SPECTROGRAPHIC DETERMINATION OF BORON IN PLANT TISSUE;
SURVEY OF THE BORON CONTENT OF KANSAS
GROWN ALFALFA AND WHEAT

by

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At the present time, literature shows that approximately 60 elements have been identified positively in plants (29). Many of them are, of course, present in such extremely minute quantities that it is difficult to quantitatively determine the amount present. There are some elements that are present in slightly larger quantities but still require special methods of analysis. These are the minor elements.

The minor elements in plants may be divided roughly into two classes, essential and nonessential. Although there is a difference of opinion as to the elements that should be in each class, it is accepted generally that the minor elements of great agricultural importance are those that usually are recognized as essential, such as boron, manganese, iron, zinc, and copper. The effect of this group of elements on plant growth is fairly well known. Into the second group falls elements such as titanium barium, strontium and lead. Very little is known about the effects of those in the latter group.

Until about two decades ago, the importance of the minor elements was generally overlooked. Recently, however, the significant role played by these elements in the plant metabolism and plant growth has been realized to such an extent that the so-called trace or minor elements are receiving considerable attention from soil scientists and plant physiologists. Indicative of this interest is the element boron.

Boron was first discovered in plants nearly a century ago by Wittstein and Apoiger (33) who found boric acid in the seed of maesa picta. Years later boron was found in the tissues of many plants. Since that time the interest in this essential minor element as a nutrient growth factor of major importance has expanded greatly, as has been shown by over 600 listed publications on the subject. The majority of these papers have been published in the past 10 years. They show the problem of boron deficiency to be rather widespread, 31 states in the United States and 6 provinces in Canada have reported boron deficiency symptoms in over 40 crops (24). The areas known to be deficient in this country include all the states east of the Mississippi river and those in the Pacific Northwest. The middle states and those of the southwest, however, have apparently been considered to be supplied adequately with the boron necessary for normal plant growth. Although there has been much survey work done in the generally accepted deficient areas in this country, few data have been obtained in Kansas or in the surrounding states. Because of this existing situation, it was felt desirable to make a survey of the boron content of the important crops grown in this state.

Among the crops known to be affected by a deficiency of boron, alfalfa is one of the most important. Legumes apparently require more boron than other crops, and consequently boron deficiency symptoms in any given area are more likely to appear first in alfalfa or clover. In the past few years, alfalfa has

become of greater economic importance to Kansas because of the
relatively greater quantities being grown in the state. Its
value as an excellent and cheap source of protein and vitamins
for livestock and poultry is well known. With the increased
dehydration of the crop, alfalfa assumes an even more signif-
icant place in the Kansas economy and consequently is a factor
in the industrial development of the state. This makes it
even more important that Kansas maintain its position as a
producer of high quality alfalfa, and indicates why it was
felt advisable to make alfalfa the object of the first study
in connection with boron content of Kansas crops.

At least seven different symptoms of boron deficiency are
known to affect the alfalfa plant (19). They are abnormal
foliage coloration, terminal "dieback", rosetting, multiple
branching, defective inflorescence, stripping and seedling
death. Of all these signs probably the most common is the
first, usually known as alfalfa "yellows" or yellowtop. Un-
less the topmost leaves of the plant are discolored, it is not
a deficiency symptom, but is due probably to other causes, e.g.,
potato leaf hopper which manifests itself in discoloring the
whole plant.

Besides correcting the above abnormalities of the alfalfa
plant, boron has been reported by several authors (6, 11, 19)
to greatly increase the yield and the duration of the stand of
the crop. Some workers (10, 11, 19, 27) report that a large
increase can be obtained in the seed production when the proper

amount of boron is added to the deficient soil. Piland and
Ireland (26) reported that the proper application of boron
on a deficient soil during the time of planting resulted in
good setting of the seed. Boron also has been reported to
increase the carotene and chlorophyll content (28); give
elasticity to the cell membranes and aid in cell division
(12); prevent swelling and blocking of the roots (30); aid
nitrogen metabolism and the development of nodules and
nodule bacteria (4); and also reduce the effects of drouth
(5).

SURVEY OF THE LITERATURE

One of the most important problems in connection with
this type of survey is the availability of a simple method
of analysis of adequate precision. Since boron in plant
material is present in micro amounts, in the range of from
10 to 60 parts per million, the main difficulty concerning an
analysis of this type is to have a method that will be sen-
sitive enough not only to detect the small amount present
but also distinguish between small changes in the concentra-
tion of the material in the plant. Two general methods of
analysis were immediately suggested, colorimetric and spectro-
graphic.

By far the majority of the work reported in the literature
on the boron content of plant materials has been done by the

use of quinalizarian as a color reagent. Berger and Truog
(1, 2), in particular, have done a considerable amount of
work in the development of this procedure. Extracts of
curcumin and turmeric (22) also have been suggested as color
producing reagents for the determination of boron. Some work
on the development of a spectrographic method of analysis for
boron in plant materials has been done by McHargue, et al.
(16); Parks (25) has reported on the spectrographic determina-
tion of boron in synthetic soil mixtures; and Melvin and
O'Connor (15) have done work on the spectrochemical analysis
of fertilizers for boron and other trace elements.

Although giving fair results, most of the above men-
tioned procedures have certain disadvantages. For example,
the quinalizarian reaction must be carried out in 98 per cent
sulfuric acid, and the acid concentration is quite critical.
The shift in the absorption maximum of the reagent, which is
produced by the boron, is quite small, about 40 mμ (31). This
could lead to difficulty with certain filters in the usual
type of colorimeter employed for this purpose.

