a few dominant types of Psuedomonads. The lobster shell, as shown by other investigators, was stimulatory to the actinomycete population. When Diazinon and lobster shell were applied together, the lobster shell effect appeared muted, and the selective effect of Diazinon induced the Psuedomonad population at the expense of the actinomycetes

whose numbers were subsequently restricted. Thus, it would appear that these materials must be considered for their individual characteristics in the stimulus of unique populations which their joint use may mask. In view of the impact these materials make on the soil microflora, and the exclusion mechanisms they generate for such large sectors of the soil population, their further potential in building desired ecosystems would appear to merit concerted investigation. One further observation, with respect to Diazinon, needs mention, and that is the stunting effect, presumably hormonal, observed on new turf stands at high rates of application. The physiological changes induced in the plant of importance in themselves (practically, perhaps, as a means of regulating clipping periodicity) suggest that these two may be operative in determining the susceptibility or resistance to disease.

Most striking, however, was the clarity of the ecological determinants which emerged in the Fusarium blight of Merion Kentucky bluegrass. It was found, first, that microbiological activity was greatest in the absence of Trichoderma spp. With the exception of Fusarium, whatever the soil moisture, the invasion of other organisms was restricted. Again, no matter whether Fusarium was present, and whatever the soil moisture regime, in the absence of Trichoderma spp. infectivity did not result. Infectivity, in fact, was restricted to a very narrow set of conditions:

1. The restriction by Trichoderma spp. of a microflora which could compete with Fusarium, or otherwise be inimical to it.

2. A soil moisture regime of less than 40% moisture equivalence was significantly involved in the disease state.

Under the foregoing circumstances in which the plant was under stress, Fusarium in effect protected by Trichoderma, to which it is itself immune, was able to invade and proliferate in the susceptible plant tissues.

Couch and Bedford (21) during their investigation of Fusarium blight stated that soil under three soil moisture regimes: field capacity, 1/2 field capacity and permanent wilting percentage, no significant differences were found, and soil moisture per se was not a contributing factor in disease incidence. Their investigations were evidently conducted with a single-fungal parasite, and without regard to the possible role of other organisms. Since most soil and plant ecosystems, on both macro- and micro- levels, are usually continuously dependent on many natural circumstances for proliferation or survival, it is unlikely that any one organism is self-determined or wholly self-dependent. Therefore, one could postulate that either one or several contributory organisms, or particular environmental factors. either simultaneously or in a stepwise degree, are necessary for an organism to attain or maintain the virulent status found in a blight or epiphytotic. Darpoux (24) stated there are various phases of fungal and bacterial interactions

which either lead to the disease state or inhibit the development of it.

It was observed in the general investigation of Fusarium blight that the mere presence of Fusarium roseum did not lead to a disease state. In the field plots on the football field under natural conditions, Fusarium roseum condia, chlamyclospores and mycelia were found in fairly large numbers in combination with other organisms; Trichoderma viride notably was absent. These plots were well watered and maintained at approximately field capacity at all times. Garret (32, 33, 34), in his extensive studies of pathogenic organisms, stated that more than moisture relations were involved in the excitation of an organism into the highly virulent stage. As has been shown in the work reported, moisture is only one of a number of stress situations of which the prevailing microbial interrelations are the second group of determinants.

It was noteworthy that throughout the soil moisture experiments plants inoculated with Trichoderma, or with Trichoderma-Fusarium together, were slightly chlorotic. This condition observed by other investigators (Wright, 1956) has been attributed to the large amounts of Gliotoxin produced by Trichoderma spp. within the rhizosphere.

Treatment with fungicides failed to arrest the disease and may in fact have contributed to the development of the disease by restricting fungi which are susceptible to such drenches (to which Trichoderma is notoriously resistant).

Such fungi may have been withdrawn from the pool of competitors to Fusarium, itself resistant, so that the ecological balance may have been further weighted in favor of the pathogen. It would therefore appear, that as with Diazinon and lobster shell, the application of fungicides must be seen in terms of their total impact on the soil microbiota rather than as restricted to any particular species where eradication is sought.

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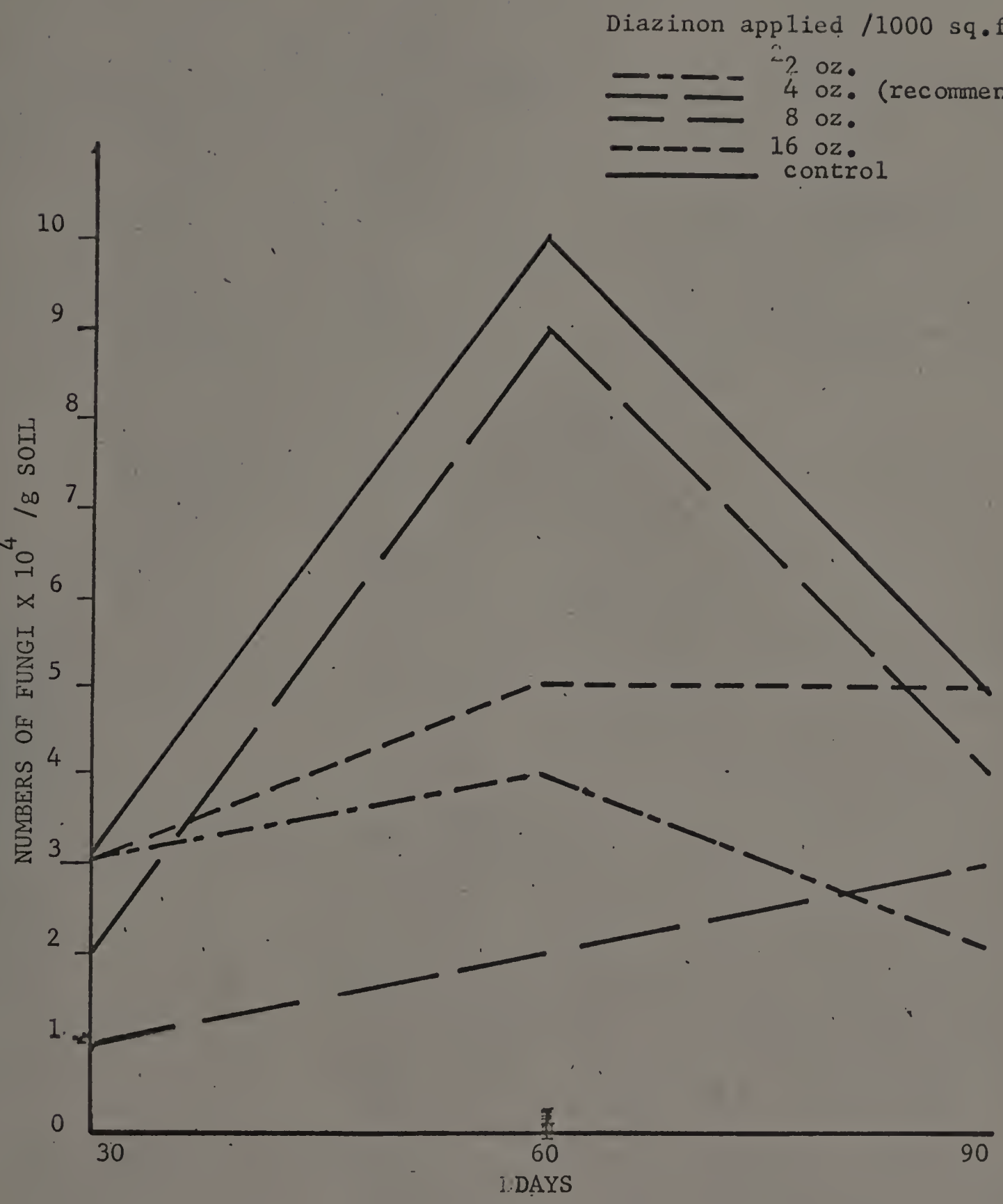

Figure 14: Influence of Diazinon on fungi of an established turf.

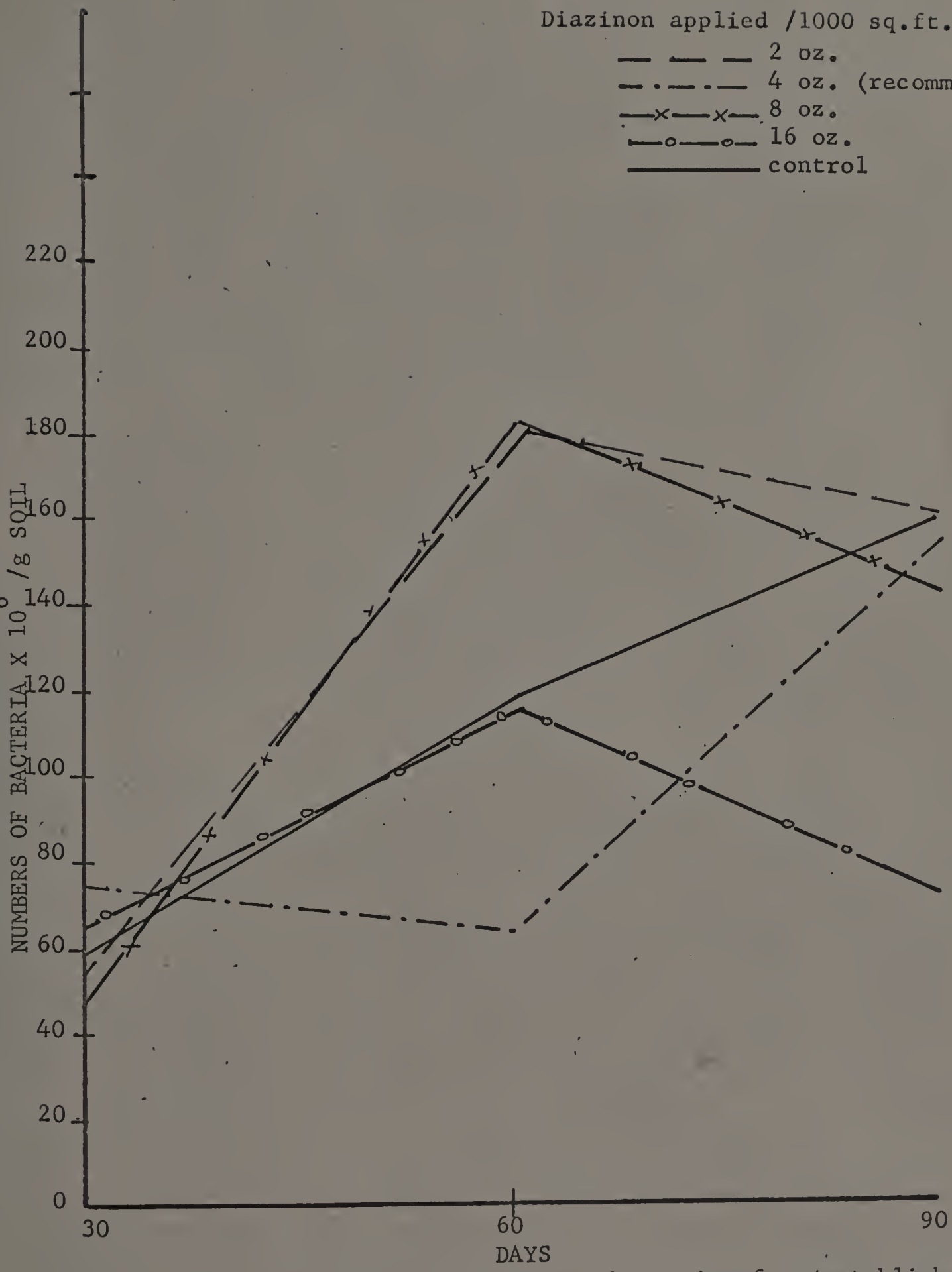

Figure 15: Influence of Diazinon on bacteria of an established turf.

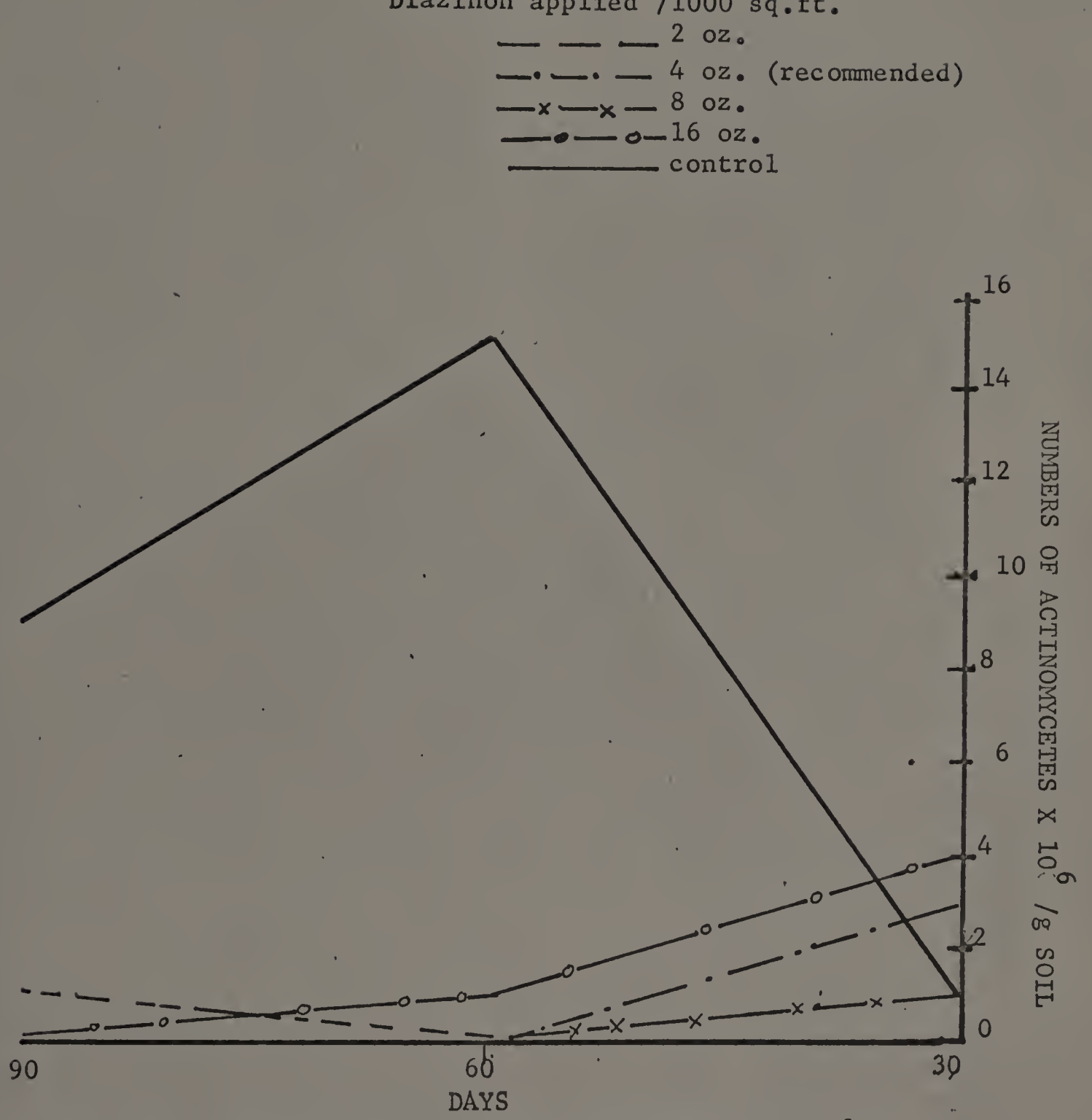

Figure 16: Influence of Diazinon on Actinomycetes of an established turf.

