ternal cooling of the leaf. If such an effect existed it should have been greatly reduced following illumination with 2,600 fc of blue light, for the blue filter (10 mm of 20% aqueous CuSO₄) reduced the external heating effect of the light markedly and should have reduced the internal heating even more. Average external leaf temperatures were as follows: white light—30.1°C, blue light—27.5°C, dark—26.5°C.

The initial and subsequent rates of respiration (based on the slopes of segments a and b as shown in figure 1) of a leaf of hybrid tobacco were measured for 20 consecutive cycles of light and dark during which white light at 2,800 fc and blue light at 2,600 fc were alternated. The mean a rates (in mg CO₂/min) were as follows: white—0.1437 ± 0.026 (standard error), blue—0.1348 ± 0.026. The slight difference between mean rates could have occurred by chance alone oftener than once in twenty trials and is considered fortuitous. The mean b rates were: 0.0345 and 0.0339, respectively. The ratios of mean a to mean b were: 4.7 and 3.98, respectively. These results show that the respiratory deceleration pattern could not have been the result of internal cooling of the leaf. The experiment was repeated through 20 cycles with each of two other plants from the same seed lot and planting with almost identical results.

Subsequent experiments have shown that the CO₂ evolution decelerated for approximately 6 to 9 minutes following darkening and that the final stable dark rate was about 75% of that shown for segment b. Thus the mean initial rate was approximately 5.4 times greater than the basal dark rate.

Obviously more work is needed before an explanation will be found which will demonstrate the compatibility of these findings and those of Brown (1).

LITERATURE CITED


EFFECT OF BORON DEFICIENCY ON NICOTINE FORMATION IN TOBACCO

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Connecticut Broadleaf tobacco plants have been grown in ten liter water cultures deficient in micronutrients for many years (5). A preliminary study of the effects of micronutrient deficiency on nicotine formation was included among the biochemical determinations made with these plants. The micronutrient deficiencies checked were iron, zinc, copper, manganese, boron and molybdenum.

Single plants for assay were selected at random from each group of triplicates. Usually the triplicates were quite uniform visually in every respect. However, only plants showing marked and characteristic symptoms of deficiency were used for analysis. Five series of tests in all were made over a period of more than a year.

A different procedure was followed in sampling in the first trial than in subsequent tests. The method used in this initial experiment was selection of a portion from the middle of the stalk, several leaves midway up the stalk, and the entire root of the plant for analysis. These were dried in a current of air at 80°C for several hours, ground and bottled separately. Each sample was assayed for total alkaloid by Dawson's spectrophotometric method (1, 2). Total alkaloid is equivalent to better than 90% nicotine in the tobacco variety employed. The results of the several analyses for each plant have been combined and are expressed as percentage and milligrams of total alkaloid for the dry plant as a whole. All other samplings included all the leaves, the entire stalk and the whole root of the plant being assayed.

The first series of determinations revealed significant differences in alkaloid content only with manganese and boron deficiencies. Results on leaves of plants deficient in iron, zinc, copper and molybdenum varied little from those with the control. Examination of table I will show, however, that the minus boron plant displayed a four-fold increase in alkaloid concentration expressed as percent of total dry weight and an increase in quantity of nicotine of about 60%. This took place despite a 60% loss in dry-weight yield of the boron-deficient plant. Decreased alkaloid in the minus manganese plant seemed to be a direct consequence of decreased yield, which was equivalent to but 20% of the control.

As can be seen by comparison with the last group

1 Received August 13, 1954.
of plants, the method of sampling employed in the first experiment led to results in no way essentially different from those where the whole of the plant parts were included in the sample. Percentages of total alkaloid on a dry weight basis at the early flowering stage of the controls in spring of 1951 were 0.13, 0.50 and 0.08% with partial plant samples for control, minus boron and minus manganese. In spring of 1952 with whole plant samples, the corresponding values were 0.12, 0.44 and 0.15%. Agreement was satisfactory in view of the difficulty usually experienced in close control of deficiency levels with micronutrients.