The use of curcumin and turmeric has been shown to give
results which do not agree well with other procedures, par-
ticularly at low concentrations (2). Results obtained by
the titration procedure of Wilcox (32) have been shown to be
relatively high on small quantities of boron.

The procedure of McHargue et al. (16) consisted in con-
verting the boron in plant material to methyl borate, dis-

tilling off the methyl borate and determining the boron in the
distillate spectrographically. They also reported the deter-
mination of boron directly in the plant ash but without the
added controls of spectroscopic buffer and internal standard.
Obviously, the advantages of spectrographic procedures sug-
gested that a further study of this method might lead to
other procedures for stabilizing the boron line and inhibit-
ing the effects of the extraneous elements. The results of
this study have led to the following spectrographic procedure
which shows quite high accuracy and reproducibility with a min-
imum of preliminary work. Results compare favorably with the
quinalizarin procedure. Furthermore, although boron was made
the object of the study, the conditions under which the spectra
are produced will permit the simultaneous estimation of phos-
phorus, manganese and magnesium with no further change in con-
ditions.

EXPERIMENTAL PROCEDURE

Description of Apparatus

The spectrograph used in this research was a Bausch and
Lomb large Littrow instrument with a quartz optical system.
This instrument has a wave length range of 2100 to 8000 Ang-
strom units and a linear dispersion of 2.5 Angstrom units per
millimeter at a wave length of 2500 Angstroms. The quartz

prism is 57 millimeters high with a refraction face 95 milli-
meters long, the reflection coating being of the evaporated
aluminum type. The lens in front of the prism has a focal
length of 1827 millimeters and is provided with two stops for
reducing the aperture.

The illuminating system consists of the electrode holders,
a spherical condensing lens, and a rotary sector disk.

Line densities were obtained by means of an Allied Re-
search Laboratories-Dietert densitometer of late design.

All samples were excited with a direct current generator
at a potential of 120 volts and a current of eight amperes.
A ballast resistor was placed in series with the generator to
stabilize the current.

Preliminary Work

The line of the boron spectrum considered to be most suit-
able for analysis is the one located at a wave length of 2497.73
Angstroms. Although the data given in Brode (3) show that
there are interfering lines from iron, molybdenum, vanadium,
tin and tungsten there was no difficulty in this respect for
probably two reasons: first, the interfering elements were not
present in sufficient quantity to give visible spectral lines
in this region; and second, the dispersion of the instrument
in this range is sufficient so that no difficulty was exper-
ienced. Proof of this latter reason is shown in the example

of the tin line located at 2495.7 Angstroms. This line was
separated from the boron line on the plate a distance of 0.3 mm
and was used as the reference line of the internal standard,
Plate I. Table 1 shows a list of the possible interfering
lines with their wave lengths and relative intensities.

Since the spectrum length of the large Littrow spectro-
graph is about 80 cm it is possible to photograph only a cer-
tain portion at a time. There are nine different positions
of the plate and prism necessary to photograph the entire
spectrum length. Position seven was used to photograph the
boron line at 2497.73 Angstroms because the line appeared near
the center portion of the plate.

Because of the possibility that the instrument was not in
the same adjustment as it was when it left the manufacturer,
it was thought advisable to check the positions of the focus
and tilt prescribed by them. This was done by photographing a
series of iron spectra with the tilt set at the prescribed
value and then varying the focus a few divisions on each side
of the given value. Examination of the plate after development
showed the focus to be best at 154. Making a similar plate by
setting the focus at 154 and varying the tilt, the tilt was
found to be best at 204. These settings were used for all
analyses.

EXPLANATION OF PLATE I

A typical spectrographic plate used in making analyses for boron. In locating the lines referred to, the scale will have to be read backwards. The line located at 84.5 is the tin line 2495.7 Angstroms, used for internal reference. The next line at 85.5 is the boron line at 2497.7 Angstroms used for the analysis of the boron. The two pairs of lines located at 100 and 107 are lines that can be used for the simultaneous determination of phosphorus. The magnesium line located at 164 and the manganese line at 156.5 can be used for the simultaneous determination of these elements. The five top spectra are for the working curve, the remainder are unknown samples.
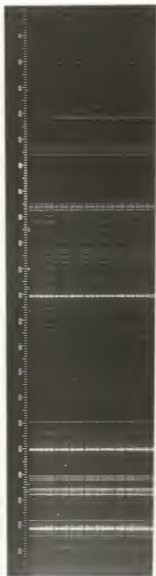
PLATE I

Table 1. Possible interfering lines that appear in the
region of the boron line located at 2497.73
Angstroms.

Element	:	Line	:	Relative line intensity
		: Angstroms:		
Boron		2497.73		500
Iron		2496.99		20
		2497.82		15
Molybdenum		2481.9		50
Vanadium		2483.1		20
		2503.0		7
Tin		2483.4		125
		2495.7		100
Tungsten		2495.3		20
		2496.6		10
		2497.5		10
		2499.2		2

Calibration Curve for Position Seven

Because the dispersion of a prism is not linear, it was
necessary to prepare a calibration curve for each position to
locate the spectral lines appearing in that position. To do
this, a spectrographic calibration curve was made by printing
a scale on the plate and directly below this an iron spectrum
and then below this a spectrum of the spectrographic standard
used in the analyses. After development of the plate, the wave
lengths of the iron lines were determined by comparison with
the iron spectrum charts compiled by Brode (3). The scale

readings corresponding to the line wave lengths were plotted using the scale reading as the abscissa and the wave length as the ordinate to obtain the curve shown in Fig. 1.

