MINERAL NUTRITION

OF

THE BLUEBERRY.

By

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INTRODUCTION

The blueberry is a native of America, and various species range over the entire continent (58). Much interest for commercial planting has been shown in the highbush, or swamp blueberry (Vaccinium corymbosum L.), and the lowbush species (V. pallidum and V. angustifolium)have drawn some attention as soil conserving crops, and as sources of supplemental farm income. However, it was only comparatively recently that the blueberry plant has assumed an important place in cultivated acreage, largely as a result of the lifelong efforts of Coville (56), who developed, through breeding and selection, a number of named, large fruited horticultural varieties of the swamp blueberry.

Ecologically, the swamp blueberry is unique as a cultivated plant, and as such, has been surrounded with mystery, particularly as to its soil requirements. The frequent failures in attempts at its cultivation, as well as the mystery, have been due in large measure to lack of basic knowledge of its normal requirements, which in some respects may be fundamentally different from those of most cultivated plants. It was thought possible through this study, by adding to the fundamental knowledge of blueberry requirements, to diffuse some of the mystery. Upon such basic data, intelligent farming practices may be recommended which would improve the chances for the successful blueberry culture under a wide range of conditions, both intensively, and for purposes of soil erosion control.

In approaching the problem, it was thought that a study of mineral deficiencies would be valuable, since both wild and cultivated blueberry plants often exhibit certain symptoms which, in the absence of a pathogen, appear to be caused in part at least by some soil nutrient deficiency. If under controlled conditions of sand culture in the greenhouse, such symptoms could be easily defined and ascribed to the lack of single mineral nutrients, then the diagnosis and cure of these deficiencies in the field would be greatly facilitated. When it soon became apparent that nitrogen was the element required in the largest quantities, and its deficiency was by far the first to appear, a study of nitrogen uptake and movement in the plant was undertaken.

Closely related with mineral nutrients are the characteristics of the culture medium. The frequent occurrence of blueberries on peat soils led to the investigation of some of the chemical and physical factors of peat and other media, and their relation to blueberry growth. Since the beneficial effect of peat is sometimes attributed to the presence of various growth promoting substances, their role in the complex was also to be considered.

During the process of the investigation it was found that the hydrogen-ion concentration of the blueberry plant sap is strikingly high. This characteristic promised to provide a diagnostic tool, and to produce data that might explain in fundamental terms some of the uniqueness of blueberry plant behavior.

REVIEW OF LITERATURE

This review was limited to the available literature on the blueberry, and to the more recent publications on factors affecting the acidity of plant fluids, and on the effect of mineral nutrients on nitrogen uptake and translocation. The literature on controlled nutrient culture work with blueberries consists of one report by Doehlert and Shive (70). Reference will be made to recent comprehensive reviews, especially for those topics which will not be covered in detail.

Blueberry Nutritional Factors. As early as 1921, Coville (54) advanced the following three requirements for successful blueberry culture: (1) acid soil, (2) good aeration, and (3) moderate moisture. Of hundreds of species of plants listed by Spurway (170), the acid tolerance of the highbush blueberry was found to be greatest, and approximated only by some pitcher plants (Serracenia spp.); nevertheless, the question of the acidity requirements of blueberries in general is not yet settled. Merrill (124) obtained best highbush blueberry growth in peat soil at a pH of 4.4, and Bailey (6) found that increasing the greenhouse soil pH from 4.5 to 5.0 by liming decreased linear growth by 55 percent. Coville (55) found that blueberry seedlings growing poorly in ordinary soil could be revived by acidifying the medium with aluminum sulfate. The results of Perlmutter and Darrow (141) indicate a similar beneficial effect from large applications of aluminum or ammonium sulfate. On the other hand, Chandler (37) in field investigations with the lowbush

blueberry (V. angustifolium) obtained a beneficial response from lime applications which brought the soil pH up to as high as 6.6. Kramer, Evinger, and Schrader (111) working with the dryland blueberry (V. pallidum), presented indirect evidence to show that lime applications would be beneficial on tight soils, largely through improvement of the physical condition of the soil. The results of the greenhouse investigations of Stene (172) were inconclusive, showing good and poor growth over a wide range of pH. Considering the swamp blueberry (V. corymbosum), the overwhelming evidence is in favor of an acid soil requirement in the neighborhood of pH 4.5. Other species, however, may have a wider or higher range of pH, as in a like manner, the dryland species appears to have greater resistance to drouth (54) than the swamp blueberry.

The soils on which the swamp blueberry is grown commercially are peat soils in Michigan (105), and light sandy soils mixed with peat in New Jersey (20). The beneficial effect of peat has been generally recognized. Perlmutter (140) obtained best growth of blueberry seedlings in a medium composed of half peat and half sand. Kramer, Evinger, and Schrader (111) reported a doubling of the yield of highbush blueberries where a peat-mulch was applied, and almost complete elimination of plant loss when peat was trenched in alongside the root zone of both highbush and lowbush plants. Root development under peat mulch was very extensive horizontally, but was almost entirely limited to the peat layer itself. This beneficial effect of peat may be due to the improved

aeration which the peat provides simultaneously with improved moisture conditions (188).

The occurrence of the highbush blueberry in swamp soils suggests a tolerance for low oxygen levels. Coville (54), however, pointed out that even though the blueberry grows in swamps, it is usually located on a tuft of moss or sedge. Moreover, active root growth did not begin until the summer, when top growth was almost completed, and the water table was below the root zone.

Fertilizer recommendations have varied with the region. On the Michigan (105) soils that are high in organic matter, there has been little response to nitrogen application and potassium fertilization is stressed. In New Jersey (19), on the other hand, nitrogen is the chief component of the fertilizer recommended, and best yields have been obtained from the use of complete fertilizers. Coville's assertion (54) that root activity does not begin until summer, is substantiated by Doehlert's results (69), where fertilizer application in June was as effective as an April application. Bailey and Hverson (7) reported that blueberries recovered from a chlorosis most rapidly when ammonium sulfate was applied. Peat and aluminum sulfate also improved the color. Iron sulfate spray turned the leaves green only in the spots where the spray material came in direct contact with leaf tissue. Further investigations led Bailey, Franklin, and Kelly (8) to conclude that the chlorosis was caused by iron deficiency. Doehlert and Shive (70) found that a high level of nitrogen and a comparatively low level of potassium and phosphorus gave the best blueberry growth in sand culture.

Because of their natural occurrence under forest canopies,

blueberries have been considered to be low temperature, shade-loving plants. Perlmutter (140), however, showed that blueberry plants grown in the greenhouse benefitted from the addition of four hours of light, and Bailey and Jones (9) reported consistent increments in total linear growth with increase of temperature from 55 to 90 degrees Fahrenheit. When temperatures become exceedingly high, however, blueberry plants are injured; thus Jonathan (105) reported tip burning and even death apparently caused by high temperatures in localized areas in the field. Coville (53) demonstrated that blueberry plants go into dormancy at warm temperatures, but remain dormant longer than plants subjected to a period of freezing temperatures. Kramer and Posey (112) reported that heavy shade produced by an undisturbed forest canopy was beneficial for the perennial vegetative existence of lowbush plants, but that considerable thinning must be done if the plants are to be brought into bearing.

The chemical composition of the vegetative and fruiting parts of the blueberry has not been thoroughly investigated. Waksman (198) reported that the natural Vaccinium humus has a pH of 4.6, and contains 1.7 percent nitrogen. Romell (155) gave the content of the following elements in milligrams per gram, as found in blueberry bushes growing in the northern spruce woods of Sweden: K_2O - 10 mg., CaO - 10 to 16 mg., MgO - 4 mg., P_2O_5 - 2 to 5 mg., and N - 9 to 23 mg. The low value for nitrogen was obtained for leaves that were about to fall, and the high value for leaves in June. Merriman and Fellers (123) found the fruit to contain 85 percent moisture, 6 to 12 percent fiber, 1.25-1.50 percent ash, and 4 percent nitrogen. There were small quantities of vitamins

A and C. Murri (129) reported that 70 percent of the dry matter in the blueberry fruit is carbohydrate. Other substances found in the fruit were ash, tannins, citric, malic, and oxalic acids, and 25 bio-units of vitamin C in 100 grams. The seeds contained 30 percent fatty oils.

Deficiency Symptoms. A general discussion of plant deficiency symptoms has been given by De Turk (67). In a paper containing a very extensive list of references, McMurtrey (122) has described the deficiency symptoms of tobacco and other plants, classified the deficiencies, and presented a key to aid in diagnosis.

The diagnosis of any mineral nutrient deficiency may be carried beyond the mere observation of the visible deficiency symptoms. Carolus (36) and others developed rapid chemical tests of the plant parts for the various elements, since the deficiency of a particular element usually resulted in a low level of that element in the plant tissue (17). Thomas and Mack (185) have offered their foliar diagnosis method, emphasizing that the proportion of the various elements in the plant tissue is important, and not only the absolute concentration of the elements. Hill and Loach (94) have proposed what is probably the most direct method of diagnosing a nutrient deficiency. They injected the element, suspected of being deficient, directly into a sample plant, and if that plant, or the part treated, returned to normalcy, the deficiency was successfully diagnosed.

Nitrogen Uptake and Translocation. More recent publications stress the biological nature of inorganic-ion absorption

by plants. It is no longer considered that salts are taken up passively as a consequence of an (H^+) or (OH^-) gradient, or by mechanical osmosis alone. Lundegarth (117) in his comprehensive discussion of inorganic-ion absorption, stated that absorption, accumulation, and exudation are aspects of one mechanism having aerobic, energy expending processes at its disposal. He proposed a constant (K) which is equal to the osmotic work required to absorb one unit of ions, the energy being furnished by respiration. Hoagland and Broyer (97), and Arnon and Hoagland (5) have stressed the complexity of the problem of ion intake, and the essential requirement of air and light. A comprehensive review of nitrogen uptake, assimilation, and metabolism in plants has been given by Nightingale (130).

Nitrogen has frequently been reported as the first of the mineral nutrients to become limiting for plant development (118). It has been found in the greatest concentration in the leaves in spring (93), or early summer (118). It has been shown that nitrogen is depleted in the wood during the summer by movement into the leaves (39), but a large part returns before leaf fall (118), and thus senescence of leaves is accompanied by loss of nitrogen (150). During winter, nitrogen is stored in the older wood (128), and also to some extent in the roots (209).

Judging from various results there exists a positive gradient for all nitrogen fractions, i. e., the concentration of nitrogen increases from the base towards the growing point.

Ingard (80) is of the opinion that this gradient is simply the manifestation of the ratio of living protoplasm-containing cells to non-living protoplasm-lacking cells. Vladescu (193) carried the gradient study into the leaves, where he found that the gradient continued there, with the nitrogen content increasing from the center to the border of the leaves, and from the lower to the upper side of the leaves, except for ammonia nitrogen, which showed a higher concentration on the lower side of the leaf. The protein content (194) showed similar gradients in the stem and leaves, whereas secondary roots contained more protein than primary roots, and the flowers were richest in protein.

A scheme for nitrogen metabolism in higher plants has been presented by Murneek (126). Briefly, the inorganic nitrogen is absorbed by the roots, reduced to ammonium, and then synthesized into asparagine, while the carbohydrates are oxidized to organic acids and form ammonium salts, which combine with asparagine to form amino acids which in turn combine into proteins. Once the proteins are formed, a certain amount of respiration energy is required to maintain them (148). Hevesy, et. al. (90), by use of heavy nitrogen, found that leaves constantly break down and renew proteins. The renewal is more rapid in the new leaves, but goes on even in old leaves which had already completed their growth.

The most important single condition concerned with the penetration and accumulation in the plant of any element is the concentration of that element in the culture medium (17). Thus, to cite a few examples, increasing the nitrogen content of the medium resulted in an increase of nitrogen in the plant tissues

of oats (1), apples (14, 175), corn (18), sugar cane (26, 51, 57), tomatoes (35), citrus (42), wheat (65, 178), rice and flax (165), and cotton (195). Similarly, nutrient solutions deficient in nitrogen caused tremendous reductions in the nitrogen content of tomatoes (12), oats (21), barley (150), etc.

Although luxury consumption is usually associated with the potassium-ion, excessive quantities of nitrogen may also be taken up from a nitrogen rich substrate (144, 165, 175). Waltman (203) reported excessive applications of nitrogen beneficial to the peach tree, but not to apple tree growth, and Stuart (175) obtained actual nitrogen injury on apple trees. Carolus (35) found that luxury consumption of nitrogen by tomato plants had no toxic effects, but Chapman (42) did find potassium injury symptoms on citrus trees receiving excessive amounts of nitrogen. Demidenko and Barinova (65) obtained greater yields of wheat from low nitrogen application; however, the wheat plants grown with low nitrogen were lower in percent nitrogen.

For diagnostic purposes, it is interesting to note that Doby (68) found that normal soybean plants had 80 percent of their nitrogen tied up in organic compounds, while fully 90 percent of the total nitrogen of nitrogen starved plants was in the organic form. Similarly Stuart (175), Schropp and Arenz (160), Phillis and Mason (144), and Cooke (51), obtained relatively more inorganic, and less organic nitrogen in apple trees, field beans, cotton, and sugar cane respectively, when nitrogen was abundant in the substrate. Turner and Henry (190) used the presence of inorganic nitrogen in the leaves as an indication of excess nitrogen.

Phosphorus deficiency in apple and peach trees has been described by Waltman (203) as almost as severe in its effects on growth as nitrogen deficiency. Lack of phosphorus apparently has interfered with nitrogen metabolism by causing a reduction in respiration rate (148), and in reducase activity (78), which causes the accumulation of sugars (67), nitrates (36, 67), and other inorganic fractions, and a reduction in protein content (150, 178). Phosphorus may also be taken up in "luxury" quantities (144), and its concentration in the plant is therefore related to its concentration in the medium (14, 67).

The dependence of nitrogen utilization upon the presence of phosphorus is indicated by the results of Brewer and Davis (28) who found that the nitrogen content of legumes increased with applications of phosphorus. Sircar and Nirad (166) obtained similar results with rice. Conversely, a deficiency of phosphorus caused a reduction in nitrogen content of oats (21), and corn (67). Alten, Rauterberg and Loofman (1) found that total content, but not percent of phosphorus increased with increasing nitrogen in the oat plant. On the other hand, Bartholomew, Watts, and Janssen (12) obtained no reduction in percent nitrogen of tomato plants when phosphorus was omitted from the nutrient solution. Beckenbach, Robbins, and Shive (17, 18), working with corn, and Parker and Truog (136) working with a large number of species, found no relationship between nitrogen and phosphorus contents. An actual increase in growth response of peach trees to nitrogen additions, regardless of phosphorus content (205), and an increase in

phosphorous content upon a reduction in nitrogen (14) in apple trees have been reported recently, as well as an increase in percent nitrogen when phosphorus was deficient (40). Although the percent, or concentration is confusing, the absolute quantities indicated that there is a positive relationship between phosphorus and nitrogen, i.e., an increase in the content of the one element favors an increased absorption of the other.

The position of potassium in the plant is unique in that it has been found to exist entirely in a water soluble form in the plant sap (91, 146). A thorough discussion of the role of potassium in plant metabolism has been given by Hoffer (99), who suggested that potassium may have some function in the transformation of light energy, since of the elements essential to plant growth, it is the only one which is radioactive. Potassium has been reported as favoring swelling of plant membranes, as having a role in the absorption of other minerals, in sugar and starch formation, in regulation of respiration and transpiration, and in enzyme activity. The large number of symptoms resulting from its deficiency is an indirect indication of its many functions. Carolus (35) contended that potassium requirements of the plant are very critical, and too much or too little potassium is equally troublesome.

Potassium is required for nitrate reduction (79) and for normal respiration (148, 174). Wall (201) has stated that when potassium is deficient, nitrogen metabolism is affected

before carbohydrate metabolism. He also reported that potassium-deficient plants, grown in a medium containing nitrate as the source of nitrogen, exhibited the expected symptoms gradually, but when similar plants were grown in a medium where ammonium was the nitrogen source, they showed no symptoms for some time, and suddenly developed leaf breakdown which was apparently caused by high concentrations of ammonium, amide, and amino nitrogen fractions. The relative amount of protein in the ammonium-fed plants was correspondingly lower. Further evidence of accumulation of ammonia, amide, and nitrate nitrogen, and reduction in protein content of plants lacking potassium, is given by Richards and Templeman (150), and Burrell (32). Similarly application of potassium increased the total amount of nitrogen in the plant, and especially the protein fraction and at the same time reduced the amount of amide, and ammonium nitrogen (1, 17, 18, 39), thus indicating that the addition of potassium improved the utilization of nitrogen.

In storage organs, such as the sweet potato root (177), and wheat grain (178), however, increase of potassium caused a decrease in protein content. These results may also indicate the greater utilization of nitrogen for growth rather than for storage.

Although the adequate presence of potassium has improved total nitrogen absorption, the percent of nitrogen in the plant may not necessarily be increased (21, 92, 110, 136). In fact, upon extreme deficiency of potassium, nitrogen

relatively appeared to accumulate (12, 57, 200), and inversely, when there is excessive "luxury" consumption of potassium, the nitrogen percent may become lower (1, 35).

The potassium-ion may be taken up by plants with great ease, and consequently there is usually a close relationship between the potassium available in the substrate and its presence in the plant (1, 14, 36, 57, 91, 165, 206). When the nitrogen content is low, there can be little improvement in plant growth if potassium is added; however, if potassium is low, applications of nitrogen may sometimes be effective to a limited degree (14, 195). When nitrogen is applied in excess, potassium has been reported as highly beneficial (26).

Nitrogen-potassium relationships are strongly affected by the factor of light. Apparently maximum quantities of potassium are required when days are shortest, and maximum quantities of nitrogen when days are longest (190). This situation is explained at least in part by the results of Böttticher and Behling (27), who found that potassium may be lost in the dark, and reabsorbed in the light, and Borden (26) who reported a decrease in percent nitrogen in plants subjected to long days. When light was limiting, Borden (25) found that the percent of nitrogen in sugar cane plants increased to such an extent that it caused reduction in yield. Additional potassium under such conditions did not bring back the yield, but did improve the sugar content. This is in agreement with Arnon and Hoagland (5) who concluded that nutrients cannot compensate entirely for a deficiency of light.

The results of Sugawara (176) that application of potassium increased the ascorbic acid content of potatoes, might indicate that there exists, either a direct or an indirect relation between potassium content and photosynthesis, since ascorbic acid has been associated with the green plant pigments, and is developed only in the presence of light (31). Vinson and Sartor (191), however, failed to obtain increases in ascorbic acid from high applications of potassium.

Not only are there nutritional relationships between nitrogen and phosphorus, and nitrogen and potassium, but there may be a mutual relationship between the three elements in their use by the plant. Thus De Turk (67) observed that adequate phosphorus and potassium brought out nitrogen deficiency more rapidly, and conversely, Waugh, Cullinan, and Scott (205) found that phosphorus and potassium deficiencies could be noted only when there was an adequate supply of nitrogen. This interrelationship of the three elements is the salient feature of the "foliar diagnosis" of Thomas and Mack (135). Bartholomew, Watts, and Janssen (12) showed that phosphorus and potassium deficiencies, alone and in combination, were not effective in reducing the percent of nitrogen in tomato plants, while the omission of nitrogen, alone and in any combination, reduced the percent of nitrogen drastically.

Calcium is used by the plant for the middle lamella of new cells, and for combination with protoplast materials which in the absence of calcium, accumulate as granular pro-

teinaceous substances (131). Calcium may also be utilized to neutralize plant acid (136), particularly oxalic acid (134), although Dunne (73) showed that the acids may be neutralized readily by potassium. Although the quantity of the combined fraction of calcium has appeared to be very constant, at least in the cotton plant (146), the uptake by various plants has varied greatly, some plants taking up three to five times the relative quantities of calcium that other plants absorb (48). The relative requirements of various plants for calcium may be expressed as a calcium:nitrogen ratio (136). According to Steward (174) calcium salts decreased the rate of respiration and protein synthesis. Hoffer (99) stated that calcium retards swelling of plant membranes, thus acting in opposition to potassium.

Parker and Truog (136) found a close relationship between calcium and nitrogen content within various species. Those species having a high nitrogen;calcium ratio had low calcium requirements. Olsen (134) suggested that those plants in which much of the calcium is tied up as calcium oxalate have low calcium requirements, and grow best at very low calcium concentrations.

Nightingale, Addoms, Robbins, and Schermerhorn (131) found carbohydrate accumulation in calcium deficient tomato plants. They attributed this condition to the lack of nitrate absorption and assimilation. Skok (167) demonstrated that calcium participates in nitrate reduction, when he showed that

calcium deficient plants grew much better when supplied with urea than when supplied with nitrate as the nitrogen source. Burrell (32) also found more nitrate but much less insoluble nitrogen in calcium deficient plants. Brewer and Davis (28) reported increased nitrogen content as a result of lime applications on legumes, but Demidenko and Barinova (65) found that calcium content of wheat decreased when nitrogen applications were increased. Carolus (35) showed that the relative contents of both calcium and nitrogen are reduced when potassium is taken up in excess, but Hibbard and Grigsby (92) found that calcium deficiency had no effect on the percent of nitrogen in pea plants.

Magnesium has been found to be essential not only for chlorophyll but also forvitamin C production (156). Its absorption by various species varies considerably, as does the absorption of calcium (48). The concentration of magnesium apparently has no profound effect on nitrogen uptake. Both slight increases (21), and slight decreases (17, 36), as well as no effect (18) on nitrogen uptake, have been reported as resulting from magnesium deficiency. Large nitrogen (65), and potassium applications (35) suppressed magnesium uptake. Burrell (32) reported that there is less insoluble and more soluble nitrogen in magnesium deficient soybeans, again pointing to interference in protein synthesis.

The role of sulfur in the plant is discussed by Balks (10), who pointed out that sulfur is an essential component of plant proteins, and such substances as glutathione, vitamin B_1,

and mustard oil. He made the general statement that plant requirements for sulfur are about the same as for phosphorus. Although Beckenbach, Robbins, and Shive (18) stated that they found no correlation between the sulfate content of the nutrient solution and nitrogen metabolism, they found (17) that a high sulfur content in the plant tissue was correlated with a low nitrate content. Conversely, sulfur deficiency was found by Haton (77) and Champan and Brown (41) to result in nitrogen accumulation, and the addition of nitrogen (11) to a nutrient solution increased the protein-sulfur content. These latter results tend to agree with Balks (10), indicating that sulfur is perhaps as important as phosphorus for nitrogen metabolism and for general plant welfare.

