

FOREST RESEARCH BRANCH



ESTABLISHMENT REPORT

EFFECT OF TYROSINE ON TREE GROWTH AND RESISTANCE TO FROST

(Project Q-113)

by

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EFFECT OF TYROSINE ON TREE GROWTH AND RESISTANCE TO FROST

by J.D. Gagnon^{1/}

INTRODUCTION

Up to recently, green plants had been thought to be capable of assimilating directly only inorganic substances. However, during the last decade, many investigations have shown that, if plants do utilize primarily mineral substances, this does not imply that they cannot also utilize directly some organic substances. For instance, the work done by Flaig (1959) and by Winter (1960) in Germany has indicated that some humic molecules can play a direct role in growth besides the one they play as the basis ionic exchange. Other studies also have indicated that some organic compounds, such as amino acids and also proteins (Bradford and McLaren, 1961), can be absorbed as such by plants and play a particular metabolic role. We know, for example, after a relatively recent work (Gagnon and Bernier 1963) that tyrosine can serve as precursor in the biosynthesis of lignin which can play a specific role upon the resistance to frost by plants. More recently, results of an investigation on the effects of tyrosine on tree growth and resistance to frost (Gagnon 1964) has revealed that under controlled conditions the addition of tyrosine had a marked beneficial effect on height growth of two-year-old black spruce and increased their resistance to frost.

It is the objective of this study to appraise in the field the beneficial effect of tyrosine on tree found in greenhouse studies. Since tyrosine must be supplied to the organism with adequate nutrient in order to favour the rate of their formation (Chibnall 1939; Fisher 1948; Tambo-

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line, 1953; Kalinkevich and Udovenko 1959; Pleshkov and Fowden 1959; Glose 1960), it was found necessary to add complete fertilizers to soil to meet what is called adequate nutrient supply. In view of future study in that line leading to optimum growth at minimum expense, it was also judged preferable to vary the doses of fertilizers and tyrosine to two levels, which are believed to be roughly the minimum and maximum for that kind of experiment. To meet the practicality of the project, in one area tyrosine in solution and in powder was added to soil, and in another area tyrosine in powder was added. The former area was known as an area where trees are likely to suffer from frost.

This report summarizes the work carried out on both areas, the result obtained after one growing season, and outlines plans for 1965.

METHODS

Experimental Design

The experiment consists of a randomized block arrangement with 6 replications of 9 treatment combinations (Figure 1). In each plot, 9 white spruce seedlings (2-2 stock) were planted at 3 feet spacing. Seedlings from one ^{were} plot 6 feet distant from seedlings of the adjoining plot.

In the nursery, two experiments, one with tyrosine in solution, the other with tyrosine in powder, were established.

In the "frost pocket" area, difficult of access, only one experiment with tyrosine in powder was established.

Description of Nursery Area

a) Site Preparation:

This part of the nursery was formerly used as a garden for growing potatoes and had not yet been used as nursery. In spring 1962, the soil was ploughed down to 6 inches and, in spring 1963, the graminæa residue was incorporated in the upper layer by harrowing.

b) Soil Analysis:

Ten samples of the first 6 inch layer of soil were collected at random from the experimental area and analyzed for texture and nutrient content (Table 1).

Table 1. Results of analyses of soils from the first 6 inch layer of Valcartier nursery soils

Mechanical Analysis				Total N %	Available P ppm	Exchangeable K ppm
Sand %	Silt %	Clay %	pH			
54	43	3	5.05	.242	10	26

The soil texture appeared to be satisfactory for good development of seedlings. The pH was at proper level for conifers, the total amount of nitrogen, although above average for nursery soils, seemed to be low for satisfactory growth of 2-2 seedlings, especially if we consider the fact that when sampled soil nitrogen was at its peak due to incorporation of graminæa residue. Levels of phosphorus and potassium were quite low when compared with similar soils supporting the same size of trees.

c) Climatic Conditions:

The number of rainy and frosty days, the average rainfall from the date of planting to the end of August, also the mean minimum and maximum temperature for the same period are shown in Table 2.

