THE STANDARDIZATION OF POA ANNUA AS AN INDICATOR OF SMOG CONCENTRATIONS. I. EFFECTS OF TEMPERATURE, PHOTOPERIOD, AND LIGHT INTENSITY DURING GROWTH OF THE TEST-PLANTS [1]

MARCELLA JUHRÉN, WILFRED NOBLE AND F. W. WENT

LOS ANGELES COUNTY AIR POLLUTION CONTROL DISTRICT, LOS ANGELES, AND THE EARHART PLANT RESEARCH LABORATORY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Of the plants commonly found in Los Angeles County whose responses to smog [2] have been under extensive observation, *Poa annua* (annual bluegrass) was selected as one worthy of intensive study. It was early reported as one of the most sensitive of the plants observed in the field (11). Because it has a regular habit of growth, can be easily and quickly grown in small containers, and because the band of damage caused by smog is measurable, *Poa annua* lends itself to quantitative work. It was therefore thought that it might be standardized to serve as a biological indicator of smog concentrations. A plant indicator has a unique value, since it measures smog in terms of the damage potential to the living cell. It also gives a visible record of the effects of smog conditions at the time and place at which it was exposed.

The technique of filtering from the atmosphere its plant damaging components by means of activated carbon filters was devised in 1950, in a project sponsored jointly by the California Institute of Technology, the University of California, and the Los Angeles County Air Pollution Control District (4). Comparison could then be made between plants grown in air containing smog, and those grown in the same conditions in pure air. It became evident to many observers that certain portions of the mesophyll tissues were acted upon selectively by smog, and that damage was usually found in areas of rather young tissue.

Noble (12) described the pattern of damage found in many species, including *Poa annua,* with special attention to the location of damaged areas as a function of the maturation of the leaf. Bobrov (3) made an anatomical study of *Poa annua,* in which she showed by photomicrographs the relation of susceptible portions of the blade to cells in the young-mature stage. Older cells were shown to be more heavily suberized.

Loftfield (9) in 1921 had shown that alfalfa was susceptible to sulfur dioxide only during those hours in which the stomata were open. Middleton, Kendrick and Schwalm (11) described the microscopic appearance of damaged areas on the leaves of a number of broad-leaved crop plants, noting that damage occurred especially in the mesophyll in the region of the stomates. Bobrov (2, 3), using sections of fresh tissue, showed that the bands of damage (fig 1) found on the blades of *Poa annua* correspond in position to the region of young mature cells whose stomates have newly become functional.

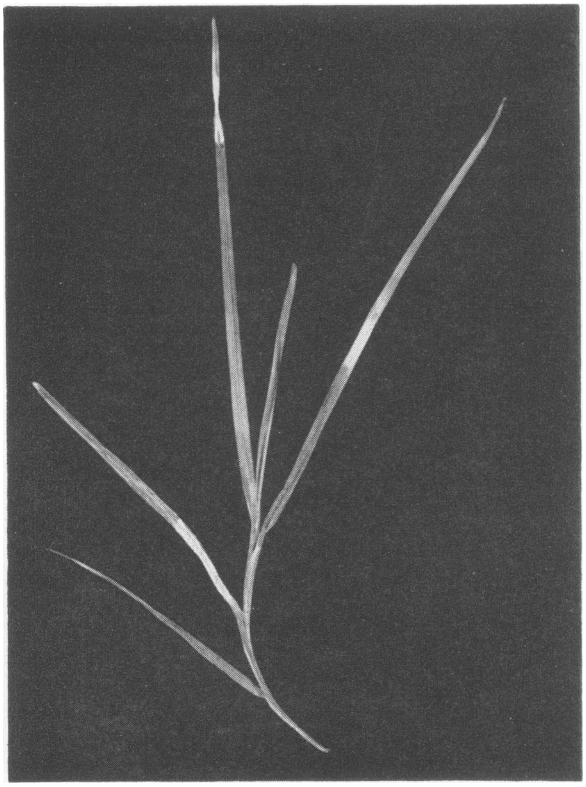


FIG. 1. *Poa annua* plant 4 wks old, after a 1-day exposure to a heavy smog. The light-colored band on each blade, in which the chlorophyll was destroyed, resulted.

Stomatal behavior had been studied also in a series of investigations begun in 1949, sponsored jointly by the California Institute of Technology and the Los Angeles County Air Pollution Control District. In the course of these, Koritz and Went (8) found that the stomata of tomato plants closed as soon as fumigation began, and after repeated treatments closed permanently in many cases. Transpiration rates dropped after each fumigation in every case, even in plants whose stomata were closed at the start of fumigation. It was concluded that although conditions favoring the opening of stomata are also those in which plants are most susceptible to smog damage,

[1] Received April 23, 1957.

[2] The term smog in this article will refer to the air pollution existing in Los Angeles County during 1955 to 1956, chiefly the partial oxidation products of unsaturated hydrocarbons.

there was no conclusive evidence that the extent of damage was determined by the degree of opening of the stomata. The fact that further injury is inflicted by smog when the stomata are closed, would indicate that penetration can occur during closure.

It was then found by Hull and Went (6) that the stomata of oats closed tightly when fumigation began, as did those of tomato plants, but those of endive remained wide open during a 2.5-hour fumigation period. Transpiration dropped during fumigation in both species. Some effects of different pretreatment were observed; with 5 crop plants, smog damage was more severe if they had been grown in natural daylight rather than in artificial light, except in the case of alfalfa; and more severe if they had been well supplied with water than if watered only enough to prevent wilting.

Studies were made on the smog response of the Pinto bean by Middleton, Kendrick and Darley. They concluded that it was a suitable indicator plant for the presence of oxidants, inasmuch as damage from ozone could be readily distinguished from damage due to oxidants; and in 1954 used the plant in a study of the relation of plant damage to oxidant level as measured by the release of iodine from potassium iodide. Plants were exposed at 5 stations in the Los Angeles Basin; the percentage of leaf area injured was estimated and given a rating called the plant injury index, or quantitative estimate of damage. Little significant correlation was found between the plant injury index and the oxidant maxima, or between the plant injury index and the oxidant 24-hour mean. The percent of plants damaged was termed the qualitative estimate of injury, and this estimate did show a significant correlation with both oxidant 24-hour mean and oxidant maximum values. The studies were believed to support the thesis that the potassium iodide method of measuring oxidant was measuring, not the phytotoxicant itself, but a concomitant condition.