Boron deficiency symptoms were found by Schropp and Arenz (159) to be similar to ammonia poisoning. Since boron deficiency was associated with a greater relative uptake of nitrogen (158), while protein-nitrogen was proportionally lower (161), they suggested that lack of boron was interfering with carbohydrate synthesis, which in turn caused an accumulation of ammonia from protein decomposition consequent upon lack of carbohydrates. Schropp and Arenz (160) also found that boron deficiency caused a reduction in the relative amount of nitrogen when potassium was increased, and that best utilization was made of both potassium and nitrogen when boron was adequate. The results of Demidenko and Barinova (64), that application of boron reduced total percent of nitrogen, but increased the

content of protein nitrogen, are in agreement with those of Schropp and Arentz.

There seems to be little direct relationship between iron content and nitrogen uptake, but there are undoubtedly indirect effects through the influence of iron on chlorophyll formation (135), and carbohydrate balance (133).

Manganese on the other hand, has a very definite role in nitrogen assimilation. There is general agreement that manganese aids in ammonium uptake, but whereas Burstrom (33), Noack and Pirson (133), and Wlassuk (210) reported an improvement in both nitrate and ammonium assimilation in the presence of manganese, Hoagland and Arnon (95), and Arnon(2) found that nitrate-grown plants received no special benefit from the presence of manganese. Wlassuk (210) stated that manganese has a reducing effect on nitrate, and an oxidizing effect on ammonium. Hoagland and Arnon (95), however, mentioned only the oxidizing effect on ammonium. Arnon (2) added that ammonium-fed culture plants benefited equally from the presence of manganese and aeration. Burstrom (33), using excised roots, concluded that the improvement in nitrate absorption brought about by the presence of manganese could not be duplicated by any other cation, or sulfate, carbonate or phosphate, salt. Noack and Pirson (133) on the other hand concluded that the same effect may be induced by the use of a catalyst not containing manganese as the heavy metal. Manganese uptake by various plants has been shown to be extremely variable (48), some plants taking up sixty times as much as others. The only

other ion showing such a range in uptake was sodium. It has been demonstrated that nitrogen uptake is of course closely, though indirectly, related to chlorophyll formation (50). In fact, Noack and Pirson (133) measured nitrate assimilation by chlorophyll content. Since vitamin C is also closely related to chlorophyll (31), it is interesting to note that manganese appears to be essential for vitamin C formation (89, 191).

Other factors besides mineral nutrients have their effect on nitrogen uptake. The interrelationship of nitrogen, potassium, and light has already been discussed (cf. p. 14). A decrease in soluble nitrogen content following an increase in length of day indicated better and more rapid nitrogen utilization (208).

Temperature effect on rate of solute accumulation is considered similar to many metabolic processes (29). Not only is nitrogen uptake delayed at low temperatures (15), but the nitrogen is translocated slowly, if at all (15, 132).

Aerobic conditions are deemed necessary for accumulation of solutes (29), for nitrates are taken up readily at low oxygen tensions and reduced rapidly in the plant, whereas at high oxygen tensions the nitrates tend to accumulate more. It has been shown that ammonium absorption is facilitated by high oxygen tensions (2, 163).

Other Mineral Nutrient Interactions. A distinct similarity in the symptoms of potassium and iron deficiencies has been observed by De Turk (67), Rohde (154), and others.

Thus a relationship between the above two ions in plant nutrition, suggests itself. Hoffer (99) has demonstrated that in the absence of potassium, iron is precipitated in the nodes of the corn plant, and Rhode (154) also pointed out that the translocation of iron in plants is facilitated by the presence of adequate potassium. Loew (116) reported that iron in the ferric form cannot be taken up by plant roots, and according to Rhode (154), it is the ferric ion that is precipitated in the absence of potassium, which acts as a reducing agent and in the presence of manganese, which acts as the oxidizing agent. Similarly, Loehwing (114) found that in the presence of excessive quantities of potassium, so much of the iron was in the soluble form, that the plants actually suffered from iron toxicity. Wadleigh (196), however, found that a high potassium content was usually associated with a low iron level.

Calcium and boron deficiencies have also been reported as exhibiting similar symptoms (164). Maximum accumulation of calcium by soybean plants was reported by Minarik and Shive (125) to have occurred when the boron supply was at the optimum concentration, but Marsh and Shive (120) found no relation between the boron concentration of the nutrient solution and the uptake of calcium by corn plants. Drake, Sieling, and Scarseth (71) emphasized the importance of the calcium:boron ratio in the substrate, stating that 1300 parts of calcium to one part of boron was satisfactory, but that 1500 parts of calcium to one part of boron in the substrate resulted in boron starvation. Shive's (164) explanation of

this calcium-boron relationship was that boron controls calcium mobility in the plant, since in the absence of boron, the calcium content of plants is largely insoluble. In the presence of boron the calcium in the plant was found to remain available even if no fresh supply of calcium were provided (120).

Hydrogen-ion Concentration of the Substrate. Recent publications have emphasized the unimportance of the pH of the substrate per se. "(H^+) or (OH^-) gradients do not determine salt uptake", stated Hoagland and Broyer (97). Arnon (2) pointed out that the pH effect of the nutrient solution is interrelated with season, form of nitrogen, aeration, and concentration of micro-elements. If other factors are favorable, normal growth may be secured over a wide range of pH, and actual detrimental effects of hydrogen-ion or hydroxyl-ion concentration were obtained only at the extreme pH values of 3 and 9 (34, 95), when an irreversible disorganization of the protoplasm took place, and all absorption ceased (34).

New Jersey workers (45, 46, 62) have frequently reported that the nitrate-ion is more readily taken up at a low pH of the nutrient substrate, while the ammonium-ion is absorbed to greater advantage at a pH approaching the neutral point. Their results on peaches and strawberries are in agreement with the results of Demidenko and Barinova (65) on spring wheat. Kapp (108), however, found both nitrate and ammonium equally beneficial for the growth of rice at a comparatively high pH, and Hoagland and Broyer (97) observed rapid nitrate uptake at a wide range of pH. Arnon (2) showed that the presence of manganese and aeration exert important influences on the

relative merits of ammonium and nitrate as sources of nitrogen at different values of pH. The peculiarities of the rice plant further complicate the nitrate-ammonium situation for apparently the rice plant benefited more from ammonium than from nitrate, independently of pH (60), and conversely, had a preference for approximately neutral pH independently of the source of nitrogen (52, 59). The work of the above Indian workers (52, 59, 60), however, is not entirely convincing, since most of it was done with single salt nutrients. Kapp (108) on the other hand found that nitrate and ammonium are both beneficial to the growth of rice plants provided the culture is not at an extreme pH.

Potassium was reported by Demidenko and Barinova (65) to be taken up best at an approximately neutral reaction of the medium, but Hoagland and Broyer (97) have stated that potassium may be readily taken up from a solution both more acid and more basic than the plant sap. Phosphorus absorption has been facilitated in a slightly acid medium (65), and was rendered unavailable at a high pH value (95). Calcium (67, 95) and magnesium (95), on the other hand have been taken up readily at high pH levels, and became less available at low pH levels. Hoagland and Arnon (95) suggested that the adaptability of acid-loving plants to their environment may be due to their low calcium requirements. Iron (95, 107), manganese (95, 34), and aluminum (95) were all more available at a low pH range, the latter two ions especially may be absorbed in excess, and cause toxic effects at low values of pH (95).

Authoritative statements on the effect of the pH of the

substrate on the pH of the plant sap have been very contradictory, and therefore confusing. A survey of some of the published data, however, clearly indicates that the substrate pH has a considerable effect on the pH of the roots, but very little (though possibly significant) effect on the juice extracted from plant tops. Thus insignificantly small differences in the pH of tops as influenced by the medium pH have been reported for corn (30, 197), Nitella (98) strawberries (46), barley (3), legumes (30, 192), and other plants (109, 182). Data showing considerable effect of the substrate pH on the root sap pH, however, were presented for corn (30), barley (3), legumes (30, 192), and other plants (109, 182). The change in pH of the plant sap was of course in the direction of the substrate pH, the effect being proportional to the difference between the hydrogen-ion concentration of the medium and that of the plant; at the same time, the greater the original difference between the pH of the plant and the medium, the greater was the final disparity (192).

The pH of the substrate is in turn influenced by the plant, directly, and indirectly through the effects of aeration (97, 49) and temperature (97), by the differential absorption of the medium itself (203), and by the composition of the nutrient solution. The increase in pH of soils through the application of lime is well known (37, 67). The use of ammonium sulphate as the source of nitrogen has caused a reduction in the pH of the medium, since ammonium is taken up in greater quantities, leaving the acid sulfate residue

(26, 97, 107, 140, 180, 187). The use of a nitrate salt has had the reverse effect (26, 97, 140, 180, 187). A reduction in the pH of the medium has also been effected by the use of aluminum sulfate (55, 141). In general, exeessive uptake of anions resulted in a change of the medium to a higher pH (97, 165), and excessive uptake of cations has had the opposite effect (97). The nutrient solution pH may be radically changed by the physical characteristics of the medium. Thus Waltman (203) reported that the pH of the nutrient solution changed rapidly from the original 4.2 to 7.0, and then to approximately 3.5 because of selective absorption by the sand itself, since the same trend in pH occurred whether plants were growing in the sand or not. The omission of nitrogen from the nutrient solution resulted in an increased pH of the leachings, while the omission of phosphorus had the opposite effect.

Trelease and Trelease (187) made use of the opposite effects of ammonium and nitrate uptake on the pH of the substrate to develop a nutrient solution which remains at a fairly constant pH by manipulating the ratio of ammonium to nitrate nitrogen used in the nutrient solution. Similarly, Eaton (76) suggested the use of nitric acid to control both pH and nitrate content in nutrient solutions, while Talley and Blank (180) used a carbonate salt to delay the acidifying effect of ammonium.

Hydrogen-ion Concentration of the Plant. It has long been known that plants do not have the same hydrogen-ion

concentration throughout. Theories, mostly discredited by now, of nutrient absorption, and other plant processes have been based on the observed pH gradients in plants (151, 97). Nevertheless pH gradients do exist across the cambium region, the xylem elements being more acid, and the phloem elements more basic (138), across cytoplasmic membranes (43), and in stems, the younger portions usually being more alkaline (83, 153). Potato sprouts (153), and actively growing potato warts (207) are exceptional, in that they have a lower pH for the young growth. In very acid tissues the acid is localized in special cells, and not in contact with the protoplasm, or enzymes that might be inactivated by such high acidities (103).

That the pH of the plant sap is not profoundly affected by the environment is believed by Hoagland and Broyer (96, 97) and Arnon (3), to be due to the electrostatic balance maintained in plants by production of required amounts of organic acids. Effects of ion absorption on the pH of plant sap have been reported by a number of workers, however. Where there existed a nitrogen deficiency, the addition of nitrogen has caused an increase in the plant pH of wheat (102) and field beans (160). Ammonium salts raised the plant pH higher than did nitrate application (16, 47). Phosphorus deficiency also caused a reduction in the pH of plant saps (63, 73, 78), as did lack of potassium (73, 160). Data on the effect of calcium on the pH of the plant are not in agreement. No influence from calcium deficiency or application were reported for buckwheat (73), wheat (102), tomatoes (131), and other plants (47). Loehwing

(115), however, reported that liming raised the pH of wheat, and other plants (114). The key to the controversy may have been supplied by Dustman (74) who found that when calcium applications were varied, the highest pH was associated with the most vigorous plants. It is therefore likely that generally a mineral deficiency will cause a reduction in the plant pH, but if the element is not actually deficient, its application will cause no further increase in plant pH.

Thornton (186) found that carbon dioxide raised the pH of plant tissues in the presence of oxygen, and lowered the pH if oxygen was lacking. Hoagland and Broyer (97), however, reported no effect of carbon dioxide atmosphere on plant pH.

Of the climatic factors affecting the plant pH, light has received most attention. The small diurnal changes occurring in pH of plants, where the lowest pH is in the morning, the highest in the afternoon or evening, have been explained as a light effect. Clevenger (47) reported that plant acids accumulate at night, and are destroyed during the day, and Loewhing (115) found no diurnal change in pH under continuous illumination; moreover plants which were etiolated as a result of shading showed a lower pH. Blackman and Templeman (22) obtained gross reduction in carbohydrate content of shaded plants, while the organic acid content remained constant. As a result, the pH of these shaded plants was lowered because of the increased ratio of organic acids to total carbohydrates. Hurd-Karrer (102), however, obtained lower pH values for wheat plants when day length was increased, and no effect on pH

from varying light intensities. Dunne (73) also found little effect of light on plant pH, and he questioned the results of others where temperature was not controlled. Only Hurd-Karrer (102) reported the effect of temperature on pH in which high temperatures reduced the pH of wheat plants.

As indicated above (cf. p. 27), the pH of the plant may be associated with its vegetativeness. It also appears to be related to the age of the plant and incidence of disease. Increase of acidity with senescence was reported for cotton (61), and wheat (102), while higher pH values have been associated with active growth (101, 207) and high catalase activity (207). Pheophytin production also has been related to high pH (173). Diseased plants frequently have lower pH values, especially when the disease is caused by a virus. Thus, yellows of walnuts (84), tobacco mosaic (87), stem rust of wheat (100), cabbage yellows (184), virus (153), and wart (207) disease of potatoes, were all associated with lower pH values of the respective plants.

The resistance of plant tissues to changes in pH exerted by external influences is made possible by their buffer systems. Reproductions of titration curves with various substances known to be present in the particular plant have been used to arrive at some idea of the composition of the buffering materials in the plant tissues. Thus Hurd-Karrer (101) was able to match the titration curve of wheat juice by using water, sodium malate for the acid reserve, phosphate as neutral, and asparagine, leucin, glucose, and sodium hydroxide for the alkaline reserve. Martin (121) found

that changes in the buffer capacity of the broad bean were associated with phosphate content, and knowing that oxalates and malates were present in the plant, he concluded that all these, along with some additional substance which stabilizes the pH level at above 6.0, comprise the buffering system of the broad bean. Dunne (73) reproduced the titration curves of a number of plant species by using organic acids, phosphates, and glucose. He was fairly certain that the acid side of the plant buffer systems was composed chiefly of organic salt radicals, but along with Martin did not know the composition of the alkaline side of the buffer system. Additional evidence that organic acids participate in the buffering action is presented by Olsen (134), who demonstrated that in many plants, there was a direct correlation between calcium uptake and the quantity of oxalic acid. In beech leaves a gradual increase in pH was accompanied by a compensating decrease in malic acid content. Similarly, Wadleigh and Shive (197) showed that an increase in the pH of the substrate is followed by an increase in the organic acid content of corn plants. Shive (163) observed that organic acid accumulation was associated with anaerobic conditions and a high rate of nitrate absorption and reduction. Such reduction leaves a basic residue, which, according to Haagland and Broyer (96, 97), is neutralized by organic acids, which may have been formed by the very same oxygen released by the nitrate radical. Blakman and Templeman (22) obtained a significant correlation between nitrogen, especially nitrate uptake, and formation of organic acids.

Iso electric Point. Robbins (151) postulated the existence of an isoelectric point for plant tissue as a whole, and suggested that it is determined by one or a combination of proteins whose single or combined isoelectric point is in the vicinity of pH 6. His theory did not stand up under the criticism of Youden and Denny (211) who pointed out that the so-called isoelectric point, as determined by Robbins almost invariably coincided with the actual pH of the particular plant sap. They also found that it was not proteins, but materials which readily passed through collodion that were responsible for the pH and buffering power of plant tissues.

Regardless of the existence of an isoelectric point for plant tissue as a whole, Pearsall and Ewig (137) and Chibnall and Grover (43) were among the first workers to demonstrate that the isoelectric points of most plant proteins are between pH of 4.0 and 5.0, and consequently the amphoteric proteins exist as anions in the great majority of plants. On the basis of such data, Pearsall and Priestly (138) developed their theory of synthesis, which is briefly as follows: a pH gradient exists across the cambium, with the pH at the cambium at approximately the isoelectric point of the proteins of the protoplasm. Since proteins at this isoelectric point show least affinity to water, the cambial protoplasm loses water to the surrounding cells, and this loss of water is conducive to synthesis, as opposed to hydrolysis. Such a view finds some support in the findings of Wasteneys and Borsook (204) that peptic synthesis occurred at the most rapid rate at a pH of 4.0. The exactly opposite stand was taken by Dastur and

Hensinaki (61), who found that the apparent isoelectric points of the cotton plant proteins were at a lower pH level than the corresponding plant sap pH throughout the life of the plant, except close to the end of the life cycle, when the apparent isoelectric point practically coincided with the pH of the plant sap. Their conclusion was that, at senescence, the pH of the plant sap was equal to the isoelectric point of the proteins of the protoplasm, and the proteins thus lose their combining power, coagulate and bring about the death of the plant.

Physical Effects of Media. That lack of optimum aeration is the limiting factor in growth of plants on some soils is generally recognized, but Arnon and Hoagland (5) found that the oxygen supply may be limiting even in sand culture. Since absorption of solutes is linked with aerobic respiration (117), aerobic conditions have been considered as required for the uptake (174), and accumulation (29) of both cations and anions. The greater effectiveness of nitrate over ammonium applications under low oxygen conditions have been found (2, 163) to be apparently due to the oxygen content of the nitrate radical (95), which may be reutilized by the plant. Manganese apparently plays an important role in freeing the nitrate oxygen (33, 117), as well as in reutilizing it for the oxidation of ammonium ions thereby preventing ammonium toxicity (2, 163). (cf. p. 19). Low oxygen tensions have been associated with high organic acid accumulations (163), while application of root growth promoting substances raised the oxygen consumption (183).

Moisture content has been usually negatively related to aeration, since both moisture and air occupy the pore spaces of the medium. Conway (49) in her extensive review of aeration and plant growth in wet soils, pointed out that submerged soils are low in oxygen, and high in manganese, ferric iron, ammonium, and sometimes even free hydrogen, thus indicating that reducing conditions are prevalent. Water plants can tolerate such conditions because of their possession of aerenchyma, and it is easy to understand how beneficial nitrate application would be under such conditions (cf. p. 31). Other plants not possessing aerenchyma may evade the reducing conditions by means of a very superficial root system, or by withstanding only temporary periods of submergence, at which periods root growth does not take place. Since the pH of wet soils increases with depth, those plants having the shallow root systems exist at the lower pH values, where there is comparatively more oxidation.

Some of the factors involved in the benefit derived from the use of peat in the medium have been summarized by Tukey and Brase (188) as being: (1) better contact with soil moisture, (2) improved aeration, (3) easier penetration and less run off of rainfall, and (4) easier penetration of roots because of the decreased density.

Effect of Growth Substances. There is general agreement that plant growth substances have their practical applications, especially in the rooting of cuttings, and the growth of excised plant parts. Pearse (139) presented a comprehensive

review of plant hormones, emphasizing their practical importance in horticulture, and gave an extensive list of results of the use of various growth substances on a large number of plant species. Thus far, however, only negative results were obtained when growth substances were applied to blueberries, except for the one report by Scholz (157) who found that the rooting of green cuttings of highbush blueberries was raised from zero to fifty percent with the use of indole-3-acetic acid. Perlmutter (140) reported no significant effect from hormone applications on the growth of blueberry seedlings, and Chandler and Mason (38), and Johnston (106) both reported negative results with various commercially prepared and pure growth substances on the rooting of cuttings.

The value of these substances in improving the growth of entire plants growing under favorable conditions, however, is at present the subject of much disagreement. Bonner and his co-workers (23) claimed to have demonstrated the favorable effect of a number of growth substances on the growth of green plants. Dennison (66), Eaton (75) and Swartz (179) were also among those who reported some favorable responses to the application of some of the so-called plant hormones. Arnon and Hoagland (5), however, stated that the need for organic constituents (hormones) for favorable growth responses of entire plants has not been satisfactorily demonstrated. Arnon (4), Borden (26), Hamner (85), and Templeman and Pollard (181) are among the many workers who obtained no beneficial effects from applying these growth substances to

higher plants growing under favorable conditions.

The effectiveness of the growth substances may depend upon some particular factors, whose effect must first be exerted before the substance application becomes beneficial. Thimann (183), for example, found that root promoting substances increased oxygen consumption. Thus if the oxygen supply were limited, those substances would not have been effective. Rakitin and Jarkovaja (147) found certain growth substances to be most effective at low levels of pH. The acidity of the medium might therefore limit the effectiveness of some substances. Other data indicated that some substances may be effective only when the nutrient supply is unbalanced; thus, Eaton (75) found that indoleacetic acid compensated to some extent for boron deficiency and Swarts (179) reported a similar effect of napthaleneacetic acid when potassium was deficient. Bonner and Greene (24) found that the response of plants to B_1 applications was negatively correlated with the concentration of the vitamin in the plant.

Shoot:Root Ratios. Tremendous volumes of data have accumulated, particularly in horticultural literature, on shoot-root ratios, and their significance. Some of the factors reported as influencing the shoot:root ratio of plants are nutrients, light, moisture, composition of the medium, and the age of the plant. A decrease in the concentration, or changes in the pH of the nutrient solution, on the other hand, were reported as having no effect on the shoot:root ratio (189).

Liberal applications of nitrogen generally have favored high shoot:root ratios (42, 82, 189). Clark (46), however, found

that the tops of strawberry plants fared equally well under nitrate and ammonium nutrition, but root growth was favored by the use of nitrate in the nutrient solution. An adequate supply of phosphorus is reported by Goedewaagen (82) and Sommer (169) to favor shoot over root growth. Waltman (203), however, showed that phosphorus deficiency in peach and apple trees caused a drastic reduction in root growth, and that there is additional response in root growth from high applications of phosphorus. Potassium application was reported by Phillis and Mason (145) to decrease the weight of leaves and increase the weight of the remainder of the plant, thus favoring root growth.

Increase in day length (140) and moisture (86, 188) favored shoot over root growth, as did the incorporation of peat or muck in the soil (140), or the use of mulch (88).

MATERIALS AND METHODS

In order to arrive at a more accurate evaluation of the influence of the various nutritional factors on blueberry growth, it was decided to carry out the experiments under the controlled conditions of the greenhouse, in substrates of known composition, rather than under the variable conditions found in the field. Since individual blueberry plants vary tremendously, it was considered imperative that the experiments be designed in such a way that significant results could be definitely verified by statistical analyses. To this end, use was made of the pot culture method with addition of nutrient solutions, as well as small soil plots in ground beds in the greenhouse.

