THE EFFECTS OF PEAT AND SAWDUST MULCHES AND THEIR LEACHATES ON THE GROWTH AND CERTAIN METABOLIC REACTIONS OF THE HIGHBUSH BLUEBERRY (Vaccinium corymbosum L. var Coville)

BY

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ABSTRACT

Greenhouse, field and laboratory experiments were conducted to ascertain some growth and metabolic responses of the Coville blueberry variety, to sawdust and peat mulches, and the leachates of these mulches.

From the greenhouse experiments it would appear that in some manner the mulches in question increased the metabolic activities of the plants. The increased activity was reflected by increased growth of the mulched plants, a higher ash content and a higher total nitrogen content of the leaves.

A greater concentration of free amino acids occurred in the unmulched plants than in the mulched ones. This indicates that the nitrogen metabolic activities were hastened by the mulching.

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INTRODUCTION

Experiments in blueberry culture were initiated by F.V. Coville in 1906. He selected and crossed wild species of high and low bush form and from 1921 to 1939 produced 18 named varieties. In 1949 these 18 varieties constituted the entire commercial acreage of highbush blueberries in America with the exception of Rubel, a variety selected from the wild by Miss E.C. White of Whitesborg, New Jersey.

At his death in 1937 Dr. Coville left many thousands of seedlings which have given rise to further superior varieties.

Today there is a total of about 4,000 acres of the cultivated highbush blueberry in the United States. In Canada the industry is small but is rapidly expanding in the Maritimes, Ontario, and British Columbia. There are now some 800 acres of blueberries planted in the lower mainland coastal area of British Columbia.

Because of the availability of large acreages in British Columbia potentially suitable for blueberry production, together with a brisk and increasing demand for the fruit, it was felt that a contribution in the field of blueberry nutrition would be of value in fostering this neophyte B.C. Industry.

In certain blueberry growing areas the cultivated blueberry appears to suffer from various nutritional disorders. Poor growth has been attributed to such factors as magnesium, iron and

manganese deficiencies, unfavourable pH and nitrogen relationships. A number of remedies have been tried with varying degrees of success. On mineral soils the use of mulching materials has in certain cases, stimulated growth and improved the health and vigour of the plants.

It was decided to investigate the effect of peat and sawdust mulches on the growth and vigour of the blueberry. Furthermore, as an indication of the nutritional status of the plants it was considered adviseable to ascertain the effect of the mulches on the moisture content, total mineral content (ash) and total nitrogen content of the leaves. It was also felt that a study of the free amino-acids in the leaves would be helpful in understanding the metabolic activity of the plant.

REVIEW OF LITERATURE

Some Effects of Mulches on Blueberries.

A comparison of the effect of sawdust and straw mulches with a buckwheat cover crop and clean cultivation was made by Shutak and Christopher (46) in New Jersey using the Pioneer variety of blueberry. They found that consistently higher yields were produced by bushes mulched with sawdust as contrasted to clean cultivated plots which produced the lowest yields. The sawdust mulch had very little effect on the pH of the soil but soil temperatures were lower in spring and summer and higher in the fall under the mulch. A larger and more fibrous root system developed under the sawdust mulch than with the other treatments. These workers also reported that weed control was greatly simplified by the use of the sawdust mulches.

Chandler (18) reported that under conditions in Maine, the soil mulched with sawdust under blueberry plantings, retained more moisture and maintained lower temperatures than in unmulched plantings. The mulch increased growth of plants in clay and loam soils, but on the other hand reduced growth of plants growing in sandy soils.

In Georgia, Savage (45) found that looser forms of mulches such as rye, straw, and oak leaves were better than clean cultivation, but not as good as sawdust.

Kramer (33) in Maryland reported increased yields of both high and dryland blueberries were obtained with various mulches

although mulching decreased survival of the dryland blueberries.

Mulching with sawdust in Connecticut gave greater growth and better yields than either clean cultivation or hay mulch according to Griggs and Rollins (26). The different types of soil management however did not affect the moisture content or the ascorbic acid content of the berries.

Several workers (2, 23, 47, 48) have reported that fresh sawdust depressed available soil nitrogen, but that rotted sawdust was an excellent soil addition.

Johnston (29) found that sawdust alone used as a surface mulch depressed nitrates in the soil slightly for the first year and further depressed it for a second year but when the sawdust was incorporated with the soil the nitrates were depressed for 18 months after which time they accumulated.

According to Turk (47), in Michigan an average sawdust contains 4 lbs. of nitrogen, 2 lbs. phosphorous pentoxide and 4 lbs. of potash per ton of dry material. This is about one-third the amount of nutrients in the same weight of wheat straw.

Comparing the effects of clean cultivation, sawdust mulch, oak leaf mulch, and rye straw mulch on blueberry plants, Savage and Darrow (45) found the plants from mulched plots were superior to those from clean cultivated plots and that sawdust was superior to either oak leaves or rye straw. On the basis of plants surviving at the end of the third year the sawdust mulch was superior to rye straw

and oak leaf mulch. The plants in the clean cultivated plots were so small that, commercially their productive capacity was regarded as valueless.

Anderson (1) reported that the addition of straw and cellulose depressed available nitrogen in the soil. Turk and Partridge (47) found that nitrification was slower in mulched than in unmulched soils, but due to leaching losses in the unmulched plots, the total nitrate accumulation was the same in all plots at the end of his experiment.

Shutak (46) found that sawdust mulches increased the water holding capacity of the soil in blueberry plots but that the increase was not as great as when straw mulch was used.

Turk (47) reported that the effect of sawdust is largely physical, resulting in a better water holding capacity, decreased soil losses due to erosion and the prevention of a hard crust on the soil surface limiting the capillary movements of water.

Johnston (30) found that a surface mulch of sawdust increased the soil moisture and that the soil temperature was more uniform under the mulch.

In order to eliminate any effects of the increased moisture holding capacity of the soil due to the mulch Harris (28) provided uniform moisture in all plots with irrigation and found that a sawdust mulch markedly increased the yields of strawberries in a sandy soil compared to that of unmulched plants.

Blueberry Nutrition.