Subsequent tests were divided into early and late samplings. The object was to determine the relation, if any, that increased nicotine synthesis might have to stage in development of minus boron symptoms. Visual boron deficiency symptoms in tobacco have been described by McMurtrey (3). In these experiments they began with excessive branching of the fibrous roots (fig 1) and inhibition of stalk growth, followed very quickly by formation of asymmetric apical leaves. Shortly thereafter basal blackening and death of the apical bud took place. Axillary buds also blackened and died subsequently. Van Schreven (6) has described the internal symptoms of boron deficiency in tobacco. Similar, even though not identical symptoms of boron deficiency, have been studied microscopically and described by Warington for other crops (7, 8). The phenomenon of excessive root branching was noted by each of these investigators. Van Schreven moreover found that the first symptom of boron deficiency in tobacco occurred in the root. Figure 1 illustrates the difference in appearance of the fibrous roots detached at the point of emergence from a tap root in control and minus boron plants. Solt and Dawson (4) found that nicotine formation was confined largely to the apices of shoots and roots. Though boron deficiency in tobacco resulted in death of almost all apices in the shoots, the concomitant large increase in number of root tips resulted in a large net increase in growing points for the plant as a whole.

The first early samplings were made with plants grown in winter. The poorer light at this season of the year was, as usual, reflected particularly in decreased growth of controls. It can be seen also that no rise in alkaloid was evident at the bud blackening stage of the minus boron plant when grown in winter. Three weeks later, however, the picture was almost identical with the first series, except for a minor difference caused by the much poorer growth of the control.

Another repetition of these tests in the spring of 1952 again gave similar results. The earlier samples were taken just prior to the necrotic or bud blacken-
ing stage of boron deficiency. Excessive root branching and leaf asymmetry symptoms were present but had not reached their maxima. Nicotine content in the minus boron plant had increased about 50% in quantity on a whole plant basis even at this very early stage before development of symptoms of necrosis. Three weeks later the sampled plants again showed the same relationships found during the two previous series of tests.

The ratio of root to total plant dry-weight varied consistently with type of deficiency in these experiments. At time of flowering it ranged from 8.9 to 11.8% in the controls; from 11.4 to 17.0% in minus boron; and from 3.7 to 7.3% in minus manganese plants. The average values at this stage of growth were 10.7, 14.1 and 5.9%, respectively, for these three types of plants.

It would appear, therefore, that these data correlate well with the findings of Solt and Dawson (4) that nicotine formation is associated primarily with apices of shoots and roots. Nicotine began to accumulate rapidly shortly after excessive root branching had begun (prior to bud necrosis) in the minus boron plant, and increased rapidly with further aggravation in symptoms. The final result was a rapid and parallel increase in number of root-tips and quantity of nicotine produced that was strikingly greater than for either control or minus manganese plants.

The determinations of total alkaloid discussed herein were made by Dr. R. F. Dawson. The writer wishes to express his appreciation of Dr. Dawson's kindness in both this respect as well as for aid given in preparation of this manuscript.

LITERATURE CITED


THE ROLE OF 2,4-DICHLOROPHENOL IN THE DESTRUCTION OF INDOLEACETIC ACID BY PEROXIDASE

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An enzyme system capable of destroying indoleacetic acid (IAA) has been reported to occur in some plants (4, 10, 12). Further reports (1) have shown that this activity may be reproduced by a crude or crystalline preparation of peroxidase from horseradish, together with added H2O2; evidence is presented that the indoleacetic acid oxidase of pea seedlings consists of a peroxidase with a physiological source of peroxide.

The present paper will report results which indicate that a cofactor, mediating the destruction of IAA by peroxidase, is a requirement for activity of certain horseradish preparations. This unknown cofactor may be effectively replaced by 2,4-dichlorophenol (DCP). It will also be shown that an effective peroxidase from Xanthium leaves is incapable of destroying IAA even in the presence of DCP, indicating a difference in the nature of the peroxidase obtained from horseradish and Xanthium.

An extract containing active peroxidase was prepared from dehydrated horseradish obtained from a local food market. The dehydrated powder was ground with a mortar and pestle in water (20 mg/ml) and the particles centrifuged off. The resultant aqueous extract was tested for peroxidase activity by the method of Siegel and Weintraub (8). The enzyme preparation and peroxide were added to potassium phosphate buffer (pH 6.0; final conc. 0.05 M) in a colorimeter tube and the transmission set at 100%, using a 420 mµ Evelyn filter. At 0-time, 0.2 ml of freshly prepared 5% pyrogallol was added to a final volume of 10.0 ml and the rate of color development measured. Control tubes, lacking H2O2, indicated a very slow autooxidation and no oxidase enzymes active on pyrogallol. The rate of enzymatic oxidation of pyrogallol is initially linear, but falls off as the pyrogallol accumulates. The horseradish prepared as above was found to have a high peroxidase activity (table I).

1 Received September 30, 1954.
2 Predoctoral Fellow, National Science Foundation.