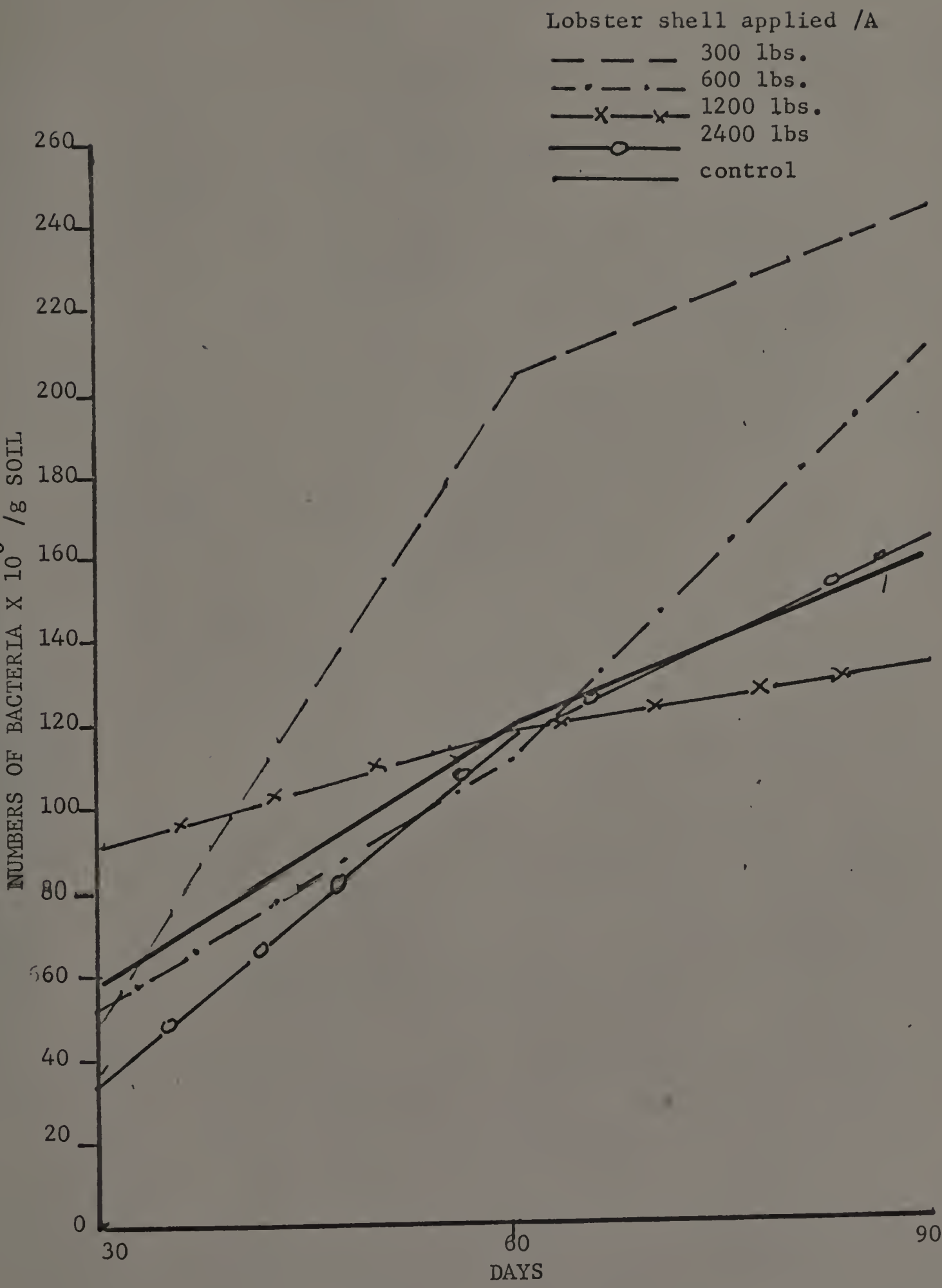

Figure 17: Influence of ground lobster shell on bacteria of an established turf.

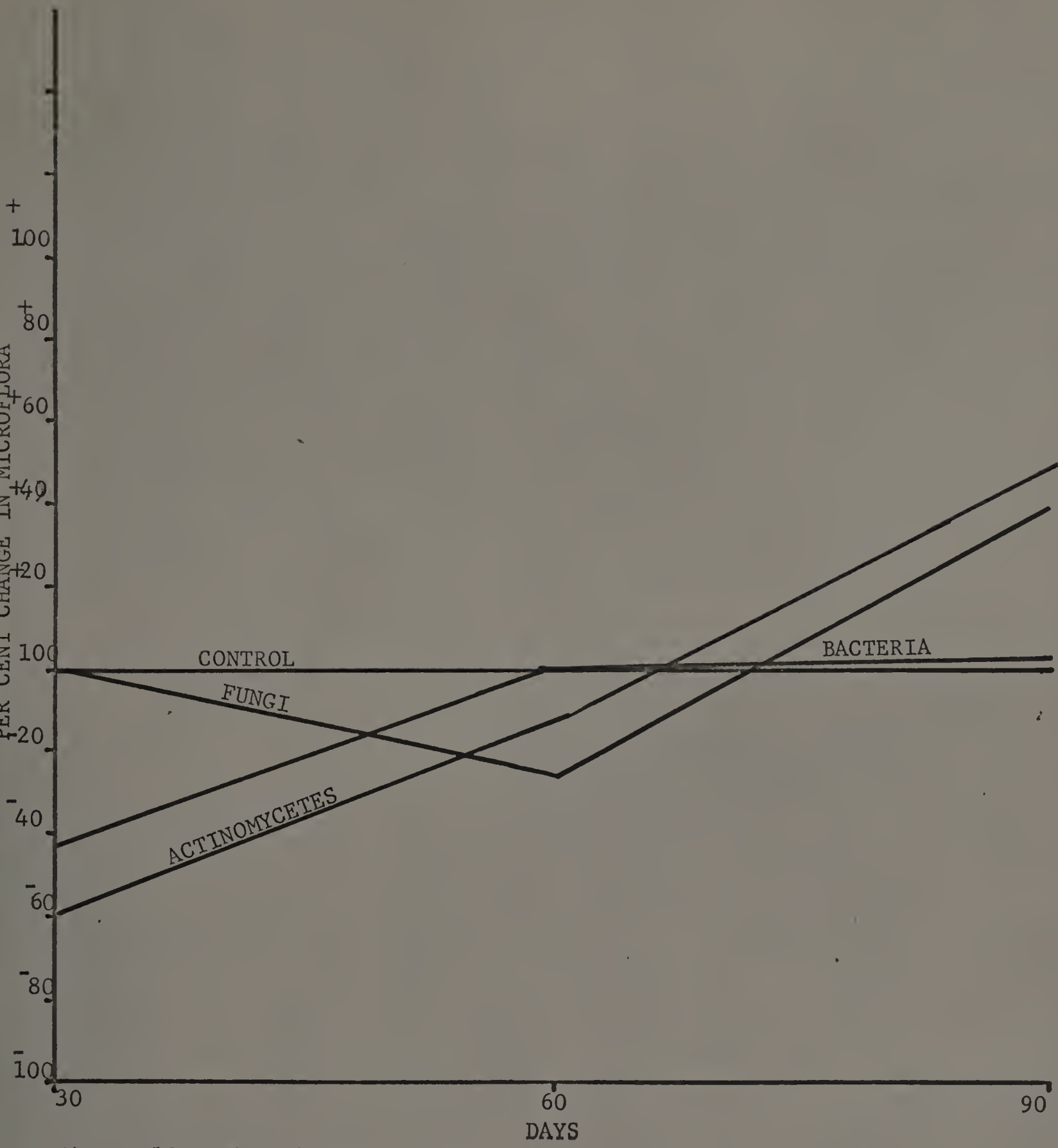

Figure 19: The effect of lobster shell at 600 lbs./A on soil microflora of an established turf.

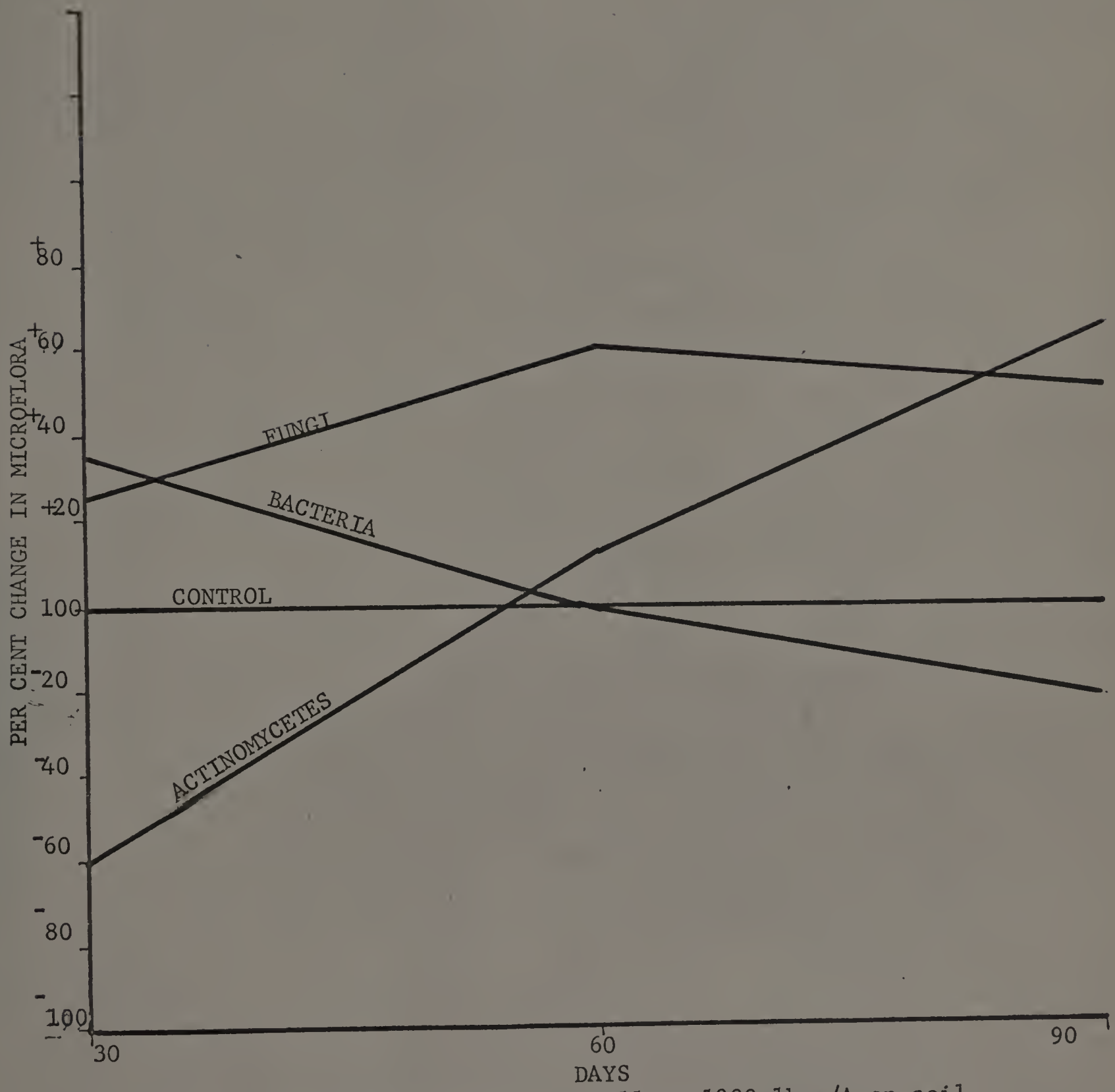

Figure 20: The effect of lobster shell at 1200 lbs./A on soil microflora of an established turf.

