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ATMOSPHERIC POLLUTION BY AEROALLERGENS

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ABSTRACT

Research on ragweed pollen as a major air pollutant has been conducted during the past year on a wide front by an interdisciplinary team of allergists, botanists, meteorologists, and public health statisticians working in close cooperation. New knowledge has been gained on the plant emission of pollen, its atmospheric transport, and allergic response to it.

The botanical research has emphasized studies of the mechanism of pollen discharge from the anther, of the characteristics and distribution of the ragweeds and especially of the newly discovered hybrid perennial ragweed, Ambrosia intergradiens, and of pollen destruction by microorganisms. Over 3000 ragweed plants were grown preseasonally and brought to pollination in June by manipulation of the photoperiod for use in a field experiment on the atmospheric dispersion of pollen. An extensive bibliography on the botanical, medical, and meteorological aspects of ragweed plants and their pollen has been completed and will be published. Research plans for the coming year are presented.

In the medical studies, research on the symptomatology, both subjective and objective, of selected hay fever and asthma patients in the State Prison of Southern Michigan at Jackson has been continued. A primary purpose of the investigation was to clarify certain apparent anomalies found during the previous season. It has been confirmed by objective tests that hay fever symptoms reach their maximum in the morning before the pollen peak is reached, whereas the maximum in asthma symptoms follows the pollen peak as would be expected. No satisfactory explanation of this anomalous hay fever symptomatology has yet been found. Other medical research has been concerned with pollen recovery from human lung tissue, the source of antigenicity of ragweed pollen, the neutralizing and eliciting activity of altered ragweed antigen, and the histopathology of nasal tissue exposed to ragweed pollen. The pollen test chamber for medical studies will be completed and in use in the near future.

The meteorological investigations have been primarily concerned with atmospheric dispersion of pollen from the preseasonal ragweed plants mentioned above which were set out in a circular plot on open farmland of the Jackson Prison. Most of the observational data taken in late June and early July have been abstracted and analysis has commenced. A preliminary theoretical analysis of the observations is presented. An observational program during the regular pollen season was also carried out. Continuing development of sampling methods has been pressed, with improvements made in the gravity-slide technique and in the 6-head millipore filter sampler. A high-volume moving tape impinger of novel design has been constructed, as well as an electronic pollen counter. Other research includes an experimental study of reflotation of ragweed pollen

and a theoretical investigation of the collision process of small particles in a turbulent atmosphere.

Statistical guidance in the design of experiments and in data processing has been given, but additional assistance in the statistical phases is required.

A list of papers published or in press is presented and acknowledgment made to the staff of the State Prison of Southern Michigan at Jackson for their active cooperation.

OBJECTIVE

The atmosphere is contaminated by two groups of substances, natural and artificial. 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, 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*

1.1 INTRODUCTION

In 1957 the botanists continued to focus their attention primarily on the biology of ragweeds. The major work of this year was to complete the studies of perennial ragweeds in Michigan and to initiate new experiments concerning the pollen-discharge mechanism. In addition, much effort was expended in the laboratory study of destruction of wind-borne pollen grains by fungi in the attempt to obtain more precise data on what happens to pollen grains which fall upon the soil or upon surfaces of bodies of water. A first test on annual phenology was carried out, but conditions were such that we considered the results atypical and not applicable to our aims. Further work on the bibliography of the biology of ragweeds has brought this aspect of the project near completion, as will be discussed later.

No less important, however, has been our collaboration with the other groups of this project. Our extra-seasonal experiment of 1958 repeated the cooperative efforts of the previous year's work; the botanists prepared the plants for the experiment and attended to whatever botanical details were necessary for its successful completion. At all times we have maintained communication with the medical and meteorological members of the project, in our large meetings and in smaller conferences, freely exchanging ideas and suggestions which we hope will contribute to the overall progress of our knowledge.

1.2 PREPARATION OF PLANTS FOR THE OUT-OF-SEASON EXPERIMENT

Once again we collected a large stock of seeds (achenes) in the fall of 1956 to augment our supply. This additional collection was very fortunate because the demands of the new experiment designed for this year called for over 3500 plants, that is, over 25 times as many plants as we used previously. Thanks to the kind cooperation of the officials of The University of Michigan Botanical Gardens (Dr. A. G. Norman, Director, and Mr. W. F. Kleinschmidt, Superintendent), this big order was taken care of, even though it became necessary to build a special area in which to mature the plants. During April the seeds were treated to stimulate them to germinate until they were ready for the short-light (long-dark) period to induce flower formation. Maturation of the flowers occurred in the third week of June. The entire collection of plants (with the

*By W. H. Wagner, Jr.

exception of the all-pistillate specimens) was carried in flats, by a semi-trailer truck, to the experimental site on the Jackson Prison grounds, some 30 miles from Ann Arbor. There the plants were arranged in a nearly circular pattern and produced apparently normal flowers and pollen for the required period of study.

1.3 DISCHARGE OF POLLEN

No further work was carried out on floral anatomy during 1957 but the study will be resumed in 1958 in connection with the study of discharge of pollen. We did make a number of new observations of pollen discharge, however, and our former opinion that the flower of ragweeds is a mechanism which is capable of ejecting pollen forcibly has not withstood the test of closer scrutiny. It now appears that, when pollen is discharged, the flower protrudes above the level of the other flowers in the involucre and it may readily be seen, although, because of its small size, a hand lens may be required to obtain a clear view of it. Evidently the anthers or pollen sacs, which cannot be examined directly because they are included in the petal tube, become torn open so that their pollen is free. The pistillodium, in some unknown way, must play some role in the pushing out of the grains. The small projections at the tips of the anthers are stretched out, and the petal lobes become pushed up with the mass of pollen. The pollen grains aggregate in a large mass at the top of the flower. Although they are, no doubt, "forcibly" pushed out (i.e., by processes in the living flower, we would now consider it misleading to describe them as "forcibly ejected." That interpretation was based upon the distances that clumps of pollen could be found from pollen-producing plants. It is now our belief that the distances were really the result of small air currents in combination with the very light weight of the pollen clusters. The clusters apparently "float" for some distance, so long as there is any atmospheric motion; but they will fall nearly straight down when the air is completely still. It should be emphasized that we still consider pollen production to be an active and not a passive process of the living flowers. Pollen is released, so far as we can determine, not by mere drying out as it is in most anthers of flowering plants, but by a life process involving a structural mechanism of the flower.

A series of simple experiments was designed and carried out by Mr. R. T. Hanlin to test pollen-discharge factors. If plants of low ragweed are collected and left to dry in a laboratory, no pollen is discharged; if they are, instead, placed in jars of water rather than being allowed to dry out, some pollen is obtained, but in far lower amounts than in nature or from the living plants growing normally in pots. These facts suggest that the internal water relationships of the ragweed plant are important in pollen discharge and these, of course, can be maintained only in the living plant.

A cubicle in the Botanical Gardens of The University of Michigan was cleared of other plants, and sheets of black paper marked off in 10-cm squares were placed under the experimental plants. The clumps of pollen grains could

easily be seen on the black paper, and this also provided a rough quantitative record of pollen discharge. The ventilator at the back of the cubicle was wired shut and the only air coming into the cubicle was from the top. This was closed before counting started around 4:30 a.m. and opened again at noon, preventing the pollen from being blown around by air currents. By opening it at night, normal drop in temperature and humidity increase were permitted. A hygrothermograph was used to keep a constant record of the temperature and humidity. The door to the cubicle was kept closed at all times, except when entering or leaving the room.

One interesting fact noted was that, as temperature rose and humidity decreased, the average size of the pollen clumps became smaller. When the atmosphere became very dry the clumps broke up upon hitting the paper, scattering the grains and making it difficult to count individual clumps. The data of several experiments indicate that relative humidity is important in pollen discharge: the periods of maximum pollen discharge are correlated with the dropping-off periods after maximum humidity. This leads to the hypothesis that, in the normal diurnal cycle, the plant returns to a turgid condition in the evening after wilting in the sun the previous afternoon. Sometime late during the night the turgor pressure of the inflorescences reaches its peak. At this time, those flowers which are ready to discharge their pollen the next day begin to push out beyond the adjacent ones. As dawn approaches, the pollen mass is pressed out into the upper portion of the anthers. When the sun hits the flower, the apical structures—the tips of the anthers, the pistillodium, and the petal lobes—all extend outwardly, perhaps by drying action, and the mass of pollen aggregates at the top of the flower. The separation of the mass into clusters of pollen grains depends on drying as the humidity decreases in the morning and as breezes come up; and clusters will tend to fly off more or less whole or they will break apart into separate grains. If this overall hypothesis proves to be true, rainy days or cloudy and humid days will both tend to upset the normal cycle, and an abnormal pollen-discharge pattern will be observed.

It is planned to focus especially on the physical factors of the environment and the mechanical details of pollen discharge in 1958 in the attempt to establish more concretely the true nature of the pollen-release process. For this purpose we hope to have a trained physiologist join the botany group and to have carefully controlled environmental conditions studied. The following factors are among those suggested: (1) correlation of humidity and temperature with the discharge process; (2) effect of light (directly or indirectly) on discharge; and (3) effect of "stickiness" of pollen clumps on dispersal.

1.4 THE PERENNIAL RAGWEEDS OF MICHIGAN

The earlier studies of Michigan ragweeds as discussed in our Progress Report No. 1 (pp. 4-12) proved to possess so many interesting facets that we continued the investigation into 1957 with profitable results. Mr. T. F. Beals

joined the senior author this year to investigate particularly the pollen grains and chromosomes. The final draft of our report "Perennial ragweeds (Ambrosia) in Michigan, with the description of a new, intermediate taxon" has been accepted for publication in Rhodora but will not appear in print for at least several months. Accordingly, we shall summarize some of our major findings here.

It is now clear that in discussing the perennial ragweeds of Michigan we are dealing not with one type of plant but two. The field researches of 1957 have shown that the perennial hybrid ragweed of our last report, known then from only two localities, is unexpectedly frequent, as will be described later. We have, therefore, given this newly discovered ragweed the scientific name, Ambrosia X intergradiens (because of its intergradient nature, between the low annual ragweed, A. artemisiifolia, and the perennial species). The eastern perennial ragweed which occurs in the Great Lakes area we are now tentatively designating A. coronopifolia rather than as a variety (var. coronopifolia) of A. psilostachya. (Typical A. psilostachya of the west seems at present to differ sufficiently from our eastern plant to be maintained as a distinct species.)

1.4.1 Eastern Perennial Ragweed, Ambrosia coronopifolia

A number of new facts modify our previous opinion concerning this plant. The extensive stands of clones of this species result from underground vegetative reproduction not by structures which are morphologically stems but by roots instead. The underground axes of the perennial ragweeds are most commonly referred to in literature as "rootstocks" or "rhizomes" (i.e., underground stems), but this is not accurate. The vegetative reproductive organs appear to be root systems, and it is hoped that a detailed report on the process of vegetative propagation can be published in the future. It is this capacity of vegetative reproduction which makes the perennial ragweeds unique among ragweeds, and which permits them to become locally serious weeds that are difficult to eradicate once they become established. Removal of this type of ragweed probably cannot be accomplished by merely pulling up the plant, an effective method with annual ragweeds; it will be necessary to kill the underground axes as well.

