INFLUENCE OF LEAF DESTRUCTION BY SULPHUR DIOXIDE AND BY CLIPPING ON YIELD OF ALFALFA

GEO. R. HILL, JR. AND M. D. THOMAS

(WITH SIX FIGURES)

Introduction

Exposure to a sufficiently high concentration of sulphur dioxide for a long enough period of time always injures the leaves of alfalfa. Almost invariably the fully grown, highly functional leaves show the injury first, and are more severely injured than the rest. If the concentration is high enough, for example, to produce markings within an hour, after possibly three-fourths of this time has elapsed the leaves become noticeably stiffened, as though the cells had greatly increased in turgidity. The turgidity seems to increase until certain areas in the leaf (those along the margins and between the veins, where the intercellular air spaces are most abundant) suddenly become flaccid and take on a water-soaked aspect, giving the leaf a mottled appearance. If the fumigation be continued, additional marginal or interveinal areas on these leaves already affected, and corresponding areas on hitherto unaffected leaves, become similarly flaccid.

With a few hours of sunshine after the fumigation, these flaccid areas bleach almost to an ivory color. The areas of each leaf which do not become flaccid remain green. Usually the green areas remaining after a heavy fumigation lie along the midrib and the principal veins of the leaf. They are separated sharply from the bleached areas.

The marked areas of an alfalfa leaflet after any fumigation may vary from one spot the size of a pin-point to several larger ones, which may involve 50 per cent. or more of the area of the leaflet. A given fumigation may destroy more than 50 per cent. of the green tissue of some leaflets and yet leave 50 per cent. of the leaves entirely unmarked. Basal leaves are not often marked, and leaves that have not attained their growth only rarely. In all of our experience with alfalfa, a marked stem has never been found. When a majority of the green tissue of a leaflet is destroyed by sulphur dioxide, the leaflet curls and frequently drops from the plant.

This type of injury is called "acute." Occasionally another type of sulphur dioxide injury on alfalfa leaves is encountered, a type that mani-

1 Contribution from the Department of Agricultural Research of the American Smelting & Refining Company, Salt Lake City, Utah.

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fests itself several days after the fumigation. This is evidenced by a rather rapid disappearance of chlorophyll from parts of an otherwise apparently normal leaf, as if the chloroplasts in certain areas of the leaf, three or four days after the fumigation, had ceased to manufacture that unstable pigment. This type of injury is called "chlorotic" (chronic injury by Stoklaska 8).

What is the effect upon yield of a fumigation which destroys a portion of the leaf tissue? Does the green tissue that remains after the fumigation continue to function, and if so, at how nearly normal a rate? If there is a decrease in yield following a sulphur dioxide fumigation, is that decrease proportional to the leaf area destroyed?

In this paper the fumigation apparatus and the methods of growing, fumigation, measuring the injury, and harvesting the plants are described; also yield data as affected by sulphur dioxide lesions and by clipping are presented. The influence of various environmental and physiological factors on leaf destruction by sulphur dioxide, and upon the absorption of that gas by plants, will be considered in subsequent papers.

**Historical review**

There are many papers, mostly foreign, on the relations of industrial wastes, particularly sulphur dioxide and sulphur trioxide, to agricultural production. The problem has long been recognized as one of considerable scientific and economic interest. The European investigations have been summarized by Haselhoff and Lindau (1) and by Stoklaza (8). In this country the principal report of the problem is by the Selby Smelter Commission (2), which also contains an extensive bibliography with abstracts of most of the earlier papers. Verplancke (14) has discussed the subject in a general way; and in a review of the literature by Johnson (3), reference has also been made to recent investigations.

The earlier investigators gave descriptions of the lesions produced on many different plants, and called attention to the acute type and to the chronic, or chlorotic type of SO₂ markings. They also suggested an order of magnitude of the exposures to sulphur dioxide which might cause injury to vegetation. The Selby Commission developed a technique for fumigating plants with sulphur dioxide similar to that subsequently improved upon by O'Gara, described later, and carried out an extensive study of the action of SO₂ on barley. The Commission's report for the first time gave quantitative information as to the effect of concentration and duration of fumigation upon leaf destruction. They showed that "severe" lesions might reduce the yield of grain as much as 40 per cent., depending on the stage of growth when the fumigation occurred, and that it was somewhat proportional to the intensity of the fumigation. They found no reduction in yield
when the leaves were injured only slightly or not at all. Similarly Johnson (3), working on the problem of invisible injury, found no measurable injury to wheat even after very prolonged exposure to sulphur dioxide but at concentrations too low to produce visible markings. So far as the writers are aware, there are no investigations in the literature on the relation of leaf destruction by sulphur dioxide to yield of alfalfa.

