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ATMOSPHERIC POLLUTION BY AEROALLERGENS

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## ABSTRACT

The ragweed research program undertaken at The University of Michigan has continued to be conducted during the past year by an interdisciplinary team of allergists, botanists, meteorologists, and public health statisticians working together in close cooperation. This team effort has proven very useful, as it has in past years, in gaining new knowledge of the several areas under investigation by project personnel.

Botanical research has been concerned with the evolution and migration of ragweeds, ecological and phenological studies, observations on floral development and function, and experiments on pollen grains, including the clumping phenomenon and the processes of germination and respiration.

Medical research has continued in four main areas started in previous years. These are hemagglutination tests with serum of pollen-sensitive patients, studies on the allergenicity of tapetal fluid, effects of air ionization on passive anaphylaxis in guinea pigs, and recovery of pollen grains from respiratory tissues. In addition, five new projects have been initiated during the past year. These include studies on the rate of adsorption of isotopically-tagged antigens incorporated in various types of respiratory emulsions, the effect of pH upon skin-sensitizing antibody, the adsorption of pollen allergens on glass surfaces, the effect of normal human serum and gamma globulin on passive transfer tests, and observations on the incidence of ragweed and other allergies in a large foreign student population as a means of evaluating methods for determining the genetics of atopy.

Meteorological research during the past year has consisted of nine interrelated studies. Pollen sampling techniques, and instrumentation developments in pollen sampling, have been continued from work started in previous years. Reports are given on extensive sampling experiments that were carried out both prior to and during the natural ragweed pollen season. Further analysis and conclusions from a 1958 experiment on urban-rural spatial variations in ragweed pollen concentrations is presented. Meteorological factors influencing the release and transport of pollen are discussed and tentative conclusions drawn. Flotation and reflation of pollen is a problem that is approached by means of a mathematical model to explain the several phenomena

that control the processes involved. Research on methods for predicting the daily ragweed pollen concentration resulted in the development of a day-to-day pollen prediction graph for one locality. The question of the removal of ragweed pollen from the atmosphere by falling rain is being studied with the aid of newly-developed instrumentation. Finally, progress is reported on the operational tests made on the pollinosis test chamber recently completed during the past year.

The activities of the statistical group during the past year consisted of providing statistical consultation in the design, collection, and analysis of data from several studies made by the botanical, medical and meteorological groups. An example of the detailed statistical results possible by use of high-speed electronic computers is presented.

A list of papers published, accepted, or submitted for publication by project personnel is included.

## OBJECTIVE

The atmosphere is contaminated by two groups of substances, natural and artificial, depending upon their method of production and introduction into the air. Among natural contaminants are the aeroallergens, air-borne substances such as pollens, spores, rusts, and smuts which induce allergic reactions in sensitive individuals. There is evidence that some of these aeroallergens, notably ragweed pollen, which is one of the worst offenders, are becoming more widespread and more serious public health problems as a result of man's use of land.

A comprehensive program of research on an aeroallergen such as ragweed pollen requires an integrated study of the plant and its pollen, of the means by which the pollen is dispersed in the atmosphere, and of the fundamental nature and cause of the physiological reaction of sensitive individuals to it. The present investigation represents a fundamental attack on the problem in all its phases by specialists in allergy, botany, meteorology, and public health, all working in the closest cooperation.

## 1. BOTANICAL PHASE

by

W. H. Wagner, Jr.

### 1.1 INTRODUCTION

The botanists have been associated in consultation and conference with the work of the meteorologists, allergists, and public health workers during 1959-1960 as in previous years. The problems which are specifically botanical will be reported on here. These include research on evolution and migration of ragweeds, ecological and phenological studies, observations on floral development and function, as well as experiments on pollen grains, including the clumping phenomenon and the processes of germination and respiration. The report on the botanical phase is a summary of the work covering a number of biological aspects of the ragweed plant. Unless otherwise indicated, the investigations described below pertain to the common or low ragweed, Ambrosia artemisiifolia.

### 1.2 THE CULTURE OF EXPERIMENTAL PLANTS

For the extra-seasonal experiments, the culture of plants followed the lines of previous years, but the plots were smaller and there were two plots each with approximately 500 plants. One of the plots was used for the pollen source and the other, located nearby, was used for making meteorological and botanical observations. The plants were arranged in the plots in the form of a circle, and the results of these studies will be reported by the meteorologists.

For sources of pollen and for comparative studies, a very large number of new plants were introduced into the Botanical Gardens of The University of Michigan. The perennial species were planted in pots and also in soil out-of-doors; and seed collections were made for annual species. A large colony of Ambrosia coronopifolia and its relatives was established, and it is expected that these will prove valuable in future studies of the ragweeds as well as for analysis of relative antigenicity of their pollens.

### 1.3 RESEARCH ON EVOLUTION AND MIGRATION OF RAGWEEDS

Studies on ragweed evolution, distribution and migration have been conducted by Willard W. Payne as a basis for his doctoral dissertation. In the course of his 1959 studies he visited 23 states and collected 1100 specimens for the herbarium, as well as numerous fixed and living specimens. A number of herbaria and universities were visited for the purpose of studying specimens and discussing ragweed problems with other botanists.

It was determined in these studies that common ragweed (Ambrosia artemisiifolia) is much less common in the Northern United States and Canada, and though still a common weed there, it does not compare in abundance to that found further south. Ambrosia trifida is consistently less common than A. artemisiifolia except possibly in some of the more southerly states (e.g., Indiana, Illinois, and lower in the Mississippi Valley), where, if not actually as abundant, the giant ragweed contributes probably more to the total ragweed pollen load.

From evidence gathered in 1959, it may be suggested that both low and giant ragweeds have probably invaded the Southeastern United States in fairly recent times. According to plant taxonomists who have spent some time in the area, the weeds have been abundant enough to cause hayfever only in the past three decades or so. Ambrosia trifida has evidently not yet reached Florida or Georgia in any abundance. Ambrosia coronopifolia seems to be increasing in the Northern States, but it has been established there, at least as a casual introduction, for some time. Collections from the New England States, from New York, and from Michigan, have been made since the earliest years of the present century.

Two other ragweed species are of relatively minor importance. Ambrosia bidentata, the southern ragweed, is the most restricted of the common species, and it occurs only from mid-Illinois and Indiana to northern Louisiana, and from there westward. It is not as abundant as the low ragweed except in the central parts of its range, but is commonly associated with disturbed habitats, as are the giant and low ragweeds. Ambrosia hispida is much restricted in range because of its growth form and habitat preferences. It is perennial by aerial shoots that would be killed by frost, and it grows only in saline and calcareous soils in the Florida Keys and the extreme southeastern coast of Florida.

A number of tentative conclusions and hypotheses, including the following, are under study:

a. The genus Ambrosia is closely related to the genus Franseria of the Southwestern United States. From a correlation of characteristics, it seems fairly obvious that the genus Franseria is the older one, and it probably gave rise to Ambrosia. The species-groups of annual ragweeds, Ambrosia trifida, A. artemisiifolia, and A. bidentata, are fairly natural and appear to have originated in the Mississippi River watershed. The perennial ragweeds are not a natural group; many are only distantly related, and may have originated independently.

b. The southern forms of Ambrosia artemisiifolia and A. trifida are different in many ways from the northern forms, as discussed in earlier reports of this project, and they may constitute distinct species. These forms may, of course, be merely varieties, and the fact that intergradation occurs in the Central States seems to support this conclusion. More data are being accumulated to clarify this problem.

c. Hybridization has probably played an important role in the evolution of certain ragweeds (as discussed in earlier reports). It may also bear on the interpretation of the giant and common ragweed complexes.

d. Migration of ragweeds probably followed various directions from Central North America under natural conditions. However, man's influence has greatly stimulated their spread. Early invasion of the Northeastern United States and Canada is indicated in the development of the Ambrosia artemisiifolia complex. Ragweeds in the Southeastern United States probably arrived later. The only areas which are not well supplied at present with common ragweed (with the exception of desert regions and marine islands) are the Western States. Common ragweed has been rarely reported in California, and is only now invading Oregon. It is almost certain that all parts of the United States which are suitable for its growth will someday be infested by common ragweed.

e. The giant ragweed (Ambrosia trifida) complex underwent migration similar to that of common ragweed, except that it has not spread so far. It is still rare in the Southeast, especially Florida. Its adaptive potential may be nearly as great as common ragweed and someday its range may be as extensive. Southern ragweed (A. bidentata) seems to have less adaptability than the other North American annual ragweeds. If it does become more capable of spreading, it will be particularly troublesome since it is adapted to survival in pastures and meadows. The western perennial ragweed complex (A. psilostachya in the broad sense), probably because of adaptation to sandy, more arid areas, is most abundant in the Pacific Coastal States, especially California. The easternmost

variety, A. coronopifolia, of the perennial ragweeds, follows sandy areas, and is known to hybridize with common ragweed. In time it may acquire increased tolerance and a broader range.

f. Most of the ragweeds common to North America have been introduced into Europe. The so-called "European ragweed," Ambrosia maritima, is the only species believed to occur naturally outside the Americas. However, it seems to be indistinguishable from the common ragweed, A. artemisiifolia. It may actually be derived from early introduction of the latter species into Europe. Thus it is possible that no species of ragweed occurs natively outside of the New World, and that even the "European ragweed" was naturalized there from America. Hybrids between supposed European and American ragweeds are healthy and vigorous; tests are being made to determine whether the  $F_2$  generation will also be fertile.

g. Cuttings from most species of ragweeds will root under favorable conditions, but with difficulty. Thus the possibility of dispersal by vegetative propagules exists, but such propagation is unlikely. However, further experiments are being conducted to determine the actual method of geographical dispersion of ragweed.

h. Seeds of common ragweed are capable of being disseminated by birds. The seeds pass, apparently without damage to viability, through the animals' alimentary canals. Since few other possibilities for natural dispersal seem to exist, this can be assumed to have been the important means of dispersal prior to the arrival of civilized man in North America.

#### 1.4 ESTIMATION OF LOCAL RAGWEED POLLEN POTENTIAL

In connection with the Tecumseh Community Health Study Program a method was devised to make a census of ragweeds as an estimate of local ragweed pollen potential. Such a survey was requested by officials of the School of Public Health because of a differential that was discovered in the Health Study Program between the incidence of ragweed hay fever within and outside the town of Tecumseh. The ragweed survey was designed and conducted as follows:

The study area was divided into plots according to road boundaries, political boundaries, and rivers and streams. Every plot was given three scores, each score ranging from 0 to 10, and each number representing approximately the nearest ten per cent. A set of scores might be 2, 3, 7. The first indicates that some 20 per cent of the land in the plot was ragweed-bearing land. The second refers to the actual density of plants on the land suitable



for their growth; the score of 3 states that approximately 30 per cent of the ragweed-bearing land was actually covered with ragweed plants. The third number refers to the average vigor of the plants in the plot, a parameter necessarily an estimate, because all populations are not, of course, alike; the plants of different populations ranging from small (with only one or a few pollen-producing spikes) to large specimens over four feet tall (with hundreds of spikes). Extreme populations of the former type were scored 1 and the latter 10. The least pollen potential in a given plot would be 0,0,0; the greatest 10, 10, 10. Multiplication of the scores gives pollen index estimates ranging from 0 to 1000.

Using this system, two researchers independently made their own subjective estimates of pollen potential, and their independent judgments were in very close agreement. Only the area of coverage in the final data was decided jointly. The results of this survey are intended to be used by the Public Health workers in seeking the reasons for the differences in ragweed hay fever incidence in town and in country. The method seems to be a useful one where gross estimates of local pollen potential are required.

#### 1.5 THE ECOLOGY AND PHENOLOGY OF RAGWEEDS

The question had been raised previously whether ragweeds brought to flowering in June would be able to produce an additional crop of pollen. To answer the question, some of the remaining plants at the Willow Run experimental area were cut at ground level, some at about one-fourth the distance from the ground level to the top, and some were left untouched. By the middle of August, it was seen that those plants which had not been cut off were no longer producing new flowers and were mostly brown and dying. Those which had been cut at the ground level produced a small amount of growth, and a few flowers. Those which had been cut higher, however, had produced excellent growth and a full complement of new spikes, showing that a single plant, treated as described, was induced to produce two loads of pollen, one in June (grown out of season in the greenhouse) and another in August (after decapitation out-of-doors). Some herbarium sheets were preserved to illustrate these results.

Preliminary ecological observations that were made included field studies on common ragweed. These were made on selected plants within 16 sample plots in an area near the Willow Run office of the Meteorological Laboratories. The study was conducted by the meteorologists who also equipped the sample area with instrumentation for measuring temperature, relative humidity, precipi-

tation, and soil temperature during the time of the observations. The ecological field study was a corollary of the phenological research by the meteorologists. The same sixteen meter-square plots were observed that were used for the phenological study. The objectives of this study were as follows:

- a. To determine variability of Ambrosia artemisiifolia within the sampled area.
- b. To determine how density of the ragweed is related to the presence and abundance of other species.
- c. To determine how the phenology of ragweed is related to density and floristic composition of the sample areas.
- d. To determine whether the ragweeds occur in definite patterns of association with other species or whether they occur in random mixtures.
- e. To determine in what ways the phenology of ragweed may be influenced by stages in phenology of the other species in the sample area.

The methods adopted were as follows:

- a. Early in the summer each plot was surveyed for its floristic composition.
- b. The abundance and sociability of each species was recorded using the scales recommended by the Zurich-Montpelier school of phytosociologists. Total plant cover in the plot was also noted as well as any additional observations that seemed pertinent, e.g., evidence of animal activities or recent disturbances of any kind in the plot.
- c. A second series of surveys, distributed in time throughout the summer months was made using a prepared form for recording data (which will be described in a later report). These forms provide the same kind of information obtained in the earlier study plus additional information of a more specific nature.

As yet, no analysis of the data has been made, but a few generalizations about the results of the ecological study can be presented:

- a. The total number of species varies from approximately 15 to 30 species per plot.

b. The density and vigor of ragweed plants seems to vary considerably among the various plots. This variation seems to coincide with variations in the total cover and the vigor of the other plant species.

c. The competition of the ragweed plants with other species seems to vary throughout the growing season. Thus, the growth success of the ragweed plant may involve different plant competitors at different stages in the life cycle.

d. In moderately dense meadow stands, ragweed plants seem to grow only in small islands of otherwise unoccupied surface. Even in the areas densely covered with perennial grasses, the associated plant is often another annual species or a vegetative plant of one of the perennial grasses.

Further observations on the relative roles of growth period and photoperiod in bringing ragweeds to flower were made at the University Botanical Gardens. R. P. Wodehouse has asserted (Thirteenth Annual Meeting Northeastern Weed Control Conference Proceedings, pp. 26-32) that city lamps may keep ragweeds from flowering: "All the weeds beyond the influence of the light, about 18 feet, flowered at the appointed time, and by the end of September had ripened their seeds and the plants were dead and dried up. Not so those under the street lamps. . . . . When the first killing frost arrived on the 11th of November they were still green and with the flower buds beginning to show."

On 15 April, plants of ragweed for treatment with lights were started at the same time as those used in the extra-seasonal experiment. The plants, about 50 in all, were kept in the light every night. The lights were turned on each evening at 1600 EST and turned off at 0900 each morning. Two ordinary flood-bulbs, 150W-120V, were used, exposed 28 inches from the plants. The flowering was considerably delayed in comparison to the extra-seasonal plants, and the first definite flower spikes were noticed on 1 August. By the middle of August flowers were producing pollen. By 21 September, the end of the experiment, the plants were still forming flowers, unlike those out-of-doors, which were turning brown and dying. Furthermore, the plants had a peculiar aspect -- the entire plant was attenuated, 5-6 feet tall, the leaves were long with narrow segments, and with many branches, the latter still producing primordia. The flower spikes were extremely attenuated.

It should be noted, then, that the plants did produce pollen at approximately the regular time; and that the time of starting the plants was approximately the normal time when they start in the field. Thus, the natural growth period, which runs around 80 to 90

days up to pollen production in this area, was sufficient to bring flowering to take place, even under constant light conditions. However, the light did have a peculiar effect in modifying the shape of the plant and its flower spikes and in prolonging flower production over a longer period than is normal.

The continuation of ecological research will include, at Willow Run, an expansion of the plant population studies, the phenological studies, and the ragweed seed plantings, and, at the new University of Michigan Botanical Gardens site near Dixboro, Michigan, various experimental phytosociological manipulations which will be carried out as a doctoral research program by Mr. A. I. Gebben.

## 1.6 FLORAL DEVELOPMENT AND FUNCTION

The studies of ragweed flowers included field studies at Willow Run and field collections of material at various localities. Most of the work was conducted, however, in the Department of Botany laboratories.

It was noticed for the first time that there is a tendency for the involucre (cups in which the pollen flowers are borne) to open and close during the day-night period, the closure being especially noticeable during the night. The work on the flower was focused especially on the stages of development and the tissue structure of the mature flowers. New observations were also made on the pistillodium, the function of which has been somewhat in doubt, as described in previous reports.

The observations at Willow Run were devoted largely to the closing of the flower and the withdrawal of the pistillodium. The pistillodia appear in the morning about 1100-1130 and are especially numerous around 1300-1400 EST. Most of them have withdrawn into the flower by sundown, although many pistillodia still remain extended and can be seen in the process of withdrawal straight through the night. The collections of material in the field were focused on two problems: obtaining female flower material of two types, normal and mutational (the female flowers forming the entire spike); and collections of vegetative apices for studies of the transition from vegetative growth to flower growth.

In the laboratory, the pollen-flower slides were reviewed in detail in both cross-section and longitudinal section. A survey of some 1000 slides was made and notes kept on all the stages. The stages were diagrammed, and it was noted that the stages of

tapetal development were particularly clear. The development of the pollen grains was also examined in detail. The processing of new material (mainly of the female flowers) involved embedding, microtoming, staining, and mounting; and some 500 new slides were prepared. Survey of the female-flower material involved ascertaining the important steps in the ontogeny of the mature structure and diagramming the major changes.

Answers to a number of questions were sought during the 1959 research, and these are enumerated as follows:

a. Do involucres open and close? There is no question that in the field the young involucre of the extra-seasonal experiment do open and close. However, this turned out not to be a general phenomenon with the older involucre. It therefore appears to be correlated with the age of development of the involucre.

Observations made during September of the growing ragweed plants showed that during the night the two to five pairs of top leaves, i.e., those which had not yet completed their growth and development, would also close upward during the middle of the night, opening again in the morning. Thus the opening and closing observed in young involucre may well be related to the same phenomenon in young vegetative leaves at the apex of the plant at any earlier stage of growth.

b. How do the pistillodia extend? Measurements were taken of the pistillodia at various lengths, and it was discovered that the number of cells does not increase. There is a notable increase, however, in the lengths of the cells (but a lesser increase in the breadths of the cells). The mechanism of extension, then, appears to be cell elongation (through turgor pressure) and this is supported also by anatomical studies of the structure of the component cells which indicate their plasticity.

c. What is the function of the pistillodium? These organs were observed over periods of time, and it was concluded that they do act as "pollen pushers." The stages were noted by dissecting flowers. By the time that pollen is discharged the pistillodium is only one-half to three-fourths of the way out. The residue of the pollen which does not drop when the sacs dehisce is literally pushed out by the pistillodium.

d. When does the vegetative apex first become changed to a reproductive or floral apex? This turned out to be much earlier than was originally supposed. A collection which was made on 7 July revealed that the transition to flowers was already under way, so that the change must begin normally sometime in the week before.

The mechanism by which the flowers (which will shed their pollen on a particular day) push their way above the others seems to be by special thin-walled cells at the base of the flower which become extended at the time of anthesis. The dehiscence of the pollen sacs appears to be correlated with special mechanical tissues in the walls of the anther. This will be studied further. The opening and closing of young involucre seems to be related to special "bubble-like" cells. It is concluded that each of the floral functions is related to structural characteristics of the parts of the flower; and it is hoped that each of the different tissue and cell types can be described in relation to their functions.

## 1.7 BIOLOGY OF THE POLLEN GRAINS

Pollen for medical research was collected from the plants at The University of Michigan Botanical Gardens. The pollen was gathered from various lots of plants started at different times so that there would be pollen available whenever needed. (New lots were also started during the fall and winter, so that this work could continue at all times.) All of the pollen collections were made between 0730 and 0900 LST. During the early part of the summer the pollen came from potted plants indoors, but later (during the regular season) plants growing outside were used. A vacuum pump with a sintered glass filter connected by a rubber hose was used to obtain the pollen and the filter was held near the spikes and moved in a vertical direction parallel to the inflorescence axis. Gently bumping the inflorescences resulted in easy removal. Pollen loads were taken approximately 15 times, for several project uses.

During the summer of 1959, project technicians of the meteorological group were trained in the identification of pollen grains and spores by specialists of the botanical staff. In addition, useful literature bearing on these problems was reviewed.

### 1.7.1 Humidity Effects on the Size of Pollen Clumps

The clumping of pollen has been noted previously in our studies and has been published [1]. It was the purpose of Bianchi's experiments during 1959 to determine whether or not humidity had any effect on the size of pollen clumps as they fall from the flowers.

It had been observed that the pollen as it emerges from the anthers drops in large clumps, possibly because of adhesion of

pollen grains to each other by actions of tapetal fluid, or by mere compression of the pollen grains while in the anther sacs. Preliminary observations made in 1956 suggested that the size of the clumps depended on relative humidity, the clumps being larger under more humid conditions. If this were the case, then the time necessary for the reflation of the pollen would be less on dry days because the clumps would be smaller and fall apart more quickly. Conversely, the reflation might be prolonged on damp days.

Plants of Ambrosia artemisiifolia were grown under greenhouse conditions, out of season, at the Gardens. In experiments using intact plants, the main spike was submerged in water the evening prior to experimentation. At the time of testing, the plants were put into a walled glass chamber and the humidity of this chamber was controlled by mixing moist air with air that had been dried by passing it through a column of silica gel. When the plant was dissected, the entire main spike of the plant was removed and attached to the upper lid of a battery jar (see Figure 1). At the base of the battery jar was placed a container with appropriate salt solutions to create the desired humidity in the jar. A sheet of black paper was placed at the bottom to serve as a means of collecting the clumps of pollen as they fell from the flowers. The jar was sealed with petrolatum and a glass plate. The detached spikes were submerged in water prior to experimentation to prevent release of pollen before the desired time. A control chamber with no liquid solutions, the latter with unknown relative humidity, was used as a reference.

This experiment indicated that there was no correlation between the relative humidity and the size of the pollen clumps (see Table 1). The experiment should be repeated and amplified before concrete conclusions are reached about the clumping phenomenon. Some of the factors which should be included in further experiments would be a test of the flowers from one plant under the various conditions. In the experiments described here, each of the spikes in the jars had come from a different plant. Various temperatures should be considered also in future experimentation on clumping. The present study was made at a temperature of 24.5C only.

### 1.7.2 Pollen Germination Studies

Results from research on the germination and respiration of ragweed pollen confirm the conclusions of earlier studies. The family Compositae, to which the ragweeds belong, has pollen grains which, in the past, have proven difficult to germinate under ex-

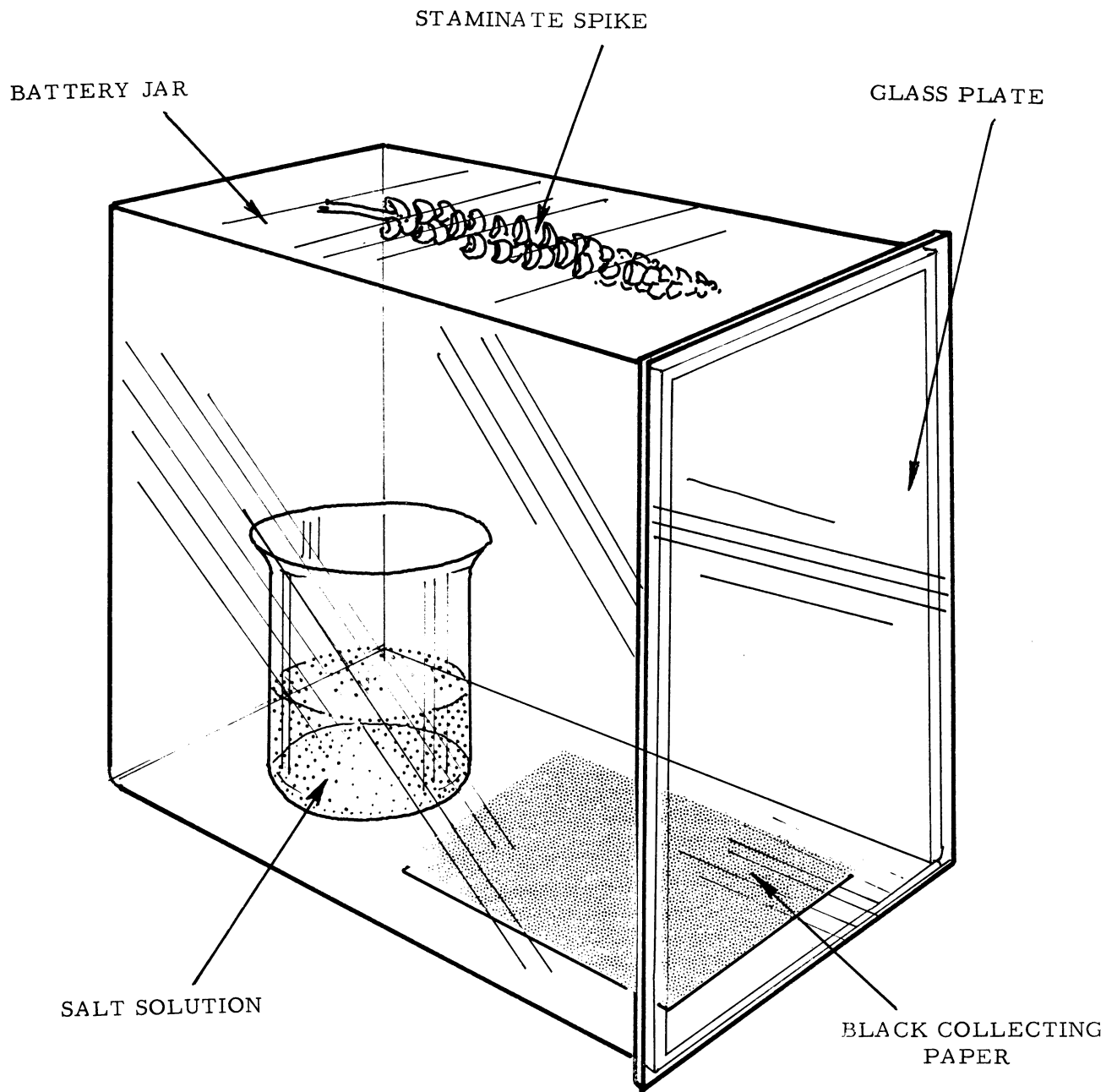


Fig. 1. Apparatus for determining effects of humidity on pollen clumping.



perimental in vitro conditions. The purposes in the latest studies were (a) to define an experimental environment that would induce germination of Ambrosia pollen, and (b) to survey the effects of varying environment on the germination process.

TABLE 1

Relative Humidity and Pollen Clump Size

Relative Humidity per cent	Average Clump Diameter, mm	Range in Clump Diameter, mm
31	0.42	0.10 - 1.00
43	0.39	0.20 - 1.15
52	0.47	0.20 - 1.00
68.6	0.42	0.10 - 1.40
79.3	0.36	0.10 - 1.10
88.0	0.40	0.10 - 1.40
100	no clumps	

Percentage of pollen germination was determined by "dusting" pollen with a small camel-hair brush onto an agar surface. Five ml. of the melted nutrient agar solution was poured into small (4.5 x 3.0 cm) petri dishes and allowed to solidify. After suitable incubation, the germination was terminated by the addition of 0.3 ml. of 10 per cent formalin solution. Approximately 300-400 pollen grains were examined by placing the petri dish directly on the microscope stage.

The following results were obtained: In preliminary experiments, pollen was taken at undefined times after the dehiscence of the flowers. Attempts to germinate this pollen on a wide variety of simple and supplemented media were unsuccessful or inconsistent. It was later observed that pollen taken within a few hours after dehiscence would germinate on a simple sucrose-agar medium. Using pollen harvested within approximately one hour after dehiscence, the components of this medium were examined in detail. When the sucrose concentration of the environment was varied, a concentration of approximately 15 per cent sugar proved to be the most favorable. Almost no germination was found with concentrations up to 5 per cent sucrose. Although a well-defined optimum was noted

for 15 per cent sucrose, the total germination was not high. Results of a typical experiment are compiled in Table 2.

TABLE 2

Influence of Sucrose Concentration on Germination  
(per cent)

Incubation Time (min)	Sucrose Concentration (gm/100 ml.)					
	0	5	10	15	20	30
30	0	0	7	10	7	1
90	0	0	11	18	8	3
150	0	2	11	33	18	9

The above results were adduced with sucrose solutions solidified with 2 per cent agar. The influence of agar concentrations on germination was examined using a 15 per cent sucrose solution. The results of this study are illustrated in Figure 2. No germination occurred when the pollen grains were floated directly upon the sucrose solution, but the addition of enough agar to form a firm gel greatly stimulated germination. Optimum germination was obtained using an 0.7 per cent gel. Germination as high as 70 per cent was obtained with this concentration of agar. In subsequent experiments a concentration of 0.7 per cent agar was used to solidify the 15 per cent sucrose solution.

A consideration of the influence of pH on germination revealed an optimum at 6.8-7.0 when Sorensen's phosphate buffer was employed. Almost no germination took place below a pH of 5.7 or above 8.0. These results are summarized in Table 3. Although an optimum is evident, the total germination is not as high as might be predicted from the results of the experiments on agar concentration. If a sucrose-agar medium is prepared, using various dilutions of the phosphate buffer, it becomes evident that the buffer is inhibitory to germination. In Figure 3, it can be seen that germination is almost completely inhibited by 0.03 M phosphate buffer. (In previous experiments in which the media contained this buffer, the low percentage of germination may have been due to the buffer rather than other factors; at first it was not realized that phosphate buffer would be inhibitory.)

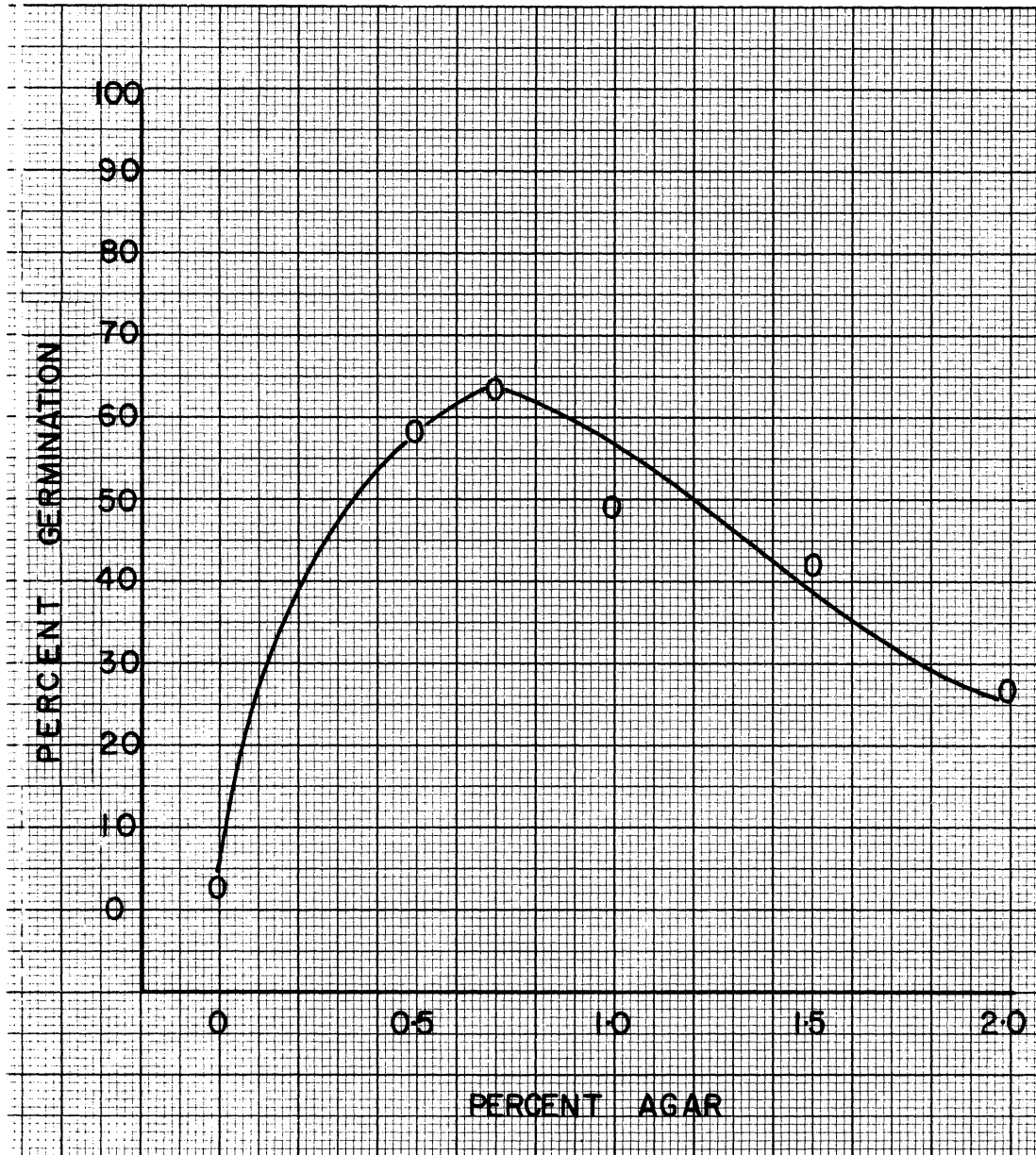


Fig. 2. Influence of agar concentration on germination

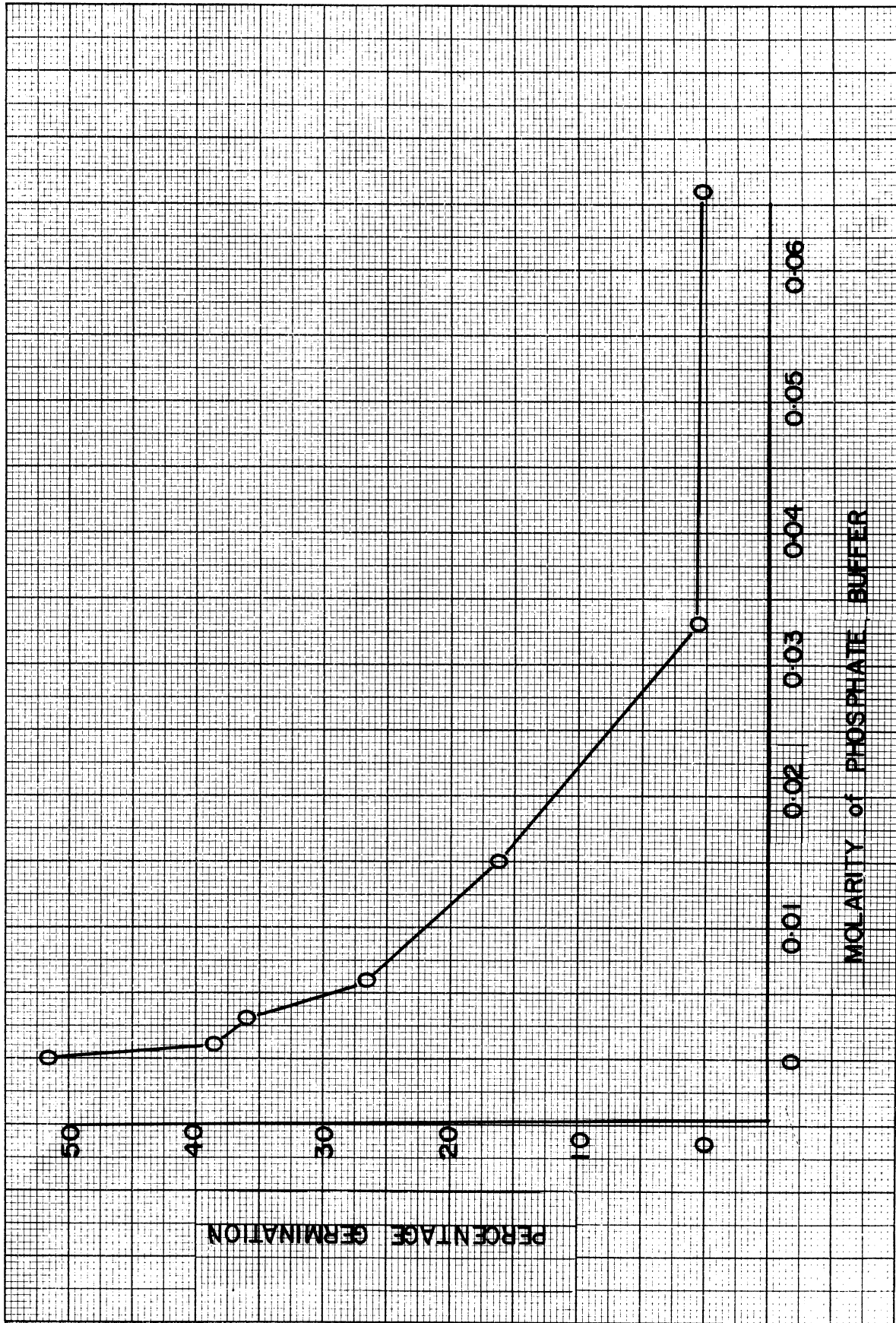


Fig. 3. Influence of phosphate buffer on germination of Ambrosia pollen.

TABLE 3

## Influence of Hydrogen Ion Concentration on Germination

Per cent Germination	pH of Medium (adjusted with 0.008 M buffer)					
	4.8	5.7	6.4	6.8	7.3	8.1
	0	1	25	30	5	0

The high concentration of sucrose needed to promote optimum germination might suggest that the role of the sugar is to provide a proper osmotic environment rather than a suitable substrate. It was therefore interesting to obtain information as to the specificity of sucrose. The germination obtained on equimolar concentrations of several sugars is summarized in Table 4. Sucrose promoted the best germination of the various sugars, maltose and galactose were next, and the poorest results were obtained with fructose and glucose.

TABLE 4

## Influence of Different Sugars on Germination

Sugar Employed	Per cent Germination
Sucrose	51
Maltose	41
Lactose	18
Glucose	5
Fructose	2
Galactose	35
Mannose	1
Mannitol	1

Because the germination of pollen from many plant species is stimulated by the addition of boron salts, the influence of boron on ragweed pollen was studied. Boron, however, added as boric acid in concentrations up to 200 mg per l., did not clearly stimulate germination. In an unbuffered medium some stimulation was noted at

a boron level of 1, 5, and 10 mg per l.; but total germination in control cultures was very low, and a clear interpretation was impossible.

Indolacetic acid had no effect in concentrations of 0.5 to 5.0 mg per l. Concentrations up to 10 mg per l. almost completely inhibited germination. As with boron, the results are complicated by low germination in the controls.

The release of pollen by the common ragweed has been demonstrated by us earlier to take place with a very definite diurnal rhythm. Pollen was collected in the morning soon after dehiscence of the flowers and stored in vials at room temperature. At measured time intervals, a sample of pollen was removed and placed on buffered sucrose sugar. After an incubation of one hour at 27C, the percentage germination was determined. The viability of pollen under these conditions is greatly influenced by the time interval between dehiscence of the flower and the inoculation of the sucrose-agar, called the "age" of the pollen. Shortly after dehiscence the viability is low, it increases to a maximum after about two hours, and then slowly declines. After eight hours of storage, almost no germination is observed. These results are illustrated in Figure 4.

Storage of pollen at different temperatures influences the duration of the ability to germinate. The results summarized in Figure 5 are from an experiment in which pollen was taken approximately two hours after dehiscence of the flowers and then stored at the temperatures indicated. After the proper time interval, the pollen was placed in the sucrose-agar and incubated for one hour at 27C. Exposure to 37C rapidly inactivated the pollen. Storage at 4C preserved to some extent the viability of the pollen, while the usual slow decline was observed at 27C.

### 1.7.3 Pollen Respiration Studies

Standard manometric techniques were utilized in the studies of pollen respiration. The pollen was weighed on a Roller-Smith balance and then suspended in a buffer or sugar solution and an aliquot containing 5 mg of pollen added to each Warburg flask. All experiments on respiration were carried out at a temperature of  $26.4C \pm 0.2C$ .

Pollen suspended in phosphate buffer demonstrated an active oxygen uptake that was easily measured in the standard Warburg apparatus. The  $Q_{O_2}$  of grains (i.e., microliters of oxygen per

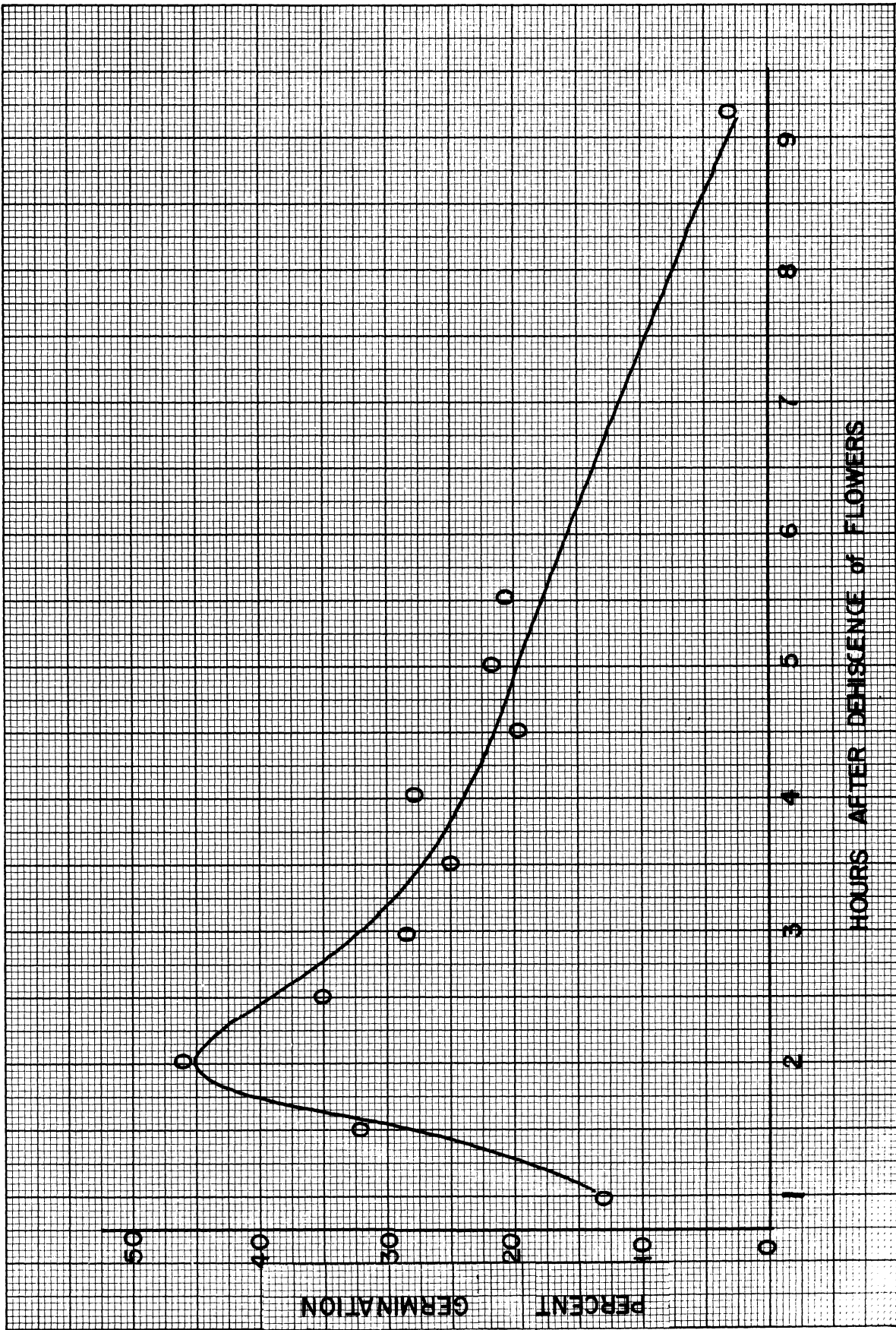


Fig. 4. Effect of pollen "age" on germination.

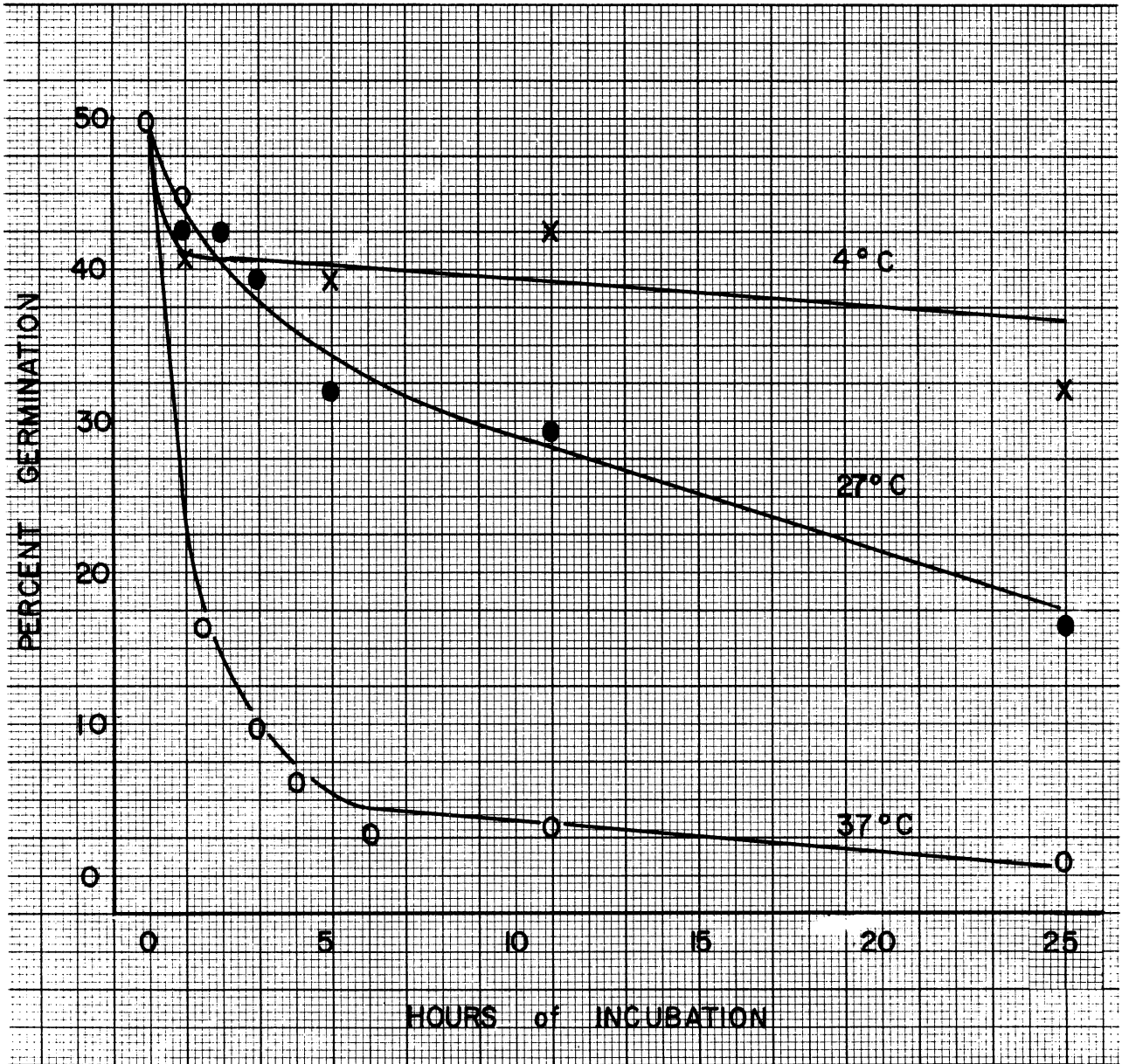


Fig. 5. Germination obtained after storage of pollen at different temperatures. Pollen was stored at temperatures indicated above at indicated times, and then incubated for 1 hr at 27C to determine percentage germination.



milligram fresh weight of pollen) varied from day to day with different collections. Values between 1.92 and 9.04  $\mu$ l. of oxygen consumed per milligram of fresh weight were observed. In most cases the gas exchange was almost linear for the first hour and declined only slightly after two hours incubation. The addition of sugars to the pollen suspension had some effect upon oxygen uptake. A slight stimulation of respiration was noted for sucrose, in one case raising the  $Q_{O_2}$  from 1.92 to 2.16. Glucose seems to have no effect, while a definite reduction of the oxygen uptake was noted in the presence of mannose. A summary of the data from Warburg experiments is presented in Table 5.

An attempt was made to determine whether the presence of an agar surface has any influence on respiration, as it does on germination. Pollen suspended in 0.4 M sucrose solution was solidified with 0.7 per cent agar. In control vessels the pollen was tipped into liquid sucrose. After determination of gas exchange, the contents of the flasks were examined for germinating pollen grains. The rate of oxygen uptake by pollen in both situations was identical, exhibiting a  $Q_{O_2}$  of 1.55 after the first hour. In the flasks with agar, germination approximated 50 per cent whereas there was no appreciable germination in flasks without agar.

A sample of pollen was stored in 0.0013 M phosphate buffer, pH 6.9, for 4 hours at room temperature and 20 hours at 4C. The  $Q_{O_2}$  was then determined for this sample of pollen and compared to an aliquot which was utilized on the same day as the collection. The  $Q_{O_2}$  was found to decrease from 2.50 to 1.97 after 24 hours. Thus the pollen retained 79 per cent of its respiratory activity after 24 hours (Table 5).

The results as outlined above indicate that if three conditions are properly manipulated, ragweed pollen can be induced to germinate. These three conditions are (a) the provision of a proper surface, (b) a supply of a suitable carbohydrate, and (c) pollen of the proper "age."

The role of the agar surface is not understood. When pollen is placed in a sucrose solution, it floats very readily but does not germinate. The presence of an agar surface greatly stimulates germination. It seems unlikely that the role of the agar is in the improvement of aeration. No increase in oxygen uptake could be demonstrated when pollen on agar was compared to pollen floated on a sucrose solution. The presence of trace nutrients in agar that stimulate germination must be considered. The utilization of

TABLE 5

## Summary of Data from Warburg Experiments

Medium in vessel	Gas Phase	Gas Ex- changed	$Q_{O_2}$ or $Q_{CO_2}$ *				
			Experiment Number				
			2	3	4	5	6
.133% phosphate buffer, pH 6.8	Air	O <sub>2</sub>		1.92	2.50	1.97**	4.04
0.4 M sucrose	Air	O <sub>2</sub>	1.55	2.16			
0.4 M mannose	Air	O <sub>2</sub>	1.55	1.03			
0.4 M glucose	Air	O <sub>2</sub>		1.92			
0.4 M maltose	Air	O <sub>2</sub>		1.83			
0.4 M fructose	Air	O <sub>2</sub>			1.67		
.133% phosphate buffer, pH 6.8	Air	CO <sub>2</sub>			2.50		3.64
0.4 M sucrose	Argon	CO <sub>2</sub>					7.00

\* All  $Q_{O_2}$  or  $Q_{CO_2}$  values are in terms of  $\mu$ l. gas exchanged/mg fresh weight pollen/hour usually taken after the first hour when rates became constant.  $Q_{O_2}$  and  $Q_{CO_2}$  values on dry weight basis are approximately 13% greater.