Controlled Pot Cultures

Nutrient Deficiencies, Media, and Growth Substances. On March 1, 1940, sixty, two-gallon coffee urn liners (crocks) were filled with quartz sand, and sixty with a three-inch depth of German peat above the sand. Good drainage was obtained by covering the drain hole with glass wool. The media were then leached for two weeks with heavy applications of tap water.

Dormant, rooted four-inch cuttings of the Cabot blueberry obtained from a commercial nursery were weighed individually and placed in the media, three per liner, on March 15. Tap water was added for ten additional days until the cuttings began to leaf out, after which time various nutrient solutions were applied automatically and continuously by du Buy's method (72). Twelve liners, six with peat-on-sand, and six with sand only, were used for each nutrient solution.

The full nutrient solution contained the following chemically pure salts, based on the results of Doehlert and Shive (70): 150 parts per million of mono-potassium phosphate; 600 p.p.m. of calcium nitrate; 150 p.p.m. of magnesium sulfate; and 275 p.p.m. of ammonium sulfate. In addition, 2.8 p.p.m of boric acid; 1.8 p.p.m. of manganese chloride, and 5 p.p.m. of iron citrate were added to the nutrient solutions, while two parts per million each of copper and zinc sulfate were applied at weekly intervals.

The following modifications of the full nutrient solution were made in order to obtain the various mineral deficient solutions:

Deficiency	Omission	Substitution
Nitrogen	Calcium nitrate, Ammonium sulphate	Calcium sulphate, Sodium sulphate
Potassium	Mono potassium phosphate	Mono sodium phosphate
Calcium	Calcium nitrate	Potassium nitrate
Magnesium	Magnesium sulphate	Sodium sulphate
Sulphur	Magnesium sulphate Ammonium sulphate	Magnesium chloride
Phosphorus	Mono potassium phosphate	Potassium chloride
Boron	Boric acid	
Iron	Iron citrate	
Manganese	Manganese chloride	

Once weekly, two of the sand cultures and two of the peat-on-sand cultures of each of the ten treatments received 500 milliliters of 1×10^{-8} thiamin chloride (Vitamin B_1) solution; similarly, two sand cultures and two peat-on-sand cultures of each treatment received 500 milliliters of 1×10^{-6} thiourea, and likewise distilled water was used to serve as a check. These applications, besides supplying the substances, also served to leach out possible salt accumulations during the week. Since the tap water con-

tained considerable quantities of nutrients[1/], distilled water was used after April 1.

Six weeks after planting, on the twenty-ninth or thirtieth of April, one of the three plants in each liner was removed. The roots were washed free of adhering sand and peat particles, allowed to drain on a wire screen for several minutes and weighed. After obtaining total fresh weight the current growth was pruned off and weighed separately. All the plant parts were then placed in individual manila bags and dried in an oven at 70° C. for three to four days until constant weight was obtained. A second plant of each liner was removed between the tenth and fourteenth of June, and treated similarly. The fullnutrient solution was then applied to all the remaining blueberry plants (one plant in each container) with the exception of those not receiving manganese. Nutrient deficient solutions were not applied after this date, other than to the minus manganese cultures. A daily record of symptom deficiency development was maintained throughout the experimental period.

Nitrogen Intake and pH of Plants in Nutrient Deficient Media. In December, 1941, 74 coffee urn liners were prepared with quartz sand, as in the 1940 nutrient experiments (cf. p.36). Dormant rooted blueberry cuttings were weighed individually and two cuttings were planted in each liner on January 7, 1942.

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1/ Analysis of the tap water showed the following in parts per million: silicon dioxide-12, iron-.02, calcium-8.6, magnesium-2.3, sodium-4.4, potassium-1.5, carbonic acid-23, sulfate-15.0, chloride 4.8, nitrate-1.6. Total dissolved solids-60.

Tap water was added for the first ten days. Because of the larger number of treatments, and the smaller number of liners in each treatment compared to the 1940 experiment, the nutrients were applied by hand. Six liners containing twelve plants were used for each treatment except the minus-N-P-K treatment for which only two liners were used.

The full nutrient solution, and those modifications that were used in the 1940 cultures to provide for the various deficiencies, were used again here, except that the manganese deficiency was omitted (cf. p. 37). In addition, calcium and boron were simultaneously omitted from the nutrient solution applied to one set of plants, as were potassium and iron, potassium and phosphorus, and nitrogen, potassium and phosphorus. The following modifications of the full nutrient solution were made to provide solutions deficient in the above combinations of ions.

Deficiency	Omission	Substitution
Calcium and boron	Calcium nitrate, Boric acid	Potassium nitrate
Potassium and iron	Monopotassium phosphate, Iron citrate	Monosodium phosphate
Phosphorus and potassium	Monopotassium phosphate	
Nitrogen, potassium and phosphorus	Monopotassium phosphate, Calcium nitrate, Ammonium sulphate	Calcium chloride

One half-liter of the respective nutrient solution was applied daily to each liner. Distilled water and chemicals of c.p. grade were used throughout. The liners were flushed with at least one liter of distilled water every two weeks in order to prevent salt accumulation. As an added precaution each solution was carefully adjusted to a pH of 4.8-5.0. by the use of hydrochloric acid.

The plants came out of dormancy slowly, so that it was not until January 30 that half the plants had at least one bud breaking. On February 22, four plants from each treatment were selected at random as samples for the determination of green and dry weights, pH, and total nitrogen. The total green weight of each plant was recorded immediately. Each plant was then separated into the current top growth, the old wood, and the roots, and the fresh weights of the separate parts were obtained.

The plants were dried as in the 1940 experiment, and the dry weights of the separate parts recorded. The dried current top growth, and root, samples, were ground in a Wiley mill without difficulty. The old wood, however, was so hard that a preliminary grinding in a pencil sharpener had to be resorted to before that material could be ground satisfactorily in the Wiley mill. Total nitrogen determinations were then made on the ground and dried material by the Kjeldahl method as modified by Wurneek and Heinze (127).

A second group of four plants from each treatment was removed on March 15, and the last group on April 10, 1942.

These plants were analyzed exactly as those removed on February 22.

Determinations of the pH of the plant sap were made by means of Coleman pH electrometer (model 3). The plant juice for the pH determinations was prepared by grinding a representative part of the current leaf and stem growth, immediately after removal of each plant, in a mortar with a few milliliters of distilled water and quartz sand, and decanting the supernatant liquid into the cuvette of the pH apparatus.

For the titration curves data, obtained in March, two grams of fresh leaves of the respective plant species growing in the greenhouse were thoroughly macerated with 100 milliliters of water in a Waring blendor, transferred, and made up to a volume of 250 milliliters with additional distilled water. A 50 milliliter aliquot was removed, to which increasing quantities of N/15 hydrochloric acid were added (table 11), with the pH being measured following each addition of the acid. Another 50 milliliter aliquot was similarly treated with increasing additions of N/15 sodium hydroxide.

In order to separate the water soluble constituents of the blueberry leaf tissue from the residual solids, additional blueberry leaves were blended in April, and filtered through a number 1 Whatman filter paper, and made up to 250 milliliters. The residue was then dispersed for several minutes in additional 250 milliliters of distilled water. Only 25 milliliter aliquots were used for these titrations and con-

sequently the titration curves thus obtained are not directly comparable to those obtained for the material titrated in March.

The isoelectric point of the soluble proteins in the blueberry tissue was determined by adding 25 milliliter aliquots of the filtrate obtained in a similar manner from twenty grams of fresh leaf tissue and made up to a volume of 500 milliliters, to each of a series of beakers containing varying amounts of hydrochloric acid or sodium hydroxide. After the aliquot was added to a beaker the pH of its contents was determined, and it was then filtered and the precipitate dried down to constant weight and weighed. In this manner the weights of the soluble proteins precipitated at various pH values were obtained.

Plants Grown in Soil Media

Although stress was placed on the controlled pot culture method as a means of studying the nutritional requirements of the blueberry, it was considered advisable to make some studies with various soil types representing extremes that might give some lead as to the effect of certain physical aspects of soils as well as the role of growth substances under such soil conditions. No attempt was made to go into detail on such specific factors as aeration, etc.

Soil Types. Four ground beds were prepared during February, 1940, from four different soil types:[2/] (1) a red

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2/ These soil types have been identified as: (1) Collington silt loam, (3) Portsmouth loam, and (4) Sassafras silt loam.

silt-clay loam from the U. S. Soil Conservation Service tract at Beltsville, Maryland, upon which blueberries occurred naturally, (2) a gravelly loam from a stream bank in College Park, Maryland, (3) a soil from a low spot at College Park having the characteristic bluish cast of a swamp soil, and (4) a brown silt loam from a field at College Park which had been under cereal and hay crop cultivation for many years. The area of each bed was 15 x 9 feet, and the depth one foot. The subsoil consisted mostly of gravel, and additional drainage was provided by an adequate system of tile pipes.

Samples for mechanical soil analyses, taken on August 7, 1940, were analyzed in the soils laboratory of the University of Maryland through the courtesy of Dr. R. P. Thomas, by the hydrometer method.

On March 23, 1940, eighteen dormant rooted blueberry cuttings were planted in each bed, each plant occupying 2.5 x 3 feet. Six plants in each bed were fertilized with potassium chloride, and six with sodium acid phosphate at the rate of 200 pounds per acre, while the remaining six plants in each bed remained untreated. All the beds were watered according to normal greenhouse practice.

During August 27 and 28 the top of each plant was removed and its fresh weight recorded. Each root system was then carefully washed out with a stream of water, and a record was taken of the spread of the roots in four horizontal directions, and the depth to which the deepest root penetrated. For statistical purposes these measurements were combined into one cubic

figure as the volume of soil penetrated by the root system by multiplying the square of the average of the four horizontal measurements by the vertical measure of the deepest root. 3/

After the root measurements were obtained, the excess moisture was removed from the roots by means of blotting paper, and the fresh root weights recorded.

Addition of Growth Substances. Ninety-nine ten-inch clay pots were filled with silt-clay loam soil 4/. The pots were arranged in a ground bed in the greenhouse in eleven rows of nine pots each. On March 21, 1940 one dormant rooted Cabot blueberry cutting was placed in each pot. To ten of the rows of pots, ten different substances were applied in three concentrations as follows:

Substance	Concentration		
	High	Medium	Low
Naphthaleneacetamide	1×10^{-5}	1×10^{-6}	1×10^{-7}
Naphthaleneacetic	1×10^{-5}	1×10^{-6}	1×10^{-7}
Indoleacetic acid	1×10^{-6}	1×10^{-7}	1×10^{-8}
Indolebutric acid	1×10^{-6}	1×10^{-7}	1×10^{-8}
Indolepropionic acid	1×10^{-6}	1×10^{-7}	1×10^{-8}
Indoleacetamide	1×10^{-5}	1×10^{-6}	1×10^{-7}

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3/ It is recognized that such a figure cannot and should not be taken as the actual volume of soil thoroughly penetrated by roots. The figures thus obtained are used only for purposes of comparison.

4/ Same as soil number 1 in the soil type experiment cf. p. 42-43.

Photo Sen Sin	1×10^{-4}	1×10^{-5}	1×10^{-6}
Thiourea	1×10^{-5}	1×10^{-6}	1×10^{-7}
Ascorbic acid	1×10^{-5}	1×10^{-6}	1×10^{-7}
Thiamin chloride	1×10^{-7}	1×10^{-8}	1×10^{-9}

Thus each concentration of each substance was applied to three pots with a total of nine pots for each substance. The plants were dipped in a solution of the respective substance before planting, and the various solutions were then applied once weekly at the rate of one-half liter per pot. The remaining row of nine pots was treated with similar quantities of distilled water, and served as a check plot. The plants were examined frequently for any visible evidence of plant response.

After about five months of treatment, all the 99 plants were removed on August 28-30, 1940. A quantitative measure of growth response was obtained by recording top and root weights, and root measurements, as was done in the soil type experiment (cf. p. 43).

Statistical Analyses

Wherever possible, the data on growth responses, plant weights, nitrogen contents, and pH determinations were analyzed by the use of analysis of variance and adjusted for variations in the original weights of the cuttings by the use of analysis of covariance as suggested by Mahoney and Baten (119). Where one to three plants were missing, the data were treated according to the method proposed by Baten (13).

In the February 22, 1942 group of plants, the removal was so arranged that all the missing plots of that experiment would occur in that group, and as a result eleven plants were missing, and consequently Snedecor's method of unequal numbers was used (168). The size of the dried and ground samples of this same group of plants was so small, that the replicates had to be grouped together in order to provide samples of sufficient size for the nitrogen determinations. Thus only one nitrogen determination could be made for each plant portion of each treatment. Since there were no replicates, a statistical analysis was impossible. In the other removals, it was still necessary to combine all the ground material from the four replicate plants in order to have sufficient quantity for duplicate samples. Differences between such duplicates, however, may be used to show the accuracy of the method, and not the variability, in percent nitrogen (44) between replicate plants. In the final removal, however, there was enough material from the full nutrient plants for individual plant nitrogen determinations, and the standard deviation in percent nitrogen between plants was determined. Total nitrogen contents of plants were calculated on the basis of the adjusted plant weights.

The pH data were not first transformed to hydrogen ion concentrations as suggested by Steinbauer (171), because the linear pH data were considered more useful for correlation and covariance purposes than the curvilinear hydrogen-ion concentration values. In most instances the correlations reported were calculated directly from the covariance data.

RESULTS

Under the pot-culture conditions with full nutrients, the blueberry plants grew rapidly and developed vigorous new growth and large green leaves similar to growth under optimum outdoor cultural conditions (fig. 2a). However, in the medium of peat-on-sand the plants were somewhat more vigorous, which held true for all nutrient treatments, except minus potassium.

The full-nutrient plants grown in the 1942 experiment developed more slowly than similar plants in the 1940 pot-culture experiment. The difference in growth rate may have been due to length of day, since the 1940 plants were grown from March until June, while the 1942 plants were grown from January until April. At the cessation of the experiment, on April 10, 1942, the full nutrient plants were apparently at the very beginning of a rapid period of growth, as evidenced by the comparatively large root system, and the many swollen buds about to leaf out.

Responses to Mineral Deficiencies

As detailed later, nutrient deficiencies for certain elements did not appear as quickly in peat-on-sand cultures as in sand cultures, and with minus-calcium and minus-sulphur no deficiency symptoms occurred with peat-on-sand cultures during the experiments. The addition of growth substances had no effect on these results with nutrients.

Deficiency Symptoms, (fig. 1). Deficiency symptoms appeared in the following order in the 1940 cultures. Kramer and Schrader (113) presented color illustrations of these same deficiencies.

Fig. 1.- Leaf deficiency symptoms of the Cabct blueberry (letters below leaves are the symbols for the respective mineral nutrients omitted)

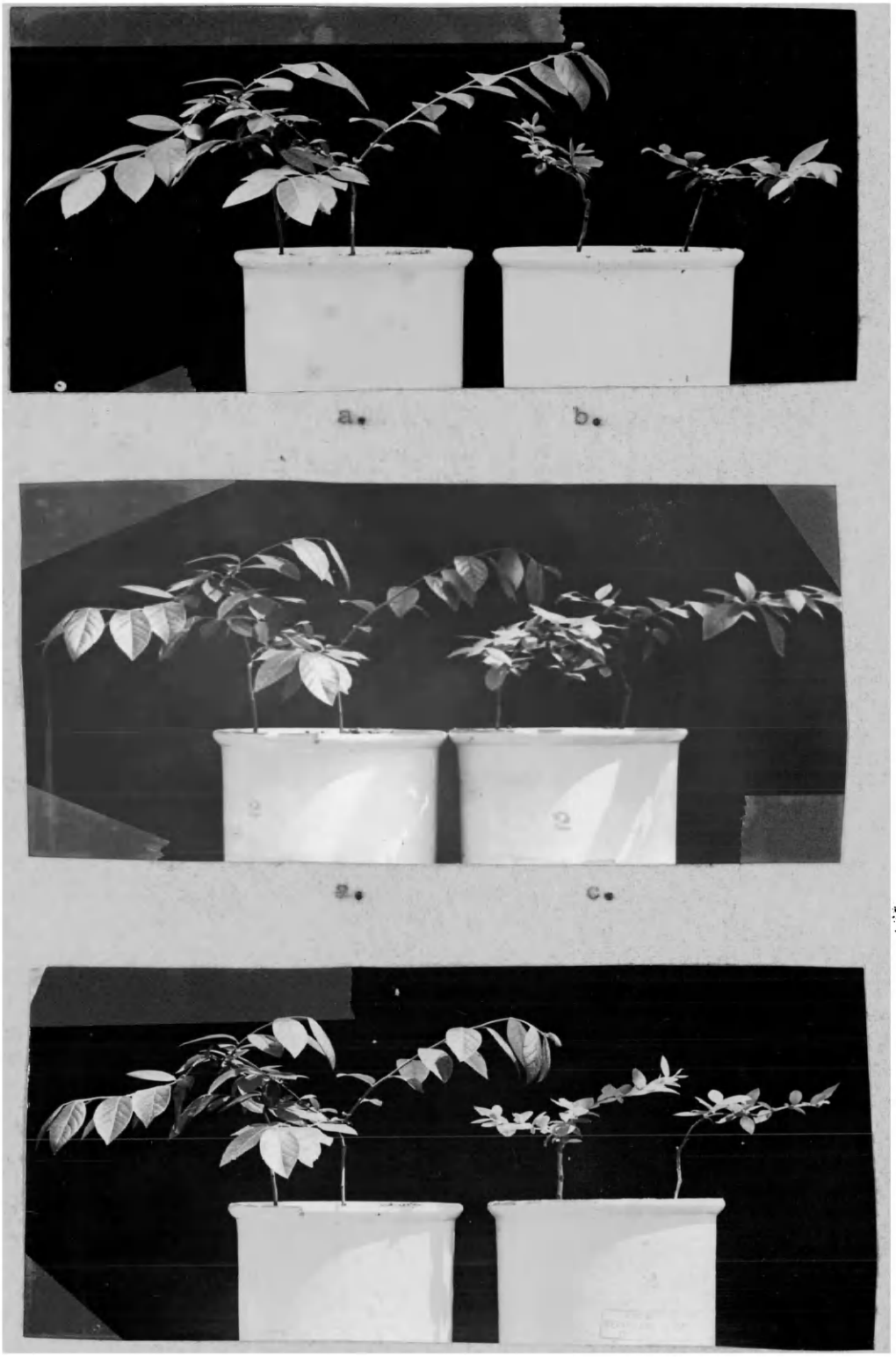

Fig. 2.- Relative growth of Cabot blueberry cuttings in substrates deficient in various mineral nutrients. a- full nutrient, b-minus nitrogen, c- minus potassium, d- minus sulfur

Nitrogen deficiency (fig. 2b) symptoms appeared during the first week of April, only ten days after the buds began leafing out. By April 30, the plants were so severely affected that small quantities of calcium nitrate had to be added to keep them alive. The early symptoms of uniform yellowing of the entire leaf surface was followed by a reddening of the leaves, and dying. All the leaves were affected. The entire plants, tops and roots were severely stunted.

Potassium deficiency (fig. 2c) was next to become evident, being readily discernible by April 20. The sequence of symptom expression was apparently complicated by the phenomenon of periodic abortion of the terminal growing points, a phenomenon which seems to be characteristic of most Vaccinium species in the field as well as in the greenhouse. Consequently the more severe symptoms of marginal scorching and development of necrotic spots throughout the leaves appeared first on the older leaves, while interveinal chorosis, similar to what has been described as iron chlorosis (7, 8), appeared later on the new growth arising from axillary buds stimulated into growth after the terminal growing point aborted.

Sulphur deficiency (fig. 2d) was apparent only in the pure-sand cultures. Symptoms were similar to those caused by nitrogen deficiency in the early stages, except that the yellowing had a more bleached appearance. Whereas the plants lacking nitrogen turned red at a later stage, those lacking sulphur turned a faint pink. The older leaves retained their green coloration.

Calcium defieiency (fig. 3b) symptoms also appeared in the sand cultures only. The leaf symptoms were similar to those induced by potassium deficiency on the younger growth (iron deficiency ?), but the regions adjacent to the veins that remained green were considerably narrower. The veins proper remained green, in contrast to the plants lacking sulphur. In advanced stages, it was difficult to distinguish between the leaf symptoms of the calcium and the sulphur deficiencies. Both tops and roots of the calcium deficient plants were not as severely stunted as the sulphur deficient plants.

Boron deficiency (fig. 3c) symptoms appeared with amazing abruptness. On May 8, all plants appeared to be healthy, while on the following day all the blueberry plants growing in the sand medium were decidedly affected. Plants growing in peat showed no deficiency symptoms until June 7. The first evidence of deficiency was a bluish color of the terminal growing point, followed by chlorotic spotting of the young leaves directly below the terminal. As the deficient condition progressed, the young leaves became badly blotched and misshapen.

The necrosis of the terminal growing points caused by boron deficiency apparently stimulated the axillary buds into growth, and the symptoms first evidenced at the terminal growing points were repeated at each axillary growing point. These symptoms may be clearly distinguished from the natural

a. b.

c. c.

a. d.

Fig. 3.- Relative growth of Cabot blueberry cuttings in substrates deficient in various mineral nutrients a- full nutrient, b- minus calcium, c- minus boron, d- minus phosphorus

abortion of the terminals, since the latter is definitely limited to the growing point, and the young leaves remain entirely normal; moreover, the discoloration of the naturally occurring abortion is brown, not blue.

Phosphorous deficiency (fig. 3d) symptoms were not readily discernible until June 5, when they appeared as a slight purpling of the leaves and stems. Dwarfing of the plants, however, was noticeable as early as May 5. The color of the leaves was decidedly dull in contrast to the bright, glossy color of healthy blueberry leaves.

Magnesium deficiency (fig. 4b) did not cause as severe dwarfing as phosphorus deficiency, but it exhibited a very distinct symptom by May 15. The leaf margins became uniformly chlorotic while the leaf area near the midrib retained the normal green color. At a later stage, the chlorotic area became red and necrotic, while the rest of the leaf area remained green. The older leaves were affected.

Iron deficiency (fig. 4c) in contrast to expectation, appeared as late as May 15, and then in the sand cultures only, as interveinal chlorosis of the younger leaves, very similar, but not as severe as the potassium deficiency symptom on the younger leaves.

Manganese deficiency (fig 4d) did not become clearly apparent throughout the experimental period, although some manganese deficient cultures were continued until the end of July. 1/ There was some indication that lack of magnanese

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1/ The nutrient solutions were made with tap water after June 15.

a. b.

a.

a. d.