In 1914 the American Smelting & Refining Company established a Department of Agricultural Research to study the smelter smoke problem. This Department, under the leadership of the late Dr. P. J. O’Gara, carried out a considerable number of experiments during a period of nine years, the details of which, for the most part, remained unpublished in 1923, when O’Gara became ill and was unable to participate further in these researches. Some of the results of these studies, however, were published in a paper by Wells (15), who discussed the conditions favoring the dispersion of sulphur dioxide from a smelter. Swain (9) and Thum (13) have summarized the smoke litigation in Salt Lake Valley, Utah, giving some details of O’Gara’s work, including photographs of the apparatus and of the experimental plots in the field. O’Gara discussed some of his conclusions in 1917 (4), and also before the American Institute of Chemical Engineers (5). Only an abstract of the latter paper was published. Further, O’Gara circulated in a limited way a list which indicated the relative resistance of a number of plants to sulphur dioxide.

**Fumigation apparatus and technique**

The plan of procedure developed by O’Gara in his closed-cabinet fumigation experiments has been largely followed in these studies. O’Gara used small field plots 5 feet square, suitably spaced in the field so that they were readily accessible. He fumigated these plots with various synthetic mixtures of sulphur dioxide and air, under a cabinet 180 × 180 × 120 cm., made of celluloid mounted on a wooden framework. The sulphur dioxide was delivered from a bottle through a needle valve to an empirically calibrated capillary flowmeter, which indicated the rate of flow of the gas. An adjustable water trap before the needle valve enabled the operator easily to obtain a controlled and very steady stream of gas, which was sent into a fan delivering about 10,000 liters of air per minute. The gas mixture was then conveyed through a 15-cm. pipe to the center of the top of the fumigation cabinet, where radial baffle plates distributed the stream uniformly downward to all parts of the cabinet. As the cabinet was not gas-tight and had no specific outlet, the displaced air-SO₂ mixture leaked out wherever it could. The concentration of SO₂ in the air in the cabinet was determined intermittently by the starch-iodine method of Marston and Wells, as described by the Selby Commission (2). This method consisted
of drawing a definite volume of the \( \text{SO}_2 \)-air mixture into a partially evacuated 20-liter bottle containing a starch-iodine solution of known strength, absorbing the \( \text{SO}_2 \) by vigorous agitation, and titrating this solution with iodine or sodium thiosulphate to a standard light blue color. The amount of \( \text{SO}_2 \) thus determined by analysis usually agreed well with the values calculated from the volumes of \( \text{SO}_2 \) and air as indicated by the \( \text{SO}_2 \) flowmeter and a pitot tube in the air line. Humidity readings were taken at intervals with the whirling psychrometer, and the condition of the plants was observed after the fumigation.

The writers planned to control the humidity and light in the fumigation cabinet, and to measure continuously the sulphur dioxide in the cabinet and also the amount of it absorbed by the plants. O'Gara's apparatus was therefore modified with these objectives in mind.

The fumigation cabinet was of similar type, consisting of a light steel framework 195 × 195 × 150 cm., covered with celluloid, and made nearly gastight. It was mounted on a galvanized iron base provided with a 20-cm. outlet pipe on one side, and a trough on top in which a water seal with the bottom of the cabinet could be made. It was found later that a more satisfactory seal could be made by using a pad of felt 2 cm. thick in the trough, instead of the water. The base was placed around the plot, made level, and its lower edge sealed with soil. When the cabinet was placed on this foundation, anemometers in the intake and outlet pipes of the system indicated that about 80–100 per cent. of the air which entered the cabinet went out by the outlet pipe.

The air was analyzed for sulphur dioxide continuously and automatically by means of a sulphur dioxide autometer, as described later. This machine sampled the air on a 2-minute schedule alternately from a point just below the delivery pipe in the top of the cabinet and from the outlet pipe. The unaccounted-for air was assumed to have escaped at the average concentration of intake and outlet gas. Sulphur dioxide was added to the system with an arrangement similar to that used by O'Gara and already described, except that the apparatus was inclosed and thermostated. The intake of the fan was provided with a shutter so that the volume of air delivered to the cabinet could be varied as desired.

The cabinet was wired so that lamps and reflectors could be mounted above the plants. When the cabinet was covered with canvas, definite light intensities could be maintained, depending on the number and size of the lamps employed. It has not yet been practicable, however, to attain light intensities approaching sunlight. For determining light intensity, the cabinet was provided with three thermocouple light meters. These thermopiles had sixteen junctions and were constructed of 5-cm. pieces of no. 24 nickel-chromium : constantan wire. They were mounted in a wooden box
about 12.8 cm. square and 2.5 cm. thick. One set of junctions was spread out in a row in a section of the box provided with a glass window, the junctions being painted black and the interior of the compartment being painted white. The cold junctions were placed in a dark but ventilated section of the box. This arrangement gave a reading of about 50 millivolts with full sunlight intensity. More recently, the new Weston "photronic" cell shunted across a resistance box has been used with excellent satisfaction. With the proper shunting resistance, the cell and thermopile gave nearly identical readings in the range from about 10 to 100 per cent. of full sunlight intensity. At lower light intensities the accuracy and sensitivity of the thermopile decreased, whereas the photronic cell remained useful, even in dense shade. The cell was also practically instantaneous in its action, and readily indicated differences resulting from the angle of incidence or clouds in the sky.