A complete review of air pollution as affecting plants may be found in an article, with a bibliography, by Donald F. Adams (1); also in section 9, by Thomas and Hendricks, of "The Air Pollution Handbook" (10).

In 1952 test boxes were designed by Noble in which plants could be exposed to smog in an airstream of controlled rate. Species which might be good indicators of smog concentrations were studied by him as to their endurance in test box conditions. Since differences in environmental factors were known to affect the sensitivity of plants to smog, plants were grown in controlled conditions in the Earhart Laboratory, then set out in the test boxes. Spinach, endive, alfalfa, sugar beet, oats, and annual bluegrass were studied in this manner throughout the year.

Of these, the annual bluegrass proved hardiest. It had the added advantage of being so common locally that it may easily be found in the vicinity of any test box, when field observations are desired as a check on test box findings.

Test boxes were put in operation in September 1954, at 12 locations over the county, usually at farms or nurseries. Since then, a set of 10 *Poa annua* plants has been placed in the test chamber each day in each location.

Petunia "Rosy Morn" has also been placed in the boxes, for comparison. As it is a plant of quite different characteristics, it seems unlikely that both species would become insensitive or ultra-sensitive due to the same climatic factors.

Daily, the damage to *Poa annua* has been measured and on this basis the responses to smog have been judged light, medium, or severe. Results are studied in connection with chemical methods of determining smog concentrations, with meteorological data, and with damage to plants growing in the fields nearby. A complete description of the test boxes and their operation has been prepared, together with data showing the correlation of test plant injury with field injury (13).

METHODS

Poa annua was grown in various combinations of day and night temperature, photoperiod, and light intensity in the Earhart Laboratory at intervals of approximately 6 weeks throughout the year. At the age of 4 weeks the plants were taken out and exposed to smog in standard test boxes. Damage to sets of plants from the different conditions was assessed and compared. The plants were also measured to obtain growth rates. In some cases fresh and dry weights were taken, or weights were taken of similar sets. Plants of different ages were also compared in the same manner.

The Earhart Laboratory has been fully described elsewhere (15).

Temperatures employed were the following:

DAY TEMP., °C	NIGHT TEMP., °C
17	11 (cold)
20	14 (cool)
23	17 (moderate)
26	20 (warm)
30	24 (hot)
23	14
26	14
26	17

These were combined with natural daylight and artificial light. Several light intensities were produced by shading plants in daylight with copper screen, and by using artificial light of 700 ft-c and of 1000 ft-c. Most of the above temperatures were combined with 8-, 12-, and 16-hour photoperiods, and two light intensities were tried with each of these combinations.

At the same time, sets of plants were tested which had been grown in a greenhouse built by the Los Angeles County Air Pollution Control District for the regular production of plants for the air pollution test box. This greenhouse, referred to as the "A. P. Greenhouse," is located at the Los Angeles State and County Arboretum in Arcadia. It has a high degree of control of environmental factors. In winter the temperature is 17° C at night, and rises to a maximum of 27° C at midday. The maximum temperature lasts about 2 hours, the average for the day being 26° C. The heat sum per 12-hour day is from 286 to 290 degree hours, averaging 288. This is the same as in a day of warm conditions at the Earhart Laboratory, the main difference being that in the A. P. Greenhouse, the temperature rises gradually to a peak, as it does outdoors, and gradually declines, instead of holding a constant level throughout the day, as in the Earhart Laboratory. In summer, in the A. P. Greenhouse, the slightly higher range of 19° at night to 30° C in the daytime is maintained, the sum for a 12-hour day being about 300 degree-hours, and for the night, 230 degree-hours.

The temperatures and humidities in the A. P. Greenhouse are recorded on a hygrothermograph so that conditions are known for periods during which each experimental group is being grown. Light intensity is very close to that of outdoors. Supplementary lighting is given in winter to complete a 16-hour photoperiod, and is also supplied on cloudy days. Light intensity is reduced as needed by window-screening placed across the roof. Humidity is not allowed to fall below 60 %. Smog is removed by passing the air through activated carbon filters.

The smog to which the plants were exposed was that of the atmosphere at Arcadia. The test boxes change air 4 times per minute. They have control chambers in which air is forced through a filter of activated charcoal. Thus, in cases of doubt, smog damage may readily be distinguished from injury due to other causes. The plant compartments of the test-boxes have glass walls, and the boxes may be oriented so that the plants are in sunlight all day, or so that they are in the sun morning and afternoon, but are shaded by the opaque door at noon. In these experiments, plants were placed in the test-box before 10:00 A.M. and were removed at 4:00 P.M.

An attempt was made to expose them, so far as possible, on days of comparable smog attacks. During each of the exposures, there was mild sunshine, the temperature was between 60° and 65° F, and reduced visibility and eye-watering were observed in the early afternoon.

After exposure to smog, the grasses were placed in the A. P. Greenhouse for three days to await full development of damage, after which time the extent of the injury was estimated.

For estimating damage, the following method was used: the length of each of the 3 uppermost mature leaves was measured. (The youngest visible blade has been observed to incur damage only after it is at least half as long as the next-older blade. When shorter than this, it is not considered as one of the 3 uppermost mature blades.) The length of the band of damage was also measured and expressed as percent of the blade length. The damage to the 3 blades was then averaged, giving a score for the plant; finally the scores for all the plants in a group, or statistical unit, were averaged, giving the final score in terms of percent of damage per blade per plant. Analyses of variance made on a number of runs indicated that 10 plants are sufficient to give statistically significant values. However, not less than 3 cups with 10 plants in each were used as a unit in these studies.

The depth, or severity, of the smog damage was also recorded, in the following terms: light, if there were only flecks or streaks of white; moderate, if there was no green color left within the band of damage except for a line along the midrib; severe, if all chlorophyll within the band of damage was destroyed.

Usually, when a large proportion of the plants was severely damaged, the bands of damage were long, so that good correlation was found between the two manifestations of injury. But this was not invariably the case. The more consistent results were obtained by measuring the length of the bands of damage. It was felt that until the significance of the severity of damage is better understood, it was best to simply record it, without attempting to integrate it with the measurements.

Usually, the demarcation at the end of a band of severe damage is clear; but sometimes the chlorosis shades out gradually above and below the severely damaged area. In such case, the measurement was made to include, besides the severely damaged part, approximately half of the lightly damaged portion.