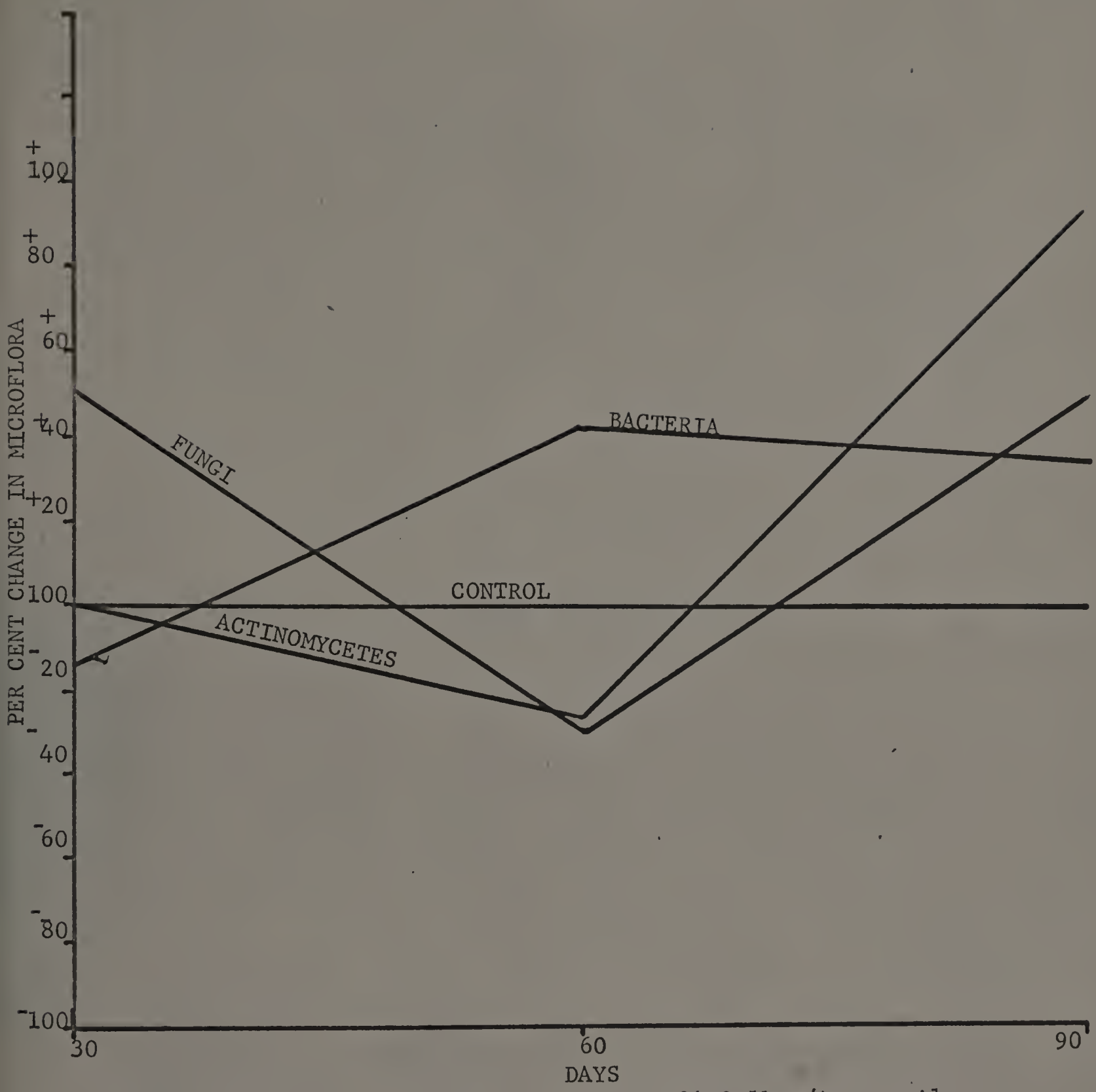

Figure 21: The effect of lobster shell at 2400 lbs./A on soil microflora of an established turf.

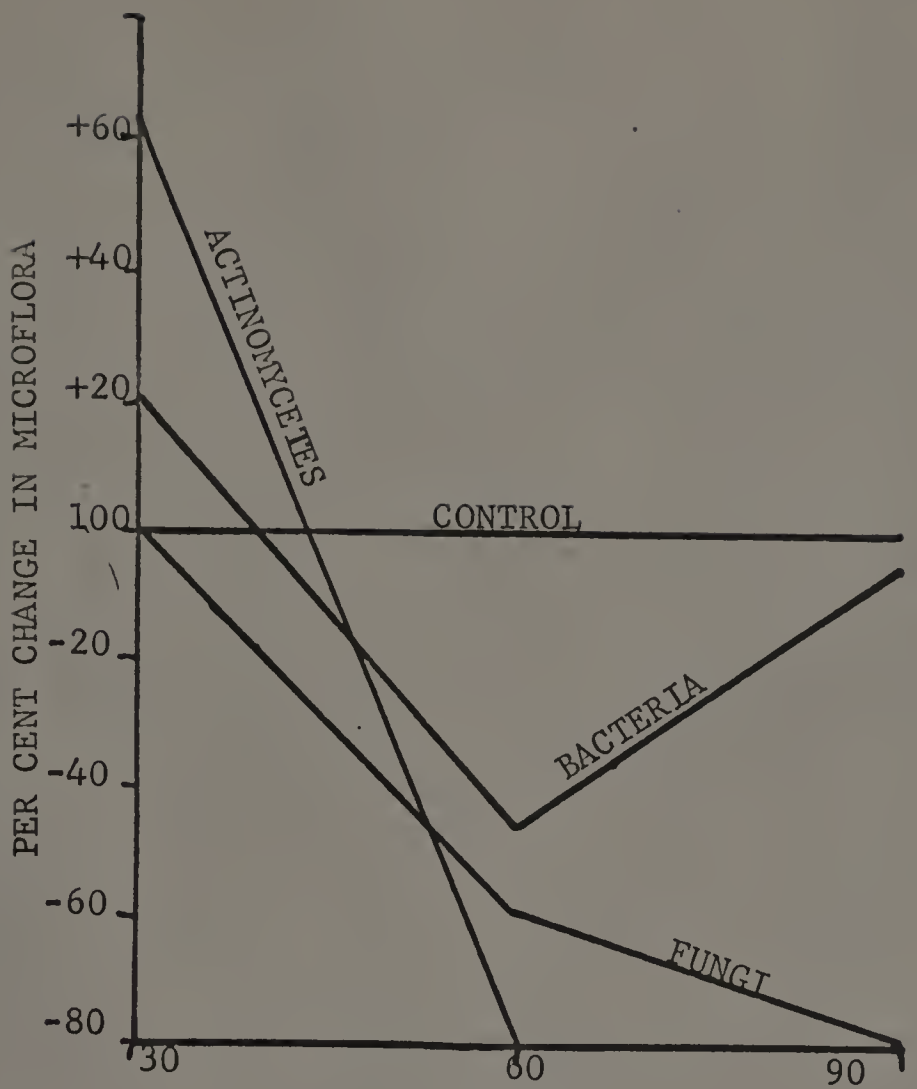

Figure 22: Effect of Diazinon at 2 oz./1000 sq.ft. on soil microflora of an established turf.

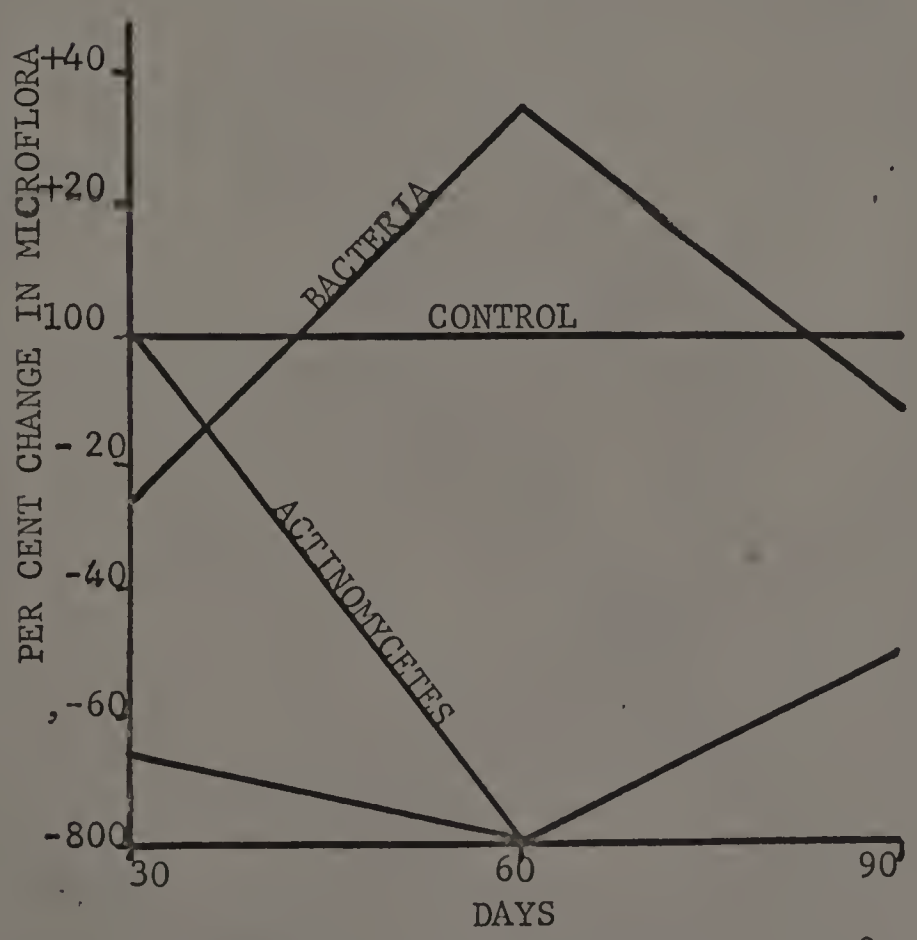

Figure 23: Effect of Diazinon at 8 oz./1000 sq.ft. on soil microflora of an established turf.

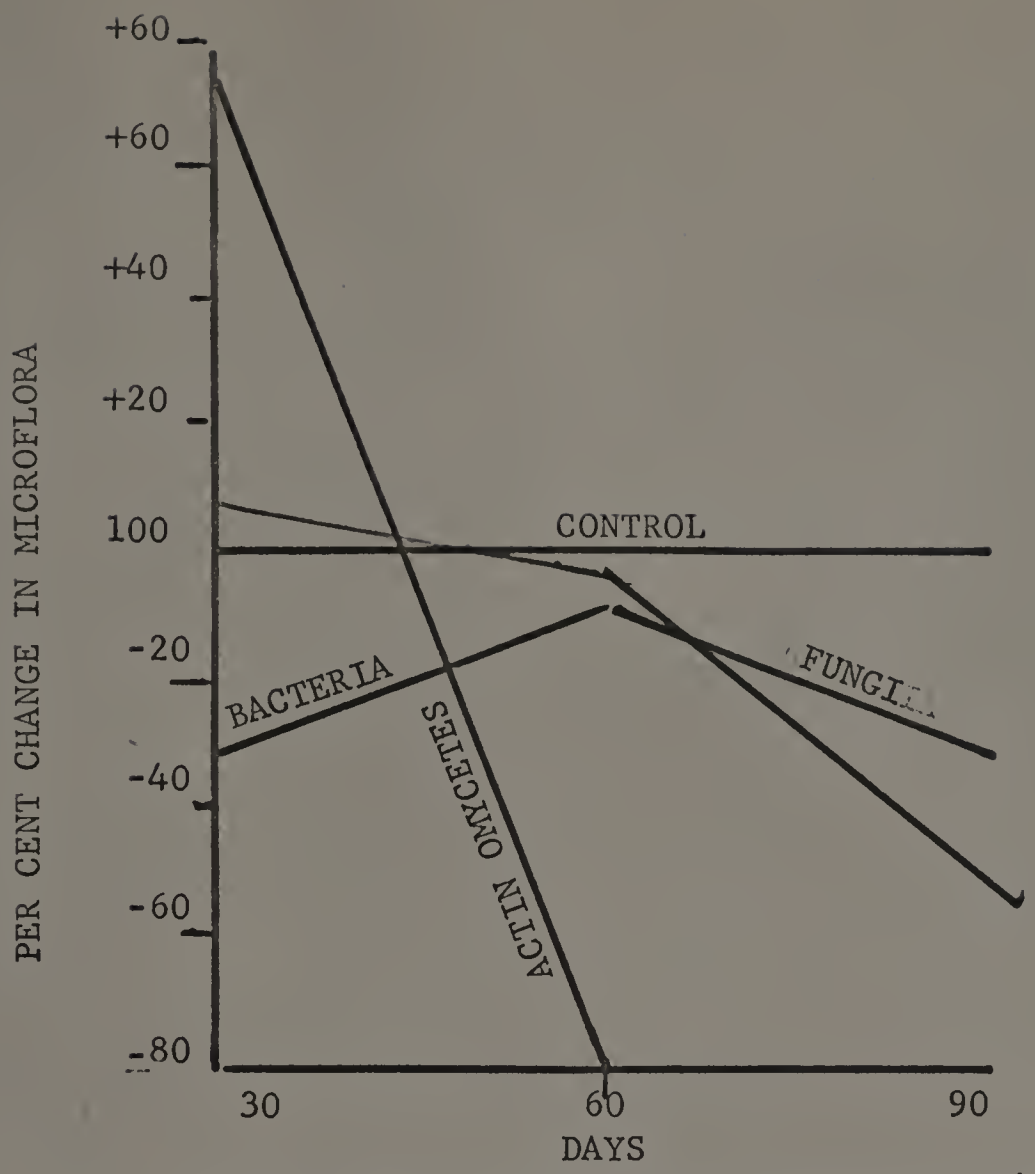

Figure 24: The effect of Diazinon at 4 oz/1000 sq.ft. (recommended) on soil microflora of an established turf.