For making humidity determinations the cabinet was provided with wet and dry bulb thermometers. One pair of thermometers was mounted in the upper part of the cabinet and was provided with a small fan to cool the wick of the wet bulb. Another pair was mounted in the base near the outlet pipe where the movement of the air in the pipe furnished sufficient draft to cool the wet bulb. The relative humidity was maintained at any desired percentage by blowing an atomized spray of water into an intake pipe leading to the fan and also by introducing steam into the same intake pipe. The spray alone was found to be insufficient to maintain the relative humidity on dry summer days above 60 to 70 per cent.; but when this supply of moisture was augmented by steam, the humidity could be maintained between 95 and 100 per cent. Use of the water spray alone ordinarily causes the temperature of the cabinet to fall from 1° to 4° below the outside temperature. While such lowering of the temperature might have a slightly adverse influence on the experiment, it was also advantageous in preventing moisture from condensing on the walls of the cabinet. Silica gel was used to lower the relative humidity.

A continuous, automatic analytical method for determining sulphur dioxide in air was a fundamental requirement for this work. This was partly attained in 1927, when a machine was constructed (10) which automatically drew a measured volume of gas through a measured volume of standard starch-iodine solution at a uniform continuous rate and discharged the solution into a bottle every two minutes, ready for titrating the excess of iodine. In 1928 this apparatus was modified (11) so as to absorb the gas in a slightly acidulated solution of hydrogen peroxide, which oxidized the SO₂ to sulphuric acid. The acid thus produced made the solution a better conductor of the electric current, and it was possible to follow the absorption with an electrical conductivity recorder, thus obtaining a con-
tuinuous record of the concentration of the sulphur dioxide. The apparatus has been further modified (12), so that it is now well adapted to determine with great precision the absorption of the gas by plants in a fumigation cabinet at both low and high concentrations.

The alfalfa plants used were as uniform and comparable as it was possible to secure them. Twenty uniform vigorous seedlings of "common" alfalfa were selected for each plot, in which there were four rows with five plants in each row. The plants thus spaced, when mature, produced approximately fifty stems to the plant. When in the early blooming stage, these stems had approximately fifty leaves (150 leaflets) to the stem. The plots were irrigated by the flooding method often enough to keep the soil well supplied with moisture.

A considerable number of fumigations of alfalfa were carried out under diverse conditions, which produced leaf destruction to different extents. The percentage of leaf destruction was estimated for each plant, either by counting the leaves that were marked and measuring the marked area, or by comparing with a set of standard marked plants, the percentage of bleached area of which had been carefully measured. The estimates of the individual plants, made independently by different observers, were seldom found to differ by more than 10–15 per cent., and the average values for the plot were found to agree within 4 per cent.

At harvest each plant was cut and tied separately, and its green weight obtained immediately. The plants were then placed on end in the greenhouse until air-dry, and again weighed individually, samples being taken to determine the oven-dried weight of the material. A number of individual plants from various parts of the room were weighed at intervals during the time the other plants were being weighed, and were found to vary only to a negligible extent. Accordingly the air-dried weights of each group of plants were considered to be comparable among themselves.

In working out the yield data, the yield of a treated plot was compared with the yield of the same plot on preceding and succeeding untreated crops during the same year, thereby eliminating any influence of variation between the different plots. The yield of successive crops of alfalfa decreases rather regularly during the season (6, 7). Factors were, therefore, determined for each crop by means of untreated check plots scattered throughout the field. By multiplying the yield of each of the crops from each plot by its corresponding factor, the yields of the different crops were placed on a comparative basis. The factors for the 1929 yields were found to be as follows: first crop 1.00, second crop 1.46, third crop 2.00, fourth crop 2.75; and for 1930: first 1.00, second 1.51, third 1.61, and fourth 1.99. It was then possible to compare the yield for any treated plot with preceding or subsequent yields of the same plot when untreated. The yield of a treated
crop divided by the expected yield of that crop gave the percentage of yield as a result of the treatment. This method of calculation was complicated by the fact that, in case of the very severe treatments, the next crop subsequent to the treatment might show a reduction in yield also. In such case comparison was made on the basis of the preceding crop, or of the second subsequent crop. Not more than two of the four crops on any plot were treated during a season.

**Alfalfa yield data**

A. O'Gara's data.—O'Gara conducted many experiments in which he sought to determine the effect on yield of sulphur dioxide fumigations of varying degrees of severity. He recorded the extent of leaf destruction, but in qualitative terms only. It is therefore impossible to evaluate the experiments in which appreciable markings were observed, although they apparently agree in order of magnitude with our own. Accordingly this material is not considered further.