Fig. 4.- Relative growth of Cabot blueberry cuttings in substrates deficient in various mineral nutrients a- full nutrient, b- minus magnesium, c- minus iron, d- minus manganese

resulted in a breakdown of the axillary buds.

In the 1942 experiment all the plants grew more slowly, and consequently the deficiency symptoms (fig. 5) were delayed in appearance. Nitrogen, potassium, and sulfur deficiency symptoms were practically identical to the symptoms observed on similarly treated plants in 1942. Some of the phosphorus deficient plants showed some mottling in addition to the dull color. Magnesium symptoms appeared to be more severe in 1942, while calcium and boron deficiencies were only beginning to appear at the time of the last removal.

Omission of both phosphorus and potassium (fig. 5a) apparently resulted in greater injury than the single deficiency of either element. These plants were severely stunted, mottled, and scorched, and exhibited much purple discoloration particularly in the leaf petioles.

The simultaneous omission of potassium and iron (fig. 5b) from the nutrient solution, on the other hand, resulted in less injury than the omission of potassium alone, in that there was little evidence of scorching, but there was considerable mottling, more than was evident in the minus-iron plants.

The combined omission of calcium and boron (fig. 5c) differed little from either single omission. Boron deficiency symptoms were only beginning to be apparent on April 10.

When nitrogen, phosphorus, and potassium (fig. 5d) were all omitted, the plants seemed to fare better than the minus-nitrogen plants, in that they did not become chlorotic

Fig. 5.- Relative growth of Cabot blueberry cuttings in substrates deficient in various combinations of mineral nutrients. (letters below illustrations are symbols of mineral nutrients omitted)

as quickly as the minus nitrogen plants, but the green color faded out very gradually and uniformly.

Fresh and Dry Weights. Inasmuch as growth substance application in the 1940 pot culture experiments had no significant effects, the six plants of each nutrient treatment for each medium which received these growth substances, were included in studies of fresh and dry weights, as affected by nutrients and media.

The fresh weights of the 1940 plants removed after six weeks' growth are given in table 1. At this early stage, only nitrogen, potassium, and phosphorus deficient plants showed a significantly lower top weight; and only potassium and nitrogen deficient plants showed a significaty lower root weight than the plants receiving the full nutrient solution and growing in the peat-on-sand medium. In the sand medium, the top weight of the calcium deficient plants was also significantly low, while that of the potassium deficient blueberry plants was not, and only the nitrogen deficient plants had a significantly lower root weight than the full nutrient plants.

By June 10-14, after twelve weeks of growth, additional differences became apparent (fig. 6, table 2). At this time the factor of media was responsible for very significant differences in both top and root weights, as indicated by the values of F for media in table 2. All but the potassium deficient plants made better growth in peat-on-sand than in sand.

Table 1 — Fresh weights and percent dry weights of blueberry plants removed April 29 - May 1, 1940. 1/

(Weight in grams, average of six plants)

Deficiency	Fresh weights								Percent dry weight			
	Current top growth		Root growth 2/		Total Growth		Shoot-root 2/ ratio		Current top growth		Total plant growth	
	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand
None	2.43	2.17	10.59	8.32	13.02	10.49	.23	.26	31.0	34.3	28.5	41.7
Manganese	2.14	1.96	9.41	7.39	11.55	8.37	.23	.27	31.2	33.3	29.3	45.5
Iron	2.40	2.02	7.70	8.06	10.10	10.08	.31	.25	28.8	32.7	33.2	40.8
Sulphur	1.92	1.71	12.61	7.98	14.53	9.69	.15	.21	31.0	33.0	24.0	44.0
Calcium	1.96	1.45	10.88	10.06	12.84	11.51	.18	.14	30.5	29.3	29.5	38.8
Boron	2.27	2.15	9.61	8.76	11.88	10.91	.24	.25	29.3	32.8	29.0	35.8
Magnesium	1.96	2.04	10.59	8.57	12.55	10.61	.19	.24	31.8	32.0	27.8	41.3
Phosphorus	1.67	1.65	8.75	7.63	10.42	9.28	.19	.22	31.8	33.0	26.5	40.3
Potassium	1.61	2.25	6.51	7.67	8.86	9.82	.25	.29	31.5	32.7	31.2	42.0
Nitrogen	1.06	.89	5.56	5.59	6.62	6.48	.19	.16	34.5	35.6	41.5	45.8
Difference required for significance 3/	0.64		2.95						2.61		4.56	
F values for:												
Nutrients	5.36**		4.03**						5.31**		6.03**	
Media	0.056		7.08**						18.67**		256.66**	
Substances 4/	2.61		0.78						1.93		.90	

1/ All fresh weight values adjusted for variations in original weight of cuttings by the use of analysis of covariance.

2/ Entire weight, minus the current top growth, included in "Root growth".

3/ Upon the suggestion of Dr. A. F. Brandt, Acting Chief, Experiment Station Division, Soil Conservation Service, the accuracy of this value was tested by analyzing separately data of individual deficiency treatments against the data of the full nutrient treatment. Of the treatments tested by this more precise method, no additional significant values were obtained, and the values found to be significant by the more general method, remained significant when treated by the more precise method.

4/ Values for growth substances are not given, since they caused no significant differences when the data were treated by the more general and the more precise methods of analysis.

** Significant beyond the one percent point.

Table 2 — Fresh weights and percent dry weights of blueberry plants removed June 10-14, 1940. 1/

(Weight in grams, average of six plants)

Deficiency	Fresh weights								Percent dry weight			
	Current top growth		Root growth 2/		Total growth		Shoot-root 2/ ratio		Current top growth		Total plant growth	
	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand
None	12.33	8.63	22.13	14.38	34.46	23.01	.56	.60	35.5	39.2	22.5	29.3
Manganese	15.07	7.71	18.08	11.90	33.09	18.81	.84	.65	30.2	39.3	24.0	31.3
Iron	16.53	6.48	15.73	11.82	32.26	18.30	1.05	.55	30.0	40.5	24.7	30.3
Sulphur	10.41	3.41	20.16	9.24	30.57	12.65	.52	.37	33.7	53.0	20.8	38.5
Calcium	14.38	6.73	15.16	9.98	29.54	16.71	.95	.67	29.8	42.7	22.8	33.2
Boron	14.09	3.27	13.82	10.16	27.91	13.43	1.02	.32	31.0	48.8	23.5	33.5
Magnesium	11.85	6.13	12.87	13.60	24.72	19.73	.92	.45	31.7	39.7	25.2	27.5
Phosphorus	5.50	2.86	11.38	10.07	16.88	12.93	.48	.28	42.8	58.2	27.3	37.3
Potassium	5.27	6.82	8.10	11.50	13.37	18.32	.65	.59	39.7	42.0	28.7	31.2
Nitrogen 3/	4.01	1.94	7.97	7.01	11.98	8.95	.50	.28	45.0	48.8	27.3	42.7
Difference required for significance	3.64		5.54						5.78		4.57	
F value for:												
Nutrients	12.38**		4.73**						13.36**		5.93**	
Media	87.45**		16.64**						126.37**		148.91**	
Substances	.15		.11						2.86**		.64	
Nutr. X Media	4.31**		2.32*						4.33**		4.75**	

1/ All values adjusted for variations in original weight of cuttings by the use of analysis of covariance.

2/ Entire weight, minus the current top growth, included in "Root growth".

3/ Calcium nitrate added in small quantities from April 30, on.

* Significant beyond the five percent point.

** Significant beyond the one percent point.

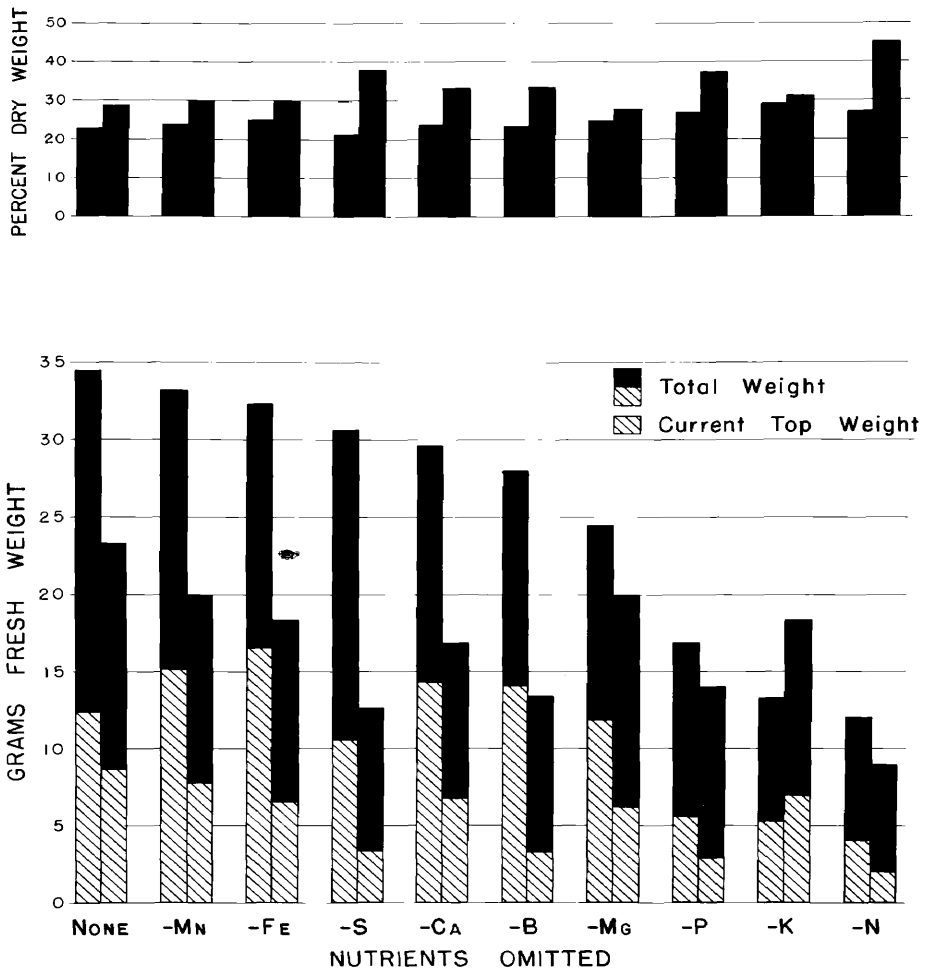


Fig. 6.- Fresh weights and percent dry weights of Cabot blueberry plants grown from rooted cuttings, March 15 to June 10, 1940. (Growth in the peat-on-sand medium is shown in the left of of each pair of columns, and growth in sand in the right)

Considering the blueberry plant as a whole, the various deficiencies as they appeared after twelve weeks of growth may be grouped into three catagories according to total weight of plants; namely, those that were not significantly poorer than the plants receiving the full nutrient solution, those significantly poorer, but as yet making some growth, and those so severely affected that they could make no further growth unless the deficiency were supplied promptly. Of the plants growing in the peat-sand medium, those receiving a nutrient solution deficient in manganese, iron, sulphur, and calcium fall in the first group; boron and magnesium in the second; and phosphorus, potassium, and nitrogen in the last catagory. In the sand medium, potassium and magnesium would move up to the first group, sulphur and boron would drop down to the last group, and calcium would remain alone in the middle group.

Medium	Not significantly lower than full nutrient 1/	Significantly lower but not severe	Severely affected
Peat-sand	Manganese Iron Sulphur Calcium	Boron Magnesium	Phosphorus Potassium Nitrogen
Sand	Manganese Iron Magnesium Potassium	Calcium	Sulphur Boron Phosphorus Nitrogen

1/ Although the plants deficient in the elements listed in this column did not have a significantly lower weight than the plants receiving all the nutrients, all but the manganese deficient plants exhibited definite deficiency symptoms. (cf. pp. 47-54). It is of interest to note that in the sand medium, only the anion deficiencies caused severe reduction in growth.

When top and root growth are considered separately, the results vary considerably (table 2). It appears that when manganese, iron, calcium, or boron was withheld from blueberry plants growing in the peat-sand medium, those plants actually made greater top growth than plants receiving the full nutrient solution. The iron-deficient plants made a significantly greater top growth, while the others approached a significantly higher difference. Phosphorus, potassium, or nitrogen deficiency, on the other hand, resulted in a significant decrease in top weight. In the sand medium, the full nutrient plants made the greatest top growth, and the nitrogen, phosphorus, sulphur, and boron deficient plants were significantly low. As for root weights, all full nutrient plants, the peat-sand and the sand grown, showed the greatest weight, being significantly higher than all but the manganese and sulphur deficient plants in peat-sand, but significantly higher than only the nitrogen deficient plants in sand.

These variations in shoot and root response are also expressed in terms of shoot-root ratios (tables 1, 2). Thus, the manganese, iron, calcium, boron, and magnesium deficient plants growing in peat-sand had a much higher ratio than the full nutrient grown plants, while the sulphur, boron, phosphorus, and nitrogen deficient plants growing in sand had a markedly lower ratio than the blueberry plants receiving the full nutrient solution.

The percent dry weight of the 1940 culture plants was not greatly influenced by the various nutrients during the

first six weeks' growth (table 1). Only the nitrogen deficient plants, which were already severely affected tended to have a higher proportion of solids. The dry weight of the top growth was about 31 percent in the peat-sand, and 33 percent in the sand medium. The entire plants averaged about 28 percent in the peat-sand, and 40 percent in the sand. In the peat-sand medium the tops contained a greater percent dry weight than roots, while in the sand medium the roots had the greater percent dry weights, compared to tops.

After twelve weeks' growth it became clearly apparent that those plants that made the greater fresh weights also had the smaller percentage of dry weight (fig. 6). When calculated, this relationship resulted in a negative correlation of -.71. Such a highly significant correlation indicated the possibility that the difference in fresh weight given in tables 1 and 2 mightbe due to variations in moisture content alone. However, dry weight data analyses showed essentially the same relationships as were shown by the fresh weight data (compare tables 2 and 3), except that the differences between the treatments were greater on a fresh weight basis,and therefore more distinct.

The fresh weight determinations for the plants grown in 1942 are given in figure 7, and tables 4, 5, and 6 for the first, second, and third removals respectively. Whereas the 1940 plants leafed out within ten days after planting, only one-half the total number of the 1942 plants had one or more open buds by January 30, although they were planted on January 7, 1942. The F values for total, current top growth, old wood, and roots

Table 3 — Dry weight of current top growth of plants removed June 10-14, 1940. 1/

(Weight in grams, average of six plants)

Deficiency	Peat	Sand
None	4.27	3.24
Manganese	4.50	3.03
Iron	4.93	2.59
Sulphur	3.32	1.74
Calcium	4.25	2.58
Boron	3.98	1.64
Magnesium	3.70	3.06
Phosphorus	2.10	1.73
Potassium	2.23	2.34
Nitrogen	1.62	1.04
Difference required for significance		1.04
F values for:		
Nutrients		11.04**
Media		51.68**
Substances		.22
Nutr. X Media		2.45*

1. All data adjusted for variations in original weight of cuttings by the use of analysis of covariance.

* Significant beyond the five percent point.

** Significant beyond the one percent point.

Table 4 — Fresh weights and percent dry weights of blueberry plants removed February 22, 1942. 1/ (Weight in grams, average of four plants grown from rooted cuttings placed in sand culture January 7, 1942)

Deficiency	Fresh weights				Shoot root ratio 2/	Percent dry weight			
	Total	Current top growth	Old wood	Roots		Total	Current top growth	Old wood	Roots
one	6.42	1.05	3.12	2.27	0.46	31.8	25.4	46.6	14.2
itrogen	6.63	0.42	2.71	3.50	0.12	35.8	37.7	59.3	17.4
hosphorus	6.38	0.83	3.32	2.23	0.37	33.3	25.4	47.6	22.3
otassium	6.70	0.82	3.38	2.50	0.33	32.5	25.4	47.3	14.7
alcium	5.73	0.80	2.79	2.14	0.37	28.6	25.7	49.0	16.6
agnesium	7.74	0.78	3.23	3.73	0.21	32.1	19.1	47.9	21.1
ulphur	8.23	1.21	4.20	2.82	0.43	34.0	27.5	48.2	15.8
oron	7.50	0.90	4.43	2.17	0.42	36.9	26.2	49.1	16.7
ron	6.91	1.04	3.43	2.44	0.42	33.8	25.3	47.4	18.3
a and B	8.45	1.50	4.99	1.96	0.76	36.7	28.3	47.3	16.2
and Fe	6.81	1.10	3.21	2.50	0.44	33.5	24.3	48.3	18.5
and K	7.34	0.89	2.93	3.52	0.25	30.5	20.8	44.1	21.7
, P, and K	6.54	0.48	4.46	1.60	0.30	42.9	50.0	48.2	27.3
value	0.42	1.64	1.28	0.78					
ifference required or significance	3.04	0.77	1.78	2.38					
orrelation with ith original plant eight	.964	.812	.856	.909					

/ All fresh weight values adjusted for variations in original weight of cuttings by the use of analysis of covariance.

/ Current top growth divided by root growth.

Table 5 — Fresh weights and percent dry weights of blueberry plants removed March 15, 1942. 1/

(Weight in grams, average of four plants, grown from rooted cuttings in sand culture, January 7, 1942).

Deficiency	Fresh weights				Shoot root ratio 2/	Percent dry weight			
	Total	Current top growth	Old wood	Roots		Total	Current top growth	Old wood	Roots
None	10.37	3.25	3.47	3.65	.89	31.1	25.9	46.1	21.6
Nitrogen	5.81	1.30	2.74	1.77	.74	35.5	30.1	46.8	21.9
Phosphorus	6.79	1.82	2.83	2.14	.86	34.0	29.3	47.6	19.9
Potassium	7.07	1.85	3.27	1.95	.95	35.2	31.0	48.1	18.1
Calcium	8.33	2.11	3.53	2.67	.78	31.2	26.7	44.4	17.5
Magnesium	10.58	1.96	3.18	5.44	.36	28.1	27.6	46.8	17.3
Sulfur	8.30	1.90	4.19	2.21	.86	37.3	31.8	49.4	19.4
Boron	7.46	1.55	2.60	2.31	.67	26.9	26.6	46.7	16.7
Iron	8.31	2.33	2.49	3.49	.67	30.6	26.4	47.2	21.3
Ca and B	7.22	2.12	2.84	2.26	.93	31.3	27.8	46.0	15.8
K and Fe	8.23	2.43	3.47	2.33	1.04	34.0	29.9	46.8	19.4
P and K	6.82	2.10	2.78	1.94	1.08	32.8	27.6	47.1	18.2
F value	1.17	1.15	0.82	2.42*					
Difference required for significance	3.99	1.30	1.56	1.80					
Correlation with original plant weight	.838	.824	.759	.757					

1/ All fresh weight values adjusted for variations in original weight of cuttings by the use of analysis of covariance.

2/ Current top growth divided by root growth.

* Significant beyond the five percent point.

Table 6 -- Fresh weights and percent dry weights of blueberry plants removed April 10, 1942. 1/ (Weight in grams, average of four plants, grown from rooted cuttings planted in sand culture, January 7, 1942).

Deficiency	Fresh weights				Shoot root ratio 2/	Percent dry weight			
	Total	Current top growth	Old wood	Roots		Total	Current top growth	Old wood	Roots
None	18.12	4.48	4.04	9.60	.47	27.6	30.3	42.5	20.1
Nitrogen	5.62	0.55	3.09	1.98	.28	35.5	33.6	42.8	24.5
Phosphorus	7.03	2.04	2.56	2.43	.84	33.1	34.4	45.7	18.7
Potassium	10.19	3.08	3.99	3.12	.98	31.9	29.7	45.7	16.5
Calcium	11.93	3.13	3.47	5.33	.58	29.8	29.1	44.8	20.5
Magnesium	12.29	4.05	3.98	4.26	.95	29.8	29.3	46.8	14.5
Sulfur	11.98	2.80	3.56	5.58	.50	30.1	34.4	45.7	18.0
Boron	11.63	3.95	3.76	3.92	1.01	30.4	28.6	47.1	16.2
Iron	13.23	4.92	3.59	4.72	1.04	27.4	28.7	43.2	14.1
Ca and B	15.69	3.59	5.93	6.17	.58	31.6	31.8	47.2	16.5
K and Fe	12.62	4.18	4.08	4.36	.96	32.7	30.3	47.7	21.1
P and K	6.45	1.71	2.37	2.37	.72	34.0	34.6	47.6	20.1
N, P. and K 3/	7.05	1.00	3.22	2.83	.35	38.8	41.3	49.0	26.3
F value	3.23**	5.00**	2.06	3.67**					
Difference required for significance.	7.06	1.60	1.83	3.11					
Correlations with original plant weights	0.712	.693	.695	.699					

1/ All fresh weight values adjusted for variations in original weight of cuttings by the use of analysis of covariance.

2/ Current top growth divided by root growth.

3/ One plant only.