Table 2. Climatic data obtained in the vicinity of the experimental area

Period	Number of rainy days	Number of frosty days	Total prec. inches	Mean min. T. (°F.)	Mean max. T (°F.)	Monthly mean (°F.)
June*	4	0	1.27	49.1	75.8	62.4
July	14	0	4.26	55.7	76.5	66.1
August	18	0	6.72	50.1	67.8	58.9

* Data compiled from June 10, date of planting.

Attention must be drawn to the fact that planting took place during a very dry period. The period from June 6 to 18 was rainless and, therefore, water held by soil particles certainly was at a minimum.

Description of Frost Pocket

a) Site Preparation:

Before scarification the soil was covered with a continuous carpet of polytrichum sp. moss with an overstory of spiraea sp. (Figure 2). In spring 1962 the soil was scarified: a pulverizer equipped with its own motor pulled by a carterpillar 6 (Figure 3) was used to till the top 5 to 6 inches resulting in a loose mixture of fine detritus of polytrichum and spiraea and top mineral soil (Figure 4). This machine, which can scarify and pulverize at the same time completed its work over the three acre area in less than two hours.

b) Soil Analysis:

One year after scarification and pulverization ten soil samples collected at random in the first 6 inch layer of soil were analyzed for texture and nutrient content (Table 3).

Table 3. Results of analyses of soils from the first 6 inch layer of the frost pocket

Mechanical Analysis				Total N %	Available P ppm	Exchangeable K ppm
Sand %	Silt %	Clay %	pH			
54	41	5	4.61	.315	6	84

The soil texture is about the same as in the nursery and is typical of Valcartier formerly cultivated soils. Greater acidity, greater content of nitrogen and potassium than in the nursery soils are due to the influence of lesser vegetation of which pulverization has accentuated their

decomposition resulting after one year in a temporary increase in the pH and amount of nitrogen and potassium. In fact, before scarification the pH of the soil was found to be 5.01, and the potassium content 11 parts per million. At the level of 6 parts per million, available phosphorus in soil is considered to be low.

c) Climatic Conditions:

The same climatic data recorded for the nursery have been recorded in the frost pocket and are shown in Table 4. The frost pocket being located within 3 miles of the Valcartier Forest Experiment Station where precipitation is recorded, it was not found necessary to install a raingauge on the experimental area. However, temperature records shown in the table are from thermographs installed in the experimental area.

Table 4. Climatic data obtained in the frost pocket experimental area

Period	Number of rainy days	Number of frosty days	Total prec. inches	Mean min. T. (°F.)	Mean max. T. (°F.)	Monthly mean (°F.)
June *	4	2	1.27	41.1	75.0	58.2
July	14	0	4.26	49.3	73.9	61.6
August	18	5	6.72	42.5	65.0	53.7

* Data compiled from June 17, date of apparatus installation.

Planting took place on June 11.

Planting

Planting holes were dug with the Saguenay planting tool and local 2-2 white spruce seedlings of about similar size were planted according to the disposition shown in Figure 1.

Application of Treatments

NPK treatments and tyrosine in powder were incorporated into the first inch of top soil and within a 6 inch radius around each tree.

Tyrosine in solution was also added to the soil within a 6 inch radius around each tree. Addition of tyrosine in solution was made 3 times a week at the rate of 5 oz. each time, from June 11, time of planting, to August 30, end of growing season. Trees in the control plots were irrigated with an equal quantity of water.

RESULTS AND DISCUSSION

When dealing with height growth response to treatments applied on a newly established plantation more conclusive results can be expected after the end of the second growing season where young trees have overcome the planting shock, and where stored food reserves laid down in the previous year have been absorbed by trees. However there are cases where current growth factors play a capital role so as to influence current growth as this experiment demonstrates.

At this stage of the experiment it is not intended to discuss thoroughly the results obtained during the first growing season of the plantation, but to rather bring the attention to those of special interest. These are presented in two sections. The first one deals with mortality in the nursery and in the frost pocket, the second with the effect of treatments on growth in both places.