Preparation of the Spectrographic Standard

One of the requisites for the standard in an analysis of this nature is that it be quite similar in composition to the material being analyzed which in this case is the plant ash.

The spectrographic standards used in the analysis of flour by Morris et al. (20), with a few modifications, were found to be quite satisfactory. Close examination of the spectral lines that appear in the alfalfa ash and the spectrographic standard showed that practically the same lines appeared in both spectrograms in about the same ratio.

A series of standards were made by adding varying amounts of the spectrographic standard solution to the buffer solution and the internal standard solution as shown in Table 2. All chemicals used in these analyses were checked spectrographically to show boron was not present as an impurity.

Selection of the Internal Standard

The internal standard method of control, first used by Gerlach and Schweitzer (9) to compensate for variations likely to occur in the arc during the excitation of the sample,
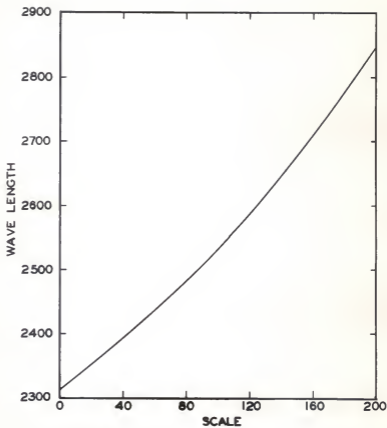
FIG.1. DISPERSION CURVE.

assumes that any variability in the arc will affect the excita-
tion of the unknown and the reference element in the same
manner. The difference in densities of the two lines also is
assumed to be proportional to the logarithm of the ratio of the
intensities of these lines in the source. The first assumption
has been shown to be reasonably justified in many cases. In
some, however, a change in conditions will materially change
the relative intensities of a pair of lines. The second
assumption holds only for the straight line portion of the
characteristic curve for the photographic plate. In the case
of the analyses found in this paper, it should be noted that in
all cases the quantity of boron was such that the line fell well
within the straight line portion of the plate calibration curve.

Table 2. Dilutions used for making a series of spectrographic
standards.

Standard number	Spec. std. solution	Buffer solution	Int. std. solution	Amt. boron B/4 ml on electrode	
		ml		ppm	μg
1	0.10	2.90	1.00	1.0	0.025
2	0.25	2.75	1.00	2.5	0.0625
3	0.50	2.50	1.00	5.0	0.125
4	0.75	2.25	1.00	7.5	0.1875
5	1.00	2.00	1.00	10.0	0.25
6	1.50	1.50	1.00	15.0	0.375
7	2.00	1.00	1.00	20.0	0.50
8	3.00	0.00	1.00	30.0	0.75

If a series of exposures were made for separate portions of a given sample, the line densities of the spectra obtained will vary over a considerable range. The chief reason for this lies in the fact that one cannot accurately control the rate of vaporization of the sample material. During the course of an exposure the temperature of the source changes as the vapor atmosphere changes. Therefore, the probability of excitation for a vaporized atom will change with time. The material used for an internal standard should therefore possess the following characteristics:

1. The internal standard line should have the same excitation voltage as the analysis line, so that changes in temperature will affect both lines in the same manner.

2. The internal standard element should be vaporized at the same rate as the constituent sought in the analysis, and their boiling points should be approximately the same.

3. The internal standard should be present in such concentration that the line density is about the mean density found for analysis lines in the concentration range investigated, so that the lines to be compared will be as nearly as possible of the same density.

4. The internal standard must be homogeneously mixed with the analysis sample.

5. The internal standard line should be in the same spectral region as the line of the element sought.

In practice not all of these characteristics are found gen-

erally in the element to be used as the internal standard. In this case, tin fulfilled most of the requirements. Both tin and boron have excitation voltages that are relatively close together, being 7.30 and 8.29 electron volts, respectively. The two elements have nearly the same boiling points, tin 2260° C. and boron 2550° C., and thus would be vaporized off at about the same rate. The third characteristic was rather well satisfied as can be shown by referring to Tables 3 and 4 where data are shown for two wide variations in the boron content of some alfalfa. The percent transmission of density measurements appears under the columns headed Sn and B. The fourth requirement was met in that the samples were made up in solution and mixed thoroughly. The tin line used for reference and the boron line are only about two Angstroms apart in the spectrum thus meeting the conditions of the last characteristic.

There have been a number of lines suggested for use as an internal standard for the determination of boron. Melvin and O'Connor (15), in their analysis of trace elements in fertilizers, used beryllium as the internal standard. Parks (25) has reported the tin line at 2495.7 Angstroms as being satisfactory and possessing characteristics desirable in an internal standard when the excitation source is a high voltage a.c. arc. His work was verified for the d.c. arc used in this research; consequently tin was selected and used in the method presented.

The internal standard was prepared by dissolving one gram of reagent grade tin in enough hydrochloric acid to keep it in

solution. This was then diluted to 500 ml after which it was mixed one to one with the double strength buffer solution to obtain a final concentration of one milligram of tin per milliliter.

Selection of the Spectroscopic Buffer

Because photoelectric densitometry has greatly increased the precision of measurement of spectral line intensities, small variations in these intensities can be detected. Several factors contribute to these variations which affect analytical data. Among these variations is the effect of extraneous elements in the arc. This condition is due at least in part to the transport mechanism of ions across the arc. Transport phenomena in arc sources may be the result of an equilibrium between thermal diffusion of material from the region of the electrode and a migration of ions of the sample due to electrical forces. Langstroth and McRae (14) have investigated transport phenomena and reached the following conclusions:

1. Lines of different elements have, in general, different intensity distributions along their lengths.

2. The relative distributions depend on ionization potentials and masses of the elements, and on the ionization potentials of other atoms present in the discharge.