Doehlert and Shive (22) working with both sand cultures and field plot experiments to determine the nutritional needs of the cultivated blueberry, (Vaccinium corymbosum), reported that the best nutrient solutions for the blueberry were low in phosphorus and high in nitrogen. Nitrate nitrogen appeared to be of greater value than ammonium nitrogen for this particular species. Good agreement was obtained between the results found in the sand cultures and those found in the field plots. The blueberry was shown to be sensitive to deficiency of boron and manganese. Cain (16) reported that ammonium nitrogen was superior to nitrate nitrogen for blueberries in contrast to the results found by Doehlert and Shive (22). Plants which received nitrate nitrogen showed iron deficiency symptoms more readily than those which received ammonium nitrate although the iron content of the leaves in both cases was similar. He concluded that ammonium nitrogen was in some way associated with iron nutrition.

Perlmutter and Darrow (40) under the conditions of their experiment, did not obtain any benefit from applications of various forms of nitrogen fertilizers.

They also concluded that soil pH may not necessarily be a direct controlling factor in iron absorption because plants growing on soils of relatively high pH often contained as much iron in their foliage as those growing at a lower pH.

In a study of a chlorotic condition of blueberry leaves, Mikkelsen and Loth (37) found that green leaves contained 350 ppm.

of soluble magnesium as compared with 30 ppm in the chlorotic leaves.

McHargue (36) reported that manganese is concerned in nitrogen assimilation and in the synthesis of proteins and that it functions as a catalyst in plant metabolism and also functions with iron in the synthesis of chlorophyll.

Importance of pH in Blueberry Culture.

A number of investigators (8,19,27, and 30) have stressed the importance of soil acidity for blueberry growth, suggesting a range of pH of 4.0 to 5.2 above or below which blueberries may not be expected to survive. Boller (13), however claimed that soil structure, aeration, organic matter and water are the most important factors in blueberry culture and that if these conditions are satisfactory, plantings could be maintained over a wide pH range.

Cain (17) concurred with Boller and reported successful plantings on soils above pH 6.0 in the field and at pH 6.5 in nutrient solutions when an adequate nutrient supply was maintained. He considered that soil pH was not necessarily a direct controlling factor in iron absorption because plants growing on soils of relatively high pH often contained as much iron in their foliage as those growing at a lower one.

Spraying the foliage with soluble iron salts was recommended by Blasberg (10) to correct chlorosis associated with high pH soils, but only as a temporary corrective measure because the chlorosis usually reappeared in new growth made after the spray application.

Bailey and Everson (6) corrected chlorosis with soil applications of ammonium sulphate or aluminum sulphate. T hey found soluble iron available in the soil as a result of their treatments.

Beckwith (7) suggested that ammonium sulphate gave better growth of blueberry plants in soils above pH 5.5 and Doehlert (21) recommended its use in connection with lime on commercial blueberry plantings.

Johnston and Ware (31) performed experiments using sawdust as mulch; and they reported that differences in pH of soils mulched with sawdust and not mulched were small.

Manganese in Blueberry Nutrition.

McHargue (36) reported that manganese is concerned in nitrogen assimilation and in the synthesis of proteins; and that it functions as a catalyst in plant metabolism and also functions with iron in the synthesis of chlorophyll. He considered also that since low manganese affects the production of dry matter, the indications are that it has some effect upon carbon assimilation.

Typical symptoms of manganese deficiency in plants are:- the terminal bud remains alive and leaves do not wilt; chlorosis is present with spots of necrotic tissue scattered over the leaf; the small veins tend to remain green giving the leaves a checkered or reticulated effect (36).

On the other hand manganese excess is typified initially by a yellowing of the leaves accompanied by slight upward curling of the upper leaves. The chlorosis continues until the newer

leaves are almost white. The leaves then curl down and the midribs often darken and break down. Large necrotic areas finally develop in the chlorotic leaves and this is accompanied by the breakdown and death of the stem (36).

Magnesium in Blueberry Nutrition.

Mikkelsen and Loth (37) established the fact that the soluble magnesium contents of green and chlorotic leaves were different. Normal green leaves contained 350 ppm of magnesium as compared with 30 ppm. in the chlorotic leaves. Soil samples collected near affected plants contained only a trace of available magnesium.

Kramer and Schrader (33) studied the effects of mineral deficiencies on the growth of the Cabot blueberry in sand cultures. They described magnesium deficiency as a marginal leaf chlorosis of the basal leaves extending intra-veinally with green midrib. The late stages of this deficiency are characterized by the chlorotic areas becoming red. This characterization of magnesium deficiency has been observed in the field on the Jersey, June, Cabot, and Stanley varieties in New Jersey, except that necrosis has been rare.

Very few data are available on the rates of soil application of magnesic fertilizers necessary to correct magnesium deficiency. Mikkelsen and Doehlert (37) reported that magnesium deficiency in blueberries was corrected by applying magnesic materials to the soil. Fall applications of 70 to 300 pounds of MgO per acre supplied as Epsom salts and hydrated dolomitic lime, respectively, corrected the deficiency during the following season. Magnesic fertilization increased the magnesium content of the leaves and elevated

the milliequivalent cation composition of them, while phosphorus, iron and manganese composition was relatively unaffected.

The foregoing indicates that there is disagreement in the value of mulches used in blueberry culture and that the evidence conflicts as to the various causes of poor growth.

The following is a review of chromatography methods used for the detection of free amino-acids.

Qualitative Paper Partition Chromatography.

Consden, Gordon, and Martin (20) have shown that good separation of amino-acids can be obtained on filter paper by allowing a suitable solvent, which has previously been saturated with water to flow over the paper in a closed container, the air in which was saturated with the vapours of water and the solvent.

Filter paper contains 20-25 per cent water under these conditions, and separation depends upon the differences in partition coefficient of the amino-acids between the stationary water phase and the moving solvent. It has further been shown that for any individual amino-acid the value:

$$Rf = \frac{\text{distance moved by the amino-acid}}{\text{distance moved by the advancing front of liquid}}$$

is directly related to the partition coefficient, true absorption by the cellulose playing little part.

Qualitative Analysis of Amino-acids.