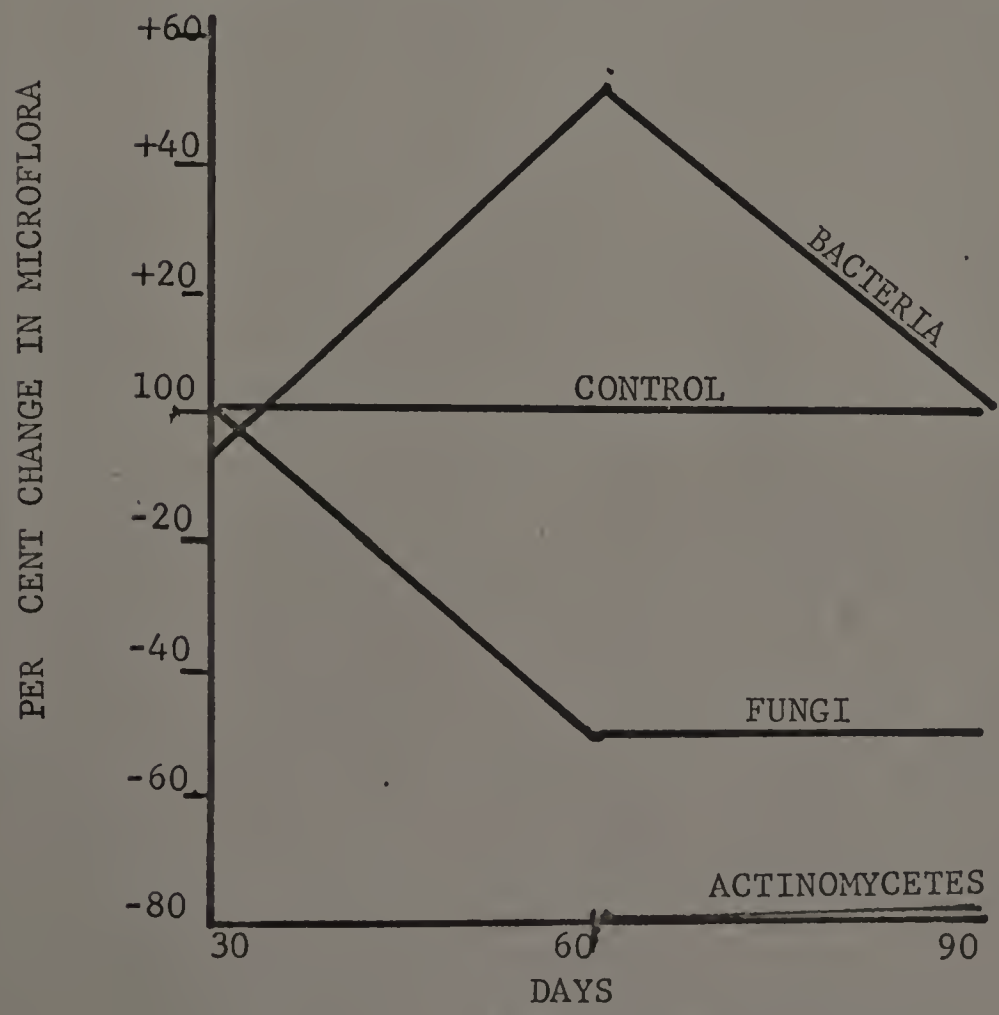

Figure 25: The effect of Diazinon at 16 oz./1000 sq. ft. on microflora of an established turf.

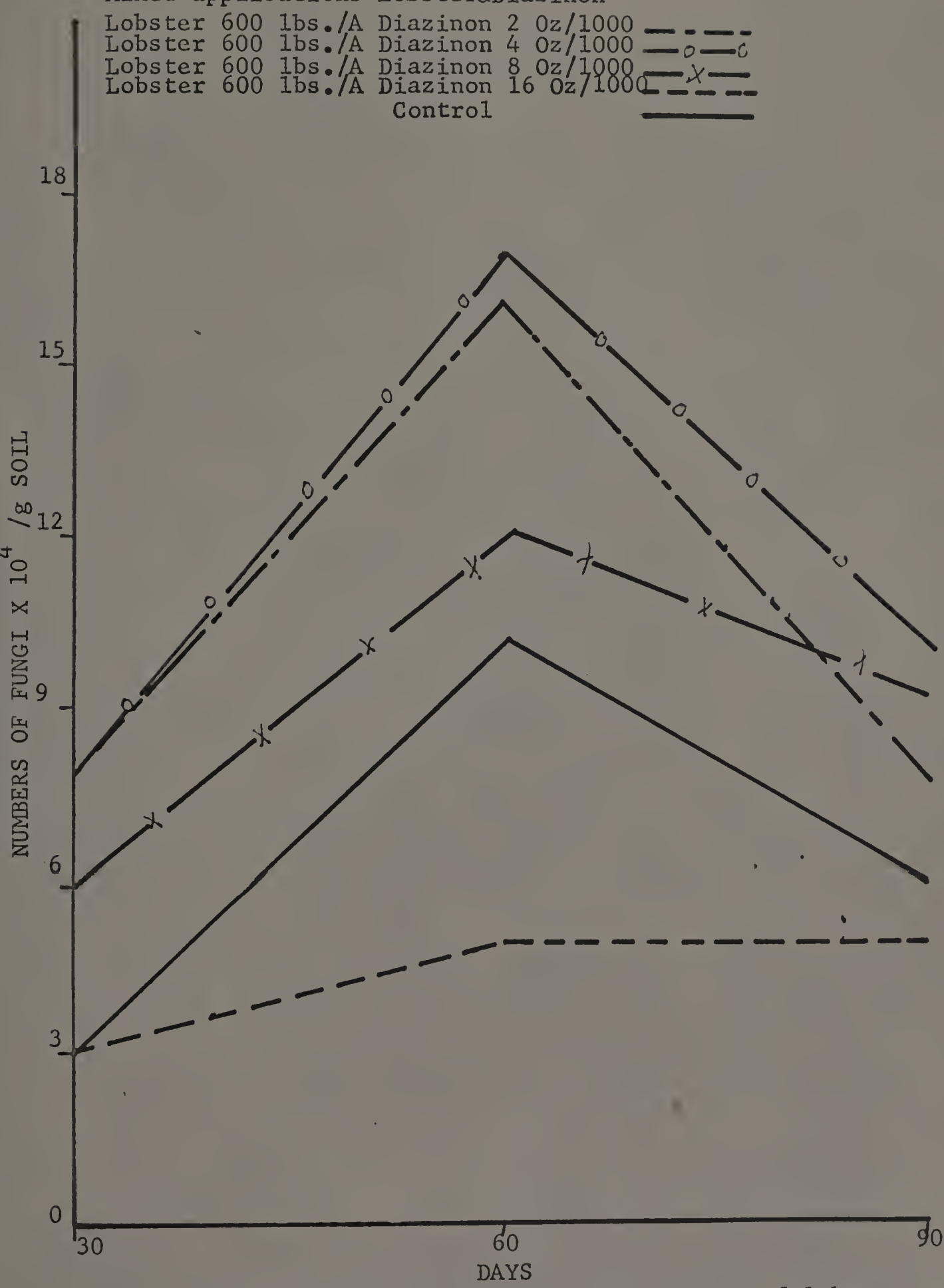

Figure 26: The influence of mixed applications of lobster and Diazinon on fungi of an established turf.

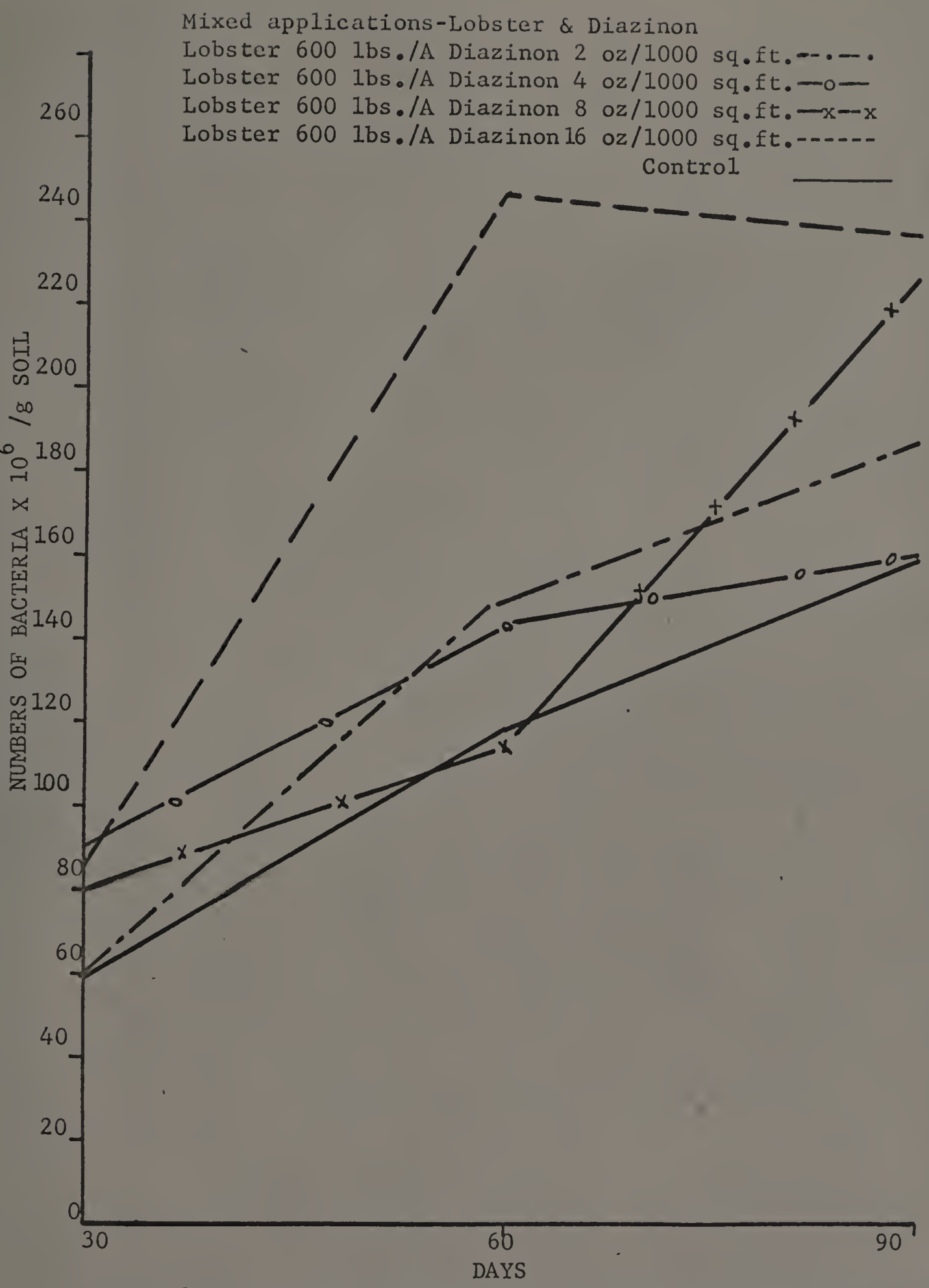

Figure 27: The influence of mixed applications of lobster and Diazinon on bacteria of an established turf.

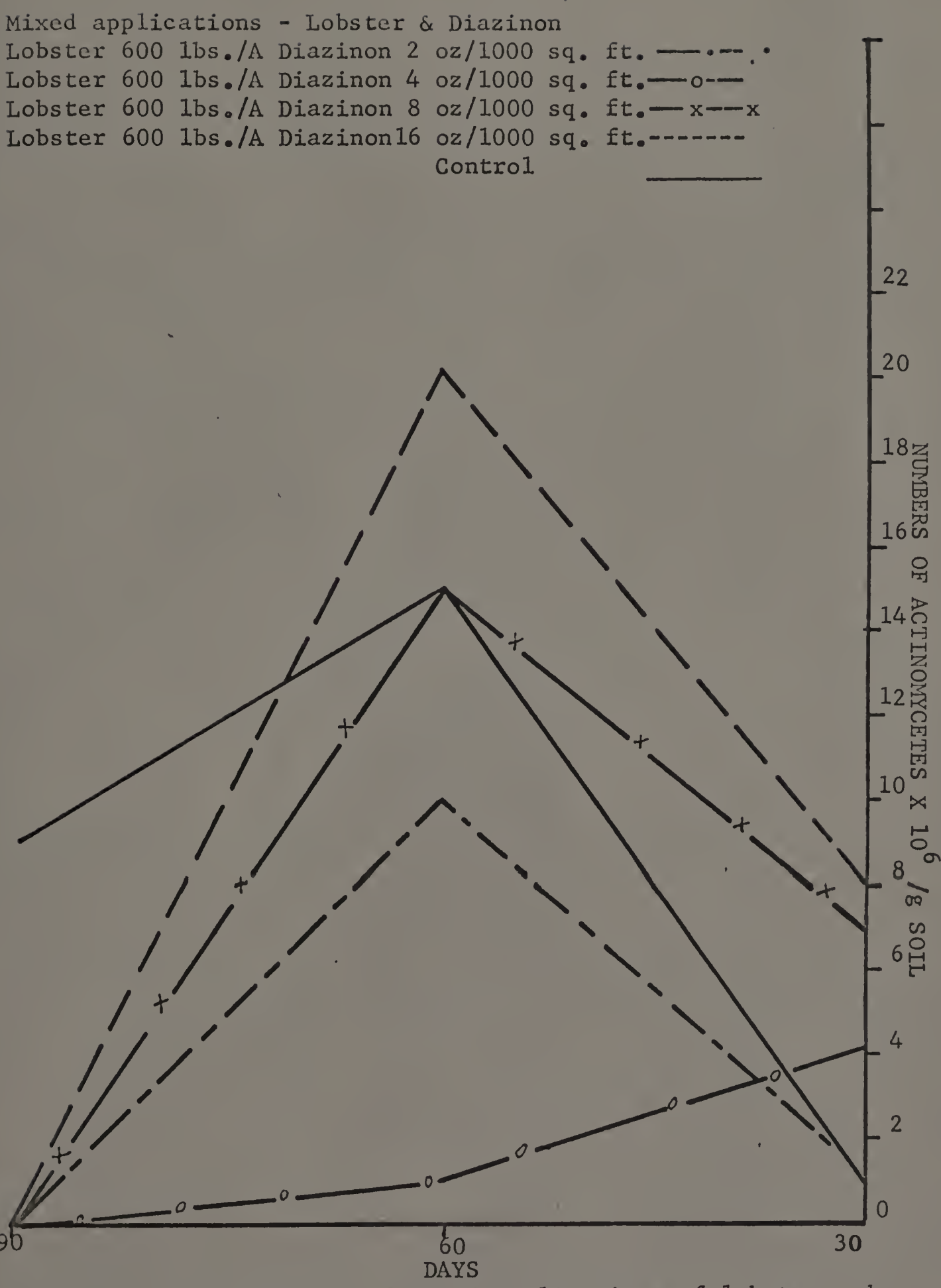

Figure 28: The influence of mixed applications of lobster and Diazinon on Acinomycetes of an established turf.