O'Gara also carried out two important experiments with the idea of determining the yield as influenced by repeated low concentrations which did not produce markings. He fumigated three pairs of plots simultaneously, with and without sulphur dioxide, 30 minutes daily for 27 days. On the subsequent crop he reversed the treatment on 42 days. He expressed the yield of the crops fumigated with SO₂ as the average percentage of the yield of the check plots for both crops. This method tended to eliminate plot differences. Table I has been compiled from O'Gara's unpublished data. This table indicates that so long as fumigations do not produce more than traces of markings, there is no significant reduction in yield resulting from a considerable number of short fumigations.

B. Yield of alfalfa as affected by acute sulphur dioxide markings.—During the season of 1929, a great number of plots were given single fumigation treatments on chosen crops, in which varying percentages of leaf tissue were destroyed. The results are shown in figure 1, in which the yield is plotted against the percentage of leaf area destroyed. Since the number of fumigated plots is so large, it is not practicable to present the individual yield data in a table. In figure 1 the various crops which were treated are shown by different kinds of points, as indicated in the legend. The equation of a straight line through these points, worked out by the method of least squares, is \( y = 98.6 - 0.265x \) . . . . . . . . . . . . (1a) in which \( y \) is the yield of dry matter expressed as percentage of the check, and \( x \) is the percentage of the total leaf area destroyed. The number, \( n \), of plots fumigated in this experiment was 80, and the standard deviation, \( S_y \), of the individual plot yield from the line is 5.85 per cent. The somewhat high value of the coefficient of correlation, \( r = 0.715 \pm 0.055 \), indicates
<table>
<thead>
<tr>
<th>Date</th>
<th>Crop</th>
<th>No. of Fumigated Plots</th>
<th>No. of Check Plots</th>
<th>No. of Fumigations per Plot</th>
<th>Duration of Fumigation</th>
<th>Concentration of SO₂</th>
<th>Yield Dry Weight per Plant (Percentage of Check)</th>
<th>Leaf Destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/3–9/4</td>
<td>2nd</td>
<td>3</td>
<td>3</td>
<td>27</td>
<td>min.</td>
<td>30</td>
<td>0.3</td>
<td>2.0</td>
</tr>
<tr>
<td>9/8–10/3</td>
<td>3rd</td>
<td>3</td>
<td>3</td>
<td>41</td>
<td>30</td>
<td>0.4</td>
<td>2.3</td>
<td>6.2</td>
</tr>
<tr>
<td>8/3–9/4</td>
<td>2nd</td>
<td>3</td>
<td>3</td>
<td>27</td>
<td>30</td>
<td>0.9</td>
<td>4.1</td>
<td>1.82</td>
</tr>
<tr>
<td>9/8–10/30</td>
<td>3rd</td>
<td>3</td>
<td>3</td>
<td>42</td>
<td>30</td>
<td>0.7</td>
<td>3.2</td>
<td>1.21</td>
</tr>
</tbody>
</table>
that considerable confidence may be reposed in equation (1a) as expressing the relation between yield and leaf destruction for a single fumigation. The difference between the origin of this curve at 98.6 per cent. yield and at 100 per cent. is without significance, considering the large value of the standard deviation of the yield from the curve. The equation indicates that a fumigation which destroys all of the fully matured leaf tissue of a plant at any given time could be expected to reduce the yield about 28 per cent., and that the destruction of any given percentage of leaf tissue could be expected to reduce the yield by that same percentage of 28 per cent.

**Fig. 1.** Effect of the destruction of various percentages of leaf tissue by a single sulphur dioxide fumigation on the relative yield of alfalfa. Each point represents the yield of the fumigated crop from a plot as compared with the untreated crops from the same plot; 1929 data.

In 1930 the attempt was made to produce the same percentage of leaf destruction on certain crops in each of one, two, and three fumigations. The percentage of leaves which were marked was determined, as well as the percentage of the leaf area destroyed. In the multiple fumigations these values are expressed as the average of the individual percentages for each fumigation. These data are presented in figures 2 and 3.

In the upper chart of figure 2 are given the 1930 data. These duplicate the 1929 experiments recorded in figure 1, except that the points are segregated as to stage of growth at time of fumigation, instead of as to the crop fumigated. The equation of the best-fit straight line is:
FIG. 2. Effect of the destruction of various percentages of leaf tissue by one, two, and three sulphur dioxide fumigations on a single crop on the yield of alfalfa. Yield of fumigated crop is expressed as percentage of yield of untreated crops on the same plot; 1930 data.
Fig. 3. Effect of the destruction of various percentages of leaf tissue by one, two, and three sulphur dioxide fumigations on a single crop on the yield of alfalfa. Leaf destruction is shown as percentage of the total number of leaves which were marked in any degree. Yield values are the same as in fig. 2; 1930 data.
\[ y = 99.5 - 0.302x \quad \ldots \quad (1b) \]
\[ n = 96 \]
\[ S_y = 7.35 \text{ per cent.} \]
\[ r = 0.64 \pm 0.060 \]

It is apparent that these 1930 data confirm those of 1929 in a very satisfactory manner.