1. Mortality

a) Nursery:

Whether tyrosine was added to the soil in solution or in powder, the mortality in the nursery was less than 1%. The cause for mortality could not be determined. Considering the fact that planting

took place during a very dry period and rain fell only 8 days after planting, it appears that in sandy loam soils, near the Boreal Region, white spruce seedlings can endure severe drought for a long period before being affected. That constataion raises this question: "To what extent must soil moisture be depleted before it seriously affects coniferous tree growth?"

b) Frost Pocket:

Less than 2 % of the trees planted in the frost pocket died during the growing season. As in the nursery, the mortality could not be related to treatments. Periodic observations were made, especially days after frost had occurred and mortality could not be attributable to frost. It must be said that the 2 days of frost recorded after planting in June were not severe frosty days since the temperature was equal to, or slightly below, 32°F and this for only one hour at 4 a.m. It is generally admitted that frost occurring in spring, just after buds open, are more disastrous than late frost. Therefore the 5 days of frost recorded in August when most growth is completed (Gagnon 1963) would not have had much effect on the newly established plantation.

Comparison of mean minimum temperatures shown in Tables 2 and 4 clearly indicates that the choice of the frost pocket to test our hypothesis that tyrosine increases resistance to frost was judicious and conclusive results may be expected next spring.

2. Effect of NPK and Tyrosine Treatments on Growth

In the expectation of obtaining more accurate results when calculating growth responses, it was found desirable to use terminal growth expressed as a percentage of height based on size of seedlings when planted.

a) Nursery:

While growth could not be related to NPK treatments, it was statistically significant when tyrosine in powder was added to the soil. However, the addition of tyrosine in solution had not produced noticeable growth (Table 5).

Table 5. Variance ratios for growth attributable to tyrosine in powder and tyrosine in solution; nursery.

Source of variation	D.F.	Variance ratio		Snedecor's "F" values	
		Tyrosine in powder	Tyrosine in solution	p=.05	p=.01
Replications	5	1.97	4.54	2.45	3.51
Treatments	8				
NPK	2	1.95	2.55	3.23	5.18
Tyrosine	2	<u>8.73</u>	1.31	3.23	5.18
NPK x Tyrosine interaction	4	.48	.78	2.61	3.83
Error	40				
Total	53				

The significance of the tyrosine in powder on growth implies that different levels of tyrosine produced significantly different effects. The relative efficiency of various levels of tyrosine in powder was determined by the "t" test and the critical difference between two treatment means revealed that the response was less pronounced with higher level of tyrosine in powder, thus indicating limitation in the quantitative use of tyrosine as a growth stimulant. Similar results were obtained in greenhouse studies.

Lack of significant growth response to addition of tyrosine in solution prompted us to study the top:root ratio in that block (Table 6).

Table 6. Variance ratios for top:root ratios attributable to tyrosine in solution.

Source of variation	D.F.	Sum of squares	Variance ratio	Snedecor's "F" values	
				p = .05	p = .01
Replications	5	25.27	9.90	2.45	3.51
Treatments	8	21.78	5.28	2.18	2.99
NPK	2	2.20	2.15	3.23	5.18
Tyrosine	2	16.43	<u>16.12</u>	3.23	5.18
NPK x Tyrosine interaction	4	3.15	1.55	2.61	3.83
Error	40	20.36			
Total	53	67.41			

The relative efficiency of various levels of tyrosine in solution to produce different top:root ratios was determined by the "t" test and the same results as for growth were obtained: the addition of .1 gm produced greater top:root ratio than the addition of .5 gm.

b) Frost Pocket:

In the frost pocket area none of the treatments produced any significant growth response. However the response to addition of

tyrosine in powder was more pronounced than the addition of NPK (Table 7).

Table 7. Variance ratios for growth attributable to treatments; frost pocket.