In an experiment on the qualitative micro-analytical technique for amino-acids, Consden, Gordon and Martin (20) reported on:

1. Rate of diffusion of Amino-acids in various solvents.

A number of solvents have been tried and abandoned. Of these ethyl acetate, methylethyl ketone, aniline, cyclohexanol, cyclohexane, quinoline and a light aniline fraction b.p. 174-180 were unsatisfactory as the amino-acids ran too slowly, whereas with methyl acetate, acetone, sec-butanol, pyridine, the picolines and the lutidines, the bands of amino-acids either moved too fast or were unduly broadened. Hydroxy-amino-acids move more slowly than the corresponding amino-acids in phenol, but in collidine the rates are similar.

Ammonia selectively slows the movement of aspartic and glutamic acids and hastens that of basic amino-acids. Acid has the reverse effect.

2. Additions of Acids Bases and Salt Hydrolysates, or mixtures in which the ratio of soluble inorganic salts of amino-acid were high, gave unsatisfactory chromatograms. The salt absorbed water from the atmosphere and the solvent and caused local water-logging of the paper. The amino-acids were not readily washed from these regions and the resulting bands were grossly distorted. This salt effect was eliminated by impregnating the paper with salt and using the solvent and atmosphere equilibrated with saturated salt solution instead of with water.

Micro Estimation of Amino-nitrogen.

Pope and Stevens (43) described a method for determining amino and peptide nitrogen. Woiwood (50) successfully adapted this method to the determination of micro amounts (1 - 25 ugm) of α-amino-nitrogen. The essential points of the method were that after

the amino-acid was allowed to react with copper phosphate, the soluble copper complex in the filtrate was decomposed by means of sodium-dithio-carbamate, the resultant characteristic golden colour was extracted in amyl alcohol and the amount of copper determined absorptiometrically. The theoretical value of 0.44 for the ratio of α - amino-nitrogen to copper in the complex A_2Cu was not obtained under the experimental conditions used, it was essential to construct a standard curve for each individual amino-acid.

Such curves were reproducible, and over their linear portion (10 - 25 ugm, α - amino-nitrogen) the amino-acids methionine, hydroxyproline, aspartic acid, leucine, isoleucine, histidine, phenylalanine, valine, glutamic acid, tryptophane and tyrosine gave ratios lying between 0.50 and 0.55; alanine, glycine, threonine, serine, cystine and cysteine ratios between 0.55 and 0.80; and lysine, arginine and glucosamine ratios between 12.5 and 1.6.

Quantitative Paper Chromatography.

Quantitative Application of Paper Chromatography to Amino-Acids.

One of the two major problems in developing a method for quantitative determination of amino-acids by paper partition chromatography is the measurement of the amino-acids after they have been chromatographed. A number of procedures have been proposed which include colorimetric and polarographic methods as well as the use of photoelectric densitometer.

Polson et al (42) were among the first to report a

quantitative procedure for determining amino-acids after separation by paper partition chromatography. The technique included the development of a colour with amino compounds and ninhydrin on the paper, the extraction of the colour with acetone and the measurement of the colour. Aside from the fact that the acetone is a poor solvent for the coloured product, these authors apparently found other disadvantages to this procedure because it was abandoned for one in which comparisons were made with standard amounts of amino-acids chromatographed simultaneously with unknowns.

This technique has the disadvantage that it requires a large number of standards with each sample, or set of samples, and it depends on a subjective test which can only be approximately quantitative.

Several subsequent methods have utilized one-directional chromatography, formed a colour with ninhydrin, and measured the colour by the density or size of coloured areas. Fisher et al (24) measured the area of the spot formed by the ninhydrin reaction, and found that the size was proportional to the logarithm of the concentration of the amino-acid. The concentration of an amino-acid in a sample can then be determined by comparison with standards run simultaneously. One technique they used was a photographic method for determining a spot size.

Bull et al (15) determined the light transmission of the coloured compound on the paper at different points along the spot

and in the direction of phenol movement. By integrating the area under the curve relating colour density and distance, an estimate of quantity was made. Block (12) used a similar method except that the amino-acids were first separated into acidic, basic, and neutral amino-acids on ion exchange resins before chromatography. In the case of two-directional chromatograms, Block (12) determined the quantity of amino-acid by multiplying the maximum colour density of the area of the spot.

Block (12) has developed this method further in order to obtain the percentage composition of an amino-acid mixture. The method is more adapted to relative rather than absolute values. The maximum intensity of a given spot was found to give an estimate of the quantity of each amino-acid. The maximum colour density of the spot obtained for each amino-acid was determined at the same molar concentration.

The ratio of the colour density for a particular amino-acid to the sum of the colour densities of all the amino-acids constitute a "standard colour ratio for the amino-acid in question". An "experimental colour ratio" for each amino-acid is determined in the same way in a sample of the mixture to be analysed.

The "experimental colour ratio" divided by the "standard colour ratio" gives the molar ratio of each amino-acid in the mixture. Analysis of casein by the empirical method agreed closely with published values although a large number of chromatograms (11)

were necessary to achieve this.

Rockland and Dunn (44) used a densitometric method for glycine and alanine. The amino-acids were separated on one dimensional chromatograms and the colour produced by ninhydrin. By measuring the density of an area larger than the biggest spot it was possible to measure the density due to amino-acid by difference.

In the analysis of amino-acids in urine by chromatography, Berry and Cain (9) utilized visual comparisons of spots. Owing, however, to the effect on the chromatography of water-soluble materials in the urine, the standards with which comparisons were to be made were prepared by adding known amounts of amino-acid urine. Although the authors claim 10 to 15 percent accuracy of their methods, the recovery of added acid varied over as wide a range as 63 to 160 percent.

A novel method somewhat analogous to that of Bull et al (15) was introduced by Keston et al (32). The amino-acids were made to react with a compound containing radio-active iodine and the resulting compounds were chromatographed in n-pentanol saturated with 2N ammonium hydroxide. The radioactivity was measured in the direction of solvent movement, and specific compounds were located and estimated by the local intensity of radioactivity. The methods cited to this point have, in general, estimated the amino-acids directly on the paper. These methods have the disadvantage that most utilize only one-directional separation of amino-acid which

is often inadequate. If two-directional chromatography is used, large numbers of chromatograms must be made in order to obtain satisfactory data. This is probably due to insufficient control of the variable involved in the chromatography. Other methods locate amino-acids on the paper, then remove the amino-acid from the paper and complete the quantitative estimation in vitro.