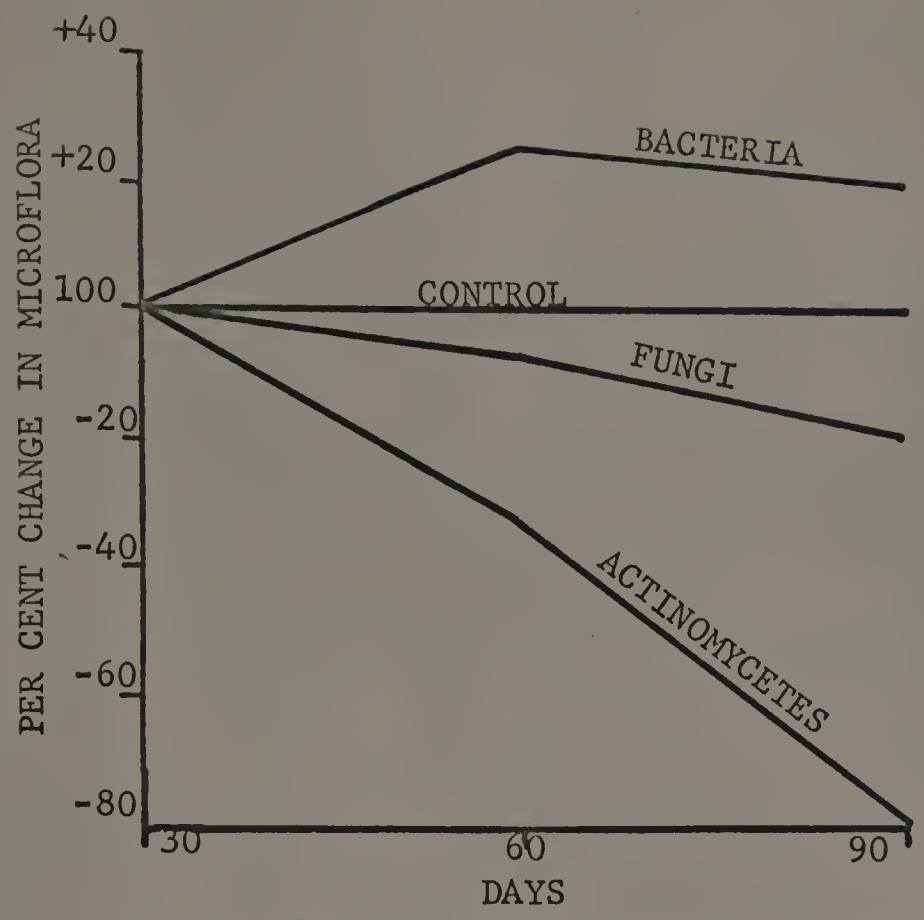

Figure 29: Influence of lobster at 600 lb/A and Diazinon at 2 0z/1000 sq. ft. on microflora of an established turf

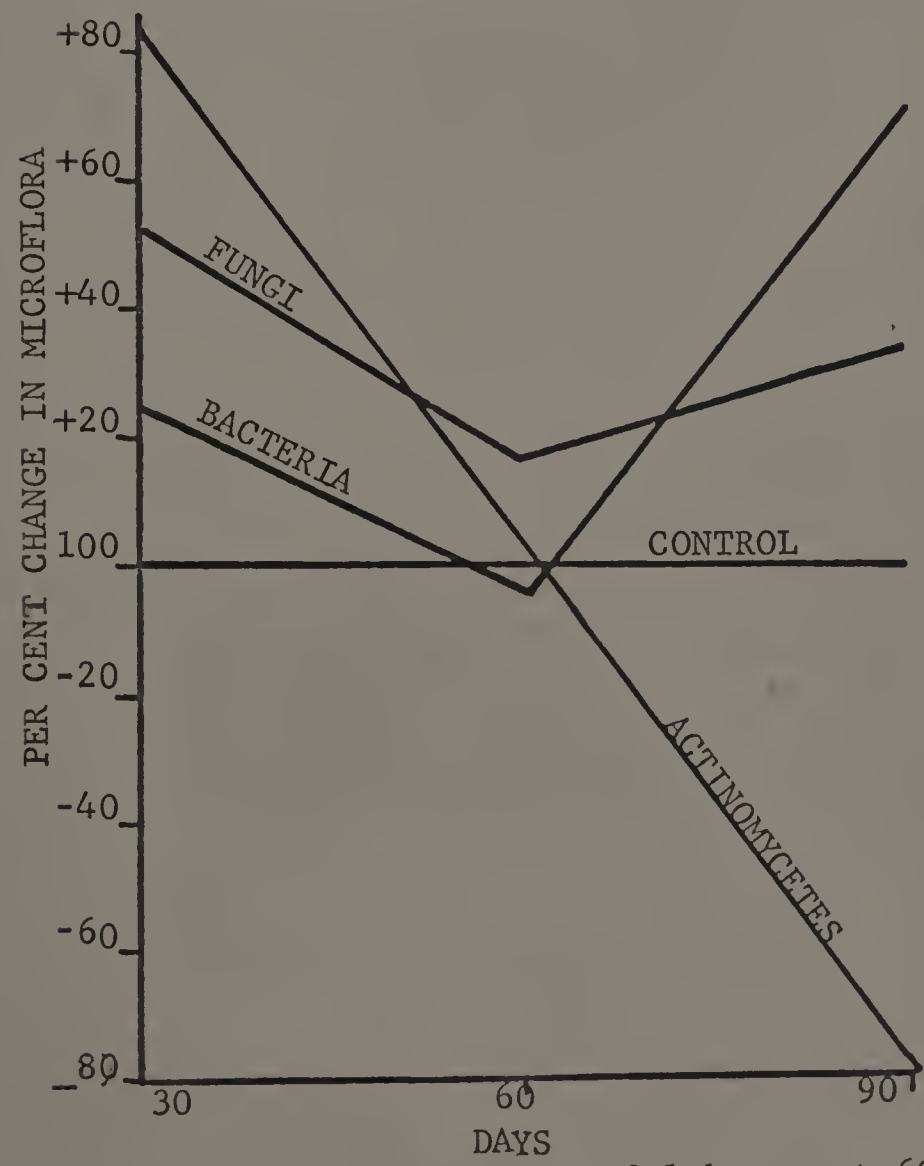

Figure 30: Influence of lobster at 600 lb/A and Diazinon at 8 oz/1000 sq. ft. on microflora of an established turf.

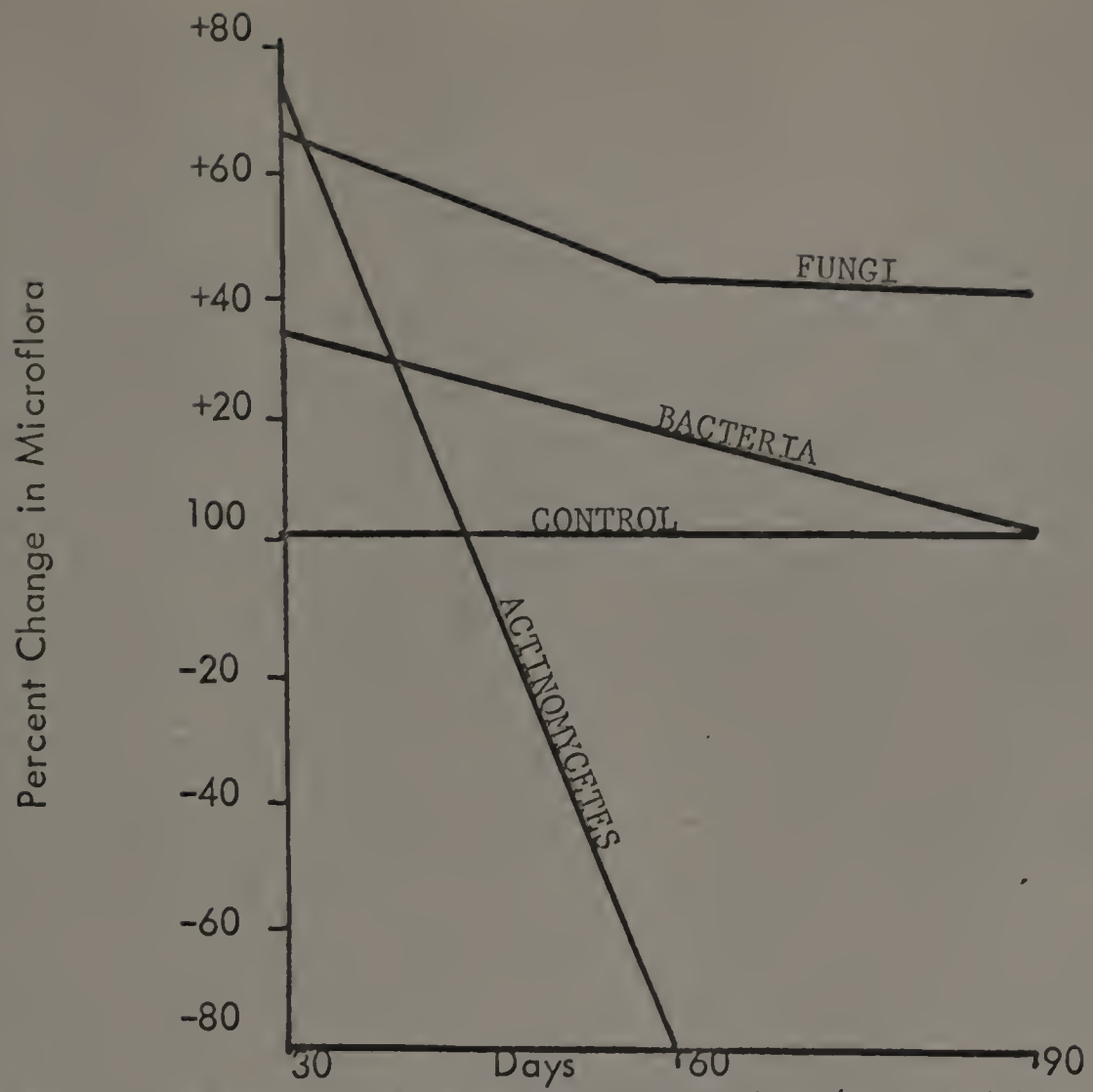

Figure 31: Influence of lobster shell at 600 lbs./acre and Diazinon at 4 oz./1000 sq. ft. on microflora of established turf.

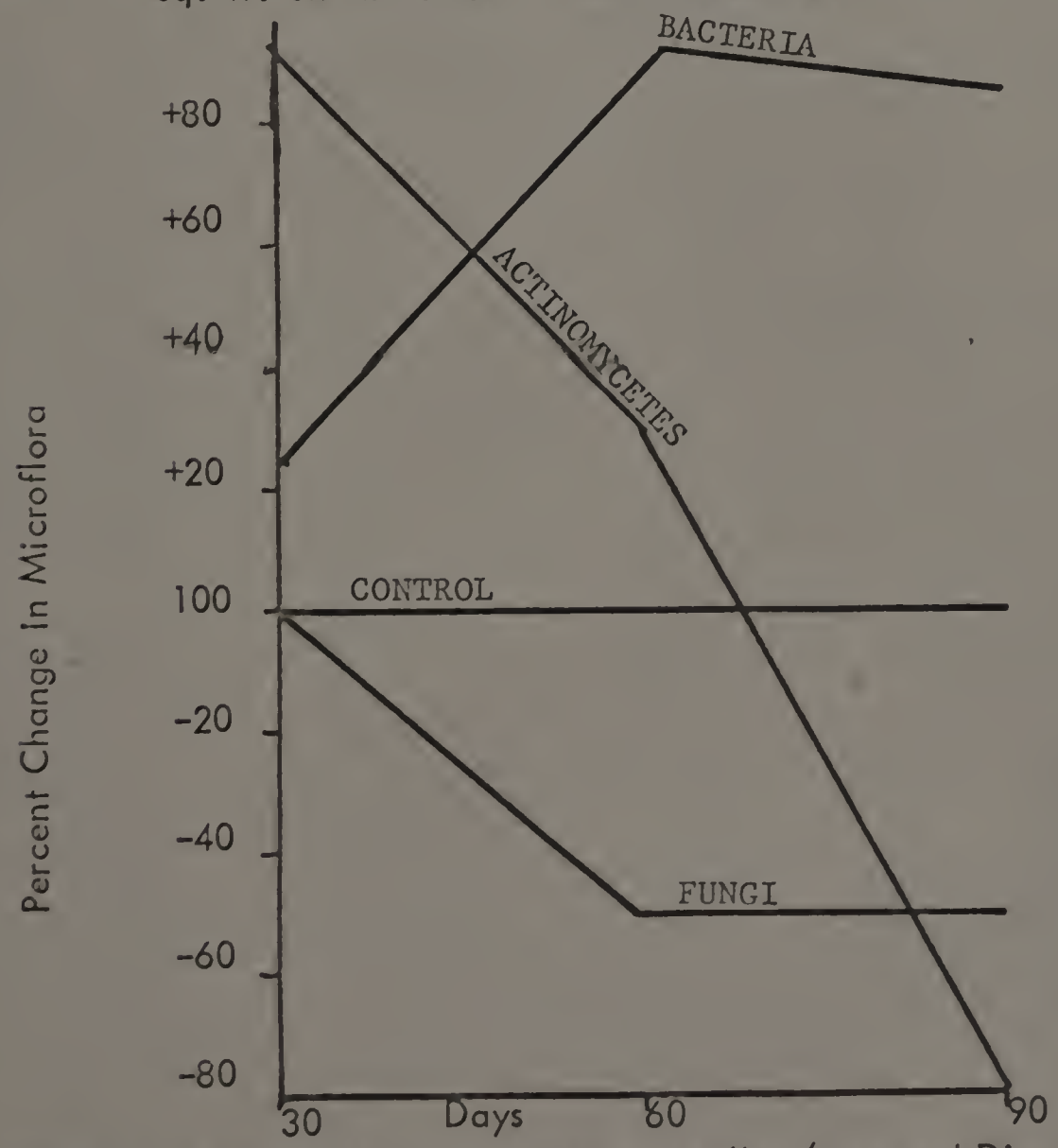

Figure 32: Influence of lobster shell at 600 lbs./acre and Diazinon at recommended rate.