From figures 2 and 3 it is shown that a double or a triple fumigation produces greater reduction of yield than a single fumigation. The equations and statistics of the straight-line relations between the percentage of leaf area destroyed and yield are as follows:

For the double fumigation:

\[ y = 95.5 - 0.489x \quad \ldots \quad (2) \]
\[ n = 34 \]
\[ S_y = 8.15 \text{ per cent.} \]
\[ r = 0.79 \pm 0.065 \]

For the triple fumigation:

\[ y = 96.6 - 0.754x \quad \ldots \quad (3) \]
\[ n = 12 \]
\[ S_y = 4.1 \text{ per cent.} \]
\[ r = 0.975 \pm 0.014 \]

The value of the yield in the case of two complete leaf destructions on one crop is thus about 47 per cent.; and in the case of three complete leaf destructions, about 21 per cent. While neither of these curves strikes the origin at 100 per cent. yield, they both strike within the standard deviation of the values of the yield. The effect of the injury is shown only roughly in both the double and triple fumigation treatments, in spite of the higher values of the coefficient of correlation, \( r \), since the data are hardly sufficient to characterize the curves with a high degree of precision.

In figure 3, which shows the yield as affected by the percentage of leaves which show markings, rather than by the percentage of leaf area destroyed, a straight-line function does not fit the data, and a logarithmic equation has therefore been worked out by the method of least squares. The reason for this is that there is a much greater difference between the percentages of leaves marked and the leaf area destroyed with low percentages of the leaf destruction than with high percentages. The equations and statistics of these three curves are as follows:

For the single fumigation:

\[ y = 28.6 \log (110-x) + 40.5 \quad \ldots \quad (4) \]
\[ n = 96 \]
\[ S_y = 7.36 \text{ per cent.} \]
\[ r = 0.59 \pm 0.067 = \text{index of correlation} \]
For the double fumigation:

\[ y = 48.0 \log (110-x) - 1.0 \]  \hspace{1cm} (5)

\[ n = 34 \]

\[ S_y = 7.4 \text{ per cent.} \]

\[ r = 0.835 \pm 0.052 \]

For the triple fumigation:

\[ y = 75.5 \log (110-x) - 54.3 \]  \hspace{1cm} (6)

\[ n = 12 \]

\[ S_y = 5.25 \text{ per cent.} \]

\[ r = 0.96 \pm 0.023 \]

In figure 2 the point designations refer to early, medium, and late stages of growth respectively, and in figures 1 and 3 similar designations refer to first, second, third, and fourth crops.

These data indicate that any given percentage of leaf destruction of alfalfa is accompanied by approximately the same percentage of decrease in yield, regardless of the stage of growth or whether it is the first, second, third, or fourth crop, providing time is allowed for the treatment to take effect. Of course a very late fumigation, just before harvest, could hardly be expected to affect the yield; but in these experiments the crop was not harvested until at least seven to ten days after the last fumigation, thus allowing the influence of the fumigation to be fully reflected in the yield. Similarly the alfalfa had been growing a week before the experiments were begun.

The six curves shown in figures 2 and 3 are brought together in figure 4, in order to compare and contrast, in their effect on yield, the different percentages of leaf destruction and the percentages of leaves showing markings. A horizontal line, drawn from any point on any one of the dotted-line curves to the point that it intersects on the corresponding straight-line curve, will indicate the percentage of leaf destruction to which any chosen percentage of leaves showing markings corresponds. Thus, if 50 per cent. of the leaves show markings in one fumigation, approximately 29 per cent. of the leaf area of the plot will have been destroyed, thereby lowering the yield by approximately 9 per cent. Similarly, if 75 per cent. of the leaves show markings, nearly 55 per cent. of the leaf area will have been destroyed, with a corresponding reduction in yield of about 17 per cent. It is interesting to note also that a reduction in yield of 30 per cent. is indicated with a single 100-per cent. leaf destruction, or a double 52-per cent. leaf destruction, or a triple 35-per cent. leaf destruction.

These yield data are based on the average leaf destruction and average yield for plots of about twenty plants. The individual plants in any plot, however, vary greatly at different times, and also vary greatly from one
another in their ability to absorb sulphur dioxide. While this subject will be treated at length in a subsequent paper, it is desirable to call attention here to the fact of considerable differences between the percentages of markings on the different plants in a plot following a given fumigation. Table II

**TABLE II**

**Percentage of leaf reduction on nineteen different plants in plot 51–2, fumigated August 2, with an average leaf destruction of 16.2%, and again on August 11, 1930, with an additional average leaf destruction of 38%**

<table>
<thead>
<tr>
<th>Plot 51–2, August 2</th>
<th>Plot 51–2, August 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Row 1</td>
<td>Row 2</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
</tr>
</tbody>
</table>

has been compiled to illustrate these differences. As will be noted in this table, the plants in plot 51–2 were fumigated twice during the same crop, on August 2 when the stems were 16.5 inches tall, and on August 11 when
the same stems averaged 19.8 inches in height. Comparison of the percentages of leaf destruction of different plants in the two fumigations will illustrate the variability in sensitiveness of the individual plants, as well as of the plants in the plot.