\*\* This  $Q_{O_2}$  was obtained with the same pollen used in Experiment No. 4 but which had been aged 24 hours at 26.4C.

washed agar preparations or other substrates such as silica gels would be useful in this respect. It is also possible that the agar somehow combines with an inhibitor of germination present in or around the pollen grain. If such an inhibitor is present, it might be possible to extract the substance from agar that has supported the germination

Since sucrose does have some specificity in the promotion of germination, a role as an actual metabolic substrate might be suspected. However, the high concentration necessary for optimum

germination is puzzling. The very slight respiratory response of pollen to added sucrose would suggest that there is an almost optimum supply of endogenous substrates. A study of the uptake of sucrose labeled with  $C^{14}$  would help to define the role of sucrose in the germination process.

The most important factor regulating the germination of Ambrosia pollen seems to be the age of the pollen. For a few hours after release from the flowers a low incidence of germination is observed. Changes which occur during this ripening period are unknown. Either a synthesis of an essential metabolite, or the destruction of a germination inhibitor may be involved. The influence of flower extracts upon germination may help to answer this question.

The results of this investigation suggest that pollen one day old is inactive with regard to germination. Preliminary observations of the effect of age on actual germination on the stigmatic surface of female flowers indicate that this decline in viability occurs under natural conditions. The data on the oxygen uptake of pollen one day old demonstrate that pollen respiration is still present. It is possible that changes in the membranes or wall of the pollen physically prevent the emergence of the pollen tube, although the grain remains still active metabolically. This loss in viability may have some interesting effects upon the hybridization between widely separated populations of ragweed plants.

## 1.8 PLANS FOR 1960-61

The work on evolution and migration of ragweeds as well as the research on ecology and phenology will be continued during 1960-61. The experimental phytosociological experiments at the Botanical Gardens will involve growing plants under different conditions (dry upland woods, dry grassy meadow, dry herbaceous meadow, open marsh, and shaded marsh). In each community, plants will be established and carefully followed in their development to determine what effects the environment can produce. Experiments on the influence of shade will be carried out at the same time, using artificial shade provided by saran screening. The study of floral development and structure will especially focus upon the relationship of function to the structure of cells and tissues. A motion picture film will be prepared during this year to show the floral function and pollen discharge in common ragweed. During the preparation of this film it is expected that new facts as well as confirmations of previous ideas regarding the stages of ragweed floral activity will be obtained. The research on the biology of the

pollen grains is being discontinued during 1960 because of the lack of appropriate personnel.

## 1.9 SUMMARY AND CONCLUSIONS

In addition to consultation and conference with project associates of other disciplines, the botanical group attacked the specific problems described below.

a. Research on evolution and migration of ragweeds involved on-the-spot studies in 23 states as well as numerous field collections. These investigations yielded a clearer definition of abundance and range of the species than was heretofore available.

b. In connection with the Tecumseh Community Health Study Program, a subjective method of estimating local pollen potential was devised and carried out. The method seems to be useful where gross estimates of local pollen potential are required.

c. The 1959 studies suggest the following hypotheses: The genus Franseria seems to be more primitive than, and possibly ancestral to, Ambrosia. The southern and northern forms in the Ambrosia artemisiifolia and A. trifida complexes are either distinct species or varieties. Hybridization has evidently played a role in the evolution of ragweeds. Migrations probably began in central North America under natural conditions, but man's influence has stimulated the spread greatly. The ragweed believed to be native in Europe, A. maritima, may actually represent an early introduction from America by man. The dispersal of ragweeds by fragmentation of plants seems much less important than by seeds eaten and dispersed by birds.

d. Ecological studies at Willow Run Meteorological Station were conducted to determine especially (i) variability of ragweed; (ii) the relationships of ragweed density to presence and abundance of other plant species; (iii) the relationship of ragweed phenology to other, associated plant species; and (iv) whether ragweeds have definite associations with other species or merely random associations. Experimental cutting of pre-season plants that flower in June showed that the new growth would flower again in August.

e. A test to determine whether ragweeds would flower under constant light was made, and the results were positive. Although there was a delay in comparison to wild plants, the constantly illuminated plants did flower, and there is thus no evidence that city lights can prevent pollen production.

f. Research on floral development and function showed that (i) the involucre open and close to some extent; (ii) that the extension of pistillodia and stamens is due to enlargement of cells and not to rapid cell division; (iii) that the pistillodium literally does push out the pollen that remains in the opened pollen sacs; and (iv) the vegetative apex becomes converted to an embryonic floral axis as early as the first week in July.

g. The biology of pollen grains was studied especially from two standpoints (i) the clumping of pollen grains; and (ii) the processes of germination and respiration. The clumping of pollen grains was examined under different relative humidities. The experiment, though not entirely conclusive, indicates that there is no correlation between relative humidity and the size of pollen clumps.

h. Pollen of Ambrosia artemisiifolia will germinate on a sucrose-agar medium. Germination is inhibited when the sucrose solution is not solidified with agar. A high concentration of sucrose, approximately 15 per cent, is needed for optimum germination. Sucrose supported the best germination of the 8 sugars tested; maltose and galactose were also effective, but germination on glucose was poor.

i. The time interval between dehiscence of the flower and inoculation of the pollen on sucrose-agar has a definite influence upon germination. Germination is low immediately after dehiscence, rises to a peak after approximately two hours, and then declines. Frequently, no germination at all is observed after ten hours.

j. The influence of pH and phosphate concentration on germination was studied, as well as the effect of storage at different temperatures. Preliminary observations on the respiration of Ambrosia are also reported.

k. Plans for future botanical research include additional studies on evolution, migration, and ecology of ragweeds, the development and structure of the flower, and a motion picture film on the functioning of the flower. The work on pollen biology will be discontinued because of lack of appropriate personnel.

## 2. MEDICAL PHASE

by

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### 2.1 INTRODUCTION

During the past year, work has continued on four projects (reported upon in previous progress reports): (a) hemagglutination tests with serum of pollen sensitive patients, (b) studies on the allergenicity of tapetal fluid, (c) effects of air ionization on passive anaphylaxis in guinea pigs, and (d) recovery of pollen grains from respiratory tissues. In addition, five new projects have been instituted in the past year: (a) studies on the rate of adsorption of isotopically tagged antigens incorporated in various types of repository emulsions, (b) the effect of pH upon skin-sensitizing antibody, (c) the adsorption of pollen allergens on glass surfaces, (d) the effect of normal human serum and gamma globulin on passive transfer tests, and (e) observations on the incidence of ragweed and other allergies in a large foreign student population as a means of evaluating methods for determining the genetics of atopy.

Proposed studies on the immunochemistry of ragweed pollen extracts have been delayed pending procurement of an adequately trained immunochemist. The fact that more than ten months of active effort were required before a suitable and available person could be located points up the shortage of well trained persons in this field. We are pleased to report, however, that we procured the services of a well trained person on 1 July 1960.

### 2.2 FURTHER STUDIES ON HEMAGGLUTINATION TESTS EMPLOYING SERA OF TREATED AND UNTREATED POLLEN-SENSITIVE SUBJECTS

Work on the possible application of hemagglutination methods to the in vitro detection and quantification of skin-sensitizing antibody has been continued. As reported previously, the methods employed have been bis-diazotized benzidine (BDB) hemagglutination tests by the technique of Gordon, Rose, and Sehon (with slight

modifications), the tanned cell antiglobulin (TCAG) test by the technique of Mathews, and passive transfer (Prausnitz-Kustner) tests. Major attention has been focused on the question of whether or not the hemagglutinins are identical with skin-sensitizing antibodies (SSA).

### 2.2.1 BDB Tests

Sera of 26 additional untreated pollen sensitive patients have been tested by the BDB hemagglutination method since 1 July 1959. Twelve of these patients gave positive results in titers of 1:20 or higher. Although this represents a considerably lower percentage of positive results than has been attained in some other laboratories, we have adopted conditions of testing in which controls with normal sera are consistantly negative, while in some other laboratories more than 50 per cent of normal serum controls are positive. The specificity of some of the positive tests has been confirmed by neutralization tests, the hemagglutination reactions being inhibited by preaddition of homologous antigen directly to the serum but not inhibited by heterologous antigen. Positive results have been obtained both with water and buffered saline pollen extracts.

Positive results have been obtained in BDB tests with serum of all of 12 patients who had received hyposensitization therapy with pollen extracts. The titers of these post-treatment sera generally were higher than those obtained with sera of untreated patients.

As an important approach towards trying to answer the major question of whether the hemagglutinating antibodies are identical to skin-sensitizing antibodies, adsorption experiments were carried out on sera of 14 patients. The effects of adsorbing these sera for 1 hr with cells sensitized with ragweed pollen extract (by BDB) are shown in Table 6. The passive transfer titers represent geometric mean titers on 2 to 7 recipients and in some instances were read "blind." The BDB agglutinin tests of paired adsorbed and unadsorbed sera were done simultaneously, employing the same lot of sensitized rabbit cells. The post-adsorption PK and BDB tests were done on aliquots of the same adsorbed serum. In two instances, repetition of the entire adsorption experiment after a period of many months yielded satisfactorily reproducible results. To prevent hemolysis when the human sera were preliminarily adsorbed with washed red cells from the specific rabbit to be used in a given experiment, the human sera first were adsorbed with zymosan. It was shown that zymosan adsorption does not alter the PK titer of human sera.

TABLE 6

Sera Adsorption Effects  
Using Ragweed Pollen Extract Sensitized Cells  
(Reciprocals of Titers)

Serum	Avg. PK Titer before Adsorption	Avg. PK Titer after Adsorption	BDB Agglu. Titer before Adsorption	BDB Agglu. Titer after Adsorption	Cell/Serum Volume for Adsorption
<sup>1</sup> D. Wi.	80	80	80	<10	1
<sup>2</sup> P. G.♣	160	160	40	<10	1
<sup>3</sup> D. Wa.	40	20	160	<10	1.75
<sup>4</sup> E. M.	80	<20	80	<10	2
<sup>5</sup> G. N.	40	<20	160	<10	3
<sup>6</sup> F. P.	160	20	40	<10	4
<sup>7</sup> M. L.	1280	640	320	<10	0.75
<sup>8</sup> S. M.	1280	160	320	20	4.5
<sup>9</sup> R. W.	160	20	160	40	3
<sup>10</sup> E. G.	320	80	320	80	3
<sup>11</sup> P. E.*	160	80	80	<10	2.5
<sup>12</sup> M. DeF.*	160	160	320	<10	3
<sup>13</sup> G. R.* ♣	640	160	320	<10	3
<sup>14</sup> P. B.*	1280	1280	1280	<10	3

\* Post-treatment sera.

♣ Water-soluble ragweed extract used.

The sensitized cells employed in the adsorption procedure were washed three times and used immediately. Appropriate experiments were conducted to exclude the possibility that the experimental results might have been influenced by soluble antigen derived either from inadequate washing or elution of antigen from the sensitized cells. It was shown that no soluble antigen was present even with less washing or much longer storage of the sensitized cells (up to 24 hr). The volumes of sensitized cells required to adsorb the pollen antibodies in general were large, suggesting



that the cells did not have large amounts of all the important pollen antigens on their surfaces. The values given in the last column of Table 6 are not strictly comparable, since the cells were sensitized with different amounts of pollen antigen in different experiments depending on the conditions which were optimal for testing purposes with each lot of rabbit cells. Also, the values given do not necessarily represent the minimal amount required for adsorption. In some additional experiments, sera 10 and 13 were adsorbed with varying amounts of the same lot of cells. It was found that a four-fold increase in the amount of cells used for adsorption beyond a certain optimal quantity yielded no further reduction in the passive transfer titers.

Concentrated pollen extract dialysates were employed as a neutralizing antigen in further studies designed to distinguish possible differences between SSA and hemagglutinins. Concentrated (15 per cent) pollen extracts were dialyzed for 72 hr, and the dialysate was concentrated five- to six-fold by pervaporation in Visking tubing. This material was used to neutralize sera of untreated patients, and BDB and PK tests were carried out on these "neutralized" sera. It can be seen from Table 7 that the BDB tests showed essentially no change in titer among nine sera studied in this manner. On the other hand, there appeared to be a substantial decrease or complete loss of PK activity following neutralization of sera of six untreated patients with concentrated dialysate. However, additional studies on sera of three patients sensitive both to ragweed and grass revealed that in two of three instances there was a non-specific decrease in reactivity of passive transfer sites to grass pollen extract as well as to ragweed following in vitro neutralization with ragweed extract dialysate. This non-specific effect is known to occur in PK neutralization tests and is presumably due to temporary depletion of the skin histamine stores at the site of injecting antigen-antibody mixtures (large immediate reactions occurred at the time of planting our ragweed dialysate-serum mixtures). Although this evidence of non-specific desensitization of the PK sites limits conclusions which can be drawn from these data, the very fact that immediate reactions and site exhaustion occurred (especially with fairly high serum dilutions) in itself suggests the occurrence of a reaction between the dialysate and the serum. Additional studies in progress on the effect of concentrated ragweed extract dialysate upon PK titers of sera containing SSA to grass, but not to ragweed, should help to clarify this problem.

An obvious approach to the relationship between SSA and BDB hemagglutinins is the correlation between the titers of these two

antibodies in a series of sera. The correlation coefficient between SSA titers (by PK dilution tests) and BDB titers in the first 10 sera giving positive results in the latter test was found to be 0.38.\* However, as is apparent from inspecting Tables 6 and 7, there are a number of rather definite individual exceptions to this trend.

TABLE 7

Neutralization Tests with  
Concentrated Ragweed Pollen Extract Dialysates  
(Reciprocals of Titers - Sera All from Untreated Patients)

Serum	Avg. PK Titer before Neutrali- zation $\bar{x}$	Avg. PK Titer after Neutrali- zation $\bar{x}$	BDB Agglu. Titer before Neutrali- zation	BDB Agglu. Titer after Neutrali- zation
P. G.	160	<10	80	160
D. Wi.	80	<10	80	160
	$\nabla$ 160	$\nabla$ 160		
D. Wa.	40	10	160	80
F. P.	320	<10	40	40
	$\nabla$ 80	$\nabla$ <10		
E. M.	80	<20	80	40
	$\nabla$ 80	$\nabla$ 20		
R. W.	160	<10	40	40
E. G.			40	20
S. M.			320	320
M. L.			160	160

$\bar{x}$  Mean titers on 2 to 5 recipients.

$\nabla$  Sites challenged with grass pollen extract.

\* The reciprocals of the titers were converted to a geometric scale for purposes of calculating this correlation coefficient.

### 2.2.2 TCAG Tests

After using TCAG tests for almost two years without difficulty, during the summer of 1959 a number of positive control tests were encountered. The difficulty was incomplete suppression of non-specific uptake of normal human serum globulin by the tanned cells. Considerable effort was expended on trying to resolve this problem. New lots of cells, Alsever's solution, distilled water, tannic acid, normal rabbit serum, and Coombs' serum were tried. Different methods of inactivating the human sera were reevaluated including the use of heat, zymosan, EDTA, and  $\text{NH}_4\text{OH}$ . The only factors found to be relevant to the difficulty were the distilled water and the age of the human serum. In agreement with the work of Jandl, the presence of certain metallic cations in water was found to exert a profound influence on the uptake of protein by the cells. It also was found that non-specific protein uptake occurred more readily from fresh serum or serum stored in a frozen state than from serum which had aged several months at 4C. Since passive transfer titers are maintained well under such conditions of storage, recent experiments have been limited largely to older sera.

All of 14 normal sera gave negative TCAG tests with ragweed allergen in dilutions down through 1:5 or 1:10.

In addition to the 27 tests reported last year, TCAG tests have been done on sera of 19 more untreated ragweed or grass sensitive patients. Of these, one was definitely positive and five questionably positive. The lower percentage of positive reactions this year may be related, in part, to the necessity for using aged serum in the recent tests.

Sera of all of eight treated ragweed or grass sensitive patients gave positive reactions in the TCAG test. The titers generally were slightly higher than with the BDB test. The sera were neutralized by the addition of homologous antigen but not heterologous antigen.

Adsorption tests were carried out with ragweed extract sensitized tanned cells in a manner analogous to the procedure used in the BDB adsorption tests. Adsorptions were carried out for 1 hr at 37C with three times washed, sensitized cells. The results of these tests are shown in Table 8. Again, the post-adsorption PK and BDB tests were done on aliquots of the same adsorbed serum, and some of the PK tests were read "blind."

TABLE 8

Sera Adsorptions for Three-Times-Washed Sensitized Cells  
(Reciprocals of Titers)

Serum	Avg. PK Titer before Adsorption*	Avg. PK Titer after Adsorption*	TCAG Test Titer before Adsorption	TCAG Test Titer after Adsorption	Cell/Serum Volume for Adsorption
D. Wi.	160	80	80	<20	1
P. G.	320	320	160	<20	0.75
D. Wa.	320	320	320	<20	1.5
			320	20	1
E. M.☞	160	160	320	80	0.75
			320	20	1.5
			320	<20	3
G. N.☞	320	40	320	40	0.75
			320	<20	1.5
			320	<20	3
F. P.			80	<20	0.75
M. L.☞	1280	1280	1280	40	3

\* Geometric mean titers on 2 recipients.

☞ Post-treatment sera.

### 2.2.3 Formalin-Treated Cells

In working with tanned cell techniques, one needs daily to wash the cells three times, tan them, wash again, sensitize with antigen, and again wash. Since this becomes very tedious, some preliminary observations were made on the use of formalin-treated red cells sensitized with allergens, since such cells sensitized with ordinary antigens are said to be usable for several months.

Human type O red cells were treated with formalin by the McKenna method. On one run, such cells when freshly tanned and sensitized with egg albumin gave titers identical to those obtained

with ordinary tanned cells in tests with rabbit anti-egg albumin serum by the direct-tanned cell technique of Boyden. In limited observations, formalin treated, freshly ragweed sensitized cells were equally unsuccessful in demonstrating hemagglutination in human sera by the Boyden method as were ordinary-sensitized tanned cells. When the formalin treated cells were sensitized with thyroid antigen, agglutination was obtained with sera of patients having thyroid disease, though the titers were somewhat lower than those obtained with ordinary red cells in a limited number of observations.\*

When used in the TCAG test, in three experiments freshly sensitized formalin treated cells gave titers identical to those obtained with ordinary cells when exposed to sera of ragweed sensitive patients. Fifteen-day-old ragweed-sensitized, formalin-treated cells still gave the same titers as freshly sensitized tanned cells, but cells 3.5 months old gave lower titers than freshly sensitized cells. Inhibition of non-specific uptake of normal serum globulin in the TCAG test was the same with formalin treated cells as with ordinary cells.

#### 2.3.4 Discussion

In interpreting the results of these studies, major consideration must be given to the fact that multiple antigens are present in the crude pollen extracts. Pollen antibodies of varying specificity probably are present in the human sera. In spite of this complexity of the system being studied, the hypothesis that all the skin-sensitizing antibodies to ragweed-extract antigens are agglutinins in the BDB test appears untenable in view of such results as were obtained with sera of D. Wi., P. G., D. Wa., M. L., P. E., M. DeF., G. R., and P. B. Indeed, failure of the passive transfer titers of sera to fall to any major extent after complete removal of the agglutinins by adsorption indicates that no type of SSA could have contributed to a major extent to the agglutinin titer of these sera, particularly since the sensitivity of the two tests is of the same order of magnitude. On the other hand, it has been shown that the adsorption of at least some sera with a sufficient volume of cells will remove SSA as well as agglutinins (sera of E. M., G. N., F. P., S. M., R. W., and E. G.). Among possible explanations for this variability, an attractive hypothesis is that BDB links to cells small amounts of certain pollen

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\* These tests were run by the Thyroid Research Laboratory, The University of Michigan.

extract component(s) which are homologous only to certain species of SSA.\* Sera in which these particular types of SSA are dominant can be adsorbed by the sensitized cells, while sera containing predominantly other species of SSA are not adsorbed. There must be hemagglutinating antibodies other than SSA in the latter type of sera and very possibly in all these sera, whether from treated or untreated patients. In fact, the antigen-antibody system(s) involved in the hemagglutination reaction might be entirely separate from SSA and their antigens. This is not an unheard of possibility, since there are recent reports on the incidental presence of hemagglutinins to food antigens even in sera of normal persons. The lack of complete identity of SSA and BDB test hemagglutinins is also supported by inspection of their comparative titers in our sera and the results of the neutralization tests with concentrated pollen extract dialysates (Table 7).

With the TCAG technique, adsorption tests (Table 8) indicate that the agglutinins being measured by this method also are not identical to SSA for reasons entirely analogous to those just discussed in connection with the BDB test.

## 2.3 STUDY OF THE ALLERGENICITY OF TAPETAL FLUID

### 2.3.1 Introduction

About two years ago, an attempt was made to obtain tapetal fluid by inserting a small glass capillary into an anther sac of a ragweed flower and then applying suction. The attempt failed, perhaps because of the great viscosity of the fluid. This capillary was withdrawn, and though no fluid could be seen in it, it was rubbed in a drop of saline applied to a prick on the arm of a ragweed sensitive patient. A four plus reaction occurred. This suggested that tapetal fluid might be extremely allergenic and might in fact be the major source of pollen allergen. It therefore appeared important to determine more precisely what the allergenicity of the fluid might be.

Tapetal fluid surrounds and bathes pollen grains during their course of development. It comes from a conspicuous layer of cells in the anther sac called the tapetum or tapetal cells. The fluid is believed by some to be a nutrient for the developing pollen grains, and some believe that it is the tapetal fluid that causes the clumping of the pollen grains.

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\* Possible interpretations of this work and comparison with the results obtained in other laboratories will be presented at more length in the published report.

Obtaining the fluid for study presented a major technical problem because of the small size and volume of the ragweed anther sacs and the great viscosity of the tapetal fluid. Also, to isolate its contribution to allergenicity, it is necessary to separate the tapetal fluid from the pollen grains.

### 2.3.2 Attempts at Separating Tapetal Fluid by Differential Centrifugation

The first attempt to obtain the fluid involved dissecting the anthers and slitting across the sacs on a glass slide under a dissecting microscope. The slit sacs ( $3.75 \times 10^4$  in all) were placed in ether, since water was known to extract pollen. The sacs were shaken vigorously and centrifuged at 3000 rpm for 1 hr. It had been hoped that a layer of the fluid would form between the sacs' debris and the ether layers, but this did not occur. Probably the small volume of tapetal fluid was trapped in the debris.

The sacs were firmly crushed to squeeze out more fluid and were then recentrifuged, but still no layer appeared. Higher speed centrifugation was considered but could not be tolerated by the glassware being used, and the plastic cups ordinarily used in the higher speed centrifuges tend to be softened by ether.

### 2.3.3 Estimation of Allergenic Activity by Difference between Whole Anther Sac and Pollen Grain Allergenicity

After pouring off the ether, the packed anther sacs were divided approximately in half. A measure of 1.8 ml. saline was added to half of the anthers and also to one tube containing old pollen\* and to another containing fresh pollen in quantities estimated to be roughly similar to the amount of pollen in  $1.875 \times 10^4$  anther sacs (see Appendix). An extraction period of 28 hr was allowed for each mixture which then was centrifuged and used in scratch tests. Four plus reactions were elicited with all three extracts on a ragweed sensitive subject. After Swinny filtering and culturing the extracts, intradermal tests were done, and these end points of activity were found: the limiting ten-fold dilution of anther extract was  $1:10^4$  producing a strong positive reaction, the old pollen extract  $1:10^6$  gave a weak positive reaction, and the fresh pollen extract  $1:10^7$  produced a weak positive reaction. Thus, there was no evidence of allergenic activity of the tapetal fluid, since the anther sac activity

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\* Undefatted old pollen collected in 1955 was used throughout.

could easily be accounted for by its pollen content. Indeed, the anther extract was somewhat less active than might have been expected from its pollen content alone. Less favorable mechanical conditions for extraction, the rough nature of the calculations, and possible loss of active substance into the ether which had been used in the previous step are possible explanations for the discrepancy. That the latter factor was not of major importance was suggested by the fact that scratch tests with the decanted ether gave reactions similar to those provoked with normal ether. In any event, these observations did not reveal the presence of highly allergenic material in tapetal fluid.

#### 2.3.4 Other Methods to Attempt Separation of Tapetal Fluid from Pollen Grains

Slit anther sacs were pressed onto filter paper which, it was presumed, would absorb tapetal fluid. After drying, the pollen and debris were brushed off and the filter paper extracted for allergenic activity. It was discovered that many pollen grains remained adhered to the filter paper in spite of rather vigorous brushing; consequently, the method was abandoned.

Another approach to the problem was tried as follows: fresh and old pollen grains were extracted with volumes of fluid which could simulate the amount of tapetal fluid in the anther sac. Assuming ragweed pollen grains to be perfect spheres, the ratio  $R$  of the volume of an anther sac full of pollen to the space occupied by the actual pollen grains is given by:

$$R = \frac{n d^3}{1/6 \pi d^3 n} = 1.9$$

where  $n$  is the number of grains in the sac, and  $d$  is the diameter of each grain. The actual count of pollen grains per anther indicates substantially lower numbers than the possible maximum regardless of whether the sac is considered a cylinder or a double cone. With a maximum observed count of 220 grains per anther sac and assuming a double cone shape, it was found that, at most, only 23 per cent ( $220/944$ ) of the anther sac capacity for pollen grains is utilized. Dividing by  $R = 1.9$  gives only 12.2 per cent of the sac volume actually occupied by pollen grains. Presumably the remaining volume is tapetal fluid. To obtain a maximum estimate of tapetal fluid allergenic activity which might result simply from extraction of allergen from the cells into the fluid, fresh and old pollen grains were extracted in a 22 per cent volume ratio in water.



In the same manner, old and fresh pollen were placed separately in similar volumes of water and centrifuged immediately so that the extraction period was only 10 min in the case of old pollen and 15 min for fresh pollen.

A small capillary was placed in each supernate, withdrawn and blown through to rid the capillary of visible fluid. Each capillary was then rubbed in a drop of saline over a prick on a sensitive patient's arm. Impressive reactions occurred in all four instances, suggesting two conclusions:

a. Only minute amounts of pollen extracts are necessary to give impressive skin reactions (just the small amount of fluid remaining on the capillary after blowing through it).

b. Some pollen antigen is extracted by water in a very short time (10 to 15 min). The first conclusion suggests that tapetal fluid might not have been unique in producing the four plus skin reaction noted two years ago, since water in contact with the pollen grains under somewhat similar circumstances can do the same.

Pure tapetal fluid still had not been obtained for study until the Botany Department provided larger flowers called Prairie Docks (Siliphium terebinthinoceum) from the family Compositae, which are closely related antigenically to ragweed. These were found to be rich in tapetal fluid which was easily observed to be sticky and viscous, resembling vasoline. Attempts to aspirate this material through capillaries inserted in anther sacs were unsuccessful. The sacs were then crushed thoroughly on a slide, the debris and pollen scraped lightly off, and finally the remaining partially-dried tapetal fluid was scraped off. Both pollen and tapetal fluid were collected at once and to each was added 0.5 ml. saline.

Scratch tests were done on a ragweed sensitive patient, with both full strength solutions, and four plus reactions occurred. In 1:10 dilutions, a four plus reaction occurred with the pollen extract and a three plus reaction occurred with the tapetal fluid extract.

Using intradermal titration tests, end points of activity were found. On both of two patients, the pollen extract produced positive skin tests in ten-fold higher dilutions than the tapetal fluid extract. Considering the fact that the original extracts were made from 10.1 mg pollen and 5.3 mg dried tapetal fluid, the pollen extracts were approximately five times more active than the tapetal fluid extracts on a weight to volume basis.

If the tapetal fluid extract had been much more active, it might have been considered that tapetal fluid, dried on the surface of the pollen grains, was responsible for the activity of pollen; however, for tapetal fluid to be a major source of pollen allergen, it would have to be many times more active than the pollen extract.

The main conclusion reached from this study is that tapetal fluid is not a major source of pollen allergen. The relatively modest allergenic activity which it possesses might be extracted from pollen grains or might be derived from the tapetum just as other parts of the plant (e.g., stems, seeds, and leaves) are said to possess a certain amount of the ragweed allergen.

#### 2.4 POLLEN RECOVERY FROM RESPIRATORY TISSUES

In 1956 a method of decomposing respiratory tissue and recovering the pollen was developed in the Allergy Research Laboratory of The University of Michigan. It consisted of boiling the tissue in 10% KOH solution and filtering the solution through a millipore filter. The procedure has been described in detail elsewhere [2]. However, it was soon found that over 0.1 gm of tissue would result in such a heavy residue (undissolved tissue debris) that details were obscured. Also, the appearance of many of the pollen grains was altered in such a way as to make identification difficult.

In 1958 an attempt was made to improve the technique. It was found that by lowering the temperature of the KOH solution and increasing the digestion time, the sample size could be increased several fold and the appearance of the pollen was improved. The improved technique is as follows:

1. Place the tissue in 90 ml. of 10% KOH solution at 85C for 1 hr, agitate constantly.
2. Cool flask under running water and centrifuge in special flask for 15 min at 2000 rpm.
3. Carefully pipette off the KOH and suspend in 10% NaCl solution.
4. Centrifuge in same flask for 30 min at 2000 rpm.
5. Carefully pipette off the NaCl solution, resuspend in fresh NaCl solution, and filter through a millipore filter (include the washings).

6. Dry, mount and stain filter.

7. Count pollen.

The constant agitation during the time of digestion greatly aids the digestion. Samples of guinea pig lung as large as 5 gm have been treated in this manner, and the amount of tissue debris in most cases did not seem greatly to hinder the counting of the pollen. However, it does take about 2 hr to count a 47 mm diameter filter pad.

It was found in control tests that the number of pollen grains recovered from normal lung tissue sometimes exceed the number added to the tissue in vitro. A complete check of the technique revealed that the reagents, especially the 10% NaCl solution, was contaminated with ragweed pollen. Subsequently, a reagent control was run with every tissue sample. Under satisfactory conditions, 80 to 100 per cent of pollen grains added in vitro to lung tissue in control experiments could be recovered by the described technique.

The initial application of the technique was in studies in which guinea pigs were exposed in a chamber to high concentrations of ragweed pollen in the air. The results are shown in Table 9.

Human autopsy material was collected during the peak of the 1958 ragweed season and the results are shown in Table 10.

A major problem in using human autopsy material arises from the large amount of carbon contained in many lungs, which made it very difficult to count the pollen on the filter. The sample size had to be decreased to about 2 gm, and still the counting of the filters was very tedious. Random and variable contamination makes much of the data questionable. Many of the controls were very high even after many precautions were taken to prevent contamination. However, it appears that only a small number of pollen, if any, reach the lung and remain there for any length of time. The high concentration of pollen in human lung reported elsewhere [3] was probably due to undetermined sources of contamination.

A second method for determining the amount of pollen in respiratory tissues is being developed. It is similar to the technique used by plant histologists in "clearing" leaves for microscopic study and also similar to the "clearing" of embryos for studying the bone structure.

TABLE 9

Number of Ragweed Pollen Grains Recovered from  
the Lung and Trachea of Guinea Pigs

Number	Time Interval Before Pig Killed (After removal from pollen chamber) (hr)	Ragweed Pollen per Grams Tissue			Grains per 5 gm sample	
		Actual Trachea	Specimen Lung	Count Reagent Control	Trachea	Lung
1	0	11/0.45	20/6.1	5	122	16
2	0	27/0.5	23/3.8	0	270	30
3	1	46/0.7	17/3.6	5	329	24
4	1	7/0.35	3/4.61	1	100	3
5	0	3/0.3	9/2.0	5	50	23
6	0		17/3.76	12		23
7	3.5	1/0.55	5/4.37	0	9	6
8	3.5	2/0.3	10/2.95	3	33	17

The best results so far have been obtained by the following method:

1. A piece of lung or trachea is placed in an aqueous 2% KOH solution and left for one or two weeks.
2. The tissue is then washed in tap water until all traces of the KOH are removed.
3. The tissue is dehydrated with alcohol and finally stored in xylene.

If the KOH solution gets dirty or cloudy it is replaced with fresh solution. The clearing process seems to be speeded up if the solution with tissue is placed in an incubator at 37C. It is difficult to tell when the clearing is complete while the tissue is in an aqueous or alcoholic solution. Sufficiently cleared tissue turns almost transparent when dehydrated and placed in xylene. If the tissue is cloudy when placed in xylene, it may be hydrated and placed back into a KOH solution for further clearing. This process renders the tissue very fragile and jelly-like.

TABLE 10

Number of Ragweed Pollen Grains Recovered from  
Human Lung Tissues during the Ragweed Season

Sample Number	<u>Lung</u>		Reagent Control	<u>Muscle Control</u>	
	Total Count	Grains per gm		Total Count	Grains per gm
1	81	16	8		
	9	2	43	1	2
	23	5	2		
2	112	22	1	60	128
	91	20			
3	51	12	3		
	24	7	9	12	12
	38	7	2		
4	0	0			
	0	0		2	1
	2	1	1		
5	1	0.4			
	4	1.7		1	1.3
	1	0.4	1		
	3	1.3	1		
6	9	3			
	5	2			

Pieces of tissue a half-inch thick have been cleared by this method. With a dissecting microscope small bronchi in the interior of the lung are clearly visible. However, in order to identify ragweed pollen it is necessary to use a magnification greater than 100 times. Unstained ragweed pollen have been injected into cleared tissue and viewed through a stereoscopic microscope with 144 power magnification. Even at this magnification it is very difficult to determine if a small sphere is really a ragweed pollen. Another department at The University of Michigan has ordered a lens system that will give a magnification of 216 power. When this becomes available the tissue and pollen will be studied with it. Perhaps a staining technique will have to be devised to aid in locating pollen in the tissue.

## 2.5 THE EFFECT OF ARTIFICIAL UNIPOLAR AIR IONIZATION ON HYPER-SENSITIVITY PHENOMENA

### 2.5.1 Introduction

It has been postulated that an increase of negative ions over the level normally present in outdoor air may affect, by physical and/or chemical means, microscopic airborne contaminants such as dust, spores, bacteria and pollen. Increased ciliary beating of animal trachea under negative ionization has been observed by others, with an accompanying increased rate at which mucus moves through the trachea under these conditions.

The objective of this work was to study the effects on human pollenosis by alterations in atmospheric variants including air ionization. A preliminary pilot project was initiated to test possible effects of artificially-altered ion concentrations in ambient air on guinea pig anaphylaxis, since this is a well-studied, readily-reproducible phenomenon in the laboratory. If air ions influence this immunologic event in the animal world, human pollenosis may likewise be affected to a significant, measurable degree.

### 2.5.2 Materials and Methods

To measure and maintain significant altered ion densities within the confines of a small space, a wooden chamber was constructed with a glass front so that events within could be constantly observed. Air ions of either positive or negative polarity were passed through ports from Wesix ionizers utilizing a tritium source.

a. Ionizers. Tritium foil and a charged plate are mounted in a small plastic protective holder. The tritium emits beta particles of low energy, which, in turn, create both positive and negative air ions on collision with air molecules. The charged plate serves as a polarizing electrode and is connected to a small rectifier. If the plate charge is negative, positive ions are attracted to it, allowing negative ions to be conveyed out of the ionizer head by following lines of electrostatic force.

b. Ionization Monitor. A Wesix Mark IV Ion Collector was operated at a collection potential of 224 volts, and a Hewlett-Packard Model 425 A microammeter was used to measure the collected ion current. The following equation was used to compute the ion density.

$$N = \frac{I}{q v A}$$

where

N = number of ions per cc of air

I = ion current in amperes

q = charge per ion =  $1.6 \times 10^{-19}$  coul

v = air velocity in collector = 75 cm/sec

A = area of plates =  $49 \text{ cm}^2$

This formula assumes that the material ionized is air and that each ion is only singly ionized.

c. Guinea Pigs. Female guinea pigs weighing 200-275 gm were used in the passive anaphylaxis experiments.

d. Chamber. Guinea pigs were challenged in a wood chamber having dimensions of 24 x 8 x 8 in. The front wall of the chamber was glass, and the heads of 3 ionizers were sealed into the rear wall of the chamber at uniform intervals. The geometry was such that the animals could not possibly be farther than 9.5 in. from one of the ionizers, and the average distance to the nearest ionizer would be four to five inches. One to four guinea pigs were used in the chamber at once. The chamber temperature was found to remain quite constant at about 25C. Aerosolized antigen was introduced at one end of the chamber and an exhaust opening was provided at the opposite end.

e. Antigen. Three times crystallized hens' egg albumin (Armour) was used both for sensitizing animals and for challenging. For the latter purpose a 0.5 per cent solution of egg albumin (Ea) in saline was aerosolized in a DeVilbiss No. 40 nebulizer and introduced into the chamber at the rate of 1.8 ml. per min.

f. Antiserum. On 30 July 1959 five guinea pigs were given three subcutaneous injections of 0.5 ml. of an emulsion of Ea solution (6.25 mg Ea per ml. saline) in equal volume of Freund's complete adjuvant; a single additional injection of 0.5 ml. of this emulsion was given one week later. Bleedings from the heart were commenced 20 August 1959. The pooled serum was found by the standard quantitative precipitation techniques of Heidelberger and Kendall to contain about 480 mg antibody N per ml.

### 2.5.3 Results

a. Physical Measurements. The three ionizers used in the chamber throughout the study were tested individually for ionizing activity. The head of the ionizer was inserted into a circular hole in the side of an additional section of ducting, approximately six inches long, attached to the inlet of the Wesix Ion Collector. The following measurements are not absolute but do have relative validity:

<u>Ionizer</u>	<u>Collector Current</u>	<u>Equivalent Ion Density</u>
1	$170 \times 10^{-12}$ amps	$2.9 \times 10^5$ ions/cc
2	$100 \times 10^{-12}$ amps	$1.7 \times 10^5$ ions/cc
3	$40 \times 10^{-12}$ amps	$6.8 \times 10^4$ ions/cc

For measurement of chamber ion density the Wesix Mark IV Ion Collector inlet was inserted through a matching aperture centrally located in a side wall of the chamber. With test animals in the chamber and the polarizing current turned off, the measured ion density was  $6.8 \times 10^3$  ions per cc. With the polarizing current on, the figure was  $1.7 \times 10^4$  ions per cc, a net increase of approximately  $10^4$  ions per cc in the chamber. It is likely that the test animals were subjected to ionization levels higher than the figures cited above because their average distance to the ionizer heads was shorter than the path length from ionizers to ion collector. The ion collector was disconnected from the chamber during the actual experimental runs since its fan withdraws a substantial amount of ions and aerosol antigen from the chamber.

When egg albumin or histamine solutions were nebulized into the chamber containing animals, and with ionizers functioning, the ion densities attained the following values:

<u>Egg Albumin at</u>	<u>Ion Density</u>
0.18 ml./min	$8.5 \times 10^5$ ions/cc
<u>Histamine at</u>	
0.18 ml./min	$1.5 \times 10^5$ ions/cc

Since these values of ion density were greatly in excess of those measured without nebulization of histamine and egg albumin solutions, a limited series of tests were run to check the results.



It was found that if the rate of nebulization increased, the ion current increased, and if nebulization was stopped, the ion current decreased to the previously determined value. Furthermore, if nebulization was present but the ionizers were off, the measured ion current returned to within about 25 per cent of the background value. These results were repeatable and thus tend to absolve the ion collector of at least gross measurement artifacts caused by the presence of nebulized materials.

b. Biological Experiments. Guinea pigs were passively sensitized by intracardiac injections of varying amounts of guinea pig anti-Ea serum. The following day the animals were challenged by placing them in the chamber and introducing aerosolized Ea antigen. In experiments calling for air ionization, the guinea pigs were put in the chamber and the ionizers turned on for 10 min before the aerosolized Ea was introduced. The animals were observed until death occurred or for a period of 30 min. Most animals showed signs typical of anaphylaxis, and death occurred after 3 to 17 min exposure. When death occurred, its anaphylactic nature was confirmed by finding marked distention of the lungs upon autopsy. Surviving animals could easily be killed by the intravascular injection of Ea. The results of these experiments are summarized in Table 11.

TABLE 11

Results of Tests on Passively Sensitized Guinea Pigs

Pooled Guinea Pig Anti-Ea Serum per kg Recipient Guinea Pig (ml.)	Air Ionization	Number of Animals	Anaphy- lactic Deaths	Deaths per Number Tested
4.0	control	3	3	3/3
3.0	control	2	2	2/2
2.0	control	10	9	9/10
1.4	control	12	9	9/12
1.4	negative	12	8	8/12
1.4	positive	12	8	8/12
1.0	control	5	0	0/5

As is evident, experiments on control animals showed that 2.0 ml. of the anti-Ea serum per kg of recipient guinea pig constituted approximately one lethal dose ( $LD_{100}$ ) under the conditions of these experiments. Working at less than one  $LD_{100}$  dose of antiserum, it is seen that the results in the presence of positive or negative air ionization are essentially the same as the control group.

Some preliminary studies, in which normal guinea pigs were exposed to an aerosol of 0.375 per cent histamine solution, showed no significant differences between the time of putting the animals in the chamber and the time of death among six animals exposed with negative air ionization, six with positive ionization, and five controls. The same ionizers and chamber were used for this work as for the passive anaphylaxis experiments.

Observations also were made on the effect of air ionization on the induction of asthma in a ragweed sensitive dog. Patterson has shown that this dog's asthma immunologically and physiologically closely resembles human bronchial asthma. The threshold concentration of ragweed pollen which would induce asthma in this dog upon a 15 min exposure had been firmly established by repeated exposures at suitable time intervals over a period of several months. Exposures were made in a 24 x 18 x 12 in. chamber with ice under the flooring to maintain the temperature at 25C. Ragweed pollen suspensions of varying concentration were delivered into the chamber through a DeVilbiss No. 40 nebulizer at a rate of 0.18 ml. per min. The dog's respirations were recorded on a kymograph. It was found that the threshold concentration necessary to produce asthma increases if experiments are run on successive days. This rise in threshold was not permanent, however, since resting the dog for seven days results in a lowering of the threshold to the level which produced the attack the previous week. Thus, it was possible to establish a reproducible threshold concentration of pollen which would precipitate asthma by weekly exposures. Two runs were made in which the dog was exposed to negative air ionization (by means of three ionizers in the rear wall of the chamber) for 30 min before and during exposure to the ragweed antigen. In both instances negative air ionization failed to raise the threshold concentration of pollen required to produce asthma, nor did it reduce the severity or duration of the dog's asthma or delay its onset.

#### 2.5.4 Discussion

Although there may be some immunological differences between human atopy and animal anaphylaxis, the latter has been widely used as an experimental model for studying immediate type hypersensitivity reactions. In addition, there is recent evidence indicating physiological similarities between the respiratory disturbances in guinea pig anaphylaxis and human bronchial asthma. Guinea pig anaphylaxis simulates human asthma most closely when the antigen is administered by aerosol, and since the reaction develops more slowly under these conditions, experiments involving the production of anaphylaxis by aerosolized antigen would seem to provide favorable conditions for demonstrating possible effects of air ionization on a hypersensitivity reaction. Since actively sensitized animals are known to exhibit marked variations in their degree of hypersensitivity, passively sensitized animals were selected for the type of quantitative work called for by these experiments. This enabled us to test the effects of air ionization under conditions which are demonstrably minimal for producing anaphylactic death. Although death was regarded as the only reliable endpoint in these experiments, careful records were kept as to the time of appearance of various signs of anaphylaxis, and no differences in the course of sublethal anaphylactic reactions could be discerned in the presence of positive or negative air ions.

Of major concern in the design and execution of these experiments was the possibility that the introduction of aerosolized antigen might in itself produce important quantitative or qualitative changes in the air ions produced by the ion generators. As indicated above, the introduction of Ea aerosol into the chamber with the ionizers running resulted in about a fifty-fold increase in the ion density. The aerosol itself (without the ionizers running) produced only about a 25 per cent increase over the background values. It seems likely that ion production and recombination rates are involved in the increased collection current observed with the ionizers running in the presence of the aerosol. The large, low mobility ions which result in the presence of the aerosol tend to increase the average ion density (perhaps by slower recombination rates).

Although the air ion densities observed in these experiments may appear large, they constitute almost an infinitesimally small portion of the estimated  $2.7 \times 10^{19}$  molecules per cc of air. On the other hand, the ion densities employed in this work are higher than values shown by Krueger and Smith [4] to be minimal for affecting trachea ciliary activity, though there no doubt are qualitative differences in the ions involved in the two studies.

Nevertheless, the present work of course does not exclude the possibility that higher air ion density levels might influence guinea pig anaphylaxis, and even the same or lower ion densities might influence other hypersensitivity phenomena. Very important, too, is the possibility that ionized particles of different sizes and mobilities than those produced in the presence of our aerosolized antigen might have greater biological effects. Longer exposure to ionized air before challenging the sensitized animals also might possibly have yielded different results.

#### 2.5.5 Summary

Uniploar artificial air ionization failed to influence guinea pig anaphylaxis under the conditions described. It also failed to influence the development of asthma in a ragweed sensitive dog.

### 2.6 STUDIES ON REPOSITORY ANTIGEN PREPARATIONS

#### 2.6.1 Retention of Antigen at the Injection Site\*

Although there is great current interest in the clinical use of repository injections of pollen extract, relatively few laboratory data are available on this general subject. The purpose of this work is to study certain phases of repository antigen administration in animals. We have used an isotope method, a modification of a technique used by Talmage and Dixon [5], to compare the relative efficiencies of various repository media.

a. Materials. Male albino rats were fed a standard rat diet with the addition of Lugol's solution to their drinking water to prevent iodine deficiency. The rats were randomly numbered, grouped, and assigned to a particular type of treatment.

Sterile radio-iodinated ( $I^{131}$ ) human serum albumin (RISA) was used as the antigen. The radioactivity of each dose was such as to give about 7000 gamma emission counts per minute. Previous work has shown that dissociation of tracer amounts of  $I^{131}$  from serum proteins is negligible both in vitro and in vivo [6].

Emulsifiers and mineral oils were used in the preparation of emulsions. The emulsifiers used were Falba (Phaltz and Bauer, Inc.)

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\* Published in its entirety in The University of Michigan Medical Bulletin, 26: 138, 1960.

and Arlacel A (Atlas Powder Co.). The mineral oils used were Atreol No. 9 (Atlantic Refining Co.) and Drakeol 6VR (Pennsylvania Refining Co.). The mineral oil-emulsifier mixtures were prepared according to currently used ratios (3 parts Atreol to 2 parts Falba; 9 parts Drakeol to 1 part Arlacel) and sterilized by autoclaving.

Detection of gamma radiation was accomplished by a scintillation detector probe with a 0.75 in. sodium iodide crystal. A 2 cm straight bore collimator was attached to the detector probe.

b. Methods. The following solutions, mixtures and emulsions were prepared for injection:

- Solution 1: 0.5 ml. RISA plus 4.5 ml. isotonic saline
- Solution 2: 0.5 ml. RISA plus 4.5 ml. normal human serum albumin
- Solution 3: 0.5 ml.  $I^{131}$  plus 4.5 ml. isotonic saline
- Mixture 4: 0.5 ml. RISA plus 2.0 ml. isotonic saline plus 2.5 ml. Drakeol-Arlacel mixture. (Materials in this mixture were not emulsified but only mixed.)
- Emulsion 5: 0.5 ml. RISA plus 0.3 ml. isotonic saline plus 4.2 ml. Atreol-Falba mixture
- Emulsion 6: 0.5 ml. RISA plus 2.0 ml. isotonic saline plus 2.5 ml. Atreol-Falba mixture
- Emulsion 7: 0.5 ml. RISA plus 2.0 ml. isotonic saline plus 2.5 ml. Drakeol-Arlacel mixture
- Emulsion 8: 0.5 ml. RISA plus 0.3 ml. isotonic saline plus 4.2 ml. Drakeol-Arlacel mixture
- Emulsion 9: 0.5 ml.  $I^{131}$  plus 2.0 ml. isotonic saline plus 2.5 ml. Drakeol-Arlacel mixture.

Water-in-oil emulsions were prepared using the technique described by Mitchell [7] in which two 10 ml. Luer-Lok syringes are attached to a Tomac three-way stopcock. One syringe was loaded with the aqueous phase and the other with the oil-emulsifier phase. Emulsification was obtained by stoking the contents of one syringe through the stopcock into the other and back again over a two min period. The completeness of the emulsification was checked by gross and microscopic examination, water drop testing and centrifugation.

The rats were randomly assigned to groups of three, and one of the nine solutions, mixtures or emulsions were given to the members

of each group. A total of nine groups (27 rats) were injected. The right hip served as the injection site, and the area was delineated with indelible ink. Using the scintillation detector probe at a constant distance from the injection site, one-min counts were obtained immediately after injection and at varying time intervals thereafter. Background counts were also performed, and after correction of radioactive decay, it was assumed that the corrected count at the injection site represented the relative amount of antigen remaining at the injection site.

c. Results. Measurements of radioactivity retained at the injection site which reflect the amount of retained antigen are shown in Table 12 and Figure 6. Three distinct categories may be identified: (i) the group injected with  $I^{131}$  in saline shows a very rapid disappearance of the radioactive tracer with a local site half-life of less than 15 min; (ii) the groups injected with RISA in saline or human serum albumin, and RISA in unemulsified combination with Drakeol-Arlacel show a moderately rapid disappearance of antigen with the local site half-life less than 12 hr; (iii) the groups given emulsified antigen injections have a very slow rate of local antigen disappearance with local site half-life varying from more than one to more than 22 days.

d. Discussion. In general our results confirm those of Talmage and Dixon [5] who used radio-iodinated bovine gamma globulin emulsified with incomplete Freund's adjuvant and injected it into rabbits. Our use of different adjuvant media has shown the superior total retentive powers of currently used repository preparations, although our semi-logarithmic plots show antigen regression curves similarly shaped to those of Talmage and Dixon in that antigen is released relatively rapidly from the site of emulsion injection during the first week and thereafter the release occurs at a slower linear logarithmic rate. Extrapolation of data shown in Figure 7 suggests the time interval to regress to 1 per cent of the initial injection of antigen is from 69 to 164 days, varying with the type of emulsion used. Under the conditions of the experiment, our results show that the Drakeol-Arlacel preparation promotes better retention of antigen than does Atreol-Falba.

Clinical inference from these studies should be based on the observation of increased rate of local site antigen release from an emulsified injection using a small molecular weight antigen. Since the very active ragweed antigen fractions probably lie intermediate in molecular weight (in the range of 5000) between  $I^{131}$  and RISA, one may expect the injection site half-life of ragweed antigen in Drakeol-Arlacel emulsion to be on the order of 5 to 10 days.