** Significant beyond the one percent point.

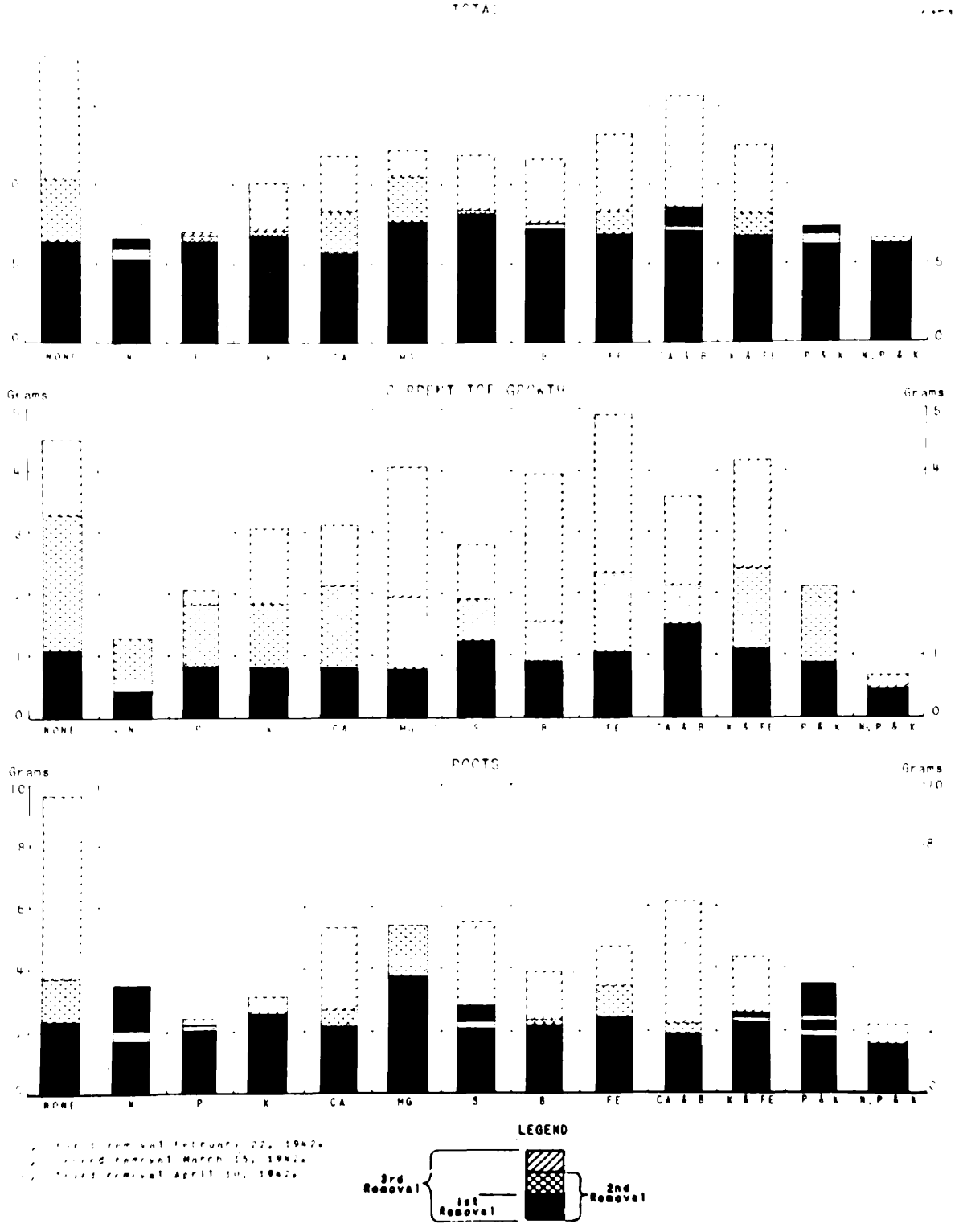


Fig. 7.- Fresh weights of Cabot blueberry plants grown from rooted cuttings, January 7 to April 10, 1942. (letters below columns are symbols for respective mineral nutrients omitted)

all show that there were no significant differences between the fresh weights of the plants that were removed on February 22 (table 4). The very low weights for the current top growth of the minus-nitrogen and the minus-N-P-K plants, however, suggest that had there been more replications, the minus-nitrogen plants would have shown significantly less new growth development.

The only significant F value in table 5, is that for the fresh root weights, where the nitrogen deficient plants have significantly less weight of roots than the full nutrient plants. By April 10, however, when the last removal was made, significant differences (table 6) were obtained for all fractions of the plant, except the old wood. Nitrogen, phosphorus, sulfur, phosphorus-potassium, and N-P-K deficient plants showed significantly low current top weights, thus comparing favorably with the 1940 results for deficiencies of nitrogen, phosphorus, and sulfur in the sand medium. All the deficiencies showed significantly lower root weights than the full nutrient plants, the root weights of the minus nitrogen, phosphorus, phosphorus-potassium, and N-P-K deficient plants being especially low.

The relative growth increments during the entire period of the 1942 experiment are graphically shown in figure 7, for easy comparison of plants at all three stages of development. It may be readily seen that there was an actual loss in weight of the minus-nitrogen, and the minus phosphorus-potassium plants as the season progressed, while the minus phosphorus, and the minus N-P-K plants were at practically the same weights at the first and third removals. Although all plants continued to

make some gains in the weight of their current top growth, the severe losses in the root weights of some of the plants more than offset the slight gains made by the tops of those plants which showed a net loss in total weight. Observations of the plants upon their removal indicated that a major part of the original root system of all the plants disintegrated, and where vigorous root regeneration did not occur, there was a loss in root weight.

Nitrogen Uptake and Translocation. Total nitrogen data, expressed as absolute amounts, and as percent of dry weight, are given in tables 7, 8, and 9 for the plants sampled at the first, second, and third removals respectively, during 1942. Total nitrogen values in milligrams per plant are condensed for all three dates in figure 8, and the percent total nitrogen, in figure 9. As with the fresh weights the total nitrogen content of the minus-nitrogen, and minus N-P-K plants actually decreased between the first and third harvests for the entire plants, while the nitrogen content of the roots decreased also for the minus-phosphorus and minus-phosphorus-potassium plants. It is interesting to note that despite some increase in the fresh weights of the current top growth of the nitrogen deficient plants, their nitrogen content nevertheless decreased.

In the plants removed on February 22, 1942, the percent nitrogen (fig. 9) showed no wide difference as a result of the nutrient deficiency treatments. The nitrogen concentration in the old wood and roots remained about as it was at the end of the

Table 7 — Percent and total nitrogen, and pH of blueberry plants, removed February 22, 1942.

(Average of four plants grown from rooted cuttings planted in sand culture, January 7,1942)

Deficiency	Total Nitrogen (milligrams per plant)				Percent nitrogen (of dry weight)			pH	
	Total	Current top growth	Old wood	Roots	Current top growth	Old wood	Roots	Leaves	Leachings
None	24.32	7.79	11.34	5.19	2.92	0.78	1.61	3.66	4.4
Nitrogen	24.16	5.13	12.70	6.33	3.24	0.79	1.04	3.69	6.0
Phosphorus	27.13	6.31	14.85	5.97	2.99	0.94	1.20	3.70	4.4
Potassium	21.70	6.39	10.39	4.92	3.07	0.65	1.34	3.67	4.2
Calcium	—	6.85	10.39	—	3.33	0.76	—	3.90	4.5
Magnesium	27.44	5.00	12.53	9.91	3.35	0.81	1.26	3.78	4.4
Sulfur	27.90	9.28	13.36	5.26	2.78	0.66	1.18	3.67	4.5
Boron	—	—	16.10	3.35	—	0.74	0.92	3.90	4.6
Iron	26.05	7.66	13.17	5.22	2.91	0.81	1.17	3.65	4.2
Ca and B	29.58	11.93	14.16	3.49	2.81	0.60	1.10	3.95	4.3
K and Fe	23.51	6.79	11.63	5.09	2.54	0.75	1.10	3.58	4.5
P and K	21.66	7.61	9.95	7.10	2.49	0.77	0.93	3.72	4.1
N, P, and K	—	—	—	—	—	—	—	4.42[1]	
F value	—	—	—	—	—	—	—	8.37**	
Difference required for significance								0.15	
Correlation with original plant weight								-.121	

** Significant beyond the 1 percent point.

[1] Only one plant — not included in statistical analysis.

Table 8 — Percent and total nitrogen, and pH of blueberry plants removed March 15, 1942.

(Average of four plants grown from rooted cuttings planted January 7, 1942)

Deficiency	Total nitrogen (milligrams per plant)				Percent nitrogen (of dry weight)			pH		
	Total	Current top growth	Old wood	Roots	Current top growth	Old wood	Roots	Young leaves	Old leaves	Leachings
None	42.9	22.6	11.7	8.6	2.69	0.73	1.09	3.45	3.70	4.01
Nitrogen	13.5	5.1	6.7	1.7	1.31	0.52	0.44	3.56	3.67	5.93
Phosphorus	24.2	11.7	8.4	4.1	2.20	0.62	0.96	3.55	3.74	3.98
Potassium	30.4	14.6	11.5	4.3	2.56	0.73	1.18	3.75	3.96	4.02
Calcium	30.6	14.0	11.1	5.5	2.50	0.71	1.18	3.81	4.26	4.25
Magnesium	39.5	16.4	14.9	8.2	3.03	1.00	0.87	3.80	4.17	4.31
Sulfur	28.0	12.3	12.6	3.1	2.05	0.61	0.73	3.70	3.86	4.08
Boron	24.5	10.2	9.1	5.2	2.48	0.75	1.33	3.50	3.85	4.13
Iron	31.6	15.1	8.7	7.8	2.44	0.74	1.06	3.64	3.82	3.99
Ca and B	32.3	14.1	11.5	6.7	2.39	0.88	1.50	3.63	3.94	4.06
B and Fe	35.7	16.7	11.2	7.8	2.29	0.69	1.26	3.65	4.00	4.01
Ca and K	29.0	14.6	10.6	3.8	2.52	0.81	1.09	3.50	3.68	3.98
F value								1.25	3.90**	146.4**
Difference required for significance								0.305	0.272	0.04

** Significant beyond the one percent point.

Table 9 — Percent and total nitrogen, and pH of blueberry plants removed April 10, 1942.

(Average of four plants grown from rooted cuttings planted in sand culture, January 7, 1942).

Deficiency	Total nitrogen (milligrams per plant)				Percent nitrogen (of dry weight)			pH	
	Total	Current top growth	Old wood	roots	Current top growth	Old wood	Roots	Young leaves	Old leaves
None	79.0	35.6	15.8	27.6	2.62	0.92	1.43	3.43	3.77
Nitrogen	10.2	2.3	5.6	2.3	1.23	0.42	0.47	3.44	3.67
Phosphorous	37.3	19.2	12.4	5.7	2.73	1.06	1.25	3.77	4.45
Potassium	47.3	21.7	17.7	7.9	2.37	0.97	1.54	3.59	4.31
Calcium	51.8	24.1	14.0	13.7	2.65	0.90	1.25	3.57	4.06
Magnesium	55.0	29.2	16.0	9.8	2.42	0.86	1.58	3.65	4.07
Sulphur	39.4	16.0	12.5	10.9	1.90	0.77	1.09	4.00	4.40
Boron	52.6	30.5	13.3	8.8	2.54	0.75	1.39	3.53	3.87
Iron	58.2	31.7	14.9	11.6	2.35	0.96	1.74	3.50	3.75
Ca and B	60.2	28.2	20.4	11.6	2.49	0.73	1.14	3.57	3.86
K and Fe	55.1	29.0	14.0	12.1	2.13	0.72	1.32	3.60	4.03
P and K	27.1	12.4	9.7	5.0	2.13	0.86	1.06	3.47	3.63
N, P, and K[1/]	15.3	4.5	6.9	3.9	1.15	0.44	0.53	3.67	3.70
F value								2.85**	2.37*
Difference Required for significance								0.268	0.466

1/ One plant only.

* Significant beyond the five percent point.

** Significant beyond the one percent point.

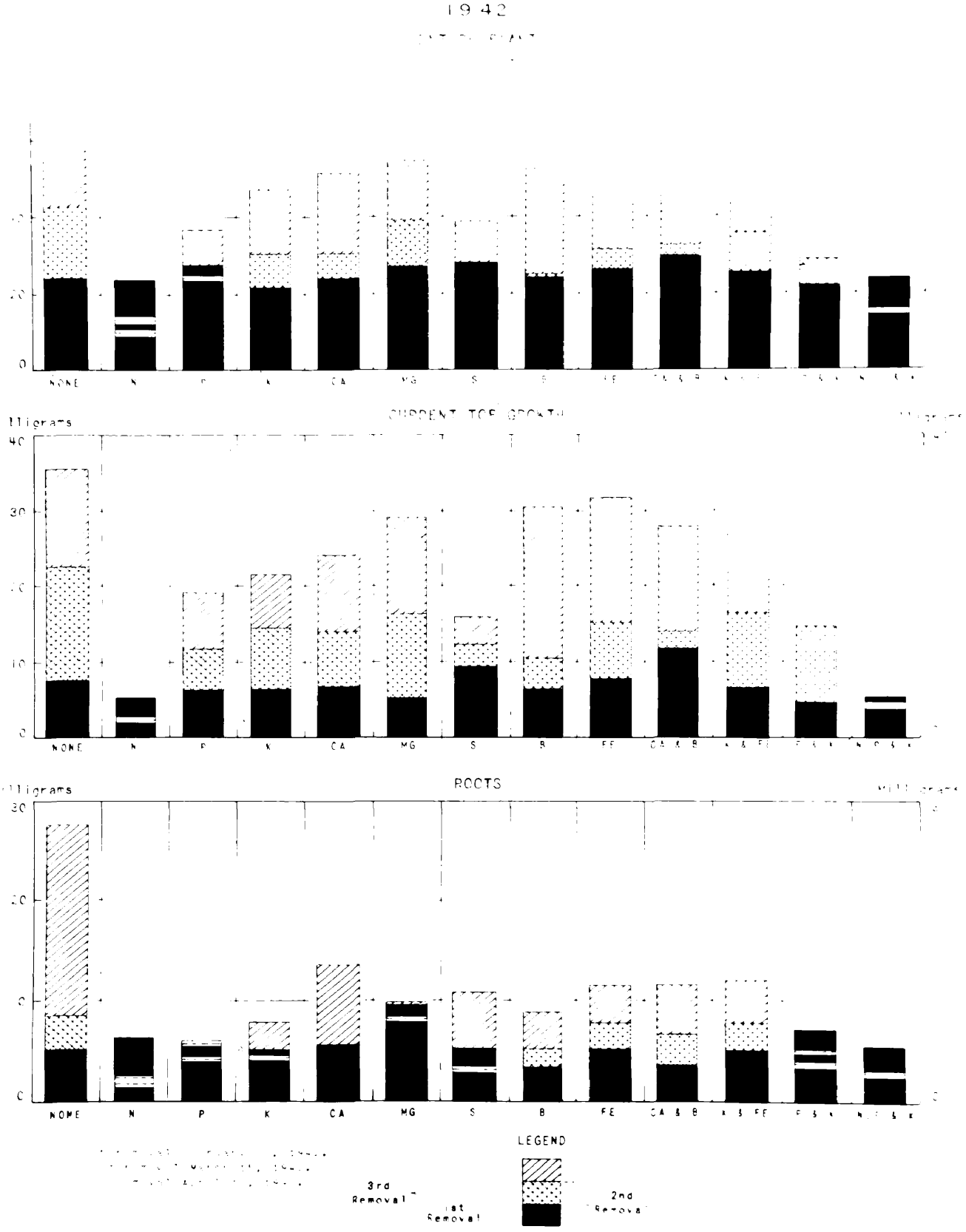


Fig. 8.- Total nitrogen content in milligrams per plant of Cabot blueberry plants grown from rooted cuttings, January 7 to April 10, 1942. (letters below columns are symbols for respective mineral nutrients omitted)

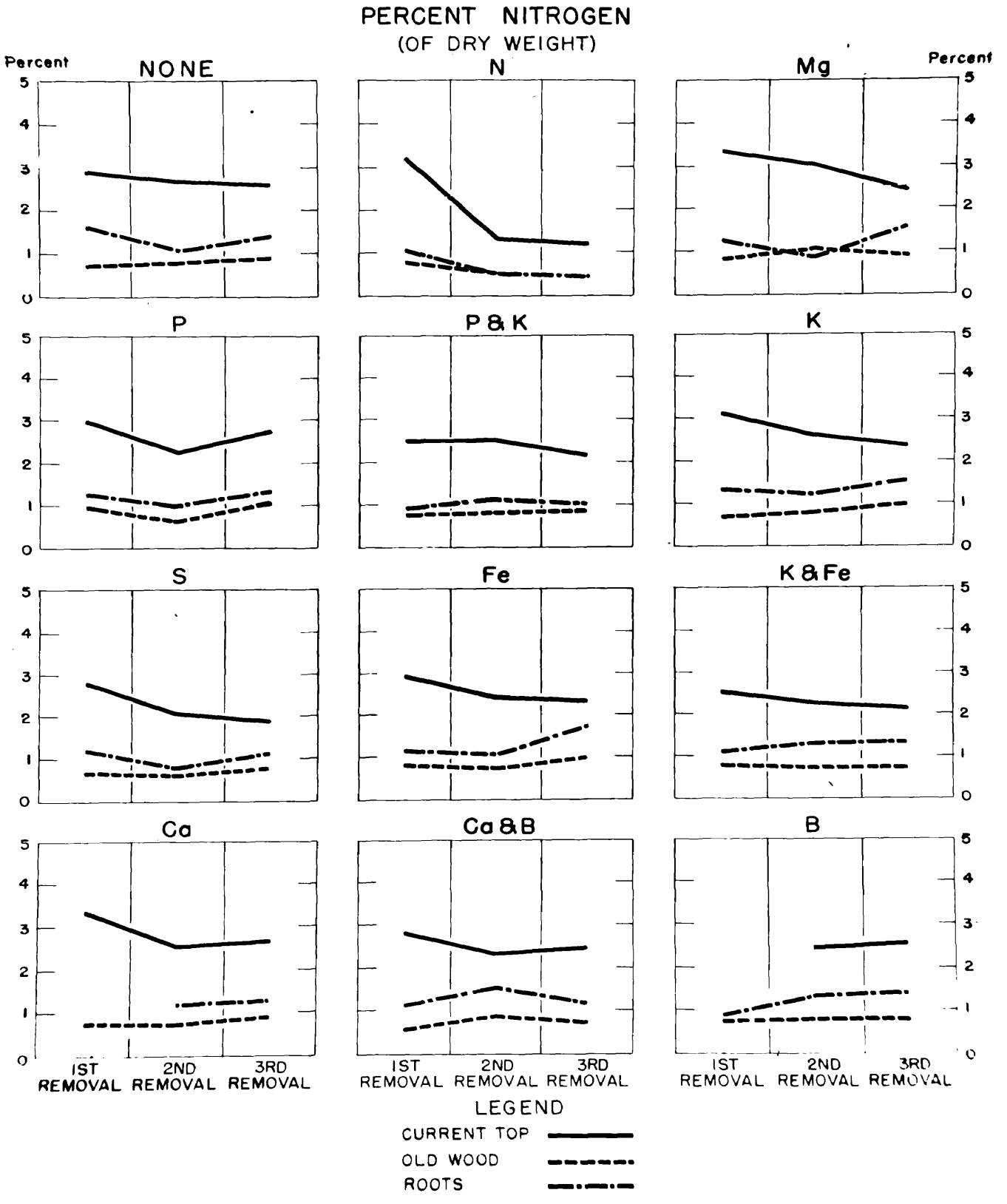


Fig. 9.- Trends in percent nitrogen (dry weight basis) of Cabot blueberry plants grown from rooted cuttings, January 7 to April 10, 1942. (letters above each block are symbols of respective mineral nutrients omitted)

dormant period, on February 1, 1942, at approximately 0.75 and 1.0 percent respectively, and about 3.0 percent for the new top growth. As the season progressed, the percent nitrogen in the current top growth of the full-nutrient plants decreased slightly, probably because of the increasingly larger proportion of wood to leaves, while the nitrogen percent of the old wood increased slightly. The nitrogen-deficient plants of course showed the greatest decrease in percent nitrogen in all parts, reaching the low levels of 1.23, 0.42, and 0.47 by April 10, 1942, for the current top growth, old wood, and roots respectively. The minus-N-P-K plants were also reduced to similar low nitrogen levels. The omission of sulfur from the nutrient solution also caused a marked reduction in the nitrogen level of plants so treated, while phosphorus-potassium, and potassium-iron deficient plants also had lower nitrogen levels in their current top growth. Some slight reductions in percent nitrogen were found when potassium or iron were omitted from the nutrient solution. Phosphorus deficient plants began showing a low level of nitrogen, but recovered to more than the full nutrient equivalent by the time of the third removal. None of the deficient plants showed consistent accumulations of nitrogen above the level of the full nutrient plants, but calcium, and particularly magnesium deficient plants, had higher nitrogen levels in the early stages of their growth.

There was no apparent tendency for nitrogen to move from one fraction of the plant to another when its concentration in the plant was reduced as a result of some mineral

nutrient omission. There was some indication, however, that nitrogen accumulates in the roots when potassium, or magnesium, or especially iron, is omitted from the nutrient solution.

In a comparable experiment with one year old field grown plants, the percent nitrogen on October 30, 1941, as the leaves were about to fall, was 1.31 for the leaves, 0.48 for the old wood, and 0.88 for the roots. When planted in glazed crocks, and supplied with the full nutrient solution, the percent nitrogen in the old wood rose to 0.74, and to 1.19 in the roots, at a stage when the buds of the plants were about to break. Besides showing the low nitrogen level of blueberry plants in the fall, these results also indicate that the nitrogen content of these older plants is similar to that of the cuttings.

As some index of the validity of the above conclusions, it may be possible to use the measure of variability in nitrogen contents among individual plants as determined in the last sampling date. Thus the standard deviations in percent nitrogen between individual full-nutrient plants removed on April 10, were 0.07 for the current top growth, 0.11 for the old wood, and 0.18 for the roots.

Hydrogen-ion Concentration. (tables 7, 8, 9). The pH data in tables 8 and 9 clearly indicate that the younger blueberry leaves are more acid than the more mature leaves. The difference between the acidity of the young and old leaves was least in the nitrogen deficient plants. In the first stage of growth the leaves of the minus-calcium, boron, and calcium-boron plants were significantly higher in pH value than the

full-nutrient leaves. At the second removal, calcium deficient leaves were still significantly less acid, as were the minus-magnesium leaves, while the pH values for the minus-potassium, and potassium-iron leaves approached a significant decrease in acidity. At the last removal, calcium, magnesium, and potassium-iron deficient leaves remained less acid, though not significantly less than the full-nutrient leaves, and phosphorus, potassium, and sulfur deficient leaves were all significantly higher in pH value than the full-nutrient leaves. In general, those parts of the leaves directly affected by lesions caused by some deficiency, were least acid, the pH values of such leaf areas reaching as high as 5.0 in some individual cases.

The leachings from all the pot cultures clustered about the pH value of 4.4 at the first removal, and 4.0 at the second removal, with the exception of the minus-nitrogen cultures, where the leachings averaged close to pH 6.0.

The pH titration curves for the leaf sap of blueberries and other small fruits are given in figure 10. The actual pH values are given in tables 11 and 12. It is obvious, from an examination of figure 10a that not only is the actual acidity of blueberry leaf sap strikingly greater than that of the other plant species, but the acid reserve of the blueberry is also much higher. The data presented in figure 10b indicate that practically all the acidifying substances are water soluble.