3. When easily ionized atoms are present in the dis-

charge, the distribution of an element relative to that of a more easily ionized element of comparable mass falls off more sharply toward the unloaded electrode than it does when easily ionized atoms are absent.

The purpose of the spectrographic buffer is to minimize variations in the transport mechanism from spectrum to spectrum. Requisites of an acceptable buffer are that it does not emit a complicated spectrum of its own, and that it has a low ionization potential.

Parks (25) has reported on a number of different buffers for use in the determination of synthetic soil mixtures. He has shown the necessity of careful selection of a good buffer in order to limit the effects of extraneous elements and give suitable calibration curves. Brunstetter and Myers (5), however, report satisfactory results on boron and other elements in plant tissue without buffers or internal standard. Since it is generally accepted that proper selection of buffer and internal standard gives more satisfactory results, this procedure was adopted.

Data of Parks (25) were confirmed regarding the effects of aluminum chloride, sodium chloride and ammonium chloride. Calibrations or working curves of standard solutions using these materials and sodium sulfate as buffers are shown in Fig. 2. It is shown that straight line calibrations did not occur with aluminum chloride and sodium chloride. The other two salts were better. Calcium chloride, as a buffer, markedly suppressed the
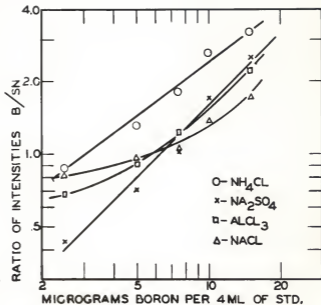
FIG. 2. STANDARD CURVES TO SHOW EFFECT OF
DIFFERENT SPECTROSCOPIC BUFFERS.

boron line and was rejected. Further work in this regard led to the use of lithium chloride as a suitable buffer. This material, in addition to having properties which produce a straight line calibration with boron, has the added advantage of helping maintain a steady arc during excitation. Its hygroscopic tendency was overcome by removing the electrodes from the oven a few at a time. Since this material, as a buffer, seemed to possess several of the characteristics desired, it was chosen for use in this procedure. A typical calibration curve is shown in Fig. 3.

The buffer for the data reported in this paper was prepared by making a 10 percent solution of reagent grade lithium chloride using hydrochloric acid (1:2) as the solvent. This was diluted half and half with spectrographic standard, internal standard solution and water to give respectively the solutions used to make the final series of spectrographic standards.

Determination of Exposure

In order to observe correctly the amount of a material in a sample or the standard it is necessary that there be a complete arcing of the sample; that is, all of the sample should be vaporized and excited. The rate at which a material will be vaporized will depend upon the temperature of the arc and the nature of all the materials on the electrodes.

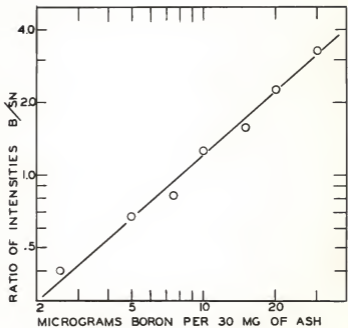By making a moving plate spectrum of a sample of the

FIG.3. WORKING CURVE FOR BORON.

largest spectrographic standard used for preparing the working curve, it was found that all of the boron was burned off of the electrodes in approximately 25 seconds. In order to account for any variations that might occur the samples were excited for 45 seconds.

It was found that after a 45-second exposure, the spectrum of the sample had a small excess of background, and the boron line at 2497.7 was too opaque for best results. To correct this condition, the rotating sector was used. This allows a spectrum of the sample to be taken during the entire excitation of the sample, but cuts the exposure an amount proportional to the sector opening.

The blackening or intensity of the image produced on a photographic plate is proportional to the product of the intensity of the radiation and the time. If the total exposure is made up of a small number of short exposures a condition known as the intermittancy effect is produced. If the number of interruptions exceeds 50, the effect becomes negligible. In all analyses reported here, the number of interruptions greatly exceeded the minimum of 50 reported by Brode (3).

Preparation of Electrodes

Since the standard commercial electrodes cannot be easily purified of boron, it was found advantageous to obtain the special, high grade commercially purified graphite electrodes

from the National Carbon Company. These were prepared for use by cutting the 5/16 inch carbons into 7/8 inch lengths and shaped so that the anode had a cone shaped crater about 1.5 mm in depth. The cathode was shaped similar to the round nose of a bullet. In the early part of the research the cathode electrodes were shaped with sharp points like a pencil, but it was found that the arc had an unsatisfactory tendency to wander with this type. On using the round nose electrodes, it was observed that the arc was less likely to wander.

The electrodes were prepared for the sample solutions by filling the crater of the anode to excess with a saturated solution of carnauba wax in carbon tetrachloride. They were then dried in an air oven for about 15 minutes at a temperature of 100° C. After cooling, the electrodes were then ready to receive the sample solutions.

Spectrographic Procedure

Two grams of the dry plant material were ashed in a porcelain crucible. The percent of ash was noted, and 30 mg of this material was weighed into a small glass vial. One or two milliliters of 1:1 hydrochloric acid were added and the solution evaporated to dryness. The residue was cooled and taken up in three ml of the spectroscopic buffer solution and one ml of the internal solution. Solution was mixed thoroughly.

With a serological pipette, 0.10 ml aliquot of the unknown

or standard solution was placed on each of two prepared electrodes. A duplicate set of electrodes was needed because of the double exposure used. A series of standards were run on each plate for a working curve.