For planting, plastic cups 3 inches in diameter and 6 inches deep, with holes for drainage, were used as containers. The planting medium was vermiculite, and seeds were sown on the surface. Nutrients were supplied by watering to field capacity once a day with a modified Hoagland's solution. During the exposure to smog, the cups stood in trays containing half-strength nutrient solution to a depth of half an inch.

Preliminary tests were carried out to determine satisfactory planting media, proper planting depth, and number of plants to a cup. These will not be reported in detail, but data are available upon request.

The stock of seed came from a strain of *Poa annua* which had been standardized by the U. S. Soil Conservation Service. Although *Poa annua* is not apomictic as are most members of the genus, this strain had been grown for many years in an isolated field plot at the U. S. Soil Conservation Service nursery formerly at San Fernando, and is as nearly homozygous as could be obtained.

Plants grown under different conditions varied in form to such an extent that it was sometimes difficult to be sure which plants were the larger, without weighing them. Since this meant uprooting them it could not always be done, so the weights of duplicate sets of 60 to 90 plants were taken.

For obtaining growth rates of living plants, a figure was obtained by multiplying the length and width of the longest blade and the number of blades. This gave good correlation with dry weight. It was less satisfactory after lateral shoots developed. Simply the length of the longest blade times its width was a good index, at the age at which the plants were used for testing. To get the average for a group of 90 plants in units of 10 per container, the length and width of the longest blades were measured on any 3 plants taken at random from each container. When there were as few as 30 plants in a group, all were measured.

Stomatal opening was studied by means of xylene infiltration, porometer readings, and the ultrapak microscope. When xylene was used, it was applied to four blades of at least 10 plants in each condition. Thirty plants were used when available.

To determine whether the wave-like spread of the xylene was due entirely to infiltration through the stomata, or whether the xylene might be spreading through the interior of the leaf also, grass blades were coated with silicone over part of their length, and xylene was applied at a little distance from the edge of the silicone. There was at times some spreading of the xylene beyond the coating; but as it was relatively slow and limited in extent, it was not thought to be a source of error.

Humidity was adequate for stomatal opening in both the Earhart Laboratory and the test boxes.

Results

Germination and Early Development: Germination occurred in about 7 days, except in the cold conditions, where 18 to 22 days were required. Light shading produced a slightly higher percentage germination when the photoperiod was 16 hours and light intensity was high. In the highest temperatures (day 30° C, night 24° C) there was some loss of very young seedlings.

In the optimal or near-optimal conditions of a warm day and cool night, with a 16-hour photoperiod and natural daylight reaching a maximum of 5000 ft-c, the two-leaf stage was reached about one week from emergence. The growth rate became more rapid at about the development of the 3rd leaf. The 5-leaf stage was reached 3 weeks after emergence. Lateral shoots then began to develop. Flowering began in from 5 to 7 weeks. At the onset of flowering, the uppermost blade, which heretofore had been longer than the one preceding it, developed to only about half the expected length and showed little evidence of stomatal opening. For this reason and because of their size, plants at this age were no longer desirable as test plants. Four weeks was the preferred age.

With the same light intensity and photoperiod, young plants developed most rapidly in the warm temperatures (day 26° C, night 20° C), and in warm days with cool nights (day 26° C, night 17° C). Growth was progressively slower in the moderate, cool, and cold conditions, and was also slower in the hot ones (day 30° C, night 24° C). Relative to the length of the blade, plants in cool conditions had broad blades and long internodes. The opposite was true of plants in hot conditions (fig 2).

Sensitivity to smog developed first in the plants in the hot conditions, which incurred damage as soon as the first blade expanded. This sensitivity tended to be lost by the time they had 3 or 4 leaves. They did not become immune to smog, but suffered less damage than did plants in other temperatures. Sensitivity to smog was absent in plants in cool temperatures (day 20° C, night 14° C) at the 2-leaf stage, but increased as they grew older, up to the age of 6 weeks, when it declined. Young plants in warm and moderate conditions showed sensitivities intermediate between those of hot and those of cool ones. In the cold (day 17° C, night 11° C), plants did not become sensitive to smog until they were beginning to flower.

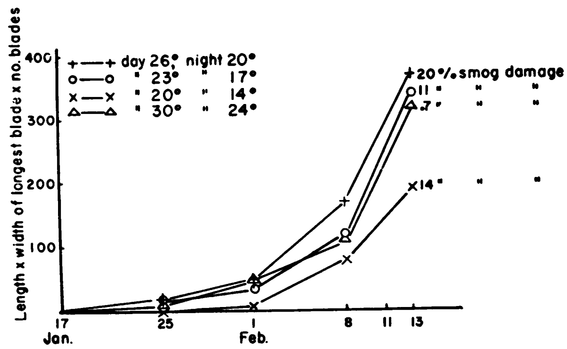


Fig. 2. Growth of *POA annua* in 4 combinations of day and night temp. The photoperiod was 16 hrs, and the light intensity up to 4200 ft-c at noon. Plants in cool temperatures have slower growth rate than those in hot ones, but suffered more damage from smog.

Interaction of Temperature and Light Intensity: Growth in 4 temperature combinations is shown in figure 2. It is typical of the growth of Poa in these temperatures throughout most of the year. In January, however, plants grown in cool and in moderate conditions made the best growth, while in August those in hot conditions did so. There appeared to be an interaction of light intensity and temperature such that plants growing in high temperatures made better growth in high light intensities, while those in low temperatures grew better in lower light intensities.

This was most clearly seen when artificial light (700 and 1000 ft-c) was combined with cool, warm, and moderate temperatures. Figure 3 shows the results of this experiment. The same response had previously been found in tomatoes (16).

In the warm day, cool night combination (day 26° C, night 17° C) the effect of light intensity was minimized; the plants weighed the same in both light intensities. There was a relative difference in wet and dry weight, fresh weight being greater at 700 ft-c and dry weight at 1000 ft-c. This combination was also optimal for growth.