It seemed desirable to recalculate some of the yield data with this variability in mind. A number of plants were therefore selected at random from all parts of the field. These plants had all had a single fumigation on one crop in 1930. They were placed in seven groups, each group representing a different percentage of leaf destruction. The yield of each plant was then calculated, using the method already described for the plot yields. Table III presents the average leaf destruction and the average yield of each of these groups of plants, together with the standard deviations of the means of the yield. A comparison of the last two columns of the

| Table III |
| Relative Yields of Groups of Once-Fumigated Individual Alfalfa Plants Selected at Random from All Parts of the Field, Based on Percentage of Leaf Destruction, Compared with Those Calculated from Equation (1b); 1930 Data |

<table>
<thead>
<tr>
<th>No. of Plants</th>
<th>Leaf Destruction</th>
<th>Yields in Percentage of Check</th>
<th>Yields Calculated from Equation (1b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
<td>%</td>
</tr>
<tr>
<td>71</td>
<td>Less than 1%</td>
<td>0.5</td>
<td>104 ± 2.3*</td>
</tr>
<tr>
<td>68</td>
<td>5-10</td>
<td>8</td>
<td>94 ± 1.6</td>
</tr>
<tr>
<td>52</td>
<td>20-25</td>
<td>23</td>
<td>94 ± 2.0</td>
</tr>
<tr>
<td>50</td>
<td>40-45</td>
<td>42</td>
<td>89 ± 2.0</td>
</tr>
<tr>
<td>29</td>
<td>55-60</td>
<td>57</td>
<td>83 ± 2.6</td>
</tr>
<tr>
<td>62</td>
<td>70-75</td>
<td>73</td>
<td>81 ± 1.2</td>
</tr>
<tr>
<td>31</td>
<td>85-90</td>
<td>92</td>
<td>72 ± 2.1</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

The table indicates the agreement between these yield values and those calculated from equation (1b). Both show very definitely that the decrease in yield of alfalfa is directly proportional to the percentage of leaf destruction.

C. Yield of Alfalfa as Influenced by Chlorotic Sulphur Dioxide Markings.—Thus far in this study, the effects of only the acute type of markings have been considered. The conditions producing the chlorotic type are not very well understood, but they have been observed four or more days subsequent to short fumigations when the light intensity was low and
the concentration of sulphur dioxide was sufficiently high so that under conditions of high light intensity, severe acute markings would have resulted. Chlorotic markings also appear not infrequently after long-continued exposures to low concentrations of sulphur dioxide which are insufficient to produce markings of the acute type. Since chlorosis from a number of widely different causes is common on plants that have not been fumigated, it is frequently uncertain whether, in certain cases, the markings on plants which have been fumigated are due to sulphur dioxide or not.

**TABLE IV**

**RELATIVE YIELD OF ALFALFA AS AFFECTED BY SULPHUR DIOXIDE MARKINGS OF THE CHLOROTIC TYPE**

<table>
<thead>
<tr>
<th>No. OF PLOTS</th>
<th>TREATMENT</th>
<th>LEAF AREA MARKED</th>
<th>YIELD</th>
<th>STANDARD DEVIATION</th>
<th>STANDARD DEVIATION OF MEAN</th>
<th>YIELD CALCULATED FROM EQUATION (1b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACUTE TYPE</td>
<td>CHLOROTIC TYPE</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>13</td>
<td>Fumigated</td>
<td>0.5</td>
<td>9.2</td>
<td>96.0</td>
<td>5.35</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table IV gives the yield of thirteen plots of alfalfa, upon the leaves of which numerous chlorotic markings were produced. While these data are insufficient to characterize the effect quantitatively, they indicate that the chlorotic lesions are not more effective in reducing yield than the same percentage of leaf area destroyed by the acute type of injury.

**D. YIELD OF ALFALFA AS AFFECTED BY CLIPPING TREATMENTS.**—In 1931, instead of destroying portions of alfalfa leaves with sulphur dioxide, the same range of percentages of leaf reduction as in the 1930 experiments was approximated by clipping off the leaves. These two treatments differed in the fact that, for a given amount of leaf destruction, whole leaves were removed by clipping and normal leaves left, whereas with fumigation, all the leaves were subjected to sulphur dioxide and the leaf destruction on individual leaves varied from zero to as much as 100 per cent. in some cases. Also the clipping treatment was applied uniformly to all of the plants, whereas with the sulphur dioxide treatments the individual plants of the plot showed widely varying percentages of leaf destruction, as indicated by table II. It is interesting to note, therefore, the close agreement in the yields between corresponding percentages destroyed by fumigation and by clipping.