The electrodes with the solutions were placed in an air oven for 30 minutes at 80° C. then after adjusting the temperature to 100° C. the samples were allowed to remain several hours, preferably overnight.

The samples were arced at a d.c. potential of 120 volts and at a current of eight amperes for a period of 45 seconds with the sector set at 5/8 open. Then without moving the plate, an identical exposure was made with the duplicate electrodes.

After exposure, plates were developed for five minutes at a temperature of 68° F. in Eastman X-ray developer, diluted one to two, fixed, dried, and the line densities determined with the aid of an A.R.L.-Dietert densitometer. Eastman 33 plates were used throughout the survey and were found to be very uniform as shown by the calibration curves taken on each plate.

The results were converted to the dry weight basis of the plant material and are reported in parts per million.

Precision of the Method

In order to ascertain the possibility of distinguishing between the small differences that were likely to occur in a survey of this type, it was necessary to determine the

precision of the method used. For this study two samples of alfalfa ash with quite different boron concentrations (36.6 ppm and 16.6 ppm) were each analyzed several times. Averages were obtained and the deviations and probable errors were calculated. Tables 3 and 4 present data which indicate the precision of the method at the two different concentrations. In the case of the sample containing the smaller amount of boron the probable error was 1.73 percent, in the second case a probable error of 1.43 percent was calculated. This also indicated that the absolute errors were nearly equal in this range of boron analysis.

An interesting comparison can be noted here as to the stability of the blunt nosed electrodes. The sample containing the larger amount of boron was analyzed in the same manner for precision using the sharp pointed electrodes. In this case, the probable error was 2.7 percent. This compares with the value of 1.43 percent shown above with the blunt nosed electrodes.

Comparison of Spectrographic and Chemical Methods

Before a spectrographic procedure can be accepted with any degree of confidence, it must undergo a favorable comparison with a well known chemical procedure. The method used for comparison was identical to the widely used quinalizarin colorimetric procedure of Berger and Truog (1, 2) modified only to

Table 3. Analyses of alfalfa from Belleville to show
precision of spectrographic method.

Sn	B	Sn/B	μg/30 mg	ppm	D	D^2
39.5	31.2	1.27	10.7	32.8	3.8	14.44
34.5	24.5	1.41	12.0	36.8	0.8	0.64
37.1	28.1	1.32	11.2	34.4	2.2	4.84
42.8	30.2	1.42	12.1	37.1	0.5	0.25
40.2	32.0	1.26	10.6	32.5	4.1	16.81
33.5	24.2	1.39	11.3	36.2	0.4	0.16
36.1	25.5	1.42	12.1	37.1	0.5	0.25
41.5	30.2	1.38	11.7	35.9	0.7	0.49
37.6	25.8	1.40	11.9	36.5	0.1	0.01
39.7	26.0	1.53	13.2	40.5	3.9	15.21
42.3	29.0	1.46	12.5	38.3	1.7	2.89
41.3	26.7	1.55	13.3	40.8	4.2	17.64
Total			143.1	439.0	22.3	73.03
Average			11.9	36.6	1.86	

s = standard deviation = $\sqrt{\dfrac{\sum D^2}{n-1}}$ = $\sqrt{\dfrac{73.03}{11}}$ = $\sqrt{6.64}$ = 2.58

Standard error = $s/\sqrt{n}$ = $2.58/\sqrt{12}$ = $\dfrac{2.58}{3.46}$ = 0.747

Probable error = (0.747) (0.7) = 0.523

Percent standard error = $\dfrac{0.747}{36.6}$ = 2.04 percent

Percent probable error = $\dfrac{0.523}{36.6}$ = 1.43 percent

Table 4. Analyses of alfalfa from a Columbus plot No. 7 to
show precision of spectrographic method.

Sn :	B :	Sn/B :	μg/30 mg :	ppm :	D :	D^2
33.4	37.4	0.89	7.1	18.1	2.1	4.42
30.8	38.8	0.79	6.2	15.8	0.2	0.04
31.4	42.1	0.75	5.9	15.0	1.0	1.00
37.5	50.7	0.74	5.8	14.7	1.3	1.69
35.6	45.7	0.78	6.1	15.5	0.5	0.25
37.1	47.0	0.79	6.2	15.8	0.2	0.04
39.0	47.4	0.82	6.5	16.5	0.5	0.25
35.0	41.6	0.84	6.7	17.0	1.0	1.00
39.2	42.0	0.93	7.5	19.0	3.0	9.00
37.5	46.2	1.81	6.4	16.2	0.2	0.04
32.0	41.8	0.77	6.0	15.2	0.8	0.64
Total				176.8	11.3	17.68
Average				16.3	1.03	

$$s = \text{standard deviation} = \sqrt{\frac{\sum n^2}{n-1}} = \sqrt{\frac{17.68}{10}} = \sqrt{1.77} = 1.33$$

$$\text{standard error} = s/\sqrt{n} = 1.33\sqrt{11} = \frac{1.33}{3.31} = 0.402$$

$$\text{Probable error} = (0.402)\ (0.7) = 0.281$$

$$\text{Percent standard error} = \frac{0.402}{16.3} = 2.46 \text{ percent}$$

$$\text{Percent probable error} = \frac{0.281}{16.3} = 1.73 \text{ percent}$$

the extent that the ash was weighed directly.