The field studies of 1957 plus further examinations of herbaria and literature confirm our conclusion that the perennial species was introduced into Michigan around the turn of the century. We still have no botanical records of Ambrosia coronopifolia prior to 1900. Our recent explorations have swelled the known county distribution of this ragweed from 30 counties to 43. The species extends across the Upper Peninsula of Michigan down to the middle of the Lower Peninsula. Further southward, i.e., in the bottom half of the Lower Peninsula, the species is frequent only in the western or Lake Michigan side of the state. It is extremely rare in the southeastern quarter of the Lower Peninsula. All we can surmise is that A. coronopifolia has spread since 1900 into a great number of localities in Michigan. It was either very rare and local, or nonexistent, in the state prior to that time. It seems likely that the bulk, if not all, of the present-day populations have been introduced from further west—

Minnesota, Wisconsin, and perhaps Illinois—where this species was probably native and well-established. An enumeration of all the counties from which A. coronopifolia is currently recorded will be published along with the year of the first collection for each.

Studies of the variation of A. coronopifolia in Michigan demonstrate that the species is by no means constant in its appearance. The leaves vary from lance-shaped in outline to triangular, from coarsely lobed to bipinnatifid, and from stalkless to short-stalked. The leaf arrangement, while usually opposite at the base of the plant and alternate above, may occasionally be whorled (i.e., with 3 leaves per node). The plant aspect varies from small and simple to large and "bushy" (depending, apparently, largely on where the plant is growing). The fruits vary from short-spiny to spineless. However, all the plants of one clonal population will tend to be alike because of their genetic identity; the variation is noticed between clones and not within clones.

1.4.2 Hybrid Perennial Ragweed

The new, intermediate ragweed, Ambrosia X intergradiens, a natural hybrid between the perennial species, A. coronopifolia, and the low annual species, A. artemisiifolia, has turned out to be surprisingly numerous in Michigan. It is estimated now that between forty and fifty different populations, large and small, have been discovered in our explorations. These were found in over twenty localities in fifteen counties: Alger, Antrim, Benzie, Charlevoix, Cheboygan, Clare, Crawford, Emmet, Grand Traverse, Kalkaska, Marquette, Mecosta, Osceola, Otsego, and Wexford (see Fig. 1.4.1). The different intermediates are quite variable among themselves, and it is conceivable that there is some degree of backcrossing taking place. The hybrid usually occurs with both

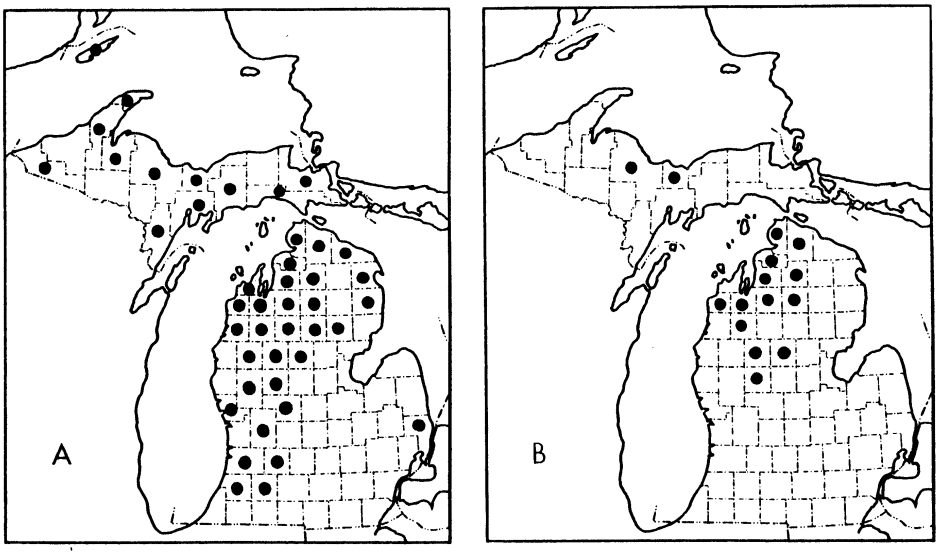

Fig. 1.4.1. County distribution maps of perennial ragweeds in Michigan: A. Ambrosia coronopifolia. B. A. X intergradiens. (Base maps courtesy of Cranbrook Institute of Science.)

parents, but sometimes one or even both are missing in a given locality. It sometimes invades plowed fields, gardens, and plantations of trees. The best localities in which to find it are grassy places along railroad tracks and sandy roads in and around towns and villages.

The intermediate is most likely to be confused with Ambrosia coronopifolia because of its perennial habit. The more or less subtle differences between their gross characteristics are as follows: hairs on stems of A. X intergradiens more spreading and more delicate; whole plant slightly greener (not whitish); leaves more divided with narrower laminar wings between the pairs of lobes and with longer petioles; fruit with more strongly developed beak and spines.

1.5 CHROMOSOMES, POLLEN, AND FRUITS OF THE RAGWEEDS

It was pointed out in Progress Report No. 1 that we hoped to carry out a study on the cytology of pollen formation in the hybrid. This has now been completed with interesting results. One of the curious facts observed in the pollen grains of the new hybrid ragweed was that, in spite of the great irregularity of nuclear divisions leading to pollen formation, the pollen grains themselves appear (superficially, at least) to be normal.

It is assumed as a general rule that, for production of normal pollen grains, the diploid or 2n chromosome set of a typical plant must undergo pairing, the homologous chromosomes lining up precisely with each other before cell division. When the cell divides up ultimately into pollen grains, the haploid or n number (i.e., the number characteristics of the sex cells) will be neatly apportioned to the nuclei of the grains. This is exactly what happens in the low annual ragweed, A. artemisiifolia with a haploid number of n = 18, and in the perennial ragweed, A. coronopifolia, with n = 36. (Our finding of the latter number as given in our previous progress report was confirmed, incidentally, a short while later by G. A. Mulligan, in his paper on "Chromosome numbers of Canadian weeds," in Canadian Journ. Bot. 35:779-789.) In each of the basic species, chromosome pairing appears to be entirely normal in contrast to their hybrid. In Ambrosia X intergradiens, pairing of chromosomes is far from regular. Unpaired chromosomes (univalents) at meitotic metaphase range from 8 to 19, pairs of chromosomes (bivalents) from 11 to 20, and triplets (trivalents) from 0 to 4. When the chromosomes of the hybrid separate to form new nuclei, many division figures show "lagging" or "excluded" chromosomes, i.e., chromosomes which lie separately in the cytoplasm and do not become integrated with the newly formed nuclei. A sample of 171 division figures showing second anaphase had 55% of the figures with one or more (up to 10) excluded chromosomes. It is very difficult to determine precisely the 2n number of chromosomes in the hybrid at the time of pollen formation, but the number can be found by making preparations of dividing cells in the roots. Our additional studies of root tips in 1957 confirm the number of 2n = 54 given previously.

Although, as indicated, the pollen grains of the intermediate seem to be

normal, a test involving the staining of the contents of the grains showed that an average of 54.9% (range 42% to 83%) of the grains of 12 collections was apparently empty. This average percentage is very considerably higher than in 9 collections of A. artemisiifolia (16%) and in 11 collections of A. coronopifolia (22.6%), but is not surprising in view of the highly irregular meiotic nuclear divisions. Additional measurements of the pollen grains of these collections confirmed that the standard deviation of diameter runs well over double that in either parent.

The fruits of all three ragweeds were studied in late September, 1957, in an area north of Stanwood, Mecosta County, Michigan, where the two species and their hybrid are common. Our comparative study revealed that the fruits of the hybrid are what would be expected in a plant of this evolutionary origin. In the parent A. coronopifolia, the terminal beaks are relatively short (average 0.6 mm), and the lateral spines are short or even absent; in the other parent, A. artemisiifolia, the beaks are much longer (average 1.2 mm) and the sides of the fruit are provided with lateral spines averaging 0.5 mm in length. The fruits of A. X intergradiens lie exactly between them in form (see Fig. 1.5.1).

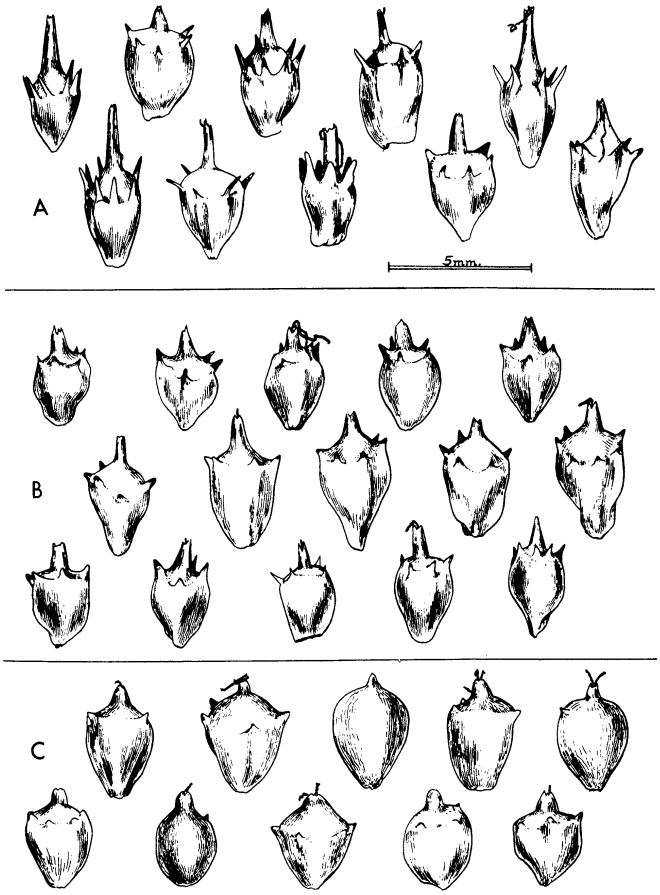

Fig. 1.5.1. Fruits of ragweeds collected near Stanwood, Mecosta Co., Mich., September 28, 1957 (specimens drawn without hairs): A. Ambrosia artemisiifolia (from several plants). B. A. X intergradiens (each horizontal row from a different clone). C. A. coronopifolia (each row from a different clone).

However, the development of fruits in the intermediate is far from perfect, and only 16% of the pistillate flowers enlarge into fruits, in contrast to 55% in A. coronopifolia and 77% in A. artemisiifolia. The intermediate is thus considerably less fertile than either parent. We have no evidence at present on whether the apparently "good" fruits will actually germinate, but a test is planned. One of the interesting byproducts of this study is the conclusion from our quantitative data that the annual low ragweed produces well over six times as many fruits per stem as the typical perennial species. This is perhaps not to be unexpected when it is realized that the former relies entirely upon seeds for survival since it is annual and must reproduce sexually again each year, while the latter is a long-lived perennial and reproduces in the main by underground axes, i.e., vegetatively.