Source of variation	D.F.	Sum of squares	Variance ratio	Snedecor's "F" values	
				p = .05	p = .01
Replications	5	2673	1.57	2.45	3.51
Treatments	8	1979	.72	2.18	2.99
NPK	2	459	.67	3.23	5.18
Tyrosine	2	734	1.08	3.23	5.18
NPK x Tyrosine interaction	4	786	.58	2.61	3.83
Error	40	13632			
Total	53	18284			

On account of current adverse temperatures prevailing in the area (Table 4), pronounced growth response to treatments was not expected this year. Furthermore, summer frost occurring in August is, according to Pomerleau and Ray (1957), disastrous for seedlings and 5 days with frost were recorded in the frost pocket. It must be remembered that this area was selected especially to test the effect of tyrosine on resistance to frost, and growth data have been recorded only for comparison with nursery trials.

SUMMARY

Two areas in the Valcartier Experimental Area were selected to verify in the field the results obtained from greenhouse tests with tyrosine. One area was located in a nursery with an ultimate aim to study the effect of tyrosine on growth; the other was located in a frost pocket and the aim was to test if tyrosine really increased resistance to frost. In both areas, 2-2 white spruce seedlings were planted in June 1963 after site preparation.

In the nursery, two experiments were established; one with tyrosine in solution, the other with tyrosine in powder. In the frost pocket, only one experiment was established and tyrosine in powder was added to soil. In each condition, the experiment consisted of a randomized block arrangement with 6 replications of 9 treatment combinations.

At the end of the first growing season, less than 1% mortality was recorded in the nursery and less than 2% mortality in the frost pocket. Significant growth response to tyrosine in powder was obtained in the nursery. Tyrosine in solution had a significant effect on top:root ratios but had no noticeable effect on growth. In the frost pocket the addition of tyrosine in powder produced no significant changes in growth.

More conclusive results can be expected after the end of the second growing season when young trees have overcome the planting shock, and where stored food reserves drawn from the site really reflects the conditions of the site.

As mentioned in the project plan, seedling dry weight, shoot: root dry weight ratio and current growth will be determined next year.

REFERENCES

- Bradfute, O., and A.D. McLaren. 1961. Uptake of enzyme molecules by plant roots. *Information techniques de microbiologie du sol*. Institut Pasteur. Vol. 1, août 1961.
- Chibnall, A.C. 1939. *Protein metabolism in the plant*. Yale Univ. Press. New Haven, Conn.
- Close, R. 1960. Free amino-acids of some fungi. *Nature, Lond.* 185, (4713) 609-610.
- Fisher, A. 1948. Amino-acid metabolism of tissue cells in vitro. *Bioch. Jour.* 43:491-497.
- Flaig, W. 1959. Soil organic matter. Mimeo: notes of conferences given at Ames, Iowa. 1959.
- Gagnon, J.D. 1963. Weekly radial increment of balsam fir in Quebec as related to McIntock's tree classification. *For. Chron.* 39: 318-321.
- Gagnon, J.D., and B. Bernier. 1963. Acides aminés libres de quelques plantes forestières. *Nat. Canad.* 40:177-192.
- Gagnon, J.D. 1964. Does tyrosine increase tree growth and resistance to frost? (Mimeo 63-Q-27).
- Kalinkevich, A.F., and G.V. Udovenko. 1959. The question of the effect of nutrient conditions on the amino-acid content of plants. *Dokl. Akad. Nauk.*, 126: 684-687. *Apud Soils & Fert.*, 22: Abst. No. 2330.
- Pleshkov, B.P., and L. Fowden. 1959. Amino-acid composition of the proteins of barley leaves in relation to the mineral nutrition and age of plants. *Nature, Lond.*, 183:1445-1446. May 1959.
- Pomeroy, R., and R.G. Ray. 1957. Occurrence and effects of summer frost in a conifer plantation. *Can. Dept. of Northern Affairs and National Resources, For. Res. Div. Ottawa, Tech. Note* 51.
- Tamboline, R.F. 1953. Nitrogen and vitamin requirements of pseudomonas hydra-
phila. *Canad. J. Bot.* 31:206-211.
- Winter, A.G. 1960. Allelopathie als stoffwanderung und stoffumwandlung. *Der. Deut. Bot. Ges.* 73:365-379.