Martin and Mittelman (35) rejected both Folin's colour reaction and the ninhydrin reaction for the quantitative determination of amino-acids after separation by paper partition chromatography. Micro-kjeldahl procedure and titration of the amino-acids in glacial acetic acid were also tried and found wanting. They finally dissolved the amino-acids from the paper, mixed them with insoluble copper phosphate and determined, polarographically, the copper which dissolved by forming a complex with the amino-acids. Woiwood (50) used this same general procedure except that he measured the copper colorimetrically. In both these procedures, one-directional chromatograms only were used and the amino-acids must be located by simultaneous chromatography of known amino-acids. Woiwood(50) amplified his earlier work to give a more detailed procedure and has also extended it to include two-directional chromatography. He used the copper phosphate procedure to test the ninhydrin reaction, but came to some misleading conclusions by the use of too dilute a solution of ninhydrin and by failing to recognize that the reaction does not proceed once the paper is dry.

Naftalin (39) and Awapora (4) located the amino-acids on

the paper by reaction with ninhydrin and then completed the colour development in a test tube.

The method of Awapora (4) was modified by Landria and Axitapara (5). This method consisted of the following.

Amino-acids were located on the paper with ninhydrin, the amino-acid spot was cut out and the reaction was completed by heating in a test tube with Moore and Stein's (48) ninhydrin solution which contained stannous chloride. Although this method gave high colour production, it has been utilized only with unidirectional chromatograms and gave erratic results, possibly because this ninhydrin solution reacted with ammonia. Fowden and Penny (25) have utilized an analogous procedure except that the amino-acids were located by their flourescence in the ultraviolet light after heating the paper to 90 to 100° Centigrade. They found that there were losses of amino-acids unless the solvents were removed by washing with ether before treatment.

MATERIALS AND METHODS

MATERIALS

Field Experiments

The University 5-year blueberry planting consisted of 18 varieties with 5 plants of each variety replicated 9 times in randomized blocks. One third of the plants were mulched with a 2-inch layer of peat, another third with a 2-inch layer of sawdust, and

the remaining third was clean cultivated. All plots received 4 - 10 - 10 fertilizer at the rate of 1000 pounds per acre. Included in the 18 varieties was the variety Coville. It was intended to use this variety for leaf and fruit analyses.

Unfortunately all plants in the field experiments were dug up and removed by a University decree in order to make 'Parking Lots'; thus depriving the writer of the use of these plants to complete this phase of the investigation.

Greenhouse Experiments

One year old rooted cuttings of highbush blueberry plants var Coville - rooted in peat - were taken directly from the propagating bed. They were selected for uniformity of size of both top and root system.

The tops consisted of 2 to 3 shoots of 20 to 25 cm. in length. The roots were washed carefully to remove as much of the rooting medium as possible and the plants were set in 10 inch clay pots containing quartz sand, one plant to each pot. The experiment was conducted in one section of the University Greenhouses.

On October 26, 1958, one hundred and five plants were set up and arranged in 3 blocks; each block consisted of 35 plants. Each block was further divided into 7 plots (5 plants per plot), and labelled A, B, C, D, E, F, and G.

A quantity of mixed sawdust (fir and hemlock) and a similar amount of peat was used as a mulch and another portion was treated

at 22 psi. for one hour. Portions of the treated and untreated peat and sawdust were placed in different 10 gallon earthenware crocks containing water and allowed to stand for several days; and these leachates were used for watering the plants.

The experiment was set up in the following manner:

Plots	Treatments
A	Peat steam treated leachate.
B	Control - Knop's solution.
C	Two inches of sawdust were applied to the surface of the pots as a mulch.
D	Sawdust steam-treated leachate.
E	Peat untreated leachate.
F	Sawdust untreated leachate.
G	Two inches of peat were applied to the surfaces of the pots as a mulch.

Weekly applications of 500 c.c. of Knop's nutrient solution were applied to the surface of each pot. Besides the weekly feedings of Knop's solution the mulched and control pots were kept moist with distilled water; to the other pots; the various leachates were added replacing the distilled water. In November, 1958, and August, 1959, samples of percolates from each pot were taken and their pH values were determined with a Beckman pH meter.

In August 1959, the accumulative height of each plant was again taken and recorded.

In order to determine the effects of peat and sawdust

mulches on the dry weight, total mineral content (ash), total nitrogen and amino-acid content of blueberry leaves, samples of the control series (B), the sawdust mulched series (C) and the peat mulched series (G), were taken at random from the plots in each of the three blocks. Each sample was divided into two parts and duplicate analyses were made on each part.

Moisture content was determined by weighing samples to a constant weight after drying in an electric oven at 70 degrees F.

Ash determinations were made according to the standard method using a muffle furnace as set out in the A.O.A.C. (3).

Total nitrogen was determined by the Kjeldahl method (3).

After trying a number of the methods indicated in the review of literature, amino-acids were eventually determined by a one-dimensional method of paper chromatography as advocated by Dent, Stepe and Steward (29) with slight modifications. Several other workers (20, 34, 41, 38) recommend this method in preference to other methods used in similar investigations. Twenty-five grams of the fresh leaf material was placed in a porcelain mortar and ground with well washed quartz sand to a homogeneous thin paste. Ten c.c. of 80 per cent ethyl alcohol was added slowly to the sample during the grinding process. This thin paste was then placed into centrifuge tubes and centrifuged for 10 minutes at the rate of 500 revolutions per minute. The samples were removed from the centrifuge and the supernatent liquid was decanted into clean test tubes. This liquid was decanted into clean test tubes. This liquid was evaporated

to dryness, under reduced pressure, without the use of heat. To the residue was added 0.1 c.c. of distilled water.

An 18" x 22" sheet of Whatman no. 1 filter paper was placed on a large sheet of brown paper on the top of the work bench, care being taken to protect the filter paper from any contact with the hands or with the laboratory bench. A pencil line was drawn about 3 $\frac{1}{2}$" from one end. Along this line short perpendicular lines at 2" intervals were drawn. At these intervals, using micro-pipettes, 10 micro-litres of the solutions for analysis were applied, drop by drop, allowing each drop to dry before the successive one was added; so that a spot of about one centimetre in diameter was obtained.

