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Effects of temperature and species on TNT injury to plants

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The studies tested the toxic effects of trinitrotoluene (TNT) to plants grown hydroponically. The first study tested the effect of temperature and TNT concentration on plant growth, and the second tested the effect of TNT on various legumes and grass. The studies showed that the tolerance to TNT is related to both the genotypic characteristics of the plant and its rate of growth. Plants growing in more optimum environments and having a greater growth rate were more tolerant to the injurious effects of TNT. TNT tolerance was greater in grasses than in legumes. The growing points of plants originatin from the crown were most tolerant to TNT injury.

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PREFACE

This report was prepared by Antonio J. Palazzo, Research Agronomist; Ronald Bailey, Biologist; and John Graham, Biological Technician, Geochemical Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory. Funding was provided by the U.S. Army Medical Research and Development Command, Ft. Detrick, Maryland.

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Effects of Temperature and Species on TNT Injury to Plants

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INTRODUCTION

The compound 2,4,6-trinitrotoluene (TNT) is used by the Department of Defense to manufacture munitions. One area of concern in this process is the effect of TNT on terrestrial plants growing where waste products are stored or where accidental spills occur. Identifying the degree of toxicity of this compound to plants is one of the initial steps in evaluating its effects in the environment.

A literature review did not locate any information about the effects of TNT on the growth of terrestrial plants (Palazzo and Leggett 1983). Studies in aquatic environments, however, have shown that discharge of ammunition wastes has a deleterious effect on downstream biological communities. Schott and Worthley (1974) found the growth of duckweed (*Lemna perpusilla*) to be depressed at TNT concentrations of 1 mg/L and above, with death occurring at 5 mg/L and above. Liu et al. (1976), Smock et al. (1976) and Won et al. (1976) found that the growth of freshwater algae was inhibited by TNT concentrations in the range of 2–15 mg/L.

In an unpublished study described by Palazzo and Leggett (1983) in their literature review, weekly applications of pink water (wastewater obtained from a TNT manufacturing plant containing about 140 mg/L of TNT) were toxic to established forage grasses and a legume grown in a greenhouse. Pink water at full, half and quarter strength was applied at a rate of 10 cm/week. Chlorotic symptoms developed and plant yields were reduced; these changes were related to the strength of the wastewater.

In a later study, Palazzo and Leggett (1986a) grew yellow nutsedge (*Cyperus esculentus* L.) in hydroponic cultures containing 0, 5, 10 and 20 mg/L of TNT. They found that the deleterious effect of TNT was rapid and occurred at solution concentrations of 5 mg/L and higher. Root growth was most affected, followed by leaf and rhizome growth. TNT and its metabolites 4-amino-2,6-dinitrotoluene (4-ADNT) and 2-amino-2,6-dinitrotoluene (2-ADNT) were found throughout the plant, with the greatest portion in the roots. No differences in growth were found between plants grown at TNT levels of 5 and 20 mg/L; therefore, it was assumed that these levels were beyond those that cause early growth inhibition in plants. To test this, a study was conducted using TNT concentrations of 0, 0.5, 2.0 and 5.0 mg/L (Palazzo and Leggett 1986b). The results showed that plant yields and rates of leaf and root elongation decreased as the level of TNT increased from 0.5 to 5.0 mg/L.

In the previous studies, plants were grown under nearly optimal conditions in a greenhouse. These conditions are seldom encountered by plants grown in the field, where conditions of light, temperature or water may restrict plant growth. These indirect stresses may accentuate the phytotoxicity of TNT.

EFFECTS OF TEMPERATURE ON TNT INJURY

Materials and methods

The purpose of this study was to assess the effects of both temperature and TNT concentration on the growth and mineral uptake of perennial ryegrass grown in hydroponic cultures.

Seeds of perennial ryegrass (*Lolium perenne* L. "Pennfine") were germinated in clean sand. Uniform seedlings approximately 5 cm tall were transferred to hydroponic cultures. The hydroponic cultures consisted of 1-L flasks that were painted black on the outside to restrict light from entering. The tops of the flasks were covered with aluminum foil, and two holes were cut in the foil. One plant was placed in each hole and supported by polyester fiber. A third hole was made for the air line.

The nutrient solution for all flasks was a modified version of that reported by Epstein (1972) and was used at half the recommended strength. The solutions had an initial pH of 6.0. The nutrients were added to distilled water that had been pumped through a Milli Q reagent water system.