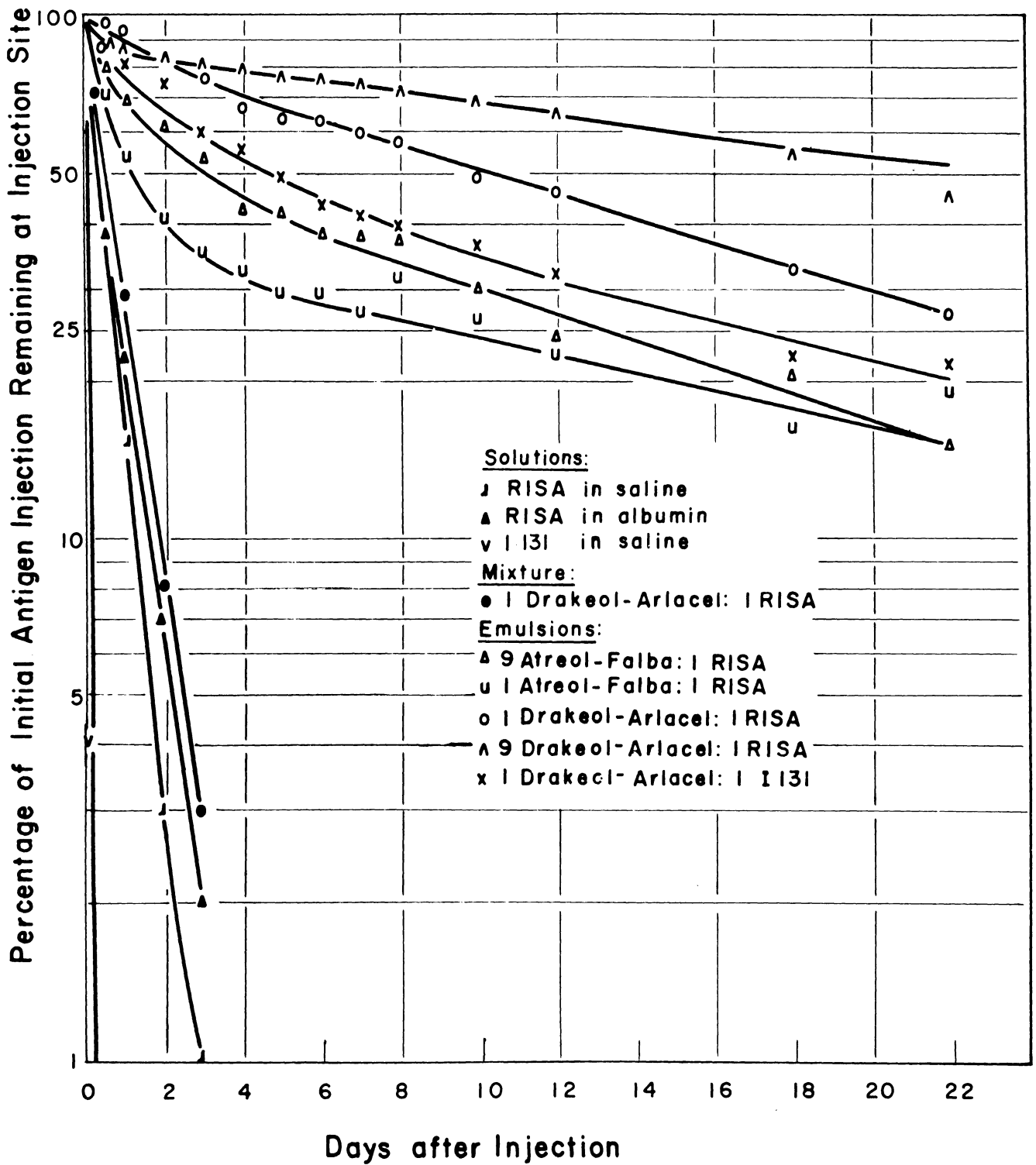


Fig. 6. A comparison of the abilities of various preparations to retain antigen at the site of subcutaneous injection as determined by residual radio-activity measurement. The curves are constructed from the means of the experimental results.

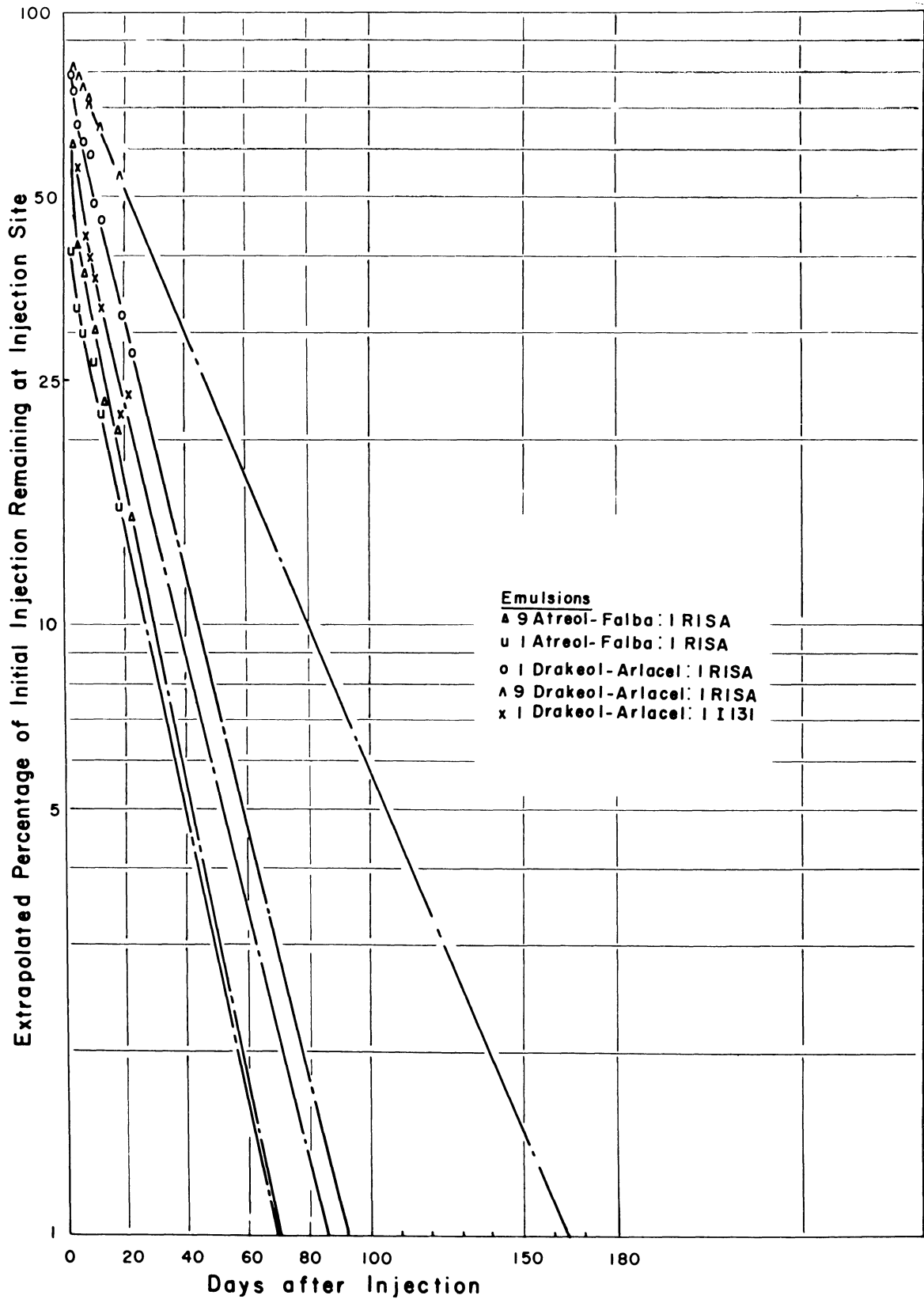


Fig. 7. Extrapolation of experimental results to infer the total time required to reduce the amount of antigen injected in various repository preparations to 1 per cent at the site of injection.



TABLE 12

Comparison of Various Preparations by Antigen Retained at the Site of Injection

Time After Subcutaneous Injection	Mean % of Initial Antigen Injection Remaining at Injection Site									
	Prepa- ration:*	1	2	3	4	5	6	7	8	9
0		100	100	100	100	100	100	100	100	100
1 hour		84	85	4	-	85	74	100	88	96
4 hours		67	71	-	-	83	74	96	80	91
12 hours		38	-	-	-	80	71	98	84	88
1 day		15	22	0	29	68	53	95	86	81
2 days		3	7		8	62	41	80	81	75
3 days		1	2		3	54	36	76	82	61
4 days		0	0		0	42	33	66	79	56
5 days						42	29	63	78	50
6 days						38	30	62	77	43
7 days						39	27	60	74	41
8 days						38	36	59	73	40
10 days						30	27	48	70	37
12 days						23	22	46	65	33
18 days						21	16	32	54	22
22 days						15	21	28	45	24

\* The preparations are as follows: (1) 0.5 ml. RISA, 4.5 ml. saline; (2) 0.5 ml. RISA, 4.5 ml. human serum albumin; (3) 0.5 ml. I<sup>131</sup>, 4.5 ml. saline; (4) 0.5 ml. RISA, 2.0 ml. saline, 2.5 ml. Drakeol-Arlacel - not emulsified; (5) emulsion of 0.5 ml. RISA, 0.3 ml. saline, 4.2 ml. Atreol-Falba; (6) emulsion of 0.5 ml. RISA, 2.0 ml. saline, 2.5 ml. Atreol-Falba; (7) emulsion of 0.5 ml. RISA, 2.0 ml. saline, 2.5 ml. Drakeol-Arlacel; (8) emulsion of 0.5 ml. RISA, 0.3 ml. saline, 4.2 ml. Drakeol-Arlacel; (9) emulsion of 0.5 ml. I<sup>131</sup>, 2.0 ml. saline, 2.5 ml. Drakeol-Arlacel.

e. Summary. In rats, systemic adsorption of subcutaneously injected human serum albumin in various water-in-oil emulsions is relatively rapid during the first 24 hr following injection but approximates a much slower linear logarithmic rate by the fifth day following injection.

From the data presented, there is suggestive evidence that Drakeol-Arlacel preparations are superior to Atreol-Falba preparations in promoting retention of antigen at the site of injection.

#### 2.6.2 Isotopic and Histologic Demonstration of the Repository Effect of Vegetable Oil - Whole Ragweed Pollen Suspensions Injected into Rats

The purpose of this experiment was to evaluate Pro-Sorb base (a vegetable oil mixture supplied by Barry Laboratories, Inc.) by the isotopic method used in the study described above and by histologic study of the injected site at various time intervals.

a. Materials. Eleven male albino guinea pigs were prepared in a manner identical to that described in the previous study. Ragweed pollen grains were tagged with radioactive chromium ( $\text{Cr}^{51}$ ) by the addition of an aqueous solution of  $\text{Cr}^{51}$  to pollen grains, incubating with agitation for 24 hr, filtering of pollen grains and washing with water until no more  $\text{Cr}^{51}$  was found in the washings. The tagged pollen grains were then suspended in the Pro-Sorb base.

b. Methods. A scintillation detector probe identical to that described above was employed for counting of gamma radiation. No controls were available for the isotopic portion of the experiment; untagged pollen grains in aqueous solutions and in a comparable concentration to that of the ragweed - Pro-Sorb suspension were used as histological controls.

The Pro-Sorb-ragweed suspension, being a solid at room temperature, was liquefied by warming in a water bath. It was then injected intramuscularly into the right flanks of the guinea pigs. Aqueous ragweed pollen was injected similarly into control animals. The sites of injections were marked and counts performed as in the previous study at varying time intervals over a seven day period. The animals were sacrificed at intervals of one, two, three, seven and ten weeks. The sections of skin, subcutaneous tissue and abdominal muscular wall were prepared for microscopic study in the usual way.

c. Results. There was excellent retention of pollen at the injection site as determined by radioactive counting in all six of the animals that received the Pro-Sorb Cr<sup>51</sup>-tagged ragweed pollen suspension. As above, there was an initial period of relatively rapid loss of local radioactivity during the first 24 hr followed by a much slower logarithmic phase. One cannot attempt to compare the repository effects of the Pro-Sorb base against the mineral-oil-emulsifier mixtures because of differences in animals, antigen and radioactive elements used in the two experiments; nevertheless, the new vegetable base (Pro-Sorb) seems promising at this time as an agent that might find extensive clinical usage whenever repository effects are desired.

The histological studies are not complete at the time of this printing and definite conclusions are not as yet appropriate.

## 2.7 THE EFFECT OF pH ON SKIN-SENSITIZING ANTIBODY

### 2.7.1 Introduction

A review of the literature revealed no study directed specifically at the effect of pH variation on skin-sensitizing antibody or other antibodies. There were several references containing data in some way related to the present subject, but only the more pertinent ones will be cited here.

In this laboratory, Patterson, Correa, and Mathews [8] altered ragweed antigen in various ways in an attempt to demonstrate haptenic activity in unmodified or modified ragweed extract. During the acid-hydrolysis study, it was noted that exposure of reagin to pH 2.0 for 1 hr neutralized or inactivated the reagin completely, in that it would no longer sensitize a passive transfer site. Also in this laboratory, peptic digestion in an acid buffer of globulin solutions containing skin-sensitizing antibody effected rapid inactivation of skin-sensitizing activity. It was noted, however, that even in the absence of pepsin, there was inactivation of skin-sensitizing activity within a few minutes at a pH as high as 4.7. By comparison, Rosenheim [9] had studied in 1937 the effect of enzymatic digestion on typhoid O and H agglutinins from horses; H agglutinin was resistant to peptic digestion at pH 4.7.

## 2.7.2 Materials and Method

a. Skin-Sensitizing Antibody. Blood specimens were obtained from two patients with good histories of ragweed hay fever and 4+ prick tests to ragweed. Using appropriate proportions of 0.1N NaCl, 0.1N HCl, and 0.1N NaOH (to maintain ionic strength of 0.1), a sample of the ragweed antiserum was brought to approximately pH 2.0; another sample to pH 3; another to pH 4, etc., through pH 12.6. Each sample was allowed to stand at its proper pH for 24 hr at 4C. Then each sample was neutralized by addition of 0.1N HCl or 0.1N NaOH. Serial two-fold dilutions of each ragweed antiserum sample were made (except that the initial dilution was 1:30, and the next dilution 1:50). Each dilution was implanted on the back of a passive transfer subject, and each site was challenged 24 hr later with 1:500 ragweed extract. Testing with ragweed extract on unaltered skin of the passive transfer subject and on a site implanted with the diluent were done only as controls.

b. Tetanus-Toxoid Antibody. Blood specimens were obtained from two subjects hyperimmunized with tetanus toxoid. In the same manner as described for skin-sensitizing antibody, a sample of the serum was brought to pH 2, another to pH 3, etc. through pH 12.6. Each sample was allowed to stand at its proper pH for 24 hr at 4C; then each sample was neutralized as described above. Serial two-fold dilutions of each antiserum sample were made starting at a dilution of 1:40. The antibody titer was determined by the tanned red-cell hemagglutination technique, using a modification of the method described by Boyden [10]. Serum from a subject not immunized with tetanus toxoid served as one control. Tanned RBC's not treated with tetanus toxoid also served as a control; anti-serum which had previously been incubated with tetanus toxoid to neutralize it served as another control; and lastly, sensitized, tanned RBC's added to the normal rabbit-serum diluent served as an additional control.

c. Typhoid Agglutination. In the same manner as previously described, antiserum samples were brought to appropriate pH, allowed to stand 24 hr at 4C, and then neutralized. Serial two-fold dilutions were made, starting at 1:40, and the standard typhoid-agglutination test was performed, using H antigen. Tubes containing only physiological saline and typhoid H antigen served as controls.

## 2.7.3 Results

Each control in the skin-sensitizing antibody system, tetanus-toxoid system, and typhoid-agglutinin system was negative. The results are summarized in Table 13.

TABLE 13

Highest Dilution at Each pH Which Gave a 1+ Reaction (except for tetanus toxoid antibody, where 2+ endpoint was used)

pH	Skin-Sensitizing Antibody H.A. Serum	Skin-Sensitizing Antibody M.A. Serum	Tetanus-Toxoid Antibody P.S. Serum	Tetanus-Toxoid Antibody A.B. Serum	Typhoid Agglutinin
1				none	none
2	none	none	1:320	1:160	1:80
3	none	none	1:320	1:320	
4	1:50	1:30	1:1280	1:5120	1:160
5	1:200	1:50	1:1280		
6	1:400	1:30	1:2560		
7	1:400	1:100	1:2560		1:320
8	1:400	1:50	1:10240	1:10240	
9	1:200	1:30	1:5120		1:320
10	1:100	none	1:5120		
11	1:50	none	1:5120	1:5120	1:80
12	none	none	1:40	1:320	1:40
12.6	none	none	none	none	none

a. Skin-Sensitizing Antibody. The serum of patient M. A. did not give high enough anti-ragweed titer to allow adequate interpretation of the results. However, the general appearance of the resulting curve was similar to that of the second patient, H. A. The samples of H. A. serum which had been exposed to pH 2, 3, 12, and 12.6 showed no reaction on challenge with 1:500 ragweed even at the lowest dilution of 1:30. As would be expected, the samples exposed to the more physiologic pH values of 6, 7, and 8 showed positive reactions at the highest dilutions (1:400 in each instance). The endpoint was reached at lesser serum dilutions at pH on either side of these three mid-zone pH values.

b. Tetanus-Toxoid Antibody. It is readily apparent that the tetanus-toxoid antibody titer, determined by tanned RBC-hemagglutination method, was much higher than the skin-sensitizing antibody titer. In general the pattern was quite similar in that the highest titers were in the mid-zone pH range with the titer decreasing as one approached either extreme of pH range. Only at pH 12.6 was no antibody activity demonstrable. Serum from subject A. B. was exposed to pH 1, and a doubtful reaction was obtained in the first two dilutions. Serum from the two subjects immunized with tetanus-toxoid in general gave very similar results.

c. Typhoid Agglutinin. A high antibody titer was not obtained by the immunization procedure. However, again positive tests were obtained in the highest dilutions at pH 7.4, with the titer decreasing toward extreme pH values. Again, no antibody activity was demonstrable at pH 12.6 and only doubtful readings at pH 1.

#### 2.7.4 Conclusions

In this study, all three antibodies demonstrated the best titers in the mid-zone pH range. There appears to be progressive interference with antibody activity (at least by the methods employed here) as the pH is varied in either direction from the mid-zone. There does not appear to be a specific point on the pH scale where antibody activity is suddenly and dramatically reduced. However, it is interesting that there was no activity demonstrable after exposure to pH 12.6. Kabat [11] in 1939, reported that horse-antipneumococcus antibody activity was destroyed and complete breakdown of the antibody molecule occurred at pH 12.4.

It would appear that skin-sensitizing antibody may well be more markedly affected by pH values at either extreme than are the other two antibodies studied, but the differences in antibody titers and techniques of measuring them make this somewhat uncertain.

## 2.8 STUDIES ON THE ADSORPTION OF RAGWEED ANTIGEN TO GLASS

### 2.8.1 Introduction

Recently there have been reports that various dilute protein solutions, such as tuberculin-purified protein derivative [12, 13] enzymes and viruses [14] show a "volume effect" or a greater inactivation or loss of potency when stored in a partially-filled vial than when stored in a completely-filled vial.

Hjorth [15] using a mixed-grass pollen extract to study this phenomenon, found as much as a ten-fold decrease in potency in dilutions not uncommonly used in clinical allergy or in experimental immunology, and he advised adding 0.01 per cent Tween-80 to allergen extracts to minimize this possible source of error.

### 2.8.2 Method

Since Tween-80 interferes with the action of such antibacterials as phenol and phenylmercuriacetate, since it conceivably may be a sensitizing substance, and since there is confusion in the literature as to whether or not this loss of potency is primarily dependent upon adsorption of protein to glass or denaturation of the protein at the air surface or the air-glass-liquid interface, the following studies were undertaken: Fifteen per cent aqueous extracts of dwarf ragweed pollen (Ambrosia elatior) and timothy pollen (Phleum pratense) were made using a non-phenolated Coca's solution. The former extract contained 0.56 mg phosphotungstic acid precipitable N per ml. and the latter 1.25 mg PTA N per ml. These extracts were diluted to 1:50 and to 1:5000 concentrations using sterile normal saline. They then were stored (see Table 14) in varying volumes in 25 ml. Ehrlenmeyer flasks, 50 ml. cylindrical multidose vials, and the latter vials filled with glass "bump" beads. The glassware was chosen to cause marked differences in the relationship between the amount of antigen and glass or air contact or air-glass interfaces. The 50 ml. multidose vials were cleansed by immersing them in concentrated nitric acid for 24 to 48 hr followed by several washings with distilled water, and the glass "bump" beads were similarly cleansed but were kept in the nitric acid for over ten days.

After the antigen extracts had thus been stored at 4C for three or more days, multiple ten-fold dilutions using sterile normal saline were made and tested in patients giving a known positive skin test to ragweed or timothy. In the case of the 1:50 dilution, this was done by prick tests on the back; for the 1:5000 dilution, intracutaneous tests on the volar surface of the forearms were used, employing glass tuberculin syringes cleansed with concentrated nitric acid for 24 to 48 hr, washed in tap water and distilled water, and then dry heat sterilized at 200C for 2 hr. The test solutions were coded to prevent bias, and ulnar versus radial placement of the two extracts being compared were alternated. Results were analyzed by the presence or absence of a wheal, the data being transposed to a log-probit graph paper as in Becker's [16] method of evaluating the relative potency of antigenic solutions. (See Figure 8 for example.) A number of the results were also checked by measuring the amount of histamine the antigen would release from the blood of humans sensitive to that antigen. (See Figure 9 for example.)

TABLE 14

## Summary of Results on Adsorption of Ragweed Antigen to Glass

Storage Conditions of Solutions Compared		Number of Subjects Skin Tested	Ratio of Potency by Skin Testing	Ratio of Potency by Histamine Release
1.	50 ml. ragweed 1:50 vs (a) 5 ml. in 50 ml. vials (b) 5 ml. in contact with rubber stopper	11 11	$\frac{1}{1}$ $\frac{1}{1}$	$\frac{1}{1}$ $\frac{1}{1}$
2.	50 ml. timothy 1:5000 vs 5 ml. in 50 ml. vials	13	$\frac{2}{1}$	Not Done
3.	50 ml. ragweed 1:5000 vs 5 ml. in 50 ml. vials	17	$\frac{3}{1}$	$\frac{2}{1}$
4.	25 ml. ragweed 1:5000 vs 2.5 ml. in 25 ml. Ehrlemeyer flasks *	12	$\frac{5}{1}$	Not Done
5.	5 ml. ragweed 1:5000 vs 15 ml. plus 35 ml. of glass beads in 50 ml. vials †	16	$\frac{1}{1}$	$\frac{1}{1}$
6.	50 ml. ragweed 1:5000 ‡ vs (a) 5 ml. ragweed 1:5000 ‡ (b) 5 ml. (in new 50 ml. vial - not acid cleansed)	9 12	$\frac{3}{1}$ $\frac{3}{1}$	Not Done

\* Flasks not cleansed with acid since flasks not previously used.

† Glass beads cleansed in acid for over ten days time.

‡ Allergy extracts stored in 50 ml. vials that had been cleansed in acid four days.

‡ Wheals from the vial containing 50 ml. of ragweed averaged 12.9 x 9.9 mm vs 11.9 x 9.3 mm for the vial containing 5 ml.



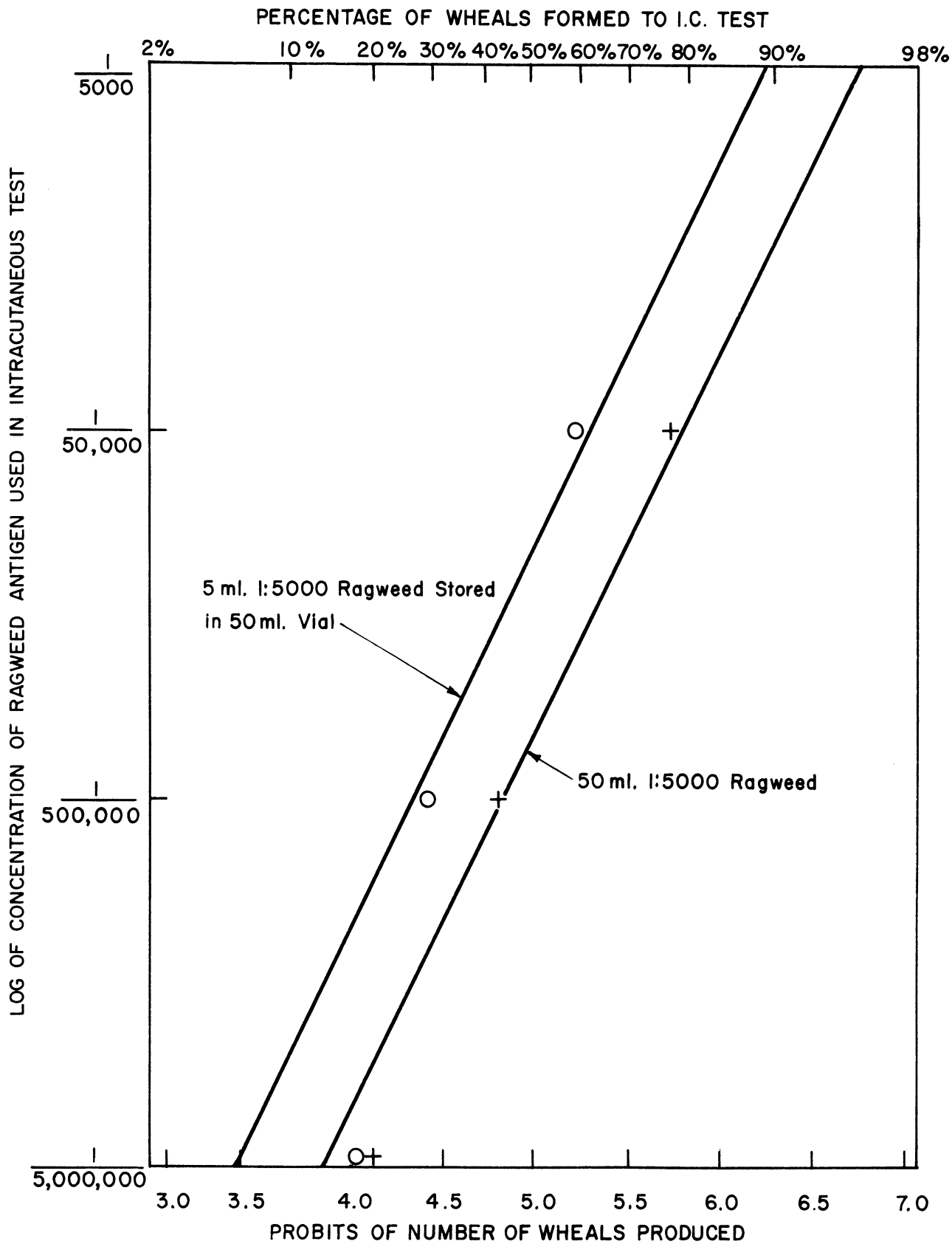


Fig. 8. Approximate three-times greater strength of 1:5000 ragweed extract after storing in full vial versus one-tenth full vial, as measured by skin testing.

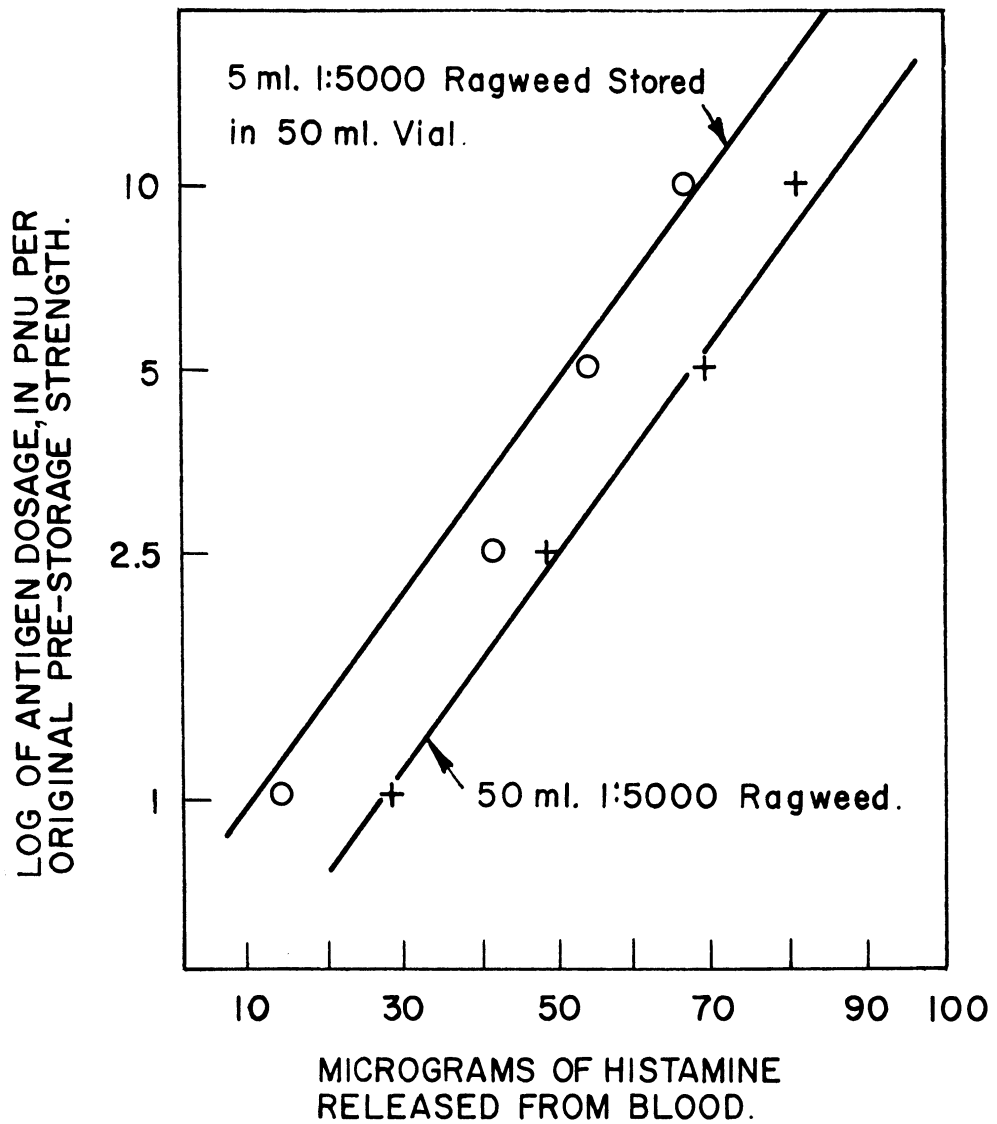


Fig. 9. Approximate two-times greater strength of 1:5000 ragweed extract stored in full vial versus one-tenth full vial, as measured by histamine release.

### 2.8.3 Results

The data accumulated to date (Table 14) confirms the fact that a volume effect takes place and that it occurs with dilute solutions of ragweed pollen extract, as well as with other dilute protein solutions. At a 1:50 dilution of ragweed antigen, the effect is not measurable by the technique used, but this is consistent with the findings of others [12, 13, 15] that the volume effect increases as the protein concentration decreases. The experiment with the antigen stored in contact with the rubber stopper of the vial was undertaken since earlier trial runs had suggested this might affect the potency of the stored antigen. However, this suggestion was not confirmed.

The ten-fold difference in potency found by Hjorth was not confirmed in these initial experiments, and no correlation could be discerned between the experimental results and the antigen-glass-air geometric relationships in the different containers. It was thought, however, that this failure might be dependent upon a decreased-volume effect produced by cleansing the glass containers in concentrated nitric acid for prolonged periods of time. In using antigen-coated glass as a specific adsorbent of antibody, Sutherland and Campbell [17] found that after several treatments of their glassware with boiling 50 per cent concentrated sulfuric acid, the glass ceased to adsorb antigen. This was completely rectified by treating the glass for 1 hr in 0.1 M  $\text{CrCl}_3$  at a pH of 3.5.

It is planned to investigate this phenomenon further by storing 50 and 5 ml. volumes of 1:5000 ragweed antigen in 50 ml. multidose vials, some of which will be acid-cleansed for four days, some acid-cleansed and treated with chromic chloride, and other vials not subjected to acid cleaning. The potency of these solutions will then be compared simultaneously in a larger group of subjects by the presence or absence of a wheal (as in Becker's method), by wheal size, and by histamine release. The results to date (Table 14) have been consistent with the hypothesis that the volume effect may depend, at least in part, upon the adsorption of protein molecules by metallic ions in the glass. If this is the case, uniform methods of cleaning the glass must be used before one could expect to correlate experimental results and geometric factors. The possibility of markedly depressing the "volume effect" by thorough acid washing of glassware has important practical implications.

## 2.9 STUDIES ON THE POSSIBLE INHIBITORY EFFECT OF HUMAN GAMMA GLOBULINS OR OF HUMAN SERUM ON PASSIVE TRANSFER TESTS FOR SKIN-SENSITIZING ANTIBODY

### 2.9.1 Introduction

In 1915 Lewis [18] showed that normal sera have an inhibitory effect on passive anaphylaxis in the guinea pig. Ovary and Bier [19] reported in 1953 that normal rabbit sera used as a diluent for rabbit antisera have an inhibitory effect on passive cutaneous anaphylaxis (PCA) in the guinea pig. Cooke and Fisher [20] subsequently confirmed the results of Ovary and Bier and also showed that normal euglobulin, when used in the same concentration as in the whole serum, inhibits the PCA reaction as much as whole serum. Albumin was found to have no activity in this respect. In 1959, Biozzi, Halpern and Binaghi [21] showed that this competitive effect of gamma globulin was a direct function of the concentration used. In 1959 also, Ishisaka and Campbell [22] were able to demonstrate competitive inhibition by gamma globulin of the in vivo blueing reaction produced by injecting suitable soluble antigen-antibody complexes into the skin of normal animals injected intravenously with a blue dye. The purpose of the research reported below was to find out if the same competitive action of whole serum or normal gamma globulin found with animal antisera could be demonstrated in man by using a ragweed-antiragweed reaginic system.

### 2.9.2 Materials

a. Sera from ragweed sensitive subjects. Titrations of three different sera were made by passive transfer tests employing progressive dilutions of each serum in buffered saline. These sera were obtained from untreated patients proven sensitive to ragweed by a positive history and positive prick test. The serum which gave the highest titer was the one used exclusively afterwards.

b. Gamma globulin. Gamma globulin was isolated from a normal human serum by Cohn's fractionation method and was lyophilized. A 1.17 per cent solution of this gamma globulin was made with buffered saline. Of this solution, 0.05 ml. was injected into the skin of a normal subject (not sensitive to ragweed by skin test), and the site of injection was challenged with a ragweed extract 24 hr later. No reaction was observed. However, three gamma globulin preparations obtained from commercial sources were tested with this technique and all gave positive reactions, thus indicating that they contained skin-sensitizing antibody (atopic reagin) to ragweed antigens.

This incidental finding was rather interesting, raising the possibility of using gamma globulins obtained from commercial sources as a source of reagins in passive transfer tests. This is a particularly attractive possibility, since it is said that one cannot transmit serum hepatitis with human gamma globulin extracted by Cohn's fractionation method. Two-fold dilutions of a concentrated solution (16.5 per cent) of gamma globulin obtained from a commercial source (Lederle poliomyelitis immune globulin lot No. 2175-344C) were made with buffered saline (pH 8). An injection of 0.05 ml. of each dilution was made at different sites into the skin of a recipient who was not sensitive to ragweed or grasses by history or skin tests. These sites were challenged 24 hr later with a 1:500 solution of short ragweed defatted pollen extract or with a 1:500 solution of June grass defatted pollen extract. The reactions are shown in Table 15.

TABLE 15

Results of Challenge by Pollen Extracts to Sites Injected with Various Dilutions of Gamma Globulin

Dilution	Antigens Used	
	Short Ragweed	June Grass
1:1 (16.5%)	3+	1+
1:10	2+	--
1:20	--	--
1:640	--	--
Control	--	--

A dilution of 1:10 represents the upper limit of the normal concentration of gamma globulins in normal serum. This means that although the concentrated gamma globulin obtained from commercial sources contains a small amount of reagin, they are insufficient to be of great practical usefulness. It also is probable that this Cohn fraction does not contain all the reagins [23].

One lot of commercial gamma globulin was also tested by the same technique with mixed grasses extract as antigen and gave positive results.

c. Whole human-serum globulins. Protein from a normal human was precipitated at 60 per cent saturation with ammonium

sulfate. The precipitate was redissolved in buffered saline, dialyzed for 48 hr against hypotonic saline, concentrated to one-third the original serum volume by evaporation in Visking tubing, and sterilized by Swinny filtration. The final pH was 6.8 to 7.0.

d. Normal human serum. This serum was obtained from a subject who was not ragweed sensitive by history or skin test.

e. Buffered saline. The buffered saline used as the control serum diluent was a phosphate buffered saline with 0.4 per cent Phenol, pH 8.

f. Ragweed antigen. The material used was a 1:500 extract of 80 per cent short- and 20 per cent giant-ragweed pollen in buffered saline.

g. Recipients. All the recipients used to perform the passive transfer tests of sensitivity to ragweed pollen had shown a negative history, negative prick tests and negative intracutaneous skin tests to ragweed pollen extract.

### 2.9.3 Methods

Two-fold dilutions of the ragweed-sensitive serum were made using two or three different diluents. In the first group of dilutions, buffered saline was used. In the second group, a 1.17 per cent solution of human gamma globulin in buffered saline was used at first. Also used in two cases were the three-times concentrated whole human globulin solution obtained as described above. In the third group, normal human serum was used as a diluent. Of each dilution of each group, 0.05 ml. was injected intracutaneously in the skin of a recipient. Each of these injected sites was challenged with 0.01 ml. of 1:500 ragweed antigen solution 24 or 36 hr later. The dilution giving the last detectable but definitely positive reaction was considered to be the end-point titer. To be able to compare the results obtained in different recipients, a ratio was established which is called the competitive ratio. This ratio is the reciprocal of the end-point titer in saline divided by the reciprocal of the end-point titer in serum or gamma globulin solution. If there is no competitive effect, this ratio is 1; if there is a competitive effect, this ratio is greater than 1.

#### 2.9.4 Results Obtained

The results of a typical experiment are presented in Table 16. In this case (recipient No. 3), the end-point titers were 1:640 in buffered saline, 1:640 in gamma globulin, and 1:640 in human serum. These give a competitive ratio of  $\frac{640 \text{ (buffered saline)}}{640 \text{ (gamma globulin)}} = 1$ . Buffered saline and human serum also give the same ratio.

TABLE 16

Sites Injected with the Indicated Dilutions of Reaginic Serum and Challenged 24 hr Later with Ragweed Extract  
(Reactions obtained from recipient No. 3)

Serum Dilution	<u>Diluents Used</u>		
	Buffered Saline	Gamma Globulins	Normal Human Serum
1:10	4+	4+	4+
1:20	4+	4+	4+
1:40	4+	4+	4+
1:80	4+	3+	4+
1:160	3+	2+	3+
1:320	2+	2+	3+
1:640	1+	1+	1+
1:1280	--	--	--
Control*	--	--	--

\* As a control the diluent used was injected into the skin and the site challenged 24 or 36 hr later with 1:500 solution of ragweed pollen extract.

Table 17 summarizes the results. Except in one case where a ratio of 3 was found between buffered saline and gamma globulin, all recipients showed a ratio of 1 or 2. A ratio of 2 is considered insignificant since it is within the limits of error of the technique used.

TABLE 17

Competitive Ratios for Various Solutions Injected  
Intracutaneously in Several Recipients

Recipient	<u>Ratios Computed</u>		
	<u>Buff. Sal.</u> Human Serum	<u>Buff. Sal.</u> Gamma Globulin	<u>Buff. Sal.</u> 3X Conc. Whole Globulin
1	2	2	
2	2	2	
3	1	1	
4	1	1	
5	1	3	
6			1
7			2

## 2.9.5 Discussion

The conditions under which normal serum or gamma globulin inhibit passive anaphylaxis, passive cutaneous anaphylaxis or blueing reactions by soluble antigen-antibody complexes suggest that they compete with antibody globulin for receptor sites of attachment to the skin or other tissues. The failure of normal serum or gamma globulin to inhibit passive transfer tests in the present study implies that human skin-sensitizing antibody (SSA; atopic reagin) becomes fixed to skin by a mechanism which differs from that binding normal serum proteins and other types of antibodies to skin. This implication is supported by the fact that sites of serum injections remain reactive much longer in the Prausnitz-Kustner test than in PCA; furthermore, SSA remains localized in skin longer than post-treatment blocking antibody. Although the question of whether all SSA is gamma globulin is still being debated, the fact that whole-serum and concentrated whole-serum globulin as well as gamma globulin failed to inhibit passive transfer tests indicates that the results cannot be explained simply on the basis that all SSA may not be gamma globulin. The nature of the binding of SSA by skin remains unknown, but the process appears to be rather unique to SSA among the serum globulins and constitutes an important characteristic of SSA.



## 2.9.6 Conclusions

Dilution of human serum containing skin-sensitizing antibody with human gamma globulin, human whole-serum globulins, or normal human serum failed to depress passive transfer titers as compared to tests done with atopic serum diluted with saline. A relatively unique mechanism of fixation of skin-sensitizing antibody to skin is implied. Commercial human gamma globulin preparations often contain small amounts of skin-sensitizing antibody to ragweed.

## 2.10] THE PREVALENCE OF RAGWEED AND GRASS POLLINOSIS IN SEVERAL GROUPS OF UNIVERSITY OF MICHIGAN STUDENTS WITH A CRITICAL ANALYSIS OF THE HEREDITARY ASPECTS OF ALLERGIC DISORDERS

### 2.10.1 Introduction

Numbers of investigators have attempted to define the genetics of atopy.\* Isolated family pedigrees, twin studies, and multiple case studies have been employed for this purpose. There is fairly general agreement that the former type of data is unsuitable for evaluating the heredity of diseases of such common occurrence as atopic disorders, and conflicting conclusions have been reached from studies of the latter two types. These discrepancies in part may be explained by variable definitions of atopy or the allergic diathesis. In addition, we have reason to believe that the development of clinically overt atopic disease is conditioned by exposure to potent environmental allergens as well as by hereditary factors. If this is true, past and continuing attempts to define the genetics of atopy purely by the multiple case study approach are doomed to failure. This work serves to document this contention by surveying the incidence of some clearly defined atopic diseases occurring among foreign and native students in an area where there is an abundance of a potent allergen (e.g., ragweed) not present elsewhere in the world. It is shown that there is a highly significant difference in the familial history of atopy between these two groups of patients suffering from the same disease.

The study also serves incidentally to provide a considerable amount of data about the incidence of atopy in an area having

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\* A complete bibliography and critical review of the papers in this field is included in Publication No. 20 on Atmospheric Pollution by Aeroallergens. See list of publications in Appendix 2.

relatively large amounts of tree, grass, and weed pollens and fungus spores in the air. The validity of these data is limited by the lack of a purely objective and reliable diagnostic test for atopy, but the method used for collecting the data and the limited definition of atopy employed in the study should provide as reliable and conservative an estimate of the incidence of these diseases as can be obtained in mass surveys at the present time.

#### 2.10.2 Methods

In 1960, 985 students from 82 foreign countries were attending classes at The University of Michigan. Selection of 344 foreign students for participation in this study was made with the only prerequisite being that a good command of the English language was necessary. A control group of 324 students of comparable age and sex distribution, but native to the United States, was used for comparison. Two other groups of native United States students were used as further controls: 170 allergic patients currently being treated in the Allergy Unit of the Student Health Service, and 200 University of Michigan medical students and nursing students in the second year of their training.

Except for the allergic patients, these groups were all approached in small groups of 10 to 20 students. The students completed an eight-page questionnaire and were urged to ask questions regarding any facet of it or diseases listed therein. Students unable to give positive information did not take part in the experiment. The survey form asked for information regarding the students' racial ancestry, age, time of arrival in the United States and Michigan, time of onset of allergic symptoms, type of symptoms, seasonal occurrence of symptoms, complications, past treatment, and results of treatment. Information on the family history of allergy included the maternal and paternal grandparents, the parents, uncles, aunts, cousins, and siblings, but in the analysis of the data given below only those students whose parents or siblings had definite allergic symptoms were considered to have a positive family history.

Specific inquiry was made into the presence or absence of asthma of the extrinsic type; of allergic rhinitis due to tree, grass and ragweed pollen, mold spores, and dust; of hives; and of atopic eczema. However, the statistical analysis given below refers only to students having definite allergic rhinitis due to the above-mentioned inhalants or extrinsic bronchial asthma due to the same factors.

### 2.10.3 Results

a. The study showed that ragweed pollinosis as well as atopy in general occurs in 18 national groups.

b. The incidence of hay fever and extrinsic bronchial asthma in the groups studied was found to be higher than anticipated. Table 18 shows the incidence in the various groups together with the incidence of a positive family history.

TABLE 18

#### Incidence of Hay Fever and Extrinsic Bronchial Asthma

	Total Number	Total Number Allergic	Incidence (per cent)	Incidence of Positive Family History in Total Group (per cent)
Foreign Students	322	68	21.1	15
University of Michigan Control Students	310	59	19.3	25
Medical and Nursing Control Students	200	49	24.3	25

It is of interest that the medically-oriented control group gave a higher incidence of allergic disease than the control group from the student body at large, though the difference was not statistically significant. Consequently, the former group was eliminated as a control for the foreign students. The 19.3 per cent incidence of extrinsic asthma and allergic rhinitis is somewhat higher than the figure of 16.7 per cent recently reported by Van Arsdel, but the latter study was carried out among students in Seattle where there is no ragweed pollen.

c. Table 19 shows a marked discrepancy occurring in the incidence of a positive family history of allergy in the atopic groups studied. The difference in the percentage of positive family histories between the first two groups is highly significant ( $\chi^2$  of 11.7 or  $P < 0.01$ ).

TABLE 19

Incidence of Positive Family History of Allergy  
Among Those with Allergies

	Number	Incidence of Positive Family History (per cent)
Allergic Foreign Students	68	33
Allergic University of Michigan Controls	59	62
Allergic Student Health Service Patients	170	64

TABLE 20

Incidence of Positive Family History of Allergy Among  
Those Sensitive to Ragweed and Grass

	Positive Family History (per cent)
Ragweed Sensitive Foreign Students	30
Ragweed Sensitive University of Michigan Controls	62
Grass Sensitive Foreign Students	41
Grass Sensitive University of Michigan Controls	69

d. A further analysis of these groups shows that the same relative incidence of a positive family history occurs when more specific atopy is considered. Table 20 shows the ragweed and grass sensitive patients with their relative positive family history.

The data in Tables 19 and 20 support the assertions made in the introductory paragraph concerning the lack of validity of using a positive family history as the criterion for establishing the genetics of atopy. The most rational explanation for these figures is that latent atopy is present in the families of many of these

foreign students but does not result in clinically overt allergic disease until some member of the family is exposed to a potent allergen such as our ragweed pollen. Other data under analysis indicate that ragweed pollen can bring out symptoms more often in foreign persons who have a positive family history than in persons who have a negative family history.

e. Of 265 cases of ragweed pollinosis among native students at The University of Michigan, 78 per cent developed ragweed symptoms prior to the age of twelve.

f. The possibility of asthma complicating ragweed pollinosis is greatest in the group whose onset of pollinosis is before four years of age. One half of the children developed asthma as a complication when their ragweed symptoms occurred before the age of three.

g. The "period of sensitization" in the ragweed-sensitive foreign student has been studied. The data show that 82 per cent of the foreign students who developed ragweed pollinosis acquired symptoms within the first two years of residence in this country.

h. Interesting studies on the incidence of urticaria in the student group, both control and foreign, show that the incidence of this entity is 30 per cent in the non-allergic group as compared to 27 per cent in the allergic group. The type of family history had no bearing on the incidence of urticaria in either group.

i. Further analyses of the considerable amount of data collected in this project are still being carried out.

#### 2.10.4 Discussion and Conclusions

Entirely reliable data concerning the prevalence of atopy and the mechanism of its inheritance probably will not be obtained until some entirely objective and reliable diagnostic test for atopy becomes available. Meanwhile, indications of the high frequency of overt allergic disease can be obtained by careful collection of data and the use of conservative diagnostic criteria. However, since significant differences in the incidence of a positive family history of overt allergy have been demonstrated in two groups of student patients with the same atopic diseases, it may be inferred that calculations based on family history alone cannot be relied upon to delineate the inheritance of atopy.

## 2.11 SUMMARY OF CONCLUSIONS

- a. Hemagglutinins measured by the bis-diazotized benzidine or tanned cell anti-globulin tests are not identical to skin-sensitizing antibodies.
- b. Tapetal fluid is not highly allergenic.
- c. Ragweed pollen grains penetrate into the lower respiratory tract in very small numbers, if at all.
- d. Positive or negative air ionization did not affect passive anaphylaxis in guinea pigs under the conditions studied.
- e. External counting over sites of injection of isotopically labeled antigens can be used to evaluate the capacity of various types of repository preparations to retain antigen at injection sites.
- f. When employed as antiserum diluents, normal human serum or serum globulins do not significantly affect passive transfer titers.
- g. Significant differences in the incidence of a positive family history of overt allergy have been found in two groups of students with the same atopic diseases. Hence, calculations based on family history alone cannot be relied upon to delineate the inheritance of atopy.
- h. Loss of potency of dilute extracts of allergens stored in glass vials has been confirmed, and data providing a possible explanation for this phenomenon have been presented.
- i. Skin-sensitizing antibodies to ragweed pollen extracts may be inactivated somewhat more readily at conditions of moderately low or high pH than are more ordinary antibodies, but the differences, if any, are only moderate.

### 3. METEOROLOGICAL PHASE

by

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#### 3.1 INTRODUCTION

Meteorological research during the past year is described under nine major headings in this section of the report. It will be clear from the frequent cross references between the sections that the nine subjects are not separate research problems, but are rather those particular aspects of a single broad problem that have received special emphasis in recent months.

Pollen sampling techniques and instrumentation developments in pollen sampling have been pursued as a natural outgrowth of work described in previous reports on this project [2, 3]. The experiments carried out before and during the natural pollen season are described in Sections 3.3 and 3.4. This experimental work is grouped as a unit in spite of the fact that the experimentation often deals with diverse aspects of the ragweed pollen problem. The methods and results of a sampling program carried out from automobiles during the previous year are also presented.

One of the important requisites to knowledge of pollen behavior in the atmosphere is an understanding of the factors that influence the release and transport of pollen. These include the influence of the wind on the flotation and reflation of pollen after the anthers have dehisced. A report is given of a simple mathematical model devised to explain some of the phenomena observed in earlier experiments.

A specific community service was rendered during the natural ragweed season of 1960 by dissemination of pollen counts made at the Meteorological Laboratories at the University. These counts were given to local radio stations, newspapers and weather services. The response to this service underscored the value of developing techniques for prediction of the daily ragweed pollen concentration. The progress which has been achieved in this regard is described in Section 3.8.

It is well known that rain has a remarkable cleansing effect on the atmosphere. In particular the question of the removal of ragweed pollen from the atmosphere by rain is being studied with the aid of newly developed instrumentation. This is the subject of Section 3.9.

The responsibility for completion of the pollinosis test chamber was assigned to the project meteorological engineers. Their progress is reported in Section 3.10. Conclusions of the various tasks and experiments complete this phase of the report.

## 3.2 DEVELOPMENTS IN POLLEN SAMPLING TECHNIQUES

### 3.2.1 Introduction

During the course of the project considerable effort has been expended in perfecting efficient and economical pollen samplers. The invention of the flag sampler for use in diffusion studies and the modification of the rotorod sampler [24] for routine sampling are probably the most noteworthy accomplishments of the project along these lines. Work on a sampler suitable for continuous operation has continued. Various devices have been proposed, but all appear to have crucial shortcomings.