As well as the solutions from the plants of the control, sawdust mulched and peat mulched plots, 0.1 M solutions of alanine, asparagine, proline, aspartic acid, glutamic acid and tryptophane were spotted. These amino-acids were selected because preliminary trials with several of the methods outlined in the review of literature had indicated that they were present in appreciable amounts.

The spots were allowed to dry and the sheets of filter paper were placed in a chromatocab.

A phenol-water solvent consisting of 80 gm. phenol + 20 c.c. water was applied to the trough and the chromatograms were allowed to run for about 20 hours. After this time the chromatograms were removed from the chromatocab and allowed to dry by hanging in a fume hood and the Rf values were recorded.

The chromatograms were then sprayed with 0.5% ninhydrin solution (1, 2, 3 - triketohydrindene hydrate) in isopropyl alcohol; and incubated at 80^{0} C to develop the characteristic amino-acid colour. They were then photographed.

RESULTS OF EXPERIMENTS

It was observed that the control plants at the end of the experiments were much smaller than those in the plots that were mulched or treated with the various leachates. Also the control plants showed a greater tendency to die back starting from the tips, than any of the treated plants. These results agree with the findings of Savage and Darrow (45).

The leaf surface area of control plants was much smaller than that of the treated plants.

Leaves of plants in the control plots were of a light green colour while the leaves of the treated plants were a dark green colour.

TABLE I

The Effect of Treatments on the pH of the percolates after passing through the growing medium.

Blocks						Plots and Treatment							
Nov. 1958	Aug. 1959	Nov. 1958	Aug. 1959	Nov. 1958	Aug. 1959	Nov. 1958	Aug. 1959	Nov. 1958	Aug. 1959	Nov. 1958	Aug. 1959	Nov. 1958	Aug. 1959
5.8	5.8	5.8	5.8	5.8	5.8	5.9	5.8	5.8	5.8	5.7	5.8	5.9	5.8
5.8	5.9	5.8	5.9	6.0	5.9	5.8	5.9	5.8	5.7	5.8	5.8	5.7	5.7
5.9	5.8	5.9	5.8	5.8	5.7	5.9	5.7	5.8	5.7	5.8	5.9	5.8	5.7

Table I indicates that the plants throughout were feeding on a nutrient solution of approximately constant pH.

TABLE II

The effect of peat and sawdust mulches, steam-treated and untreated leachates on the accumulative growth of blueberry plants (length of shoot measured in centimetres).

TREATMENTS

	PEAT			SAWDUST			CONTROL
	Mulch	Leachate		Mulch	Leachate		
	G	Steam Treated A	Untreated E	C	Steam Treated D	Untreated F	B
Block 1							
Final Growth	781	516	356	680	430	394	345
Original Growth	52	49	74	58	54	58	55
Increase in Growth	729	467	282	632	376	336	290
Block II							
Final Growth	867	537	418	605	552	552	334
Original Growth	85	61	65	66	57	69	67
Increase in Growth	782	476	353	539	495	483	267
Block III							
Final Growth	802	534	479	748	462	351	474
Original Growth	65	58	62	78	60	34	60
Increase in Growth	737	476	417	670	402	287	414

TABLE III

STATISTICAL ANALYSIS OF INCREASE IN SHOOT GROWTH
(in Centimetres)

Blocks	Plots and Treatments							Total Blocks
	G	C	A	D	E	F	B	
1	729	632	467	376	282	336	290	3112
11	782	539	476	495	353	483	267	3395
111	737	670	476	402	417	267	414	3383
Treatment Totals	2248	1841	1419	1273	1052	1086	971	9890

ANALYSIS OF VARIANCE

Factor	S.S.	D.F.	M.S.	F.	0.05	Required F 0.01
Total	508877	20				
Treatments	444219	6	74036.5	15.5	3.00	4.82
Blocks	7318	2	3649.0	.79	3.89	6.93
Error	57340	12	4778.3			

In table III the calculated value of F for the treatments exceeds the required value of F.01 so that there are highly significant differences among treatments.

Table III also shows that the calculated value of F for the blocks is lower than the required value of F.05; so that there are no significant differences among blocks.

TABLE III A

Treatments and accumulative mean shoot growth arranged in Descending order

G	Peat mulch	749.3	Centimetres
C	Sawdust Mulch	603.6	"
A	Steam treated peat leachate	473.0	"
D	Steam treated sawdust leachate	426.3	"
Untreated F	Sawdust leachate	362.0	"
Untreated E	Peat leachate	350.7	"
B	Control	323.7	"

$$LSD._{05} = 2.179 \sqrt{\frac{2 \times 4778.3}{3}}$$

$$= 2.179 \times 56.421 = 122.9$$

$$LSD._{01} = 3.055 \times 56.421 = 172.3$$

Any treatment with an accumulative mean shoot growth greater than 446.6 centimetres is significantly greater than the control ($P = 0.05$). The above data show that the treatments with peat mulch, sawdust mulch, and steam-treated peat leachate significantly increased growth.

Only peat and sawdust mulched plants showed a highly significant increase in growth over the control plants at the level of $P. = 0.01$.

The peat mulch showed the greatest increase in growth of all treatments.

TABLE IV

The effect of sawdust and peat mulches on the average moisture, ash and total nitrogen content of the leaves of blueberries. (figures represent averages of 12 determinations.)

TREATMENT	MOISTURE	ASH	TOTAL NITROGEN
	%	% F.W.	% F.W.
Control (Unmulched)	60.87	1.33	0.4005
Sawdust (Mulched)	60.43	1.51**	0.4320**
Peat (Mulched)	60.53	1.525**	0.4299**

** - significantly greater than the control at the level of P = 0.01. (see appendix)

Table IV shows that the moisture content of the leaves was not affected by the treatments. Ash and total nitrogen were significantly higher in the leaves of the plants from both the sawdust and peat mulched pots than in the leaves of the plants from the unmulched pots. There was no significant difference between the ash and total nitrogen content of the leaves from the sawdust mulched and peat mulched plants.

TABLE V

The Rf values and ninhydrin colour of amino-acids found in the leaves of unmulched, sawdust mulched and peat mulched plants compared with standard values of certain amino-acids.