When aliquots of the water soluble filtrate of blueberry leaf tissue were added to solutions of varying pH, the greatest precipitation occurred at pH 5.0, thus indicating

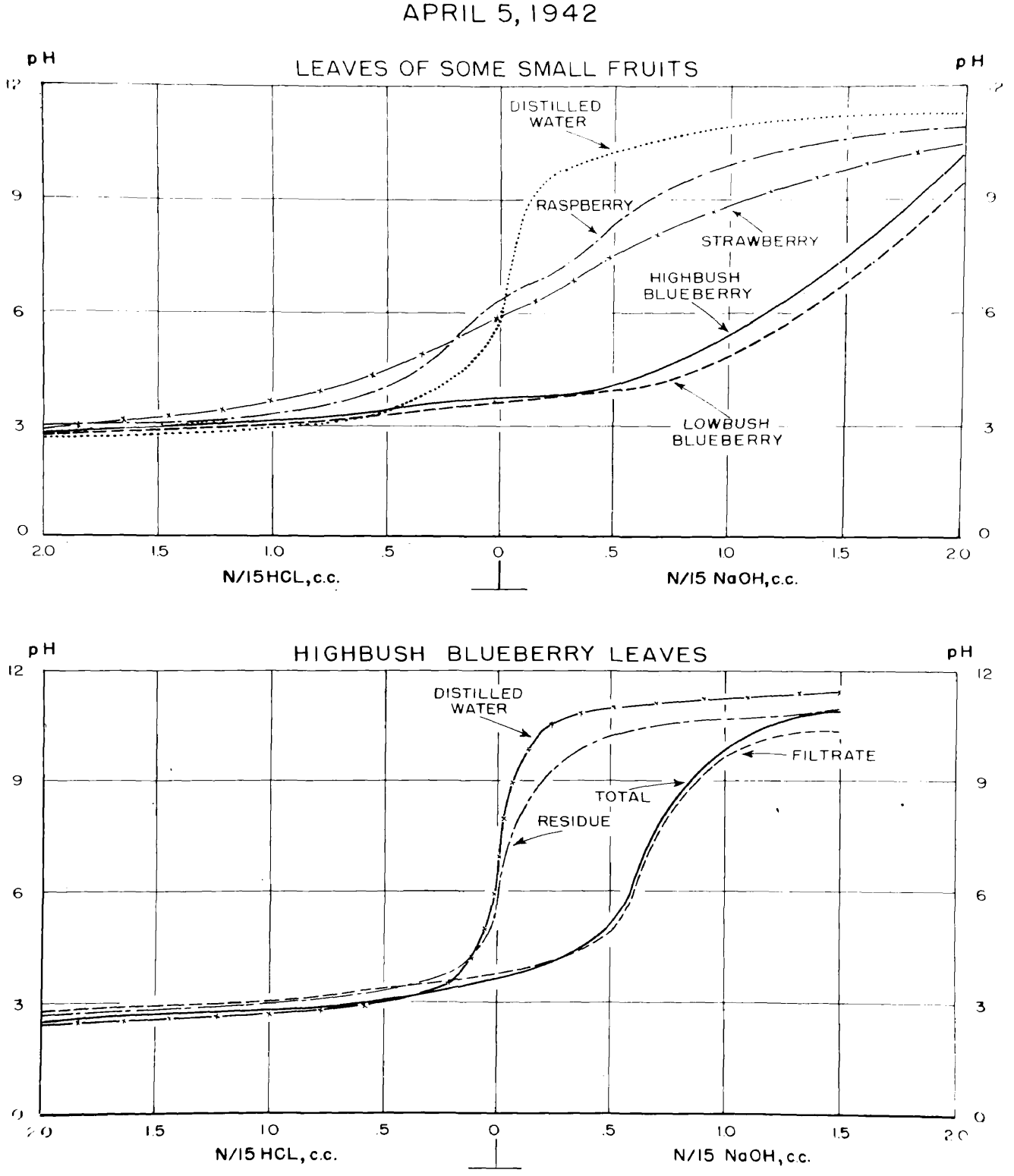


Fig. 10.- pH titration curves of leaf sap of some small fruits. a- effect of successive increments of acid and base to blueberry leaf tissue compared to other small fruits. b- effect of successive increments of acid and base to the water soluble filtrate of blueberry leaf tissue compared to the residual solids.

Table 11 -- pH values of leaf tissue of various species as affected by successive additions of acid or base to the sample.

Reagent added	Highbush blueberry		Lowbush blueberry	Raspberry		Strawberry		Tomato	Cabbage	Distilled water
	Dark leaves	Mottled leaves		Healthy	Nitrogen Deficient	Healthy	Diseased			
N/15 Na OH										
15 cc.	11.70	11.70	11.65	11.75	11.77	11.84	11.74	11.72	11.85	11.90
7 cc.	11.45	11.45	11.36	11.50	11.56	--	11.51	—	—	11.72
4 cc.	11.20	11.15	11.04	11.30	11.20	11.20	11.08	11.40	11.50	11.62
2 cc.	10.20	10.00	9.45	10.92	10.84	10.52	10.10	11.07	—	11.28
1 cc.	5.40	5.20	4.80	9.99	9.50	8.79	8.60	10.50	10.65	10.96
.5 cc.	4.18	4.06	4.00	8.36	7.54	7.53	7.52	8.73	9.90	10.35
.2 cc.	3.78	3.77	3.75	6.94	6.46	6.39	6.44	7.08	8.77	9.60
0	3.72	3.72	3.73	6.40	5.90	5.95	5.94	6.15	6.59	6.25
N/15 HCl										
.2 cc.	3.60	3.60	3.50	5.30	4.40	5.35	5.06	5.19	--	—
.5 cc.	3.42	3.55	3.30	4.00	3.54	4.41	4.20	4.23	—	—
1.0 cc.	3.15	3.15	3.05	3.35	3.14	3.53	3.45	3.45	3.30	3.06
2 cc.	2.80	2.80	2.75	3.00	2.76	2.98	2.90	3.02	2.80	2.70
4 cc.	2.49	2.47	2.39	2.53	2.50	2.57	2.53	2.58	2.50	2.45
7 cc.	2.25	2.25	2.20	2.35	2.29	—	--	--	--	—
15 cc.	2.00	2.00	2.00	2.17	2.07	2.05	2.05	2.02	2.04	2.00

Table 12 — pH values of leaf tissue of highbush blueberries, as affected by successive additions of acid and alkali.

N/15 NaOH	Blended leaves	Filtered water extract	Residue	Distilled water
1.5 cc	10.94	10.40	10.95	11.35
1.0 cc	9.91	9.68	10.67	11.24
0.5 cc	5.03	4.85	10.18	10.95
0.2 cc	3.89	3.97	9.14	10.40
0	3.59	3.78	5.35	6.35
N/15 HCl				
0.2 cc	3.37	3.54	3.68	3.45
0.5 cc	3.08	3.36	3.32	2.96
1.0 cc	2.81	3.03	3.00	2.68
2.0 cc	2.50	2.74	2.70	2.40

that the isoelectric point of the soluble proteins in blueberry leaves is approximately at that pH.

Abortion of the Terminal Growing Points. The percent of terminal growing points of the 1940 culture plants aborted by April 7, or three weeks after planting was determined (table 10)(fig. 11). A significantly greater percent of growing points aborted when nitrogen was withheld from the plants. Abortion of 60 percent of terminals of the minus-nitrogen plants in peat-on-sand and 67 percent of the minus-nitrogen plants in sand compared with abortion on other treatments ranging from 25 to 51 percent, averaging 35 percent in peat-on-sand and 33 percent in sand culture. The correlation between top weight and percent of terminals aborted was found to be -.71 for the plants growing in sand, -.47 for the plants growing in peat-sand, and -.60 for all plants regardless of media. All the correlations are significant.

Effects of Media

Addition of Peat to Sand. As has been stated above (cf. p.47), calcium and sulfur deficiencies failed to appear in the peat-on-sand cultures of the 1940 experiment, and boron deficiency symptoms were delayed in appearance (fig. 12). By May 1, 1940 (table 1), there was as yet no significant difference in top growth between the media, but there was already a highly significant difference favoring the peat-on-sand medium in respect to root growth. The sulfur-deficient plants especially made better root growth in the peat-on-sand medium than in the sand alone. By June 10-14 (table 2), after

Table 10 — Percent of terminal growing points aborted by April 7, 1940.

(average of 18 plants)

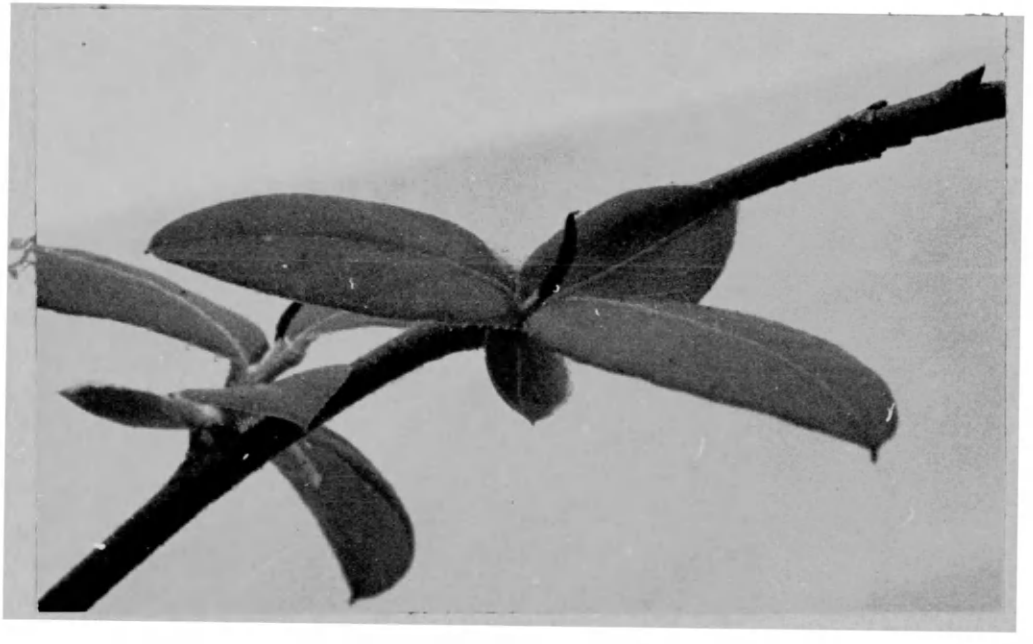
Deficiency	Peat	Sand
None	46.2	35.3
Manganese	32.7	50.7
Iron	31.5	30.5
Sulphur	34.8	24.7
Calcium	40.3	41.3
Boron	25.2	26.3
Magnesium	48.3	27.3
Phosphorus	29.0	30.8
Potassium	31.2	31.0
Nitrogen	60.5	66.7
Difference required for significance	23.92	
F values for:		
Nutrients	3.33**	
Media	.15	
Substances	.63	

Correlation between top weight and percent aborted terminals:

		all plants
-.47	-.71	-.60

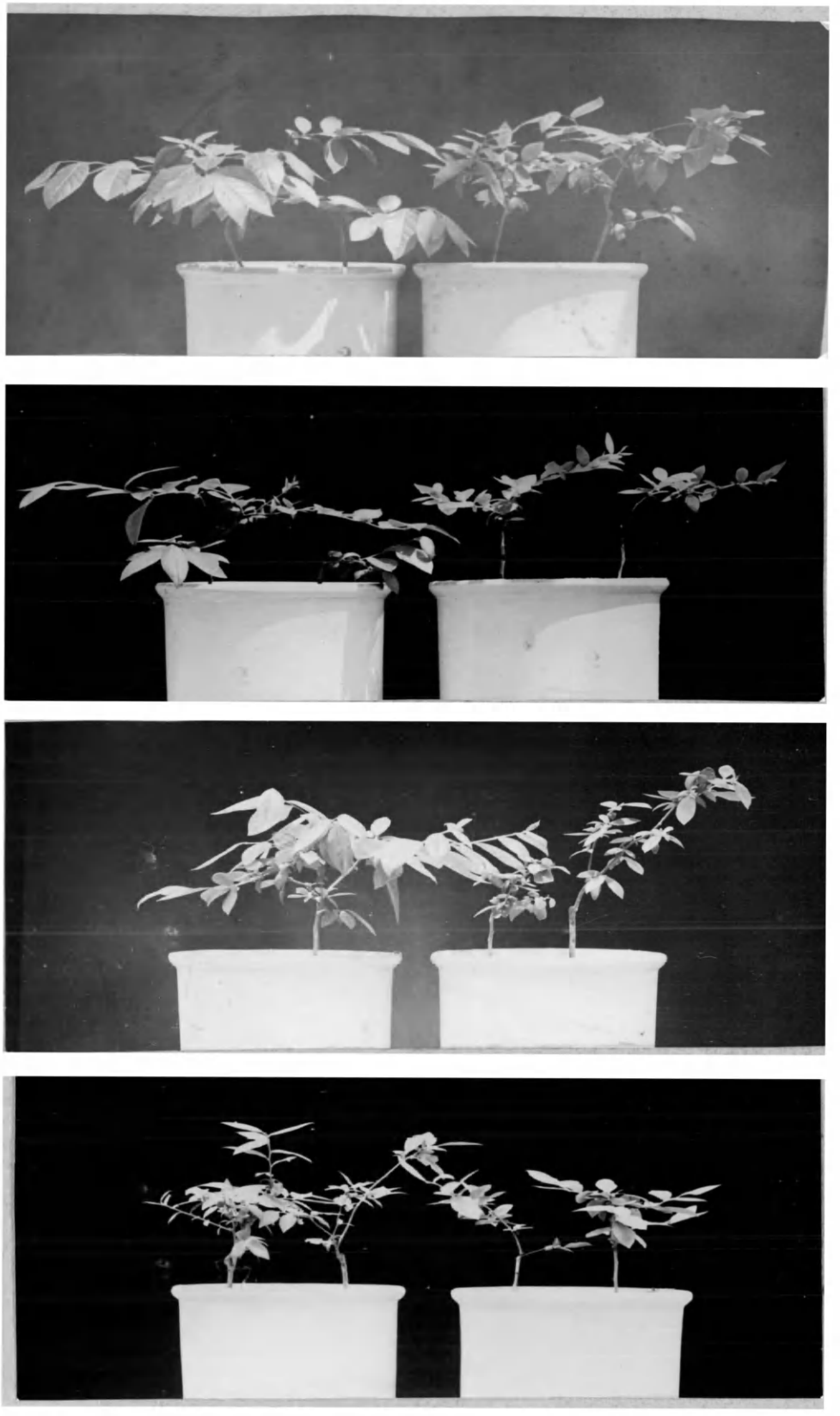
** Significant beyone the one percent point.

a.



b.

Fig. 11.- Abortion of the terminal growing points of Cabot blueberry.
a. actively growing terminal point
b. aborted terminal growing point



a.

b.

c.

d.

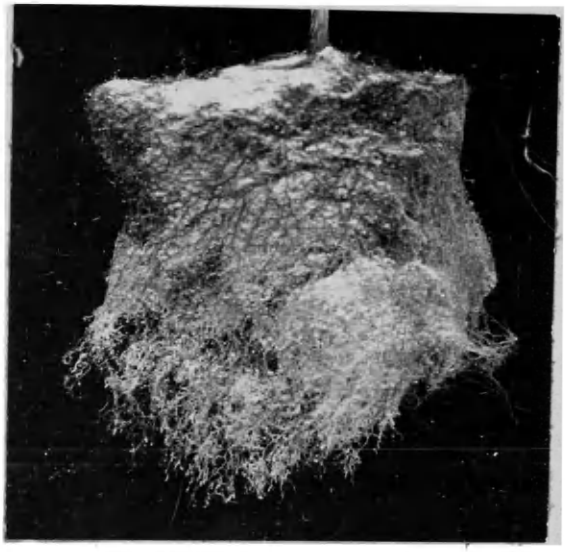
Peat-on-sand sand

Fig. 12.- Relative growth of Cabot blueberry cuttings in peat-on-sand and sand media, as affected by various mineral nutrient omissions. a- minus calcium, b- minus sulfur, c- minus boron, d- minus phosphorus

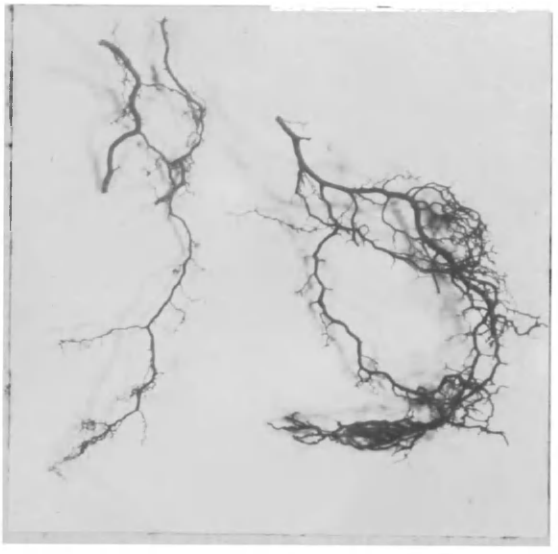
twelve weeks of growth, plants of all treatments with the exception of the minus-potassium plants showed a highly significant beneficial response to peat.

It was observed that the roots in the peat-on-sand medium tended to remain within the three inch layer of the peat itself, while roots growing in the sand medium penetrated to a far greater depth, finally reaching to the bottom of the liner (fig. 13a). After four months of growth, the roots in the peat layer were so numerous, that they formed one solid mass with the peat material. The fine roots growing in sand developed numerous laterals, at approximately every quarter inch of their length, while the roots in the peat medium elongated considerably more, at times for several inches before any laterals were produced (fig. 13b,c).

Soil Types. Data presented in table 13 indicate that there is a relationship between the mechanical composition of the soil and blueberry growth response. Thus the greatest amount of growth was made in the soil having the greatest percent clay and silt, and the poorest growth occurred in the gravelly soil. Applications of potassium and phosphorous fertilizers had no effect on the total fresh weights of the plants grown in these soils. Data in table 14 show that the fertilizer applications had no effect on root distribution, except that the addition of potassium caused a significant increase in the depth of root penetration. The effect of soil types on root distribution was similar to their effect on plant weights.



a.



b.

c.

Fig. 13.- Relative root growth of Cabot blueberry plants in peat-on-sand and sand media. a- root system of entire plant in sand medium, b- roots grown in sand medium c- roots grown in peat-on-sand medium

Table 13 — Mechanical analysis of various soil types, and their relation to blueberry growth. (Weight in grams of rooted cuttings grown in various soil types from March 28 to August 28, 1940).

Soils	Mechanical Analysis Percent				pH	Plant weights			Shoot/root ratio
	Sand	Silt	Clay	Fine Clay		Total	Tops	Roots	
1. Collington silt loam (Beltsville)	46.8	31.8	21.4	18.7	4.2	550.6	293.9	256.7	1.14
2. Sassafrass silt loam (College Park)	45.8	32.9	21.3	18.4	4.8	379.0	215.4	163.6	1.32
3. Portsmouth loam (College Park)	60.8	21.8	17.4	15.4	4.4	233.5	111.7	121.8	0.92
4. Gravelly loam (College Park)	67.2	17.1	15.7	14.4	4.5	122.4	64.1	58.3	1.10
F value						3.58*	3.85*	3.18*	
Difference Required for Significance						278.1	148.8	132.0	

*Significant beyond the five percent point.

Table 14 — Effect of phosphorous and potassium application, and soil types on root depth and distribution of Cabot blueberry plants. (Total values for six plants).

Soils	Fertilizers					
	Root Distribution*			Root Depth		
	None	Potassium	Phosphorous	None	Potassium	Phosphorous
1. Collington silt loam (Beltsville)	1012	1349	650	47	46	38
2. Sassafrass silt loam (College Park)	482	464	664	39	48	42
3. Portsmouth loam (College Park	72	1196	264	23	48	28
4. Gravelly loam (College Park)	58	371	48	21	30	18
F value soils	2.88			8.73**		
fertilizers	2.13			4.97***		
Difference required for significance	500.4			8.2		

*Value obtained by multiplying the square of the average horizontal extension of the roots by the depth of root penetration.

** Significant beyond the one percent point.

*** Significant beyond the five percent point.

Effect of Growth Substances. As stated before (cf. p. 47) weekly applications of thiourea, and thiamin-chloride to the 1940 pot cultures had no significant effect on blueberry plant growth. The F value of 2.61 for growth substances given in table 1 was not quite high enough to be significant at the five percent point. The size of the value that was obtained was chiefly due to the possible inhibition of top growth by the application of thiourea, and a possible improvement of the calcium deficient condition in the sand medium by the addition of thiamin chloride. The data in table 15 indicate that neither the two above mentioned substances nor eight others had any significant effect when applied at various concentrations to blueberry plants growing in silt loam soil.

DISCUSSION

Nutrient Deficiencies. If it may be said at all, that one element is more essential than another, then it is obvious that nitrogen is the element which is most essential for normal growth of blueberry plants. Such a conclusion is supported by considerable field data which serve as a basis for recommendation of fertilizers high in nitrogen (8, 19, 20). Greenhouse observations of Doehlert and Shive (70) and Stene (172) also point in the same direction. Similar conclusions were reached by many investigators working with different plant species (118).

Assuming that the sand medium approaches the physical and chemical condition of a poor sandy soil, the

Table 15 — Effect of some phytohormones and their concentration of Cabot blueberry growth. (Weight in grams of three cuttings grown from March 23 to August 29, 1940.

Phytohormone	Concentration		
	High	Medium	Low
Napthylacetamide	67.5	44.1	36.5
Naphthaleneacetic acid	47.4	34.7	28.4
Indoleacetic acid	64.1	45.1	79.5
Indolebutyric acid	96.3	48.4	23.8
Indoleproprionic acid	77.2	69.4	51.3
Indoleacetamide	72.7	52.9	48.7
Photo-Sen-Sin	104.2	57.3	56.2
Thiourea,	46.3	52.5	108.3
Thiamin chloride	61.7	56.1	88.1
Ascorbic acid	53.1	49.2	8.71
F value for:			
Hormones	.74		
Concentrations	1.66		
Difference required for significance	62.4		

Check (average of 9 plants x 3) = 76.4

various elements in such a soil might be expected to be required in the following order of importance: Nitrogen, phosphorous, sulphur, boron, calcium, potassium, iron, magnesium, and manganese. In a like manner, if the peat-sand medium may serve as an example of a soil high in organic matter, the order of importance in such a soil might be expected to be: Nitrogen, potassium, phosphorus, magnesium, boron, calcium, sulphur, iron, and manganese.

Earliness of leaf symptom appearance is not necessarily related to the degree of injury or stunting. Potassium deficiency symptoms, for example, appeared sooner than any except nitrogen symptoms while definite phosphorus deficiency symptoms appeared much later. Still, the minus potassium plants continued to produce new growth, and towards the end of the experimental periods their weights were far greater than those of the minus-phosphorus plants.