Figure 33: Depressed areas are first visible signs of Fusarium blight. Fungus in virulent stage gives grass within spots a wettish gray-green appearance.

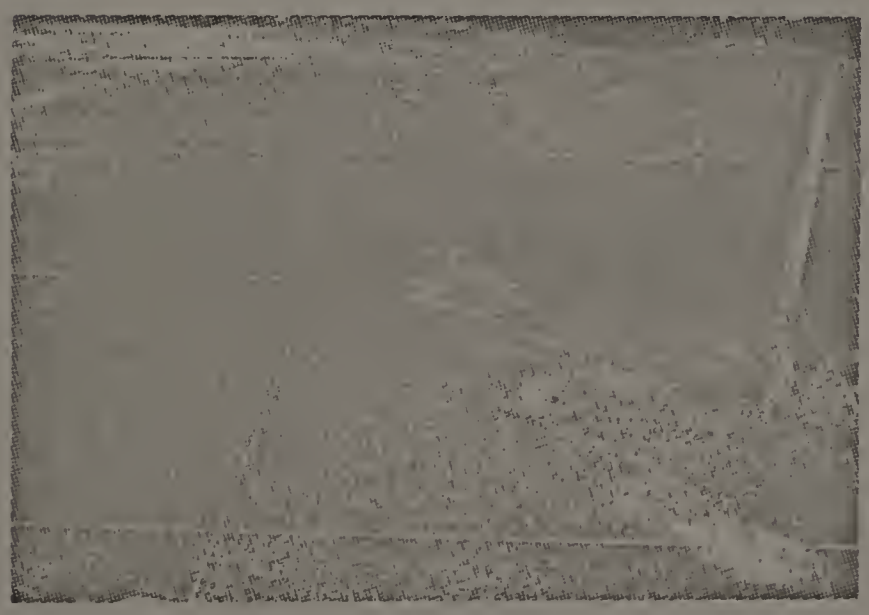

Figure 34: Light circular and crescent shapes occurring in later stages of Fusarium blight. Note dead areas occur in shade as well as in full sun. Fungal activity first observed beneath the wild dogwood tree above.

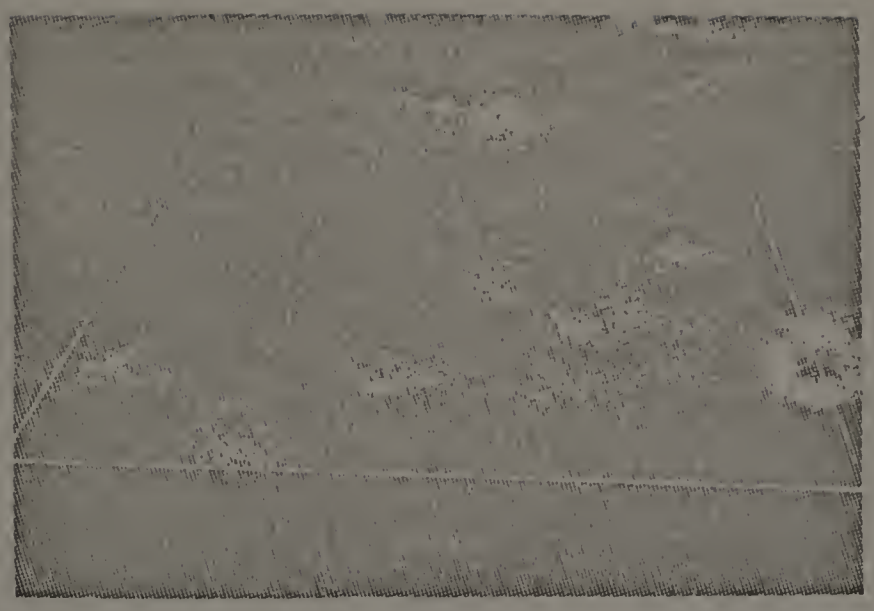

Figure 35: Typical symptoms of Fusarium blight: crescent and circular areas of killed turf.

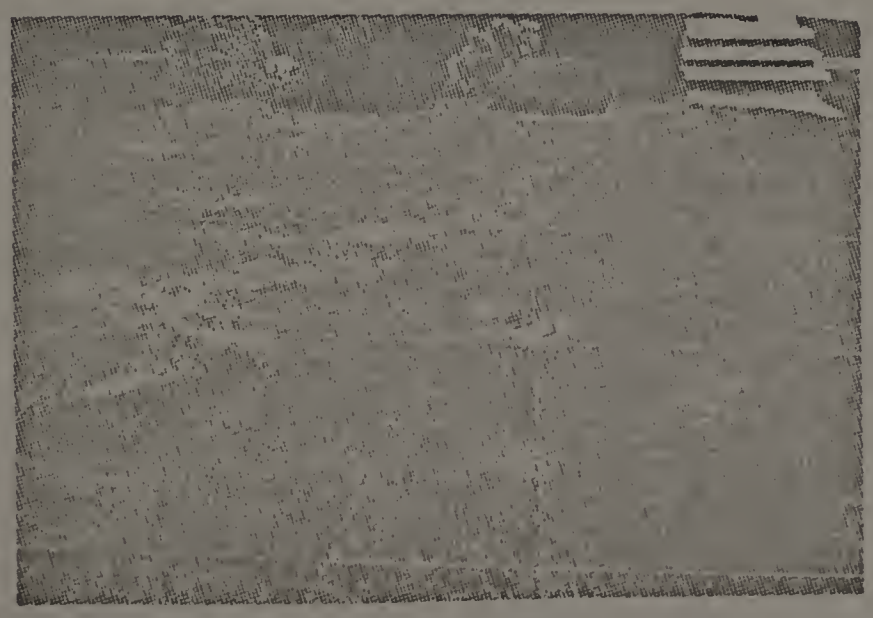

Figure 36: Later stages of Fusarium blight showing patches of dead grass coalescing into larger area.

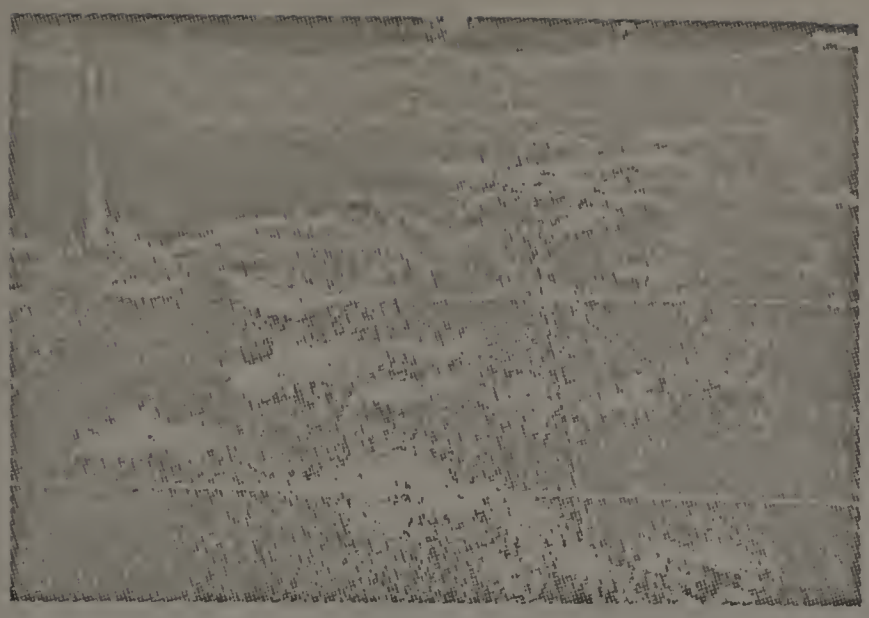

Figure 37: Fusarium blight in its most active stage.

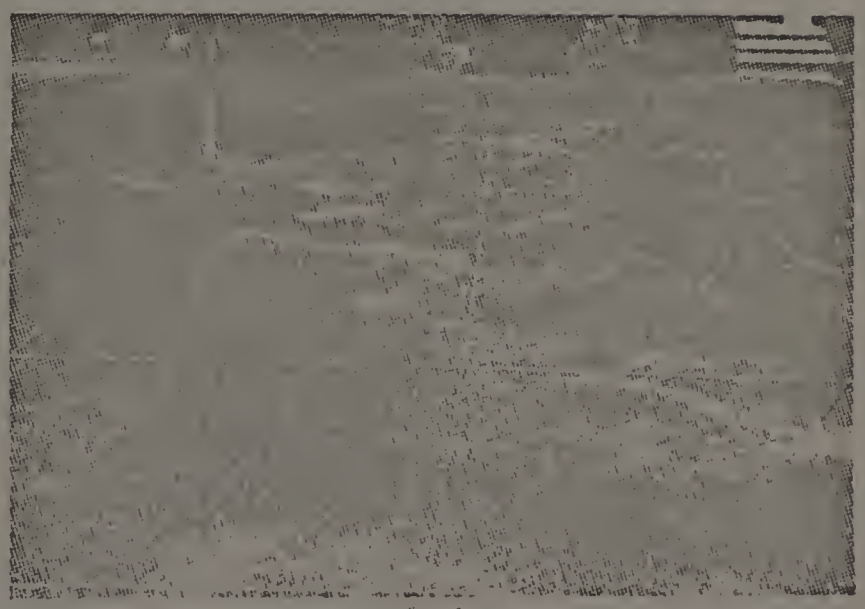

Figure 38: Expanded view of blighted area. Disease more predominant in well-drained areas of site at this stage.

Figure 39: Blight at last stage, encroaching on non-infected but droughted area.

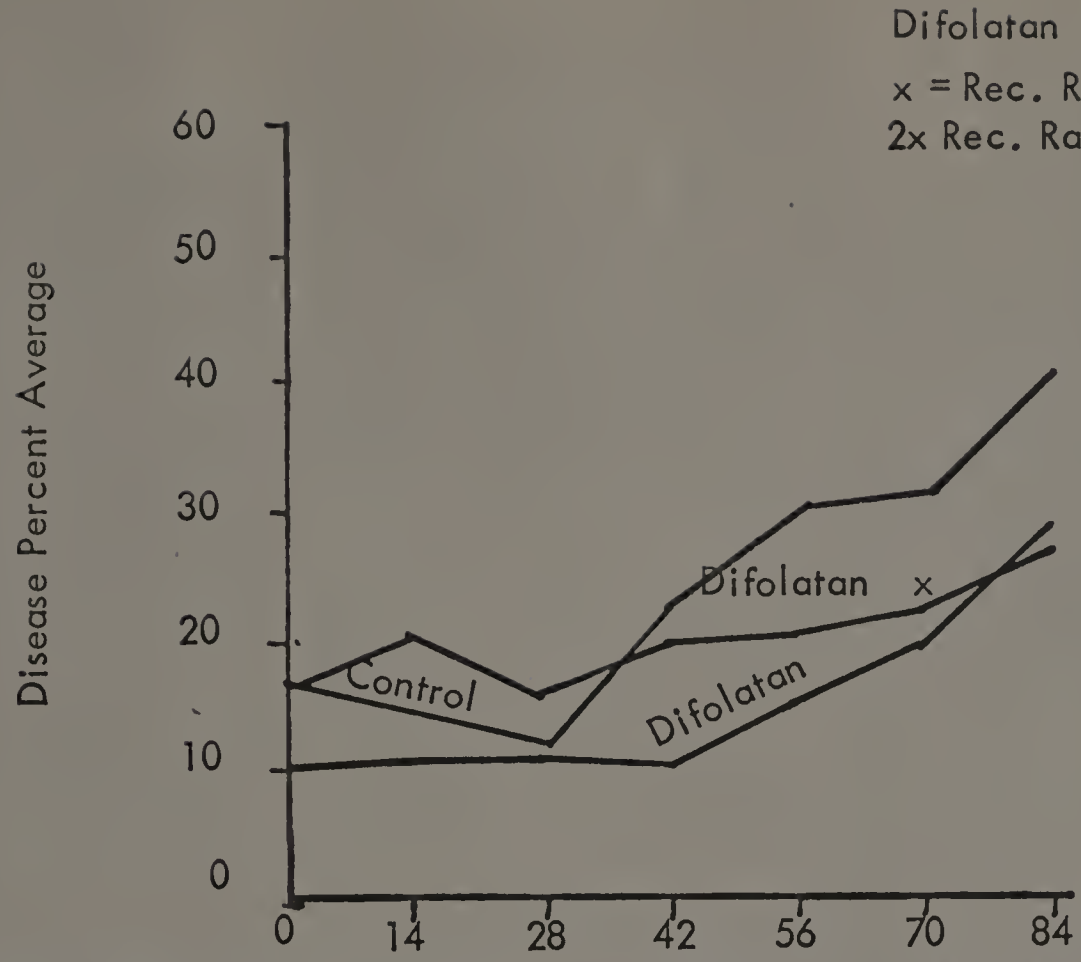

Figure 40: Effect of Fungicides on a Fusarium-infected turf site.