Temperature had a decided effect on susceptibility to smog. Plants grown in hot conditions (day 30° C, night 24° C), received much less smog damage than did those from any other condition (fig 2). This was true whether the hot-condition plants were the largest,

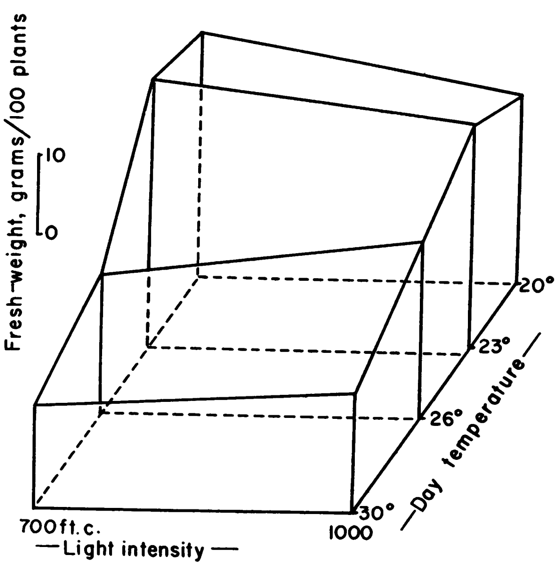


FIG. 3. Effect of various temperature and light intensity combinations on the growth of *Poa annua*. Artificial light and a 16-hr photoperiod was used. Night temp was 6° lower than day temp. In cool and moderate temperatures, growth is better with the lower light intensity; in warm and hot temperatures, with the higher intensity.

as when they were grown in high light intensity, or whether they were relatively small, as when grown in lower light intensity. In some of the trials they sustained no damage at all.

Small differences in degree of injury were always found among plants grown in cool, moderate, and warm conditions. The greatest damage from smog was incurred by plants grown in warm conditions (day 26° C, night 20° C) or in a warm day with a somewhat cooler night (day 26° C, night 17° C). This latter combination seemed somewhat better for both growth and smog sensitivity. With a still cooler night (day 26° C, night 14° C) growth was not as good.

OTHER EFFECTS OF LIGHT INTENSITY; OPTIMAL INTENSITY: When light reached the plant from all sides, as when plants were grown one to a cup, the effect of high natural light intensity was to induce early formation of lateral shoots. In an experiment in which 1, 2, 5, 10, and 20 plants per cup were grown in the A. P. Greenhouse in May, the lateral shoot development was in inverse proportion to the number of plants per cup. By 4 weeks, the area filled by the grass blades was about the same, whether the blades proceeded from one large plant or 5 to 10 smaller ones. Because of this, plants of the age to be tested had no freer air flow among their blades, nor less shading of one blade by another, when planted one to a cup than when planted 10 to a cup. With 20 to a cup, the stems were thin and weak, and the plants appeared to have suffered from crowding.

In the Earhart Laboratory, the optimal intensity did not appear to be exceeded. The largest plants were raised in warm temperatures with natural light, and under these conditions they did best in full sunlight. Plants placed next to them but shaded by one layer of gauze, did not attain the same size. The optimal intensity was then sought in the A. P. Green-

TABLE I

EFFECT OF PHOTOPERIOD ON GROWTH AND SUSCEPTIBILITY TO SMOG OF 4-WEEK-OLD POA ANNUA

DAY TEMP, °C	NIGHT TEMP, °C	PHOTO-PERIOD, HRS	LENGTH OF LONGEST BLADE, MM	WIDTH OF LONGEST BLADE, MM	NO. OF BLADES	NO. OF LATERAL SHOOTS	TOTAL DRY WT, GM/100 PLANTS	SMOG-DAMAGE: % PER BLADE PER PLANT	SMOG-DAMAGE: PLANTS WITH SEVERE DAMAGE, %
20	14	8 A	45	1.0	4.0	0	0.24	21	100
"	"	12 A	71	3.0	5.0	2	0.83	*	83
"	"	16 A	95	3.0	5.0	2	1.90	17	96
23	17	8 A	60	2.0	4.0	0	0.27	16	96
"	"	12 A	80	2.5	6.0	2	1.34	*	*
"	"	16 A	110	3.5	6.5	3.5	3.80	10	84
26	20	8 A	74	1.5	4.0	0	0.30	15	96
"	"	12 A	60	2.0	4.0	0	0.47	*	*
"	"	16 A	66	2.0	4.0	2	0.87	10	79
30	24	8 A	35	1.0	4.0	0	0.12	4	100
"	"	12 A	*	*	*	*	0.31	*	*
"	"	16 A	52	2.0	5.0	0	0.42	2	24
20	14	8 N	63	2.2	4.5	0	4.1	18	12
"	"	16 N	128	3.3	5.0	0	11.8	29	5
30	24	8 N	14	0.5	2.0	0	0.2	Traces	0
"	"	16 N	*	*	*	*	1.3	1	0

A = artificial light, N = natural light. Measurements are averages of 30 plants; wts are averages of 30 to 60 plants. Damage is termed severe when there is a band in which all chlorophyll is destroyed.

* No data.

FIG. 4. Five-week-old *Poa annua* grown in 4 temp combinations, each with 3 photoperiods.

house, where light intensities are almost as high as outdoors. Here plants did better unshaded, both as to germination and growth, up to the month of May. In May, plants shaded by window-screening weighed more. There was a higher root-shoot ratio, but the blades were also larger; the figure for length × width × number of blades being 862 for unshaded plants, 2065 for shaded ones. According to data from the nearest U. S. Weather Bureau Station, the unshaded plants received in May a total of 14,000 langleys, or a daily average of 485 lys with a maximum of 748 lys. In April, when the unshaded plants had been slightly larger, they had received 12,677 lys, or a daily average of 408 lys with a maximum of 645 lys. This probably is optimal. Data from the weather station correlated well with light meter readings taken several times a day at the greenhouse.

EFFECT OF PHOTOPERIOD: A 16-hour photoperiod produced plants that were larger in every respect than did a 12-hour period, and this in turn, than an 8-hour period. This was true in each of the 4 temperature conditions. In the hot conditions, a photoperiod of at least 12 hours was necessary for good survival as there were always many dead seedlings in the 8-hour photoperiod (fig 4). In cool conditions, the 8-hour photoperiod produced plants that were small but quite healthy.

The effect on smog sensitivity may been seen in table I. Damage was consistently greater in the 8-hour photoperiod.

When plants were grown in cool conditions and a short photoperiod, they were remarkable for the consistency of the smog damage. An analysis of variance was made, and bore out the impression that the variation in amount of damage among the individual plants was small. There was a definite pattern, with damage at the base of the third leaf in every case. The areas of severe injury were clearly demarcated.

Even the plants grown in the hot conditions showed fair sensitivity when in an 8-hour photoperiod and shaded, provided their water-supply was maintained at field capacity. Maintenance of this plentiful water supply did not produce such sensitivity in plants in a 16-hour photoperiod nor in the unshaded plants in the 8-hour photoperiod and the same temperature.