During the past year minor improvements in the preparation and use of the flag and rotobar samplers have been effected, and better determinations of their collection efficiencies have been made. In addition, several special-purpose sampling devices have been developed, generally modifications of more basic designs previously presented. Some of these are discussed below; others are discussed in Section 3.4 together with the experiments for which they were designed.

### 3.2.2 Determination of Sampler Collection Efficiency

The collection efficiency of the flag and rotobar samplers has been under study. The problem of adequate evaluation arises because the function of collection involves two components, the first of these being the impingement of the particle to be collected on the collecting surface, and the second being the adhesion of that particle on that surface. Whereas the "impingement efficiency" of samplers of cylindrical or square-rod shape is subject to quite complete treatment which has been referred to



before [3], relatively little has been determined about the "efficiency of adhesion" of the various possible surface coatings. The approach adopted for the present study of this problem has been to conduct tests in a wind tunnel.

Because there does not exist any absolute standard sampler, a problem arises in getting adequate comparisons for calibration purposes even in the wind tunnel. To meet this problem a special adaptation of the filter type sampler was arranged so as to approximate isokinetic conditions in the wind tunnel situation. A Gelman filter head was modified by adding a thin cylindrical inlet tube (1.59 cm inner diameter, 3.34 cm long) in front of the filter. This tube displaces the entry point of the air from the disturbed flow zone around the filter holder itself. A membrane high-flow filter with a pore size of about  $7.5\mu$  is used. The air flow through the filter is then carefully controlled by means of an air pump connected to the filter through a system of limiting-flow orifices and valves, so as to match the air flow in the wind tunnel and thus maintain an isokinetic condition during the calibration run. It is felt that this system provides as nearly as can be done a standard for comparison in the sampling calibration tests (see Figure 10).

Preliminary tests in the wind tunnel showed that the data from a single sampling station in the wind tunnel had to be regarded with some suspicion. As a result the tests were designed on the basis of three sampling stations spaced at equal distances across the width of the wind tunnel test section. At each of these stations a two-bladed roto-bar sampler was placed near one of the isokinetic filter samplers. The separation between these pairs of samplers was just sufficient that the disturbed air flow of one would not interfere with the other (Figure 10). The vacuum system for the filter samplers was then arranged by connecting them in parallel to a single vacuum pump through a three-way junction. Limiting-flow orifices were used, placed in the line to regulate the air velocity. The gas meter measured the volume of air; the air pressure (which could be regulated by the valve) was measured by means of the manometer. The rate of flow through the filter was then regulated by adjustment of the valve to a predetermined manometer reading. This elaborate regulating system was necessary because of the rather large variability of the high-flow filters.

Pollen was emitted into the tunnel from a dispenser similar to that described in Section 3.10 (see Figure 11). The pollen traveled through the fan, the return ducts, the honeycomb, and

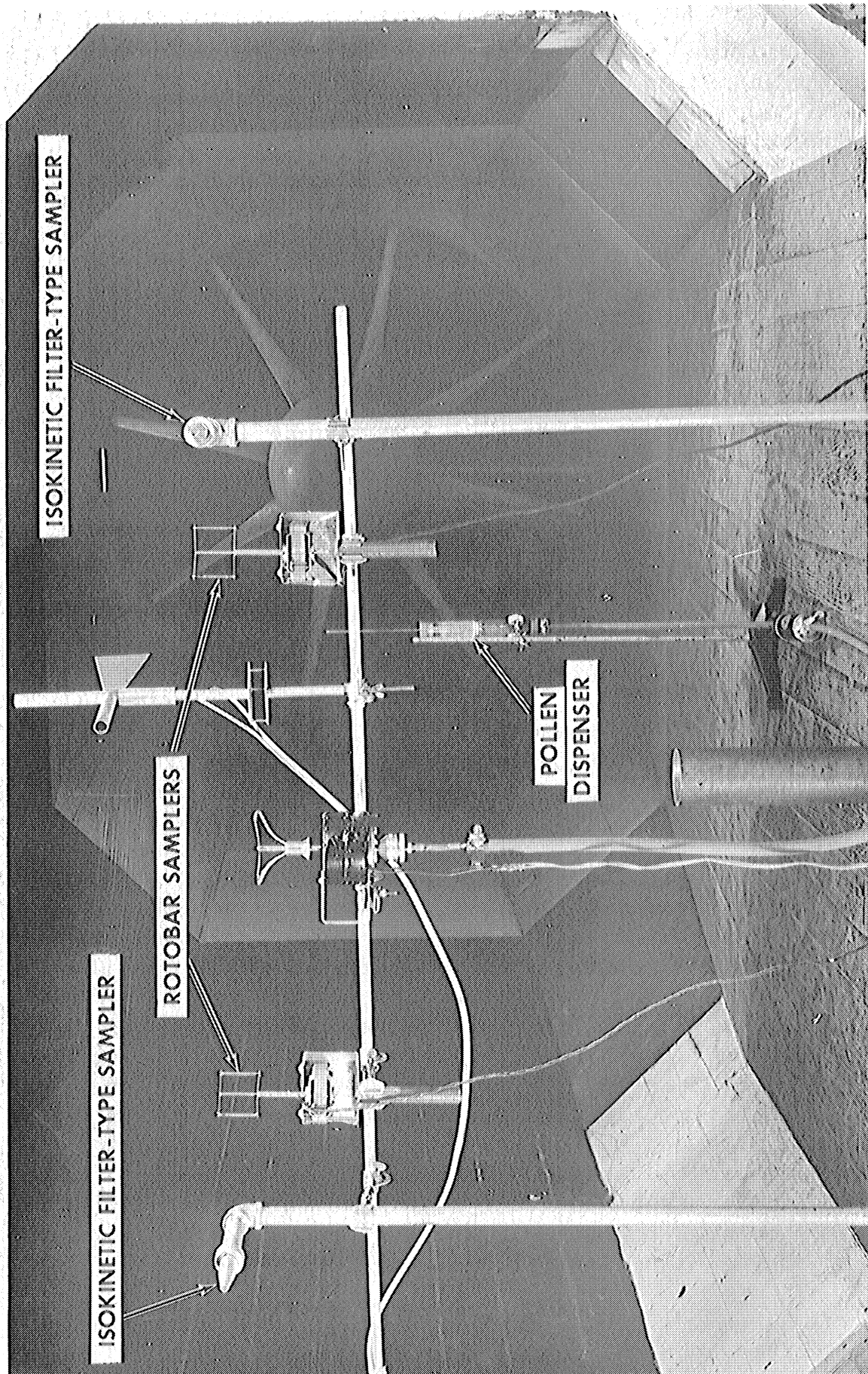


Fig. 10. Exposure of isokinetic filter-type sampler and roto-bar sampler in low-speed wind tunnel.

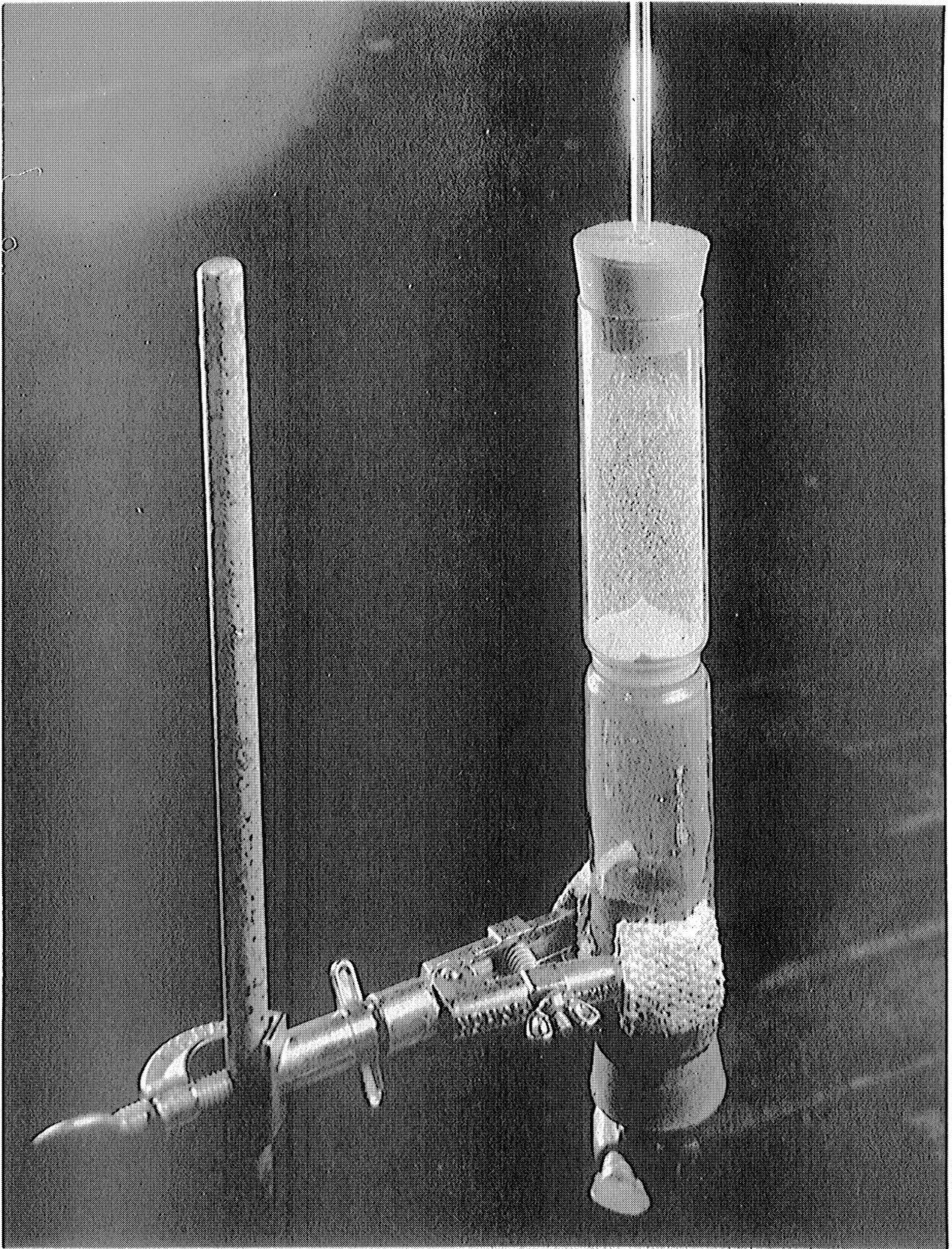


Fig. 11. Pollen dispenser unit, pollen sampler tests and pollinosis test chamber installation.

then back into the test section. It was expected that a uniform pollen concentration across the tunnel would result, except near the walls, ceiling, and floor where the concentration should decrease rapidly due to deposition on those surfaces.

The amount of pollen dispensed was controlled by holding the rate of emission roughly constant (the regulation of the flow of air to the dispenser), and by adjusting the duration of emission as desired. Following each test, air was circulated through the tunnel for an additional ten minutes to reduce the residual pollen concentration while the samplers were being changed.

Although the tunnel is equipped with a pitot-tube air-speed indicator, the readings are not dependable at low velocities. Therefore, a sensitive Thornthwaite anemometer was placed in the center of the test section 8 ft downwind from the samplers. Wind speeds in this series of wind tunnel tests ranged from about 4 to 12 mph. The data were too scattered to form a basis for firm conclusions regarding the relation of collection efficiency of the roto-bar sampler to wind speed. Further tests on this point are contemplated.

The average efficiency of the roto-bar sampler in all the tests was 68 per cent. If the impingement efficiency is taken to be 93 per cent [3], then the adhesive efficiency of the cylindrical section of rubber cement-coated scotch tape is 74 per cent. This set of tests was well adapted for statistical analysis, the results of which are presented in Section 4.2.

Before these experiments were run, a series of preliminary tests were made:

a. An experiment to determine the effect on sampling efficiency of the age of the coating. Result: no significant difference was noted in the efficiency as the age of the coating was varied from several minutes to two weeks.

b. An experiment to test the feasibility of sampling on first one edge of a DC-driven roto-bar and then reversing polarity (direction of rotation) and sampling on the other edge (see Section 3.2.3). Result: no pollen could be found on the coated trailing edges of the bars, nor on the sides of the bars, after sampling; hence the procedure is acceptable if a trailing-edge sampler does not lose material picked up during its sampling phase.

c. An experiment to measure the rate of reduction of pollen

in the tunnel after the emitter is stopped. Result: the effective pollen concentration in the test section, 10 min after stopping the emitter, was zero.

One possible source of error in all the tests described above was in the method by which the coating of rubber cement was applied to the sampling surfaces. The use of a brush for this purpose gave a highly variable coating. The coefficient of variability of samples obtained in identical pollen concentrations by similar samplers with brushed-on coatings was nearly 20 per cent, most of which must be attributed to the variability of the coatings. To meet this problem, a new procedure which uses a spray gun for applying the rubber cement has been adopted. Tests of this procedure are under way. The spray gun procedure offers the added advantage of mass-production of suitably coated samplers.

### 3.2.3 DC Rotobar Sampler

Since the requirement frequently arises for sampling pollen at locations where the usual 115V AC electrical power is not available, a battery powered DC roto-bar sampler was developed (see Figure 26). The original design had employed a DC motor, but the company manufacturing the one specified had been purchased by Barber Colman Co., and stocks were in a process of transfer. Two governed DC motors were considered: the Barber Colman types AYQM 2013 (6V) and BYQM 2020 (12V).

Over the range of voltages and loads which might be anticipated in field use, the BYQM 2020 proved to have a speed more nearly constant (see Figure 12). In addition, the cost of the motors was in a range which permitted the purchase of 24 for use on the 21-m masts of the 1959 in-season experiment (see Section 3.4.2).

The sampler and sampling technique are the same as those used with the usual AC power supply and motor, except that the radius of the sampling arm was increased to 3.56 cm, providing a peripheral speed of 20 mph at 2400 rpm, and a volume rate of sampling of  $1 \text{ m}^3$  per hr.

### 3.2.4 Whirling Arm Sampler

The whirling arm sampler was developed to measure the pollen source strength for the pre-seasonal experiments. The basic requirement of knowledge about source strength in diffusion studies

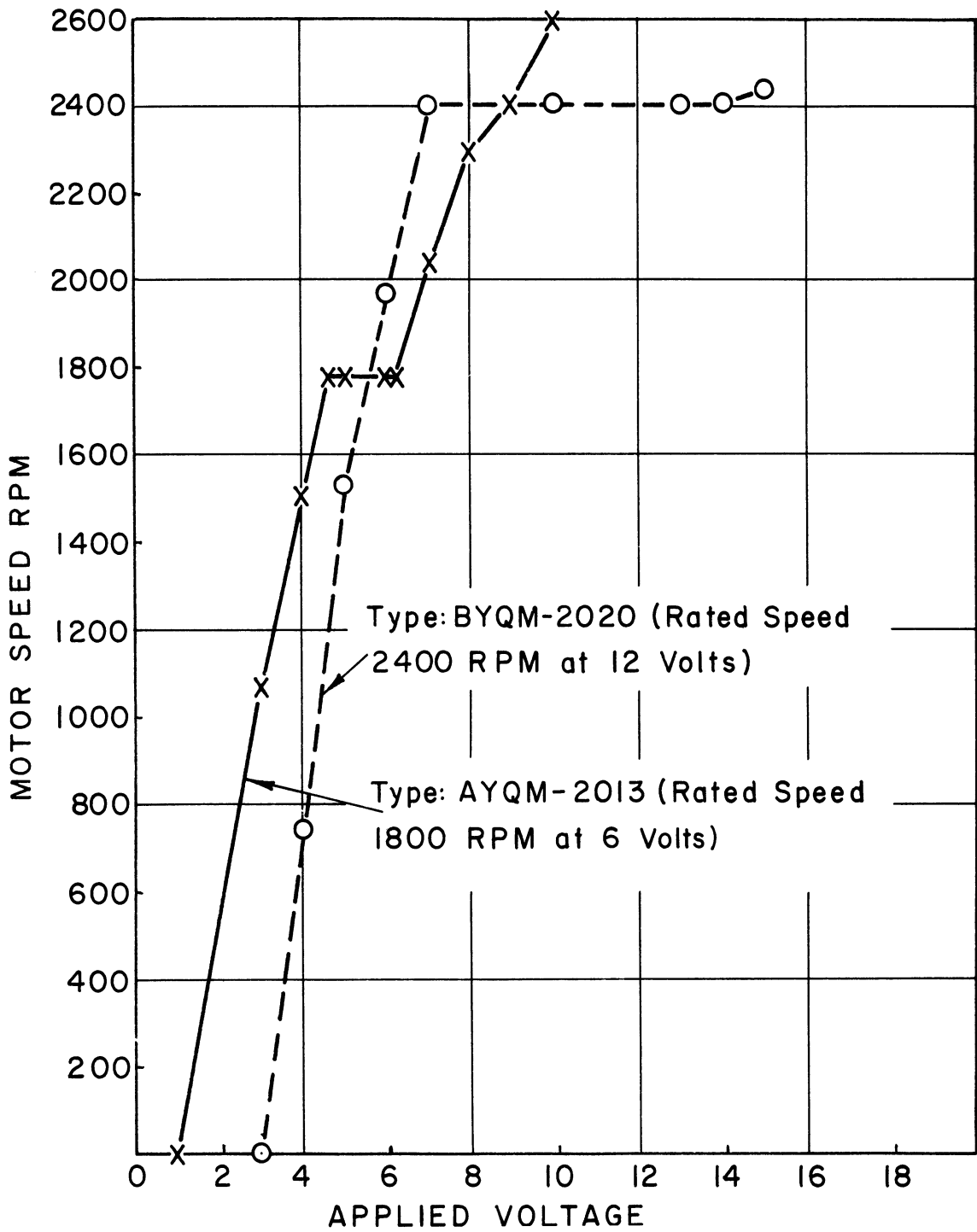


Fig. 12. Motor speed versus applied voltage for Barber-Colman governed-speed DC motors.

is apparent upon reference to the governing equations [3]. Discussion of the practical problem of measuring source strength directly led to the suggestion by E. L. Deacon that a device of this general description might work.

The whirling arm sampler consists of two vertical rods supported from above in such a way that they can be rotated rapidly about the periphery of the pollen source (Figure 13). Sampling bars identical to those used on the rotobar sampler are mounted at one-foot intervals on the leading edge of each of the vertical members. Sampling bars are also placed on the leading edge of the horizontal supporting arm to provide a measure of the amount of pollen leaving the source at a higher angle than that sampled by the vertical sampler. Rotation is produced by an electric motor mounted on a post in the center of the circular source. The motor and gear reduction box serve as the sole support for the sampler.

The dimensional characteristics of the sampler are as follows:

- speed of rotation - 58.3 rpm
- peripheral speed - 484 m per min
- radius of rotation - 4 ft
- dimension of sampling bars - 2.54 x 0.10 cm
- volume of sample (each sampling bar) - 0.67 m<sup>3</sup> per hr
- position of sampling bars
  - vertical - 1, 2, 3, 4 ft above ground surface on side one; 1.5, 2.5, 3.5, 4.5 ft above ground on side two
  - horizontal - 3 ft from center on one side; 2 ft from center on the other side.

The strength of the pollen source,  $Q$  (pollen grains per unit time), may be found from the count on the collecting bars of the whirling arm sampler according to an expression which is derived below. It is assumed that the pollen concentration  $\chi$  at any point  $P$  on the periphery of the plot is independent of the wind speed  $u$  and is proportional to the length of the wind trajectory over the plot, i.e., to  $2r \sin \theta$ , where the direction  $\theta = 0$  is oriented normal to the wind direction and  $r$  is the plot radius (see Figure 14). It is further assumed that diffusion is completely negligible in the vicinity of the plot.

On the basis of these assumptions, the number of pollen grains leaving the plot per unit time between the height  $h$  and

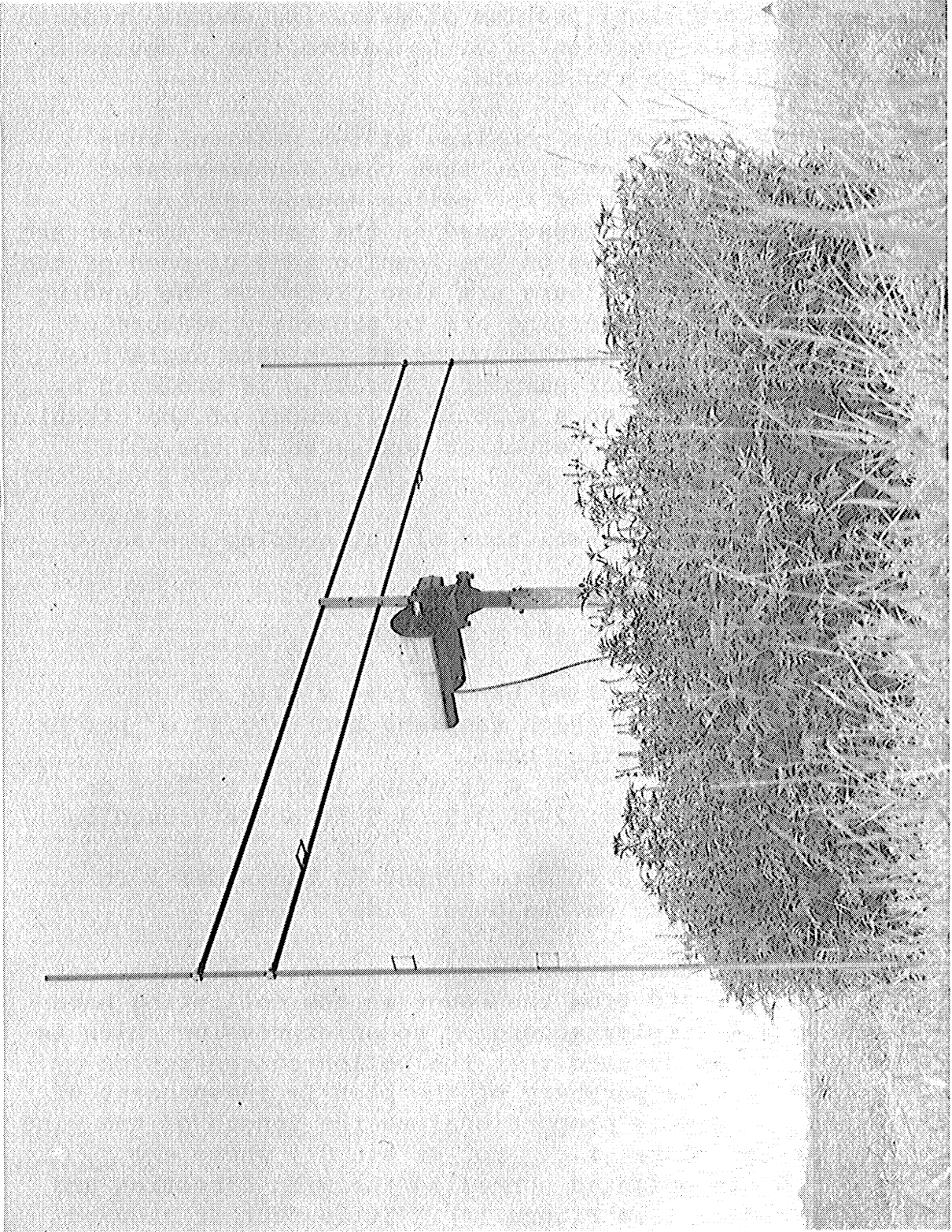


Fig. 13. Whirling arm pollen sampler.



$h + \Delta h$  and within the increment  $\Delta x$  will be:

$$Q = f(h) u(h) 2r \sin \theta \Delta x \Delta h = f(h) u(h) 2r^2 \sin^2 \theta \Delta \theta \Delta h ,$$

where the wind speed at height  $h$  above the ground is  $u(h)$ . To evaluate the source strength  $Q$  integration is necessary over only one-half the circle, since it is assumed that no pollen will leave the plot in an upstream direction. Then

$$\begin{aligned} Q &= \int_{h=0}^{\infty} \int_{\theta=0}^{\pi} f(h) 2r^2 u \sin^2 \theta d\theta dh \\ &= r^2 \pi \int_{h=0}^{\infty} u(h) f(h) dh . \end{aligned} \tag{1}$$

Note that the dependence of pollen concentration on height is vested completely in the function  $f(h)$  and that the concentration at any point on the periphery of the ragweed plot and at height  $h$  is given by

$$\chi = 2r f(h) \sin \theta .$$

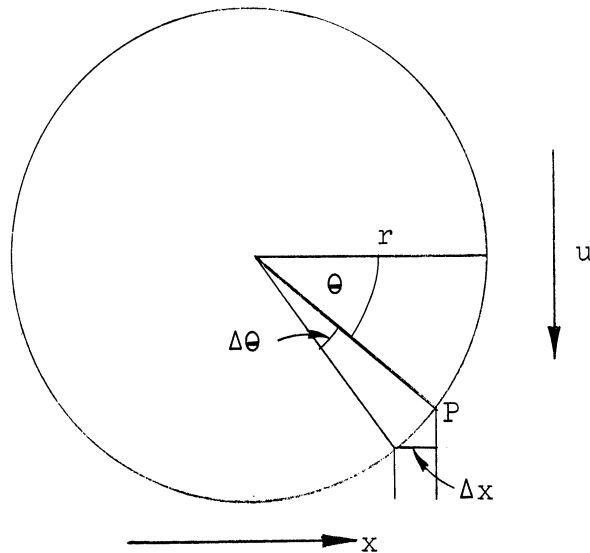


Fig. 14. Schematic of pollen plot and factors necessary for pollen strength computation.

To use equation (1) in evaluating the source strength, the function  $f(h)$  must be evaluated. The pollen counts at the various heights can be used to evaluate this function. The count on a sampling bar at height  $h$  is given by

$$C(h) = Ant \int_0^{\pi} \chi(h, \theta) r d\theta ,$$

where  $A$  is the area of the sampling bar,  $n$  is the rate of revolution of the sampler (58.3 rpm) and  $t$  is the sampling time. Then

$$C(h) = Ant \int_0^{\pi} f(h) 2r^2 \sin \theta d\theta = 4Antr^2 f(h) ,$$

And

$$f(h) = C(h) / 4Antr^2 .$$

Finally,

$$Q = \pi / 4Ant \int_0^{\infty} u(h) C(h) dh \cong \pi / 4Ant \sum_i u_i C_i .$$

One of the problems presented by the whirling arm is that it disturbs the air, in particular producing a fanning effect which draws air into the sampler at the top and bottom and throws it out at intermediate levels around the periphery. To determine whether or not this might have a deleterious effect on results, smoke was allowed to pass through the whirling arm sampler when the wind at the sampling level was between 2 and 10 mph. No major distortion of the smoke stream was apparent although some slight bending of the streamlines occurred on the margin of the plot. This, together with the fact that the dimensions of the whirling arm and samplers are small compared to the size of the plot, permit the conclusion that the error in results produced by this fanning effect is negligible.

The pollen concentrations near the margin of the plot were often extremely large. The sampling bars would have become overloaded with pollen had the sampler been allowed to operate continuously throughout the two-hour sampling periods of the experiment. Actually, the bars were changed every 15 min. The resulting loss of sampling time was not more than 5 min per hr or about 8 per cent.

If the assumption is made that the pollen concentrations at the periphery of the plot are independent of wind speed, considerable error may be introduced. Preliminary examination of the data from the alternating sampler experiment of the 1959 pre-season experiment suggests that strong winds remove considerably more pollen from the plot than do light winds. In this case the estimation of the source strength given by the above equation will be an underestimation of the actual source strength. At this point it is not possible to make a quantitative estimate of this error.

### 3.2.5 Low-Level Pollen Profile Sampler

The rate of pollen deposition on a horizontal surface is usually assumed to be a function of the pollen concentration above that surface. When the deposition rate greatly exceeds the vertical transport by eddies in the air, then the pollen concentration profile very close to the surface can be assumed to be invariant with height. On the other hand, when transport by vertical eddies predominates, the pollen concentration profile should approximate the wind speed profile [25]. In the case of ragweed pollen, the atmosphere near the ground can be divided into two layers, the first just above the surface in which gravitational settling predominates, the second above the first where turbulent diffusion predominates. Although it may be assumed that turbulent diffusion is the more important factor, no set of data exists which adequately demonstrates the theoretical profile.

The multi-level roto-bar sampler was devised to provide a pollen concentration profile for the lowest two feet of the atmosphere. The samplers are mounted on a vertical shaft driven by a 1725 rpm 1/3 hp motor.\* The motor is mounted on a heavy steel stand and the shaft is supported near the top by an angle-iron-mounted bearing (see Figure 15). The samplers are fastened to the shaft at the following heights above its base: 0, 3, 6, 12, and 24 in. The sampler mounting is similar to that of the standard roto-bar sampler except that the radius of rotation is increased to 5 cm to compensate for the slower rotational rate. Each bar sweeps out 0.828 m<sup>3</sup> per hr and two bars are mounted, diametrically opposed, at each of the sampling levels.

In operation, the sampler is set in a trench so that the lowest sampling bar is at ground level. The trench is covered

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\* General Electric model 5KH35KG122.

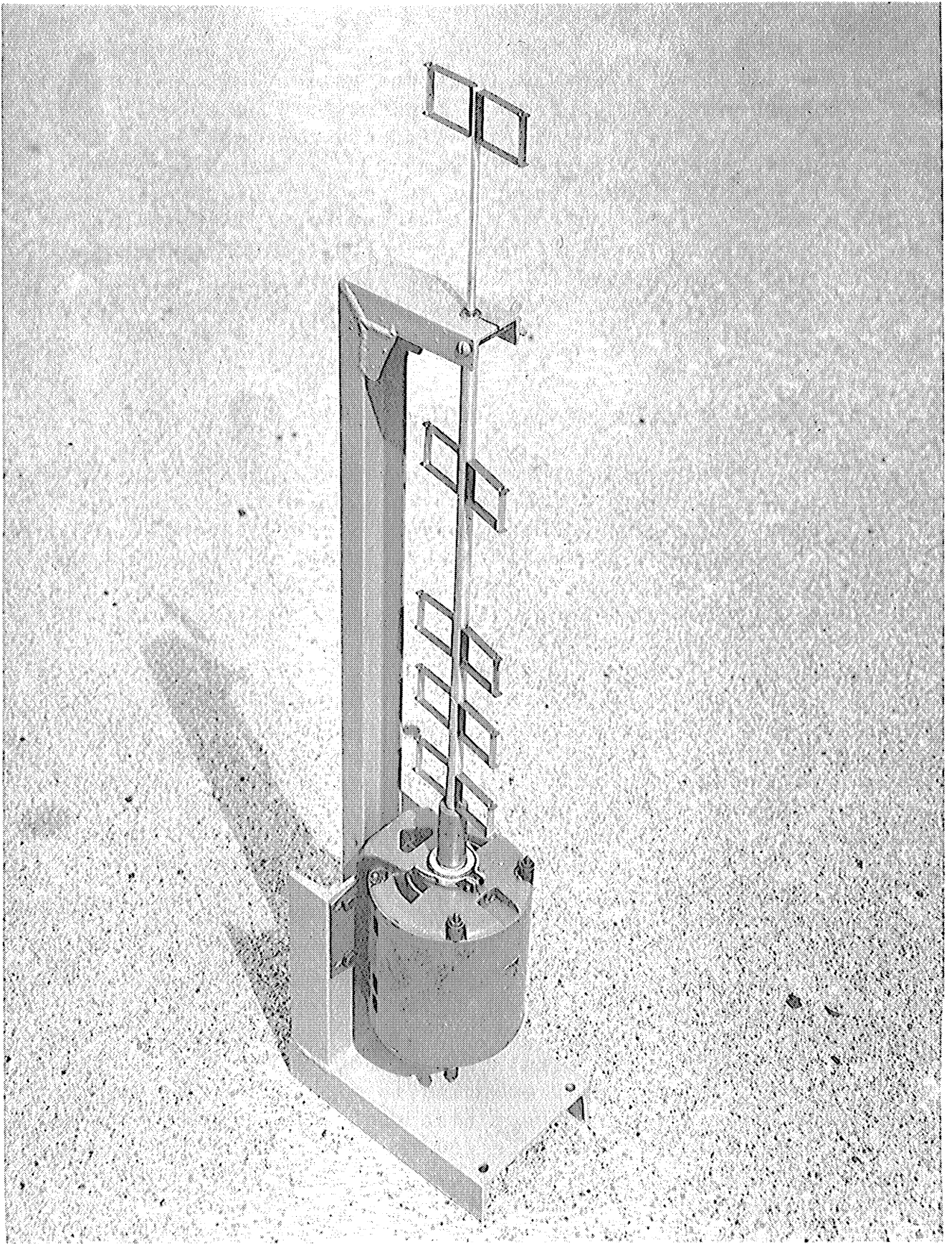


Fig. 15. Low-level pollen profile sampler.

with plywood and soil to simulate the natural surface, and the two ends of the trench are left open to allow air circulation around the motor.

The low-level profile sampler has been completed and tested only recently. Preliminary results from data taken by this sampler are in good agreement with those taken by mast-mounted rotobar samplers.

### 3.2.6 Alternating Rotobar Sampler

Because ragweed pollen depend upon the wind for their removal from the plants and flotation in the air, the speed of the wind is expected to affect directly the atmospheric pollen concentration. To study the effect of different wind speeds upon the pollen concentration, another special arrangement of rotobar samplers was devised. As an exploratory experiment, it was decided to consider the range of wind speeds according to three broad categories, light, moderate and strong, and to attempt to measure the pollen concentrations according to these categories. The rotobar sampler was chosen for this experiment because its collection efficiency is essentially independent of wind speed. Thus three rotobar samplers were arranged so that their operation would be controlled by an anemometer, the first operating at low wind speeds, the second at moderate wind speeds, and the third at high wind speeds, each being shut off when the wind speed varied outside its specified range. A photograph of the assembly is shown in Figure 16.

Operation of the samplers was determined by the wind speed as sensed by an Instruments Corporation three-cup anemometer located near the sampler units. Wind speed was recorded by a Brown recording potentiometer. Microswitches mounted directly on the indicator scale of the recorder were actuated as the pointer passed the preselected wind speed levels. The rotobar samplers and time clocks were actuated by the microswitches so that one and only one, rotobar sampler was operational during each of the chosen wind speed increments (Figure 17). The recording system was damped to prevent a short period gust from actuating the switching mechanism. At the conclusion of a sampling period, the sample bars were removed and the total operation time of each sampler was recorded. This time also represents the total accumulated time during which the wind speed stayed within the chosen class interval. During the 1959 pre-season experiment, the wind speed class intervals were 0-2, 2-6, and >6 mph.

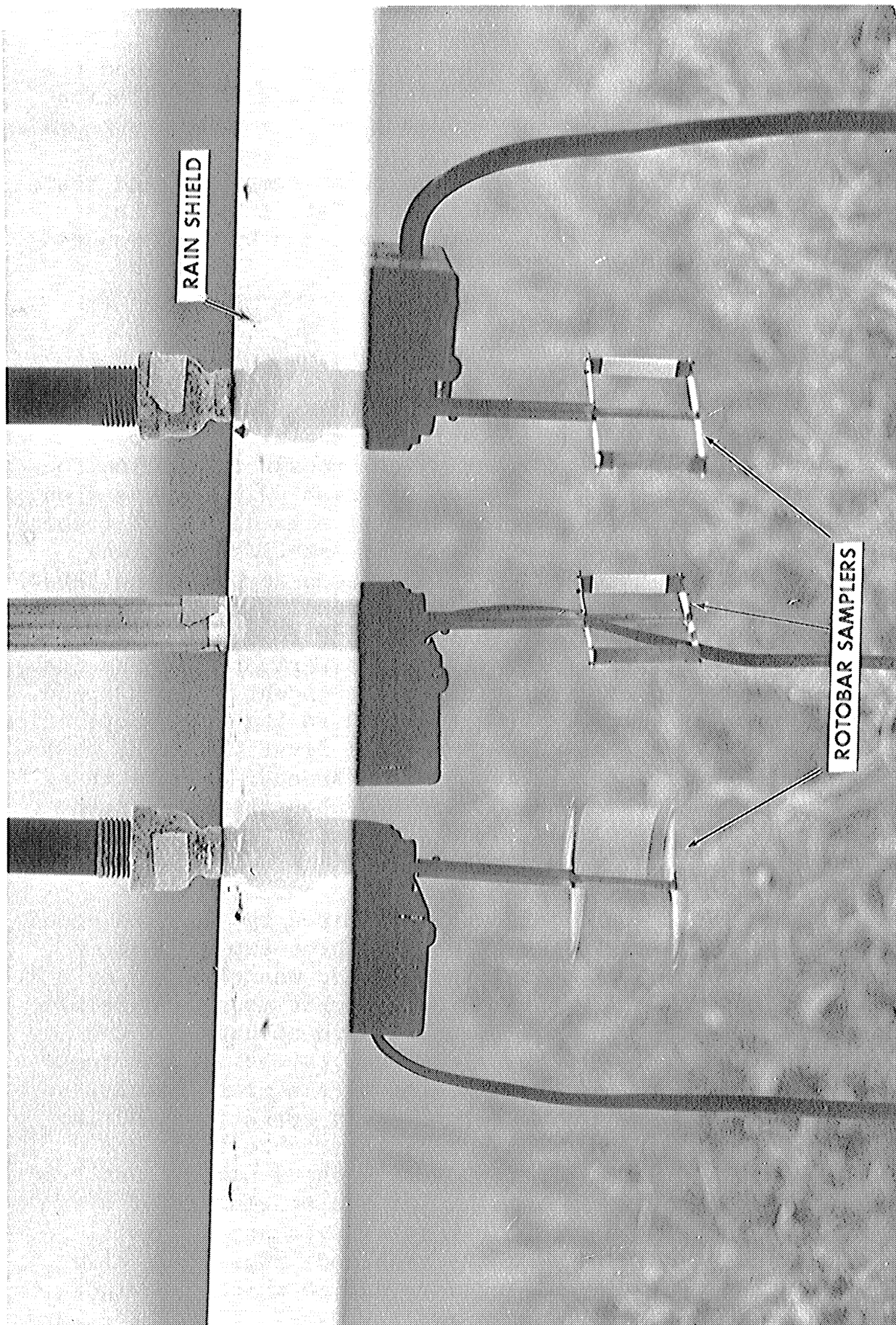


Fig. 16. Alternating roto-bar sampler.

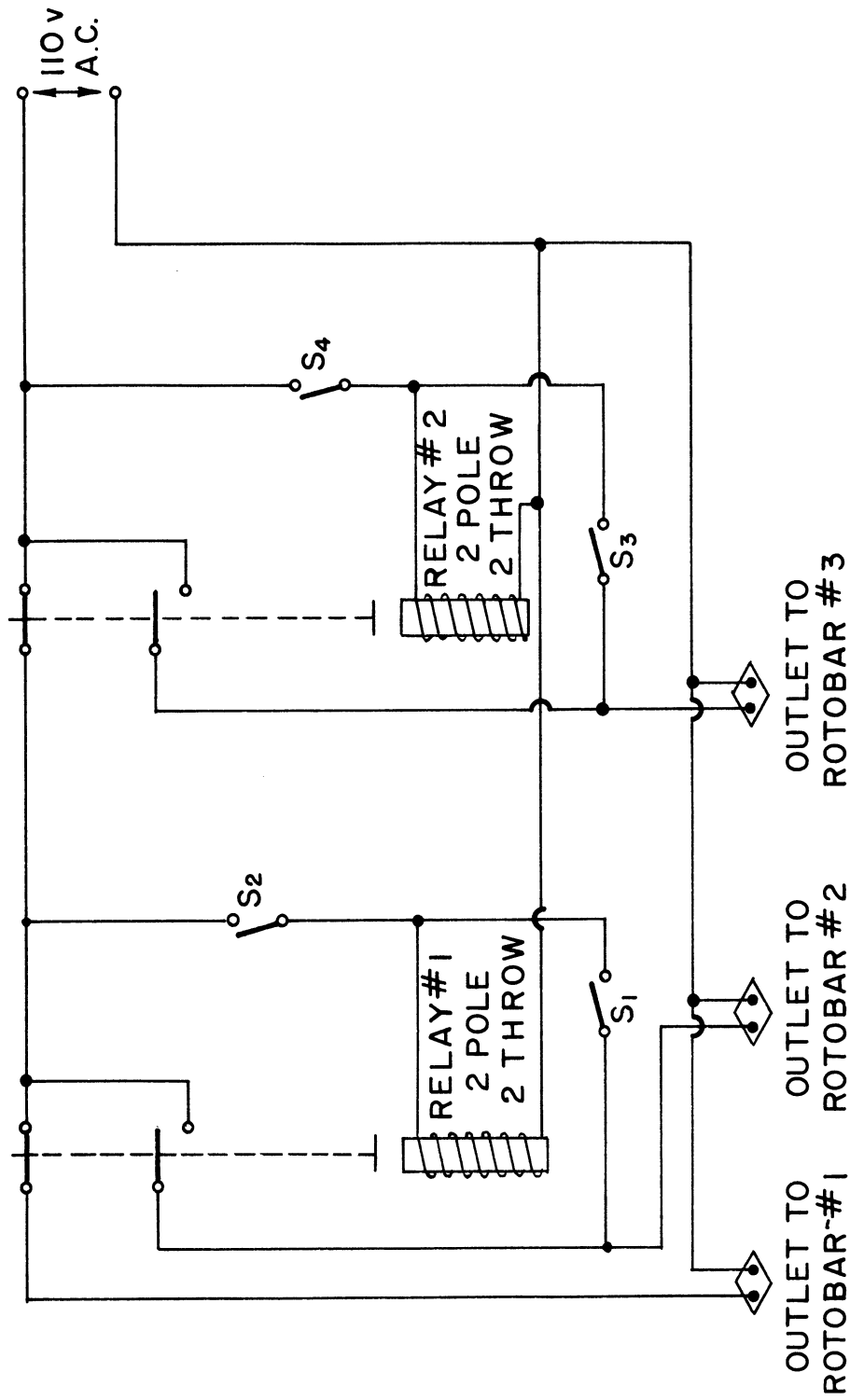


Fig. 17. Switching circuit for alternating rotobar sampler.

Mechanical specifications for this assembly include the following: (a) each rotobar sampler is driven by a Barber Colman Type YAJ 611-3, 110V AC synchronous motor; (b) the speed of rotation under sampling load was 3000 rpm giving a peripheral speed of 19.3 mph for a sampler placed 2.75 cm from the axis of rotation and a volume rate of sampling of 0.87 m<sup>3</sup> per hr for each rotobar.

A source of error of this system is found in the exposure of the sampling surfaces to airborne pollen during periods in which the rotobars are inoperative. If the sampling edge happens to have a component of the wind stream against it, sampling must occur in much the same manner as in the case of a flag sampler. For example, when the bar faces into a 5 mph wind, its efficiency is about 75 per cent. The error ratio  $R_e$  of the pollen caught by the stationary sampler to that retained by the rotating sampler is

$$R_e = \frac{75 \times 5}{92 \times 20} = 0.20 ,$$

where the efficiency of the stationary bar in a 20 mph wind is 92 per cent. Hence, the error estimate of pollen sampled by a stationary bar in a 5 mph wind is a maximum of 20 per cent when the bar edge is facing the wind. It is 47 per cent at 10 mph and 100 per cent at 20 mph. Winds were usually found to be in the 0-6 mph speed range.

Data obtained from this instrument have been employed in a study of the flotation and reflation of pollen which is discussed in Section 3.7.2.

### 3.3 PRESEASON EXPERIMENT, 1959

#### 3.3.1 Introduction

The preseason experiment of 1959 consisted of a diffusion study and a phenology study. These terms will be employed hereafter to refer to the individual experiments. Although the experiment was designed primarily to attack separately the problems of diffusion and phenology, it should prove possible to employ data collected from the diffusion experiment to study ragweed phenology, and vice versa.



The locale for the experiment was near the east parking ramp of Willow Run Airport, University of Michigan Meteorological Field Station. A schematic layout of the experiment is presented in Figure 18.

### 3.3.2 Diffusion Experiment

a. Purpose - The diffusion experiment was a direct outgrowth of similar experiments carried out in previous years. The objectives of the experiment were to:

(1) Determine the rate of emission of pollen as a function of meteorological parameters, and to verify the tentative results obtained during the 1958 experiment.

(2) Determine pollen source strength and to utilize this value together with diffusion parameters as obtained from micro-meteorological data in diffusion models.

(3) Make measurements of the diffusion rate downwind from the source for verification or modification of the models.

b. Design and Procedure - The pollen source plot of the diffusion experiment (see Figure 19) consisted of approximately 400 plants arranged in a plot of 40 sq ft. The plants, which had been brought to early maturity artificially, as in the previous preseasonal experiments, were moved to the experimental site on 10 June 1959. Prior to replanting, the soil was tilled to a depth of about 8 in. The plants, which had been grown in peat pots, were placed in the soil to a depth slightly greater than the height of the pots and soil was filled in to eliminate irregularities between pots. At the time the soil was extremely dry; moisture was not observed until depths greater than 1 ft were reached. After planting, 50 gal of water was applied to the plot each day. Accordingly, lack of moisture was never a factor in plant behavior during the experiment.

Since one of the difficulties encountered in previous diffusion studies had been inadequate knowledge of the strength of the pollen source, instrumentation was designed to measure the quantity of pollen leaving the source plot. The whirling arm sampler, Figure 13, was used; it is described on page 84.

The mast array which was established for determining the distribution of the pollen plume consisted of two arcs located at 40 and 160 ft from the center of the ragweed plot, respectively. The masts were spaced at 20 deg intervals on the 40-ft arc, and at 10 deg intervals on the 160-ft arc. Flag samplers [26] were

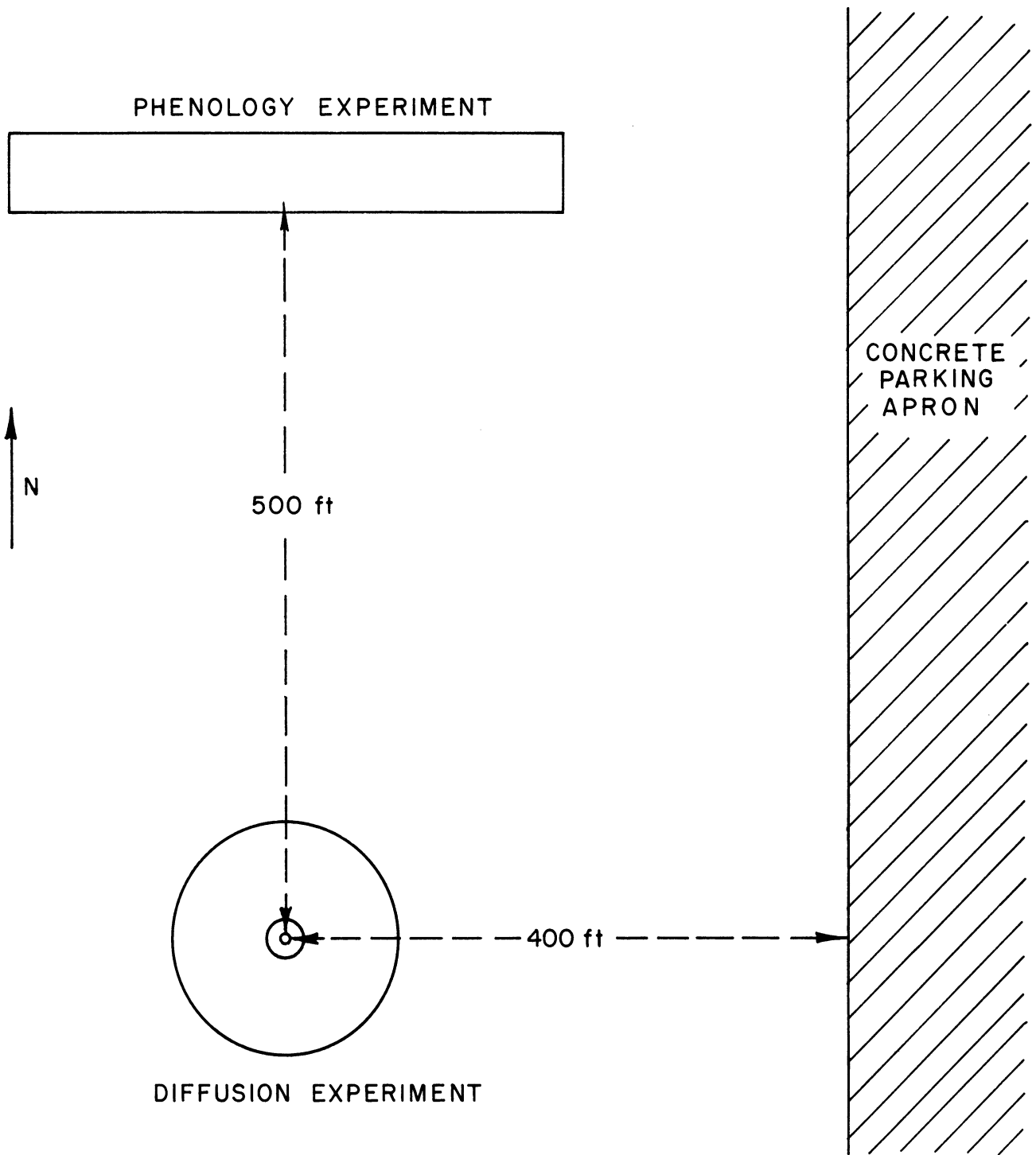


Fig. 18. Schematic layout, preseason experiment.

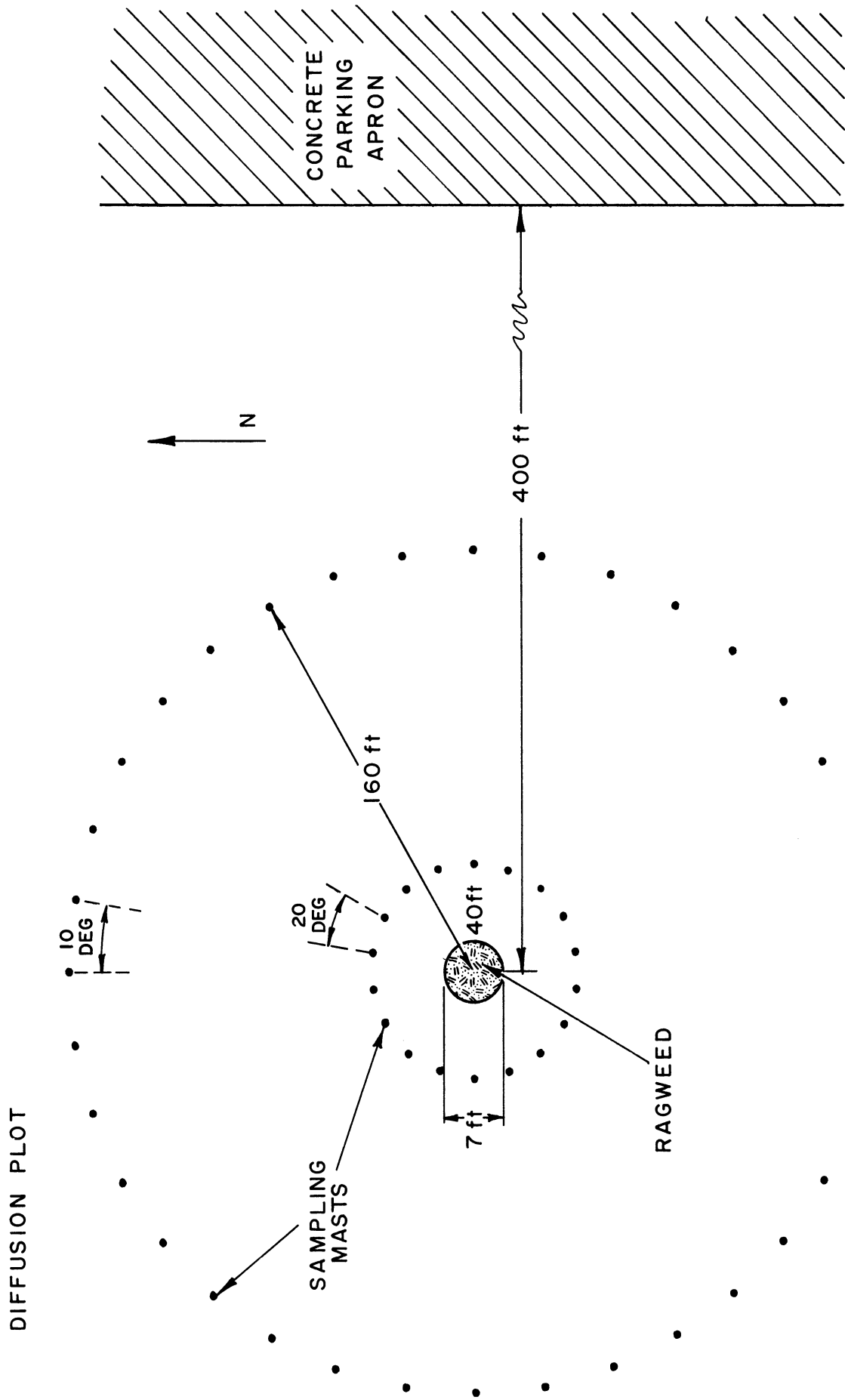


Fig. 19. Pollen source plot, diffusion experiment.

mounted on the masts at heights of 1, 2, 4, 6, 8, 12, 16, and 20 ft.