Amino Acids	Colour	Standards	Rf Values Unmulched	Sawdust Mulched	Peat Mulched
Aspartic acid	Blue	.05	--	--	--
Glutamic acid	Purple	.17	.20	.21	.20
Asparagine	Brown-purple	.33	.31	.32	.31
Proline	Yellow	.41	.40	.41	.40
Tyrosine	Blue	.48	.47	.46	.47
Alanine	Purple	.54	.52	.53	.52
Tryptophane	Brown-purple	.71	--	--	--
Phenylalanine	Blue	.83	.84	.83	.84

Table V shows that at the time of sampling the leaves of the unmulched, sawdust mulched and peat mulched plants had in the free state amino-acids, alanine, glutamic acid, proline, tyrosine, asparagine and phenylalanine.

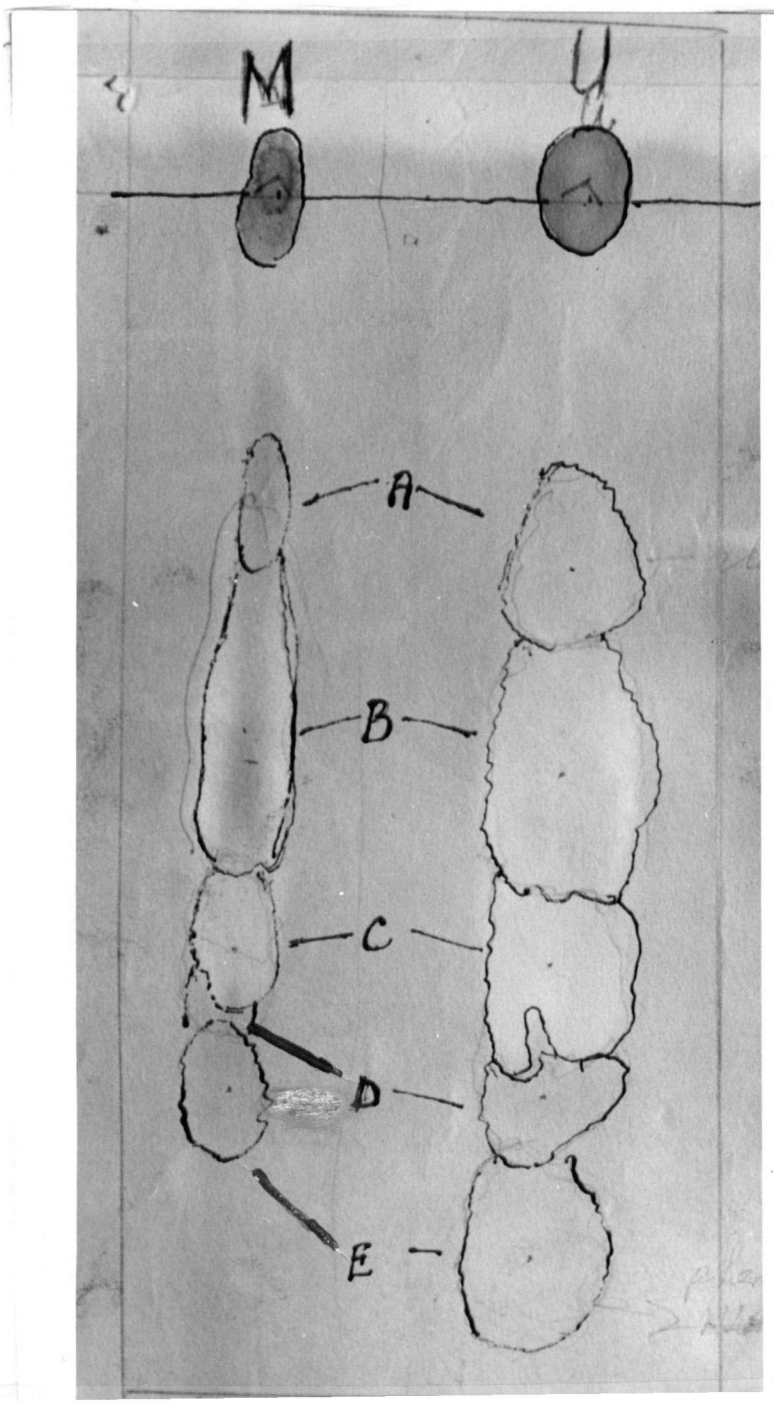
Although equal volumes of the samples were spotted, the amino-acids present in the unmulched plants covered a much larger area of the chromatogram than those present in the sawdust or peat mulched plants. This indicates that the amino-acids in the unmulched plants were present in much greater amounts than in the mulched plants.

Photograph of a typical chromatogram comparing amino-acids in samples from leaves of the unmulched and peat mulched plants is shown in Fig. I.

FIGURE I

M -- Extracts from peat mulched plants.

U -- Extracts from unmulched plants.



Amino-Acids

A - Glutamic Acid

B - Asparagine

C - Proline

D - Tyrosine

E - Alanine

TABLE VI

A relative evaluation of the nitrogen fraction found in the leaves of unmulched, peat mulched and sawdust mulched plants in the form of amino-acids

Amino-Acids	Unmulched	Mulched	
		Sawdust	Peat
Glutamic acid	++++	+++	+++
Alanine	++++	+++	+++
Asparagine	++++++	++++	+++
Proline	+++++	+++	++
Tyrosine	+++	+	+
Phenylalanine	++++++	++	++
Average total	+++++	+++	++

Table VI indicates that the greatest accumulation of amino-acids was found in the leaves of the unmulched plants, despite the fact that the greatest total amount of nitrogen was present in the leaves of the mulched plants (table IV).

Discussion of Results

The beneficial effects of the peat and sawdust mulches were pronounced.

Under field conditions these benefits have been attributed to factors such as conservation of soil moisture, the maintenance of a more uniform soil temperature, and the provision of a more suitable pH of the growing medium. However, under the uniform greenhouse conditions where watering was adequately provided at all times, and the temperature remained fairly constant, these factors were not limiting.

It would appear that in some manner, the mulches in question increased the metabolic activities of the plants. The increased activity was reflected by increased growth of the mulched plants, a higher ash content, and a higher total nitrogen content of the leaves.