EFFECT OF CHANGING PLANTS TO DIFFERENT TEMPERATURES: When plants were changed to different temperatures, the immediate result for the first 3 days was an increased growth rate, compared to the growth rate of plants which remained in the same conditions. Plants transferred from hot to cool temperatures gained in sensitivity; those transferred from cool to hot ones, lost it. After 3 days, the plants were exposed to smog, and the damage measured. The difference between each transferred group and its control group was not significant except in one case. This was the transfer from warm conditions, which produced plants of the highest sensitivity, to hot condi-

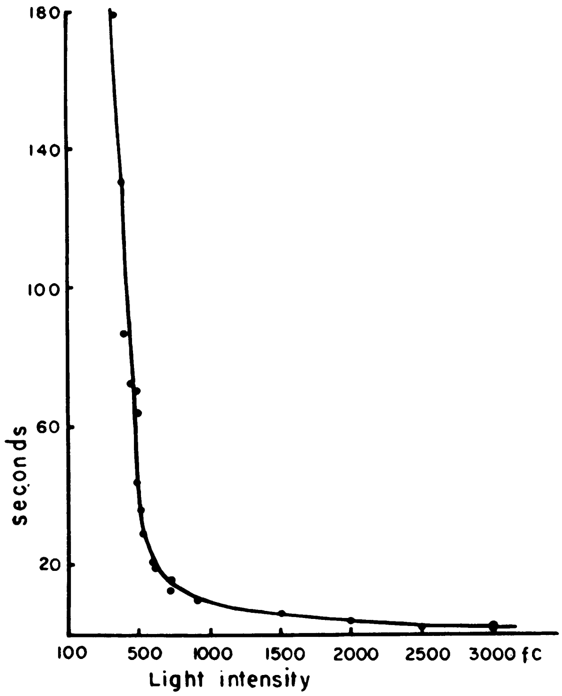


FIG. 5 A. Correlation of light intensity with degree of stomatal opening, as indicated by porometer readings, in plants grown in cool temperatures.

tions, which produced plants of the lowest sensitivity; and the reverse transfer, from hot to warm conditions.

It was not determined how long this increased growth rate would have persisted, as the plants were removed to the test-boxes after 3 days. It was observed, however, that plants which were senescent in high temperatures, became rejuvenated in the cool temperatures, and flowered again. The largest plants ever obtained by any treatment were sown in cool temperatures, given 5 cold nights one week after germination, then gradually brought through moderate temperatures to warm ones. This parallels natural conditions in a Mediterranean climate, where *Poa annua* is an autumn germinating annual.

EFFECT OF TEMPERATURE AND LIGHT ON STOMATAL OPENING: When evidence of stomatal opening was sought by applying xylene to the blades of 3- to 4-week-old plants, the color change due to infiltration appeared in bands strikingly similar in pattern to bands caused by smog damage. This had been previously noticed by Bobrov and Vasek. Bobrov (3) had correlated the damage patterns with the location of young-mature tissue, in which the stomata are most active.

Figure 5 A is based on figures obtained by estimating the areas of infiltration in terms of percent of the blade, as is done in scoring smog damage, and obtaining the average percent per blade of a large group of plants. The plants in this instance were tested at noon on a sunny day, in the Earhart Laboratory. Distinct differences in the stomatal opening of plants growing in different temperatures may be seen. Plants in the A. P. Greenhouse at the same hour on a number of days of comparable light, showed stomatal opening similar to that of the warm conditions (day 26° C, night 20° C) in the Earhart Laboratory, or a little greater.

A correlation with smog damage may be observed, in that plants from warm conditions, which have stomata open over more of the blade-length than do any other group, also incur the greatest smog damage; while hot-condition plants incur the least.

The timing of stomatal opening also differed among the 3 groups. Maximal infiltration began earlier and continued longer in the plants grown in warm conditions, than in cool-condition plants. Plants grown in hot conditions gave evidence of stomatal opening only before 9 A.M. This would be of importance in smog exposure because smog at the test site never reached damaging concentrations so early in the day.

Very young plants, in the 1- to 2-leaf stage, showed infiltration with xylene in only 3 out of 10 plants at most, in all conditions.

An ultrapak microscope and a porometer were used to try to gain an idea of the width of the opening. On days of mild sunshine, in both warm and in cool conditions, wide open stomata were interspersed with partly open ones and with tightly closed ones, in the proportion of 3:5:3. In hot conditions no fully-open stomata were ever observed.

Porometer readings were difficult to obtain in hot conditions. When areas could be found in which the stomates were open, they closed in about half an hour. Frequently by shifting to another part of the same blade, other open ones could be found, but they in turn soon closed. Figure 5 B shows a typical set of porometer readings on plants growing in cool conditions. There was mild sunshine on the day of the readings. Stomatal opening responded sensitively to light intensity. There was a 15-minute lag in response, observed also on other occasions when pass-

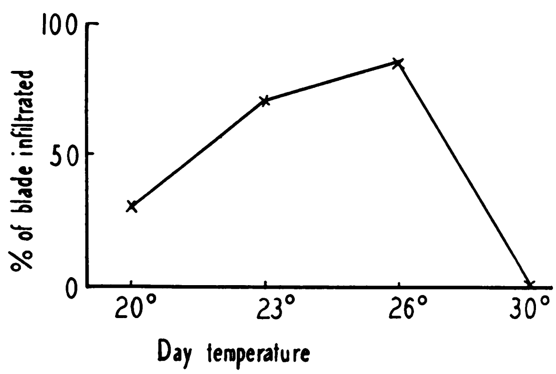


FIG. 5 B. Correlation of temperature with the leaf area over which stomates were open, in natural light at 2 P.M. on a clear day. Xylene was applied to the blades and the infiltrated areas estimated as % of blade. Averages of 10 plants. Night temp were 6° lower than day temp in each case.

ing clouds changed the light intensity. There was a threshold for opening in the morning, at about 400 ft-c.

Experiments in shaded and unshaded test boxes were carried out to see what might be the practical effect of the response to light intensity. In theory, the width of the stomatal opening should not matter, because it would follow from the principles of diffusion of gases through small apertures, that a stomate need not be fully open to permit the maximum diffusion of gases. Hence small changes in light intensity should not affect the amount of smog damage. If however the light intensity fell below the threshold of 400 ft-c, the stomata should be completely closed, and the plant be quite resistant to smog.