1.6 DESIRABILITY OF MONOGRAPHIC STUDY OF RAGWEEDS

Our researches on the ragweeds of Michigan have convinced us that monographic studies of the genus Ambrosia in North America will be a valuable contribution to the understanding of these important allergenic plants. The aims of such a study may be divided as follows: (a) to re-examine and compare the diverse types of ragweeds in detail; (b) to interpret the types in terms of their evolution and migration; and (c) to express this diversity in modern applications of the categories of species, subspecies, varieties, and hybrids. We hope that it will be possible to carry out a program along these lines in the future.

1.7 DESTRUCTION OF POLLEN BY MICROORGANISMS

One of the questions we asked earlier is "What becomes of the millions of wind-borne pollen grains which settle on the surface of the earth?" Why are they not accumulated in the soil? Are they destroyed? During the spring and summer of 1957, Mr. Solomon Goldstein carried out an investigation aimed to answer a series of specific questions bearing on these problems—for example: Are all pollens equally susceptible to attack by microorganisms? What organisms are involved in pollen destruction? Are allergenic pollens anchored to the ground soon after they are shed, or are they refloated ultimately to cause hayfever?

Mr. Goldstein collected the pollens or spores of 33 species of plants which produce wind-borne pollen, some of them known to be allergenic, including the low annual ragweed, Ambrosia artemisiifolia, as well as other plants such as corn, Zea mays, the loblolly pine, Pinus taeda (see Fig. 1.7.1), the white spruce, Picea glauca, and the cinnamon fern, Osmunda cinnamonea. The pollens he used were, in general, those which contributed most heavily to the atmospheric pollen load. The test pollens were gathered by allowing mature anthers to open over paper at room temperature. With ragweed, however, the flower parts had to be macerated with mortar and pestle to liberate the grains. The pollens

Fig. 1.7.1. Pollen of Pinus showing three infections of the grain by Rhizophidium.

were kept in capped bottles in a deep-freeze when not in use. Samples of soil gathered were from the top 2 cm of the ground; these were kept in cardboard containers until they were used in the laboratory, which usually occurred within 1-1/2 hr after collection. The soil acidity was determined by a Beckman pH meter. Each of four soil samples was divided into 33 subsamples. The subsamples, consisting of 10 gm of soil, were placed in sterile petri dishes and covered by a layer of about 0.5 cm of sterile tap water. The pollen grains were dusted on the surface of the water; one kind of pollen was added to each culture dish. Each day slides were prepared by touching a sterile microscope slide to the surface of the water, adding stain (acid fuchsin) to the adhering material, and sealing this with a cover-slip. One hundred grains per slide were examined. Other studies were made in lake water ("Third Sister Lake," near Ann Arbor) by coating petrolatum-painted slides with pollen and placing the prepared slides in "slide-traps" which were immersed in the water.

Over 140 cultures were made and maintained in the study, and the data were recorded in terms of number of grains infected per 100 grains counted per slide. The infections increased to a high point, on the average, about 10 days following inoculation; thereafter the rate of infection tended to decline. The reasons for this are not known and can only be guessed at.

The overall conclusions of this research showed that no specificity existed between any of the pollens employed and any of the fungus attackers encountered.

There does, however, appear to be a definite variation in the degree to which various pollens are susceptible to attack by the two most commonly found chytridiaceous fungi (Rhizophydium and Olpidium). For example, pollens of Chamaecyparis and Ginkgo never showed more than five infections per 100 grains, while Douglas fir, Pseudotsuga, was attacked totally (see Figs. 1.7.2 and 1.7.3).

In general the gymnosperms (i.e., conifers) are more readily attacked than angiosperms (the broad-leaved trees and herbs). In the majority of infections, the fungus seemed more involved with the contents of the grain than with the wall material, although when multiple infections of a grain occur, these would no doubt weaken (or perhaps even break up) the grain. Nowakowskiella and other polycentric or filamentous fungi may actually form sufficient vegetative material as strands or hyphae to hold down the grains. In the single fern used, the dead grains appear to be more susceptible to invasion than do the living ones. The ragweed tends to be less subject to attack by the chytrids that utilize the contents of the grains; but Nowakowskiella may possibly use its wall material since it was frequently noted in intimate association with the exine of the grain. The number of genera of fungi involved is relatively low (about five genera of any importance), but these few are found in abundance as attackers of pollen grains.

It seems patent from this study that the fungi certainly must play a role in the destruction of pollen grains. Whether the grains land on damp soil or in the water of lakes and ponds, we can expect a high degree of action on pollen grains, action which will vary in terms of the part of the grain affected (i.e., contents, or wall, or both) and intensity of attack (some species are much more readily attacked than others). It will be interesting now to continue our studies of the fate of pollens in soil and water, and especially to attempt to gain an insight into what occurs in situ under varying natural conditions.

1.8 ANNUAL PHENOLOGY OF RAGWEED

Our attempt to make a phenological study of ragweed under uniform conditions turned out, unfortunately, to be unsuccessful. The study was to have taken place in a plot set aside at The University of Michigan's Botanical Gardens, and we actually did set up the site, made plantings, and provided proper instrumentation. However, quite unexpectedly, the plot which we selected for our uniform growing over a period of several years proved to be unsatisfactory. The ground, which during the summer is reasonably dry and appears appropriate for the growth of ragweed, was found to be entirely too wet in the spring for proper preparation and planting. Another problem involved the soil itself: the substratum was much too rich for ordinary or typical low annual ragweed growth, and the plants which developed on the site after we established them grew abnormally tall with abundant branches, so that we ended up in July and August with specimens 5 to 6 ft in height and "bushy" in form which were far from representative of the normal habit of this species. We shall therefore have to determine a new site for further work on this problem.

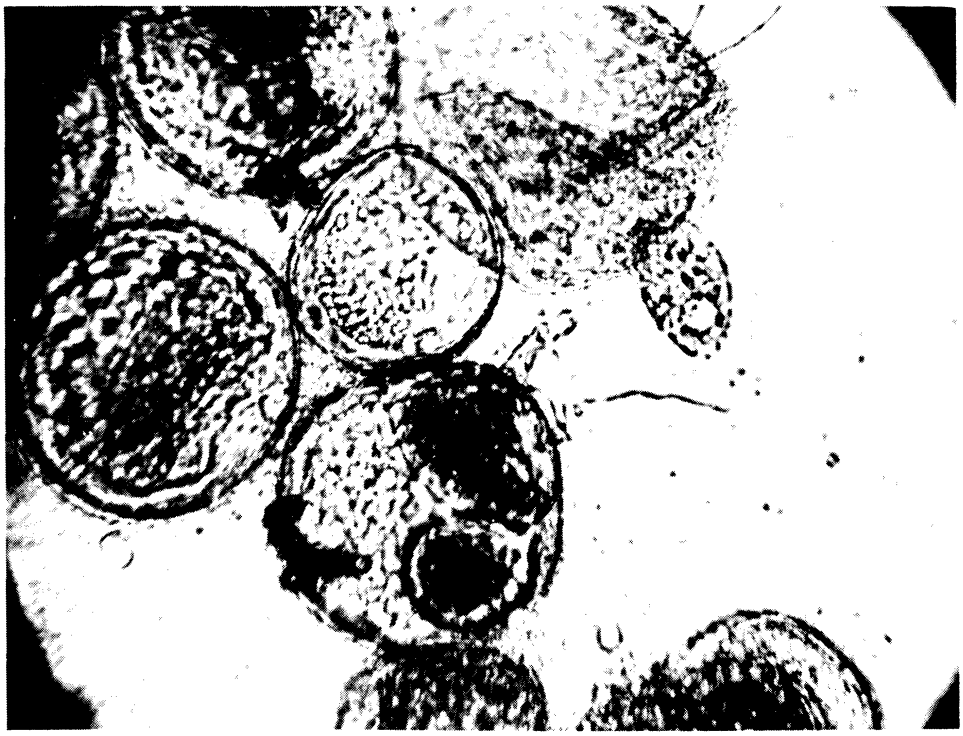

Fig. 1.7.2. Pollen of Pseudotsuga showing infection by the chytrid, Olpidium.

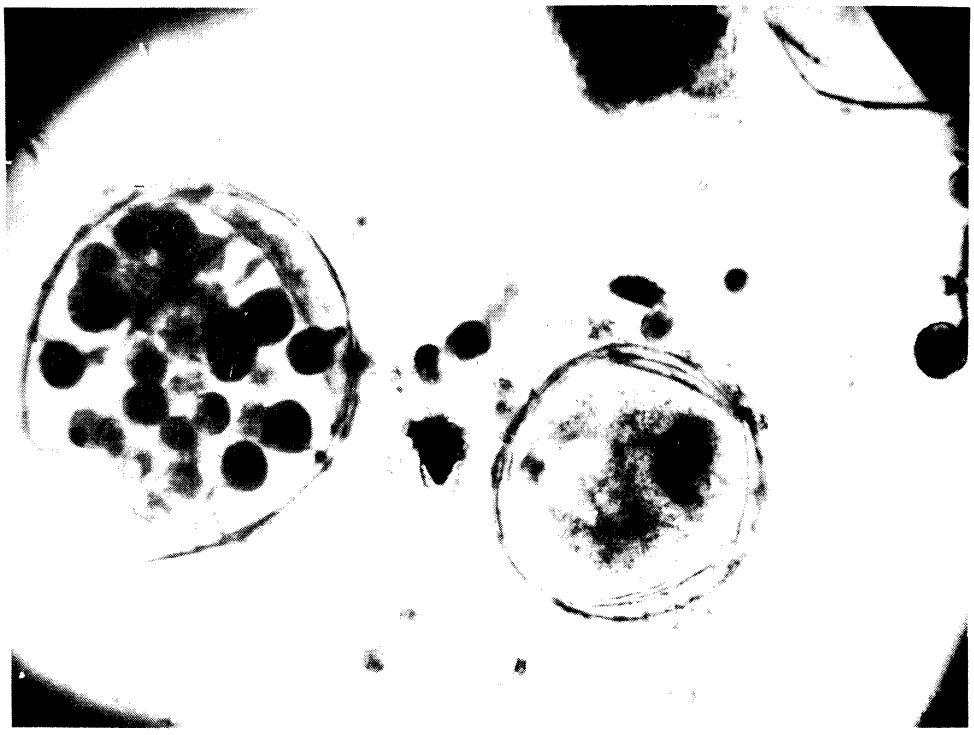

Fig. 1.7.3. Pollen of Pseudotsuga showing multiple infections by Olpidium.

1.9 BIBLIOGRAPHY ON BIOLOGY OF RAGWEED

A number of new references were added by the botanists during the summer of 1957 to our annotated bibliography; and in the late summer and fall, the bibliography was turned over first to the medical group and then to the meteorologists who checked the references and added new ones in their respective fields. Our current plan is to alphabetize all the references throughout, and to do the final editing in the near future. The abbreviations of titles of journals will follow the pattern of Chemical Abstracts. We hope to have the bibliography published in the Quarterly Review of Allergy and Applied Immunology.