Array data were taken for at least one 2-hr period on each day that the wind direction could be predicted with sufficient accuracy. Samplers were mounted on five masts on the inner arc and nine on the outer arc during operations.

### 3.3.3 Phenological Experiment

a. Purpose - The purpose of this experiment was to investigate the interrelations between various meteorological factors, plant growth, floral development and opening, and the release and flotation of pollen. In particular, verification was sought and augmentation planned for the phenological findings of the 1957 preseasonal experiment, discussed in earlier progress reports [2, 26].

b. Design and Procedure - About 450 ragweed plants of the same history as those used in the diffusion experiment were replanted, on 10 June, in a "C"-shaped plot, the dimensions of which are indicated in Figure 20. From these plants, ten with healthy, mature, well-developed spikes were selected, and individual spikes on each of these plants comprised the sample upon which observations of floral openings were made. The observations consisted of counting, at 15-min intervals during the morning hours, the numbers of individual florets which were open. Only those plants downwind from the sampling apparatus located in the center of the plot were counted on any given morning. This was done to avoid disturbing the plants which would introduce pollen into the airstream, invalidating the observed pollen count. This requirement resulted in difficulty of interpreting flower counts from day to day. Only one plant was observed on each day that operations were carried out. Observation of the flowers is a difficult task, requiring a great deal of care and perseverance. The technique of observation is illustrated in Figure 21.

The alternating rotobar sampler situated in the center of the "C"-shaped plot of ragweed was designed especially for this experiment (see Figures 16 and 22). It was calibrated in such a way that sampler 1 operated when the wind speed was less than 2 mph, sampler 3 operated when the wind speed was greater than 6 mph, and sampler 2 operated at all other times. The length of time which each sampler operated was indicated by clocks connected in parallel with the samplers. The three rotobars were

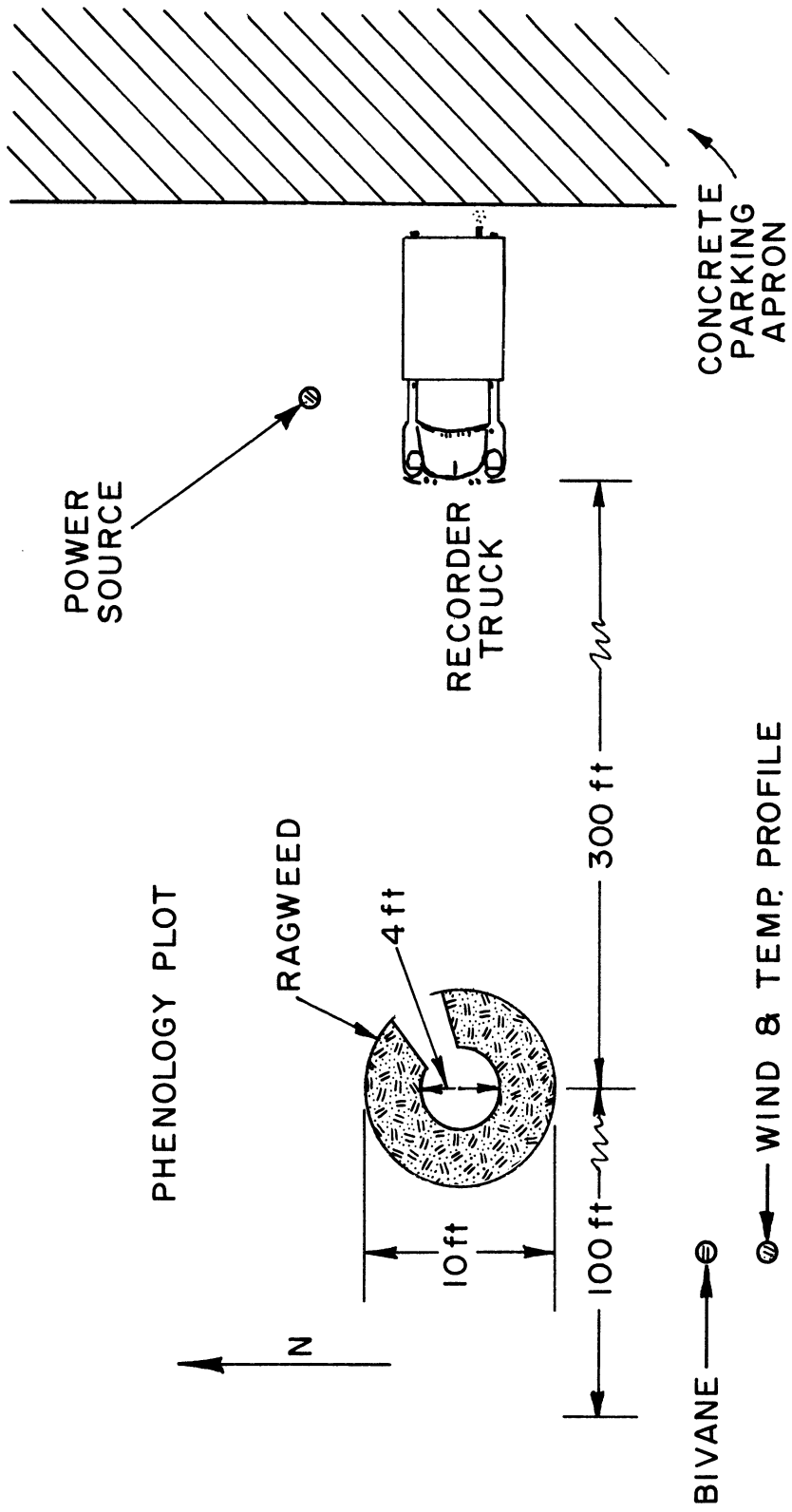


Fig. 20. Pollen source plot, phenological experiment.

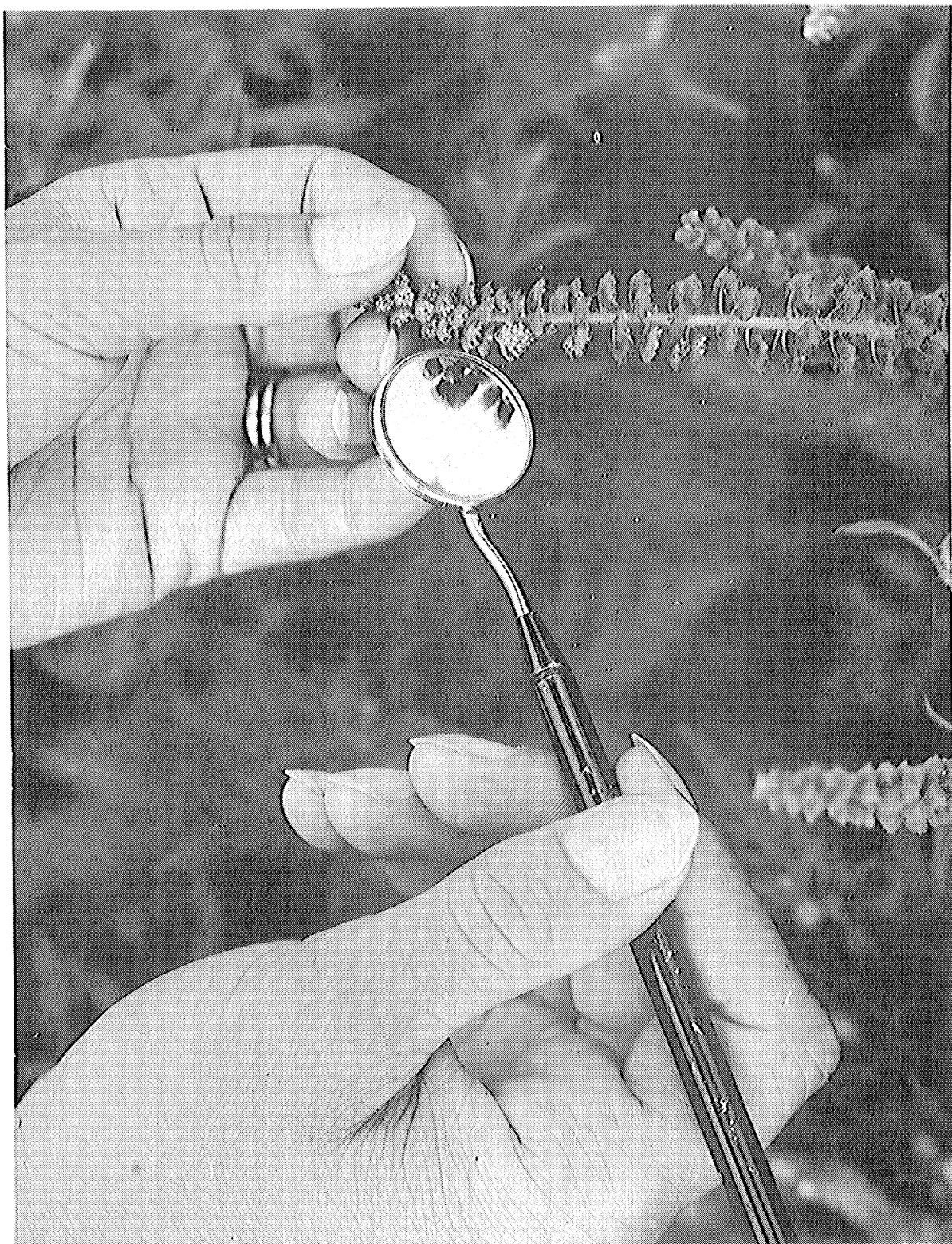


Fig. 21. Observation of individual ragweed florets.



Fig. 22. Psychrometric instrumentation and rotobar pollen samplers, phenological experiment.

changed at intervals ranging from 15 min to 2 hr, shorter intervals being employed during the early morning hours when the rate of pollen emission was expected to change most rapidly.

Certain difficulties with the experimental setup have been uncovered in a preliminary examination of the pollen counts from the alternating sampler. These arise from conditions discussed in Section 3.2.6, page 89, resulting in an erroneous count (20 per cent maximum error).

### 3.3.4 Supplementary Observations

In addition to the direct observations of the ragweed plants and the various pollen sampling devices, several parameters of micrometeorological significance also were recorded. These parameters are listed below. In addition, there were available to the project standard meteorological observations taken at the U. S. Weather Bureau Airport Office located opposite to the project site on the west side of Willow Run Airport.

a. Wind Profile - Wind speeds were measured at heights of 0.5, 1.0, 2.0, and 4.0 m above the soil surface (see Figure 23) by 4 Beckman and Whitley anemometers. Digital counters and an operations-type recorder, counted and recorded each cup revolution.

b. Temperature Profile - Copper-constantan thermocouples exposed in Thornthwaite radiation shields were located at heights of 0.5 and 4.0 m. The temperature difference between the two levels, recorded by a Bristol potentiometer recorder, gave a measure of atmospheric stability in the vicinity of the experimental plots.

c. Wet and Dry Bulb Temperatures - Wet and dry bulb temperatures were measured by a Bellaire-Tetraskelian copper-constantan thermocouple psychrometer employing an ice-water bath as thermal reference (see Figure 22, left). Autographic records were made by a Leeds and Northrup multipoint potentiometer.

d. Plant Temperature - Plant temperature was measured by insertion of a small thermocouple (36 ga) into the branch of a representative plant. Recording of this temperature was accomplished as in (c) above.

e. Solar Radiation - The output from an Eppley Pyrheliometer mounted on the roof of the recorder truck was recorded by the Leeds and Northrup multipoint potentiometer.



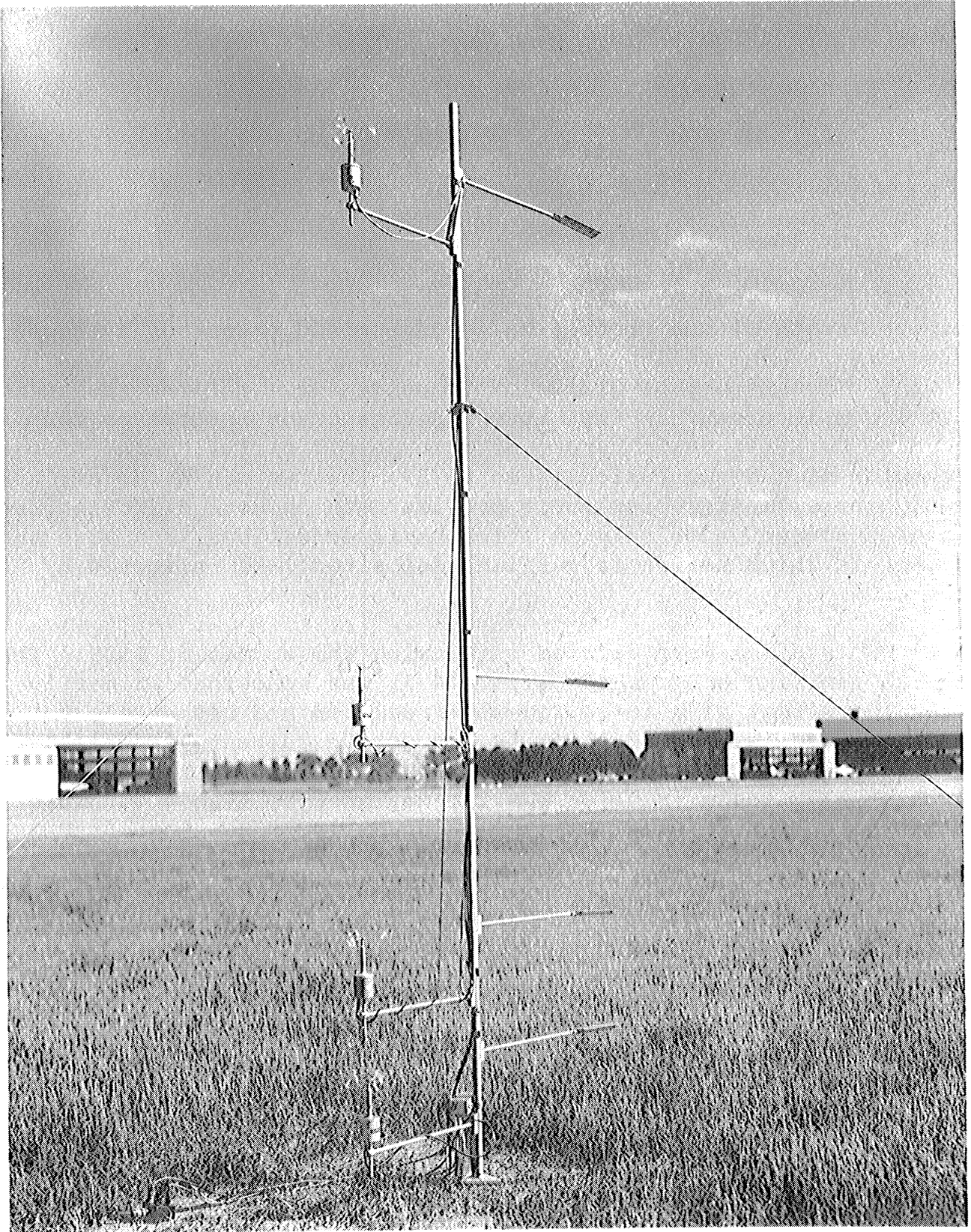


Fig. 23. Anemometer exposure, 1959 preseason experiment.

f. Wind Direction - A Gill Bivane (Figure 24) was mounted at an elevation of 4 ft close to the anemometer mast. Output was recorded on a dual-channel Esterline-Angus recorder.

### 3.4 IN-SEASON EXPERIMENTS, 1959

#### 3.4.1 Introduction

The 1959 in-season experiments were designed to afford empirical tests of the theoretically determined effect of a ragweed-free area on the pollen concentration downstream from an area source. The theory of diffusion of pollen from an area source is not a complete one, in that it makes some crude hypotheses concerning both the basic nature of atmospheric diffusion and also the behavior of ragweed pollen (see [26]). Thus it was necessary to design experiments which could provide data on both diffusion from an area source under varying atmospheric conditions, and also the manner in which pollen is carried, deposited, and refloated by the wind.

Two studies were carried out during the summer of 1959. The aerial sampling program (Section 3.4.3) was concerned primarily with the effect of a large ragweed-free area and the extent to which pollen is carried aloft by convective currents as well as by diffusion. The other experiment was a field experiment on a much smaller scale (Section 3.4.2), designed to test the theoretical model developed by Yang [26], and also evaluate the rate at which pollen is deposited on the ground and/or transported to greater heights after it leaves its source, both as functions of low level temperature and wind profile.

#### 3.4.2 Experimental Determination of the Effect of a Ragweed-Free Area on Downwind Pollen Concentrations

a. Description and Preparation of the Experimental Site - The experiment was carried out in an area lying between four runways and the east parking apron of the University of Michigan's Willow Run Airport (see Figure 25). A preseasonal survey of the area revealed that the areas indicated by "A," "B," and "C" in Figure 25 were covered uniformly with common ragweed. This area had been subjected to cultivation during previous years but had not been disturbed during the present season. The remainder of the airfield was mowed at frequent intervals and contained no

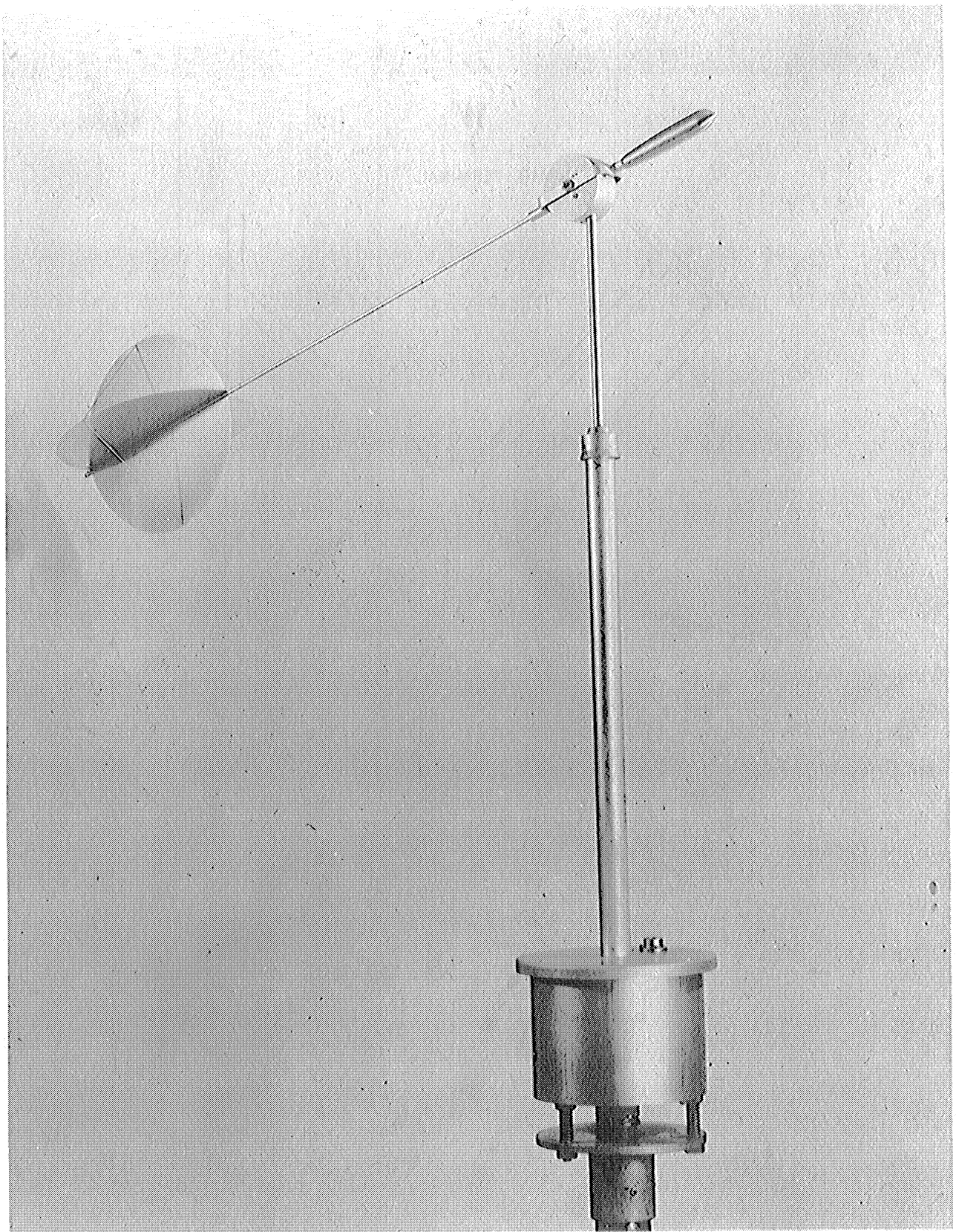


Fig. 24. Bivane utilized in 1959 preseason experiment.

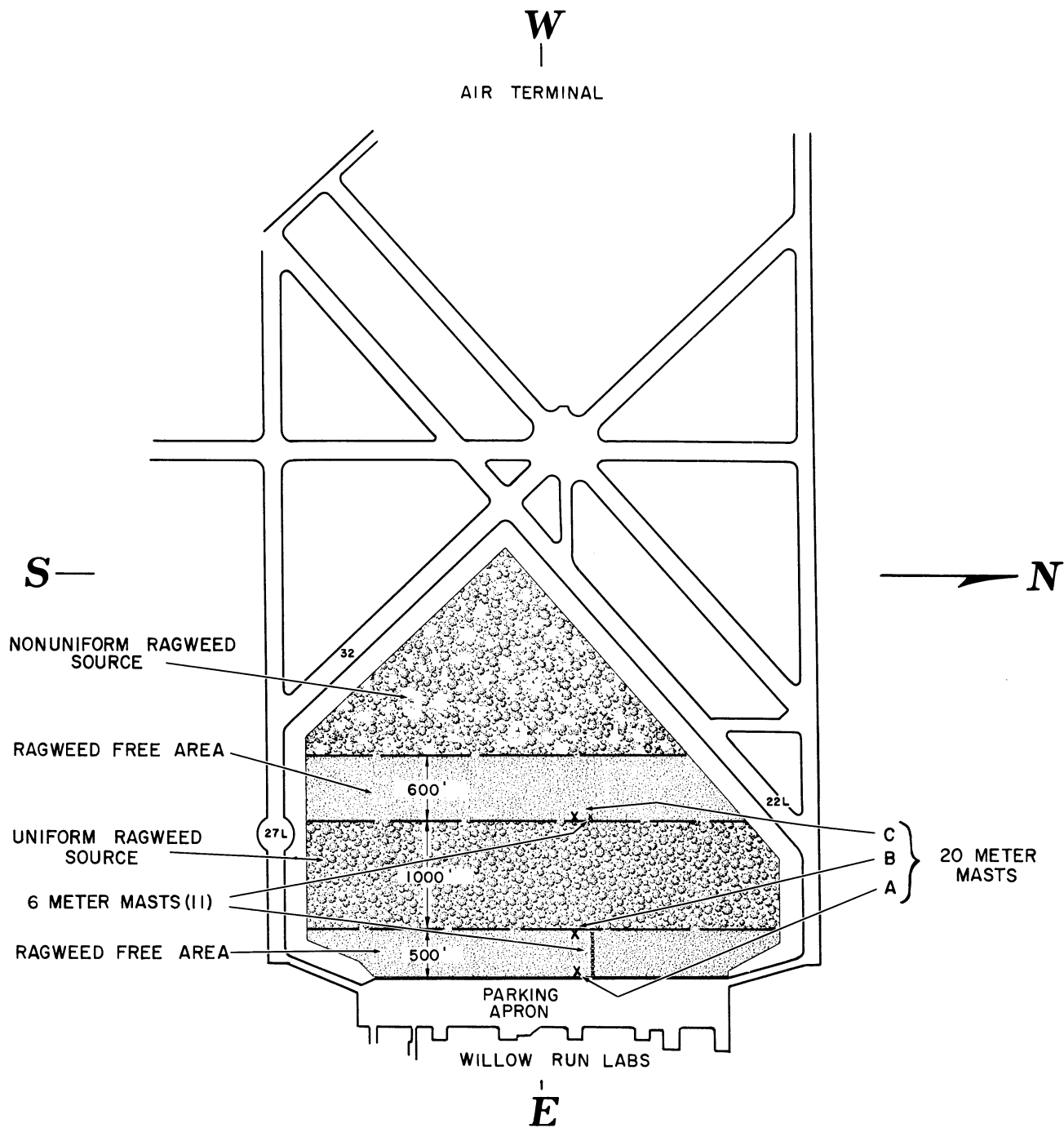


Fig. 25. In-season pollen sampling site.

ragweed with the exception of occasional isolated patches. Thus it was possible, through eradication of weeds from selected areas, to produce a rather uniform and well-defined source of ragweed pollen in an otherwise ragweed-free area. The site was also well suited to the experiment because of the large area of uniform terrain.

Area "A," a strip 500 ft wide adjacent to the concrete apron at the east end of the field, was sprayed with 2-4-D on two separate days to kill all ragweed in the area. The area had been mowed early in the season so that a uniform growth of relatively low grass remained after all broad-leafed plants had been killed.

No chemical treatment was applied to area "B," a nearly rectangular plot 1000 x 3000 ft in dimension between masts B and C. A dense and uniform growth of ragweed, intermingled with various varieties of grass, provided a uniform source of pollen.

The remainder of the area enclosed by airport runways 32, 27L, and 22L (see Figure 25) was designated area "C." Of this area, a 700 ft wide strip adjacent to area "B" was sprayed once with 2-4-D (Esterone 1010) prior to the measurement program. A survey of the area showed that plants were stunted and male inflorescence did not develop. It was observed that one application of 1 lb of active 2-4-D per acre at the time the plants are beginning to flower will satisfactorily restrict pollen production. A second application of 2-4-D at a later date affected a complete kill. All 2-4-D chemicals required for this program were donated by the Dow Chemical Company, Midland, Michigan.

Three 21 m masts were erected, one (mast "A") at the edge of the concrete apron, one (mast "B") on the line separating areas "A" and "B," and the other (mast "C") on the line separating areas "B" and "C." Ten 6 m masts were erected at intervals of 50 ft along a line 50 ft north of the large masts across area "A"; an additional 6 m mast was located adjacent to mast "C," upwind from the isolated pollen source. Data from the latter mast were used as a control in the experiment. Figure 26 is a photograph of this array of masts; it also illustrates the uniformity of the terrain.

Battery powered rotobar sampling units (see detail, Figure 27) were located at heights of 0.61, 1.83, 4, 8, 12, 16, and 20 m on each of the large masts. By reversing the polarity of the applied voltage it is possible to reverse the direction of rotation of the rotobars, hence samples could be collected on the two bars of each unit for two distinct periods of time. Standard flag samplers

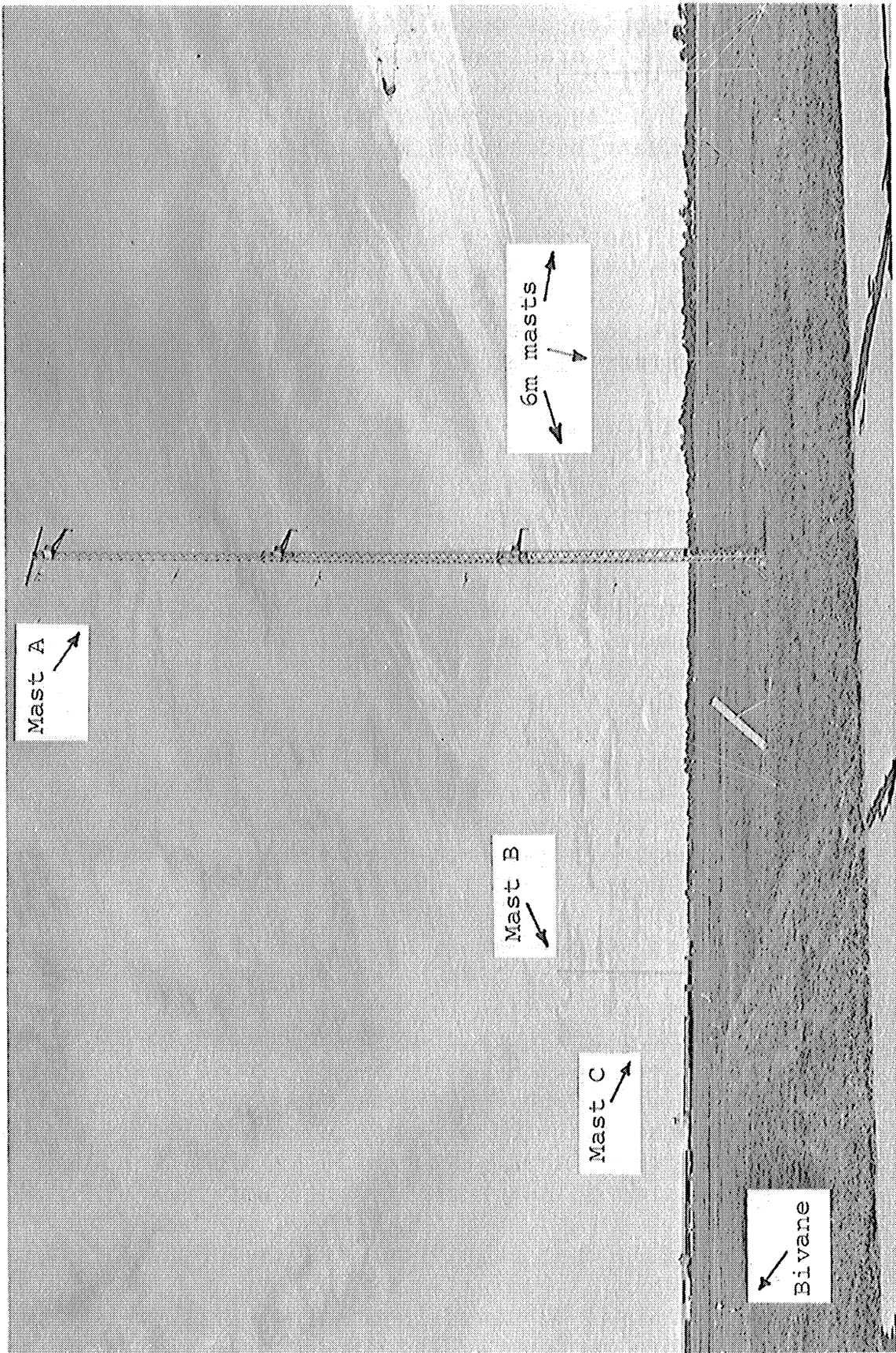


Fig. 26. Sampling mast array, in-season experiment.

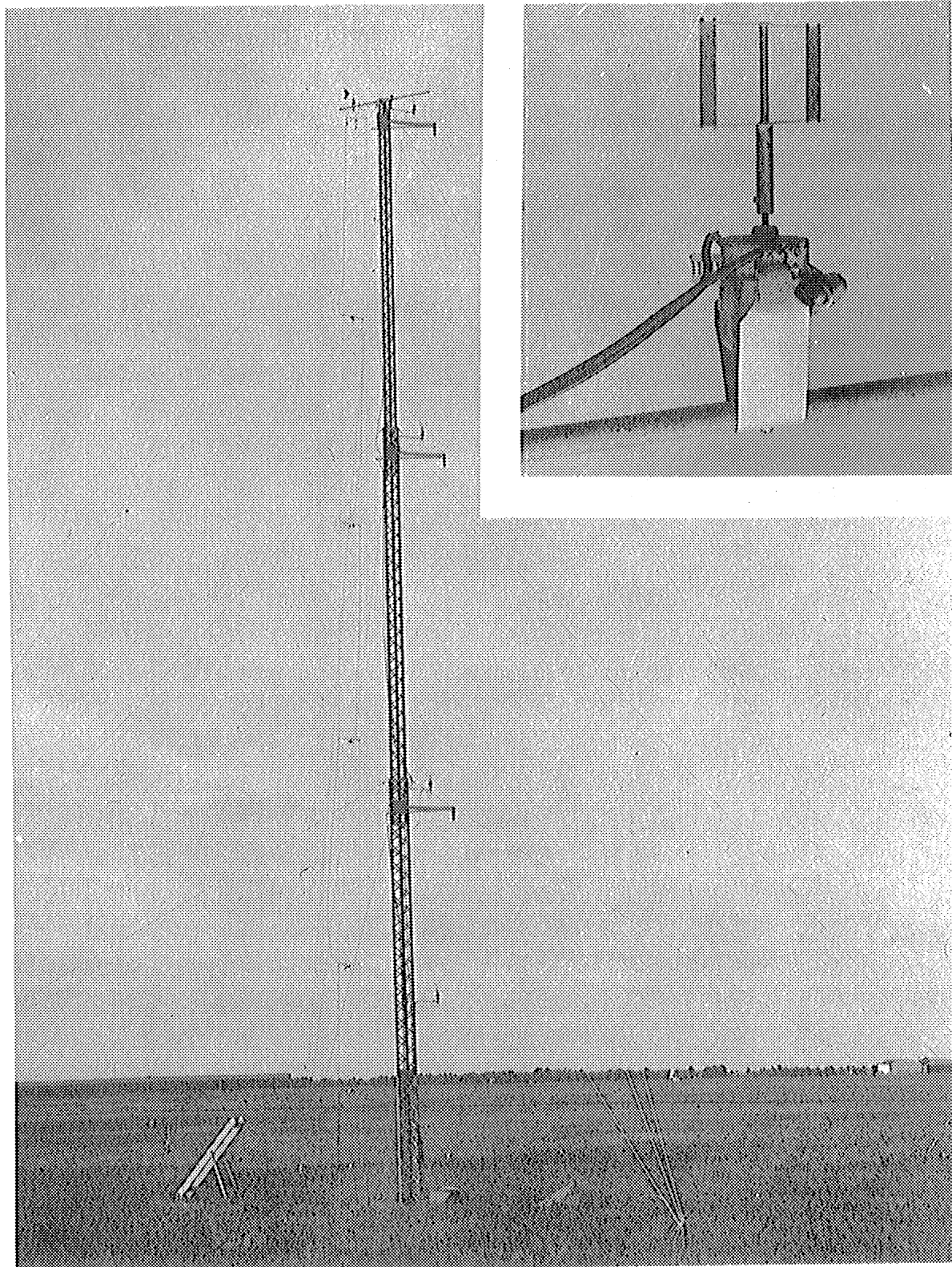


Fig. 27. Instrumentation on 21 m mast "A." Wind and temperature sensors extending at top and to right of mast; roto-bar pollen samplers supported at left of mast. Inset: detail of roto-bar sampler.

were mounted on the 6 m masts at heights of 0.3, 0.6, 1.2, 1.8, 2.4, 3.7, 4.9, and 6 m (see Figure 28).

The special low-level sampler described in Section 3.2.5 (Figure 15) was used to measure the pollen concentration profile close to the surface.

b. Meteorological Observations - Any study of atmospheric diffusion must necessarily include detailed measurements of the several related meteorological parameters. Accordingly, a system of measurements were conducted simultaneously with the pollen concentration determinations. These observations are in addition to the standard U. S. Weather Bureau observations which are made routinely on the opposite side of Willow Run Airport.

(1) Wind measurements. The vertical gradient of wind speed was measured by a system of eight low-inertia, three-cup anemometers. Beckman and Whitley anemometers were supported on 2-ft arms mounted at 5, 7.5, 11, and at the top of the 21 m mast "A" (see Figure 27). The wind speed was recorded by use of digital counters and an operation type recorder counting and recording each revolution of the anemometer cups. Four anemometers (Thornthwaite Associates) were mounted on a separate mast 4 m high and located 100 ft southwest of mast "A." These anemometers were mounted at the 0.5, 1, 2, and 4 m. Anemometer rotations were indicated on electromechanical counters and were recorded manually at 15-min intervals.

Wind direction was measured by a Beckman and Whitley vane also mounted on top of mast "A." Recording was accomplished on a standard Esterline Angus strip chart recorder. A Gill bivane was exposed at a height of approximately 1.2 m just to the northwest of mast "A," providing a record of both azimuthal and vertical wind direction near the surface.

(2) Temperature measurements. The vertical temperature gradient was measured by systems of thermocouples mounted on mast "A," and also on 4 m masts. Slow response, aspirated copper-constantan thermocouples mounted at 19.1, 12.6, and 6.5 m on the large mast were sequentially sampled once each minute and recorded to a resolution of about 0.2°C. Instrumentation detail on mast "A" is shown in Figure 29.

Arrays of fast response, 36 ga thermocouples located in Thornthwaite type radiation shields were exposed on five 4 m



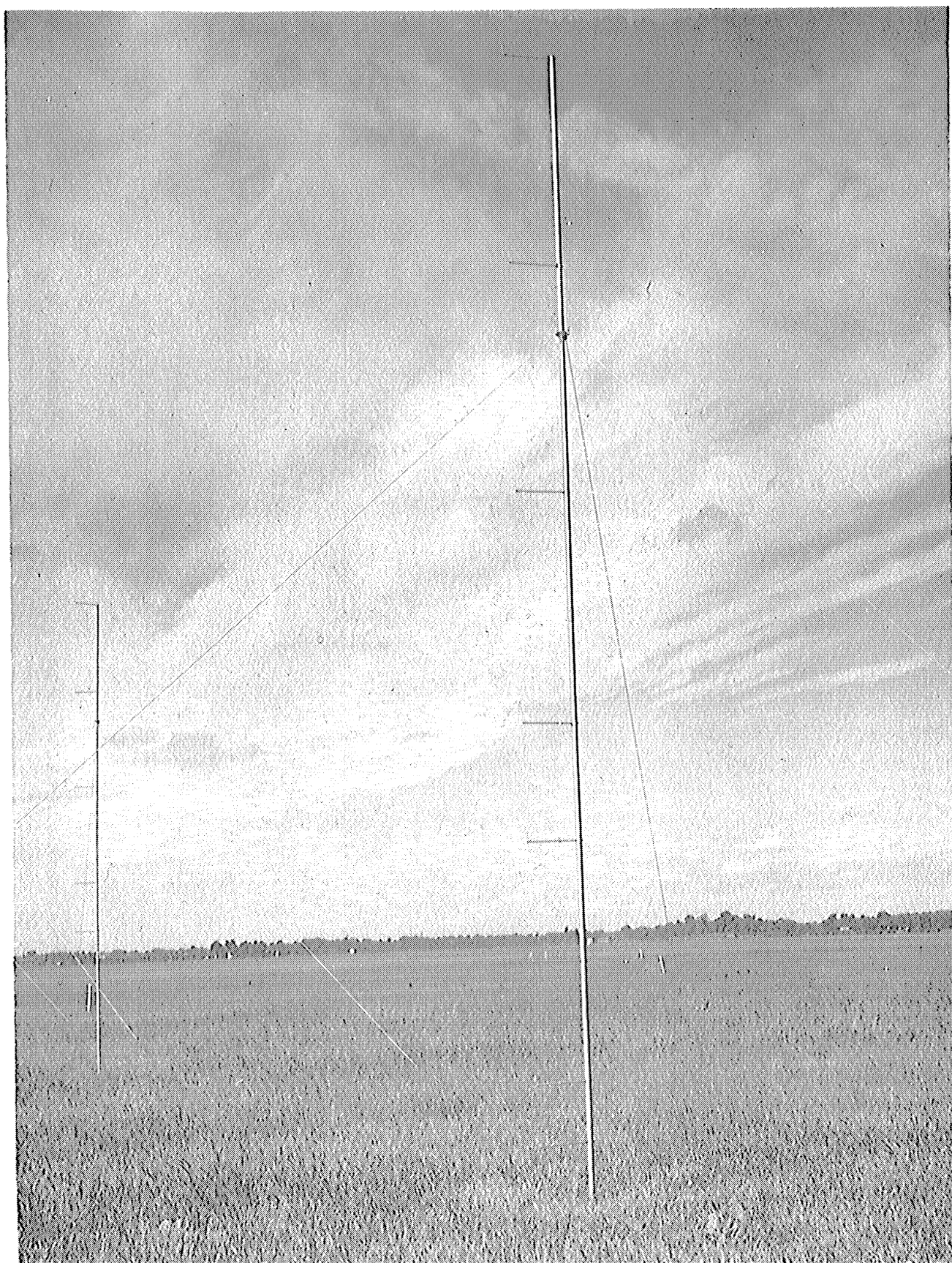


Fig. 28. Flag sampler exposure, 6 m masts.

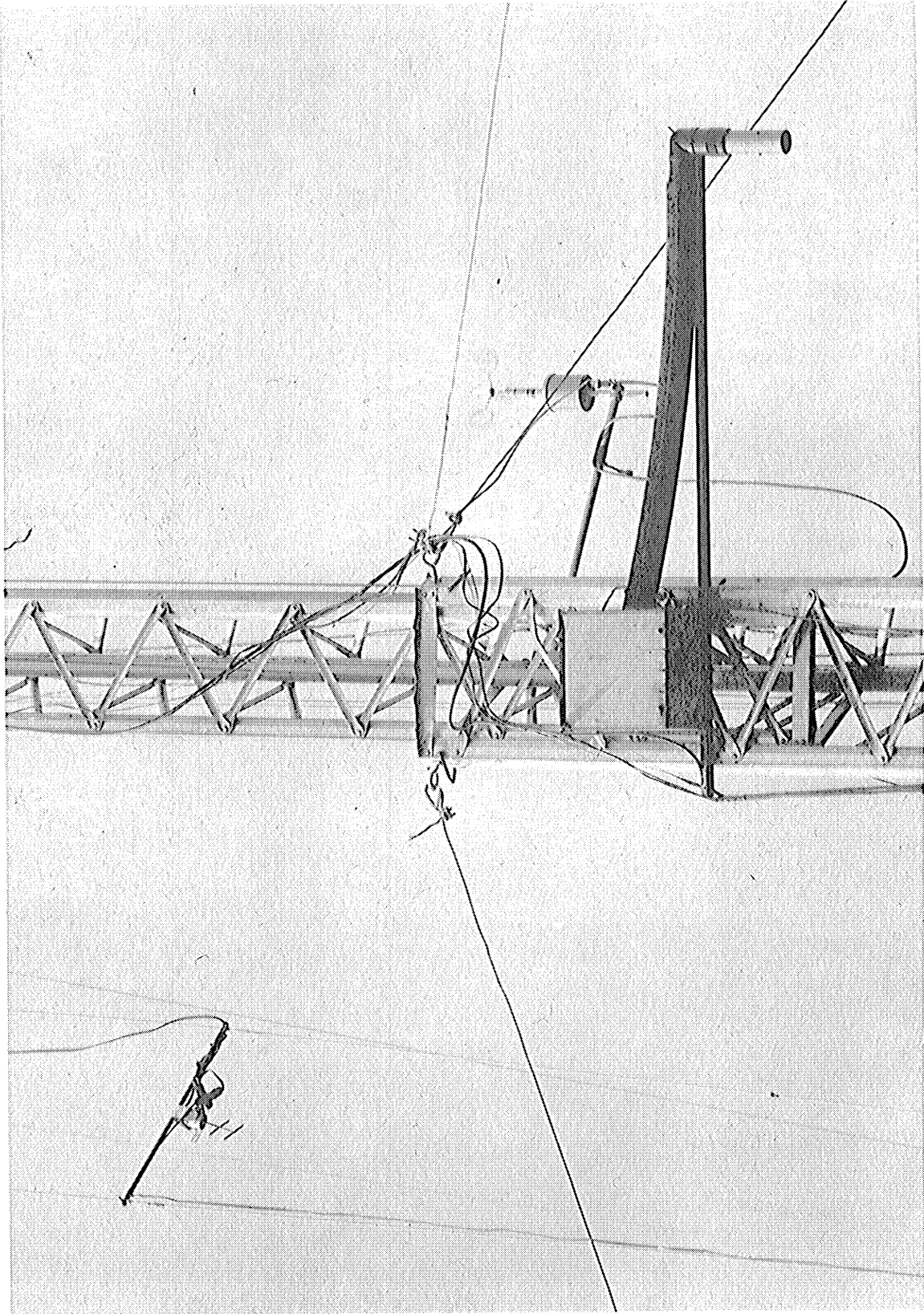


Fig. 29. Instrumentation detail, mast "A." Anemometer and aspirated thermocouple on right, rotobar pollen sampler on left.

masts at heights of 0.5, 1, 2, and 4 m. Recording was by sequential sampling each 8 sec to a resolution of about 0.1°C. The thermocouples were wired to provide fast response, space-averaged temperatures at each height.

c. Sampling Procedure - Since the array of samplers was oriented in a north-south direction, meaningful sampling could be accomplished only with westerly winds. Sampling was carried out only if the wind were in the sector from 230 to 300 deg. All flag samplers were exposed for 2 hr, while the rotobars were operated in each direction for 1 hr.

The pollen sampling program thus provided for measurements of the 2-hr mean vertical pollen concentration profiles as high as 21 m, upwind, immediately downwind, and 500 ft downwind from ground level area source of pollen. Measurements of the profile up to 6 m were also available at 50-ft increments downwind from the source. The upwind measurements were intended to provide necessary information on the background level of pollen concentration. The downwind measurements could then provide a measure of the rate of dilution due to diffusion and deposition. A total of eight periods of observations were obtained during the 1959 ragweed season. The analysis of these data is not completed; results will appear in a future report.

### 3.4.3 Aerial Sampling Program

a. Regions Selected for Sampling - Aerial sampling runs were conducted during the period of 7-11 September 1959 along the western side of Lake Michigan north of Des Plaines, Illinois. It had been planned to sample for several days on the western side of Lake Michigan in order to determine the average pollen concentration with west or southwesterly winds; following this, sampling would commence on the eastern shore of the lake in the same air mass before a frontal passage could take place. By this program, the effect of a large ragweed-free area (the Lake) could be evaluated from the measured concentrations.

The western shore flights originated from Pal-Waukee Airport which is located 5 mi north of Des Plaines, Illinois. Sampling was carried out along three paths. The first, (1) in Figure 30, was a line 10 mi long, approximately 8 mi west of Waukegan, Illinois, and about 10 mi west of the Lake Michigan shoreline. The second flight path, (2) in Figure 30, was a line 10 mi long from Kenosha, Wisconsin, to Racine, Wisconsin, about 5 mi west of the shore. The

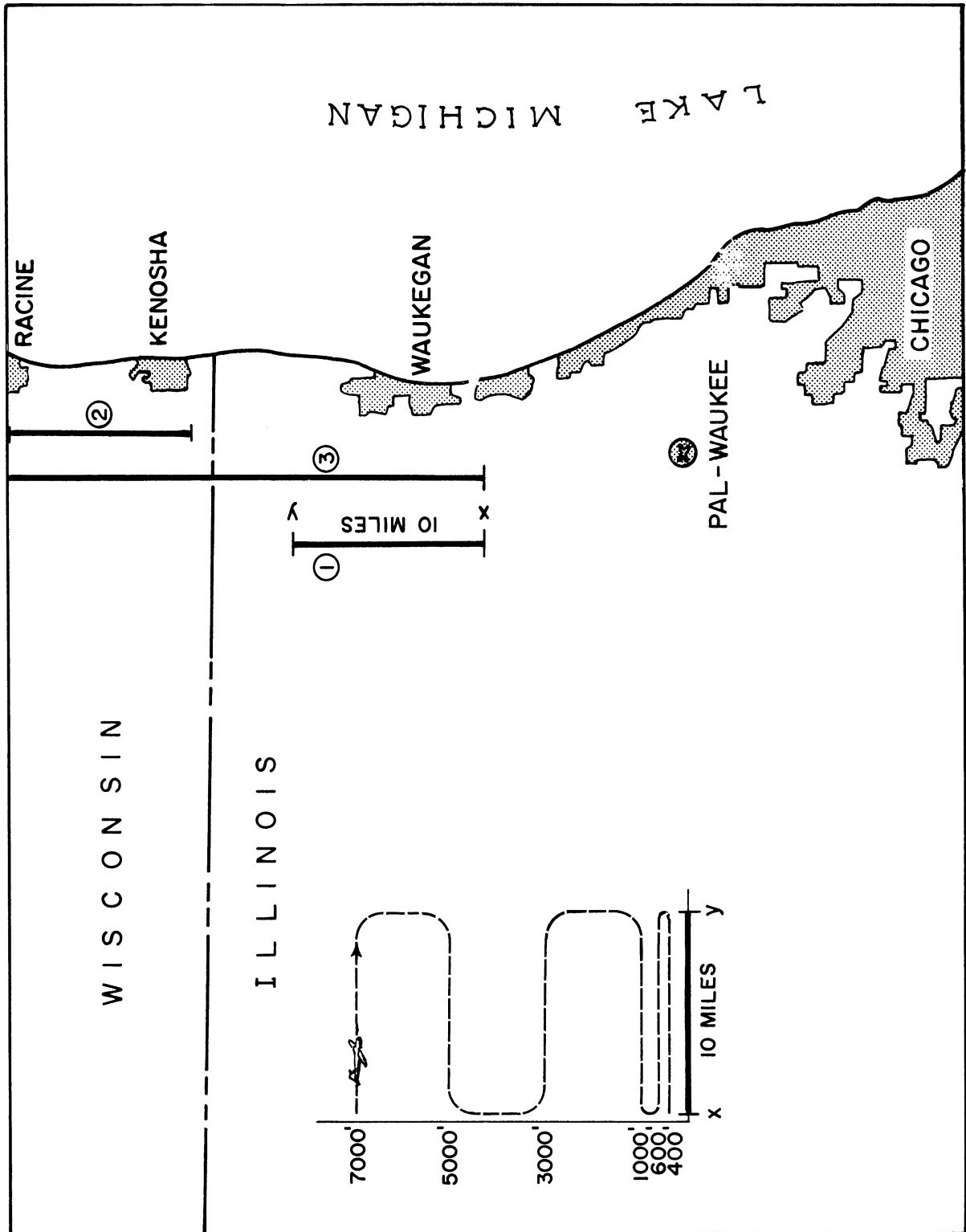


Fig. 30. Sampling paths, aerial pollen sampling program.

third path, (3) in Figure 30, extended over the region covered by the first two, but 7-8 mi west of the shore line.