NPK_0 & T_0 = zero application

NPK_1 & T_1 = 100 lbs/acre NPK & Tyrosine (.1 gm or .001M)

NPK_2 & T_2 = 200 lbs/acre " " (.5 gm or .005M)

1. NPK_0 & T_0

2. NPK_0 & T_1

3. NPK_0 & T_2

4. NPK_1 & T_0

5. NPK_1 & T_1

6. NPK_1 & T_2

7. NPK_2 & T_0

8. NPK_2 & T_1

9. NPK_2 & T_2

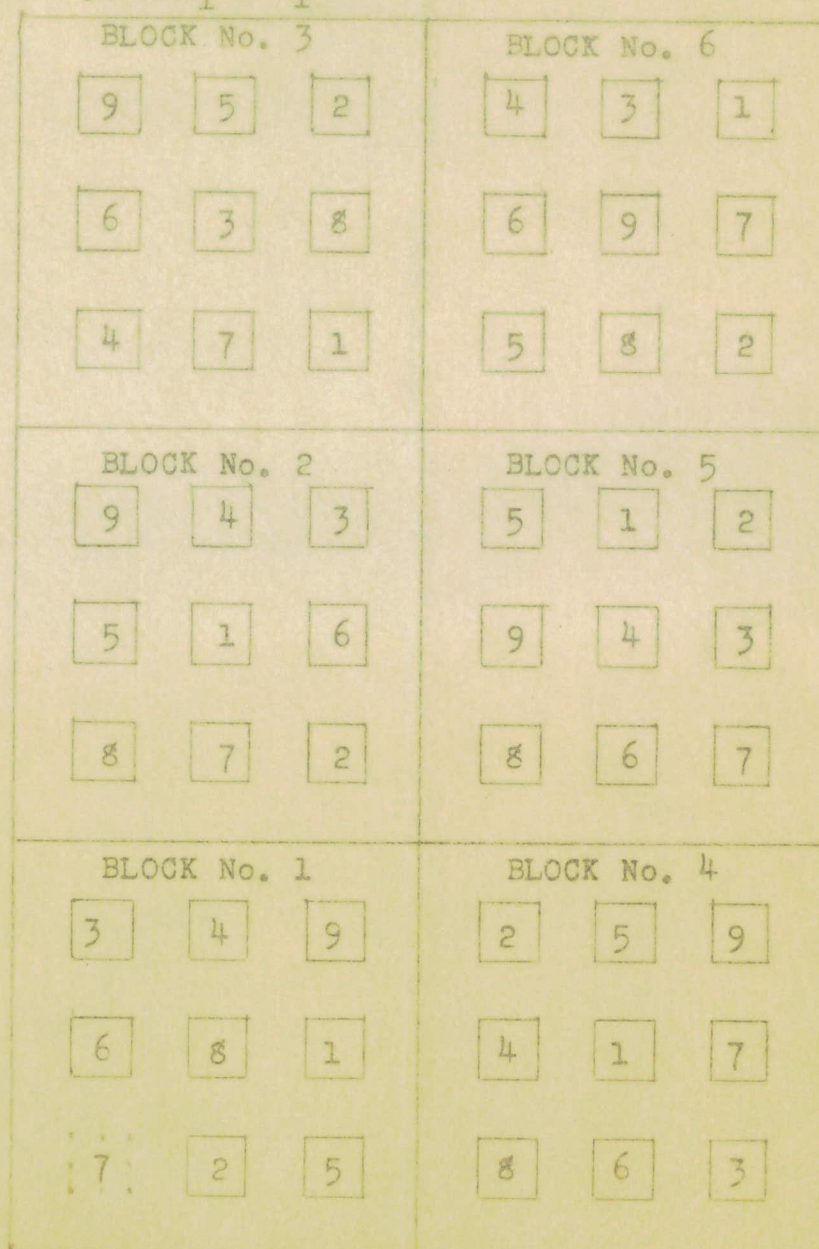


Figure 1. Experimental layout



Figure 2. Before scarification the soil was covered with spires sp. (frost pocket)



Figure 3. The soil being scarified down to 6 inches.
(frost pocket)



Figure 4. After scarification, fine detritus of polytrichum moss and spiraea shrub were mixed with top mineral soil. (frost pocket)