1.10 MAJOR EFFORTS FOR 1958

As pointed out above, the study of the floral anatomy and development will be resumed during 1958 and it is hoped that we can bring this to completion. This, of course, will relate directly to the problem of environmental factors acting on pollen discharge, the study of which, if a plant physiologist can be found to do the work, will make the second of our major efforts. A third will be devoted to experiments on the conditions of germination of the pollen of ragweed, an effort which we expect to have some bearing on the medical aspects of aeroallergens. Finally, we intend to repeat the out-of-season experiment and to grow several thousand plants, bringing them to flower roughly two months ahead of time.

1.11 SUMMARY OF THE BOTANICAL PHASE

In addition to their activities in collaboration with other members of the Aeroallergen Project, the botanists carried out the following specific studies:

1. For the experimental studies of pollen dispersal in the field out of the normal pollen season, over 3500 plants of low ragweed were grown in flats and brought to flowering in June and transported to the experimental area at Jackson Prison.

2. Our earlier belief that pollen was "forcibly ejected" from the flowers has been controverted by new observations on pollen discharge. These indicated that the pollen actually accumulates in large masses at the top of the flower where it falls or is carried away by the wind. Records of the pollen discharge through the night and day suggest a close relationship to humidity: the periods of maximum discharge are correlated with the drop-off periods after maximum humidity.

3. The study of perennial ragweeds of Michigan has been completed and is soon to be published. There are two kinds of perennial ragweeds in Michigan: the eastern perennial, which we are interpreting as Ambrosia coronopifolia, and

a hybrid with the low annual ragweed, which we have named Ambrosia X intergradiens.

4. The eastern perennial ragweed evidently reproduces not by underground stems but by proliferating roots. This enables it to form large clones, populations derived from a single plant. Our new field work has increased from 30 to 43 the number of Michigan counties in which this species is known, but we still have no evidence that it occurred in the state prior to 1900; therefore, it probably spread in from further west.

5. The hybrid perennial ragweed has turned out to be surprisingly common and between 40 and 50 populations have been discovered in over 20 localities in 16 counties. It is variable and it may be found especially in grassy places along railroad tracks and sandy roads around towns. It resembles the eastern perennial species but differs from it in a series of subtle characteristics.

6. The chromosomes do not behave normally in the hybrid as they do in the parental species. Staining the pollen grains of the hybrid shows that more than half of them lack contents and are therefore abortive; bad grains are much less common in the parents. The fruits of the hybrid are intermediate between those of the parents, but their development is much less perfect, and only 16% of the female flowers form fruits.

7. Our studies of Michigan ragweeds indicate that for the future a monographic investigation of the whole ragweed genus Ambrosia would probably make a valuable contribution to the understanding of these important allergenic plants.

8. To learn more precisely what happens to pollen grains which fall on soil and water surfaces, a laboratory study of destruction by fungi was carried out. This showed that different pollens vary in their susceptibility to infection and destruction, but a given pollen exhibits equal susceptibility. The number of genera of fungi involved is small, but these are abundant and they surely play a role in the destruction of pollen grains.

9. The attempt to follow the annual phenology of a test plot of ragweeds was spoiled by unexpectedly inappropriate conditions of the site selected.

10. The annotated bibliography on the biology of ragweed is nearing completion and it is hoped to complete the final editing soon and to submit it for publication.

11. The plans for 1958 include (a) completion of the study of the ragweed floral development and morphology; (b) investigations on the conditions of germination of the pollen of ragweed; (c) physiological research on the factors of pollen discharge utilizing precise techniques; and (d) a repetition of the out-of-season growing of ragweed plants for studies of meteorological factors in pollen dispersal.

2. MEDICAL PHASE

2.1 SURVEY OF ACTIVITIES AND OBJECTIVES

Our earliest concern was organization and analysis of the data accumulated at Jackson Prison during the 1956 season. In brief, we found that we need a great deal more statistical help than present funds permit, to analyze fully the data from 1956 and to provide adequate statistical planning of future work.

The following observations are based on limited consideration of the data and on our experience. We have not been able to improve the method of evaluating nasal smears for eosinophiles, i.e., by rating slides on a 1-to-4-plus basis. In the light of the known inaccuracy of the method, we are giving this work a low priority. There has been no change in evaluation of the pulmonary function tests from last year. From our pneumotachograms, we have calculated the average value of the ratio

$$\left[\frac{\text{Expiratory Duration}}{\text{Inspiratory Duration}}\right]$$

to be 1.2. Contrary to our first impressions, these tests may be of some help in 5 of our 11 subjects. One of these was a man who was considered to have pure hay fever, both before and after the season. However, his pulmonary function, by pneumotachogram, showed larger than average ratios during pollen peaks, or symptomatic periods. The fact that only one of the men had objective asthma of any note makes these few other abnormal results all the more impressive. We have been unable to correlate the white blood cell counts with any other events during the study.

After consideration of the above results, we believe that further extensive efforts along similar lines would not be profitable. The outcome of a test may be influenced by time of day in addition to hay fever and asthma. Thus a test which appears inconclusive might be a very sensitive indicator of asthma if conducted at some other time. This emphasizes the need for a test chamber in which it will be possible to observe one patient frequently for several hours.

Therefore, in planning for the 1957 season it was thought advisable to conserve our time, effort, and other resources for chamber work. At first we thought we would have the chamber by late fall, but at this writing it is still under construction. In addition to emphasis on the chamber, we set up certain objectives for the 1957 natural ragweed season designed to add to our seasonal

data on time of daily pollen peaks, average symptom peaks, and the time of daily symptom peaks. The last problem was to be a major part of our year's work. During the 1955 and 1956 seasons, it was noted that patients rated themselves at their worst between 4 and 8 a.m. This is before the usual daily pollen peak, observed inside the prison hospital, and it has been a common clinical observation that hay fever patients complain of great difficulty on first arising and for a few hours thereafter. Similarly, early morning attacks are common with asthmatics. There have been many attempts to elucidate the reasons for this, and some valid explanations have been presented in the case of asthma. But we are not aware of any very promising explanations for this diurnal variation in hay fever symptoms unless it is related to a greater proportion of newly released pollen in the early morning hours.*

Our plan of action for the season, then, was limited to pollen counting, patient-subjective-rating cards, and two 5-day periods of objective recording at 6:30 a.m., 10 a.m., and 1 p.m. At the same time, selected observations of the timed vital capacity and residual lung volume were to be made, and nitrogen wash-out tests were to be done. During the winter, spring, and summer the nitrogen meter was being tested and standardized in the Allergy Laboratories. It is a difficult machine to become familiar with, but by the ragweed season we felt quite confident of our results with the machine. In the last week of June, we visited the State Prison of Southern Michigan at Jackson to discuss our plans and to arrange publicity for recruiting subjects. A follow-up letter was sent out two weeks after this visit. On July 22 we started to work at the prison.

Forty-six letters of application had been written. In addition, 2 men from the 1956 group were contacted, and joined the study again. These men were interviewed and 30 were screened for skin testing. Testing for sensitivity to house dust, ragweed, and fungi narrowed our group to 19 subjects. Most of the men were simply ragweed-sensitive patients; a few had other complications (e.g., fungus sensitivity, perennial asthma) but we included them to ensure the availability of some symptomatic asthmatics to evaluate our tests. By beginning field work in the last week of July, we were able to start collecting subjective ratings on August 1, and pollen counting was underway by August 2.

We found that 19 men were about all we could handle. To render group comparisons at given times meaningful, we felt that data for all 19 should be collected within one hour. It took that long just to see and do timed vital capacity on 19 men. Not only was the time important to us, but also to the men, two of whom dropped out after the end of August because we were taking them away from their jobs too long.

During the two periods, August 19-23, and September 9-13, each man was examined at 6:30 a.m., 10 a.m., and 1:30 p.m. For the August period, timed vital capacity and one-breath nitrogen dilution tests were also done. In the Septem-

*Section 3.3.3 describes the peak in emissions of pollen observed between 4:30 and 8:30 a.m. during the 1957 preseasonal experiment.

ber period timed vital capacity and a variant of the nitrogen dilution test were done at the same hours. At longer intervals during the remainder of the season, timed vital capacities, functional residual capacities, and nitrogen wash-out tests were done on selected subjects. It should be kept in mind that we were not attempting a longitudinal study with the test this year.

The variant of the nitrogen test noted above was described by Boothby and Lundin, in Acta Allergologica. We were pleased with it at first because it presented a test that our experience with the one-breath test had suggested to us. The new test is a nitrogen wash-out test, and is the time required to obtain a mixed alveolar air sample of 2% nitrogen while breathing 100% oxygen. It was considerably easier to do with our particular equipment. We felt that our method of doing the one-breath test was open to criticism as we had neither a recorder nor flow meter. While we compensated quite well for the lack of the latter by a modification on our Vitalometer, the lack of the former is harder to compensate for, as events move quite rapidly. Despite these deficiencies, analysis of the data so far indicates that the one-breath test is the more reliable of the two.

The past year has seen further progress in the recovery of pollen from autopsy materials. This project was carried on actively before the ragweed season and was resumed after a recess during the 1957 season. It has been found unwise from theoretical and practical grounds to do this work when pollen is in the air. Results of this interesting study are reported in detail in Section 2.3 of this report.

During the year a series of experiments was carried out to evaluate the relative antigenicity of the protoplasm and cell wall of dwarf ragweed. The method of separating these two components was first worked out in 1956 and is described in the first progress report. A complete account of these experiments and the findings is included in Section 2.4 of this report.

Studies on the neutralizing and eliciting activity of altered ragweed antigen were also undertaken to explore the hypothesis that if ragweed hapten existed as such or could be produced by alteration of ragweed extracts, such a hapten could possibly neutralize reagin in the ragweed-sensitive patient without the release of histamine or histamine-like substances. Section 2.5 is an account of the work completed to data on this matter.

For the first time, during the 1957 ragweed pollen season, attempts were made to locate pollen grains in samples of nasal membrane tissue taken from a group of patients. This represents an attack on the mechanism by which the allergenic effect of the ragweed reaches the patient. One possible interpretation of the failure to find any pollen in these samples is that the whole grain may be dissolved in some manner. However, this work, reported in Section 2.6, represents only a beginning. Much more can be done along these lines.

2.2 THE JACKSON STUDY, 1957*

During the 1957 season, certain longitudinal studies of ragweed pollinosis were again carried out along the lines reported in 1955[1] and 1956.[2] We are still pursuing the question of dose relationships in pollinosis, as well as several related topics.

This year's study was planned to be limited in scope. We had four objectives in mind: to collect another season's data on subjective ratings and pollen collection, to correlate subjective with objective recordings further, and to evaluate our nitrogen meter.