A 30 mg portion of the ash already obtained for the spec-trographic procedure was weighed out and exactly five ml of approximately 0.36 N sulfuric acid were added to it and mixed thoroughly. This was allowed to stand until the insoluble materials had settled out. One ml of the supernatant liquid was pipetted out and placed in the photoelectric colorimeter tube (An Evelyn Photoelectric Colorimeter manufactured by the Rubicon Instrument Company of Philadelphia was used). Ten ml of 98 percent sulfuric acid solution containing 25 milligrams of quinalizarin per liter were then added. The tube was stoppered and allowed to cool to room temperature. The percent transmission of the tube was read and compared with a previous-ly determined curve made by reading a series of known standards.

A series of nine alfalfa samples were analyzed by this method and compared with spectrographic analyses. The results are tabulated in Table 5. An examination of the data given here will show that agreement between the two methods is quite good. The spectrographic results average approximately 6.3 per-cent less than those obtained by the colorimetric procedure, and has a maximum deviation of -16.2 percent in this series.

Table 5. A comparison of spectrographic and quinalizarin
methods of analysis of boron.

Sample	:Quinalizarin: μg/sample	:Spectrographic: μg/sample	Dif- :ference	: Percent :difference
Columbus No. 5	5.5	4.7	-0.8	-14.5
Abilene	7.4	7.6	0.2	2.7
Columbus No. 1	8.6	7.8	-0.8	- 9.3
Manhattan	9.0	8.4	-0.6	- 6.7
Burlington	8.7	7.7	-1.0	-11.5
Salina	11.5	11.7	0.2	1.7
Belleville	12.2	13.3	1.1	9.0
Topeka	13.0	11.4	-1.6	-12.3
Lyndon	16.8	14.0	-2.8	-16.2

Determinations of Other
Elements Simultaneously With Boron

Although this procedure had been developed for boron and
analyzed for the reliability of boron determinations, Fig. 4
indicates the possibility of the simultaneous determination
of phosphorus, manganese and magnesium. In this figure cali-
bration data are presented on these three elements. The same
tin line that was used for the boron analysis served as the
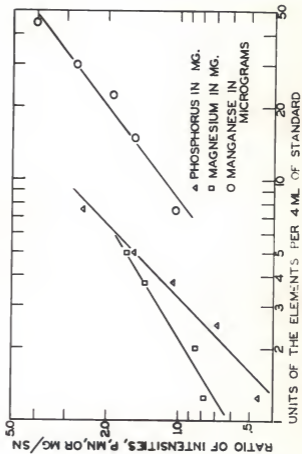internal standard for these elements. The spectral lines used

FIG. 4. WORKING CURVES FOR THE ELEMENTS PHOSPHORUS, MANGANESE AND MAGNESIUM.

were: phosphorus, 2534.0 A; manganese, 2801.1 A; and magnesium, 2790.8 A. The calibration curves were in the same range of concentrations usually found in plant material, and while it is to be expected that probable errors would be larger than in the case of boron, it is also likely that the data so obtained would be very useful.

RESULTS OF ALFALFA SURVEY

The samples used for the survey were obtained from several locations within the state. These areas were chosen in such a manner as to represent several of the different soil types found in Kansas. All samples were secured at approximately the same stage of growth, that of about one-tenth bloom. This method of sampling would tend to eliminate differences due to variations in boron content caused by collecting samples at different physiological stages of growth. Fields were sampled by obtaining small amounts of alfalfa from several places within the field and then dividing the sample in the usual manner.

A series of samples of alfalfa grown on fertilizer plots in three different places in the state were also obtained. These were secured from plots near Thayer, Coffeyville and Columbus in order to show the effects of different soil treatments on the boron content of the plant material.

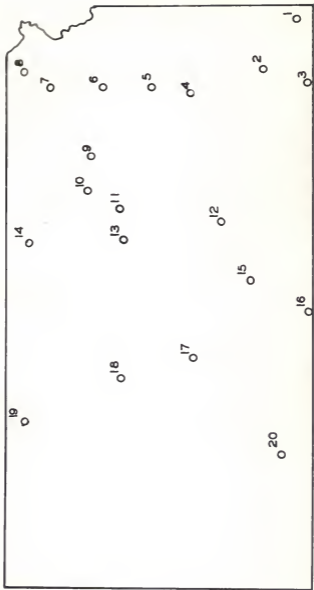An outline map of Kansas is shown in Fig. 5. The numbers

FIG. 5. KANSAS MAP SHOWING WHERE ALFALFA SAMPLES WERE OBTAINED.

of the locations given correspond to the numbers given in Table
6. The general classification of the soils in these areas, as
well as in the entire state, can be obtained readily by ref-
erence to a map of Kansas prepared by the Soil Conservation
Service (8).

The data in Table 6 represent average analyses of alfalfa
grown on untreated plots in the locations given. In general,
the southeastern locations produced an alfalfa lower in boron
content than did these farther west. Also, it may be seen
that the total mineral content is lower in these areas, as
evidenced by the lower ash content of the crop. This result
may be expected, however, on the basis of rainfall data and
the length of time the land has been farmed. These factors
would tend to reduce the readily available nutrients in these
areas and could account for such differences. Rainfall in the
Columbus area has averaged 41.8 inches annually according to
records of the weather bureau, while near Salina the average
has been 27.3 inches. This decreases correspondingly farther
west. The general soil type within these areas also may be
responsible for some of the differences observed.

The results obtained and presented in this table, however,
indicate that if the results obtained by Midgley and Dunklee (19)
are valid in this area a serious deficiency of boron does not
occur in the areas studied. These authors indicate that in the
areas studied by them in eastern United States, alfalfa con-
taining less than 15 ppm of boron will show a favorable response

Table 6. Boron and ash content[*] of alfalfa taken from untreated plots[**] in Kansas.