A 100-mg/L stock solution of TNT was prepared according to Palazzo and Leggett (1986a) by adding 0.4 g of practical-grade TNT (containing 10% water), obtained from Eastman Kodak, to 3.5 L of distilled water. Proper amounts of the stock solution were added to the flasks to produce TNT concentrations of 0, 2, 5 and 10 mg/L. The study design was completely random, with six replications of each treatment. The solutions were monitored by high-pressure liquid chromatography (Jenkins et al. 1984) to assure that the desired TNT concentrations were maintained. After 17 days all solutions were changed.

The studies were conducted in environmental chambers set at four different day/night temperatures of 12/7, 15/10, 18/13 and 21/16°C. The environmental chambers had day and night modes of 12 hr each, with both fluorescent and incandescent lighting for the day mode. The plants were misted with distilled water twice daily to prevent wilting. Chamber temperature and relative humidity were automatically recorded each hour. Two chambers were used, so two temperature regimes were tested simultaneously.

The plants were periodically removed from the flasks and the leaves and roots were measured. Visual observations of root and leaf color, vigor and other general features were recorded. After growing in the chambers for 35 days, the plants were harvested by separating leaves and roots. Dry weights were measured by oven-drying the plant tissue at 70°C for 48 hr.

An analysis of variance, to determine significance among treatments, was run on the plant weight data. A least-significant-differences test was used to differentiate among the means for each treatment (Little and Hills 1978).

Results and discussion

Effects of temperature

Perennial ryegrass grown without TNT was significantly affected by temperature (Table 1). Leaf and root weights decreased as temperatures decreased (Fig. 1 and 2). Plants grown at the lowest temperature produced only a small amount of leaf and root growth. Leaves and roots grown in TNT-free solutions at 12/7°C weighed 81% and 84%, respectively, less than those grown at 21/16°C. The optimum temperature for the growth of perennial ryegrass leaves has been reported to be in the range of 16–24°C (Beard 1973, McWilliam 1978). While declines in leaf weights were nearly linear as temperatures declined, decreases in root growth were more dramatic. At 18/13°C, root growth was 61% less than at 21/16°C, while leaf growth was only 32% less. At the two lower temperatures, reductions in growth were similar for both plant parts.

Figure 3 shows the mean leaf/root ratios of the plants grown without TNT. The ratios were similar, ranging from 2.0:1 to 2.5:1 for all the temperature regimes except at 18/13°C,

Table 1. Mean squares for leaf and root yields of perennial ryegrass.

		Mean squares	
Source	*df*	*Leaves*	*Roots*
Temperature	3	0.197*	0.03
Error A	15	0.001	0.00
Treatment	3	0.643*	0.096*
Temperature x treatment	9	0.069*	0.016*
Error B	60	0.003	0.001

* Found to have significantly different yields at the 1% level of probability.

df = degrees of freedom

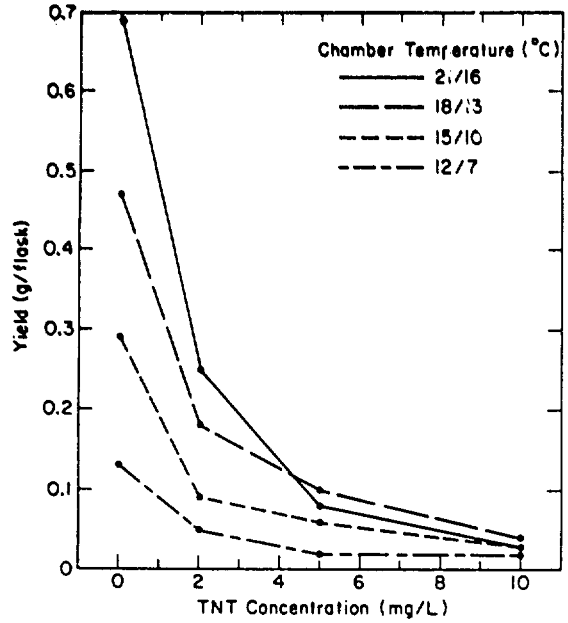

Figure 1. Effect of TNT solution concentrations and temperature on leaf yields.

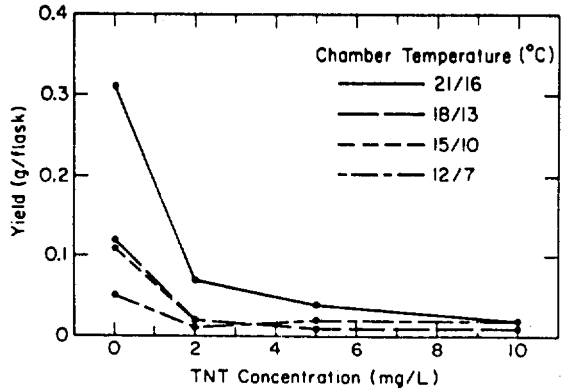

Figure 2. Effect of TNT solution concentrations and temperature on root yields.

which was nearly 4.0:1. At this temperature the ratio was higher because root yields were relatively low.