Subjects for this study were selected prisoners at the State Prison of Southern Michigan at Jackson. This locale was selected, as noted in earlier reports,[1] because we felt that the movements, food, exposure, and treatment of the population were limited. There is no hyposensitization carried out there, and patients take medications on a regular schedule, if at all. Subjects were men suffering from ragweed hay fever and/or asthma as proved by history, physical examination, hospital records, and confirmed by skin tests. Some also had fungus and dust sensitivity. The latter factor was partially controlled by use of dust casings on their bunks. Fungus was not a problem, from the histories, in our subjects.

This year we were able to collect subjective and pollen data for the complete season from August 2 until September 29. Both of these parameters were recorded by 4-hr periods starting at midnight each day.

Subjects rated themselves on a range of 0 to 2 plus, on the symptoms of stuffiness, watering of the nose, sneezing, itching of the nose or eyes, tightness in the chest, choking, wheezing, cough, "out of breath" on stairs, walking, and at rest. They marked an "S" if sleeping.

Our efforts to correlate subjective and objective evaluations took the form of two 5-day periods in which we examined each subject at 6:30 a.m., 10 a.m., and 1:30 p.m. These times were selected as being near the middle of the respective 4-hr time period. Most of the subjects arose between 5:30 a.m. and 6 a.m. This examination evaluated conjunctival color and discharge, nasal mucosal color and discharge, and airway patency and the degree of auscultatory findings in the chest.

At the times noted above, we also did timed 1-sec, and total vital capacity as well as sporadically repeating them during the season. Nitrogen meter readings of the one-breath test of uneven distribution and the wash-out test of Boothby and Lundin were also done.

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Pollen collection was done by three different methods and at three locations. A gravity slide by the standard technic[3] was collected on a high, unobstructed roof in the center of our population area. Millipore filter volumetric samplers[4] were run outside over a grassy lawn at a 5-ft level, and inside on the third floor. This latter location approximated the average sleeping "elevation" of our subjects. A new cellophane tape impinger* was also run beside the outdoor millipore sampler, and this machine is being evaluated by the meteorologists.

Nineteen subjects were recruited in the 1957 study, seven of whom were also in the 1956 group. The remaining four from 1956 were no longer in our population. Two were excluded from most of the following analyses because of unusual sleeping hours.

Figure 2.2.1 shows the average daily symptomatic peak for hay fever and asthma. The average is low because some patients always rate themselves low, and seldom did all patients have maximal symptoms at the same time of day. The highest possible hay fever rating is 8. The highest asthma rating would be 14. Note that these levels were never even closely approached. This would again require that all patients have a severe degree of all symptoms at the same time. The average objective levels on the days these were determined are also on Fig. 2.2.1.

It will be noted that hay fever starts at a level of 1.5 and rises above 2 on August 13. The peak is reached on September 4, and is again below 2 on and after September 27. The peak average symptom level is 4, or not quite three times the basal level.

The average asthma symptom level starts about 2, and on August 8 it goes above 2, but only to 2.5, except for 2.8 on August 14. The level is back under 2 by September 13, and then falls to less than 1 after September 27. It may be of no significance, but September 3 and 4 are the only two consecutive days with the average level definitely above 2.0.

This is even more disappointing than in the case of hay fever. However, there are some factors which must be considered. First of all, we had to include all patients who listed any symptoms. Thus a patient who put down a cough now and then would bring down the average. A check of the symptoms listed will indicate how many people would end up with an "asthmatic symptom" if they fill out a card daily for two months. No severe asthma was encountered either. While recognizing that averages are not always a good indication of events, we use them in the attempt to give an overall picture.

Our method of having asthma patients rate themselves seems less meaningful when the average level changes by only 25% during the season. This is partly due to difficulties in averaging asthma symptoms. Some of the patients had ranges from 0 to 8 or more.

*Operation of the impinger is described in Section 3.2.3 of this report.

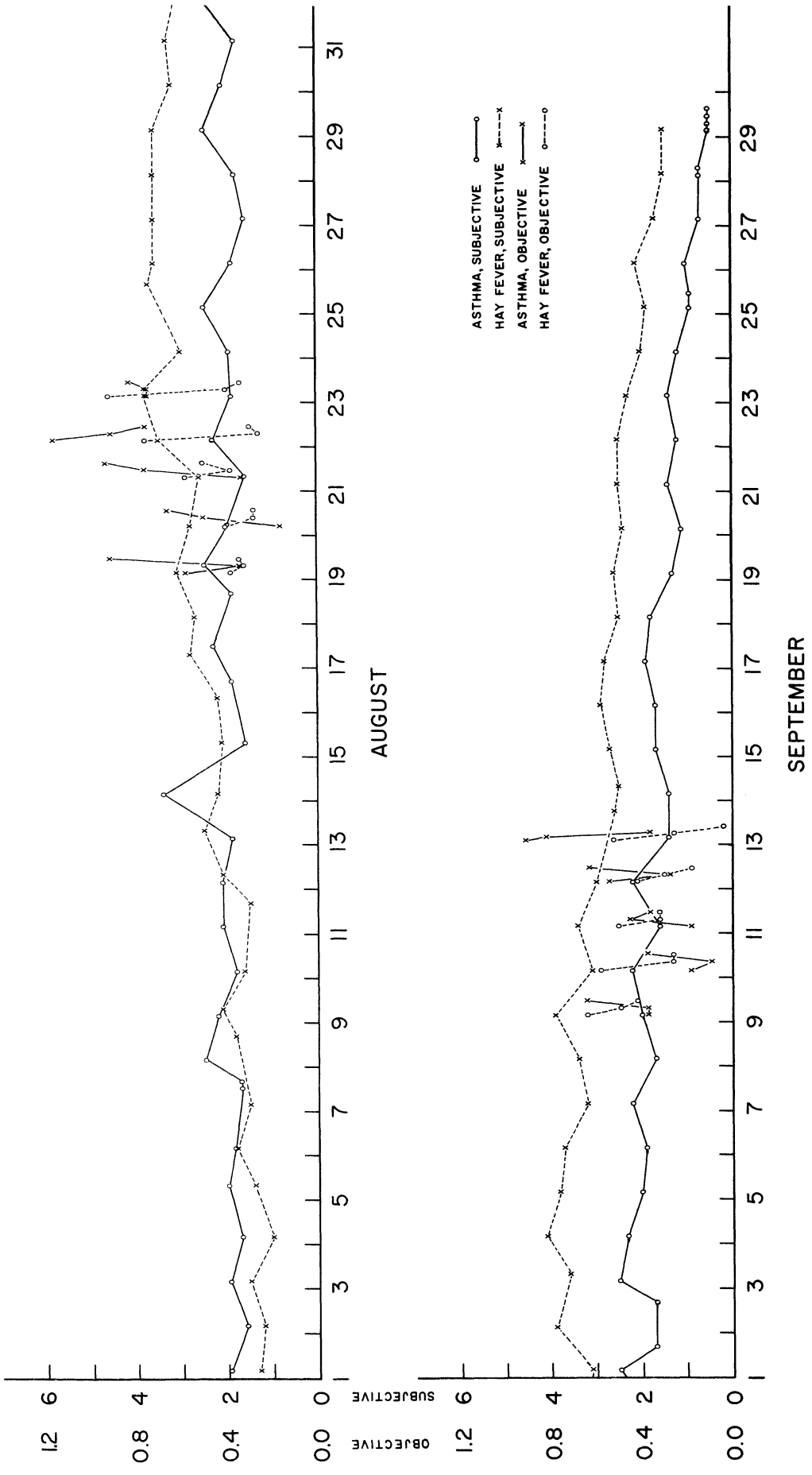

Fig. 2.2.1. Graphic presentation of daily fluctuations in subjective asthma and hay fever symptoms, August 1 - September 29, 1957, and objective signs for two 5-day periods.

As pointed out in our first paper,[2] we found a most interesting time relationship in the 1956 data. Nine of eleven subjects had the majority of their symptomatic peaks in the 4-a.m.—8 a.m. interval. At the same time, most of the pollen peaks occurred in the 8 a.m.—4 p.m. interval, with very few peaks for a day in the 8 p.m.—8 a.m. period. In the 1957 season 10 of 17 subjects had the majority of their symptom peaks in the 4 a.m.—8 a.m. interval. Again in 1957 68% of the daily pollen peaks occurred in the 8 a.m.—4 p.m. intervals. It is surprising that patients should usually feel at their worst before the maximum pollen concentration occurs.

It is important to note that of 19 patients, for whom the majority of hay fever peaks were in the 4 a.m.—8 a.m. period, 7 were on antihistamines and 12 were not. It had been suggested that a patient might feel worse on arising merely because he was only at that time not under the influence of medication. The observations presented do not support this contention.

In the light of the above findings in 1956, we decided to examine subjects more frequently in 1957. On August 19 through 23 and September 9 through 13 the objective data were collected by the method noted earlier. Our results are very interesting. Sixteen of seventeen subjects showed the great majority of their objective hay fever peaks in the 4 a.m.—8 a.m. period. Specifically, 81 peaks of 104 were in this period.

The results are quite different for asthma. Of 40 subject-days in which peaks occurred, 30% were in the 4 a.m.—8 a.m., 25% in the 8-to-noon, and 45% in the noon-to-4 p.m. period.

Thus our data force us to conclude that patients experience the greatest discomfort from their hay fever and asthma early in the morning. On most days this is well before the maximum pollen concentration is observed. We must agree with the patients regarding hay fever, but objectively their asthma seems to be greatest in the afternoon. In the case of asthma, then, the patient is objectively, or clinically, at his worst concurrent with the pollen peak of the average day.

However, there are factors in regard to asthma which suggest caution. Our asthmatics were rated on a 0 to 5-plus scale as follows:

0 - no wheeze;
1 - wheeze on forced expiration;
2 - scattered wheezing;
3 - mild, but generalized wheezing;
4 - marked, generalized wheezing; and
5 - orally audible wheezing.

We were somewhat surprised to find that there seemed to be little difference between 1 and 3-plus, in how the patient felt, or the results of ventilatory testing. Lowell has pointed out this very same thing, however.[5]

We ran the one-breath nitrogen test of pulmonary mixing[6] on all patients periodically, and three times daily during the objective examinations in August. Eighty-six results were 1.6% or over, and 1.5% is considered normal. Readings of 2% were equaled or exceeded on two or more occasions by 7 patients, all of whom were asthmatics. Readings of 2% were recorded once only, by 10 patients, 4 of whom were asthmatics; 3 others were hay fever patients. Of the 2% readings, 91% were in asthmatics.

During the September objective recording interval, we were using Boothby and Lundin's[7] modification of the nitrogen dilution test on a smaller group. Time did not allow us to do this test on the whole group. This is actually a wash-out type of test, and takes as long as 4 min for some normals. We found this test less helpful, although we did not have the opportunity to evaluate it fully.

Tabulating the one-breath test we found that 59% of the 2% or over readings came in the 4 a.m.—8 a.m. period, 25% in the 8-to-noon, and 15% in the noon-to-4 p.m. period. Of these, 65.5% were at 0 objective level (i.e., objective tests indicated zero). For comparison we analyzed our 1-sec vital capacity the same way, noting the period of lowest reading for a day, during the days of three objective readings. Here we found that there were 53 patient-day-lows of which 53% occur in the 4 a.m.—8 a.m. interval, 13% in the 8-to-noon, and 34% in the noon-to-4 p.m. interval. Of the low readings, 60% were at an objective level of 0.