This was found to be the case. When the test box was shaded so that the light intensity was only 300 to 400 ft-c, only traces of injury were found on the shaded plants, while the damage to plants in the unshaded box was 15 % per blade per plant (based on 9 containers of plants, 10 plants per container). When the test box was shaded less heavily, permitting light intensities of 900 to 1200 ft-c, damage to plants was within 2 or 3 % of the damage incurred by plants in the full light intensity of 3000 to 4000 ft-c. (This latter experiment was done 4 times with the same results; on a fifth repetition there was a somewhat greater difference between the test plants and controls—18 % damage to the former, 32 % to the latter.)

When plants were changed from cool temperatures where the stomata were open over a large proportion of the blade, to hot conditions where the stomata were nearly all closed, the stomata of the transferred plants did not at once begin to close. This was shown by xylene infiltration with groups of 20 plants on several occasions. Also, in several experiments a plant with a porometer attached to the blade was wheeled from a cool room to a hot one, both of which were sunny. The blade continued to give readings consistent with the curve already begun, and with curves obtained previously in the cool room. Conversely, plants transferred from a hot room to a cool one at the same light intensity, did not give evidence on the same day of opening their stomata over larger areas. Only after one or two days did the stomatal behavior alter until it was characteristic of plants in the temperature condition to which the plants were moved.

Lateral Shoots: Lateral shoots had about the same percent of area damaged as did the central shoots, if they were nearly the same size. When plants were grown whose lateral shoots all reached the same development, and the same as that of the central shoot, the smog damage was of the same proportion on all. Hence it was decided that the laterals were, essentially, repeating the results of their parent shoot and only the parent need be scored.

Correlation of Growth Rate and Smog Sensitivity: No correlation could be found with smog sensitivity, of either the growth rate of the plant as a whole, or of the young mature blades. So far as elongation could be detected by placing India ink

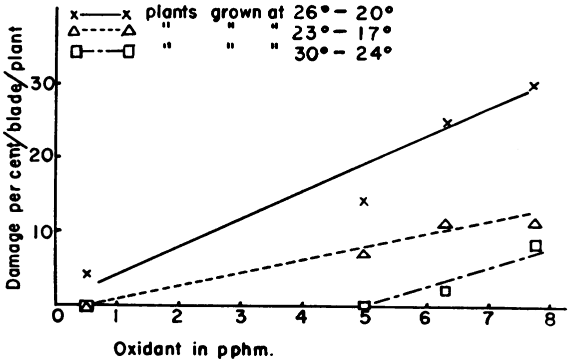


Fig. 6. Correlation of plant damage with oxidant readings at nearest station. Each point represents the damage from 1 exposure to smog (av of 30 plants). During all exposures, there were temperatures of 60° to 65° F, mild sunshine, and a peak of high oxidant readings lasting 2 hrs. All plants were grown in natural daylight with a 16-hr photoperiod.

lines 1 mm apart along the young blades, the blades had ceased to elongate by the time they became sensitive to smog. In the experiments in which growth rates were measured, the plants growing fastest during the three days previous to the smog exposure were not the ones most severely damaged. For example, plants in the 8-hour photoperiod and cool conditions were growing at a much slower rate than those in warm ones, yet damage was equally severe; conversely, when plants in hot conditions were growing fastest of all, they were still the least smog-sensitive group.

Correlation with Total Oxidant Levels: Figure 6 shows the correlation between total oxidant in the air, and the severity of damage of *Poa annua*. Each point represents the average damage caused by a single exposure to smog to plants raised in a given condition. The plants were grown in the Earhart Laboratory in natural light and 4 temperature combinations. Total oxidant reading in parts per hundred million were obtained from the nearest station. This was at the California Institute of Technology, where the phenolphthalein method was used. The figures plotted were the maximum for the day; in each case the maximum came in the early afternoon and lasted about 2 hours. Temperatures and light intensities were comparable. In these experiments the relative sensitivity of plants grown under different conditions remained the same; that is, plants grown in 26°–20° C were always more sensitive than those grown in 23°–17° C, and those in turn were more sensitive than those coming from 30°–23° C. This last condition is considered an unsatisfactory one in which to grow test plants.

Discussion

While conditions in which *Poa annua* are grown have a very strong effect on its sensitivity, it seems remarkable, compared to other plants with which we have had experience, for the breadth of the range in which it is sensitive. If one is content with a differentiation between the effects of light smog and heavy smog, only one condition need cause concern; namely, too great heat. Somewhat greater precision than this, however, can be attained in any one of a number of combinations of temperature and photoperiod. The light-optimum of *Poa annua* is rather high, but in optimal temperatures the plants make good growth and are smog sensitive over a wide range of intensities. The optimal conditions are such that they can be maintained in a relatively simple glass house such as the A. P. Greenhouse.

Petunia, barley, *Atropa belladonna,* and several other smog-sensitive plants which have been grown in controlled conditions have had narrower ranges in which they were sensitive, or in which the sensitivity was quantitatively reliable. Petunia, sensitive when grown in hot conditions, is good for use with *Poa annua.*

An apomictic species of Poa would be more uniform genetically and therefore would have advantages as an experimental plant, but so far no apomictic Poa has been found with the wide temperature-tolerances of *Poa annua,* with the possible exception of a hybrid of *Poa scabrella* and *Poa pratensis* which, with others of the genus, was tested in the Earhart Laboratory (5, 7).

The fact that the small plants from the 8-hour photoperiod and cool temperatures were as sensitive as large ones from optimal growth conditions could be accounted for by the assumption that the mature cells age more slowly than they do in warmer temperatures and with more light. There is some evidence for this, in that the old blades do not become senescent nearly as fast. If, as has been believed, the length of the band of damage in proportion to the whole leaf depends on the length of the band of newly matured tissue produced just previous to exposure, it would seem at first thought that a rapidly growing plant would have a longer band of damage than a slower growing one. However in a plant which is also aging slowly, the cells might remain longer in the stage at which they are vulnerable, and the proportion of damageable tissue be the same as in the larger plants. The length of the band would thus be determined by the balance between maturation and aging. This could explain the lack of correlation of sensitivity with growth rate.

The data afforded by the stomatal studies do not altogether account for the absence of damage to very young plants in cool conditions. Possibly this is due to the compactness of the cellular structure. While the lack of injury to plants grown in hot conditions seems accounted for by the timing of stomatal opening with reference to the onset of smog, it may also be that suberization occurs more rapidly in high temperatures. More work is needed along these lines.