TNT and temperature interaction

There was a significant temperature–TNT interaction effect on leaf and root yields (Table 1). TNT impaired leaf growth at all four temperature regimes studied (Fig. 1). The greatest declines in leaf yields occurred between the 0- and 2- and the 2- and 5-mg/L TNT treatments. At the 5- and 10-mg/L TNT levels, yields were very low. The yield reductions for perennial ryegrass with increasing TNT concentrations are similar to those found in earlier studies with yellow nutsedge (Palazzo and Leggett 1986a,b). In those studies, yields were reduced as the TNT concentration in solution increased to 5 mg/L, but no further reductions took place as TNT levels increased to 20 mg/L. In our preliminary work (unpublished data) we found that yellow nutsedge died at a concentration of 60 mg/L of TNT.

The lowest TNT concentration of 2 mg/L had the greatest differences in leaf yields among the four temperature regimes, with very little growth at the lowest temperature (Fig. 1). TNT levels of 5 and 10 mg/L dramatically impaired plant growth regardless of the chamber temperature.

TNT had a greater effect on roots than on leaves. Of the 12 treatments containing TNT (three TNT levels at four temperatures), in only one treatment was there any significant amount of root growth (Fig. 2). This was at the lowest TNT concentration (2 mg/L) and the highest temperature (21/16°C). Root growth was inhibited in all of the other treatments.

The range in leaf and root yields was greatest when only one of the experimental variables was involved. Plants affected by only one experimental variable were considered to include those grown at any of the four temperatures without TNT or at any of the four TNT concentrations and at the optimum temperature of 21/16°C (Fig. 1 and 2).

The greatest variation in leaf/root ratios caused by the changes in TNT levels occurred at the intermediate temperatures studied (Fig. 3). At both intermediate temperatures the ratios increased as the TNT level in solution increased to 5 mg/L, but they decreased at the 10-mg/L TNT level. The highest leaf/root ratio was 10:1 at a TNT concentration of 5 mg/L and a temperature of 18/13°C. The ratios at the highest and lowest temperatures reacted similarly over the four TNT levels.

The range in leaf/root ratios was greater at the 18/13 and 15/10°C temperature regimes because the roots are more sensitive than leaves to increases in TNT and decreases in temperature. At these temperatures leaf yields did not decline as dramatically as root yields as the TNT levels increased to 5 mg/L. This

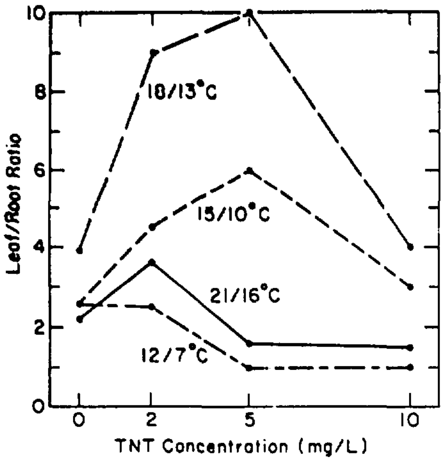

Figure 3. Effect of TNT solution concentrations and temperature on leaf/root ratios.

increased the leaf/root ratios. At the highest temperature, root yields were greater than at any other temperature, which kept the ratio low; at the lowest temperature there was very little plant growth, so there was little variation between TNT levels.

TNT influenced the rate of leaf elongation, particularly at the lower temperatures (Table 2). At the two highest temperatures the greatest differences in the daily increase in leaf length occurred between TNT concentrations of 2 and 5 mg/L. At 15/10°C the greatest difference in leaf length gains was between 0 and 2 mg/L of TNT. Therefore, at this lower temperature, leaf elongation was dramatically reduced at a low plants receiving TNT produced little or no leaf growth during the study. In contrast to leaf growth, the rate of root elongation was reduced at all TNT levels (data not shown).

Table 2. Average gains in leaf length of plants grown at various TNT concentrations and temperature regimes.