The pH values of the nutrient media ranged within the limits of pH 5.7 - 6.0 for all treatments including the control. The poor growth of the control then, cannot be attributed to an unfavourable pH. Some workers (8, 19, 27, 30) have suggested that a range of pH 4.0 - 5.2 is optimum for blueberries, and above or below this pH range, blueberries may bot be expected to survive. The present work does not support this contention, but rather, that blueberry plants may thrive equally well at the higher pH ranges of pH 5.7 - 6.0 provided that an adequate nutrient supply is maintained. This is in agreement with Boller (14) and Cain (17).

The plants mulched with peat gave a better growth response than those mulched with sawdust. It could be assumed that the beneficial effect of the peat was due to a lower C/N ratio in the peat than in the sawdust. The sawdust would then tend to depress available nitrogen

in soil. However, in this experiment nitrogen was added in adequate amounts in all cases. The presence of growth hormone substances in the peat and not present, or present in lesser amounts, in the sawdust is a possibility, but this was not investigated.

Plants mulched with sawdust showed a much greater growth than the control plants, or those to which leachates of peat or sawdust had been applied. This beneficial effect of sawdust mulch on blueberries agrees with the findings of Savage and Darrow (45).

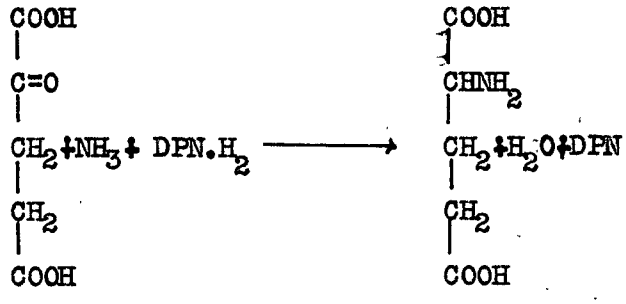
Plants to which steam treated leachates were applied grew better than those supplied with untreated leachates. It is possible that the steam treatment softened the cell walls of the peat and sawdust and thus allowed for a more efficient extraction of the water soluble nutrients present in the mulches, and so increased certain nutrient concentrations available to the plant. Further work on the water soluble nutrients of peat and sawdust at various stages of decomposition, treated and untreated with steam, is needed before a clear picture of the factors contributing to the benefits of steam treatment can be formulated.

The leaves of the mulched plants contained a higher percentage of total nitrogen but a lower concentration of amino-acids than the leaves of the unmulched plants. This may indicate that the amino-acids in the mulched plants were combining more readily than in the unmulched plants to form proteins.

Six amino-acids were identified. Of these, two, viz. glutamic acid and alanine are known to play important roles in protein and carbohydrate metabolism.

Glutamic acid is probably synthesized in plant cells by a

reaction between ammonia, often originating from the reduction of nitrates, and α-ketoglutaric acid, as follows:



α- Ketoglutaric acid Glutamic Acid.

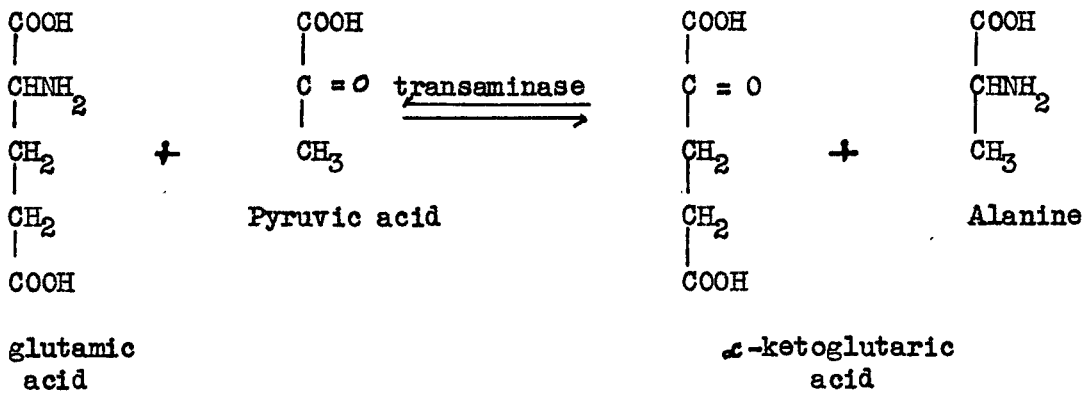
This reaction is catalyzed by the enzyme dehydrogenase, with diphosphopyredine mucleotide (DPN) as the coenzyme. This enzyme is widely and probably universally prosent in plants. Although details of this reaction have been worked out only in animal tissues (von Eiler et al), there is little doubt that this reaction also occurs in plant tissues. This type of reaction is known as reductive amination.

The other amino-acid of great significance found present in the samples was alanine. This acid may have been formed from pyruvic acid by reactions analogous to those stated above.

A close interrelation exists between the synthesis of certain amino-acids, organic acid metabolism, and the process of aerobic respiration. The reversible reactions involving the conversion of α-ketoglutaric acid to glutamic acid, alanine to pyruvic acid, and other analogous reactions which may occur can be considered as side reactions of the tricarboxylic acid cycle.

There is considerable evidence that some of the amino-acids may arise as a result of transmination reactions in which amino groups

are transferred from one kind of a molecule to another. The following is an example of such reaction:



Reactions of this kind are catalyzed by an enzyme of the type called a transaminase. Such enzymes are widely distributed in higher plants.

Due to the above reactions glutamic acid and alanine may be converted respectively to α-ketoglutaric acid and pyruvic acid and here enter in the tricarboxylic acid cycle in carbohydrate metabolism.

Thus it may be assumed that these two amino-acids were reacting more rapidly in the mulched plants than in the unmulched plants; and thereby this may account for their reduced amounts in the mulched plants.

This reaction, whereby these amino-acids enter the TCA cycle in carbohydrate metabolizm, may also be correlated with the higher percentage in dry weight of leaves in plants from the mulched plots. Assuming that the amino-acids, glutamic acid, and alanine transaminate and deaminate to form α-ketoglutaric acid and pyruvic

acid and thereby enter the tricarboxylic acid cycle, they would cause an increase in the respiration rate and subsequently protein synthesis, and finally increase the rate of growth.