Locality		Ash percent	Boron ppm	Locality		Ash percent	Boron ppm
Columbus	(1)	7.10	20.9	Abilene	(11)	11.2	28.4
Thayer	(2)	7.88	33.0	Sedgwick	(12)	10.8	37.8
Burlington	(4)	7.9	19.9	Salina	(13)	10.9	43.0
Lyndon	(5)	9.6	41.7	Belleville	(14)	9.2	40.9
Topeka	(6)	7.95	30.1	Kingman	(15)	10.6	40.7
Wetawaka	(7)	8.8	38.0	Kiowa	(16)	9.1	46.4
Hiawatha	(8)	9.4	24.0	Larned	(17)	11.4	35.7
Manhattan	(9)	11.5	32.0	Hays	(18)	12.3	56.2
Wakefield	(10)	12.9	58.4	Horton	(19)	11.9	33.5
				Meade	(20)	12.3	50.9

[*] Figures are reported on a dry weight basis.

[**] Number following locality corresponds to those on Fig. 1.

to the application of boron.

The data presented in Table 7 indicate the effect of different fertilizer treatments on the quantity of boron found in the plant material. It is evident that the addition of certain fertilizer materials reduces the concentration of boron in the alfalfa. This has been explained by some authors (7, 17, 18, 23) as being due to the fixation of boron by the added calcium, but it might also be caused by the fact that the plant has grown so fast that the mechanism for taking the boron out of the soil has failed to keep up with plant growth. It is also possible, of course, that there is not enough available boron in the soil to allow the plant to take up its full quota of the element due to increased plant growth. Of interest is the fact that the addition of the fertilizers listed in Table 7 did not significantly increase the concentration of the mineral constituents in the plant, as indicated by the ash content.

An examination of the data in Table 7 on the samples from Columbus indicates that the addition of limestone alone lowers the boron content of the alfalfa somewhat. Limestone used in conjunction with manure caused still another decrease in boron content. Likewise, when superphosphate is added with these two, the boron content is further decreased in one case and increased in the other. When rock phosphate was used with lime, an increase of the boron content was noted over the results obtained when superphosphate was substituted for rock phosphate with lime. This might be due to increased fixation of boron by the

Table 7. Boron content in alfalfa taken from treated plots at Thayer and Columbus.

Treatments[*]		Thayer Amount				Thayer Analysis			Columbus Amount				Columbus Analysis	
	L:ton	M:ton	S:lb-ton	R:lb-ton	K:lb	Boron ppm	Ash percent	L:ton	M:ton	S:lb-ton	R:lb-ton	K:lb	Boron ppm	Ash percent
None						33.0	7.77						20.0	7.35
M		8				33.8	7.51		8				19.3	7.52
L.	2	8				30.6	8.05	1.5	8				18.9	8.08
L. M.	2	8	150			30.4	7.37	1.5	8	180			18.9	7.62
L. M. S.	2	8	150			21.1	7.52	1.5	8	180			17.4	7.12
L. M. R.	2	8			1/2	20.1	7.38	1.5	8		120		13.2	6.78
L. S.	2	8	150			21.3	7.36	1.5	8	120			10.5	6.70
L. S.			1.5			21.3	7.19			1.5			13.0	6.41
			1.5							1.5	120		12.5	6.50
L. R.		8								1.5		1/2	14.5	7.14
L. 2:5 appl. bien.	2		300			22.2	7.38	1.5		300			14.4	7.15
L. S: K.	2		150		20	25.6	7.35	1.5		180		16		

* M is manure; L is ground limestone; S is 20% superphosphate; R is ground rock phosphate and K is muriate of potash (60%). The figures relate to quantities per acre and are given in pounds and tons. 2:5 appl. bien. means a double application of superphosphate applied biennially.

superphosphate or by the possibility that the rock phosphate
carried some boron as an impurity. When potash was used in
conjunction with limestone and superphosphate, the boron
content was slightly higher than without potash and almost
the same as the limestone and rock phosphate treatment. It
may be noted that the concentration of boron in alfalfa grown
at Columbus dropped below the minimum, suggested by Midgley
and Dunklee (19), in all plots where superphosphate was one of
the constituents of the fertilizer treatment, and came very
close to this level in several other instances.

The fertilizer treatments at Thayer show about the same
effect as those at Columbus, the primary difference being the
slightly higher level of boron in all samples. Manure alone
allowed the boron content to remain practically unchanged,
but the addition of ground limestone decreased the boron
content by about the same percentage as it did at Columbus.
The further addition of superphosphate caused the same magni-
tude of decrease in boron as that which occurred at Columbus.
The addition of potash also produced the same general effect
as similar Columbus samples. The only Thayer sample produced
on soil to which rock phosphate was applied did not show an
increase in boron content, nor did a double application of
superphosphate materially change the boron content of the
alfalfa. In all cases it can be seen that the boron content of
the alfalfa under the various treatments at Thayer remained
above the previously suggested minimum (19).

Table 8 presents data taken from a series of lime and phosphate fertilized plots at Coffeyville. As is true with other cases, the addition of lime decreased the boron content of the plant. The decrease is somewhat irregular; but, in general, an increase in the amount of lime decreased the concentration of boron in the plant. The boron consistently remained above the minimum level recommended in other work.

In order to show the effect of the stage of growth on the boron content of alfalfa, samples were taken at Manhattan every seven days from the time the alfalfa first showed spring growth until the time of cutting. The data obtained, which are presented in Table 9, indicated that apparently the boron content remains relatively constant during growth until the time of cutting. As the plant gets older the total mineral content decreases although the boron does not seem to decrease in the same ratio.