Now we have, in the case of asthma, the subjective and ventilatory tests indicating peak symptoms in the 4 a.m.—8 a.m. period, whereas the auscultatory findings alone would indicate more symptoms later in the day. The more reliable data, then, indicated that patients with ragweed pollinosis have more symptomatology and decrease in ventilatory ability before the maximum pollen concentration occurs. We cannot answer the logical question. Is this due to the fact that the early pollen is more potent, or to meteorological conditions, or to factors inherent in the recently awakened human being?

It is perhaps of some general interest that of three parameters applied to objective hay fever—color, discharge, and patency of the airway—we found that, as a group, subjects had a greater range of fluctuation in the color of the membranes than in the other parameters. This probably merely demonstrates that it is easier to rate a color than such rather more nebulous entities as amount of mucus or degree of obstruction of the passages.

Pollen collections showed daily patterns as shown in Table 2.2.1. It should be noted that 41% of pollen peaks fall in the time interval from noon to 4 p.m. on both samplers. Relatively few peaks are observed from 4 a.m. to 8 a.m. when hay fever symptoms are at a maximum. Somewhat different patterns of pollen release have been reported by Smith and Rooks[8] who found 53% of the total pollen deposited from 6 a.m. to noon, and in another study 56% from 10 a.m. to 1 p.m. They also state that only about 10% of the pollen is collected

TABLE 2.2.1

PERCENTAGE OF POLLEN PEAKS OBSERVED AT DIFFERENT PERIODS
(1 = 0030 - 0430, 2 = 0430 - 0830, 3 = 0830 - 1230, etc.)

Period	1	2	3	4	5	6
Inside head, %	2	16.6	22	41.6	16.6	0
Outside head, %	6	10	28	41	14	0

between 6 p.m. and 6 a.m. and that on a seasonal basis the peak incidence of pollen is reached from 11 a.m. to 12 a.m. Jones[9] also finds that 88% of the daily pollen is found between 8 a.m. and 11:30 a.m. Significantly, both these studies fix the pollen peak after our observed period of maximum symptomology, although placing the peak before noon rather than after noon.

Pollen first began to appear in quantity, 50 grains/sq cm on the gravity slide on August 13. The peak day was August 27, but a week of rain starting on the 29th ruined our slides. The volumetric counters showed the first marked rise in count on August 13 also, but the peak was September 2. The last gravity count above 50 was on September 10, while the marked fall on the volumetric filters occurred on September 12. Two or three grains were seen on the filters as early as August 2, though the first gravity grain was on August 7. Scattered grains were still being seen on the filters on September 29.

Attempts at correlation of pollen fluctuations, signs, and symptoms have led us no further than they have many others. We are more firmly convinced that a chamber wherein we can give measured doses of ragweed pollen over fixed units of time and at controlled and variable meteorological conditions, is the only way to answer the dose question. We recognize that there will be difficulties in quantifying changes in asthma, as has been pointed out elsewhere.[10] However, it seems to us that refinements in quantitative objective methods will result from test-chamber experiments. In a natural season there are just too many variables.

As this year's study was limited in scope, we did not do blood eosinophile counts. Our results during the 1956 work indicate that an elevation in eosinophiles is apt to occur in asthmatics, not in hay fever patients. We found in that group of patients that the peak of their counts occurred on or about the peak of the pollen in the air.

2.2.1 Summary and Conclusions

1. Patients' subjective ratings correlate fairly well with pollen rise

and fall over the season.

2. An asthmatic's rating of his symptoms agrees with ventilatory tests better than with our auscultatory rating.

3. Auscultatory evaluation of asthmatics can be very misleading.

4. Color of the nasal mucous membranes appears to be the most revealing sign in hay fever.

5. Our data, save for the auscultatory evaluation, indicated that most patients on most days have the peak of their hay fever and asthma before most of the pollen appears in the air.

6. The final conclusion is that, to solve many of the problems in ragweed pollinosis, a method which eliminates many of the numerous variables is mandatory. A chamber which will do just that is in process of being constructed for the use of this group.

2.2.2 References

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2.3 POLLEN RECOVERY FROM HUMAN LUNGS*

The problem of the portal of entry of a ragweed pollen into the human is not entirely understood. Many studies show symptoms within minutes of nasal insufflation, and oral ingestion has been found by some to cause symptoms.[1,2] One of us (K.P.M.) has suggested that pollen might be found in respiratory secretions, or in the pulmonary tissues.

In searching the literature for previous work on this question, we find mention of pollen in nasal secretions, sputum and sinus secretions,[3] stool specimens,[4] and nasal biopsy, and as casual findings in tissue sections.[5,6] To the best of our knowledge no one has searched the lungs for pollen.

It seems to be generally thought that pollen grains with a diameter of 18-24 microns could penetrate only to a certain size of bronchus. Toplin et al.[7] for instance, could find barium sulfate dust 2-3 microns in diameter in alveoli, but 5-10 micron particles were in larger bronchi only. This was by a microscopic technique.

When we check studies of the finer anatomy of the lung, we find that not all controversy is limited to allergy. Argument over nomenclature and debate over details of finer lung structure is evident. However, several authors, as listed by Willson,[8] give dimensions for the smallest bronchioles or alveolar ducts ranging from 200 to 400 microns. The dog bronchus has the smallest diameter of 150 microns, according to Miller.[9] Alveoli are said to range from 166 microns[11] to 50-200 microns[8] in the extremes. Innes et al.[10] report bone fragments 70-150 microns in diameter in "alveolar spaces" of animals, from their bone meal feed. Thus the dimensions of the ramifications of the bronchial tree and the alveoli themselves could apparently accommodate a ragweed pollen grain.

It has been reported that there is an initial rapid loss of dust materials from lungs which is more or less complete within 6 hr.[14] It is thought that this represents removal of dust susceptible to ciliary removal. Furthermore, the lung never comes into equilibrium with the dust of the environment during a normal life span. It has been shown that the amount of residual ash recovered

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from human lungs increased continually with age. Barnes and Rooks[12] estimate the 24-hr pollen intake for a resting adult at 150, and they state on the same basis it might be 8400 near a patch of ragweed.

We set out to see just how far the pollen does penetrate in humans. We immediately met a related and very interesting problem: where old pollen goes. Investigators have found pollen grains in rugs,[12] automobiles,[13] and the air of hotels in April, months after the ragweed season. In our own work we found that during a ragweed season we could expect to collect two grains on a 1-in.-by-3-in. glass slide while studying it under the microscope for 30 to 60 min. We also found that glassware in the laboratory on the fourth floor of a building in Ann Arbor would collect several grains in several days during October and November. Once in November 5 grains were observed on cleaned glassware that had been stored on the drying rack for 3 weeks

Our first experiments consisted of attending autopsies during August and September, 1955, and collecting bits of mucus on a round swab stick, then smearing this on a 1-in.-by-3-in. slide. We attempted to obtain specimens from the nose, posterior pharynx, tracheal stub, main stem bronchi, and whenever possible from primary and secondary bronchial branchings.

On the basis of 12 autopsies, we found that patients often had ragweed and alternaria spores in their noses, but counts on the mucus from the remainder of the tract remained at or below 2, which we came to regard as background count. Some bronchoscopic washings were studied with 0-2 grains being found. This technic was then dropped because the sampling method was felt to be very non-representative.

A method was then developed to digest lung tissue while leaving ragweed pollen unaltered in appearance. This method consists of digesting portions of human tissue obtained at autopsy in boiling potassium hydroxide for 20 min. The material is then centrifuged at 2000 rpm for 10 min, and the supernatant pipetted off. Ten percent NaCl is then used to resuspend. This last step is to dilute the KOH which was found to dissolve the filter in concentrated solution. Then the suspension is filtered through a millipore filter with suction. The filter is placed on some Calberla's fluid and the pollen grains are counted. It was soon found that over 0.1 g of tissue would result in such a heavy filtrate that details were obscured.

Samples of nasal mucosa, trachea, lungs, and in three cases exposed thoracic muscle were obtained at autopsy in the last week of August, and the first week of September, 1956. These samples were wrapped in plastic bags and kept in the deep-freeze until we could work with them. The specimens had to be stored for a while because we could not expose them to search for ragweed pollen when pollen was contaminating the air. Actually it was July and again in October and November, 1957, when we did our study.

Table 2.3.1 illustrates the results. We have presented both the actual

number of grains counted, and, to facilitate comparisons, grains per gram of tissue. The control gives some measure of the sensitivity of the techniques used. Blanks run, using just the solutions, resulted in counts of only 0 to 3 grains, the same as the controls. One blank was found to have 5 pollen grains. This was a run mentioned above where clean glassware had been sitting upside down for 3 weeks.

TABLE 2.3.1

NUMBER OF RAGWEED POLLEN GRAINS RECOVERED
FROM VARIOUS TISSUES OBTAINED AT AUTOPSIES
DURING THE RAGWEED SEASON

Sample No.	Nose		Trachea		Bronchus		Lung		Control	
	Total Count	Grains per g	Total Count	Grains per g	Total Count	Grains per g	Total Count	Grains per g	Total Count	Grains per g
1	20	400	13	11			15	25	1	0
2	15	300	34	30	9	6	7	4		
							143	292		
3	6	171	10	6			8	23	3	2
4	18	277			33	41	21	150		
							21	191		
							20	182		
							12	172		
5	14	127	3	27			9	90	0	0
	4	80					9	100		
6	1	8					0	0		
							8	100		
							1	17		

When a known number of pollen grains was added to solutions, we obtained results such as 28/35, 56/64, 7/7, and 357/330, where the numerator is the number found, the denominator the number added to the solutions. When pollen is added to tissue samples, there was a large percentage of "loss." This is easily accounted for. The filters show myriads of irregularly shaped particles, chiefly black, but some red, pink, and yellow. Some pollen grains obviously will be obscured by tissue debris. Therefore we feel that, when we can count a given number of grains on a filter, there are at least that many present.

Further studies would be of interest. It would be worthwhile to try to preserve and fix lung tissue so that we could see whether the grains are in the smaller bronchioles or in the alveoli. Study of the lymphatics of the hilum by our technique would be interesting.

Certainly, if it can be confirmed that pollen grains get into the lung itself, we have a place where pollen may be in intimate contact with capillaries, and we also have a sort of storage bin. This may bear on the fact that ragweed-sensitive patients may continue to have symptoms for a week or so after the pollen disappears from the atmosphere.

2.3.1 References

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2.4 AN EVALUATION OF THE RELATIVE ANTIGENICITY OF THE PROTOPLASM AND CELL WALL OF DWARF RAGWEED (AMBROSIA ARTEMISIIFOLIA) POLLEN*

2.4.1 Introduction

Little attention has been directed toward the anatomical localization of antigenic fractions in pollen grains, although there has been considerable research in the immunochemical field relative to the antigenic components of pollen extracts. Our interest in the possibility of localization of the active antigenic fractions in the cell wall was stimulated by recent observations in this laboratory which, at least preliminarily, suggest that tapetal fluid[1] (which surrounds pollen grains in developing anthers) may contain a relatively high content of pollen allergens.