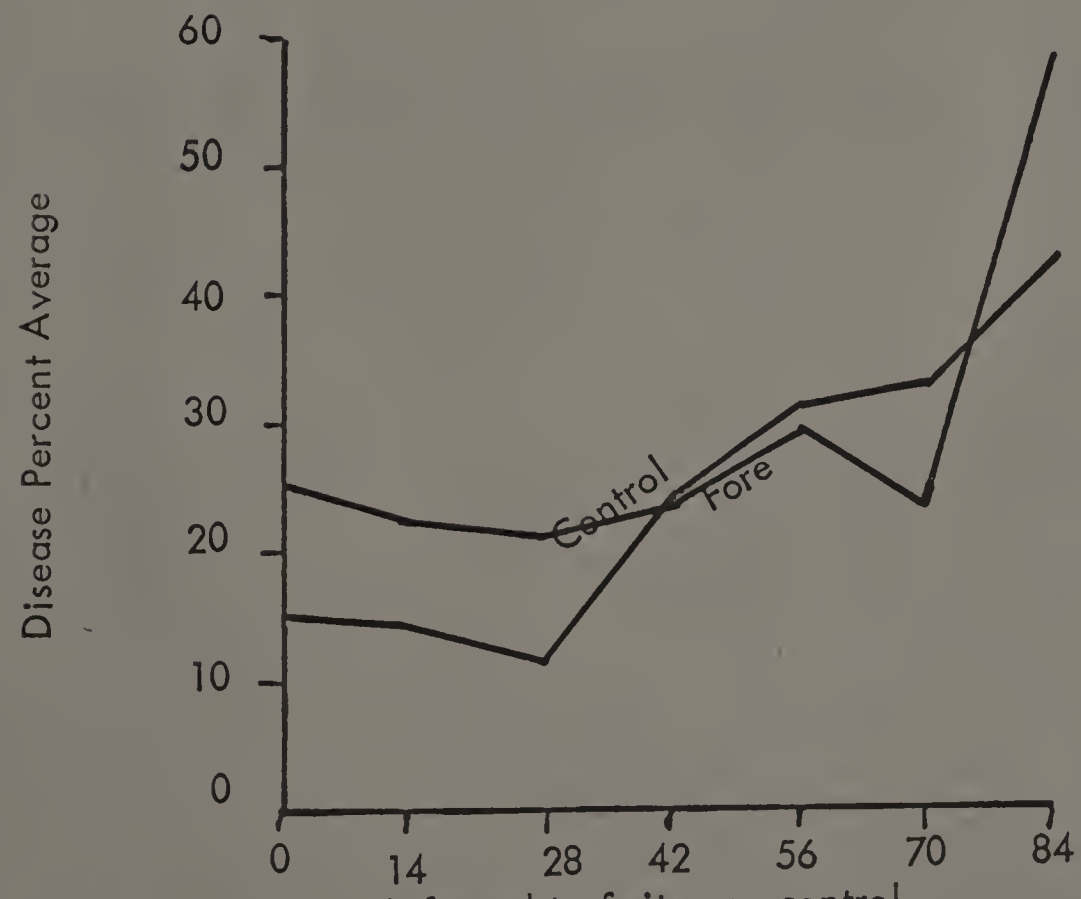

Figure 41: Fusarium-infected turf site vs. control.

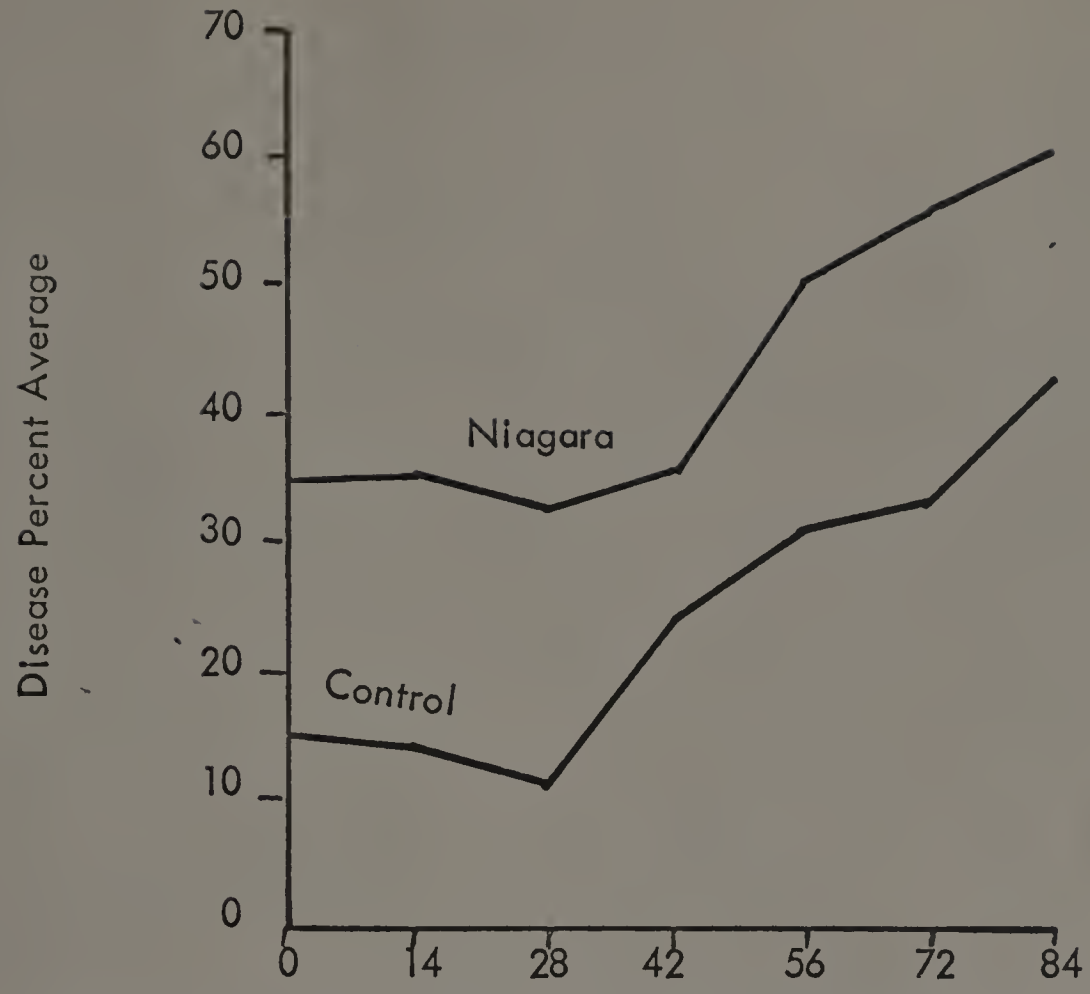

Figure 42: Effect of Niagara Experimental Fungicide on naturally infected F. roseum turf site.

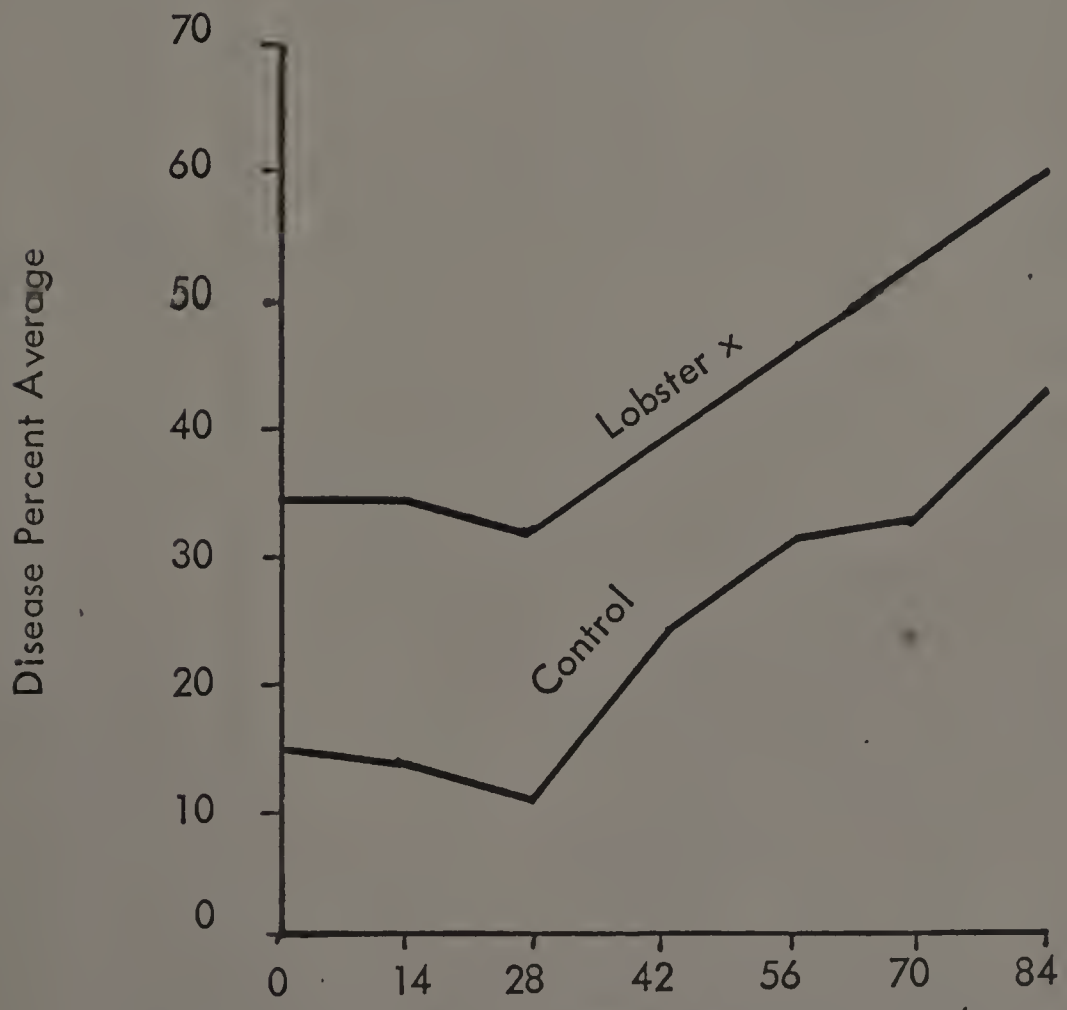

Figure 43: Influence of lobster shell at 600 lbs./acre on Fusarium-infected turf site vs. control.

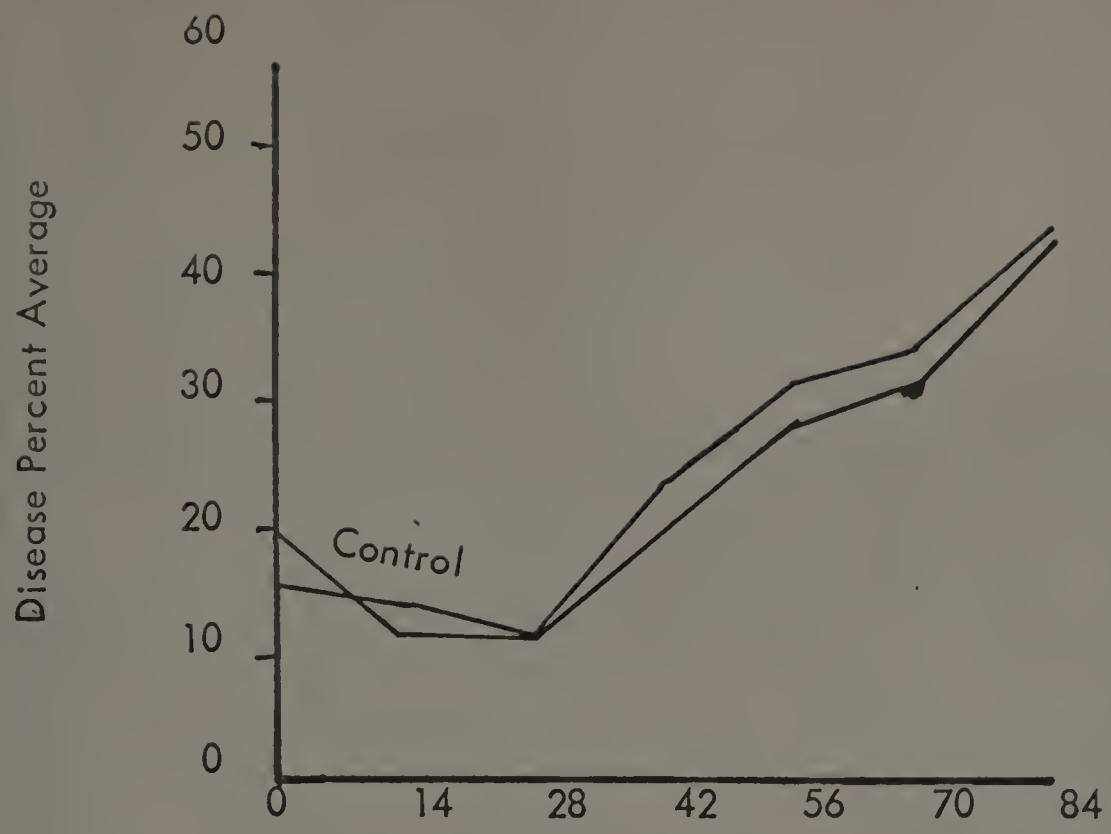

Figure 45: Influence of lobster shell 2400 lbs./acre on a Fusarium-infected turf.