Table 8.   Analyses[*] showing effect of adding lime and phos-
phate to alfalfa plots located at Coffeyville.[**]

Treatment	Ash % dry wt.	Boron ppm
No lime, 100 lbs. 18% superphosphate	9.32	37.6
2 tons lime, 100 lbs. 18% superphosphate	9.23	28.2
3 tons lime, 100 lbs. 18% superphosphate	9.55	34.7
4 tons lime, 100 lbs. 18% superphosphate	9.40	31.0
5 tons lime, 100 lbs. 18% superphosphate	9.05	22.1

[*]   Figures are reported on a dry weight basis.

[**] Location 3 on Fig. 1.

Table 9. Boron content[o] of Manhattan grown alfalfa
at seven day intervals up to time of
cutting.

Time days	Height inches	Ash percent	Boron ppm
7	5	10.6	34.9
14	10	11.4	32.5
21	14	11.0	38.5
28	17	11.3	34.2
35	21	10.5	32.8
42	24	9.7	34.6
49	27	8.75	27.1

[o] All analyses reported on dry weight basis.

RESULTS OF WHEAT SURVEY

Since wheat is the most important crop in Kansas today and will be for some time to come, it was decided to determine the amount of boron in wheat grain as a further test of the spectrographic method developed. Very little is found in the literature regarding the essentiality of boron as a factor in the growth of this crop or of the amount of boron found in the grain. According to Muhr (21), wheat reacted similarly to barley in its response to added boron in that it gave a slightly heavier growth and advanced the time of heading a few days. There are no apparent boron deficiency symptoms reported on wheat. Work at the Kentucky Experiment Station (13) has shown that wheat grain contains approximately one ppm of boron.

Table 10 shows the analyses of three varieties of wheat from 13 different localities in the state of Kansas ranging from west to east. The boron and ash contents are reported on a dry weight basis of the plant material. It should be noted that the amount of boron found in the wheat grain was very much less than that found in the alfalfa. This necessitated the using of four times the amount of plant ash for the analysis of wheat grain as compared with the alfalfa. An overall average of 1.56 ppm boron was obtained. Most of the samples were fairly uniform except those taken at Wichita and Belleville which had values of 0.84 and 2.18 ppm respectively. This represents

a two and one-half fold variation in these two locations. It
is interesting to note that while the areas of Columbus and
Thayer were considered to be rather low for alfalfa, the data
for wheat grain showed no particular variation from the average
as one would expect. The largest differences in the boron
content of the wheat grain were differences in localities, and
the variety differences of any one locality were not great. It
should be realized that the samples of wheat analyzed here
constitute only one year's crop, that of 1945, and much more
data will have to be taken before any accurate conclusions
regarding the boron status of wheat grain can be drawn.

Table 10. Boron and ash analyses* of three types of wheat grain at 15 localities in Kansas.

| Locality | Blackhull | | Tenmarq | | Turkey | | Av. boron in locality ppm |
	Ash percent	Boron ppm	Ash percent	Boron ppm	Ash percent	Boron ppm	
Tribune	1.67	1.55	1.72	1.69	1.69	1.54	1.53
Colby	1.94	1.27	2.12	1.82	1.73	1.68	1.59
Garden City	2.12	1.46	2.00	1.47	1.95	1.76	1.56
Meade	2.10	1.75	2.32	1.95	2.03	1.69	1.50
Dodge City	2.42	1.74	2.07	1.14	2.17	1.45	1.44
Hays	2.33	2.43	2.15	2.01	2.28	1.41	1.95
Smith Center	1.90	1.57	1.93	1.72	1.90	1.38	1.56
Kingman	1.61	1.00	1.39	1.24	1.70	1.51	1.25
Belleville	2.07	2.35	2.32	2.37	2.24	1.31	2.18
Wichita	1.60	0.35	1.73	0.90	1.69	0.79	0.34
Manhattan	2.17	1.55	2.12	1.92	2.11	1.39	1.62
Thayer	1.98	1.21	1.97	1.82	1.92	2.02	1.56
Columbus	2.07	1.32	2.10	1.50	2.07	1.39	1.40
Variety average		1.64		1.63		1.51	

* Analyses are given in parts per million on a dry weight basis.

CONCLUSIONS

A spectrographic procedure of good precision for the determination of boron in plant ash has been developed. The procedure makes use of lithium chloride as a buffer to suppress variations due to differences in composition and to stabilize the d.c. arc. Tin was used as an internal standard.

Analysis of the procedure has given, as a measure of the precision, a probable error of less than two percent. Agreement with the widely used quinalizarin procedure was good. In addition to boron, the procedure will permit the simultaneous estimation of manganese, magnesium and phosphorus on the same sample.

This method has been applied successfully to alfalfa plant material and wheat grain. A survey of the boron in alfalfa taken from several areas in Kansas showed that this state is well supplied with adequate boron for the growth of alfalfa in all areas except those of the southeastern part of the state which, in the case of Columbus, approached the minimum needed for normal growth. Analyses of alfalfa taken from several different fertilizer plots at Columbus and Thayer indicated that in nearly every case the addition of fertilizer tended to lower the amount of boron in the plant. Alfalfa taken at Coffeyville showed that the amount of boron in the plant was approximately decreased as the amount of lime added to the soil

was increased. Analyses of alfalfa at various ages showed
that the amount of boron remained fairly constant throughout
the entire growth until the time of cutting.

Analyses of wheat grain from several different areas in
Kansas showed that in general the boron content remained
rather constant over the whole state. The extreme variations
occurred at Wichita and Belleville. Varietal differences
within the three varieties studied were negligible. The
principal differences which occurred apparently were due to
the locality in which the grain was produced.

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