The literature[2-10] reveals that the extraction of ground pollen has been reported as early as 1906.[2] Extracts prepared in this manner generally did not prove to be conspicuously more antigenic than extracts prepared in the usual way from whole pollen grains, although they did have a higher total nitrogen and protein nitrogen content. However, all previous investigators have used either water, saline, Coca's solution, or a combination of these as the suspending medium while the material was in the grinding process. Thus the possibility of localization of the major portion of the antigenic fractions in the cell wall was not precluded by previous work, if one accepts the possibility that these fractions might be highly soluble in aqueous media. In the present work an attempt was made to separate the cell wall and the protoplasm fractions of pollen grains prior to exposing them to aqueous media. The pollen grains were usually suspended in acetone during the process of fragmentation and while attempting to separate them into protoplasm and cell wall fractions. Acetone was utilized since it is known that pollen allergens have a very low solubility in this medium. When separation into the two fractions was completed, extraction was carried out with a commonly used aqueous extracting medium, and qualitative and

*By N. K. Cohen.

quantitative comparisons of the extracts were performed by the passive transfer neutralization technic.[11-13]

2.4.2 Methods

2.4.2.1 Disruption of the integrity of the pollen grain.

Many methods* were evaluated; however, the only satisfactory process of fragmentation occurred with the use of Blendor homogenization, as follows.

A standard Waring Blendor motor base was coupled with a semi-micro container. Elevation of temperature was controlled by welding a continuous circulation-type metal water jacket to the external micro area of the Blendor container. We were therefore able to maintain a temperature at or below 10°C. Fragmentation was accomplished with this basic unit when whole nondefatted Ambrosia pollen was suspended in acetone and then mixed with Super-Brite glass beads as described by Lamanna and Malette.[14] We used a combination of 1.0 g of nondefatted pollen, 43.5 g of glass beads, and 20 ml of acetone. The disruption and fragmentation of Ambrosia artemisiifolia pollen was approximately 99% complete in 45 min with the mixture temperature at 7 - 10°C.

The use of acetone or other organic solvents necessitates frequent change of the rubber gaskets on the Blendor container.

The pollen is not ruptured in the absence of glass beads. The concentration of pollen is not critical; however, adequate beads must be present. The requirement that the viscosity of the suspension be low enough to permit flow must be considered also. Disruption of the pollen grains is apparently proportional to the ratio of beads to suspending fluid. Thus, the greater the number of beads and lesser the amount of acetone, the better the fragmentation, if mass movement is sufficient.

2.4.2.2 Separation of protoplasm from the cell wall.

After fragmentation is accomplished, it is necessary to separate the glass beads from the Protoplasm (P) -- Cell Wall (W) fractions (hereafter called P-W combination). This was performed by repeated differential centrifugations. The material was centrifuged at low speed (1200 rpm). This made it possible to remove the P-W fractions from the glass beads with a fine spatula. Further separation of P from W was attempted by repeated centrifugations at 3000 rpm. Microscopic observations revealed that the protoplasm fraction stayed at an upper level, while the cell wall material migrated to a lower level after centrifuging.

*Fragmentation attempts were made with the use of the tissue homogenizer and four different types of ultrasonic vibrator units ranging from 10 to 55 kilocycles. Whole pollen grains were utilized either dry or suspended in acetone or ether. At 55 kilocycles we were able to distort the unit shape of the whole pollen grains, although no disruption of the integrity of the cell wall occurred.

After more than 50 centrifugations, a relatively pure (approximately 99%) protoplasmic fraction was obtained. Before each centrifugation, the material was resuspended in acetone, without glass beads, by a short run in the Waring Blendor.

The procedure was repeated for the Cell Wall (W) material, but we were able only to concentrate the W material to an extent that the resultant suspension contained approximately 50% W material and 50% P material, though this is referred to, hereafter, as the Cell Wall (W) fraction. Efforts to attain better separation, by ultra-centrifugation were not satisfactory.

2.4.2.3 Extraction.

Preparation of the extracts of cell wall (W), protoplasm (P), and whole-defatted Ambrosia artimisiifolia pollen (C) was accomplished as described by Sheldon, Lovell, and Mathews.[15] Modifications were instituted as follows:

A. A Selas candle filter, size 0.02, was substituted for the Seitz filter, as listed.

B. The acetone was allowed to evaporate from the final cell wall and protoplasm preparations until a constant, dry weight was obtained. The two fractions and the whole pollen were then extracted at 1:50 concentration.

C. Sterility was tested by transfer of 0.25 ml of each extract to 15 ml of thioglycolate liquid media. This was then incubated for 7 days.

D. Semi-micro Kjeldahl nitrogen analyses were performed on each extract. The following results were obtained: Cell Wall (W) extract--0.23-mg total nitrogen per ml, Protoplasm (P) extract--0.24-mg total nitrogen per ml, and Whole Pollen (C) extract--0.32-mg total nitrogen per ml. Thereafter, we adjusted each extract to 0.15-mg total nitrogen per ml, with sterile buffered saline, as stated in E below. We labeled this as our stock extracts. These extracts were then considered as 1:100 concentration.

E. Progressive ten-fold dilutions of the stock extracts were prepared in physiologic saline solution, which was phosphate-buffered to a pH of 7.9. Each solution contained 0.4% phenol.

2.4.2.4 Passive transfer neutralization

A. Blood was obtained from three patients with a history of allergic rhinitis, symptoms of which occurred primarily in August and September. All blood donors had 4-plus scratch reactions to dwarf ragweed (Ambrosia artemisiifolia). Donors had never received hyposensitization. A specimen of each serum was tested for ste-

rility, and all sera were stored in refrigeration at 4°C.

B. The antigen-antibody mixtures were prepared by addition of 0.4 ml of serially diluted serum, from each of the donors, to 0.1 ml of each of three dilutions of the cell wall (W), protoplasm (P), or whole pollen (C) extracts. The mixtures were incubated for 24 hr at 4°C. Of the antigen-antibody mixtures, 0.1 ml was planted intradermally into the backs, arms, or forearms. Five nonatopic recipients were used. The sites were challenged 48 to 60 hr later. The serum from donor number 3 was deleted from the series after initial studies in 3 recipients showed that the un-neutralized passive-transfer control sites did not react significantly in 2 of the 3 recipients.

C. Two methods of challenging the passive transfer sites were used as follows:

1. Intracutaneous challenge of 0.025 ml of the various dilutions of cell wall (W), protoplasm (P), and whole pollen (C) extracts with antigen controls injected into unsensitized skin. Additional controls included saline injections into planted sites and antigen injections into un-neutralized serum sites (the latter representing positive controls). These reactions were read after 20 min.

2. Intramuscular challenge with 3.0 ml of 1:50 whole dwarf ragweed injected into the buttocks. The sites were inspected at 5-min intervals from 20 to 120 min after the intramuscular injection. The reactions were usually maximal at about 90 min, and fading occurred after 110 min.

2.4.3 Results

As Table 2.4.1 illustrates, the most clear-cut results were obtained with Serum II, neutralized with cell wall (W) extract. As indicated, the site planted with serum and neutralized with W 1:100 concentration extract no longer reacted when the recipient was challenged with antigen, whereas the sites neutralized with the more dilute antigen (W 1:1,000 and W 1:10,000) still reacted. The W 1:100 site is considered to be the end point of this titration.

In comparison, the Serum II sites neutralized with protoplasm (P) and whole pollen (C) extracts show complete neutralization in all the dilutions used, thereby suggesting greater antigenic activity in these extracts than in the W extract.

No satisfactory end points occurred in Serum I and therefore we could not compare antigenicity of the W and P extracts. However, the reactions in columns

TABLE 2.4.1

TYPICAL RESULTS IN A RECIPIENT CHALLENGED INTRAMUSCULARLY (IN THE BUTTOCKS) WITH 3 ML OF 1:50 WHOLE DWARF RAGWEED EXTRACT

All Sera 1:1

	Column A Neutralized $\bar{c}$: i.-5 mm e.-11 mm	Column B Neutralized $\bar{c}$: i.-5 mm e.-10 mm	Column C Neutralized $\bar{c}$: i.-0 e.-0	
Serum I	W 100 i.-8 mm e.-15 mm	P 100 i.-4 mm e.-12 mm	C 100 i.-0 e.-0	Un-neutralized Serum Control I i.-11 mm e.-30 mm
	W 1,000 i.-10 mm e.-30 mm	P 1,000 i.-6 mm e.-25 mm	C 1,000 i.-0 e.-0	
	W 10,000 i.-0 e.-0	P 10,000 i.-0 e.-0	C 10,000 i.-0 e.-0	
Serum II	W 100 i.-6 mm e.-6 mm	P 100 i.-0 e.-5 mm	C 100 i.-0 e.-0	Un-neutralized Serum Control II i.-12 mm e.-20 mm
	W 1,000 i.-6 mm e.-10 mm	P 1,000 i.-0 e.-9 mm	C 1,000 i.-0 e.-0	
	W 10,000 i.-0 e.-0	P 10,000 i.-0 e.-0	C 10,000 i.-0 e.-0	
Serum III	W 100 i.-0 e.-0	P 100 i.-0 e.-0	C 100 i.-0 e.-0	Un-neutralized Serum Control III i.-0 e.-0
	W 1,000 i.-0 e.-0	P 1,000 i.-0 e.-0	C 1,000 i.-0 e.-0	
	W 10,000	P 10,000 Saline Injection i.-0; e.-0	C 10,000	

W = Cell Wall Extract, P = Protoplasm Extract, C = Whole Pollen Extract, i. = induration, e. = erythema, 100 = 1:100 dilution of antigen used in neutralizing the serum; similarly 1,000 = 1:1,000 dilution, and 10,000 = 1:10,00 dilution.

A and B in Serum I (Table 2.4.1) all showed 4-plus reactions, although the serum neutralized with whole pollen (C) extract was neutralized completely in all three dilutions. Whole pollen (C) extract was, therefore, a more potent neutralizer of Serum I than either W or P extracts.

Serum III was eliminated following this series since no reactions occurred in the un-neutralized control or neutralized plants. The un-neutralized controls in Sera I and II were significantly reactive.

End points were not satisfactory in a number of experimental series (see Table 2.4.3). We adjusted our serums by varying the dilutions. End points were not obtained in some of the series since all the sera had been neutralized by the antigen, or since the serum controls were not reactive. Satisfactory dilutions were obtained and end points reached in only 18 of the 35 total antigen-antibody mixtures (see Table 2.4.3).

Table 2.4.2 shows typical results in a recipient challenged intracutaneously by the cell wall (W), protoplasm (P), and whole pollen (C) extracts. Columns M, N, and O refer to sites neutralized in vitro with cell wall (W) antigen. Sites in columns R, S, and T were neutralized in vitro with protoplasm (P) antigen.