The clipping treatment was applied to the second crop only. The other crops throughout the season were harvested as checks. Nine treatments
were carried out as follows: single, double, and triple clippings, in each of which about 20, 50, and 90 per cent. of the leaves were removed from each stem. In the case of double and triple clippings, the 20, 50, and 90 per cent. respectively of the leaves on each stem at the time of each clipping were removed. About forty-five plants from five different plots were subjected to each treatment. These treatments were carried out in the following order:

1. First of the triple clippings
2. First of the double clippings
3. Single clippings
4. Second of the triple clippings
5. Second of the double clippings
6. Third of the triple clippings

The triple clipping was begun about ten days after the harvest of the first crop, and was finished about seven days before the harvest of the second crop. The leaves from each plant were dried and weighed separately. The results are shown in table V, which gives the yield of the second crop with and without the leaves that were removed, and also the yield of the other three crops.

**TABLE V**

**Effect on yield of immediate and of two subsequent crops of alfalfa of clipping off different percentages of leaves; clippings made from second crop**

<table>
<thead>
<tr>
<th>No. of plants</th>
<th>No. of clippings</th>
<th>Percentage of leaves removed</th>
<th>First crop</th>
<th>Second crop</th>
<th>Third crop</th>
<th>Fourth crop</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Weight</td>
<td>weight +</td>
<td>weight +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>leaves</td>
<td>leaves removed</td>
<td>leaves removed</td>
</tr>
<tr>
<td>45</td>
<td>1</td>
<td>20</td>
<td>100</td>
<td>94.2 ± 2.26*</td>
<td>98.7 ± 2.40*</td>
<td>104.2 ± 3.17*</td>
</tr>
<tr>
<td>42</td>
<td>1</td>
<td>50</td>
<td></td>
<td>80.3 ± 2.11</td>
<td>89.8 ± 2.34</td>
<td>97.5 ± 2.22</td>
</tr>
<tr>
<td>45</td>
<td>1</td>
<td>90</td>
<td></td>
<td>68.0 ± 1.74</td>
<td>79.0 ± 1.89</td>
<td>93.5 ± 2.76</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
<td>20</td>
<td></td>
<td>85.5 ± 2.38</td>
<td>94.8 ± 2.47</td>
<td>100.6 ± 3.04</td>
</tr>
<tr>
<td>45</td>
<td>2</td>
<td>50</td>
<td></td>
<td>70.9 ± 1.68</td>
<td>88.2 ± 1.74</td>
<td>91.9 ± 1.94</td>
</tr>
<tr>
<td>46</td>
<td>2</td>
<td>90</td>
<td></td>
<td>57.8 ± 1.70</td>
<td>80.3 ± 2.50</td>
<td>81.6 ± 2.15</td>
</tr>
<tr>
<td>48</td>
<td>3</td>
<td>20</td>
<td></td>
<td>70.4 ± 1.85</td>
<td>81.0 ± 2.12</td>
<td>99.6 ± 3.15</td>
</tr>
<tr>
<td>47</td>
<td>3</td>
<td>50</td>
<td></td>
<td>61.3 ± 1.60</td>
<td>78.3 ± 1.94</td>
<td>93.5 ± 1.62</td>
</tr>
<tr>
<td>46</td>
<td>3</td>
<td>90</td>
<td></td>
<td>41.5 ± 1.24</td>
<td>62.0 ± 1.77</td>
<td>73.8 ± 2.38</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

In figure 5 the yield data for the clipping treatments have been plotted and the curves superimposed upon the fumigation yield curves from figure 2. The close agreement between these curves indicates that correspond-
Fig. 5. Effect of clipping off various percentages of leaves one, two, and three times during the growth of a single crop on the yield of alfalfa. Treatments were applied on the second crop. The yield of each partially-defoliated crop is compared with the yield of the untreated crops from the same plots. Dotted lines are fumigation yield curves from fig. 2; 1931 data.
ing partial defoliations by clipping and by fumigation with SO₂ give approximately the same reduction of yield. It should be noted that in the case of light fumigations there is little tendency to shed the injured leaves, and consequently the abscission yield data plus the weight of the leaves removed should agree more closely with the fumigation yield data at small percentages of leaf destruction. On the other hand, the abscission yield data without the weight of the removed leaves should agree more closely with the fumigation data showing large percentages of leaf destruction, on account of the tendency to shed the badly marked leaves. This agreement is shown in figure 5. The most extreme treatments appear to reduce the yield somewhat more in the case of fumigation than in the case of corresponding defoliation by clipping. The difference, although small, is possibly due to an added percentage of green tissue which is lost to the plant when the badly burned leaves are shed. The remarkable concordance between the fumigation and abscission treatments indicates that sulphur dioxide affects the alfalfa plant essentially and proportionately by removing leaf tissue. If any other effect is produced, it is of a definitely minor character.