TNT concentration (mg/L)	*Growth (cm/day)*			
	21/16	*18/13*	*15/10*	*12/7°C*
0	0.28	0.34	0.23	0.05
2	0.31	0.26	0.06	<0.01
5	0.12	0.09	<0.01	<0.01
10	<0.01	0.06	<0.01	<0.01

EFFECTS OF TNT ON DIFFERENT PLANT SPECIES

Materials and methods

The objective of this study was to test the response of several plant species to the presence of TNT. Seven species, including legumes and turf and forage grasses, were grown in hydroponic cultures containing either 0 or 3 mg/L of TNT.

Seeds of Iroquois alfalfa (*Medicago sativa* L.), Pennlate orchardgrass (*Dactylis glomerata* L.), red clover (*Trifolium pratense* L.), Ram Kentucky bluegrass (*Poa pratensis* L.), Jamestown chewings fescue (*Festuca rubra* var. *commutata* Gaud.), birdsfoot trefoil (*Lotus corniculatus* L.) and Palmer perennial ryegrass (*Lolium perenne* L.) were germinated in clean sand. Uniform seedlings about 5 cm tall were selected for study and placed in hydroponic cultures.

The plants were grown in 1-L glass flasks that were painted black on the outside to restrict light. The tops of the flasks were covered with aluminum foil. Two holes were cut in the foil. One plant was placed in each and then supported by polyester fiber. A third hole was cut for the air line connecting the air pumps to the filter stones. The nutrient solution for all flasks was used at half strength and was a modified version of that reported by Epstein (1972). The solutions were modified by substituting sequestrene Fe for Fe-EDTA. All solutions were made up with distilled water.

The eight species were subjected to TNT concentrations of 0 and 3 mg/L. There were three replications of each treatment. The TNT was added to the flasks from a stock solution prepared according to Palazzo and Leggett (1986a) by adding 0.4 g of reagent-grade TNT to 3.5 L of distilled water. To maintain TNT levels, the solutions were changed after 21 and 37 days. The plants were misted with distilled water twice daily to prevent wilting during periods of low humidity. Water was added to the flasks as needed.

After 49 days of growth, the longest roots were measured and all plants were harvested by separating leaves and roots. The visible differences between plants growing in the two solutions were noted. Dry weights were determined by oven-drying the plant tissue at 70°C for 48 hours. The results were subjected to an analysis of variance, and the Duncan's Multiple Range Test was applied to the means (Little and Hills 1978).

Results and discussion

Table 3 shows the total biomass produced by the seven species. Orchardgrass and perennial ryegrass had the highest yields among the grasses, and red clover had the highest yield among the legumes. These species also had the smallest reduction in yields in their groups. This suggests that actively growing plants are more tolerant to the toxic effects of TNT. This theory is supported by the results of our temperature study, where plants growing at a greater rate due to a more optimum temperature were less affected by TNT than plants grown at a cooler, more stressful temperature. The legumes were more sensitive to TNT than the grasses, with alfalfa and birdsfoot trefoil being reduced approximately 80% in total biomass compared to the controls. Growth in the grasses was reduced by 25–42%. Although the results are statistically significant for only one grass species, growth was reduced substantially in all grasses. The lack of significance in the other three species is due to the wide variation in yields among replications.

The effect of TNT on yields of various plant parts is shown in Table 4. For the legumes, all the reduction in yields were statistically significant. Red clover was more tolerant to TNT than the other legumes. The crown tissue of red clover was the most tolerant, with reductions of only 33%. Alfalfa and birdsfoot trefoil had reductions in yields of leaves, roots and crowns of 75% or higher. The greatest effect of TNT on the grasses was for root growth, where differences were significant in three of the four species. The injurious effects of TNT on plant roots is well documented and has been observed in yellow nutsedge (*Cyperus esculentus* L.) (Palazzo and Leggett 1986a) and in perennial ryegrass in our temperature study. Orchardgrass was the only grass to have a significant loss in leaf weight. Although the result was not statistically significant, the most tolerant species in terms of leaf growth was perennial ryegrass, with a 16% reduction in yield; reductions in leaf growth for the other grasses were similar and ranged from 35% to 42%. For all grass species, the growth of crowns was the least affected, with reductions ranging from 0% to 25%.

Table 5 shows the reductions in numbers of stems on the legumes and tillers on the grasses. The reductions were significant only for perennial ryegrass and red clover, possibly because of their growth rates. Red clover produced 3–5 times more stems in the control than the other species, while perennial ryegrass produced 1.7–4.0 times more tillers. Tiller weights were also determined in two species and were found to be insignificantly reduced when grown in the 3-mg/L TNT so-

Table 3. Total yields and reductions in yields due to TNT for seven plant species.