In the curves in which total oxidant was plotted against plant damage, the data plotted were obtained when conditions of exposure (duration of the highest oxidant readings, temperatures, light intensities) were quite comparable. The inference is not that the daily routine tests would show such a correlation; in these, a general correlation has been found (13), but complete correlation is not to be expected; obviously a number of factors could prevent this. It is felt, however, that the correlation shown in figure 6 strengthens the conclusion, drawn also from other data, that in each set of environmental conditions *Poa annua* develops a degree of smog sensitivity characteristic of that set of conditions. Between some sets of conditions, the difference is slight; between others it is quite pronounced. Proper choice of growing conditions must therefore be made, and these conditions scrupulously maintained, in order to use *Poa annua* successfully as an indicator. Furthermore, certain requirements, such as sufficient light intensity, must be provided during the exposure to smog. It is planned to present further data on test box conditions in a later communication.

It is felt that our study supports the thesis that plants, properly handled, constitute excellent test materials for smog conditions. They give a quantitative expression of smog in a visible form which may be preserved; and they give it in terms of its effect on the living organism.

Conclusions

The environmental conditions under which *Poa annua* is grown strongly affect its sensitivity to smog. Temperatures of day 26° C, night 20° C in the Earhart Laboratory, or the equivalent in daily heat sum in the A. P. Greenhouse, combined with a 16-hour photoperiod and natural light, produce plants of satisfactory sensitivity. These conditions have been adopted for the production of test plants for the daily tests carried on by the District.

Temperatures of day 30° C, night 24° C or their equivalent in daily heat sum in the A. P. Greenhouse, and with the above photoperiod and light intensity, produce plants which are resistant to smog and are considered unsatisfactory for testing purposes. Intermediate temperatures produce plants of intermediate sensitivity.

With 8-hour photoperiods and a somewhat lower light intensity, equally sensitive plants may be produced, in combination with any one of several temperatures. The interaction of temperature, light intensity, and photoperiod is such that it is not possible to consider the effect of one without the others.

A partial explanation for the differing sensitivities of plants grown in different conditions is afforded by the evidence that the stomata have a different habit of opening in each condition.

Since transfer of plants from cool to hot temperatures, or vice versa, did not result in appreciable changes in smog sensitivity in less than three days, nor in alteration other than gradual in their habits of stomatal opening, it is felt that a change in environmental conditions during the testing period does not impair the usefulness of the plants for smog testing purposes. This may not hold true if the change is extreme: hence, supplementary light for dark days, and cooling devices for periods of extreme heat, are recommended.

Small and slowly growing plants may be as sensitive as large ones, under certain conditions. These conditions—cool temperatures, shade, short photoperiod—are such that aging takes place slowly. Hence, a proportionately large amount of tissue may remain in the young-mature, susceptible stage for some time, thus compensating for the slow production of fresh susceptible tissue.

Summary

Poa annua (annual bluegrass) was grown in controlled conditions in the Earhart Laboratory in 8 temperature combinations, natural and artificial light of various intensities, and 3 photoperiods. It was also grown in the A. P. Greenhouse in which the day temperature rose gradually to a maximum lasting about two hours. Growth rates, fresh and dry weights were obtained.

For growth up to the age of 4 weeks, optimal conditions were a day temperature of 26° C, night 17° C, with a 16-hour photoperiod and a high light intensity. There was an interaction of light intensity with temperature, so that plants in high temperatures grew better with high light intensities, on the order of 12,000 ft-c at noon; while plants in low temperatures grew better with light intensities on the order of 3,000 ft-c at noon. With artificial light, this interaction with temperature was also observed, using intensities of 700 and 1000 ft-c. Plants in the warm and moderate temperatures were less affected by variations in light intensity, within those limits.

Eight-, 12-, and 16-hour photoperiods produced progressively better growth. Survival was poor in the 8-hour photoperiod with high temperatures.

Flowering was earlier and more abundant in the cool temperatures with short photoperiod.

Smog sensitivity was tested by exposing the plants to atmospheric smog for one day in test-boxes in which airflow and humidity was regulated. Three days after the smog exposure, the length of the 3 youngest leaves and of the bands of damage on them was measured and damage expressed as percent per blade per plant. A unit of 3 containers with 10 plants in each was found ample to overcome variations due to genetic or unknown factors.

Sensitivity developed at the age of 10 days from planting, even before the second blade had grown out, in plants in hot conditions; but thereafter diminished. At 4 weeks, damage in heavy smog was less than 5 % per blade per plant. Sensitivity in plants grown in cool conditions did not develop until about the 3-leaf stage, but did not diminish until the plants were more than 6 weeks old. High sensitivity (20 % to 30 % damage per blade per plant from heavy smog) was found in plants grown under several conditions: 1) optimal growth conditions; 2) 8-hour photoperiod in moderate light intensities, and cool, moderate or warm temperatures; and 3) 16-hour photoperiod in the A. P. Greenhouse, with a day temperature reaching a maximum of 27° C and a night temperature of 17° C.

Plants grown in hot conditions gained sensitivity when transferred to cool conditions. Those grown in cool conditions lost sensitvity when changed to hot ones. An appreciable gain or loss in sensitivity to smog required about 3 days.

Smog sensitivity was not correlated with the size of plants, nor with the growth rate. It was correlated to a high degree with the area over which the stomates were wholly or partly open. It was also correlated with oxidant levels, in data obtained when duration of maximal oxidant readings and climatic conditions were comparable.

The authors would like to express their appreciation to Dr. William Stewart for his kindness in extending the facilities of the Los Angeles State and County Arboretum, and for critical reading of the manuscript; also to Mr. Daniel Syrek, Data Reduction Specialist, for aid in the statistical analysis; and to Dr. C. M. Franco for adaption of the porometer for use on young grass blades. Values for the oxidant levels determined by the phenolphthalein method were kindly supplied by Dr. A. J. Haagen-Smit.