E. EFFECTS OF PARTIAL DEFOILIATION ON SUBSEQUENT CROPS.—Table V also indicates that the yield on the third and fourth crops, subsequent to clipping the leaves on the second crop, is nearly normal in the case of the less severe treatments. The yield of the third crop, however, following the triple 90 per cent. clipping on the second crop, is only about 74 per cent. of normal, and the yield of the fourth crop is only about 94 per cent. of normal. In the case of the double 90 per cent. clipping on the second crop, these values are 82 per cent. normal for the third crop and 94 per cent. normal for the fourth. This effect, which possibly is to be explained by root depletion during second-crop recovery, has also been similarly noted in the case of heavily fumigated treatments (table VI). The fumigation

### TABLE VI

INFLUENCE OF SEVERITY OF SULPHUR DIOXIDE FUMIGATIONS UPON RELATIVE YIELDS OF THE SUBSEQUENT CROP OF ALFALFA

<table>
<thead>
<tr>
<th>No. of plots</th>
<th>AVERAGE YIELD OF FUMIGATED CROP</th>
<th>AVERAGE YIELD OF SUBSEQUENT UNTREATED CROP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RANGE</td>
<td>AVERAGE</td>
</tr>
<tr>
<td>28</td>
<td>80–90</td>
<td>85.7</td>
</tr>
<tr>
<td>10</td>
<td>70–80</td>
<td>76.6</td>
</tr>
<tr>
<td>8</td>
<td>60–70</td>
<td>65.8</td>
</tr>
<tr>
<td>5</td>
<td>40–60</td>
<td>52.2</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
FIG. 6. Effect of leaf destruction by sulphur dioxide fumigation and by corresponding leaf clipping on the growth and formation of new leaves on individual stems of alfalfa.
treatments, however, were not especially planned to reveal this effect, as were the abscission treatments, and data are available on only one crop subsequent to the fumigation.

In table VI the 1930 yield data of the fumigated plots have been arranged in groups of 10 per cent. range, except for the fourth entry, in which it is 20 per cent. Tables V and VI indicate that the rates of recovery from abscission and fumigation treatments are nearly identical, and that the subsequent crop is practically normal if the treatment of the crop which precedes it does not reduce the yield more than about 15 per cent.

F. GROWTH AND LEAF PRODUCTION OF INDIVIDUAL ALFALFA STEMS.—To determine the behavior of alfalfa plants following fumigation and clipping treatments, twenty comparable vigorous stems were twice subjected to each treatment and their subsequent growth was compared with that from forty corresponding untreated stems. In this experiment the numbers of leaves were counted and the heights of the stems were measured at intervals throughout the growth period. These data are presented in figure 6. At harvest time the average dry weight of the fumigated stems was 9.8 grams, of the clipped stems, 10.1 grams, and of the untreated stems, 16.2 grams. While these observations have not been sufficiently extensive in character to have quantitative significance, they indicate that recovery of the plants from fumigation treatments is similar to that from abscission treatments, and that in both cases the subsequent production of leaves and growth of stems is comparable with that of untreated stems.

Summary

1. Sulphur dioxide lesions on alfalfa are of two types, acute and chlorotic. The former shows characteristic bleached interveinal and marginal areas in otherwise normally appearing leaves; the latter exhibits a more or less yellowed and mottled appearance and is similar to chlorosis as produced by a number of other causes.

2. The reduction in yield of alfalfa subjected to a single sulphur dioxide fumigation of one crop is in direct proportion to the percentage of leaf area destroyed. The stage of growth at which the fumigation occurs does not appear to influence the result, at least within the range of 25-75 per cent. of the total growth period of the plant.

3. If a crop is fumigated more than once, assuming at least a week to elapse between fumigations each of which produces the same percentage of leaf destruction, the reduction of the yield is also in proportion to the number of fumigations.

4. The reduction in yield is not a linear function of the number of leaves marked, indicating that the uninjured portions of the leaves continue to function in spite of bleached areas.
5. The yield-leaf-destruction curves all closely approach 100 per cent. yield at 0-per cent. leaf destruction, indicating that the gas does not reduce the yield unless it produces visible effects.

6. The reductions of yield caused by sulphur dioxide fumigation can be closely duplicated by clipping off from normal plants an amount of leaf tissue equivalent in area to that destroyed by fumigation.

7. Like acute markings, chlorotic sulphur dioxide markings appear to lower the yield in direct proportion to the percentage of leaf tissue visibly affected.

8. A severe defoliation treatment, either by fumigation or by clipping, which lowers the yield of the treated crop extensively, also reduces the yield of the subsequent untreated crop appreciably, and may even be felt slightly in the second subsequent untreated crop.

9. The subsequent growth of new leaves, and the elongation of the stems following a partial defoliation, proceed at the same rate, regardless of whether the leaf destruction was accomplished by sulphur dioxide fumigation or by clipping.

DEPARTMENT OF AGRICULTURAL RESEARCH, AMERICAN SMELTING AND REFINING CO., SALT LAKE CITY, UTAH.

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