Samples were taken at altitudes of 400, 600, 1000, 2000, 3000, 5000, and 7000 ft above the terrain. Minor changes had to be made on several days to avoid clouds, since the aircraft was operating under visual flight rules. In such cases samples were taken at the tops of the clouds, if possible, to determine the extent to which pollen had reached those levels.

The general area over which the sampling took place was flat, extensively cultivated countryside. A cursory check indicated considerable amounts of ragweed growing in the area. The flight paths were chosen so that the sampling would take place primarily over unpopulated areas. It was expected that westerly or south-westerly winds would have lengthy trajectories over areas producing a great deal of pollen.

It had been planned to locate the eastern base of operations near Muskegon, Michigan. The exact sampling areas were left to be determined on the basis of the mean wind direction during the operations of the west side of the lake.

b. Instrumentation - The aerial sampling equipment was mounted on a single-engine, high-wing monoplane, a Stinson "Flying Station Wagon." The plane operated at a speed near 90 mph, allowing it to remain airborne for about 3.5 hr.

A drum sampler and aerometeorograph were mounted beneath the right wing. Figure 31 shows two views of this equipment. The drum sampler (1) is mounted above the aerometeorograph (3) in a specially built frame (2). Both instruments are far enough out on the wing to be out of the propeller slip stream. Heavy-walled rubber tubing (4) connects the sampler to a vacuum source in the plane. Wires lead from the rotary solenoid (6) to a synchronous-motor timer also located in the plane.

An isokinetic cone (5) allows the air to be drawn through at the speed of the plane. Because there are no accelerations or decelerations of the air as it enters the cone, a truly representative sample is obtained. An average sampling rate of 50 l. per min was maintained.

The air drawn through the isokinetic cone passes through a narrow horizontal slit 6.4 x 0.73 mm and impinges on the drum inside the sampler. This drum is wrapped with double-faced

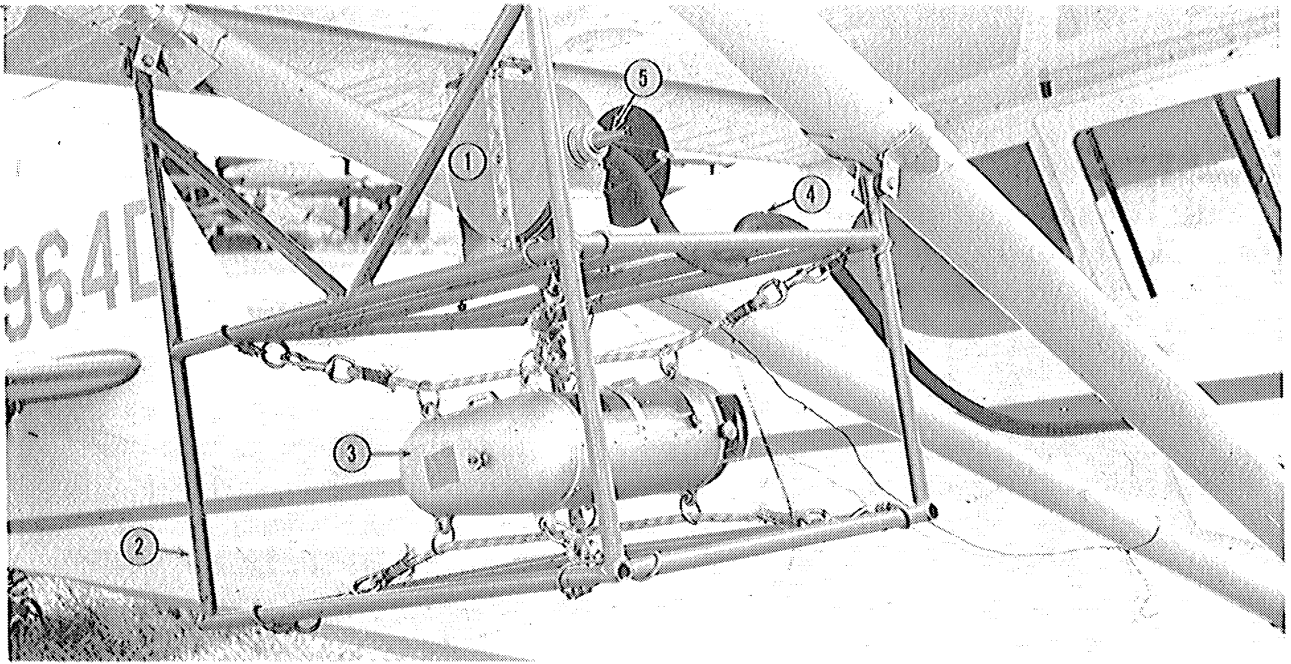


Fig. 31. Drum sampler and aerometeorograph, aerial sampling program.

scotch tape. The exposed surface is coated with a dilute solution of rubber cement. The pollen grains entering through the slit hit the tape and, by their inertia, are embedded in the rubber cement. The air in the sampler passes through a gas meter where its volume and pressure are measured.

Figure 32 shows the gas meter (1), pressure gauge (2), the vacuum pump (3), the 24V DC battery-powered motor (4), and the two 12V storage batteries (5). This equipment was located on the cabin of the plane behind the seats of the pilot and copilot. The two 12V, 72 amp-hr capacity lead storage batteries operated the vacuum pump for over 4 hr. A second set of batteries was charged while the first set was being used. Both sets were then recharged during the night.

The drum sampler is constructed so that the rotary solenoid instantly advances the drum 3 deg when an electric circuit is closed. Thus it is possible for one drum to hold 120 samples. Since one sample is lost in changing levels, the number of usable samples is generally reduced to about 100 per drum.

Six drums were prepared and kept in an airtight can to prevent any local contamination. Once a drum was used it was stored in another airtight can painted a different color so that no confusion would occur between used and unused drums.

A special microscope has been developed for counting the pollen samples collected on the drums. Figure 33 shows the microscope (1) with a drum (3) on the carriage. An ultraviolet light (2) illuminates the samples. This microscope is equipped with a micrometric-type carriage so that the microscope can be moved in a direction parallel to the axis of the exposed drum. A ratchet moves the drum at 3 deg intervals, so that the sample number on the drum can be determined easily. The samples were counted in the same sequential order as that in which they had been collected, minimizing the possibility of error in identifying the samples.

In order to keep the used drums free of contamination, the tapes were dipped in Calbera's solution immediately upon removal from the sampler. This solution stains the ragweed pollen and aided in distinguishing between those grains collected during the flight and those that may have adhered accidentally to the tape afterward.



Fig. 32. Interior-mounted equipment, aerial sampling program.





Fig. 33. Microscope for counting aerial pollen samples.

A Bendix-Friez aerometeorograph was mounted beneath the sampler ((3) in Figure 31). This instrument is insulated from the vibration of the aircraft by means of rubber shock cords and springs. It measures temperature, pressure and relative humidity by introducing air through a large slot in the front and causing it to pass over several sensing devices. The meteorological parameters are recorded by separate pens on a chart recorder. The chart drum is moved by a clock mechanism at the rate of 3.5 in. per hr. Temperature is accurate to  $\pm 0.5^{\circ}\text{C}$  and relative humidity to within  $\pm 5$  per cent. A solenoid-operated marking pen at the edge of the chart allows the observer in the plane to make the time of the beginning and ending of sampling. Height above ground was recorded by reading the airplane altimeter; the station pressure (rather than the sea level pressure) was used to set the altimeter before takeoff.

c. Flight Procedures - The flights started as early in the morning as local weather conditions would permit, usually about 0830 EST. The routine flight plan consisted of climbing to 7000 ft to reach the southern check point. At that point the vacuum pump was turned on, the gas meter read, and an air sample was taken. Normally, two 3-min samples were taken at each altitude; along flight path 3 (Figure 30) five 3-min samples were taken at each level. When the samples had been taken and the northern check point was reached, the plane reversed course and descended to 5000 ft, where the same procedure was followed. This procedure was continued, as indicated in Figure 34, until the aircraft descended to 400 ft.

As indicated earlier, it was occasionally necessary to alter this plan in the presence of clouds. The sampling times were adjusted according to the magnitude of the head- or tailwinds in order that the samples at the several levels would be one above the other. As soon as the last sample was taken, the vacuum pump was turned off. The aerometeorograph was started just prior to takeoff and turned off as soon as the plane stopped taxiing after landing. Table 21 summarizes the flights that were made. The paths designated refer to those in Figure 30.

d. Synoptic Meteorological Conditions - At the beginning of the aerial sampling program on 7 September 1959, a stationary front was oriented east-west across central Wisconsin and upper Lake Michigan at 0700 EST. The eastern United States, including the Chicago area, was under a southwesterly wind regime on the west side of a large anticyclone centered southeast of Nova Scotia.

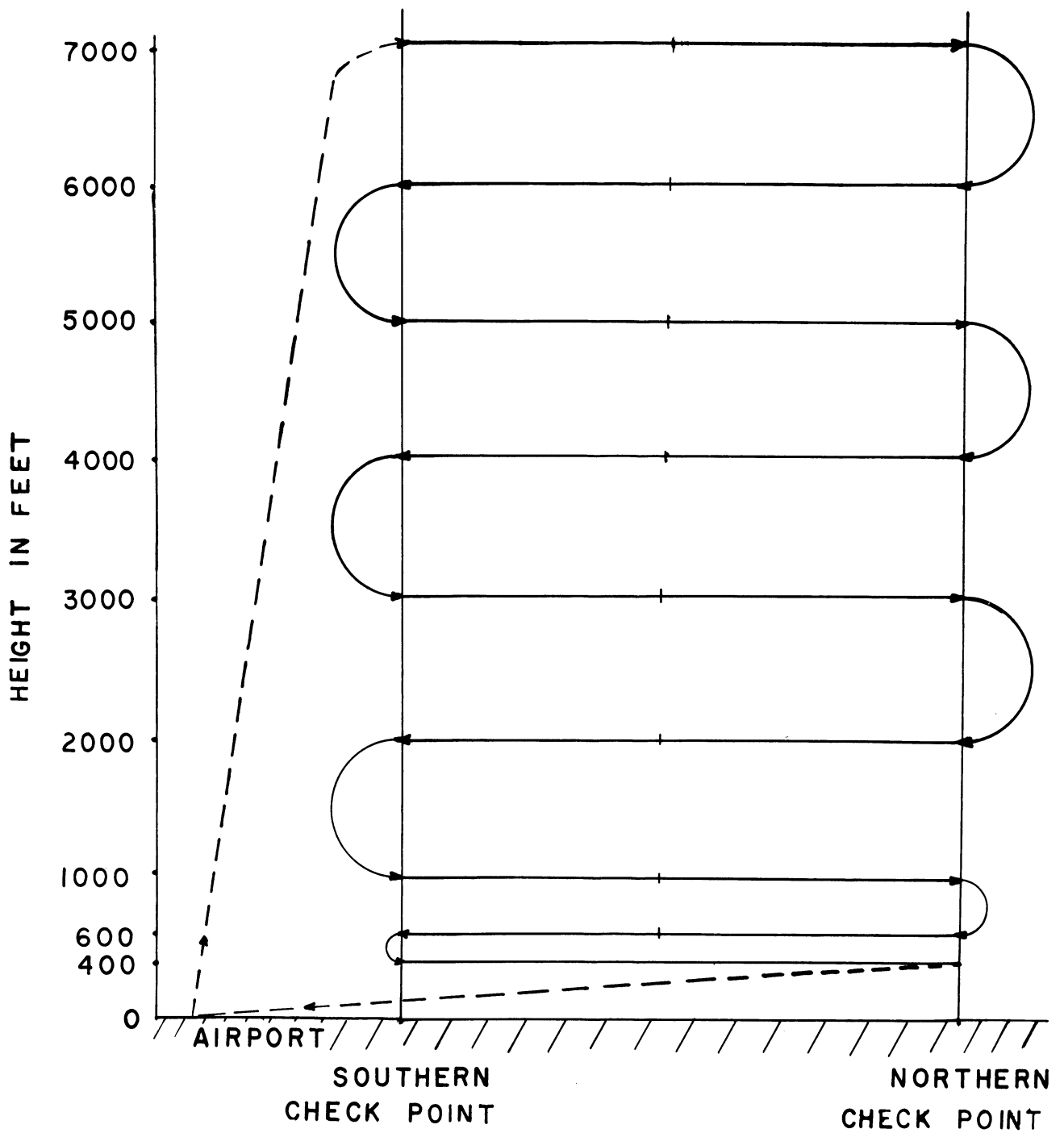


Fig. 34. Aerial sampling trajectories.

This situation persisted until the morning of 9 September, when a deepening low moved over Winnipeg with a cold front extending south-southwest through central Nebraska and then westward through central Colorado. The stationary front that had been lying north of the Chicago area had begun to move northward as a warm front. At 0700 EST on 9 September it extended from Winnipeg through Sault Ste. Marie and thence eastward through Nova Scotia.

TABLE 21

Summary of Aerial Sampling Flights Made Along the Western Shore of Lake Michigan, 7-11 September 1959

<u>Date</u>	<u>Time (EST)</u>	<u>Flight Path</u>
7	1520-1640	1
7	1724-1917	1
8	0830-1011	1
8	1100-1245	1
8	1432-1617	1
8	1647-1838	1
9	0835-1033	2
9	1058-1243	2
9	1315-1642	2
10	0831-1159	3
10	1424-1729	3
11	0927-1259	Pal-Waukeee to Ann Arbor

By the morning of 10 September the cold front had passed the Chicago area producing clear skies and northwesterly winds. The high pressure center moved rapidly to the northeast and the winds shifted to northeast. Northeast winds persisted for several days.

e. Results - As of the date of this report, the analysis of the data had not been completed. Preliminary analysis, however, makes it clear that pollen was collected at all levels, and there is no reason to believe that pollen would not have been collected at even higher levels than those traversed by the aircraft during this experiment.

The main purpose of the experiment, to test the effect of a ragweed-free area (Lake Michigan) in the light of the earlier theoretical results, was never successfully completed. Runs were made on the western shore for four days in order to establish the

mean pollen concentration. However the cold front which passed through the sampling area during the late hours of 9 September changed the air mass and the trajectory of the air, so that flights to the east of Lake Michigan would not have been comparable. The sampling carried out on 10 September indicated, as expected, that the pollen concentration was lower in the cool air than in the warm air preceding the front. If northwest winds had persisted for another day, sampling would have been carried out on the other side of Lake Michigan. The wind shift which occurred caused cancellation of these plans. Also, the pollen season was nearing its end by this time, so no further flights were made.

The sampling system and the flight procedures proved successful in the runs that were made. Under more favorable meteorological conditions the experiment could have been entirely successful. Future plans include utilization of the methods employed during this program.

### 3.5 MOBILE SAMPLING EXPERIMENT, 1958

#### 3.5.1 Introduction

Most sampling of pollen (and other particulates as well) is done from fixed sites, and indeed this has been the procedure in general in most of the research under the present project. Early in the program, however, it became apparent that the environmental variability of pollen should be known. It was for this reason that a pollen sampling experiment employing mobile samplers was undertaken. Inspection of natural pollen sources (such as those described in Section 1.5) establishes beyond any doubt that the occurrence of Ambrosia artemisiifolia is highly dependent on soil condition and botanical competition. Accordingly it is expected that the population densities of ragweed plants are not at all homogeneous. The question remains, however, on the extent to which normal atmospheric mixing will eliminate heterogeneous population densities insofar as the airborne pollen is concerned. The degree to which variability in pollen concentration exists within and between urban and rural areas was the subject of the 1958 mobile sampling program.

#### 3.5.2 Purpose and Objectives

Two mobile pollen sampling programs were designed. The first consisted of a pilot experiment to perfect the operating procedures

necessary for sampling pollen from a moving platform. This experiment is discussed in full elsewhere [26].

The objectives of the second experiment were to determine:

a. The variability of pollen concentration in the vicinity of Ann Arbor, measurements being made by 1-mi increments over a 44-mi route within a 2-hr time interval.

b. The "pollen exposure" that a person would experience by driving over a selected route. This exposure can be compared to the exposure to which he would be subjected were he to conduct all his daily activities in urban areas.

c. The temporal variations of pollen concentration at sites of different proximity to the primary pollen sources.

### 3.5.3 Procedure

a. Sampling Program - The mobile pollen sampling program was made possible by the design and installation of sampling devices on automobiles. These consisted of one flag sampler per car mounted on an aluminum rod extending horizontally 18 in. from the right front door of the car. In addition to the driver, each car carried a technician to change samplers and record operations. Samples were taken at 43 separate mile-intervals along a predetermined route in and around Ann Arbor (Figure 35). During the height of the ragweed season, the routine established was to traverse the route three times, each traverse starting at approximately 0830, 1330, and 1930 EST.

b. Survey of Pollen Sources - The variability of pollen sources was determined by counting ragweed plants in typical examples of the several kinds of surface-cover categories. Examination of the roadsides and environs of the 43-mi route revealed the following:

(1) Virtually all ragweed occurrence along road margins is in a narrow strip between the shoulder gravel adjacent to the traveled part of the road and the natural vegetation of the ditch. Roadsides which are graded in the spring but not during the ragweed season are the greatest producers of ragweed among roadsides. In no case however do the numbers of ragweed plants found along roadsides even approach the numbers found growing in cereal grain fields (paragraph 4, below).

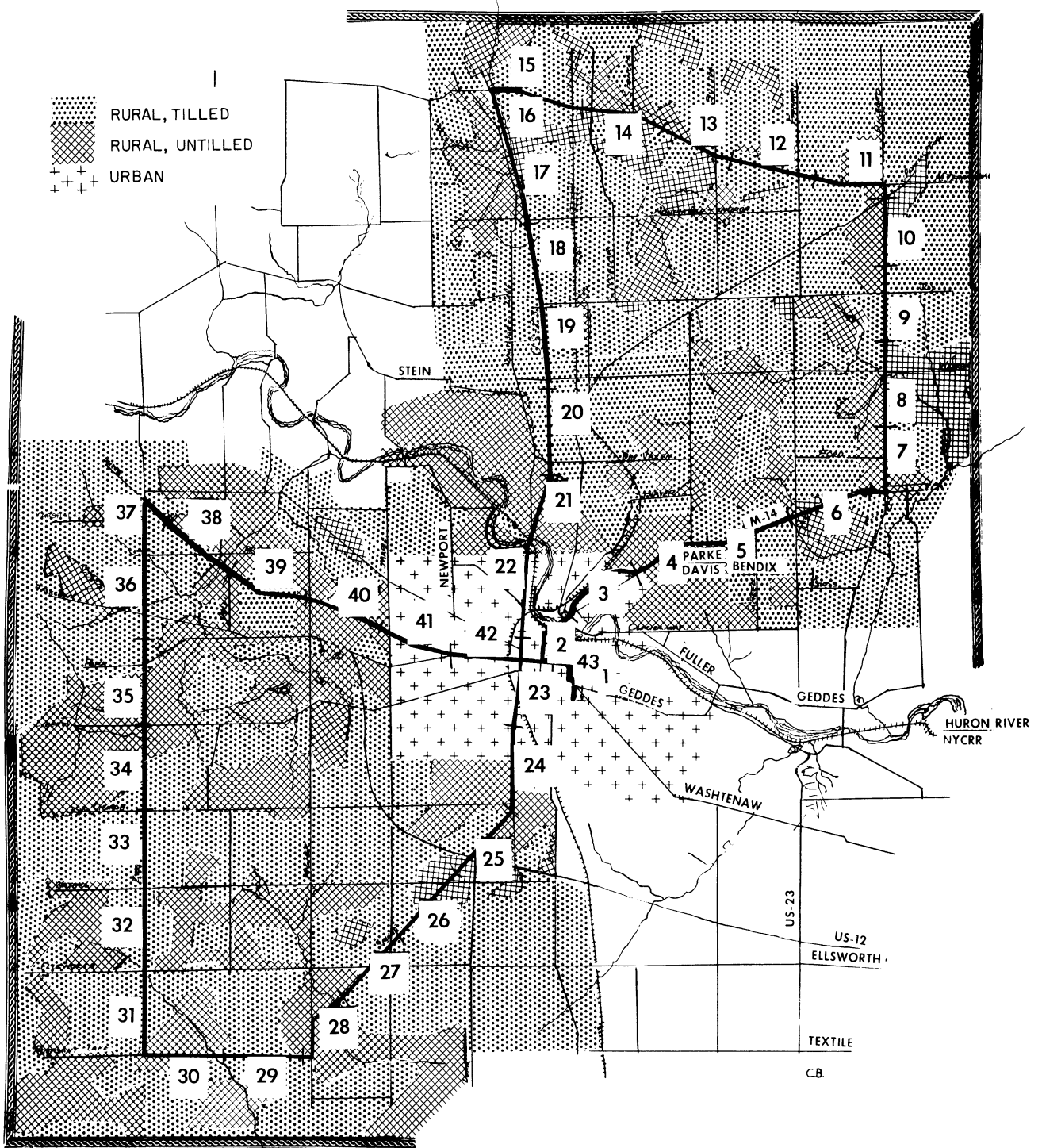


Fig. 35. Route of mobile sampling program.

(2) Very little ragweed is found along roadsides that have shoulders covered with grass which are mowed during the summer, or along roadsides that have undisturbed natural vegetation up to pavement edges or traveled portions of gravel roads.

(3) Pasturage, overgrown land, corn fields and forage crops contained very little ragweed during the period of the experiment. Average ragweed plant population for several representative fields of these four types of cover was 523 plants per acre.

(4) Cereal grain fields are the main source of ragweed. Seedlings develop during the ripening stages of the grain and grow rapidly to maturity in the stubble after the grain is harvested, between mid-July and early August. Grains which are planted with a cover crop yield one-tenth as many ragweed as do grain fields with no cover crop. Averages of ragweed plants counted in fields with winter wheat, oats and barley, and with no cover crop, were about 172,000 plants per acre--over 300 times the plant density found in pasturage. There was no evidence that ragweed was more prevalent in one type of grain field than in another.

(5) There is little or no ragweed in urban areas where the plant cover consists of lawns, regardless of whether or not the lawns are well kept. There are, however, important pollen sources within urban areas wherever large regions have been disturbed (such as in new subdivisions) and the disturbed soil left dormant for the spring and summer months. One such lot yielded 56,500 plants per acre. There were only two or three areas of this type in 1958 within Ann Arbor, and they were on the periphery of the city.

(6) The only other significant habitat of ragweed plants is along railroad right-of-ways. Although the frequency of railroads in the vicinity of Ann Arbor is low, the density of ragweed along railroad embankments is high, because of the frequent disturbances the soil undergoes during maintenance operations by railroad crews. One count yielded a density equivalent to almost 13,000 plants per acre.

On the basis of the results of this survey, the area of Ann Arbor and vicinity has been categorized as either 1) urban, 2) rural untilled (woods, pasture and brushland), and 3) rural tilled. The areas are appropriately indicated in Figure 35.



c. Wind Records - In order to determine the wind conditions during the sampling program, records from the anemograph at the Meteorological Laboratories at the University of Michigan were compared with the records obtained from the nearest first-order U. S. Weather Bureau station located at Willow Run Airport, 10.5 mi east-southeast of Ann Arbor. Since no systematic differences in either wind speed or wind direction between the two records were noted, data from the Meteorological Laboratories were used (differences in wind speed were less than 4 mph; differences in direction were generally less than 30 deg. Exposures of these anemometers are similar, 25-37 m above the surface).

Wind data were abstracted for half-hourly intervals. For the 137 intervals covering the experiment, the average wind vector was 268 deg, 9.3 mph.

#### 3.5.4 Analysis and Results

a. Areal Variations - In spite of the 300-fold differences in ragweed plant densities between areas of different histories, one would not expect to find such large differences in pollen concentrations. Indeed the observations substantiated this conclusion. On the other hand, however, there still remained a great deal of persistent variability from one area to another that was related closely to the nature of the vegetation in the area.

The largest total number of pollen for any region, detected during the 11-day test period, 18 August through 12 September, was along sector 29 (see Figure 35); the next largest was along sector 12. The fewest pollen were caught in urban areas, with the notable exception that the smallest total pollen count of all was for sector 38, a rural-tilled area. The lower counts in urban areas may be explained by the reduced local ragweed plant population, but a contributory factor may be the fact that the automobile upon which the samplers were mounted had to move slowly through the center of the city, making frequent stops. The efficiency of the flag sampler was reduced accordingly in these areas. The efficiency, although high at wind speed above 15 mph, drops to zero for calm conditions, hence, when the automobile is moving slowly the speed and direction of the wind relative to the car should be measured and recorded.

A rigorous analysis of the data collected would provide for the speed of the car, its direction, the corresponding speed and

direction of the wind, and the efficiency of the sampler. With these variables known, the actual concentration of pollen per unit volume of air could be evaluated. The relation

$$\chi = \frac{K C}{T E V}$$

can be used, where  $\chi$  is the concentration,  $C$  is the pollen count taken from the flag sampler,  $T$  is the time interval during which the sampler was exposed,  $E$  is the efficiency of the sampler (see [26]),  $V$  is the resultant wind speed past the sampler (the vector difference of the velocities of the car and wind), and  $K$  is a constant related to the units desired for the final concentration.

Because this experiment was of the nature of a trial run, all the information needed for a complete analysis of pollen concentrations was not collected on the sampling trips. Therefore, the results presented here are based solely on the raw pollen counts.

Total pollen counts over all trips for each of the 43 sectors are listed in Table 22. The counts are listed in decreasing order of magnitude under appropriate headings referring to the prevailing environmental plant cover. Route sector numbers are given in parentheses. It can be noted that the average total count in urban areas (1178) is about 30 per cent less than the average for rural-tilled areas (1658). The average total count for rural untilled areas (1309) is intermediate.

An analysis of variance indicates that the differences between the means of the three columns are significant at the 2 per cent level. Thus, categorizing the various sectors of the route provides a means for interpreting the observed pollen counts. Results indicated that, despite the natural dispersive action of the wind, differences in vegetative cover have a significant effect on the local pollen concentrations.

The area surrounding sector 29 is a case in point. This 1-mi segment had the second highest total count of all (see Table 22). Since the prevailing wind was from the west during the period, one might expect the area immediately downwind (sector 28) to have had high counts also, even though that station is surrounded by woods and pasture. It is true that the counts were high (2034), but only 65 per cent as high as that of sector 29. Upwind from sector 29, another region of woods and pasture had one

TABLE 22

Total Pollen Counts for each One-Mile Increment,  
Automotive Pollen Sampling Experiment,  
by Environmental Category

<u>Urban</u>		<u>Rural Untilled</u>		<u>Rural Tilled</u>	
Pollen Count	Route Sector	Pollen Count	Route Sector	Pollen Count	Route Sector
1488	(1)	2034	(28)	3142	(12)
1383	(23)	1685	(24)	2562	(29)
1188	(43)	1674	(16) *	2161	(15)
1099	(3)	1606	(14)	1772	(5)
1040	(22)	1574	(10)	1692	(33)
1029	(2)	1533	(9)	1678	(37)
1020	(42)	1469	(17)	1636	(18)
		1339	(6) *	1625	(11)
		1278	(7) *	1615	(25)
		1239	(35)	1489	(13)
		1206	(21)	1483	(27)
		1066	(39) *	1473	(31)
		1035	(8)	1468	(26) **
		1033	(20) *	1398	(32)
		1020	(41)	1337	(34)
		976	(30)	1311	(36)
		918	(4)	1141	(19)
		884	(40)	868	(38)
<hr/>		<hr/>		<hr/>	
1178	▽	1309	▽	1658	▽

\* 50% rural untilled, 50% rural tilled

\*\* 25% rural untilled, 75% rural tilled    ▽ Arithmetic mean

of the lowest counts (1976). Thus the data provide a guide to the evaluation of the effects on local pollen concentrations of the proximity and direction of areas cleared of ragweed, and/or of areas from which the ragweed has been eradicated.

b. Temporal Variations - Depicted in Figure 36 are pollen counts for a single 1-mi sector (No. 29) for all trips of the experiment. The temporal variations in evidence in this figure are to be taken as relatively typical of a rural area. There are large variations both from day to day and among the several trips on a single day. As expected from the fixed sampling sites of other studies, the counts are generally much higher in the morning hours than at other times during the day. The average pollen count on the morning trip was almost twice that of the midday trip, and more than four times as great as that of the evening trip. It should also be mentioned that this diurnal variation was greater in rural than in urban areas.

A peculiar feature of Figure 36 is that the largest morning counts do not necessarily take place on the same day as the largest afternoon and evening counts. Such results likely are related to local weather conditions (wind, humidity, etc.) which may be very transient. There are no available observations to explain adequately these singular observations, although it would appear that to attempt to study these phenomena and to assimilate the necessary observations would be a most fruitful objective of future research.

### 3.5.5 Conclusions

Conclusions of the mobile pollen sampling program are summarized as follows:

a. Because of the great variation in ragweed density, a person traveling through heterogeneous countryside will be subject to an average of 10 per cent variation in pollen count. Urban area counts, however, are 70 per cent below the rural-tilled pollen counts.

b. Concentrations from one particular rural area to another may differ by a factor of four when average counts are compared.

c. The difference in pollen count between urban, rural-untilled, and rural-tilled areas is significant at the 2 per cent level.

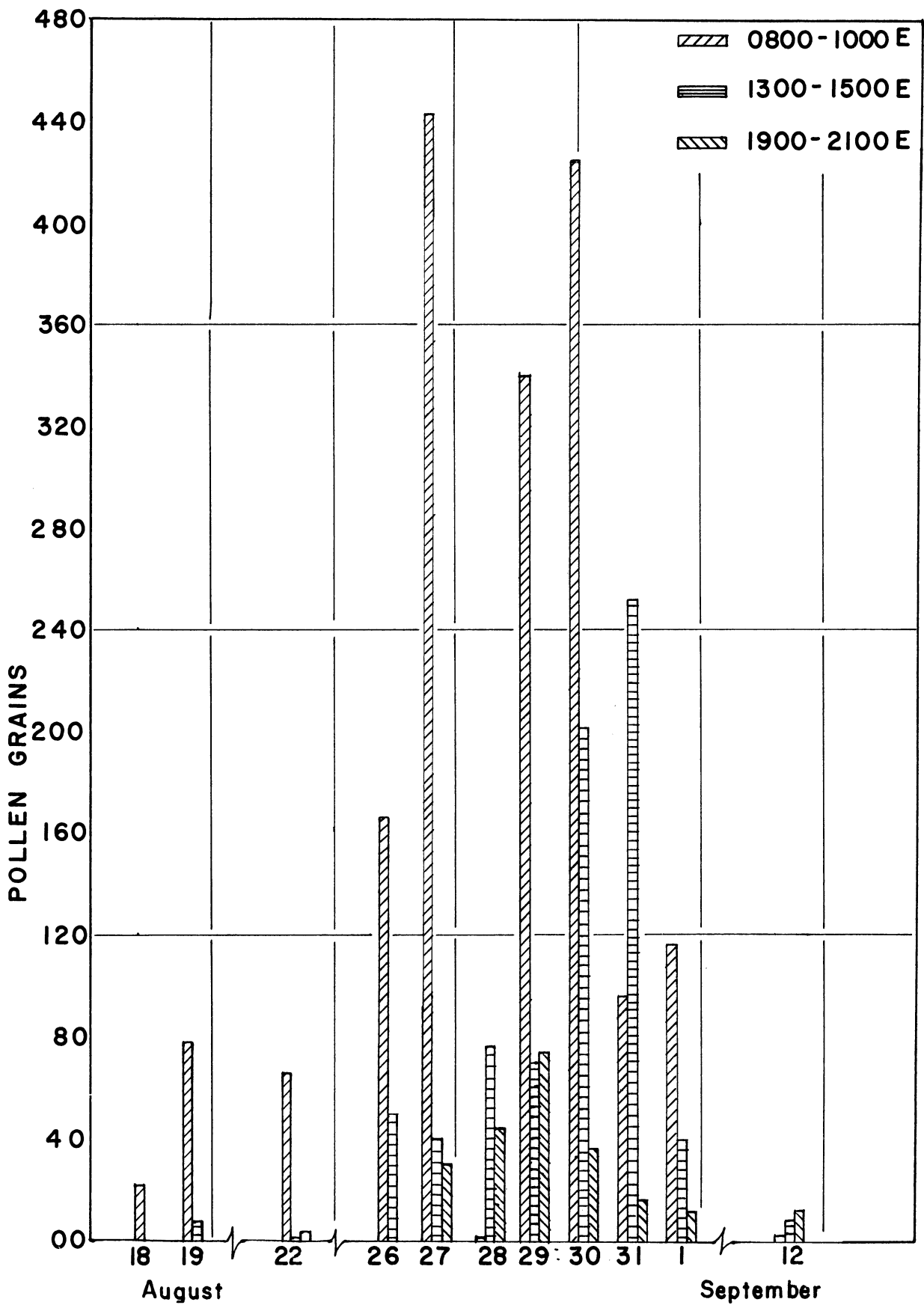


Fig. 36. Pollen counts from typical one-mile sector, mobile pollen sampling experiment.

d. The well-known morning maximum of pollen production is corroborated by this experiment, regardless of location on the 43-mi route. For an unexplained reason, the afternoon maximum is delayed over the morning occurrence of the maximum count by about two days. No such difference was noted for the evening counts.

e. Accurate determinations of pollen concentration can be computed from moving automobiles employing flag samplers if suitable supporting instrumentation (e.g., anemometry), and proper procedures (e.g., establishing time intervals within sampling stations) are established.

### 3.6 FACTORS INFLUENCING THE RELEASE AND TRANSPORT OF POLLEN

#### 3.6.1 Introduction

Although earlier analyses of the data collected during the 1957 preseasonal experiments have provided a great deal of information on pollen release and transport, it has always been asserted that additional knowledge, particularly in regard to short range meteorological influences on pollen emission, could be gleaned from the data through additional study.

The experiment has been described elsewhere in detail [26], but for this discussion it is summarized briefly. Three thousand ragweed plants, previously brought to early maturity in the greenhouse, were replanted in a plot of 13 ft radius on State Prison Farm property near Jackson, Michigan. Two gravity slides were placed near the center of gravity of each quadrant of this central plot, one at ground level and one at a height of 0.6 m. Gravity slides were also placed at the 0.6-m level at 20 deg intervals on circles of 20, 40, 80, 160, and 320 ft. The eight slides within the plot were changed at 2-hr intervals; those outside the plot were changed every 4 hr.

Research in particulate sampling has shown that the gravity slide as a device for sampling pollen concentrations yields only the coarsest first approximation. Hence, it was realized that any conclusions drawn from this experiment would have to be regarded as tentative. Nevertheless, considerable insight was gained that has proven invaluable in more recent studies, particularly the prediction of ragweed pollen concentrations,

and the construction of a mathematical model for the process of flotation and reflation of pollen (Sections 3.8 and 3.7 of this report).

### 3.6.2 Transport of Ragweed Pollen

Two questions fundamental to the study of ragweed pollen transport are: 1) What is the fraction of the pollen produced that leaves the source? 2) How far is the pollen transported by the wind? An answer to the first question can be approximated by computation of the following ratio:

$$R_1 = \frac{\text{Total pollen count at ground level in plot}}{\text{Total pollen count at 0.6 m level in plot}}$$

where the numerator is given by a sample of pollen which has been deposited and is not available to be transported out of the plot, and the denominator is given by a sample of the pollen which is being transported by the wind away from the source. The average value of  $R_1$  is about 16. Hence,  $R_1^{-1}$ , the fraction of pollen produced that leaves the source, is approximately 6 per cent.

Three other ratios  $R_2$ ,  $R_3$ , and  $R_4$  may be defined. They are:

$$R_2 = \frac{\text{Total pollen count at 0.6 m level in plot}}{\text{Total pollen count on 20-ft arc}}$$

$$R_3 = \frac{\text{Total pollen count on 20-ft arc}}{\text{Total pollen count on 40-ft arc}}$$

$$R_4 = \frac{\text{Total pollen count on 40-ft arc}}{\text{Total pollen count on 80-ft arc}}$$

Frequency distributions of these ratios were computed. Interpretation of  $R_2$  was difficult due to the different numbers of samplers at the two locations. However,  $R_2$  fell within the range of 0.6 to 1.5 in most cases. The median value was 1.1, and the maximum was 80. The ratio  $R_3$  had a median value of 4; the maximum value of  $R_3$  was 10.  $R_4$  had a much broader frequency distribution (explained by the very low pollen counts--zero in one case--observed on the 80-ft arc on many occasions) and was bimodal. The median value of  $R_4$  was 8. It is of interest that the median values of  $R_3$  and  $R_4$  were in the ratio 1:2, the same as the ratio of the distances.

Some attempt was made to assess the role of wind speed on the transport of pollen from the source, although this was done with considerable reservation, because of the large effect of wind speed on the efficiency of the gravity slide as a sampler. It was noted, however, that in the period from 0400 to 0800 EST pollen counts at the 20-ft arc were highest for wind speeds of 5-6 mph at the 0.6 m level. This relationship was less pronounced at the 40-ft arc, and was not in evidence at the 80-ft arc.

### 3.6.3 Diurnal Predictors

The subject of prediction of ragweed pollen concentrations is considered in detail in Section 3.8 of this report. Prior to that study, a pilot investigation was undertaken, and has provided some guidance for the choice of predictors in the development of the comprehensive prediction scheme.

On the basis of the data collected in the 1957 preseasonal experiment, an attempt was made to isolate these meteorological parameters which influence the emission of pollen. Examination of the data led to the suggestion that high temperatures on one day may reduce the pollen maximum on the following day. No relationship between pollen emission and low level atmospheric stability was evident, other than that which would be anticipated in view of the strong diurnal periodicities of both pollen emission and stability. Vapor pressure deficit (saturation vapor pressure minus actual vapor pressure), a measure of the tendency for pollen drying on emission was also studied, but it showed no relationship to the actual emission. Apparently any relative humidity below 95 per cent permits pollen emission; on the other hand, fog, with humidities near 100 per cent delays pollen emission. This is a confirmation of earlier botanical findings [26]. Peak emission days are likely to be clear days; cloudiness (and the consequent suppressed solar radiation) reduce pollen emission. Other parameters were considered, but the relationships, if any, were either too complex or too weak to show up. Study of the effect of stability was particularly hampered by the inadequacy of the data.

The "normal" diurnal cycle of the ragweed pollen emission was investigated. Previous average counts by 2-hr intervals at the 0.6 m level with a central plot have been reported [3]. The most prominent feature of the daily pattern found in that study was the early morning maximum. However, a slight secondary rise was in evidence also between 2000 and 2400 EST. This secondary maximum is small on the average curve, but examination of the data shows it to



be a very persistent feature. In one case this secondary maximum was as great as 20 per cent of the primary morning maximum of the same day. Since this secondary peak does not always occur at precisely the same time, its effect is spread out on the average curve, and its magnitude is consequently diminished.

This phenomenon may be solely a manifestation of the manner in which the efficiency of the gravity slides varies with the wind speed, a very considerable dependence. However, in the absence of any positive proof that the secondary maximum of pollen count is due to variations in the efficiency of the gravity slide, it may be profitable to attempt to explain this phenomenon on some other basis, in the hope of shedding additional light on the phenological behavior of ragweed. Indeed, careful examination of the data in an effort to uncover some relationship between the low level wind speed and the time and extent of the secondary maximum did not reveal any substantial evidence that these maxima could be attributed to variations in wind speed to affect the efficiency of the gravity slides [27]. The suggestion may be made (but without any impelling justification, and only as a possible initial hypothesis from which further research may proceed) that the secondary maximum of pollen count is indeed a secondary maximum of pollen emission coincident in time with the period in the diurnal plant cycle when the plants are physiologically ready for the florets to open, but that dehiscence is retarded until morning by the low temperatures and high humidities. Additional data are required to resolve these questions completely.

### 3.7 A MATHEMATICAL MODEL OF THE FACTORS INFLUENCING THE RELEASE, FLOTATION AND REFLotation OF POLLEN

#### 3.7.1 Introduction

The first data from the 1959 preseasonal experiments (Section 3.3) to be analyzed in detail have been the counts of open Ambrosia flowers. Although these data lend themselves to a wide range of studies concerning the phenology and physiology of ragweed, the objective of the present investigation was to ascertain the role of the wind in removing pollen from the immediate vicinity of the plants. The wind may act in two ways other than by any direct effects on the plants themselves: 1) the wind may carry the pollen away from the plant immediately after it is released (flotation), or 2) the pollen may first fall to the leaves and the ground in the immediate vicinity of the plants and only after some period of time be picked up by the wind and carried away (reflotation).

Presumably both of these mechanisms are operative. It is the primary aim of this study to determine the extent of the role that each plays.

### 3.7.2 Data

Three basic sets of data have been employed in the study: the counts of open flowers, the records of the alternating rotobar samplers, and wind speed records taken at a height of 0.5 m (approximately plant height). The flower counts, as originally made, consisted of the numbers of flowers on selected spikes that were observed to be open at a particular time. For the purposes of this analysis, the required information was the number which actually opened in each 15-min interval. Thus, differences were taken between successive counts. The numbers derived in this way are given the symbol  $F$ .

It is often difficult to distinguish unopened flowers from those which had undergone on that day a complete cycle of opening, stamen extension, and subsequent stamen retraction and closing. Therefore, late in the morning, when few flowers are opening for the first time, the counts of total opened flowers occasionally decreased. When such was the case the number of newly opened flowers in a particular period was taken to be zero.

The alternating sampler experiment was designed to give the pollen concentrations within a short increment of time under different wind conditions. Interest was only in the average pollen concentration during the time interval, without regard to variations of wind speed within each time interval. Thus the numbers of pollen collected on the three rotobars have been summed, and this figure divided by the time of the run in minutes, giving what is equivalent to an average pollen concentration. This variable has been assigned the symbol  $C$ .

Plots of the variation of  $F$  and  $C$  during the various mornings on which observations were carried out have been made. Curves of  $F$  and  $C$  are roughly parallel in many instances, but there are also some considerable differences (see Figure 37). The most apparent difference indicated that pollen continues to be sampled long after the flowers have ceased releasing pollen. This is, in itself, direct evidence that reflation of the pollen does occur.

### 3.7.3 The Model

The model which is described below is an attempt to account quantitatively for the differences between the curves of  $F$  and  $C$ . First, a number of quantities will be defined:

- $C_i$  the number of pollen collected on the rotobars during the time interval  $i$  (proportional to the mean volumetric pollen concentration).
- $E$  the ratio between the expected number of pollen grains available for sampling during a given interval of time, and the number caught on the samplers.
- $F_i$  the number of flowers observed to have opened during the time interval  $i$ .
- $G_i$  the number of pollen grains resting on the leaves, ground, etc., within the plot, at the end of the time interval,  $i$ .
- $H_j$  a dampness factor representing the ease with which pollen can be reflowed when it had been released from the flowers  $j$  time intervals previously.
- $K$  a constant whose value is initially unknown, related to the effectiveness of the wind in picking up pollen.
- $P$  the average number of pollen grains released by each opening flower.
- $R_{i,j}$  the number of pollen grains released during the time interval  $i$  which are expected to be remaining on the leaves and ground within the plot  $j$  time intervals later.
- $S$  a sampling factor, the ratio of the total number of plants (or spikes, or flowers) in the plot to the number actually observed.
- $V_i$  the average wind speed at plant height during the time interval  $i$ .
- $\phi_i$  the ratio of the number of pollen immediately carried away by the wind to the total number released during the time interval  $i$ , a function of the wind speed.
- $\psi_i$  a ratio representing the effectiveness of the wind in reflowing pollen which are on the leaves and ground during the time interval  $i$ , a function of the wind speed.

In terms of the above symbols, the number of pollen grains released in interval  $i$  is  $SPF_i$ . Of these,  $SPF_i \phi_i$  are immediately available for sampling and  $SPF_i(1-\phi_i)$  fall to the ground or subjacent leaves. During this same time interval  $SPF_i(1-\phi_i)H_0\psi_i$  grains are refloated and are also available for sampling. Then  $R_{i,0} = SPF_i(1-\phi_i)(1-H_0\psi_i)$ . Similarly, it can be seen that during the next time interval a fraction of these  $(H_1\psi_{i+1})$  grains will be refloated, so that  $R_{i,1} = SPF_i(1-\phi_i)(1-H_0\psi_i)(1-H_1\psi_{i+1})$ . In general,

$$R_{i,j} = SPF_i(1-\phi_i) \prod_{k=0}^j (1-H_k\psi_{i+k}) .$$

The number of pollen caught on the samplers will be  $E$  times as great as the number available for sampling, or

$$C_i = ESPF_i\phi_i + \psi_i \left[ ESPF_iH_0(1-\phi_i) + E \sum_{j=1}^{i-1} H_j R_{i-j,j} \right] .$$

Substituting,

$$C_i/ESP = F_i\phi_i + \psi_i \left[ H_0F_i(1-\phi_i) + \sum_{j=1}^{i-1} \left\{ H_jF_{i-j}(1-\phi_{i-j}) \prod_{k=0}^{j-1} (1-H_k\psi_{i-j+k}) \right\} \right] .$$

In a similar manner, we can also write

$$(G_i - G_{i-1})/SP = R_{i,0} - \psi_i \sum_{j=1}^{i-1} H_j R_{i-j,j} .$$

If we assume  $G_0 = 0$ , and know (or assume values for)  $F$ ,  $\phi$ , and  $\psi$ , it is then possible to compute  $C_i/ESP$  and  $G_i/SP$  for as long a time as the independent variables are given. In the computations which have been carried out on the IBM 704, it

has been assumed that  $\phi_i$  and  $\psi_i$  are functions of the wind speed in the form  $V_i^2/(K^2+V_i^2)$ . Values of the constant,  $K$ , need not be the same for both  $\phi$  and  $\psi$ , and various combinations (25 in all) have been tried, with  $K = 0.5, 1.0, 3.0, 5.0, \text{ and } 7.0$ .

It should be noted that certain restrictions must be placed on  $H_j$ , specifically, that  $H_0 \leq 0.5$ , and  $H_j \leq H_{j+1} \leq 1$ . If  $H_0 = 0.5$ , and all  $H_j = 1$  for  $j \geq 1$ , this corresponds to a case where all pollen is considered to be equally susceptible to being refloated, regardless of the length of time it has been on the ground. That is to say, any effects of initial dampness, stickiness, and tendency for clumping which the pollen may have, is neglected. If all  $H_j$  are set equal to a given set of numbers, other than 1, it implies a constant drying effect independent of external meteorological conditions. If the values of  $H_j$  are made functions of the relative humidity (and perhaps also of wind speed, insolation, temperature, etc.) between time intervals  $i$  and  $(i-j)$ , this would correspond to asserting that these meteorological factors influence the degree of refloation. In the preliminary computations only the first two cases were studied. For the second case the values of  $H_j$  were taken to be 0.05, 0.20, 0.40, 0.70, for  $j = 0, 1, 2, 3$ , respectively, and 1.0 for  $j \geq 4$ . Each time increment is 15 min.

#### 3.7.4 Preliminary Results and Conclusions

Only a limited number of computations have been carried out to date. Analysis of these has produced some interesting results, however, and the direction of further analysis is indicated. The particular case which is discussed here is that of 26 June 1959. Plots of  $F$  and  $C$  for that day are shown in Figure 37.

The computer program yields numbers which should be proportional to the quantities of pollen sampled, for each of the two sets of

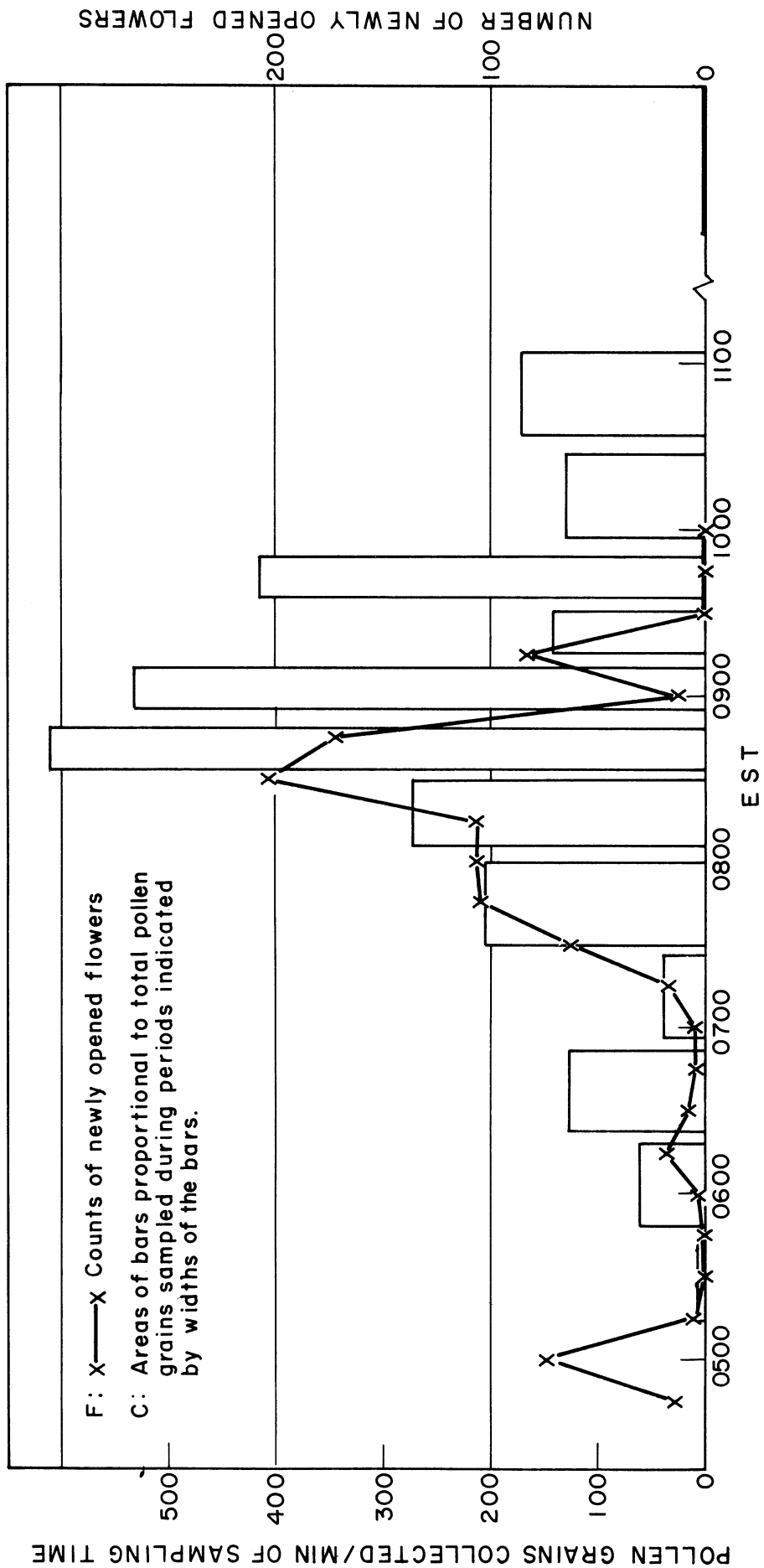


Fig. 37. Pollen grains sampled (bars) and flower opening counts (curves) as a function of time.

values of  $H_j$ , and for each of the 25 combinations of values of  $K_\phi$  and  $K_\psi$ , for each 15-min increment of time. To simplify the comparison between the computations and the observations, two statistics have been selected:  $\rho_1$ , the percentage of pollen expected to be sampled after the last flower has opened (approximately 0930 EST on 26 June), and  $\rho_2$ , the percentage expected to be sampled in the first five 15-min periods following the opening of the last flower. These percentages have been plotted on a graph whose axes are  $K_\phi$  and  $K_\psi$ , and isopleths drawn to connect equal values of the appropriate variables. The resulting families of curves are shown in Figure 38.