Literature Cited

1. Adams, Donald F. The effects of air pollution on plant life. Amer. Med. Assoc., Arch. Ind. Health 14: 229–245. 1956.
2. Bobrov, Ruth Ann The effect of smog on the anatomy of oat leaves. Phytopathology 42: 558–562. 1952.
3. Bobrov, Ruth Ann The leaf structure of *Poa annua* with observation on its smog sensitivity in Los Angeles County. Amer. Jour. Bot. 42: 467–473. 1955.
4. Haagen-Smit, A. M., Darley, E. F., Zaitlin, M., Hull, H. and Noble, W. Investigation on injury to plants from air pollution in the Los Angeles area. Plant Physiol. 27: 18–34. 1952.
5. Hiesey, Wm. M. Growth and development of species and hybrids of Poa under controlled temperatures. Amer. Jour. Bot. 40: 205–221. 1953.
6. Hull, Herbert M. and Went, Frits W. Life processes of plants as affected by air pollution. Pp. 122–128. Proc. Natl. Air Pollution Symposium, 2nd Symposium, 1952.
7. Juhrén, Marcella, Hiesey, Wm. M. and Went, F. W. Germination and early growth of grasses in controlled conditions. Ecology 34: 289–300. 1953.
8. Koritz, Helen G. and Went, F. W. The physiological action of smog on plants. I. Initial growth and transpiration studies. Plant Physiol. 28: 50–62. 1953.
9. Loftfield, J. V. G. Behavior of stomata. Carnegie Inst. Wash. Publ. No. 314. 1921.
10. Magill, P. L., Holden, F. R. and Ackley, Chas. Air pollution Handbook. Sect. 9, pp. 1–44. McGraw-Hill Co., New York 1956.
11. Middleton, John T., Kendrick, J. B., Jr. and Schwalm, H. W. Smog in the south coastal area of California. Calif. Agr. 11: 7–10. 1950.

12. Noble, Wilfred M. The pattern of damage produced on vegetation by smog. Agr. and Food Chem. 3: 330–332. 1955.
13. Noble, Wilfred M. and Wright, Lloyd A method for measuring the damage potential of smog by the use of plant indicators. Jour. Agron. (In press).
14. Rogers, Lewis H., Renzetti, N. A. and Neiburger, Morris Smog effects and chemical analysis of the Los Angeles atmosphere. Air Pollution Control Assoc. 6: 165–170. 1956.
15. Went, F. W. The Earhart plant research laboratory. Chron. Bot. 12: 89–108. 1950.
16. Went, F. W. Plant growth in controlled conditions. V. The relation between age, light, variety and thermoperiodicity of tomatoes. Amer. Jour. Bot. 32: 469–479. 1945.

PHYSIOLOGY OF THE CELL SURFACE OF NEUROSPORA ASCOSPORES. IV. THE FUNCTIONS OF SURFACE BINDING SITES [1,2]

ALFRED S. SUSSMAN, B. von BÖVENTER-HEIDENHAIN and R. J. LOWRY

Department of Botany, University of Michigan, Ann Arbor, Michigan

The previous paper in this series (6) has disclosed that the surface of Neurospora ascospores can serve as a reservoir of cations which can enter the cell after adsorption. For example, Ag^{+}, UO_2^{++}, Cu^{++} and other cations are removed from solution by dormant live ascospores and by killed ones as well. Furthermore, the kinetics of uptake and the fact that these materials can be eluted readily suggest that they are localized on the cell surface. If the dormancy of such "coated" ascospores is broken they fail to germinate due to the presence of the toxic cations on their surface. This effect has been demonstrated in the case of organic bases like Polymyxin-B (5) as well as for the substances mentioned above. These observations suggest, therefore, that the cell surface is the source of cations which are excluded from vital centers in the spore until germination is induced.

However, the fact that such absorbed ions can penetrate does not establish that this route is obligatory. It is also possible that the cations must be eluted before they enter and that the adsorption sites merely concentrate them; or, alternate means of penetration may exist which are independent of preliminary surface localization.

That surface adsorption of cations is widespread is demonstrated by its occurrence in bacteria (7, 9), erythrocytes (1), fungi (10, 13) and higher plants (8, 17). Therefore, an investigation of the relation of adsorption to penetration was undertaken in order to define the role of this ubiquitous phenomenon.

Materials and Methods

Ascospores of *Neurospora tetrasperma* were obtained, stored and prepared for use by the methods described previously (12). The latter paper also describes the techniques used in the heat-activation as well as the germination of the spores. The concentration of these cells was determined by the use of a Klett colorimeter with a blue filter (Klett # 42) whereby a reading of 180 corresponded to a concentration of 1 mg (dry wt) per ml of spore suspension.

[1] Received April 25, 1957.

[2] This work was made possible by grants from the Rackham Graduate School, University of Michigan, and from the American Cancer Society.

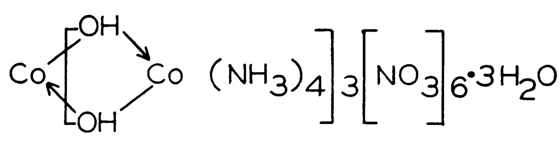
"Coated" ascospores were prepared by mixing equal volumes of suspensions containing 1 mg per ml of ascospores and 2×10^{-2} M of the unbuffered "coating" material. This mixture was incubated at 20° C on a reciprocal shaking machine for 24 hours whereupon the supernatant fluid was decanted after centrifugation and the ascospores washed in 4 changes of water. The "coated" ascospores were resuspended in water and stored at 4° C until used.

The measurement of oxygen uptake was performed by the standard manometric procedures outlined in Umbreit et al (15). Unless otherwise noted, the Warburg vessels contained 0.5 ml of the spore suspension, 0.5 ml of a solution of the cation to be tested, or an equivalent volume of water, and 0.2 ml of KOH in the inset as an absorbent for carbon dioxide. All respirometric experiments were carried out at 26° C at a shaking rate of 120 oscillations per minute. The dry weight of the spore suspensions used was obtained by keeping aliquots in an oven at 105° C overnight.

Ag^{110} was determined by means of an end-window Geiger tube and scaler. As before, aluminum pans containing 0.2 ml of the solution were used and enough counts were recorded to assure less than 2 % counting error.

Hexol nitrate (see list of abbreviations) was prepared according to the method of Werner (16), as modified by Sutherland (14). The cobalt hexammine salts were generously provided by Prof. Robert W. Parry of the Department of Chemistry, University of Michigan. List of abbreviations:

1. PCB: phenylmercuribenzoic acid.
2. Hexol nitrate: hexa-ethylenediamino-hexoltetracobaltic nitrate whose chemical formula is



This and the subsequent description of cobalt coordination compounds is used as provided in Sidgwick (11).

3. Cobalt hexammine: hexammine cobalt (III) chloride whose formula is $Co(NH_3)_6Cl_3$.