The better utilization of nitrogen in the metabolic processes of the blueberry as a result of mulching is of especial interest and warrants further investigation as to the mechanism involved.

CONCLUSIONS

From the results obtained, it may be concluded that blueberry plants grow at a faster rate when mulched with sawdust or peat than when unmulched. Free amino-acids are used up much faster in the mulched plants than in the unmulched ones; resulting in a higher protein content in the mulched plants than in the unmulched plants.

The application of mulches in blueberry culture on mineral soils can contribute to increased yields. Although only 6 amino-acids were recognized, this does not indicate that there are only 6 free amino-acids present in the blueberry plant. At the time of sampling translocation of amino-acids to various meristems may have occurred.

The application of peat or sawdust mulches to blueberry growing on mineral soils would appear to be a sound cultural practice.

SUMMARY

Experiments involving the use of Coville blueberry plants were conducted in the field, greenhouses, and laboratories of the Division of Plant Science at the University of British Columbia to determine the effects of various mulches and their leachates on the growth and certain metabolic responsis of highbush blueberry plants.

The results observed were as follows:

(1) Plants which had 2" of sawdust or peat applied as a mulch showed a much greater increase in growth than the other treatments.

(2) The greatest increase in growth was obtained from plants which were mulched with peat.

(3) The least increase in growth was obtained from the control plants, i.e. those which received water and nutrient solution.

(4) Steam treated sawdust leachate, untreated sawdust leachate, and untreated peat leachate caused an increase in growth but this was not significant.

(5) Plants to which the steam treated peat leachate was applied showed a significant increase in growth over the control plants.

(6) There was little variation in pH between various treatments throughout the experiment. The pH ranged from 5.7 to 6.0.

(7) Free amino-acids phenylalanine, proline, alanine, tyrosine, glutamic acid and asparagine were found in both mulched and unmulched plants.

(8) A much greater concentration of free amino-acids occurred in the leaves of unmulched plants than in those of the mulched plants.

(9) The percentage of oven dried weight and ash content of leaves from the mulched plants was higher than in leaves from the unmulched plants.

(10) The percentage of crude protein in leaves from the mulched plants was higher than in leaves from the unmulched plants.

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APPENDIX I

Analysis of the effect of sawdust and peat mulches on the moisture content of the leaves of blueberry plants (each figure for the samples represents the average of 2 determinations).

B = Control, C = Sawdust mulched plants, G = Peat mulched plants.

SAMPLES	TREATMENTS B	C	G	TOTALS
1	60.82	60.81	60.00	181.63
2	60.85	60.84	60.00	181.69
3	60.85	60.83	60.03	181.71
4	60.91	60.90	60.11	181.92
5	60.92	60.94	60.05	181.91
6	60.92	60.91	60.14	181.97
TREATMENT TOTALS	365.27	365.23	360.33	1090.83

Total S.S. = $60.82^2 + 60.85^2 + \cdots 60.05^2 + 60.14^2 - 1090.83^2 = 3.29$

Between Treatments S.S. = $\frac{365.27^2 + 365.23^2 + 360.33^2}{6} - \frac{1090.83^2}{18} = 2.71$

Error S.S. = 3.29-2.71 = .58

Analysis of Variance

Factor	S.S.	D.F.	M.S.	F.	Required F 0.05	0.01
Total	3.29	17				
Between Treatments	2.71	2	1.355	3.57	3.68	6.36
Error	.58	15	.38			

$LSD_{.05}$ = 2.131 .38 x 6 x 2 = 4.62

APPENDIX II

Analysis of the effect of sawdust and peat mulches on the total mineral (ash) content of the leaves of blueberry plants. (Each figure for the samples represents the average of 2 determinations).

B = Control, C = Sawdust mulched plants, G = Peat mulched plants

SAMPLES	TREATMENTS			TOTALS
	B	C	G	
1	1.29	1.53	1.52	4.34
2	1.28	1.51	1.52	4.31
3	1.28	1.54	1.52	4.34
4	1.37	1.50	1.53	4.40
5	1.36	1.55	1.53	4.44
6	1.38	1.50	1.54	4.42
TREATMENT TOTALS	7.96	9.13	9.16	26.25

Total S.S. $= 1.29^2 + 1.28^2 + 1.28^2 \cdots 1.53^2 + 1.53^2 + 1.54^2 - 26.25 = .17$

Between treatments S.S. $= \frac{7.96^2 + 9.13^2 + 9.16^2}{6} - \frac{26.25^2}{18} = .16$

Error S.S. $= .17 - .16 = .01$

Analysis of Variance

Factor	S.S.	D.F.	M.S.	F	Required F	
					0.05	0.01
Total	.17	17				
Between Treatments	.16	2	.08	114	3.68	6.36
Error	.01	15	.0007			

$LSD_{.05} = 2.131 \quad .0007x6x2 = .192$

APPENDIX III

Analysis of the effect of sawdust and peat mulches on the total nitrogen content of the leaves of blueberry plants. (Each figure for the samples represents the average of 2 determinations).

B = Control, C = Sawdust mulched plants, G = Peat mulched plants.

SAMPLES	TREATMENTS			TOTALS
	B	C	G	
1	.4005	.4389	.4340	1.2734
2	.4005	.4382	.4340	1.2727
3	.4006	.4390	.4338	1.2734
4	.4006	.4212	.4300	1.2518
5	.4007	.4211	.4301	1.2519
6	.4007	.4210	.4302	1.2516
TREATMENT TOTALS	2.4033	2.5794	2.5921	7.5748

$$\text{Total S.S.} = .4005^2 + .4005^2 + \cdots .4301^2 + .4302^2 - \frac{7.5748^2}{18} = .0041$$

$$\text{Between Treatments S.S.} = \frac{2.4033^2 + 2.5794^2 + 2.5921^2}{6} - \frac{7.5748^2}{18} = .0037$$

$$\text{Error S.S.} = .0041 - .0037 = .0004$$

Analysis of Variance

Factor	S.S.	D.F.	M.S.	F	Required t	
					.05	.01
Total	.0041	17				
Between treatments	.0037	2	.00185	61.67	3.68	6.36
Error	.0004	15	.00003			

$$LSD_{.05} = 2.131 \quad .0003x6x2 = .12786$$