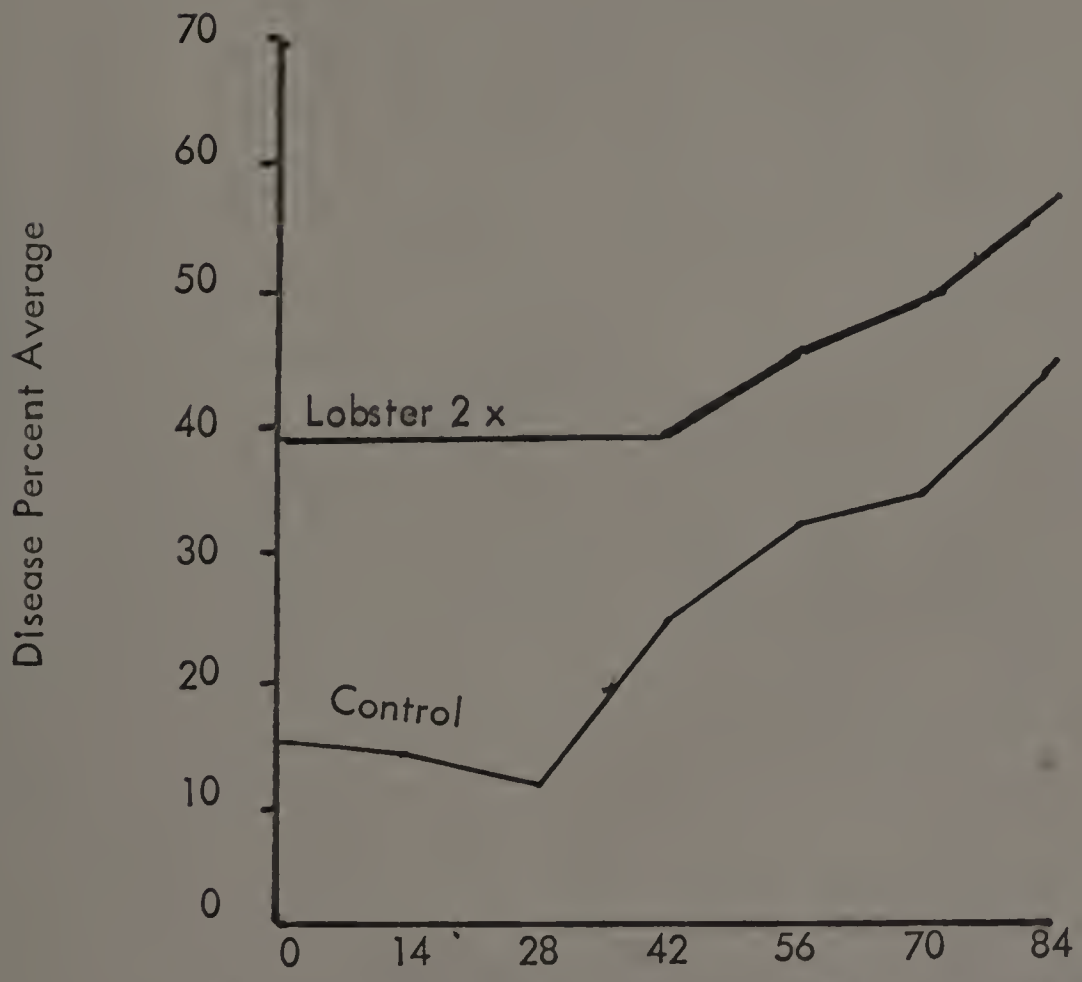

Figure 44: Influence of lobster shell 1200 lbs./acre on a Fusarium-infected turf.

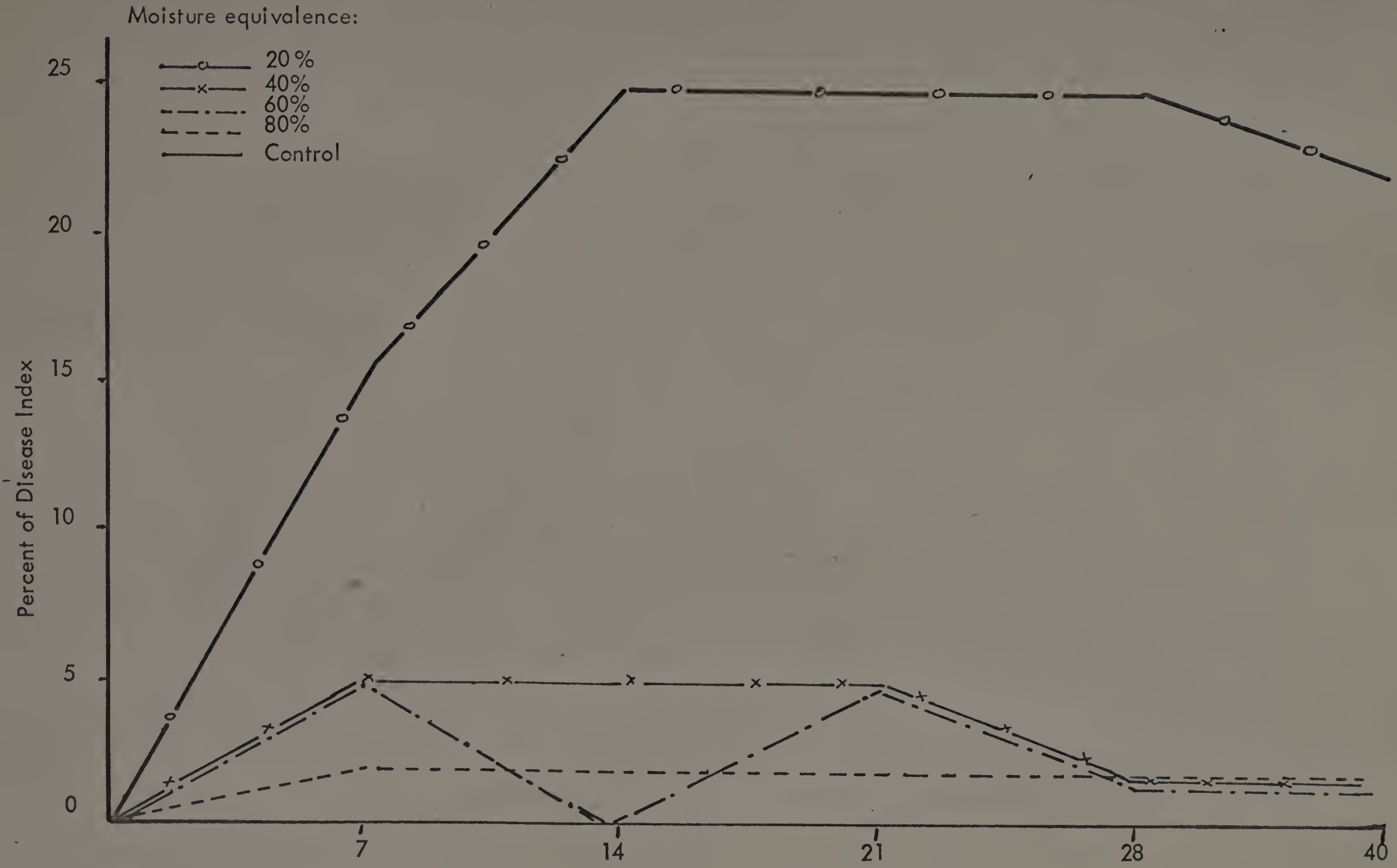

Figure 46: Effect of Soil Moisture Equivalence on Disease Incidence in Trichoderma viride Inoculated Pots.

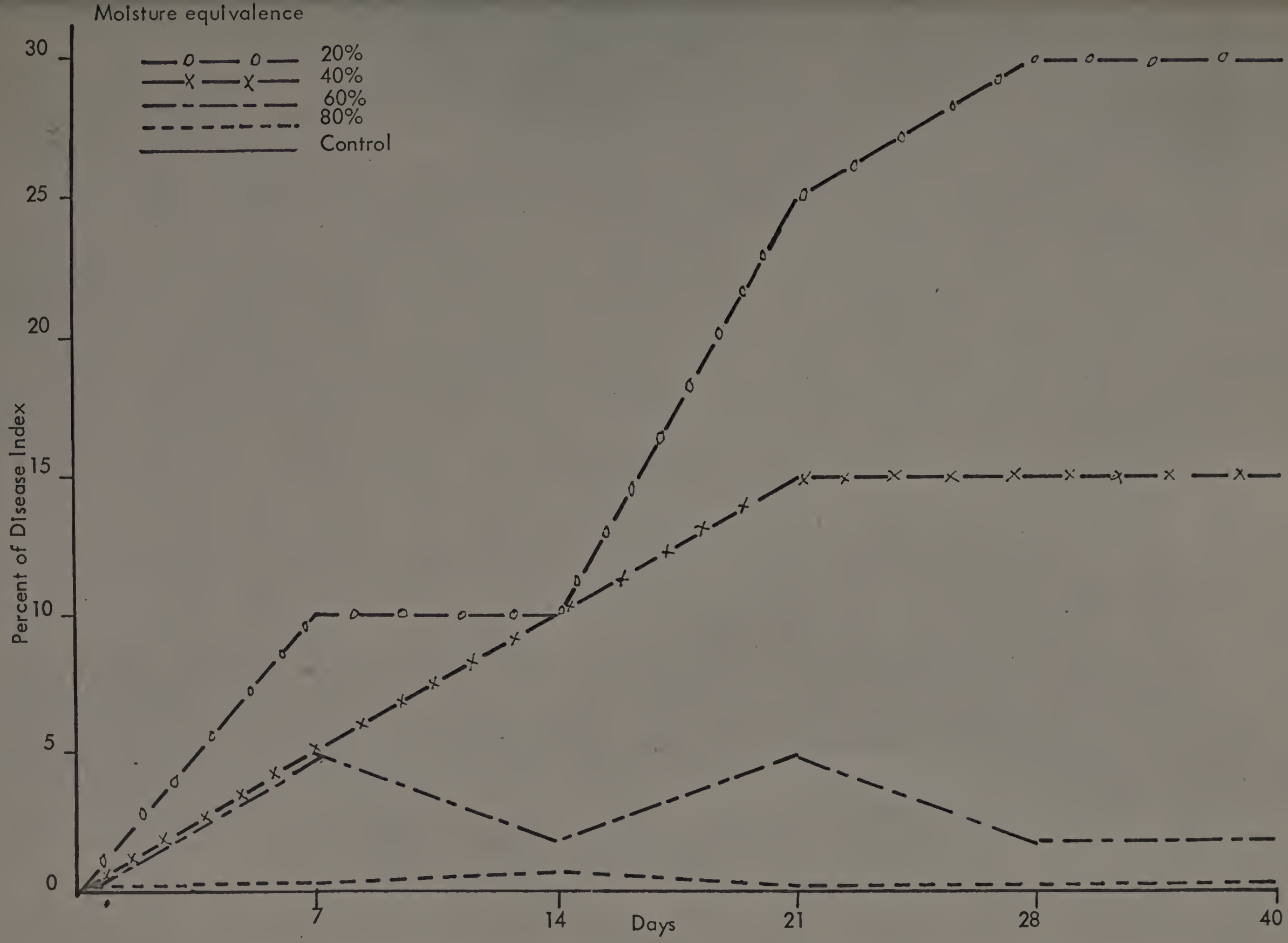

Figure 47: Influence of Soil Moisture on Disease Incidence In Pots Inoculated with Fusarium.

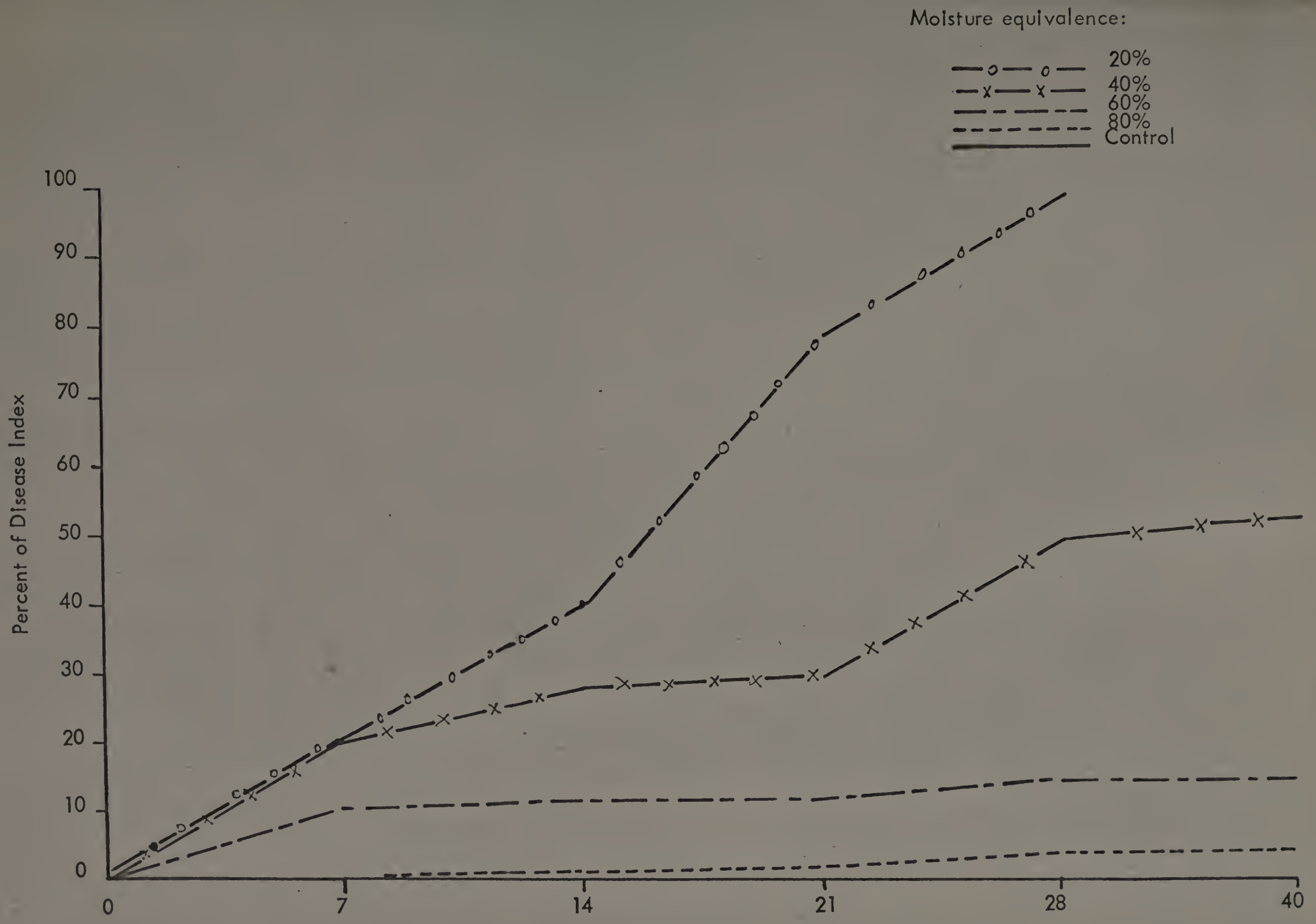

Figure 48: Influence of Soil Moisture on Disease Incidence in Pots Inoculated with Fusarium and Trichoderma.

The author wishes to express his deep gratitude to Dr. Haim B. Gunner, Chairman of the Committee, for his untiring assistance, guidance and encouragement; to Dr. Joseph Troll for guidance and encouragement throughout my undergraduate and graduate programs.

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Environmental Studies of Fusarium Blight in Merion Kentucky Bluegrass

A Thesis

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