In agreement with observations made by other workers, on different plant species (67, 154), the milder potassium deficiency symptoms of interveinal chlorosis was very similar to the condition previously described as iron chlorosis (7, 8). In view of the work of Hoffer (99), Rhode (154), and Loehwing (114), that potassium was required to keep the iron content of the plant in the reduced, and thus in the soluble form, it may well be that the potassium deficiency symptom is actually an indication of iron unavailability. The observations of Bailey (7) that application of iron sulfate spray improved the color of chlorotic blueberry leaves only where the sprayed material came in direct contact with the leaf surface, also indicates a problem of iron

availability, or translocation, rather than actual iron deficiency. The improved condition of plants receiving a nutrient solution deficient in both potassium and iron over those deficient in potassium alone, suggeste the possibility, that in the absence of potassium, the precipitated iron accumulates in the nodal regions.

A further indication that the above symptom is caused by iron unavailability was provided by blueberry plants which showed similar symptoms when grown in the greenhouse during the summer and were subjected to temperatures as high as 120° F., and high light intensities. The pH of the sap of these plants increased with increasing severity of the chlorosis from 3.5 to 4.2. Since Gericke (81) showed that high light intensity was detrimental if iron were not sufficiently available and Ingalls and Shive (104) demonstrated that the pH of plant sap increases with increased light intensity, it is possible that the high light intensity of summer days increases the pH of the blueberry plant sap to such a degree that the iron, which was available in the nutrient solution or in the soil solution, becomes unavailable in the plant sap.

That the symptom is not a simple effect of pH of the substrate is shown by the fact that those plants grown in soil having a pH of 4.4., considered optimum for blueberry growth (124), were also affected; moreover, it is not likely that iron would become insoluble in blueberry tissue because of pH alone, since even a pH of 4.2 is far below that occurr-

ing in other plants normally. Since one function of manganese in the plant was reported to be the oxidation of excess soluble iron (154), the apparently low requirement of blueberries for manganese might serve as an additional indication that it is difficult to maintain sufficient quantities of iron in the blueberry tissue in the soluble state.

Although calcium and magnesium deficiency symptoms appeared in both experiments, the calcium and magnesium deficient plants were not significantly lower in fresh weight than the full-nutrient plants at the end of both culture experiments. In fact, in the early stages of growth, the minus-magnesium plants of the 1942 experiment fared fully as well as the full nutrient plants. Knowing that the blueberry has a high nitrogen and possibly a low calcium requirement, it would of course have a high nitrogen: calcium ratio, and thus fit into Parker and Truog's (136) classification as a plant having low calcium requirements. This characteristic may be part of the explanation for the adaptability of the blueberry plant to extremely acid, swampy soils.

The anions phosphorus and sulfur, on the other hand, are apparently required by the blueberry plant in considerable quantities, since the deficiency of these two ions caused significant decreases, particularly in top growth. The results of these experiments are thus in full agreement with the results of Waltman (203) and Balks (10).

Although boron deficiency caused significant reduction in growth in the 1940 experiment, the original boron content of the 1942 culture plants was apparently sufficient

to carry them through the shorter and slower season of growth in the 1942 experiment. However, the boron deficient plants did begin to show leaf deficiency symptoms, and in view of the rapid advancement of the deficiency in the 1940 experiment, it is most probable that had the plants been grown in the boron deficient medium for several weeks longer, the symptoms would have become severe, and an abrupt cessation of top growth would have ensued.

When considering all the elements involved, it appears that when grown in sand cultures, all the cation deficient plants, though they may exhibit deficiency symptoms, still continue to survive, and their weight is lower, but not drastically lower than the weight of the full nutrient plants. The deficiency of the anions on the other hand results in severe stunting and even in actual losses in weight. This apparent preferential requirement for anion nutrients assumes considerable significance in view of the work of those investigators (3, 96, 97) who found that the pH of the plant is held fairly constant by the increase in organic acid following excessive uptake of cations. The physiological purpose of the abundant quantities of organic acid in blueberry plant tissue, therefore is beyond any amount required to neutralize the relatively low quantities of cations that are absorbed.

Nitrogen Uptake and Translocation. Undoubtedly there are many interrelationships of mineral elements, one affecting the other as to intake and translocation. The frequent symptom of chlorosis caused by a number of mineral

nutrient deficiencies, and hence their similarity to nitrogen deficiency symptoms, indicates that the deficiency of such elements may be harmful not only in directly reducing the level of the particular element in the plant, but in indirectly interfering with nitrogen intake, translocation, or metabolism.

As in a host of other plants (1, 12, 14, 17, 18, 21, 26, 35, 42, 51, 57, 65, 150, 178, 195) the omission of nitrogen from the nutrient solution caused a drastic reduction in the percent nitrogen in blueberry plants, and an actual loss of total nitrogen content. Sulfur deficiency was second only to the absence of nitrogen itself in its effects on reducing the nitrogen level in blueberry plants, which is in direct contrast to the results of Eaton (77) on the sunflower, and Chapman and Brown (41) on citrus, and might serve as an additional clue to the peculiar adaptation of the blueberry.

The phosphorus deficient plants, after first showing a reduction in percent nitrogen, later were higher in percent nitrogen than the full nutrient plants. This condition may have been caused by possible liberation of phosphorus as a result of proteolysis which may have set in at a certain point in the development of the deficiency. This liberated phosphorus may have been available for further protein synthesis, and thus hastened nitrogen metabolism, and indirectly, nitrogen intake. At any rate, these data might shed some light on the highly contradictory results reported for the effect of phosphorus deficiency on nitrogen content of plants (1, 12, 14, 17, 18, 21, 28, 40, 67, 136, 166, 205) by indicating that the effect

is dependent on the severity and duration of the deficient condition.

Hydrogen-ion Concentration. It is in this characteristic that the blueberry is most strikingly different from other cultivated plants. To begin with, the actual pH of the blueberry plant sap is two to three full points below the pH of most plants. In other words, the hydrogen-ion concentration in blueberry leaves is 100 to 1000 times greater than in leaves of most plants. Whereas in most plants the younger and more vigorous tissues are higher in pH (83, 153, 61, 101, 102, 207) in the blueberry they are lower, and conversely whereas varicus deficiencies (102, 160, 63, 73, 78, 74) and incidences of disease (84, 87, 100, 184, 153, 207), caused a decrease in the pH of most plants, such conditions usually resulted in an increase in the pH of the blueberry plant sap. All these results suggested the possibility that the plant sap of the blueberry is on the acid side of the isoelectric point of its proteins, and these ampholytes, in contrast to their behavior in most plants (43, 137) act in the blueberry plant as cations. When it was found that the isoelectric point of the soluble blueberry leaf proteins was approximately at the pH of 5.0, the above possibility was further substantiated. If this hypothesis is accepted, then many things become readily understandable. Thus if the plant proteins act as cations, there would naturally be a preferential absorption of anions, and if anion radicals are absorbed in excess, it would be difficult to keep iron in the plant in the reduced, available form. Furthermore, in the presence of large quantities of anion radicals, which

in themselves may serve as oxidizing agents, there is little need of the oxidizing effect of the manganese ion. With the blueberry, as well as other plants, the pH of the plant sap approaches the isoelectric point of its proteins with sencescence, or upon injury. Whereas most plant juices become more acid as they approach the pH of the isoelectric point of their proteins, blueberry sap becomes less acid to reach the same point.

It is interesting to observe that the pH of the minus-nitrogen plants remained low, and there was very little difference in acidity between the younger and older minus-nitrogen leaves. According to Hoagland and his co-workers (3, 96, 97), nitrogen taken up from the substrate as nitrate, is reduced in the plant to ammonium, and therefore results in an increase in the basic ions, which is compensated for by production of organic acids. Direct ammonium uptake from the substrate, if any, would of course cause an increase in the cation concentration. Thus the omission of all sources of nitrogen from the substrate would result in a low pH level in the plant sap, provided the formation of organic acids is not impeded.

Inasmuch as the buffering property of plant acids around the pH of 6.0 has been frequently attributed to a phosphate substance, the omission of phosphorus from the substrate might be expected to lower the blueberry plant pH, as it has lowered the pH of other plants (63, 73, 73). Such

actually was the case with the blueberry, in the earlier stages of phosphorus deficiency, but the significant increase in the pH of the older minus-phosphorus leaves towards the end of the experimental period, together with the accumulation of nitrogen in the same period may indicate a high accumulation of ammonia, and amide nitrogen, possibly as a result of proteolysis or some other interference with nitrogen metabolism. Such an opinion is in agreement with that of other workers (36, 67, 78, 148, 150, 178). Some similar mechanism may have been effective in the minus-sulfur plants. The significant increase in pH in the minus-calcium and magnesium plants is more difficult to explain. In view of the work of Olson (134) who showed that there was a correlation between calcium uptake and the formation of organic acids, it is possible that in the absence of calcium, and perhaps magnesium as well, the formation of organic acids was less than what would have been required to merely neutralize the calcium or magnesium taken up.

Effects of Media. Coville has long ago emphasized the special requirements of the swamp blueberry for moisture, and all observations made during the process of these experiments fully confirm his view. Plants grown in raised beds in the greenhouse grew best when they were farthest from the border where the soil was less likely to remain wet for some time after watering. Plants grown in the glazed coffee urn liners produced fifty percent more growth than plants growing in identical soil, but in ten inch clay pots. In fact, there seemed to be practically no upper limit to the growth response of blueberries to increases in moisture application. The greater growth made by plants

growing in the soils containing the higher percent clay and silt, and in the peat medium is further evidence of the high moisture requirements of the swamp blueberry.

These experiments contribute very little direct information to an understanding of the oxygen requirements of the blueberry plant. However, field experiments (111) carried on as part of the same project indicated that on tight clay soils, aeration may be a limiting factor in the growth and development of blueberries. The beneficial effects of a peat medium may be due partly to improved aeration, since Arnon (2) showed that oxygen application even to sand cultures may be beneficial. Combining the observations of Coville (54, 55) and Conway (49), it is possible to suggest a mechanism of the adaptation of the blueberry to wet swamp soils. According to Coville, the blueberry plant in its natural habitat, is frequently submerged for parts of the season, particularly during the winter and early spring. At such times there is no active root growth, but in the spring, shoot growth proceeds but root growth does not commence until shoot growth has practically ceased. By then the water table will have fallen below the root zone, aerobic conditions for root growth will have been established, and root growth commences, together with nutrient uptake which gradually replenishes the nutrient supply exhausted by the shoot growth early in the season. The characteristic abortion of the terminal growing points of blueberry shoots, which seems to be closely correlated with the vigor of the plant, may be an adaptive mechanism to prevent excessive growth of tops at a time when nutrients are not replenished. The increase in pH of leaf tissue with age towards

the isoelectric point of the proteins, may also cause a slowing down of growth rate in the tops. Since it has been shown that a reduction in pH is analogous to an increase in oxidation over reduction, and that the pH of wet soils increases with depth (49), the very shallow root system characteristic of the blueberry, particularly in organic soils, may be still another mechanism for escaping the usual reducing conditions prevailing in the natural habitat of the blueberry. On the other hand the detrimental effect of potassium deficiency in the peat medium reported here (fig. 6), together with the data of the Michigan workers which led them to recommend fertilizers high in potassium for peat soils (105), indicate that one effect of an adequate potassium level is to increase the depth of root penetration in well drained peat soils. It has been shown (table 14) that potassium application is instrumental in increasing the depth of root penetration of blueberry plants.

It thus appears that with the swamp blueberry, under natural conditions, the oxygen supply is limiting. Moreover, since effective utilization of growth substances apparently results in increased oxygen consumption (183) that is the possible reason for the almost unanimous reports of lack of response of blueberry plants to the application of growth substances. The application of many of the growth substances apparently increases the plant content of reducing substances, that is, substances that may utilize oxygen, but in the blueberry, perhaps because the amphoteric proteins act as cations, the preponderant requirement is for oxidizing substances (anion

radicals, for example), rather than oxygen consuming substances with reducing properties.

Shoot:Root Ratio. Considering Coville's (54) assertion that root and shoot growth in the blueberry proceed at different periods, and the phenomenon of periodic terminal abortion of the terminal growing points, temporary variations in shoot:root ratios should not be over-emphasized. It is quite possible that many of the large differences in shoot: root ratios obtained in these experiments, were only temporary. It was obvious, for example, that the full nutrient plants removed on April 10, 1942 (table 6)were at a point where for the time being, root growth had reached the maximum, and rapid shoot growth was about to begin, judging from the numerous swollen buds present at that time. It is probable that had these same plants been removed several weeks later, after these buds had elongated into shoots, that the shoot:root ratio would have been higher.

Climatic Factors. The possible effect of light on the pH of the plant sap, and consequently on the availability of iron has already been discussed (cf. p. 93). This effect of light, however, has not been separated from possible temperature effect, and there is therefore an urgent need for establishing the effect of light and temperature independently upon the pH of the plant sap, just as Roberts and Struckmeyer (152) showed the effect of temperature upon the photoperiodic response of plants.

The beneficial effect of partial shading on the perennial esixtence of the blueberry reported in another phase of this project (112) may be due to some extent to the reported destruction of organic acids in light (47) or the greater availability of iron and calcium, since Nightingale, et. al. (131) found that calcium is freed by proteolysis when plants are placed in the dark, and may be reutilized. Although it appears that blueberries have a low calcium requirement, in view of their large organic acid content it may be difficult to keep even the small quantities of calcium required in available form. It is also possible that the decide need for boron is due in part to the role of boron in keeping a fraction of the calcium content in a soluble form (164).

Increasing the soil temperature up to 90° F. has been shown by Bailey (9) to increase blueberry plant growth. Partial results obtained from temperature chambers set up in the greenhouse in the spring of 1941, indicated that the time required for blueberry plants to start growth from dormant buds depends entirely upon the air temperature, and is completely independent of soil temperature. This again points to the probability that blueberry shoots may begingrowth early in the season regardless of the environmental conditions affecting the roots.

An Explanation of the Unique Adaptation of the Blueberry. In an attempt to combine into one basic concept some of the results with blueberry plants when compared to results reported on other plants, especially in the light of some recent findings on the mechanism of mineral ion intake and the significance of the hydrogen-ion con-

centration of plants, the following generalization was postulated: The unique behavior of the swamp blueberry may be based upon its ability, probably developed through a long period of natural selection, to thrive under soil conditions limiting aeration, but supplying at the same time adequate moisture to meet rather exacting water requirements of the blueberry. The explanation of the mechanism for the adaptaion of the swamp blueberry to such an environment is based on the results of the present and other experiments with blueberry plants,- experiments which indicate that the blueberry has exacting moisture and oxygen requirements, high anion and low cation uptake, a requirement for highly acid soils, an extremely acid plant sap, and that the pH of its plant sap is on the acid side of the isoelectric point of its soluble proteins.

Considering then the natural occurrence of the blueberry on soils with high water tables, how can the experimental evidence be used in explanation of a special adaptation of the blueberry plant to such conditions? Because of the high moisture requirement, there is a preference for soil with high water tables. Lack of aeration in such soils is overcome not by the presence of aerenchyma tissue, but by the extremely shallow root system, and a preference for acid soils where oxidation processes, rather than reduction processes prevail. Since such soils are low in calcium, and other exchangeable bases, a low calcium and other cation requirement is necessary, while the satisfaction of the high anion requirement may increase the usually limited supply of oxygen needed for respiration and organic acid formation. The

resultant high organic acid content is responsible for the low pH value of the plant sap, which is below the isoelectric point of the plant proteins. These proteins may consequently act as cations, thus balancing the excessive presence of anions. The prevailing shade of the natural environment aids further in maintaining the low pH by preventing organic acid destruction.

When the blueberry is transplanted from its natural habitat, into ordinary cultivated soils, it probably suffers from lack of moisture, and since it is adapted to obtaining a part of its oxygen for respiratory processes from anion radicals, suffers from a lack of such nutrients, and an excess of basic nutrients. Since it has been shown by the California workers and others that pH per se is not important in nutrient uptake, it is probably the difficulty of obtaining anions from soils high in pH that causes the poor blueberry growth in soils that are not considerably acid. The destructive effect of high light intensities on the organic acid content of the plant and the consequent increase in pH towards the isoelectric point may also be a factor in poorer growth of blueberries in cultivated fields where no shade is provided.

It is recognized that much additional work is required to substantiate or reject the above explanation. To this end the the following experiments are suggested: (1) A study of blueberry plant response to variations in air and moisture levels in various media, (2) a study of plant response to nitrate versus ammonium as the source of nitrogen in substrates of various acidities, (3) a study of plant response to variations in light

periods, and intensities, and temperature, and (4) Further studies on plant responses to nutrients in various concentrations and combinations. Samples of plants used in all these suggested experiments should be analyzed for all the elements provided in the nutrient solutions, for the various organic acids, for the hydrogen-ion concentration, and for the isoelectric point of the proteins. It is believed that such studies would not only be of value in the eventual improvement in the blueberry cultural methods, and use of various species for soil erosion control, but would also be instrumental, when compared to similar work on other plants, in developing a broader understanding of many physiological processes of plants in general.

SUMMARY

Mineral Nutrients. Two series of pot cultures with rooted cuttings of the Cabot blueberry were grown in peat-on-sand, and sand media, and supplied with various nutrient solutions and growth substances.

The characteristic deficiency symptoms for each element are described and were found to be sufficiently unlike to be easily identified, particularly for nitrogen, potassium, boron, magnesium, and phosphorus. Deficiency symptoms appeared after different periods of time in both media in the following order: Nitrogen, potassium, sulfur, calcium, boron, magnesium, phosphorus, iron, and manganese.

In a second experiment, deficiency symptom expressions, and fresh and dry weight results agree with those

obtained in the first experiment except that the calcium and especially the boron deficiencies were less severe in their effects, while magnesium deficiency was more effective.

In the sand medium the omission of one of the anions:nitrate, sulfate, phosphate, or borate, caused drastic reductions in growth, while the omission of the cations caused definite but not as severe injury.

The simultaneous omission of potassium and iron from the substrate resulted in better plant growth than the omission of potassium alone, but the simultaneous omission of potassium and phosphorus resulted in more severely injured plants, than when either element was omitted singly. When blueberry plants were fed with a solution lacking nitrogen, phosphorus, and potassium, nitrogen deficiency developed more slowly than in plants lacking nitrogen only.

The shoot:root ratio of plants growing in the peat-sand medium was greatly increased when manganese, iron, calcium, boron, or magnesium was omitted, and decreased when sulphur, boron, phosphorus, or nitrogen were withheld from the sand grown plants.

There was a significant negative correlation between fresh weight and percent dry weight, but results based on dry weight were practically identical with those obtained with fresh weights, since the differences in weight were sufficiently large to be maintained on dry weight basis also.

Lack of nitrogen had a significant effect on the rapidity with which terminal growing points aborted.

There was a significant correlation between percent of terminals aborted and top weight.

Weekly applications of thiourea and vitamin B_1 had no significant effect on the deficiency symptoms, fresh or dry weights, or growing point abortion. Eight other substances applied to blueberry plants growing in silt loam soil had no significant effect.

The percent nitrogen of field grown plants in autumn was 1.31, 0.48, and 0.88 in the leaves, old wood, and roots respectively, but rose to 0.74 in the old wood, and to 1.19 in the roots when the plants were about to leaf out after being placed in glazed crocks to which a full-nutrient solution had been added. This level was approximated throughout the experimental period in the old wood and roots, while the percent nitrogen in the current top growth dropped gradually from about 3.00 to 2.60 percent. When nitrogen was omitted from the substrate the percent nitrogen dropped to 1.23, 0.42, and 0.47 in the current tops, old wood, and roots respectively. Sulphur deficiency was also effective in reducing the nitrogen level of the top growth, as were to a lesser degree deficiencies of potassium, iron, potassium-iron, and potassium-phosphorus. Phosphorus deficiency resulted in a lower nitrogen level during the first period of the experiment, but later the nitrogen level rose to slightly higher than the full-nutrient plants.

In total nitrogen content, there was an actual loss in the minus-nitrogen and minus-N-P-K plants, while the nitrogen content of the minus-phosphorus and phosphorus-potassium plants showed practically no increase throughout the experimental period.

Hydrogen-ion Concentration. The hydrogen-ion concentration of blueberry leaf sap was found to be about 3.5, 100 to 1000 times as great as in most cultivated plants. Titration curves showed the presence of a very high acid reserve, practically all of which is composed of water soluble substances. Since the isoelectric point of blueberry plant proteins was found to be about pH 5.0, it was suggested that these proteins act as cations in blueberry tissue. Older leaves had a higher pH than youhg leaves. Deficiencies of phosphorus and sulfur, and to a lesser degree potassium, calcium, and magnesium were all effective in reducing the acidity of blueberry leaf sap, while nitrogen deficiency resulted in a reduction of the difference in pH between young and old leaves.

Media for Blueberries. The beneficial effect of peat on blueberry growth was due partly to the presence of calcium, sulfur and boron in available form, since the above deficiencies failed to appear in the peat-sand medium, and partly to factors that were not controlled, since plants receiving the full-nutrient solution also made better growth, although to a lesser degree, in peat-sand than in sand medium.

In a separate experiment, blueberry cuttings were placed in four different soil types in ground beds, and fertilized with phosphorus and potassium. Best growth was obtained in the soil containing the largest proportion of clay and silt, thus indicating that moisture content was important for blueberry growth. Potassium and phosphorus applications had no effects on the weights of the plants or the volume of

soil penetrated by roots, but the application of potassium resulted in deeper root penetration.

A Suggested Hypothesis. A possible mechanism of the adaptation of the swamp blueberry to its environment, and the effect of a change in its environment upon blueberry growth is suggested, based upon the results of these and other experiments that indicate that the blueberry has high moisture, high oxygen, high anion, low cation, and acid soil requirements, and an extremely acid sap having a pH value lower than the isoelectric point of its soluble proteins.

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Table 1. Calorimetric results of the electrical-energy experiments for cyanogen.