Plant	*Total plant yield (g/flask)*		*Reduction (%)*
	Control	*TNT*	
Grasses			
Kentucky bluegrass	0.52	0.30	42
Chewings fescue	0.33	0.21	37
Perennial ryegrass	0.52	0.39	25
Orchardgrass	0.70	0.48	31*
Legumes			
Red clover	1.38	0.60	57*
Alfalfa	0.56	0.11	80*
Birdsfoot trefoil	0.59	0.11	81*

* Significant at the 5% level of probability

Table 4. Reductions in yields of various plant parts between the 0- and 3-mg/L TNT treatments.

Plant	*Reduction in yield (%)*		
	Leaves	*Crowns*	*Roots*
Grasses			
Kentucky bluegrass	42	0	58*
Chewings fescue	38	25	44*
Perennial ryegrass	16	17	56*
Orchardgrass	35*	22	40
Legumes			
Red clover	57*	33*	62*
Alfalfa	80*	75*	81*
Birdsfoot trefoil	80*	75*	80*

* Significant at the 5% level of probability.

Table 5. Reductions in number of tillers (for grasses) or stems (for legumes) between the 0- and 3-mg/L TNT treatments.

Plant	*Reduction in number of tillers or stems (%)*
Grasses	
Kentucky bluegrass	10
Chewings fescue	0
Perennial ryegrass	24*
Orchardgrass	17
Legumes	
Red clover	44*
Alfalfa	20
Birdsfoot trefoil	33

* Significant at the 5% level of probability.

lutions. For the control and treated plants, tiller weights for Kentucky bluegrass were 0.08 and 0.06 g, and for orchardgrass they were 0.13 and 0.09 g, respectively. These results agree with those found for the rhizomes (a part of the sedge plants where new growth may be produced) of yellow nutsedge reported by Palazzo and Leggett (1986a). In that study at the lowest TNT concentration of 5 mg/L, the rhizome weight was only slightly less than in the control.

The root lengths after 49 days of growth for each species and the corresponding reductions due to TNT are shown in Table 6. Plants producing the longest roots in the control solutions also produced the longest roots in the TNT solutions. Reductions in root length caused by the TNT were similar for all species, ranging from 47% to 58% of the control. Drastic reductions in root lengths have also been observed in the temperature study and in Palazzo and Leggett (1986b). Although the data are not shown here, there was good correlation (r = 0.89) between root length and root dry weight of individual treatments.

Table 6. Root lengths of the plants at harvest.

Plant	*Total root length (cm)* Control	*TNT*	*Reduction (%)*
Grasses			
Kentucky bluegrass	24	10	58*
Chewings fescue	17	9	47*
Perennial ryegrass	31	15	52*
Orchardgrass			
Legumes			
Red clover	38	18	53*
Alfalfa	25	13	48*
Birdsfoot trefoil	21	10	52*

* Significant at the 5% level of probability.

Leaf/root ratios had a narrow range of 2:1 to 5:1 (data not shown). This is in agreement with the leaf/root ratios of 3.5:1 for perennial ryegrass grown in a 2-mg/L TNT solution at a day/night temperature of 21/16°C in our temperature study.

CONCLUSIONS

Past studies have shown that TNT is injurious to plants at concentrations as low as 2 mg/L (Palazzo and Leggett 1986a,b). Roots were the most sensitive part of the plant in terms of phenotypic injury, and this was the point of accumulation of TNT and metabolites. The studies reported here tested the sensitivity of various species to TNT and the effect of temperature on their tolerance to this compound.

The rate of growth of plants is important in evaluating the extent of injury caused by TNT in solution. Faster-growing plants are more tolerant to this compound. In the species study, red clover of the legumes and orchardgrass of the grasses were the greatest yielders and among the most tolerant to TNT. In the temperature study, plants that grew faster because of a more optimum temperature were generally more tolerant to this compound.

The greatest tolerance to TNT within plants appears to be in areas where new growth is initiated, the meristematic crown tissue. This study and two using yellow nutsedge (Palazzo and Leggett 1986a,b) showed that crowns, stems, rhizomes and tillers are the least affected by TNT. In the Palazzo and Leggett studies, rhizomes were the most tolerant to TNT in solution. Since the rhizomes originate from the crown of this plant, the crowns of yellow nutsedge also must have been tolerant to this compound. Therefore, although leaf growth is hindered, the ability of the plants to produce new leaf tissue or new plants is not as sensitive. Therefore, it appears likely that plants will resume a normal leaf growth rate once the TNT is removed.

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