Referring to the alternating sampler results, it is found that a total of nearly 55,000 pollen grains were collected on the samplers between 0514 EST and the following midnight. Of these, 16,652 were collected after 0930 EST, and just over 15,000 were collected in the first 75 min after 0930. In other words, 30.3 per cent of the pollen sample was collected after the last flower had opened (in the sample observed) and 27.3 per cent in the 75 min immediately following this event. With these values available one can refer again to Figure 38, and it is seen that, although such a point is off the scale of the graph (values of  $K$  greater than 7 had not been tried), the appropriate values of  $K_\phi$  and  $K_\psi$  are fairly well indicated. In other words, in order to bring about agreement between the model and observations, one should choose  $K_\phi = 8$  and  $K_\psi = 2$ .

In view of the fact that wind speeds at the levels used here (0.5 m) tend to vary only between 0 and 3 m per sec, one can conclude that the amount of pollen which is available for sampling immediately upon being released from the flowers is small and relatively insensitive to wind speed, whereas variations in the

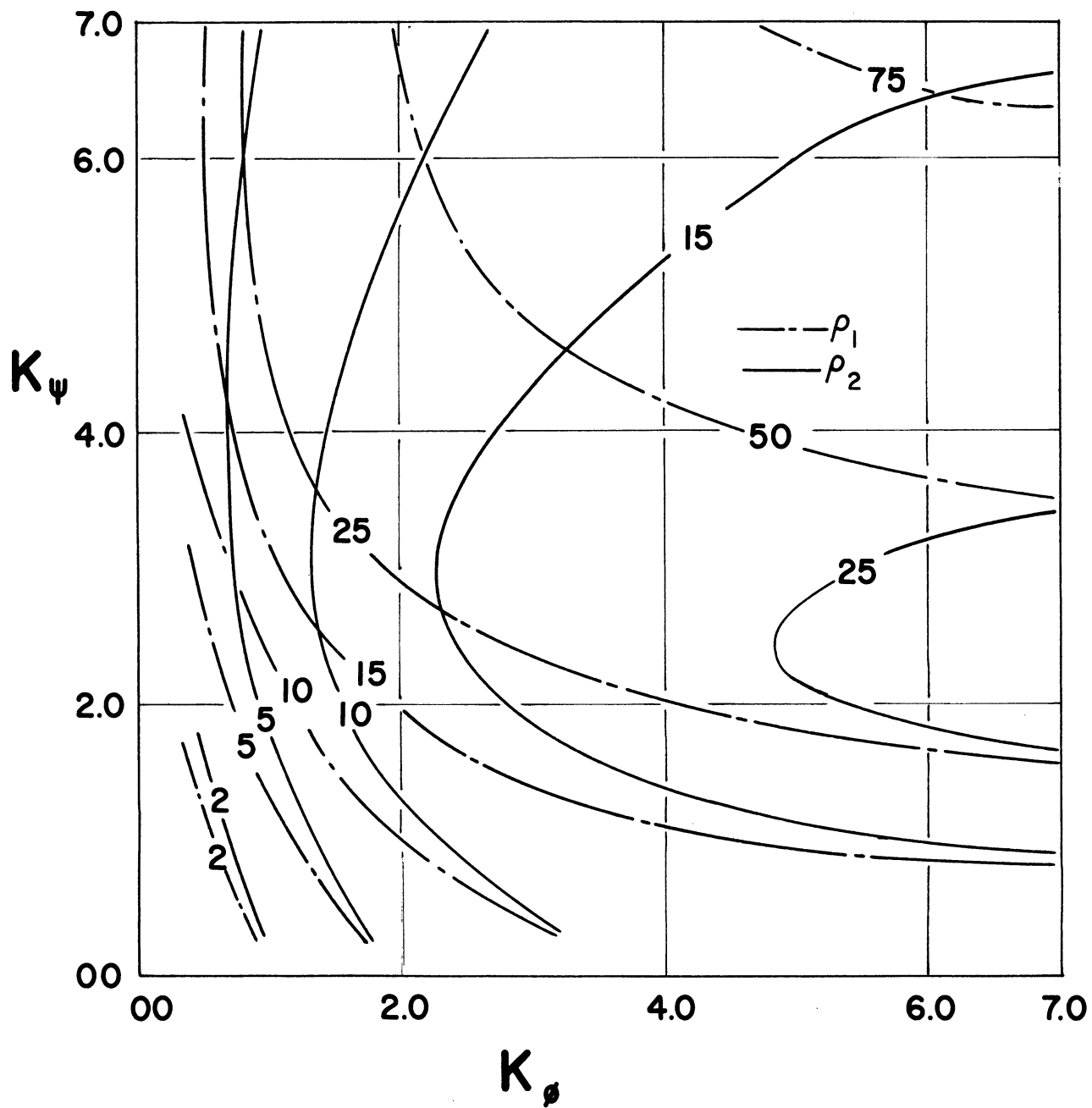


Fig. 38. Values of  $\rho_1$  and  $\rho_2$  as functions of  $K_\phi$  and  $K_\psi$ .



wind speed have a marked effect on the ability of the wind to refloat pollen that had previously been deposited on the leaves and on the ground.

The model computations of this section were based on the assumption of a fixed, but not inconsequential, drying effect. The computations indicated very little difference between the assumed drying effect and none at all. If the values of  $H$  had been smaller, the computations based on the model would have yielded smaller estimates for the values of  $K$ . It is not possible, at this time to estimate with confidence the extent to which the conclusions reached in the previous paragraph would be altered, although very great changes are not anticipated. In any case, further study along these lines should suggest not only the effectiveness of the wind in floating and refloating pollen, but also the degree to which pollen, after falling to subjacent leaves and the ground, is dried before refloatation is to be expected.

### 3.8 PREDICTION OF SEASONAL AND DAILY POLLEN CONCENTRATIONS

#### 3.8.1 Introduction

Among the numerous specialized problems within this overall program that are of specific interest to the meteorological group, and are the responsibility of that group especially, the prediction problem is probably most anticipated and least understood. It is necessary, therefore, to emphasize that, whereas prediction is a service that can be rendered within certain limits of error once the underlying processes and their interrelationships are understood, the meteorological contribution to the resolution of all of the problems under discussion here is much broader than mere prediction. It might be said that prediction and proper verification provide a kind of ultimate test of the state of knowledge and adequacy of observation of the processes involved; but in itself, prediction is only one of many potential contributions in the field of meteorology. The work of this project demonstrates this, in view of the extensive investigations on the fundamental problems of atmospheric turbulence and diffusion, ragweed ecology, phenology, air sampling, pollen emission, germination, etc., which are discussed elsewhere in this report. From the practical viewpoint, however, the predictions are expected to aid the clinician in the treatment of hay-fever and asthma sufferers, and to be of use to the patients themselves in planning their activities.

The prediction problem is logically considered in terms of components in two distinct time scales. The first of these is the long-period effect of early-season weather upon the timing of the maximum pollen release and upon the total amount of pollen produced. This long-period prediction problem has much in common with the agricultural crop-forecasting problem, and is treated similarly. The second component of the prediction problem comprises the detailed time-distribution of pollen concentrations in the air during the season of active pollination. These day-to-day and hour-to-hour variations of pollen concentrations are determined mainly by the action of specific weather occurrences upon the local ragweed population at a given location, and hence, they are predicted by the use of day-to-day weather forecasts and current weather information during the pollination season.

### 3.8.2 Long Term Effects

It is a truism among those subject to pollenosis, and their physicians, that considerable variation in "intensity" of symptoms exists from one season to another. By and large, it is assumed that this subjective "intensity" is more or less proportional to the pollen concentrations encountered. To date no substantial evidence has appeared to invalidate this broad assumption.

The immediate objective of this research was to determine whether any relationship between early-season weather and the character of the ensuing pollen season could be established. Fortunately, a consistent series of pollen counts from a Durham standard sampler [28] has been maintained at the Allergy Clinic, University of Michigan Hospital, over a 14-yr period, and these data have been made available for study. There are good reasons, detailed by Harrington, et al [29], to feel that the Durham gravity slide sampler is not completely satisfactory; however, no extensive records compiled by the use of more adequate sampling methods are available as yet. This long series of pollen records, together with weather data contained in the Local Climatic Summaries published by the United States Weather Bureau, was examined in an attempt to evaluate such relationships as might be found between pollen-season characteristics and antecedent weather.

a. The Seasonal Trend of Pollen Concentrations - For the purposes of the present study, the Durham pollen counts are assumed to be representative of airborne pollen concentrations. The seasonal distributions of these counts are shown in Figure 39

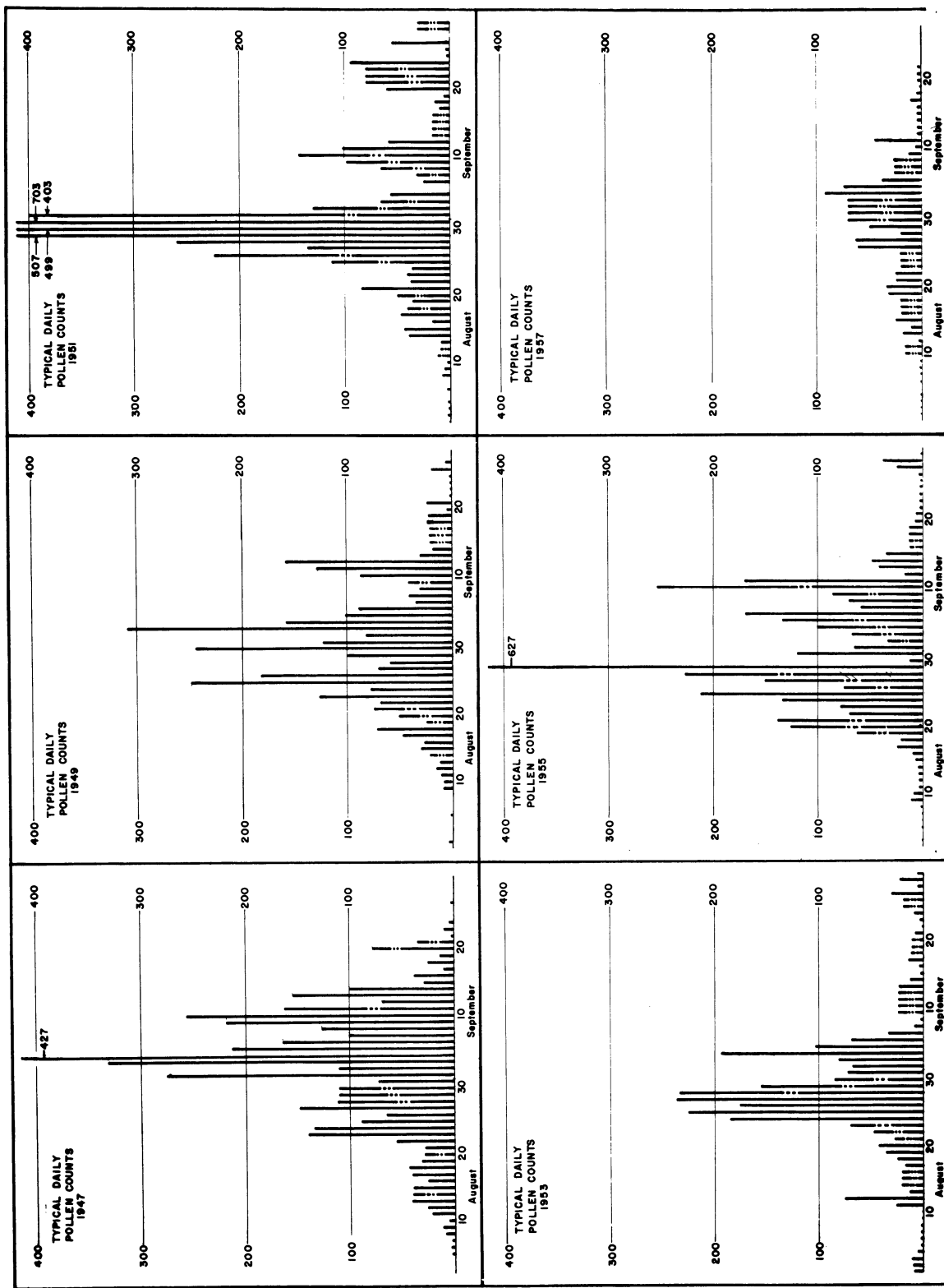


Fig. 39. Seasonal pollen counts by Durham gravity-slide sampler at Ann Arbor, Michigan, typical of 14-yr record computation.

by means of bar graphs. Because the samples were left exposed over weekend and holiday periods, it was necessary to distribute the cumulative counts for these periods among the included days. This was done as carefully as possible so that this process would not modify the general tendency of the seasonal curves significantly.

Study of these graphs suggests several characteristics that might be used to describe the seasonal distribution of pollen counts in any one year (see Table 23) The characteristics listed below are probably not entirely independent of one another, but their variability suggests the means of invoking antecedent weather parameters as prediction criteria.

(1) The maximum pollen count for the season appears to vary widely.

(2) The date of the observed maximum varied from 24 August in 1948 to 5 September in 1946.

(3) The total pollen count changes significantly from season to season.

(4) The duration of the season of significant pollen concentrations appears to fluctuate appreciably.

b. Procedure - For the long-period analysis, it is convenient first to smooth the data to reduce short-term fluctuations and bring out more clearly the features of the annual pollen season curves. A normal curve was fitted to the observed pollen concentration data for each of the 14 seasons. In this way three characteristics of each season were evaluated: (1) the total pollen yield, (2) the modal pollen count, and (3) the modal date.

These three characteristics for each of the 14 seasons were then examined in relation to the early-season growing conditions. Variables considered to determine the general growing conditions were (a) the potential evapotranspiration, (b) the rainfall, and (c) the soil moisture. All of these are derived from conventional observations of temperature and precipitation.

The potential evapotranspiration values  $E_p$  were computed by Thornthwaite's technique [30]. Temperature data from the U. S. Weather Bureau station at Willow Run Airport (10.5 mi from Ann Arbor) were used for these computations. Because  $E_p$  depends

TABLE 23

Characteristic Parameters for the Ragweed Pollen Seasons 1946-1959

Year	Maximum Observed	Date of Maximum	Total Pollen Count	Length of Season Days *	Modal Date **	Modal Pollen Count **
1946	469	5 Sept	4768	39	2 Sept	315
1947	427	4 Sept	4392	44	3 Sept	278
1948	358	24 Aug	3408	31	24 Aug	295
1949	249	25 Aug	3191	46	30 Aug	174
1950	335	27 Aug	2655	33	25 Aug	239
1951	703	31 Aug	4378	50	28 Aug	446
1952	405	1 Sept	4201	50	28 Aug	220
1953	237	27 Aug	2821	57	27 Aug	205
1954	381	4 Sept	2818	32	1 Sept	219
1955	627	29 Aug	3695	44	28 Aug	259
1956	305	13 Sept	4153	41	29 Aug	232
1957	92	3 Sept	1193	38	29 Aug	74
1958	281	30 Aug	1938	33	29 Aug	133
1959	233	25 Aug	2406	34	23 Aug	134

\* Number of days from first to last occurrence of a count greater than or equal to 10.

\*\* Determined from normal curve fitted to data.

entirely upon temperature, it is a direct temperature index. In the present work,  $E_p$  was also useful in arriving at estimates of residual soil moisture (discussed below).

Rainfall data from five nearby weather stations (Adrian, Jackson, Lansing, Flint and Ann Arbor) were averaged to obtain precipitation values,  $R$ , representative of the region that lies in the westward quadrants from Ann Arbor. It is this area that supplies most of the ragweed pollen burden that is carried to Ann Arbor by westerly winds.

Soil moisture  $M$  was estimated by assuming that all soils in the area concerned contain 4 in. of water in the plant root zone as of 1 April each year. A continuous account was computed to show the pattern of depletion of the soil moisture throughout the growing season. Typical curves are shown in Figure 40. All precipitation was assumed to contribute to  $M$  whenever  $M < 4$  in. All precipitation greater than  $M = 4$  in. was assumed to run off. Evapotranspiration losses from  $M$  were prorated on the basis of the current value of  $M$  by using the factor  $M/4$  as a weighting factor to modify  $E_p$ . In this way, the effect of reduced soil moisture on the rate of loss by evapotranspiration is accounted for.

Thirteen predictors were derived from monthly values of  $E_p$ ,  $M$  and  $R$  from the earliest time of observed ragweed germination in late March through July. A statistical screening program was developed so that the most useful predictors could be selected by means of the IBM 704 electronic computer. The first predictor was selected on the basis of the correlations with a statistic called pollen excess,  $P$ , (see Section 3.8.3 b), then partial correlations were computed to determine which of the remaining factors best explained the residual variability of  $P$ . This process was repeated until the contribution of additional factors became insignificant.

c. Results - For the prediction of the seasonal yield of pollen, the May precipitation  $R_5$ , July precipitation  $R_7$ , May potential evapotranspiration  $E_{p5}$ , and June soil moisture  $M_6$  were found to be the most useful predictors in the order of decreasing significance. Of these  $R_5$ ,  $E_{p5}$ , and  $M_6$  are positively correlated with the pollen yield, whereas the other predictor,  $R_7$ , is negatively correlated. It is interesting that May soil moisture does not enter the picture significantly, whereas in June the soil moisture is a positive predictor. One interpretation of this finding is that, generally speaking, cool

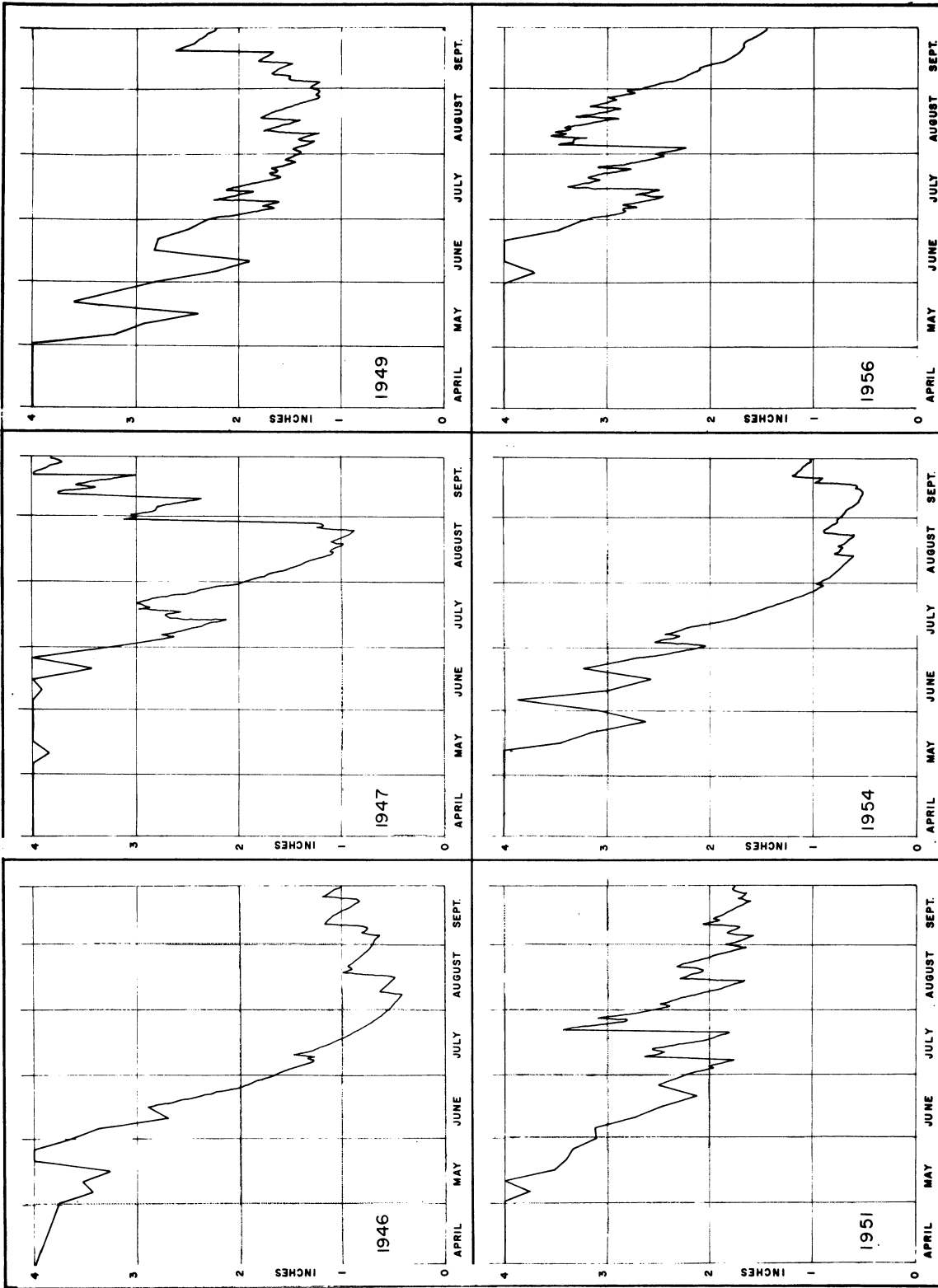


Fig. 40. Typical trends of soil moisture, Ann Arbor, Michigan.

temperatures in May tend to inhibit good growth of ragweed and to promote the most vigorous growth of competitive grasses. Therefore, relatively high May temperatures  $E_p$  are required to promote ragweed growth, but the high temperatures can operate favorably only if adequate precipitation is available to keep the upper layer of soil moist for the developing root systems. The soil moisture which characterizes the deeper soil zone is somewhat irrelevant to the health of the plants at this stage. In June, on the other hand, the ragweed root systems are well-developed and the soil moisture index, which reflects a combined cumulative temperature and rainfall effect, is of great importance. It should be noted that the June temperature is generally in the range best suited to ragweed growth in the presence of adequate soil moisture. On the other hand, excessive June precipitation should tend to promote abnormally low temperatures at the ground surface, because of both evaporative cooling and reduction of direct radiation. This moisture-temperature combination again favors the competitive grasses especially, whereas the precipitation fails to improve materially the supply of moisture to the deep-rooted ragweeds.

The negative relation of July precipitation to pollen yield may also be interpreted to mean that excessive July precipitation probably tends to favor ragweed plant competitors.

To predict total pollen yield  $Y$ , the following regression equation was computed using the first four predictors:

$$Y = 550 R_5 - 513 R_7 + 632 E_{p5} + 271 M_6 - 851$$

A comparison of predictions using this equation against the data from which it was derived is given in Table 24.

Although the tests built into the program for selecting predictors indicated statistical significance (at the 5 per cent level), for the first three predictors chosen above, the predictors chosen for neither the modal date nor the standard deviation of the fitted normal curve were so designated. This does not indicate, however, that one cannot make useful predictions on the basis of the several early-season parameters; the lack of statistical significance may be only a manifestation of the relatively short length of record, or of inadequacies in the pollen counts.

The first three parameters chosen as predictors of the date of the peak of the normal seasonal curve were respectively the



TABLE 24

Test of Total Pollen Yield Regression Equation  
Against the Data From Which It was Derived

Year	Observed	Computed	No. of Pollen	Per Cent
1946	4768	4400	368	8
1947	4392	4600	208	5
1948	3408	3700	292	9
1949	3191	3200	9	0
1950	2655	2600	55	2
1951	4378	3000	1378	31
1952	4201	3500	701	17
1953	2821	2700	121	4
1954	2818	2900	82	3
1955	3695	3700	5	0
1956	4153	4900	747	18
1957	1193	1800	607	51
1958	1938	1900	38	2
1959	2406	2400	6	0

June soil moisture  $M_6$ , July precipitation  $R_7$ , and March potential evapotranspiration  $E_{p3}$ . June soil moisture is positively correlated with the date of the peak, indicating that the ripening date of the ragweed is delayed by large amounts of moisture in the soil in June, or that if the soil is dry in June the ripening occurs earlier. It does not seem unreasonable to suppose that the higher surface temperatures that would occur, were the soil particularly dry in June, would hasten the time of ripening. It was seen above that  $M_6$  was also chosen as a predictor of the total yield, in the sense that larger values of  $M_6$  tended to induce greater total production of pollen. Combining the two effects, it appears that the soil moisture inhibits the development of ragweed; also dryness during this stage of the growing season produces lower yields and earlier ripening.

Both  $R_7$  and  $E_{p3}$  are related to the ripening date in the sense that larger values of these variables tend to coincide with earlier modal dates of the ragweed season. July precipitation was also one of the predictors selected in the case of the total yield. In discussing that selection it was suggested that excessive precipitation in July may favor those plants which compete with ragweed. If this is the case, it would not be unreasonable to presume as well that such competition would retard the development and delay the ripening of the ragweed.

The selection of the March potential evapotranspiration as a predictor of the modal date also seems reasonable. Since March is the time of year when ragweed seeds begin to germinate, one would have anticipated that warmer weather in March would permit this germination to occur earlier and that this, in turn, would be reflected in earlier maturity of the plants. This is indeed the relationship suggested by the statistical analysis.

The next two parameters selected as predictors of the ripening date,  $E_{p7}$  and  $M_7$ , do not appear to be of very great value in any final prediction scheme. Since the correlation coefficients are small, the possibility that they exhibit only a chance relationship is great. It is nevertheless of interest to note that the signs of these coefficients tend to corroborate the suggestion that warm, moist July weather delays the date of ripening, probably by stimulating ragweed competitors.

Given the modal date of the fitted normal pollen curve, and total pollen yield, only one additional parameter is required to completely specify the curve, viz., the standard deviation of

the curve. For reasons of expediency, the dependent variable introduced into the prediction program at this point was not the standard deviation, but rather the ratio yield peak, which is inversely proportional to the standard deviation. Only two predictors which gave any substantial evidence of reliability were selected. These were the May precipitation  $R_5$  and the July potential evapotranspiration  $E_{p7}$ , both of which are positively correlated with the aforementioned ratio, or negatively correlated with the standard deviation of the seasonal curve.

Interpretation of these findings is considerably more difficult. Conditions which would reduce the length of the pollen season are those which would tend to discourage the further growth and development of plants which had reached certain stages; these same conditions would have little, or even a positive, effect on other ragweed plants. Given the above findings, one could, from this point of view, find mechanisms to explain the results; however, no very strong arguments appear to be readily available at the present time.

### 3.8.3 Day-to-day Predictions

Whereas, in the consideration of long-term effects it was necessary to accept Durham gravity-slide data in order to compile a sufficient reservoir of consistent records, it is important in the consideration of the day-to-day effects within an individual season to base the study on more adequate data. The problem of obtaining a sufficiently reliable measure of airborne pollen concentrations has been treated by Harrington, et al [29]. Not until the summer of 1959 was it possible to place the roto-bar sampler in the field. Because this sampler collects particles very efficiently from a large volume of air (about  $1 \text{ m}^3$  per hr), it is allowed to sample for only 1 hr. The regular sampling periods used during summer, 1959, were from 0900 to 1000 and from 1300 to 1400 EST each day. These periods were selected on the basis of previous experience to represent, respectively, the period of diurnal maximum, and that of the diurnal average pollen concentration.

The data in Figure 41 are the ragweed pollen concentrations observed from 0900 to 1000 EST each day during the 1959 season. Gaps are attributable to the failure of equipment or personnel in one way or another. Noteworthy features of the data are (1) the wide day-to-day variability of the pollen concentrations, and

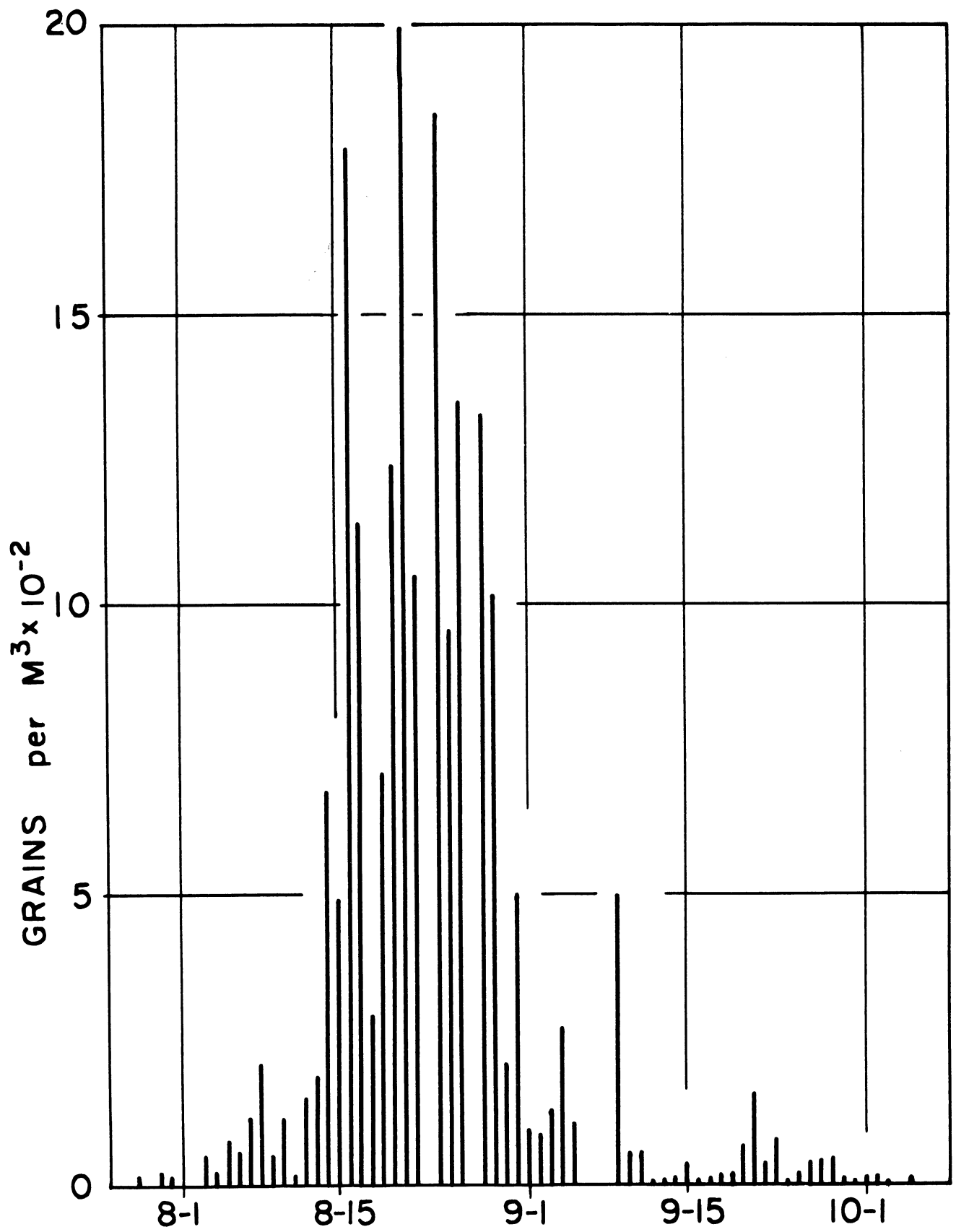


Fig. 41. Ragweed pollen concentrations, 1959 pollen season.

(2) the early-season (28 July) and late-season (9 and 21 September) peaks. An especially interesting feature of this particular ragweed pollen season is that the peak concentrations (about 2000 pollen grains per m<sup>3</sup>) were attained about 5 to 6 days earlier than appears normal from previous experience. This was also noted above in the Durham gravity-slide data.

a. Analysis - The analytical approach adopted for the day-to-day prediction problem is in some ways similar to that used for the seasonal forecast problem. Here it is first necessary to recognize that the daily counts are determined by the two interacting systems: the atmosphere, and the ragweed plant population. This leads to the first basic assumption:

(1) In the absence of short-term weather variability, the level of pollen concentrations would rise and fall with time along a smooth curve.

To determine the form and position of the idealized smooth curve, the data were smoothed by the use of ten-day moving averages. These led to the impression that a normal curve might serve to represent the idealized distribution; accordingly, a normal curve was fitted to the data (Figure 42).

Corollary to the first assumption is the second principal assumption:

(2) The departures from the smooth curve of pollen concentrations are attributable to the variable effects of weather factors.

b. The Pollen Excess - To characterize the departures from the smooth curve, the pollen excess,  $P$ , is defined. If at first, the observed pollen concentration is assigned the symbol  $X$ , and the pollen concentration indicated by the fitted normal curve is symbolized by  $X_n$ , then  $P$  may be defined by the equation:

$$P = \frac{X - X_n}{X_n}$$

The value of  $P$  for each date of the 1959 summer observations is plotted in Figure 43. Note that inherently, since  $X$  has a minimum value of zero,  $P$  has a minimum value of -1, but there is no theoretical limit to its magnitude in the other direction.

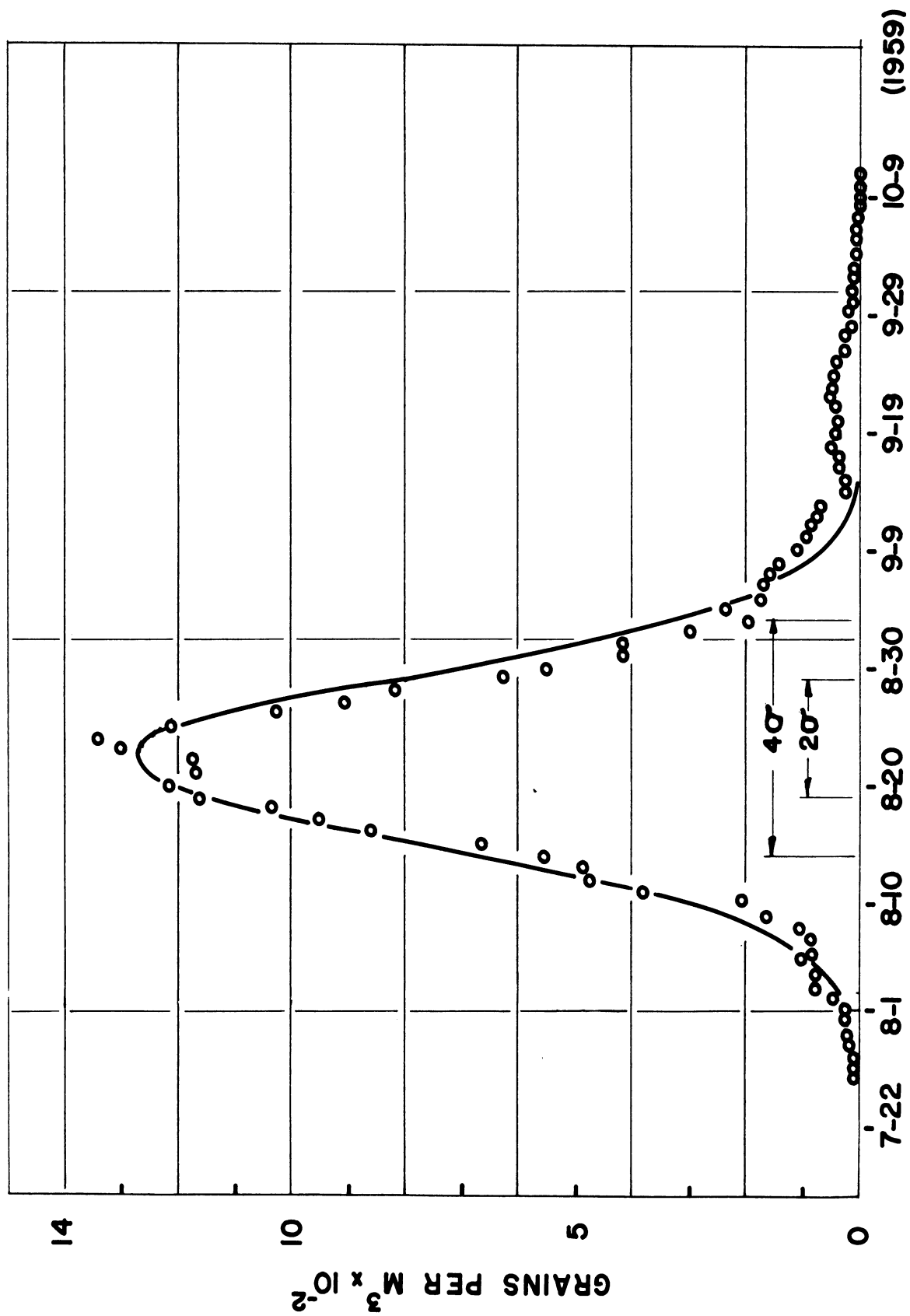


Fig. 42. Normal curve fitted to ten-day moving averages of pollen concentration, 1959 ragweed season.

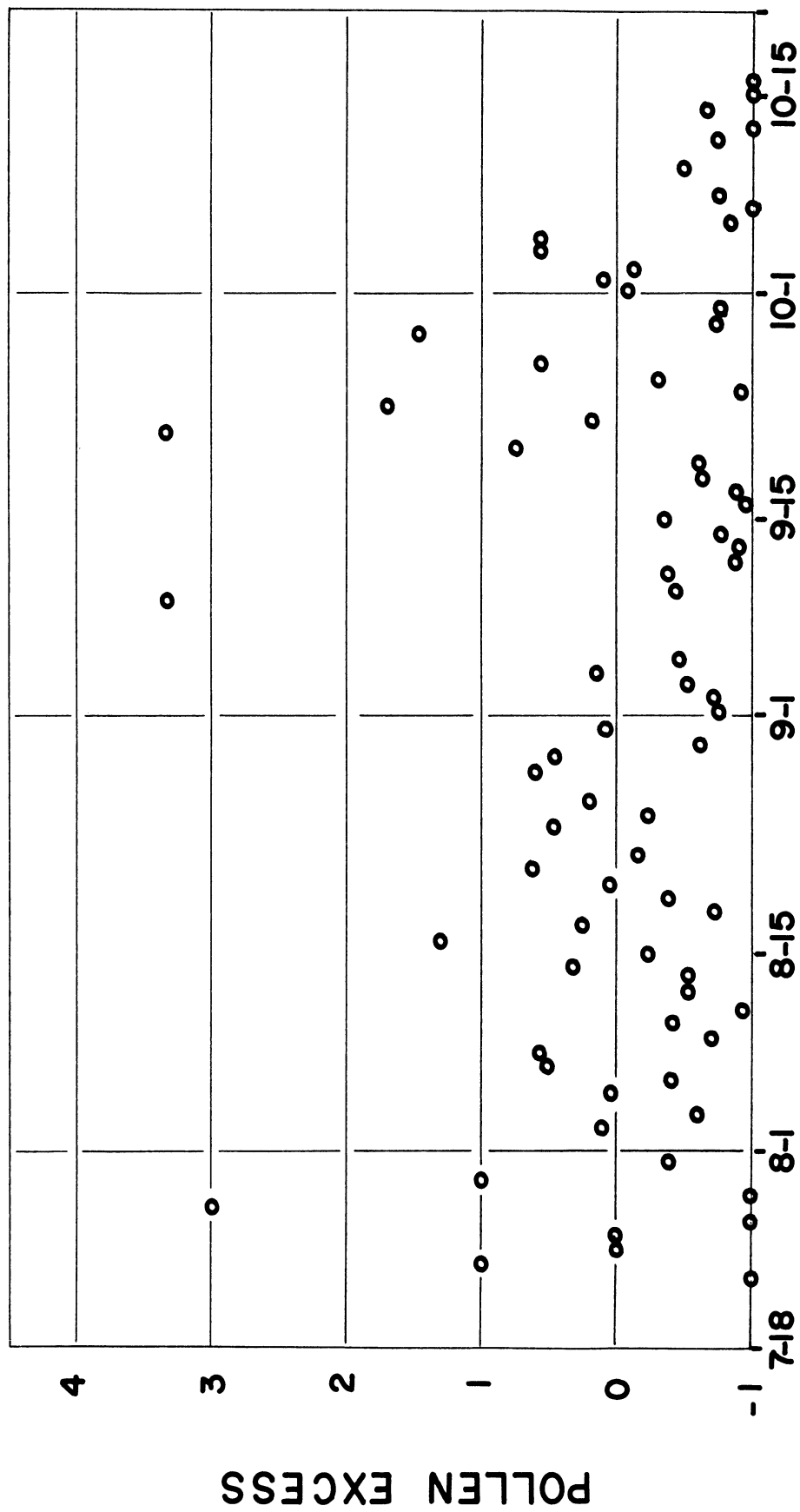


Fig. 43. Departure from normal distribution of pollen concentration (pollen excess, P), 1959 ragweed season.

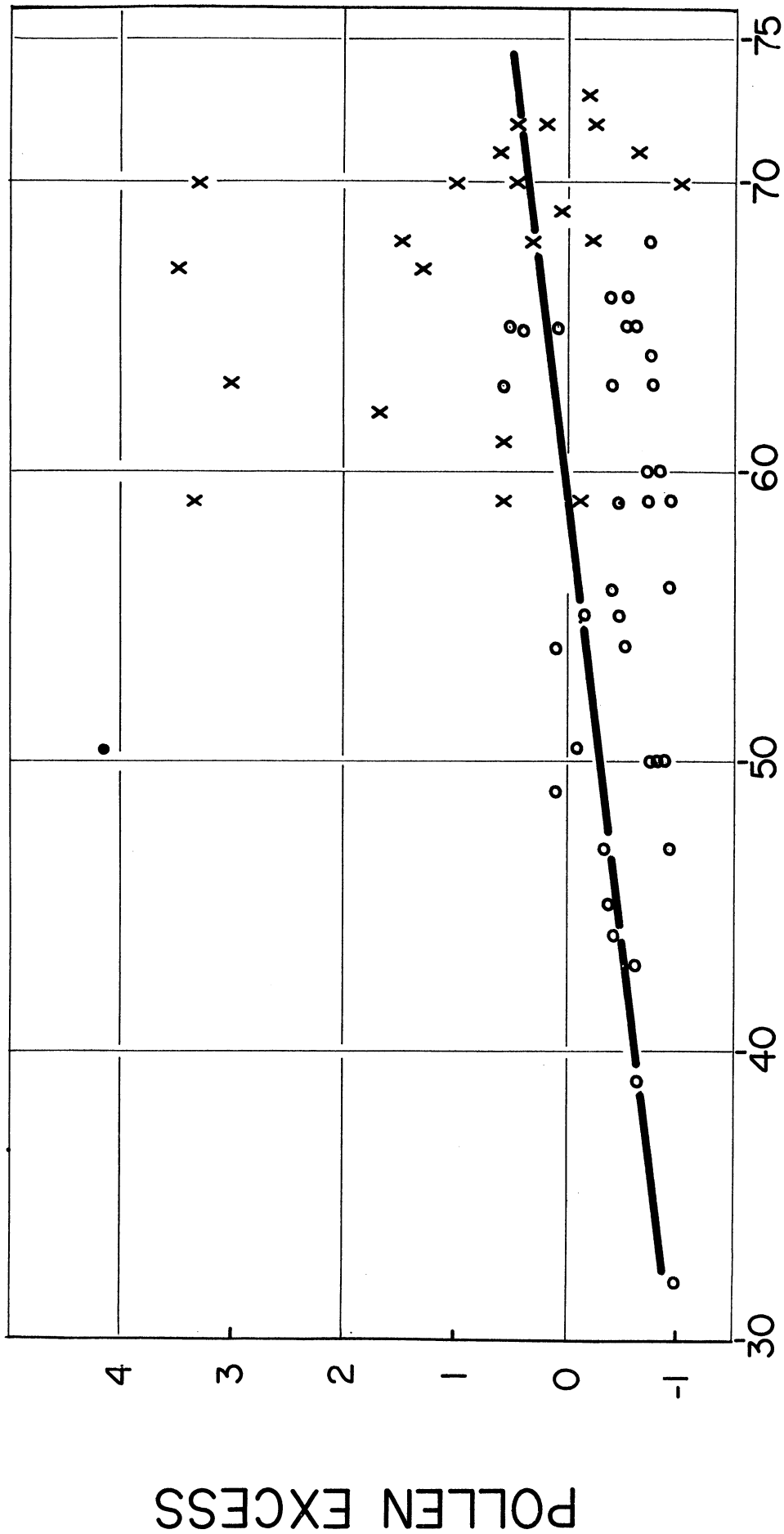
No material trend of  $P$  with time is evident from Figure 43. The highest values are found on dates which do not coincide with the "normal" pollen peak, that is, for dates on the tails of the normal curve of Figure 42. The high value,  $P = 3.0$ , on 28 July is directly attributable to the very small value  $X_n = 3$ . Thus, even though observed pollen concentration for that date is small ( $X = 12$ , as shown in Figure 41) the value of the pollen excess is extreme. The high values of  $P$  for 9 and 21 September are more directly suggested by the values shown in Figure 41.

By assuming that the plant will behave predictably under constant conditions, the variables of the problem have now been separated into two categories: (1) the inherent behavior of the plant population, represented by the normal curve  $X_n$  of Figure 41 and (2) the responses of the plants to the variable stimuli provided by the weather, represented by  $P$  (Figure 43). From this point on, the focus of attention is the value of  $P$ , in relation to environmental factors.

c. Selection of Criteria - To select the environmental factors that influence the value of  $P$ , various graphical studies were made. Laboratory experiments on the anther sacs of ragweed have shown that high humidity inhibits their dehiscence. The most direct available measure of humidity is the dew point temperature,  $T_d$ , the magnitude of which is dependent on the air mass type. Whereas  $T_d$  indicates the absolute humidity, it must be used in combination with air temperature to obtain relative humidity. The plot of  $P$  against concurrent values of  $T_d$  (Figure 44) shows only a slight relationship. In this and subsequent figures, circles represent observations taken in polar air, and crosses represent observations taken in tropical air. The heavy solid curves are lines of "best fit." The trend observed in Figure 44, however, is in the wrong direction, showing increasing  $P$  with increasing, not decreasing,  $T_d$ . This suggests that the meteorologically-observed association of high  $T_d$  values with high temperatures,  $T$ , may be an overriding consideration.

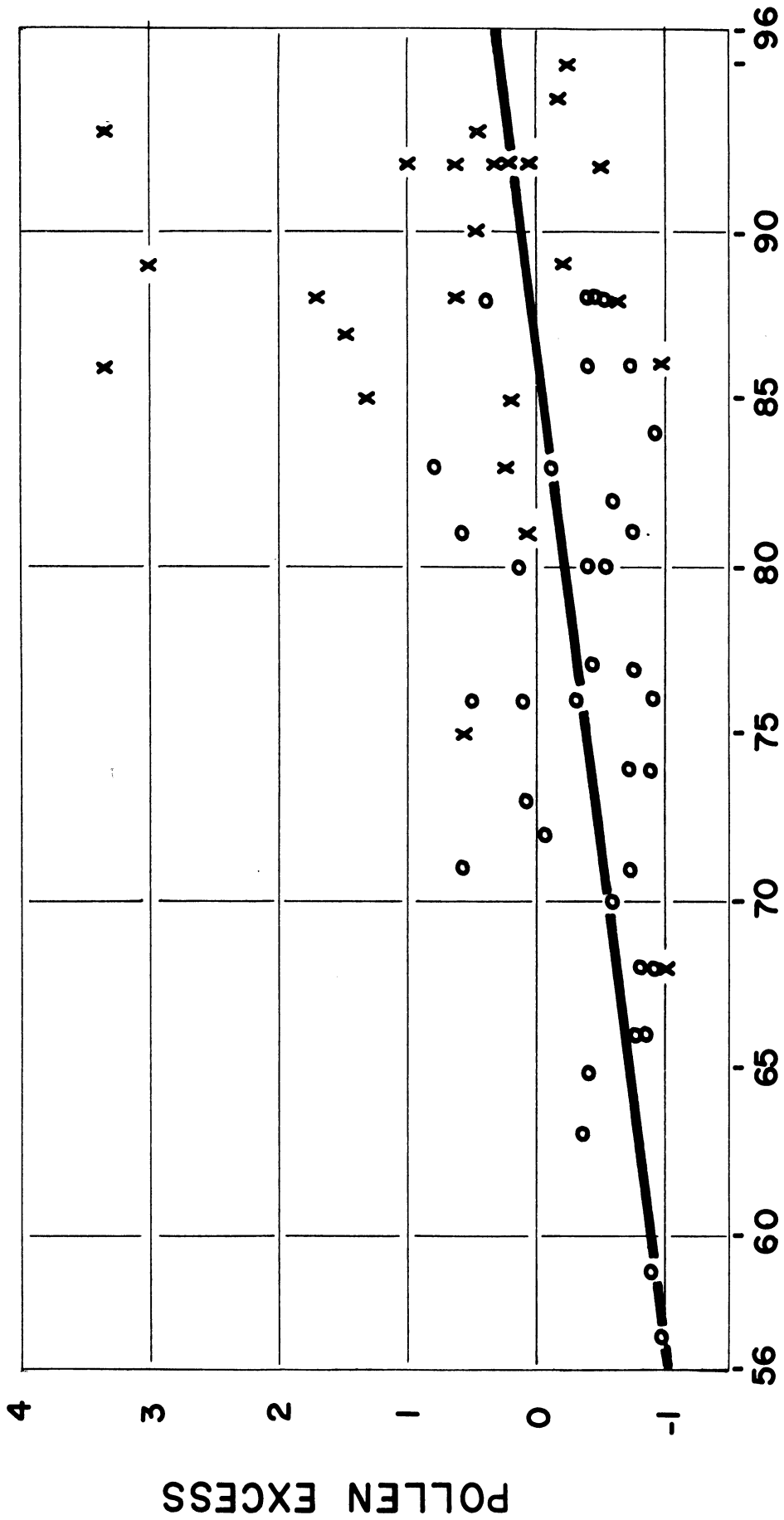
In addition, a temperature sensitivity of the plant metabolism is to be expected. The maximum temperature for the day,  $T_x$ , generally occurs 4 to 6 hr after the 0900 to 1000 sampling period, but it does indicate the relative temperature of the morning hours. The plot of  $P$  against  $T_x$ , Figure 45 leads to several interesting observations. Note that when  $T_x$  falls below 79F,  $P$  is less than zero, meaning that the value of  $X$  is less than the expected value,  $X_n$ , for the day. Note also that for all days on which  $P = 1$  or more,  $T_x$  is at least 85F. The





## DEW POINT

Fig. 44. Dewpoint temperature versus pollen excess, 1959 ragweed season.



MAXIMUM TEMPERATURE IN DEG. F.

Fig. 45. Maximum temperature versus pollen excess, 1959 ragweed season.

hay-fever patient of lower Michigan will find small solace in the fact that the accentuation of his symptoms in hot weather is not entirely psychosomatic.

The flotation of ragweed pollen from deposits on flowers and subjacent leaves appears to be at least an initial step in its transport [1]. That this requires some minimum wind speed also appears to be a reasonable inference [31]. The plot of  $P$  against the concurrent wind speed,  $U$ , (Figure 46) however, shows only a very slight trend.

It is well-known that ragweed grows abundantly for several hundreds of miles southward from Ann Arbor, but the limit of its range is much closer in northerly quadrants. The presence of Lakes Michigan, Huron and Erie also serves to prevent ragweed pollen contamination of the air as it moves from those respective directions. Hence an effect of wind direction,  $D$ , upon  $P$  is anticipated, and, in Figure 47 this is shown graphically. Principally, it is noteworthy that in all 6 cases when  $P$  exceeded 1, the concurrent wind direction was in the south to southwest quadrant.