Experiment	ΔR^1	k	K	U	Electrical energy[2]	Mass of calorimeter	Electrical energy equivalent of calorimeter system[3]	Deviation
	ohms	min^{-1}	ohms	ohms	int J	g	int J/abs. ohm	J/ohm
1	0.397063	0.001914	0.015268	0.000145	58,335.6	3602.60	152,384.1	0.0
2	.394055	.001911	.015463	.000057	57,743.1	3603.85	152,384.1	0.0
3	.393372	.001914	.015184	.000057	58,560.5	3603.86	152,367.5	-16.6
4	.393121	.001911	.015556	.000018	57,840.7	3607.53	152,359.1	-25.0
5	.392779	.001928	.015643	.000136	57,434.4	3599.71	152,357.9	-26.2
6	.393040	.001912	.015471	.000004	57,461.8	3596.16	152,350.0	-34.1
7	.393601	.001916	.015859	.000035	58,309.0	3599.36	152,356.2	-27.9
8	.396923	.001953	.015640	.000453	58,045.2	3599.52	152,437.4	+53.3
9	.395293	.001919	.015477	.000176	57,938.9	3604.35	152,435.3	+51.2
10	.394791	.001924	.015534	.000172	57,780.0	3600.26	152,409.9	+24.8
Mean							152,384.1	
Standard deviation of the mean							±10.3	

[1]The average temperature of the calorimeter was 24.98° C for experiment 4; 24.99° C for experiments 2, 5, and 6; 25.00° C for experiments 9 and 10; and 25.01° C for experiments 1, 3, 7, and 8.

[2]The time of electrical-energy input was 2280.00 seconds in each experiment.

[3]For 3600.00 g of water and an average temperature of 25.00° C.

reversing connections, so that starting and stopping the flow of current are accomplished by the same mechanical operation of the switch, cancelling out its time lag. During periods when the current was not flowing through the heating coil of the calorimeter, it was sent through an equal, external, stabilizing resistance.

The data of the electrical-energy experiments for this investigation are given in table 1. The various quantities have the same significance as above (section III). The standard deviation of the average value of the electrical-energy equivalent for these experiments is ±0.007 percent.

2. Correction Experiments

In the calorimetric combustion experiments, it was necessary to take account of the following sources or sinks of energy, which were not present in the experiments with electrical energy: (1) the spark energy, which is the energy introduced into the calorimeter when the reaction is initiated by passing an electric spark across the ends of platinum wires at the tip of the burner in the reaction vessel; and (2) the gas energy, which is the energy added to or taken from the calorimeter by the inflowing oxygen and cyanogen entering the calorimeter at a temperature different from the average temperature, with respect to time, of the calorimeter.

The spark energy was determined calorimetrically by passing the spark across the tip of the burner, with oxygen in the reaction vessel. The temperature of the calorimeter,

Table 2. Experiments determining the spark energy.

Experiment	Time of sparking	Energy of sparking	Spark energy
	seconds	J	J/sec
1	48	101.1	2.11
2	48	102.0	2.13
3	48	99.5	2.07
4	48	97.6	2.03
5	48	97.0	2.02
Average			2.07
Standard deviation of the mean			± 0.02

for these experiments, was slightly below that of the jacket and the time of sparking was about three times that employed in a regular combustion experiment. The product of the resulting small temperature rise of the calorimeter and the energy equivalent gave the amount of this sparking energy. The results of five experiments on the evaluation of the spark energy are given in table 2.

The gas energy was calculated from the known heat capacities of the gases, and the difference between their temperature (taken as that of the air near the connecting tubes leading to the reaction vessel) and the average temperature, with respect to time, of the calorimeter. For this purpose, the heat capacities of oxygen and cyanogen were taken as 29.2 and 44.6 joules per degree-mole, respectively [24].

As mentioned in section IV, besides the impurities in the combustion, which were determined and accounted for in each case by chemical analysis, another stoichiometric correction was necessitated by a small amount of cyanogen which remained unburned during the ignition and extinction processes, and which was absorbed by the carbon-dioxide absorption tube. The value of this correction was deduced from a series of eight ignitions and extinctions of the flame carried out calorimetrically and under the same conditions as in the main calorimetric combustion experiments, but with the extinction immediately following the ignition. From the evolved reaction energy, obtained from the temperature rise

Table 3. Experiments determining the amount of cyanogen remaining unburned in each ignition and extinction of the flame

Experiment	Electrical energy equivalent	$\Delta R_{corr.}$	Calorimeter energy	Spark energy	Reaction energy	Calculated mass of CO_2 formed	Observed increase in mass of absorption tube	Calculated mass of C_2N_2 unburned	Deviation from mean
	int j/abs. ohm	abs. ohm	j	j	j	g	g	g	g
1	152,540	0.001210	184.6	31.1	153.5	0.01233	0.02961	0.01728	-0.00002
2	152,540	.001824	278.2	31.1	247.1	0.01985	.03721	.01736	+ .00006
3	152,580	.001737	265.0	31.1	233.9	.01879	.03564	.01685	- .00045
4	152,580	.001590	242.6	31.1	211.5	.01699	.03513	.01814	+ .00084
5	152,650	.000936	142.9	24.9	118.0	.00948	.02658	.01710	- .00020
6	152,650	.000810	123.6	31.1	92.5	.00743	.02457	.01714	- .00016
7	152,410	.000756	115.2	24.9	90.3	.00725	.02444	.01719	- .00011
8	152,410	.001142	174.1	31.1	143.0	.01149	.02885	.01736	+ .00006

Average 0.01730

Standard deviation of the mean . . . . . ± .00013

of the calorimeter and its energy equivalent (and correcting for the spark energy), there was computed the mass of carbon dioxide corresponding to this quantity of energy, which would have been formed in the pure combustion of cyanogen. In this calculation there was used for the heat of combustion of cyanogen the value 1096 kilojoules per mole. The difference between the calculated increase in mass of the carbon-dioxide absorption tube and that actually observed was assumed to be unburned cyanogen. The data of these experiments are given in table 3, and the average value was taken as the mass of unburned cyanogen in calculating the amount of reaction in the main calorimetric combustion experiments. The uncertainty is taken as twice the standard deviation [9].

3. Combustion Experiments

The data of ten calorimetric combustion experiments are given in table 4. The various quantities have the same significance as above (section III).

In order to alter the amounts of nitrogen oxides formed in the combustion of cyanogen, a second set of calorimetric combustion experiments was performed in which the oxygen used for the combustion was saturated with water vapor at 0° C. Since this water would have to be accounted for stoichiometrially, ten experiments were performed to determine the reproducibility of the mass of water which would be added to the products of combustion in this way. The experiments were carried out by flushing the oxygen line (connected with the

Table 4. Calorimetric results of the reaction experiments for cyanogen.

Experiment	ΔR^1	k	K	U	Electrical energy equivalent of calorimeter system	Gas energy	Spark energy	Net reaction energy	Increase in mass of CO_2 absorber	Mass N_2O_3 determined	Mass N_2O_5 determined	Mass CO_2 from CO oxidation	Mass of CO_2[2]
	ohms	min^{-1}	ohms	ohms	int.j/abs. ohms	j	j	j	g	g	g	g	g
1	0.399617	0.001919	0.015576	0.000374	152,723.4	+75.0	31.1	58,488.7	4.8125$_6$	0.1062$_7$	0.0162$_5$	0.1028$_9$	4.7755$_5$
2	.397917	.001920	.014504	.000282	152,712.1	+60.0	24.9	58,410.7	4.7978$_8$	.1012$_9$	.0250$_1$	.1220$_8$	4.7734$_3$
3	.397188	.001925	.016081	.000212	152,457.0	+41.4	31.1	57,997.8	4.7563$_4$	.0997$_3$	.0309$_4$	.1319$_5$	4.7403$_9$
4	.397277	.001931	.015243	.000163	152,451.3	+ 4.2	31.1	58,258.0	4.7610$_5$	.1031$_4$	.0228$_6$	.1548$_4$	4.7725$_8$
5	.398738	.001929	.015493	.000189	152,515.4	+ 4.2	31.1	58,286.6	4.7618$_3$	.1047$_2$	.0183$_8$	.1772$_2$	4.7986$_2$
6	.397527	.001936	.014975	.000202	152,625.6	+ 6.8	31.1	58,318.3	4.7551$_1$	.1055$_1$	.0170$_8$	.1824$_4$	4.7976$_7$
7	.397478	.001931	.018540	.000000	152,626.9	- 0.2	43.5	57,792.8	4.6947$_0$	.1111$_1$	.0232$_9$	.2302$_0$	4.7731$_5$
8	.399497	.001921	.014628	.000210	152,686.1	+23.1	39.2	58,673.8	4.7887$_5$	.1022$_6$	.0207$_4$	.1692$_1$	4.8177$_6$
9	.398411	.001910	.014541	.000124	152,495.1	+35.5	37.3	58,445.5	4.7675$_8$	.1028$_3$	.0212$_6$	.1941$_3$	4.8202$_4$
10	.397811	.001930	.014097	.000288	152,405.2	+ 4.8	31.1	58,402.2	4.7605$_7$	.1012$_5$	.0140$_1$	.1951$_2$	4.8131$_4$

[1]The average temperature of the calorimeter was 25.00° C for experiments 9 and 10; 25.01° C for experiments 2, 3, 4, 5, 6, and 7; and 25.02° C for experiments 1 and 8.

[2]Includes a correction of -0.0173$_0$ g as the average amount of unburned cyanogen absorbed in each experiment.

Table 5. Experiments on the reproducibility of the mass of water collected from water-saturated oxygen used in the combustion of cyanogen under these conditions

Experiment	Mass of water collected	Deviation from mean
	g	g
1	0.0504_8	-0.0018_8
2	$.0542_0$	$+ .0018_6$
3	$.0589_5$	$+ .0066_1$
4	$.0507_7$	$- .0015_7$
5	$.0568_6$	$+ .0045_2$
6	$.0580_6$	$+ .0057_2$
7	$.0493_7$	$- .0029_7$
8	$.0495_0$	$- .0028_4$
9	$.0484_7$	$- .0038_7$
10	$.0467_5$	$- .0055_9$
Mean		0.0523_4
Standard deviation of the mean		$\pm 0.0013_7$

Table 6. Calorimetric results of the reaction experiments for cyanogen, using in the combustion oxygen saturated with water at 0° C.

Experiment	ΔR^1	k	K	U	Electrical energy equivalent of calorimeter system	Gas energy	Spark energy	Net reaction energy	Increase in mass of CO_2 absorber	Mass N_2O_3 determined	Mass N_2O_5 determined	Mass CO_2 from CO oxidation	Mass of CO_2[2]
	ohms	min^{-1}	ohms	ohms	int.j/abs. ohms	j	j	j	g	g	g	g	g
1	0.398436	0.001929	0.015599	0.000170	152,698.4	- 0.1	31.1	58,409.2	4.8660_8	0.0960_2	0.0188_4	0.0155_8	4.7039_9
2	.397995	.001937	.014992	.000158	152,665.8	+ 7.1	28.2	58,382.1	4.8474_7	$.0767_5$	$.0198_6$	$.0175_6$	4.7078_7
3	.398508	.001938	.015792	.000160	152,634.8	+ 7.5	31.1	58,238.6	4.8237_4	$.0766_6$	$.0116_1$	$.0239_4$	4.7011_5
4	.398307	.001921	.015214	.000154	152,317.8	+15.8	31.1	58,281.5	4.8588_4	$.0956_0$	$.0218_8$	$.0170_6$	4.6967_4
5	.396970	.001931	.017534	.000155	152,481.0	+12.2	35.2	57,785.7	4.8310_8	$.1039_0$	$.0182_2$	$.0137_4$	4.6544_0
6	.399237	.001930	.015243	.000092	152,761.9	+ 0.9	31.1	58,618.4	4.8946_2	$.0992_8$	$.0253_6$	$.0153_1$	4.7236_1
7	.398415	.001935	.017928	.000195	152,712.6	+ 6.8	31.1	58,041.5	4.8568_6	$.1020_4$	$.0183_5$	$.0136_8$	4.6765_0

[1] The average temperature of the calorimeter was 25.00° C for experiment 6; and 25.01° C for experiments 1, 2, 3, 4, 5, and 7.

[2] Includes a correction of -0.0173_0 g as the average amount of unburned cyanogen absorbed in each experiment, as well as a correction for the mass of water vapor introduced with the combustion oxygen (see p. 27).

reaction vessel and a water absorption tube) with dry oxygen, flowing at the rate normally used in the combustion; by-passing it for 46 minutes (the usual time of flow in the combustions) through a gas-washing bottle having a fritted-glass bubbler, and immersed in an ice bath; and again flushing with dry oxygen. The data of these experiments are given in table 5. The average amount of water absorbed in this way was $0.0523_4 \pm 0.0027_4$ g. The estimated uncertainty of the determination of the amount of reaction due to this amount of water was ± 0.057 percent.

The data of seven calorimetric combustion experiments carried out under these conditions are given in table 6. In those experiments for which the time of flow of water-carrying oxygen differed from 46 minutes, when computing the stoichiometric correction for this, the amount of water absorbed was assumed to vary linearly with the time of passage of the oxygen. The effect of carrying out the combustion of cyanogen in this manner was to reduce the amount of carbon monoxide to about one-tenth of its former value, and the fixed nitrogen to about 80 percent of its previous amount.

VI. RESULTS OF THIS INVESTIGATION

For the final calculation of the heat of combustion of cyanogen, it is necessary to identify the particular oxides of nitrogen formed in the reaction vessel during the combustion, in order that appropriate corrections for their respective heats of formation may be made to the calorimetric observations. For this purpose there must be considered nitric oxide, nitrogen dioxide (in equilibrium with its dimer, nitrogen tetroxide), nitrogen trioxide, and nitrogen pentoxide (as well as nitric acid vapor in the case of those experiments in which water vapor was present in the reaction chamber). All these substances, except nitric oxide, are absorbed by solid sodium hydroxide. Under the conditions of the present experiments, no oxides of nitrogen were detected in the exit gas from the carbon dioxide absorption tube. Hence it follows that at least half the nitric oxide issuing from the calorimeter was oxidized to nitrogen dioxide.

Nitrogen pentoxide is known to decompose completely at 25° C (p. 552 of reference [19]). According to Meller (p. 449 of reference [19]), in the gaseous state, the dissociation of nitrogen trioxide to nitric oxide and nitrogen dioxide is virtually complete. This is corroborated by a calculation based on the equilibrium data of Verhoek and Daniels [26], and according to the conditions of a typical combustion experiment of this investigation.

It thus appears that, during the combustion of the cyanogen, the fixed nitrogen present in the effluent gas from the reaction vessel was a mixture of nitric oxide and nitrogen dioxide (in equilibrium with nitrogen tetroxide). Concerning the absorption by solid alkali of these substances, Mellor reports the following: (1) nitric oxide reacts with solid potassium hydroxide in the presence of excess oxygen, to form mainly potassium nitrite, but that some nitrate is formed as well (p. 432 of reference [19]); (2) nitrogen dioxide or tetroxide should react with solid alkali to give equimolal amounts of nitrate and nitrite, but that larger amounts of nitrate may be formed (pp. 539-40 of reference [19]); and (3) nitric oxide and nitrogen dioxide in equimolal amounts are absorbed by alkali as pure nitrite. Burdick and Freed [27] state that assuming these absorptions of nitrogen oxides as pure reactions is subject to some qualification.

A reasonable interpretation of the processes occurring, with respect to the fixation of nitrogen during the combustion of the cyanogen, is the following. The primary fixation was the formation of nitric oxide, which then oxidized to the extent indicated by the analyses to nitrogen dioxide. Letting $\underline{a}$ and $\underline{b}$ be, respectively, the amounts of nitrite and nitrate determined analytically, the fixation reaction in the calorimeter was

$$[\underline{a} + \underline{b}]\left[1/2\ N_2(g) + 1/2\ O_2(g) = NO(g)\right] \qquad (13)$$

Also, the nitrogen dioxide was formed according to the

equation

$$1/2\ [\underline{a} + 3\underline{b}]\ [NO(g) + 1/2\ O_2(g) = NO_2(g)] \qquad (14)$$

The absorption of the oxides of nitrogen took place according to the reactions

$$1/2\ [\underline{a} - \underline{b}][NO(g) + NO_2(g) + 2NaOH(c) = 2NaNO_2(c) + H_2O(c)] \qquad (15)$$

$$2\underline{b}\,[NO_2(g) + NaOH(c) = 1/2\ NaNO_2(c) + 1/2\ NaNO_3(c) + 1/2\ H_2O] \qquad (16)$$

A similar interpretation holds for those experiments in which water vapor was present in the combustion chamber. In addition, however, some of the nitrogen dioxide formed reacted with the water vapor, to form nitric acid vapor. The reactions which occurred were

$$[\underline{a} + \underline{b}][1/2\ N_2(g) + 1/2\ O_2(g) = NO(g)] \qquad (13)$$

$$1/2[\underline{a} + 2\underline{b}][NO(g) + 1/2\ O_2(g) = NO_2(g)] \qquad (17)$$

$$\underline{b}\,[NO_2(g) + 1/4\ O_2(g) + 1/2\ H_2O(g) = HNO_3(g)] \qquad (18)$$

The absorption by Ascarite of these substances took place according to the reactions

$$\underline{a}/2\,[NO(g) + NO_2(g) + 2NaOH(c) = 2NaNO_2(c) + H_2O(c)] \qquad (19)$$

$$\underline{b}\,[HNO_3(g) + NaOH(c) = NaNO_3(c) + H_2O(c)] \qquad (20)$$

The calorimetric data obtained (section V) were calculated on the bases of these assumptions, and the data are presented in tables 7 and 8. The fraction of nitrogen dioxide present as nitrogen tetroxide was computed by an equilibrium calculation based on the data of Giauque and Kemp [28], and it was found to be negligible in the present experiments. For the heats of formation of the nitric oxides and of nitric acid, values were taken from the compilation of Bichowsky and Rossini [29], and the value of the heat of combustion of carbon monoxide was taken as 282.94 int kj/mole [30].

Table 7. Calculation of the heat of combustion of cyanogen from the data of table 4.

Experiment	Amount of nitrite determined	Amount of nitrate determined	Amount of NO formed	Amount of NO_2 formed	Amount of CO formed	Heat of formation of NO	Heat of formation of NO_2	Heat of combustion of CO	Corrected reaction energy	Amount of C_2N_2 burned	Heat of combustion at 25° C	Deviation from mean
	moles	moles	moles	moles	moles	int. j	int. j	int. j	int. j	moles	int.j/mole	kj/mole
1	0.002792_0	0.000300_8	0.001247_6	0.001849_2	0.002336_3	-112.7	-62.1	661.0	59324.6	0.054255_3	1093.43	-1.33
2	$.002663_2$	$.000463_0$	$.001100_1$	$.002026_1$	$.002773_9$	- 99.4	-68.1	784.1	59362.2	$.054219_8$	1094.84	+ .08
3	$.002624_0$	$.000572_8$	$.001025_6$	$.002171_2$	$.002998_1$	- 92.7	-72.9	848.3	59011.7	$.053855_0$	1095.75	+ .99
4	$.002713_6$	$.000423_2$	$.001145_2$	$.001991_6$	$.003517_9$	-103.5	-66.9	995.4	59423.7	$.054221_5$	1095.94	+1.18
5	$.002755_2$	$.000340_4$	.001207$_4$	$.001888_2$	$.004026_9$	-109.1	-63.4	1139.4	59698.5	$.054517_8$	1095.03	+ .27
6	$.002776_0$	$.000316_2$	$.001229_9$	$.001862_3$	$.004155_5$	-111.1	-62.3	1144.6	59736.6	$.054506_6$	1094.12	- .64
7	$.002923_2$	$.000431_0$	$.001246_0$	$.002108_4$	$.005229_2$	-112.6	-70.8	1479.6	59455.8	$.054228_0$	1096.40	+1.64
8	.002690$_4$	$.000384_0$	$.001153_2$	$.001921_2$	$.003849_4$	-104.2	-64.5	1069.2	59931.7	$.054734_8$	1094.95	+ .19
9	$.002705_6$	$.000393_6$	$.001156_0$	$.001943_2$	$.004411_1$	-104.5	-65.3	1248.1	59863.3	$.054763_0$	1093.13	-1.63
10	$.002664_4$	.0002594	$.001202_3$	$.001721_1$	$.004433_5$	-108.6	-57.8	1254.4	59823.1	$.054682_3$	1094.01	- .75
Average											1094.76	
Standard deviation of the mean											±0.34	

Table 8. Calculation of the heat of combustion of cyanogen from the data of table 6.

Experiment	Amount of nitrite determined	Amount of nitrate determined (= amount of HNO_3 formed)	Amount of NO formed (= amount of NO_2 formed)	Amount of CO formed	Heat of formation of NO	Heat of formation of NO_2	Heat of formation of HNO_3	Heat of combustion of CO	Corrected reaction energy	Amount of C_2N_2 burned	Heat of combustion at 25°C	Deviation from mean
	moles	moles	moles	moles	int. j	int. j	int. j	int. j	int. j	moles	int. kj/mole	kj/mole
1	$.0025264$	0.000348_8	0.001263_2	0.000354_0	-114.1	-42.4	8.0	100.2	58657.9	0.053442_3	1097.59	+0.82
2	$.002019_2$	$.000367_8$	$.001009_6$	$.000399_0$	- 91.2	-33.9	8.5	112.9	58611.7	$.053486_4$	1095.82	- .95
3	$.002016_8$	$.000215_0$	$.001008_4$	$.000543_9$	- 91.1	-33.9	5.0	153.9	58512.5	$.053410_0$	1095.53	-1.24
4	$.002515_2$	$.000405_2$	$.001257_6$	$.000387_6$	-113.6	-42.3	9.3	109.7	58537.7	$.053359_9$	1097.04	+ .27
5	$.0027336$	$.0003336$	$.001366_8$	$.000312_2$	-123.5	-45.9	7.7	88.3	58035.8	$.052878_9$	1097.52	+ .75
6	$.002612_0$	$.000469_6$	$.001306_0$	$.000347_8$	-118.0	-43.9	10.8	98.4	58862.9	$.053665_2$	1096.85	+ .08
7	$.002684_8$	$.000339_8$	$.001342_4$	$.000310_8$	-121.3	-45.1	7.8	87.9	58288.0	$.053130_0$	1097.08	+ .31
Average											1096.77	
Standard deviation of the mean											±0.30	

VII. CONCLUSION

The data of the present experiments indicate that the value of the heat of combustion of cyanogen, at 25° C and a constant pressure of 1 atm., lies between the limits 1094.5 and 1097.0 int kj/mole, or 261.6 and 262.2 kcal/mole. These limits have the following relation to the existing data, listed in ascending order of value:

Investigation	Year reported	Value (kcal/mole)
McMorris and Badger [6]	1933	251.4
Thomsen [4]	1886	259.4
Von Wartenberg and Schütza [5]	1933	261.3
Present	1941	$261.6 < x < 262.2$
Berthelot [3]	1879	264
Dulong [2]	1838	275

While the present investigation has fixed the value with an uncertainty of ±0.12 percent, it is hoped that additional experimentation with regard to the nature and amount of the products formed in the oxidation of the nitrogen will reduce the uncertainty.

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