The graphic technique is useful primarily for the preliminary studies. To evaluate the relationships between the weather factors and the pollination process, a statistical screening program, the same one employed in the study of the long term effects, was used to select useful predictors by means of the IBM 704 digital computer. Some 18 weather parameters were chosen as input data. Figure 48 shows part of the resulting matrix of correlation coefficients.

This procedure indicated that wind direction was the most important factor, and the maximum temperature of the same date was next most important, both associated positively with the pollen excess. The third and fourth factors were not significant; they were the wind speed at 0900 EST, and the dewpoint temperature, respectively. Ultimately, the method should lead to a multiple regression equation, but the inadequacies of the present data failed to justify this step. Further, it is not yet certain that the best possibilities have been treated. Rather, another prediction scheme was devised by still another technique.

d. Scatter Diagram - As indicated above, although the dew point temperature,  $T_d$ , and the wind speed,  $U$ , should be expected to have systematic relationship to  $P$ , this is not found graphically nor strongly indicated by statistical cor-



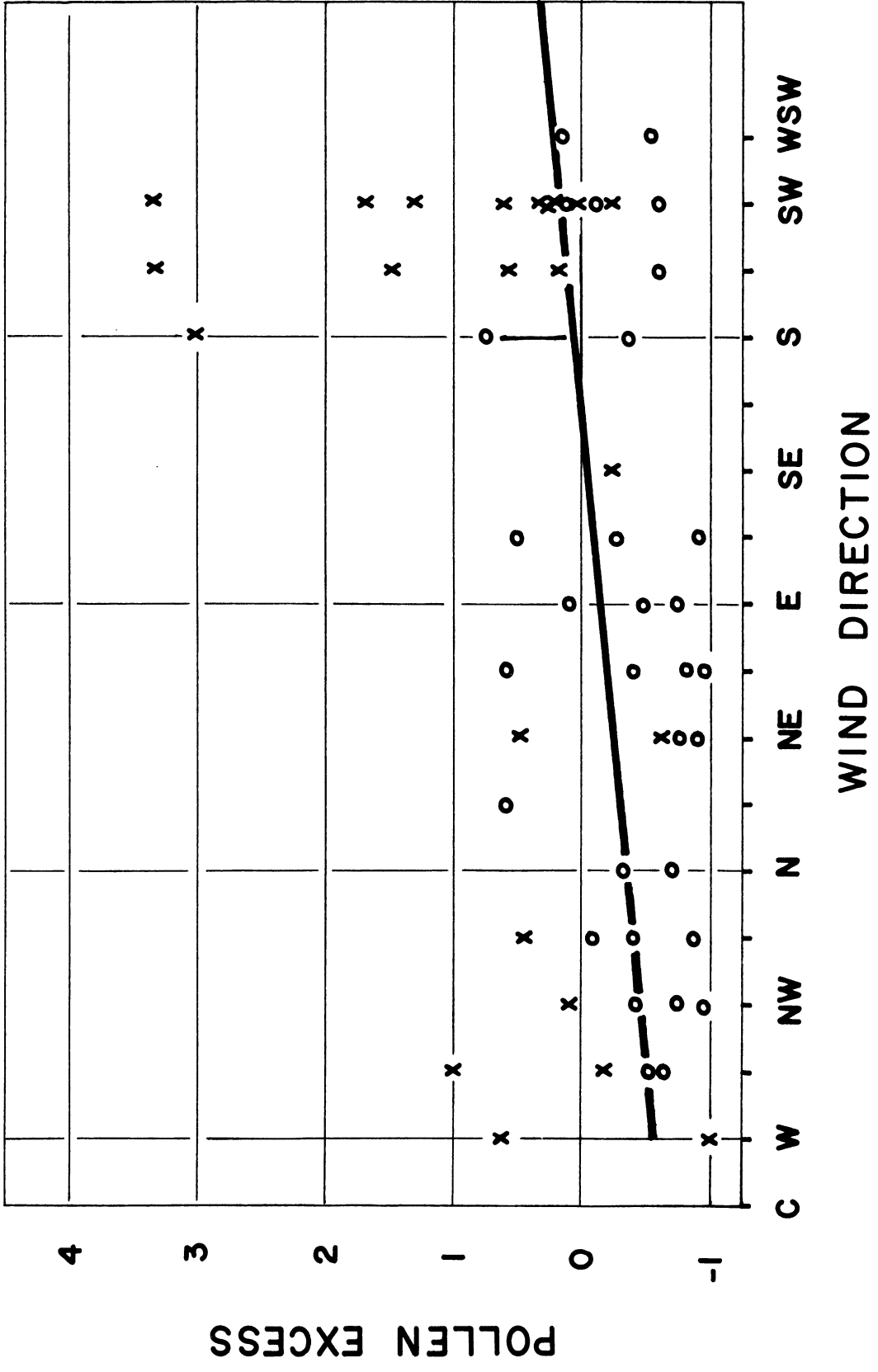


Fig. 47. Wind direction versus pollen excess, 1959 ragweed season.

Matrix of Correlations between Pollen Excess and Various Meteorological Parameters and  
Correlations between the Parameters

	Excess	Airmass	Wind Speed 0900	Wind Direction 0900	Temp. 0900	Dew Point 0900	Rain at time of Obs.	Temp. Change Min. to 0900
Excess	1.00	0.55	0.27	0.51	0.40	0.35	-0.15	0.00
Airmass		1.00	-0.06	0.45	0.62	0.64	-0.06	-0.19
Wind Speed 0900			1.00	-0.02	-0.17	-0.20	0.01	-0.06
Wind Direction 0900				1.00	0.28	0.24	-0.18	0.08
Temperature 0900					1.00	0.90	-0.17	-0.04
Dew Point 0900						1.00	0.06	-0.34
Rain at Time of Obs.							1.00	-0.527
Temp. Change Min. to 0900								1.00

Fig. 48. Matrix of correlations between pollen excess and various meteorological parameters, and correlations between the parameters.

relation. Furthermore,  $U$  and  $T_d$  are poorly correlated, hence a plot of the data on a  $U$  versus  $T_d$  grid results in a well-scattered set of points.

The value of  $P$  for each point was noted in the scatter-diagram, and simple boundaries for categories of  $P$ -values were sought. Four categories established are indicated by areas A, B, C, D, in Figure 49. Area boundaries are formed by dew point isotherms 42, 55, 61, 68 °F, respectively, and by isotachs 2.0, 5.5, and 14.5 mph, respectively. The categories are indicated by the following criteria:

(1)  $P$  is positive for all points except one. Average value of  $P$  is about +0.4, except when south to southwest winds occur 14 or more days past pollen peak,  $P_{av} = +3$ .

(2) Positive and negative values of  $P$  about equal. Further analysis using  $T_x$  and  $D$  indicates  $P = 0$  for  $T_x$  above 83F and  $D$  of south to southwest, otherwise  $P = -0.5$ .

(3)  $P$  mostly negative, average value about -0.5.

(4) No pollen, i.e.,  $P = -1$ .

From these observations, numerical factors to be applied to  $X_n$  in order to predict  $X$  (from the values of weather parameters concurrent with the predicted values,  $X_p$ ) are derived for each category and subcategory by adding 1.0 to the respective average values of  $P$ . Thus the numerical factors,  $F$ , are as follows:

(1)  $F = 1.4$  (or 4.0 for S-SW winds 14 or more days after peak)

(2)  $F = 1.0$  (or 0.5 if neither  $T_x > 83F$  nor wind from S to SW)

(3)  $F = 0.5$

(4)  $F = 0$

Table 25 gives the results of a test of these factors against the 73 observations from which they were derived.

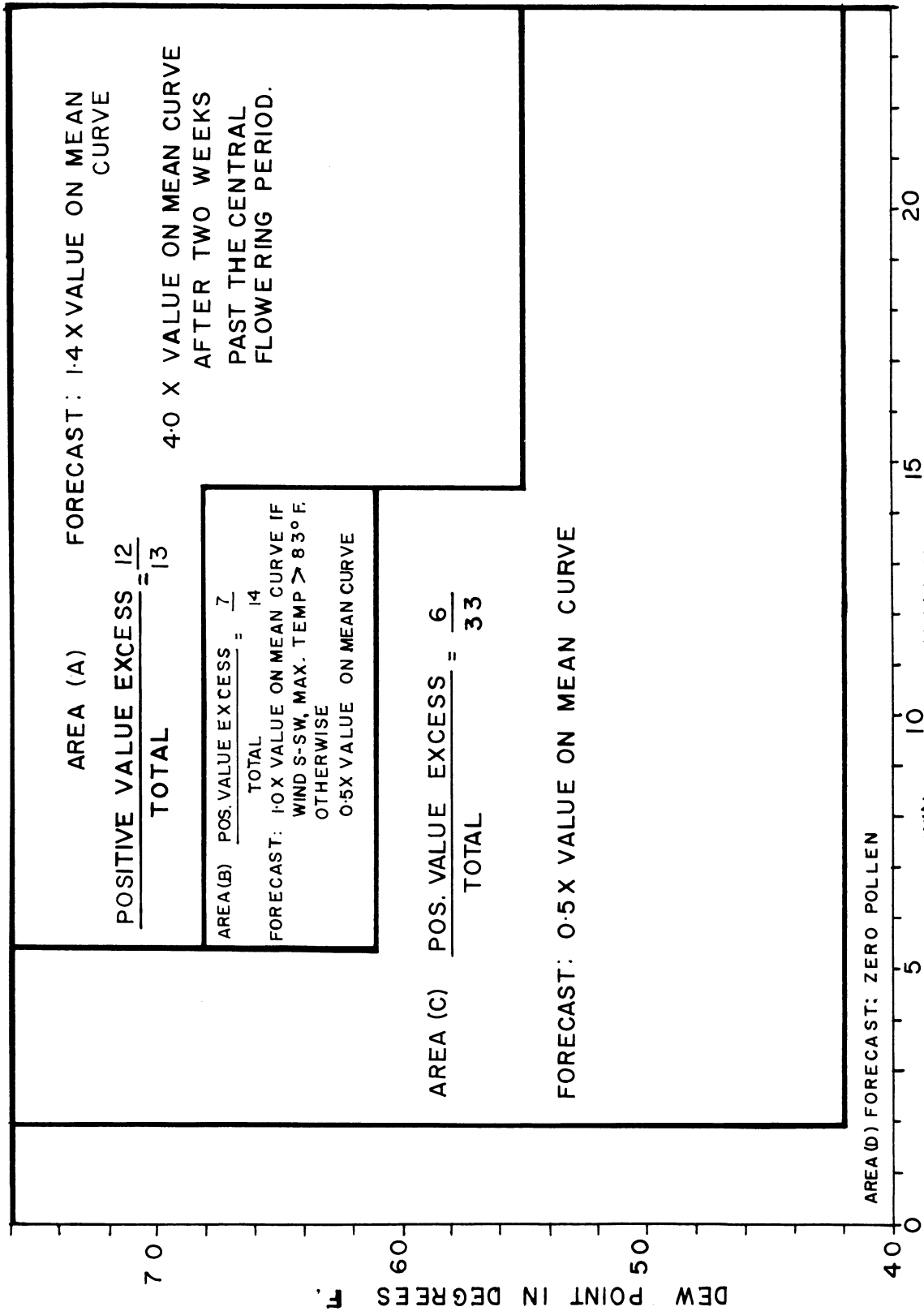


Fig. 49. Ragweed pollen prediction graph for Ann Arbor, Michigan.



TABLE 25

Test of Prediction Factors Against the Data  
from which they were Derived.

F	$\Sigma X_n$	$\Sigma X_p$	$\Sigma X$	No. of Cases
0	192	0	48	13
0.5	6729	3365	3732	37
1.0	4635	4635	4964	10
1.4	7552	10573	9987	9
4.0	182	728	770	4

#### 3.8.4 Conclusions

a. The Gaussian distribution curve may be used with some success to characterize the approximate ragweed pollen season for purposes both of predicting the general nature, intensity and timing of the season on the basis of early growing-season weather parameters, and of forecasting pollen concentrations on a day-to-day basis during the pollen season.

b. The total pollen catch (by Durham samplers) for a season is sensitive to, and hence (within limits) predictable from, the values of temperature, precipitation and soil moisture in the months of May, June and July.

c. The modal date of the idealized annual pollen distribution curve is statistically related to the soil moisture in June, the amount of precipitation which occurs in July, and the temperatures in March.

d. The ratio of the total annual pollen count to the peak value of the fitted normal curve, and hence the standard distribution of that curve, is most strongly dependent on May rainfall and July temperatures.

e. On the basis of 73 days of roto-bar sample data during summer 1959, it is determined that a crude day-to-day pollen count

prediction scheme can be devised using forecast values of dew point temperature, wind speed, wind direction, and maximum temperature.

### 3.9 RAIN SCAVENGING OF POLLEN FROM THE ATMOSPHERE

#### 3.9.1 Introduction

It is well known that rain carries with it large quantities of particulate matter suspended as dust in the air prior to the rainfall. This process is apparently one of the more important ones operating to cleanse the air of dust which would otherwise tend to accumulate in the air. In particular, particulates of the order of  $20\mu$  and smaller diameter would persist in the atmosphere for periods of weeks or months in the absence of the "scrubbing" action of rain.

Although this process has been known for some years, and has been invoked in the classical hypotheses set forth to explain the formation of raindrops themselves, quantitative estimation of its effectiveness has only recently been made possible by the work of Langmuir [32]. Greenfield [33] has now utilized Langmuir's theory and Best's [34] raindrop spectra to derive estimates of the extent to which rain is effective in removing radioactive particulates from the atmosphere. Chamberlain [35] using the Sutton [36, 37] equations together with Langmuir's collection efficiencies and Best's raindrop-size spectra, has also computed the effectiveness of rain in washing suspended particles from the air.

Experimental work on rain scavenging of particulate matter has been reported by McCully et al [38]. This group has done laboratory experimentation on both wettable and nonwettable particulates in the size range of from 0.5- to  $50\text{-}\mu$  diameter. They have also studied the washout of fluorescent dusts released from aircraft at low levels in natural rain. Their results show a considerable discrepancy between the computed and observed collection efficiencies, and they have concluded that--"these phenomena certainly merit more attention--."

Dingle [39] has reported observations of pollen concentrations in which rain was the only substantially variable weather element. In these cases, a very light rain totaling 0.03 in. had no observable effect upon observed pollen concentrations, whereas a

moderate rain of 0.21 in. reduced the pollen concentrations by about 40 per cent. This study, in common with McCully's field experiments, is difficult to evaluate with confidence because (a) the particulate sampling technique was not entirely satisfactory and (b) the measurement of rain in bulk total amounts (using rain gauge data) is not adequate for the purpose.

Promise of progress toward a resolution of these problems is offered by (a) the development of a technique which approaches isokinetic sampling of atmospheric pollen (Harrington, et al [29]), (b) the development of an instrument capable of recording detailed information on the drop-sizes of falling rain (Dingle and Schulte [40]), and (c) the development of an instrument whereby drop-size-discriminated samples of rain may be collected (Dingle and Brock [41]).

During the summer of 1960, pollen concentration data were collected by means of the rotobar sampler; raindrop-size spectrum data were collected by means of the photoelectric raindrop-size spectrometer; and bulk samples of rain, crudely sorted by drop size were collected. The bulk samples were filtered of all insoluble particulate matter by means of millipore filters. Pollen counts and identifications on these samples are being made. Reduction of the raindrop-size spectra is also in process. Observed airborne pollen identifications and counts have been mainly completed. The complete analysis of these data, in the light of existing theories of atmospheric dispersion of particulates and the scavenging action of rain is under way, and will be presented in the next report of this group.

### 3.10 STATUS OF THE POLLINOSIS TEST CHAMBER

#### 3.10.1 Temperature and Humidity Operation

Extensive tests were run on the air conditioning equipment during the summer and fall of 1959 in the completed pollinosis test chamber. When the air in the surrounding laboratory was at a temperature of 70-75F the air conditioning equipment would only modify the temperature in the chamber by  $\pm 10F$ , hence, a range of about 65-85F could be achieved. With an external relative humidity of about 60 per cent, the chamber could be varied only over the range of 45-70 per cent relative humidity. These small ranges in both temperature and relative humidity were quite disappointing to project personnel. During the warm summer days, condensation

formed on the ventilating ducts and dripped profusely into the laboratory. Also, temperature fluctuations in the adjoining laboratory were quickly reflected in the temperature of the test chamber, indicating that the plywood walls were quite transparent to both temperature and humidity.

To overcome most of these difficulties the following changes were made:

a. The rectangular ducts were thermally insulated and vapor proofed by the application of a layer of 1 in. of styrofoam (rigid type).

b. The circular ducts were covered with a 1 in. layer of fiber glass insulation.

c. The inside walls and ceiling were covered with a 1 in. layer of rigid type styrofoam followed by a special plaster. This material acted both as a thermal insulator and a vapor barrier.

In the tests conducted during the winter the following ranges of temperature and humidity were obtained:

a. highest temperature with high humidity - 89F at 58% R.H.

b. highest temperature in connection with low humidity - 74.5F at 33% R.H.

c. lowest temperature at high humidity - 56F at 89% R.H.

d. lowest temperature with low humidity - 56.5F at 45% R.H.

e. lowest temperature in any recording was 44F; lowest humidity shown was 32% R.H.

Although these results were considerably less than what was expected in the original design, they provided sufficient range for a great many experiments with ragweed pollen and hay fever patients. Why this range was not as great as anticipated is explained as follows:

When the thermostat is set for a high temperature, say 90F, with a relative humidity of 20 per cent, the compressor operates automatically in order to dehumidify the air. This is accomplished by passing the air from the chamber over the cold condenser tubes. Moisture from the air condenses on these cold tubes (about 35F)

and drips down into the reservoir and off into the sump. Simultaneously with the removal of this excess moisture, the air is cooled to approximate the temperature of the cold coils (40F). This occurs even though the thermostat is set for 90F temperature. Accordingly, the two 2500W heating elements are turned on to warm the air leaving the air conditioner. These two heaters have a capacity almost equal to the cooling capacity of the air conditioner. The net result is that the air leaving the heating coils is only slightly warmer than the air in the surrounding laboratory. If higher temperatures at low humidities are required, it appears that the simplest solution will be to increase the electric capacity of the system by several thousand watts.

If very high humidities are needed at high temperatures, then the simplest solution appears to be to use a sprayer-type humidifier in the duct rather than the present trays which are electrically heated to vaporize the water. By spraying fine droplets of water directly into the duct it should be possible to raise the relative humidity to about 90 per cent.

### 3.10.2 Ragweed Pollen Dispenser

A satisfactory pollen dispenser has been developed for the wind tunnel tests, described elsewhere [26] (see Figure 11, p. 79). This dispenser was used both for the basic testing of the various pollen samplers and for pollinosis tests on humans. Since its operation in the wind tunnel was satisfactory, it was used as the initial design for the test chamber. It consists essentially of a vertical glass tube of about 250 mm height and 40 mm diameter. A fritted glass disc is cemented about one-third of the way up the tube. Air is forced up this tube, through the fritted glass filter, through about 1.5 in. of ragweed pollen, and thence out a tube and into the air duct leading into the chamber. The air passing through the fine holes of the fritted disc bubble through the ragweed pollen, carrying a number of individual grains up into the tube and out the connecting glass tubing.

The initial installation consisted of the following items in sequence: (a) laboratory air line (25 lb psi); (b) a water trap; (c) a pressure regulator to reduce the pressure of the air fed to the dispenser down to the range of a 0-30 in. H<sub>2</sub>O gauge; (d) a duct to the base of the dispenser described above; (e) a pollen dispenser; (f) and a tube leading from the dispenser into the intake of the small blower, the latter located directly on the main air duct leading into the chamber.

The pollen was thus dispensed into the intake of this blower which was supposed to dilute the pollen concentration before it entered the duct; it could then be thoroughly mixed within the duct before entering the chamber. Several difficulties arose with this initial installation, not the least of which were the relatively noisy operation of the small separate blower and the inadequacy of the pressure it developed; this permitted some of the pollen to be blown back into the room rather than all of it going into the chamber.

The present system, incorporating a number of modifications, is illustrated in Figure 50. In this system the air supply to the pollen dispenser is very carefully regulated for pressure and volume; the flow of secondary air to dilute the pollen concentration going into the main air duct is easily regulated. The deposit of pollen along the horizontal duct leading into the main air duct is greatly reduced and the concentration within the chamber is maintained at an essentially constant level.

Three 12V roto-bar units were used to measure the pollen concentration in the chamber. These could be started and stopped by a single switch. The three samplers were suspended by wires from three of the corner ducts in the ceiling. Each was located 14 in. from the side wall and 17 in. from the end wall. A fourth unit could not be conveniently located in the fourth corner because of the door opening into the chamber. One sampler was at each of the following heights from the floor: 0.33, 1.2, and 1.6 m, providing a reasonable distribution throughout the room. In most of the tests to date the samplers were operated for a 5-min period, stopped for 1 min for sampling bar changes, and restarted for a second 5-min. Thus, complete cycles were made in 6 min.

Initial testing of the pollen dispenser has been with the air conditioner, electrostatic precipitator, humidifier, and electric heaters all inoperative. The only unit operating has been the circulating fan, to provide a vertical rate of ascent of about 0.5 cm per sec throughout the total cross section of the chamber. To determine how long the dispenser must be operating before a steady pollen concentration would be reached in the chamber, the air-circulating fan was turned on several minutes before the pollen dispenser was started. During this period, pollen concentration measurements were obtained by means of the three roto-bar samplers. Following the application of new bars to the samplers, the pollen dispenser was started, and the roto-bar started immediately thereafter.

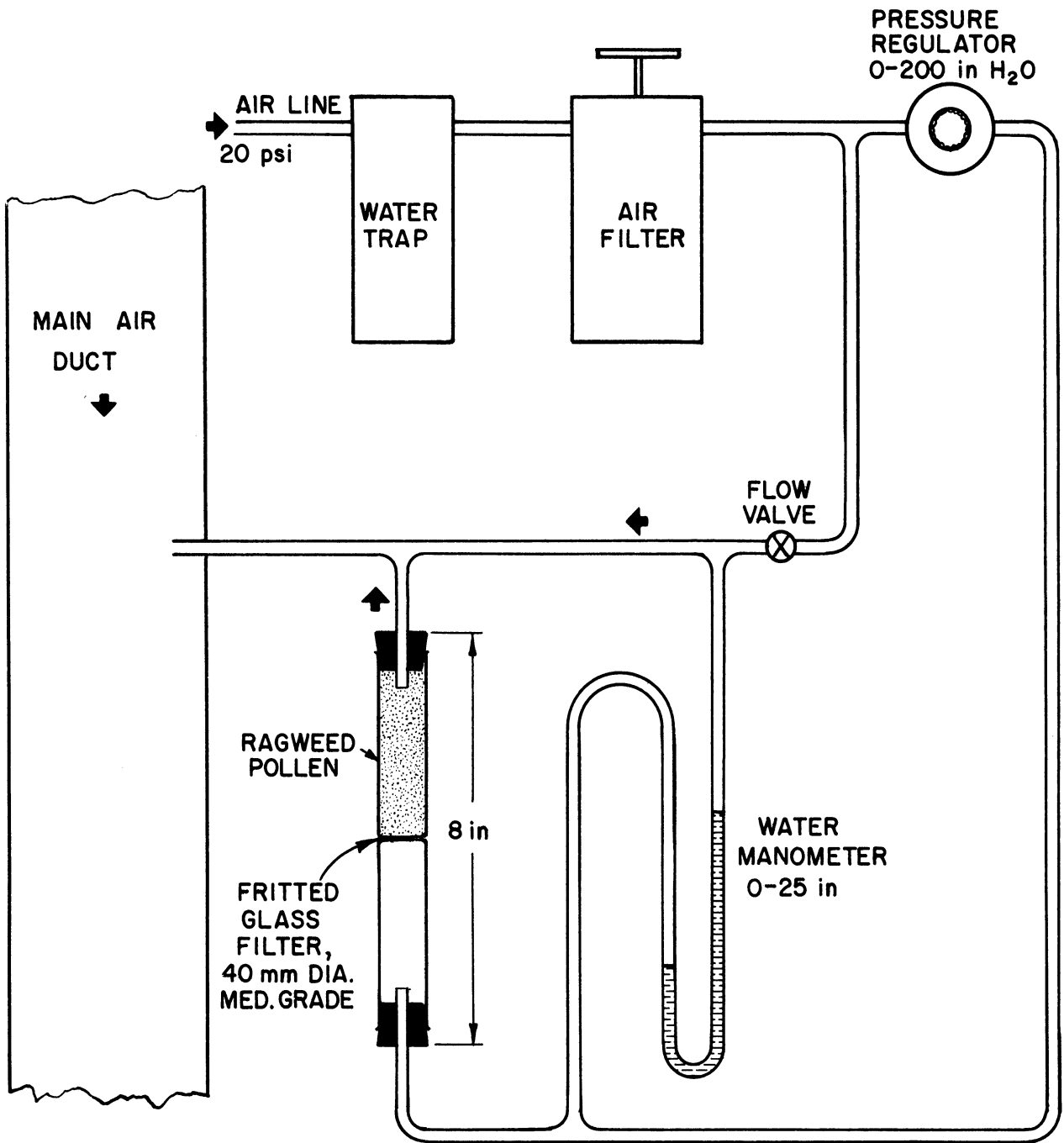


Fig. 50. Ragweed pollen dispenser system, pollinosis test chamber.

The result of one of these tests is shown in Figure 51. From this it is noted that the average pollen concentration prior to the start of the dispenser was about 40 grains per  $m^3$  and that the pollen concentration rose to about 80 per cent of its average value within 3 min after the dispenser was put into operation. The dispenser was operated for 55 min during an entire test period of 85 min. The curve also shows that the pollen concentration dropped essentially to zero within 3 min after dispenser shut-off. This was a surprising result, because it was expected that the concentration would gradually diminish to a much lower value, since the electrostatic precipitator was not operating and since there would be no moisture on the cooling coils to pick up pollen grains. Several tests were made maintaining concentrations of 1000-5000 pollen grains per  $m^3$ ; in each case the concentration has dropped off almost as rapidly as that indicated in Figure 50.

There was more dispersion in the pollen concentrations between the three samplers than anticipated, but maximum and minimum values have been randomly distributed, indicating that there must have been considerable turbulence in the chamber and fairly good mixing over the 1-hr period. Since the vertical speed was of the order of 0.5 cm per sec, it was expected that a considerable portion of the pollen would come out in small clumps. However, only about 10 per cent of the total count was found to be in clusters of two or more pollen grains. Accordingly, no reduction of fan speed (which determines the vertical air ascent rate in the chamber) is planned.

The output range of the pollen dispenser has been checked by noting various pressures indicated by the water manometer. The dispenser with the fritted glass filter has a repeatable output range for a pressure differential of 8 to 15 in.  $H_2O$ , sufficient to establish concentrations of from approximately 200 grains to 3000 grains per  $m^3$  within the test chamber. In order to lower the range of concentration, a dispenser with a 20 mm diameter fine-grade fritted-glass disc has been used; this has a range of approximately 100 to 800 grains per  $m^3$  with the same pressure differential. From the results of field studies in the area of the State Prison, Jackson, Michigan [2], it is considered that this range of concentrations will be very satisfactory for experimentation with ragweed sensitive patients.

In order to determine the effectiveness of the electrostatic precipitator, the dispenser was operated at a fixed differential pressure (approximately steady emission rate) for about 0.5 hr, then the precipitator was turned on. This was repeated at several



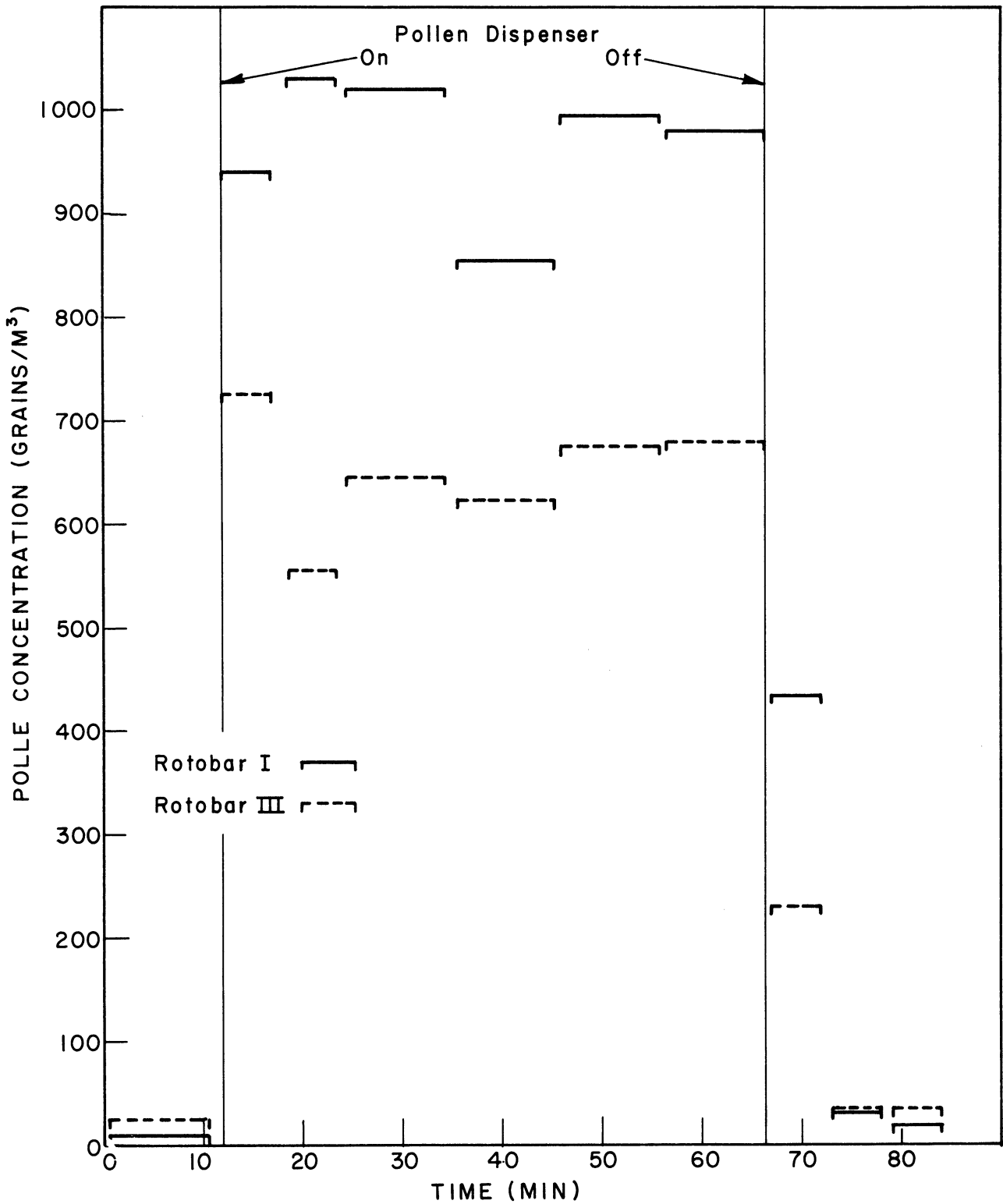


Fig. 51. Pollen concentration measured by rotobar samplers, pollinosis test chamber.

different pollen concentration values. It was found that the precipitator did not change the concentration in the chamber until the concentration reached a value of 2000 grains or higher. Until this concentration is reached, it is apparent that the fallout of the pollen is almost 100 per cent before making even one complete circuit of the test chamber. However, at higher concentrations a certain percentage does make a complete transit unless the precipitator is in operation.

Tests are currently underway to assess the effectiveness of the pollen dispenser when all auxiliary equipment is operating, i.e., under varying conditions of temperature and humidity, with and without the electrostatic precipitator.

### 3.11 SUMMARY AND CONCLUSIONS

a. An isokinetic filter-type pollen sampler was developed for calibration tests of other sampling devices. It is believed that this system, operating in uniform wind tunnel airflow, provides as satisfactory a standard for pollen sampler calibration as is presently possible (Section 3.2.2).

b. Although firm conclusions regarding the collection efficiency of the rotobar sampler with respect to wind speed are not possible, first approximations indicate its efficiency is about 68 per cent (Section 3.2.2).

c. An adaption of the rotobar sampler to operate from a 12V DC power supply was successful, making possible pollen sampling at points where 115V AC power is not available (Section 3.2.3).

d. A whirling arm pollen sampler was developed for measuring pollen-source strength. From its dimensions and specifications, a formula was derived to yield pollen grains emanating from a ragweed plot as a function of sampler characteristics, wind speed and pollen count (Section 3.2.4).

e. A multi-level rotobar sampler was devised to provide a pollen concentration profile for the lowest 2 ft of the atmosphere (Section 3.2.5).

f. A set of three alternating rotobar samplers was constructed to study the effect of different wind speeds on pollen concentration. Each sampler was actuated by an anemometer when

the prescribed range of wind speeds for that sampler was occurring. Further design is contemplated to reduce the error introduced by impaction of pollen on stationary, exposed sampling bars (Section 3.2.5).

g. A ragweed diffusion experiment and a phenological experiment were conducted in 1959 prior to the natural ragweed pollen season. Complete micrometeorological measurements were made during these experiments. Results are being sought from abstraction and analysis of the data (Section 3.3).

h. During the natural ragweed season of 1959, a field experiment was made to 1) evaluate the rate at which pollen is deposited on the ground and/or transported aloft from the source, and 2) to test a theoretical model of the effect of eradicating ragweed plants from a specified area on the pollen concentration downstream. Analyses of the data are continuing (Section 3.4.2).

i. An airborne sampling program was another in-season experiment. Its objective was to study the effect of a large ragweed-free area (Lake Michigan) on pollen distribution in the lower 2.3 km of the troposphere. Preliminary results show that pollen was collected at all levels on the upstream side of the ragweed-free area. On the downstream side no valid conclusions were reached, because of adverse air mass changes (Section 3.4.3).

j. Urban-rural spatial variations in ragweed pollen concentrations were sought by means of samplers mounted on automobiles. A 44-mi route was traversed over which the pollen count was measured by flag samplers in 1-mi sectors. Variations in synoptic pollen concentrations, and temporal variations in concentrations at different sites along the route, were determined. Among several results, it was found that 1) cereal grain fields yield a ragweed plant population 300 times greater than does pasturage, 2) there is negligible ragweed in urban areas except for large disturbed areas, such as new subdivisions, 3) airborne pollen concentration is only 30 per cent greater on the average in rural-tilled than in urban areas, and 4) for any given location, the pollen concentration is twice as high during the morning hours as it is during the afternoon, and four times as high as it is in the evening (Section 3.5).

k. In the study of the transport of ragweed pollen, the fraction of pollen produced that leaves the source at 0.6 m above the ground is 6 per cent. Peak pollen-emission days are likely to be clear with relative humidities less than 95 per cent. There

is evidence that a secondary emission maximum is reached during the evening hours (Section 3.6).

l. The extent to which flotation and reflation of pollen takes place was investigated by means of a mathematical model. The time of floret opening to pollen concentration in the air showed that pollen is sampled long after pollen release by the plant ceases. The result was interpreted to mean that the mechanism of reflation does, indeed, occur (Section 3.7.2).

m. The reflation model was tested against alternating sampler results (which showed 30 per cent of the pollen is released after the last flower has opened), and satisfactory values were chosen for reflation-effectiveness constants. The amount of pollen available for wind dispersion upon its being released by the plants is small, because of low wind speeds prevalent around the florets. Wind is a much more efficient refloating agent over the plant foliage and ground, because of the higher attendant speeds (Section 3.7.4).

n. Historical records of pollen counts show that seasonal pollen maxima vary widely in concentration; the maxima occurring between 24 August and 5 September; total pollen counts vary significantly from season to season; and the duration of appreciable pollen concentrations is widely variable. Thirteen meteorological criteria were used to establish predictors for long-term pollen concentrations. Among successful ones were 1) May- and July-precipitation, May potential evapotranspiration, and June soil moisture, for the seasonal yield of pollen; 2) June soil moisture, July precipitation, and March evapotranspiration, for the date of the highest normal seasonal pollen concentration. It was concluded that, a) both cool temperatures in May and excessive precipitation in July tend to inhibit growth of ragweed, whereas competitive grasses thrive; b) excessive soil moisture promotes early pollen ripening and low yield; c) warm March temperatures bring early maturity in ragweed plants; and d) warm moist July weather delays ripening by stimulating competitive plants (Section 3.8.2).

o. Day-to-day pollen prediction was based on assumptions of normally-distributed emission from plants, and on departures from such normalcy being attributable to variation in meteorological factors. Results of a study of weather factors and the pollination process showed that the following were in decreasing order of importance: a) wind direction, and b) maximum temperature. Wind speed and dew point temperature were found to be unimportant. Finally, a day-to-day pollen count prediction graph was developed (Section 3.8.3).

p. The problem of determining the rain scavenging action of pollen from the atmosphere depends partly on three developments: 1) an isokinetic sampling technique in the free atmosphere, 2) an instrument to yield drop-size information of falling rain, and 3) an instrument to collect drop-size-discriminated rain samples. Considerable progress has been made on all three developments. Several samples of rain-scavenged pollen have been taken, and analysis of pollen counts with other concurrent meteorological events is being pursued (Section 3.9).

q. Tests were conducted in the completed pollinosis test chamber to determine the ranges in temperature and humidity that could be produced by the control machinery. Maximum temperature and humidity produced were 89F and 89 per cent, respectively; minimum temperature and humidity were 44F and 32 per cent, respectively. It was concluded that these ranges were sufficient to conduct experiments with pollen-sensitive patients (Section 3.10.1).

r. A dispenser system was developed to introduce ragweed pollen into the pollinosis test chamber. Rotobar samplers were used to record concentration. Electrostatic precipitators were used to remove pollen from the air circulating through the chamber. It was found that pollen increased in the chamber in 3 min from 40 grains per  $m^3$  to 80 per cent of the average value after the dispenser was put into operation (1000-5000 grains per  $m^3$ ). When the dispenser was shut off, the concentration dropped to the same levels, and in the same time interval, as before the dispenser was operated. Components were developed in the system to enable the operator to select pollen concentration accurately at several different levels. Further tests under a variety of conditions are being made (Section 3.10.2).

## 4. . STATISTICAL PHASE

by

Richard D. Remington

### 4.1 INTRODUCTION

The activities of the statistical group during this period consisted of providing statistical consultation in the design, collection and analysis of data from a variety of projects of interest to the botanical, meteorological and medical groups. This consultation ranged from brief examinations of proposed projects to extensive analyses.

A major advance in statistical processing took place during this period with the acquisition by the University Computing Laboratory of an IBM 704 high speed digital computer. This machine has already been utilized by members of the meteorological group for production of results shown in Sections 3.7 and 3.8 of this report.

However, the importance of this new equipment to the entire aeroallergen research group cannot be emphasized too strongly. The group is now in a position to process on a routine basis, and at high speed, large sets of data involving complex statistical operations. Utilization of this equipment will continue to increase as additional members of the group become more familiar with the implications of high speed data-processing.

It is particularly encouraging that many of the investigators in the study are completing statistical analyses of their own data. Thus the activities of the statistical group can more properly be confined to consultation concerning experimental design, complex data handling problems and more detailed or refined analyses.

The following is a summary of some specific projects on which statistical consultation has been given during the period covered by this report. These serve as examples of the kinds of statistical activity undertaken.

## 4.2 METEOROLOGICAL ASPECTS

An experiment concerning the relative efficiency of the millipore and rotobar samplers in the wind tunnel is reported in Section 3.2. At each of three stations across the tunnel, three sampling units were installed consisting of one millipore filter and two rotobar samplers. In all cases the millipore filter was operated isokinetically. Six different tests or runs were completed using this arrangement. During each test an amount of pollen was injected into the tunnel, which was operating at a known wind speed. An initial regression analysis of pollen concentration on wind speed failed to show a significant linear relation.

The data are thus in the form of a  $3^2$  factorial arrangement with six replications. The two factors are stations (i.e., location of the sampling units laterally in the tunnel); and samplers (i.e., the difference between the three samplers at a given station). The two degrees of freedom for each of these factors were broken into single degree of freedom contrasts which were of particular interest in this experiment.

The stations across the tunnel were designated  $S_1$ ,  $S_2$  and  $S_3$ . The contrast  $S_1-S_3$  measures the extent to which pollen concentration changes laterally across the tunnel. The contrast  $2S_2-S_1-S_3$  measures the extent to which the concentration at the center of the tunnel is different from that at the sides.

If the three samplers at a given station are labelled by symbols,  $A$  for the millipore filter, and  $B_1$  and  $B_2$  for the two rotobar samplers, there are two linear contrasts which are of interest. These are  $B_1-B_2$  which measures the extent to which the two rotobar samplers differ in efficiency from each other, and  $2A-B_1-B_2$  which measures the extent to which the rotobar samplers differ in efficiency from the millipore filter sampler. These exhibit single degrees of freedom, and they can be investigated, in turn, for interaction with each other and with successive tests.

Throughout this analysis the analyzed variable was pollen concentration. Table 26 shows the analysis of variance of these data. This analysis of variance is of interest statistically because of the natural importance of the single degree-of-freedom contrasts. The statistical significance of the various items are indicated by the asterisks in the last column of the table.

TABLE 26

## Analysis of Variance of Wind Tunnel Experiment

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
1 Tests	5	731,532.83	146,306.57	475.82 **
2 Stations	2	3,197.44	1,598.72	5.20 *
2.1 2S <sub>2</sub> -S <sub>1</sub> -S <sub>3</sub>	1	2,914.08	2,914.08	9.48 **
2.2 S <sub>1</sub> -S <sub>3</sub>	1	283.36	283.36	<1
3 Treatments	2	33,708.78	16,854.39	54.81 **
3.1 B <sub>1</sub> -B <sub>2</sub>	1	318.03	318.03	1.03
3.2 2A-B <sub>1</sub> -B <sub>2</sub>	1	33,390.75	33,390.75	108.59 **
4 Tests X Stations	10	22,959.88	2,295.99	7.47 **
4.1 Tests X [2S <sub>2</sub> -S <sub>1</sub> -S <sub>3</sub> ]	5	2,148.08	429.62	1.40
4.2 Tests X [S <sub>1</sub> -S <sub>3</sub> ]	5	20,811.80	4,162.36	13.54 **
5 Tests X Treatments	10	15,362.55	1,536.25	5.00 **
5.1 Tests X [B <sub>1</sub> -B <sub>2</sub> ]	5	3,629.14	725.83	2.36
5.2 Tests X [2A-B <sub>1</sub> -B <sub>2</sub> ]	5	11,733.42	2,346.68	7.63 **
6 Stations X Treatments	4	2,365.78	591.44	1.92
6.1 Stations X [B <sub>1</sub> -B <sub>2</sub> ]	2	244.22	122.11	<1
6.1.1 [2S <sub>2</sub> -S <sub>1</sub> -S <sub>3</sub> ] X [B <sub>1</sub> -B <sub>2</sub> ]	1	3.55	3.55	<1
6.1.2 [S <sub>1</sub> -S <sub>3</sub> ] X [B <sub>1</sub> -B <sub>2</sub> ]	1	240.67	240.67	<1
6.2 Stations X [2A-B <sub>1</sub> -B <sub>2</sub> ]	2	2,121.56	1,060.78	3.45
6.2.1 [2S <sub>2</sub> -S <sub>1</sub> -S <sub>3</sub> ] X [2A-B <sub>1</sub> -B <sub>2</sub> ]	1	2,053.50	2,053.50	6.68 *
6.2.2 [S <sub>1</sub> -S <sub>3</sub> ] X [2A-B <sub>1</sub> -B <sub>2</sub> ]	1	68.06	68.06	<1
7 Tests X Treatments X Stations	20	6,149.57	307.48	
7.1 Tests X [B <sub>1</sub> -B <sub>2</sub> ] X [2S <sub>2</sub> -S <sub>1</sub> -S <sub>3</sub> ]	5	1,973.29	394.66	
7.2 Tests X [B <sub>1</sub> -B <sub>2</sub> ] X [S <sub>1</sub> -S <sub>3</sub> ]	5	1,616.84	323.37	
7.3 Tests X [2A-B <sub>1</sub> -B <sub>2</sub> ] X [2S <sub>2</sub> -S <sub>1</sub> -S <sub>3</sub> ]	5	1,159.67	231.93	
7.4 Tests X [2A-B <sub>1</sub> -B <sub>2</sub> ] X [S <sub>1</sub> -S <sub>3</sub> ]	5	1,399.78	279.96	

A - millipore filter

B<sub>1</sub> - one side of rotobarB<sub>2</sub> - other side of rotobarS<sub>1</sub> - Station 1S<sub>2</sub> - Station 2S<sub>3</sub> - Station 3\* Significant at  $\alpha = 0.05$ \*\* Significant at  $\alpha = 0.01$



The significance of the first item, tests, means that the average pollen concentration varied from test to test by more than would have been induced by random errors in sampling and counting. This may be explained simply in terms of the known variations of the ratio of the wind speed to the quantity of pollen introduced into the wind tunnel. The significance of the variations among the stations (item 2) is similarly explained in terms of a lack of uniformity of wind speed and perhaps also of pollen concentration across the tunnel. More specifically, the significance of the contrast  $2S_2-S_1-S_3$  (item 2.1) and the lack of significance of the contrast  $S_1-S_3$  (item 2.2) make it clear that the flow in the tunnel, although not uniform, is symmetrical, as should be expected.

The variance among the different sampling devices is also significantly large (item 3). This is due not to differences between the two sides of the rotobar (item 3.1), but to the fact that the average concentration indicated by the isokinetically-operated millipore filters was significantly larger than that measured by the rotobars (item 3.2). Furthermore, the evidence is substantial that the extent of the deficiency of concentration indicated by the rotobars is dependent on the factors which vary from test to test (item 5.2) and from the center of the tunnel to the sides (item 6.2.1).

If it is assumed that the millipore filter, when operated isokinetically, is a valid standard, then one must conclude on the basis of these experiments and this analysis that the efficiency of the rotobar sampler is dependent on the speed of the wind and perhaps also on the concurrent pollen concentration.

#### 4.3 BOTANICAL ASPECTS

A series of consultations were held throughout the past year with members of the botanical group and members of the Tecumseh Community Health Study. Members of the Tecumseh Study were interested in determining the distribution and concentration of various varieties of ragweed plants in Tecumseh, Michigan and vicinity. Through a series of joint meetings between the botanical and statistical groups of this project and members of the Tecumseh Study group, a scheme was devised for mapping the concentration of ragweed in this area by species. This project was successfully completed in the fall of 1959 and is more extensively described in the botanical section of this report (Section 1.4).

In addition to the activities above, another series of statistical consultations was held with members of the botanical group. These concerned experiments in the ecology of ragweed to be completed during the summer of 1960 at Willow Run Airport and at the new University of Michigan Botanical Gardens.

#### 4.4 MEDICAL ASPECTS

Two of the medical group have undertaken a study into the familial aspects of allergic disease. This study involves both native United States students and foreign students in residence at the University of Michigan. The latter presumably have had no previous experience with ragweed in their native country. A series of statistical consultations with these investigators were completed. The study is discussed more fully in the medical section of this report (Section 2.10).

An additional series of statistical analyses were performed on a set of data from an investigation of passive transfer by white cells of skin-sensitizing antibody to irradiated rabbits. The computations consisted of a series of analyses of covariance of white blood cell counts on time.

#### 4.5 SUMMARY

The statistical activities in this study during 1959-1960 have involved consultation with members of each of the three co-operating groups. Two doctoral students in Public Health Statistics, Mr. Alexander Cicchinelli and Mr. G. Stanley Woodson have recently become associated with the group. The activities of this project should be worthwhile to these graduate students in providing a series of diversified examples of biostatistical consultation at an advanced level. The acquisition of new high speed digital computing equipment on the campus of the University will greatly enhance the ability of the statistical group to handle large sets of data. This should permit a continuing, expanding range of statistical activities.

## 5. PUBLICATION RESULTS

The following papers have either been published, are in press, or have been submitted for publication.

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22. Payne, W. W.: The unique morphology of the spines of armed ragweed. (Submitted for publication to Madrono.)
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## APPENDIX

### Determination of Quantity of Pollen Employed in Allergenicity Tests

The following discussion describes the basis of the estimates of the quantity of anther sacs employed in allergenicity tests (see Section 2.3). The average length  $h$  of an anther sac is  $660\mu$ ; the average radius  $r$  is  $80\mu$ . Assuming the sac to be cylindrical, the average volume  $V$  is

$$V = \pi r^2 h = 1.326 \times 10^7 \mu^3 .$$

This means that about 770 anther sacs are required to yield 0.01 ml. of tapetal fluid if they are completely full.

Next, an estimate was made of the number of pollen grains which could be contained in one anther sac. On the assumptions that A. elatior pollen grains average  $18.3\mu$  in diameter and that they are arranged within the anther sacs in a cubical lattice, each grain would require a volume of

$$V = d^3 = 6128 \mu^3 .$$

Upon dividing this volume of one pollen grain into the volume of an anther sac, it is found that one sac might contain as many as 2164 pollen grains.

If the anther sac is considered a double cone (with common base) instead of a cylinder, its volume is found to be  $5.783 \times 10^6 \mu^3$ . Dividing this figure by the volume of one pollen grain yields 944 grains per anther sac. Since it was desired to create conditions in which there would be optimal opportunity for allergenic activity of tapetal fluid to manifest itself, it was decided to use the smaller estimate of the pollen grain content of anther sacs as a guide for selecting the amount of fresh and old pollen to be extracted for comparative purposes.

Since there were  $1.875 \times 10^4$  anther sacs used in this particular experiment, the assumption that each sac contains 944 pollen grains yields  $1.77 \times 10^7$  grains for the anther sac total. This represents 0.1 gm of dried pollen. Microscopic examination, however, revealed that mature anther sacs are not entirely filled with pollen. It was determined that, on the whole, approximately three-fourths of

each sac is filled; accordingly, 0.075 gm of dried pollen was used for comparison with a like weight of fresh pollen. Therefore, three-fourths of the  $1.77 \times 10^7$  total was used for a numerical estimate, viz.,  $1.42 \times 10^7$  grains.

At a later time, some additional data were obtained which verified the above estimates as being of the right order of magnitude. It was found feasible to count the number of grains per anther, each anther containing four sacs. The average of 6 anthers yielded 553 grains per anther or 138 grains per sac (range: 101 to 220). The original quantity of  $1.875 \times 10^4$  sacs containing 138 grains per sac would give only  $2.588 \times 10^6$  pollen grains. This amounts to one-fifth of the quantity of  $1.42 \times 10^7$  grains estimated above. The difference is probably due to the fact that the mature anther sacs are not even as full of pollen as the three-fourths estimate. If the smaller figure is used as a criterion, and if the tapetal fluid were not allergenically active, the anther extracts would be expected to be only one-fifth as potent as the pollen extract. The volume of fluid used for extraction was such that there was about 4 per cent weight by volume extraction ratio.

The application of these calculations to fresh pollen is dubious. In several experiments, fresh pollen had been removed from the flower less than 2 hr before extraction was commenced. Tapetal fluid could be seen adhering to the grain surfaces; hence, it is doubtful whether the usual figure for the number of pollen grains per gram of dry pollen is applicable here. It is likely that there were substantially fewer fresh pollen grains than old pollen grains used in preparing the comparative extracts.





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