The quantitative difference between cell wall (W) and protoplasm (P) extracts in Table 2.4.2 may be explained by comparison of columns. Column M compared with column R indicates the relative ability of cell wall (W) antigen and the protoplasm (P) antigen to neutralize serum to a challenge with cell wall (W 1:1,000 concentration) extract. The result in Serum I shows that the P extract appears to have a greater neutralizing capacity than the W extract. Although the results are not as clearly defined with Serum II, nevertheless the erythema is certainly diminished in column R as compared with column M.

Column N versus column S in Table 2.4.2 indicates the comparative ability of W and P antigen extracts to neutralize to a challenge with protoplasm (P 1: 10,000 concentration) extract. The results with both Serum I and Serum II indicate that the protoplasm (P) antigen extract has a greater neutralizing capacity than the cell wall (W) antigen extract.

Column O versus column T in Table 2.4.2 reveals in Serum I that protoplasm (P) antigen extract was a better neutralizer than was cell wall (W) antigen extract to a challenge of whole pollen (C) extract. The results for Serum II were equivocal when comparing column O with column T.

Thus, it appears from Table 2.4.2 that in 4 of 6 comparisons, protoplasm (P) extract was a more potent neutralizer than the cell wall (W) extract, when neutralizing serum to a challenge of either W, P, or C antigen extracts. The remaining comparisons were equivocal.

TABLE 2.4.2

TYPICAL RESULTS IN A RECIPIENT CHALLENGED INTRACUTANEOUSLY

All Serum I = 1:10 Dilution

	Neutralized with Cell Wall (W) Extract as Follows:			Neutralized with Protoplasm (P) Extract as Follows:		
Columns	M	N	O	R	S	T
Challenged $\bar{c}$	W 1:1,000	P 1:10,000	C 1:10,000	W 1:1,000	P 1:10,000	C 1:10,000
Serum I with Saline I.C.	i.-5 mm e.-0	i.-6 mm e.-0	i.-5 mm e.-7 mm	i.-5 mm e.-0	i.-4 mm e.-0	i.-4 mm e.-0
Control (0.025 ml) i.-4 mm e.-0	W 100 i.-5 mm e.-0	W 100 i.-4 mm e.-0	W 100 i.-6 mm e.-12 mm	P 100 i.-6 mm e.-0	P 100 i.-5 mm e.-0	P 100 i.-6 mm e.-10 mm
	W 1,000 i.-6 mm e.-21 mm	W 1,000 i.-9 mm e.-20 mm	W 1,000 i.-8 mm e.-21 mm	P 1,000 i.-6 mm e.-0	P 1,000 i.-5 mm e.-0	P 1,000 i.-9 mm e.-22 mm
	W 10,000 i.-6 mm X-e.-24 mm	W 10,000 i.-9 mm X-e.-29 mm	W 10,000 i.-11 mm X-e-27 mm	P 10,000	P 10,000	P 10,000

All Serum II = 1:5 Dilution

	Neutralized with Cell Wall (W) Extract as Follows:			Neutralized with Protoplasm (P) Extract as Follows:		
Columns	M	N	O	R	S	T
Challenged $\bar{c}$	W 1:1,000	P 1:10,000	C 1:10,000	W 1:1,000	P 1:10,000	C 1:10,000
Antigen I.C. in Normal skin	i.-6 mm e.-0	i.-4 mm e.-0	i.-6 mm e.-0	i.-4 mm e.-0	i.-6 mm e.-0	i.-5 mm e.-0
Controls (0.025 ml) W---i.-4 mm	W 100 i.-6 mm e.-7 mm	W 100 i.-6 mm e.-18 mm	W 100 i.-7 mm e.-11 mm	P 100 i.-6 mm e.-6 mm	P 100 i.-6 mm e.-0	P 100 i.-10 mm e.-12 mm
1,000 e.-0 P-----i.-5 mm 1,000 e.-0	W 1,000 i.-10 mm e.-41 mm	W 1,000 i.-8 mm e.-18 mm	W 1,000 i.-11 mm e.-20 mm	P 1,000 i.-11 mm e.-13 mm	P 1,000 i.-8 mm e.-12 mm	P 1,000 i.-11 mm e.-21 mm
C-----i.-5 mm 10,000 e.-0	W 10,000 XXi.-14 mm e.-51 mm	W 10,000 XXi.-10 mm e.-30 mm	W 10,000 XXi.-12 mm e.-29 mm	P 10,000	P 10,000	P 10,000
			Saline injection into Serum II i.-5 mm e.-0			

All sites, as indicated in this table, were challenged with 0.025 ml of antigen extract or saline, as specified by letters. W = cell wall extract, P = protoplasm extract, and O = whole pollen extract of dwarf ragweed. The letters i. = amount of induration, and e. = amount of erythema in mm. X = un-neutralized Serum I controls and XX = un-neutralized Serum II controls. The numbers 100 = 1:100 dilution of antigen used in neutralizing the serum. Similarly, 1,000 = 1:1,000, and 10,000 = 1:10,000 dilution of antigen used in neutralizing the serum. These preceding numbers are also used to indicate the strength of the challenging injection into normal skin, as indicated.

TABLE 2.4.3

SUMMARY OF RESULTS OF TABLES 2.4.1 AND 2.4.2

Total No. of Titrations	35
Unsatisfactory End Points, Total No.	17
Satisfactory End Points, Total No.	18
Protoplasm better neutralizer than cell wall extract, Total No.	14
Protoplasm equal to cell wall extract neutralizing capacity, Total No.	2
Cell wall better neutralizer than protoplasm extract Total No.	2

Table 2.4.3 summarizes the results obtained in all the recipients. There were 18 end points. In 14 instances protoplasm (P) extract appeared to neutralize better than cell wall (W) extract. Protoplasm (P) extract was equal to neutralizing ability of cell wall (W) extract in 2 titrations, and W extract neutralized better than P extract in 2 titrations.

Many of the titrations without end points showed a definite trend toward greater neutralization with protoplasm (P) extract than with cell wall (W) extract. This was indicated by the size of induration and erythema.

In addition to the quantitative types of comparison between the antigenicity of the cell wall (W) and protoplasm (P) extracts just described, it is also of interest to see if there are qualitative differences between these antigens. In other words, does either of these extracts contain antigenic fractions not present in the other? Such comparisons as columns M versus N and R versus S in Table 2.4.2 serve to answer this question. However, preliminary to making such comparisons, it should be noted that it is essential that the concentration of the three antigens used for challenging the sites have equal capacities of eliciting skin reaction. This was established by preliminary titrations (not recorded) in which varying concentrations of W, P, and C extracts were used to challenge un-neutralized passive transfer sites. These titrations indicated that cell wall (W 1:1,000 conc.), protoplasm (P 1:10,000) and whole pollen (C 1:10,000) extracts had approximately equal activity, which was the reason why these concentrations of antigen were used to challenge the sites shown in Table 2.4.2.

A comparison of column M with column N in Table 2.4.2 shows no qualitative differences between W and P antigen extracts since they both had the same end points. This is true both of serum I and serum II. However, it is possible that differences would have been apparent if smaller than ten-fold increments of antigen had been used in neutralizing the sera.

Column R versus column S in Table 2.4.2 shows that inconclusive results were obtained with Serum I. With Serum II, column R versus column S shows an interesting qualitative difference, since W extract elicits a reaction where neutralization has been accomplished with P extract. An identical finding was obtained with similar tests on another recipient. This may indicate that some antigen is present in the cell wall (W) extract that is not present in the protoplasm (P) extract.

Comparison of column M with column O in Table 2.4.2 indicates that in Serum I whole pollen (C) extract appears to contain antigen not present in cell wall (W) extract. Although this was not borne out with Serum II, results of the type obtained with serum I were achieved in another recipient.

Comparison of column S with T in Table 2.4.2 reveals that in serum I there is some apparent qualitative difference between whole pollen (C) and the protoplasm (P) extracts, since C extract still elicited reactions after complete neutralization with P extract. This was confirmed with serum II and similar results were also obtained with both sera I and II in another recipient.

2.4.4 Discussion

In preparing the cell wall fraction used in these studies, it was desired to disrupt the pollen grains in such a manner that microscopically recognizable fragments of the cell walls would remain. The method described accomplished this purpose very satisfactorily. However, the subsequent separation of the cell wall and protoplasmic fractions by repeated centrifugation proved tedious, and the large amount of handling of both the W and P fractions may in part account for differences between both of these fractions and whole pollen extracts. There is no doubt that the W fraction was contaminated with a considerable amount of protoplasmic material (though the converse was not true). It was reasoned that if a major portion of the ragweed allergen is located in the cell wall of the pollen grain, the P fraction should be clearly less active than the W fraction. This did not prove to be the case, but the contamination of the W fraction with P leaves unsettled the question of whether the cell wall of ambrosia pollen simply contains less allergen than the protoplasm or whether it contains no allergens. Further studies with a more purified cell wall would be needed to settle this point.

The finding that most of the allergenic activity of ragweed pollen resides in the protoplasmic fraction is in agreement with the *a priori* assumption of most allergists. As mentioned previously, however, it became of interest to be more certain about the anatomic localization of pollen allergens in view of the possibility that allergenic material might be deposited on the surface of pollen grains by the tapetal fluid bathing pollen in the developing anthers.

The passive transfer neutralization test was chosen as the best generally available procedure in assaying the relative activities of the W, P, and C

fractions,[16] but the limitations of the method are well known. The present work reconfirmed the desirability of having a satisfactory, quantitative, in vitro method for measuring allergenic activity. Nevertheless, it is felt that the results of this study are significant, particularly considering the fact that only ten-fold differences in neutralizing capacity were considered. Also, the results of the passive transfer neutralization tests are supported by the observation that the W extract was less active than the P or C extracts in direct intracutaneous testing of passive transfer sites.

2.4.5 Conclusions

1. A method for fragmentation of pollen grains has been outlined.

2. The relative antigenicity of two fractions, the cell wall and the protoplasm of Ambrosia artemisiifolia pollen has been evaluated by passive transfer neutralization procedures. Fourteen of eighteen satisfactory end points indicated that the protoplasmic extract was capable of greater neutralization of serum than was the cell wall extract when both were challenged either by the cell wall, protoplasmic, or whole pollen extracts.

3. The protoplasmic extract fraction revealed greater antigenicity than did the cell wall extract fraction of Ambrosia artemisiifolia when used to challenge un-neutralized serum.

4. The cell wall extract fraction contains a qualitatively different antigenic fraction not present in the protoplasmic extract fraction of Ambrosia artemisiifolia.

5. Whole pollen extract may contain antigenic fractions that are not possessed by either the protoplasmic or cell wall extract fractions of Ambrosia artemisiifolia as prepared in this laboratory.

2.4.6 Acknowledgement

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2.4.7 References

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2.5 STUDIES ON THE NEUTRALIZING AND ELICITING ACTIVITY OF ALTERED RAGWEED ANTIGEN*

2.5.1 Introduction

The possibility of application of experimental knowledge regarding hapten immunochemistry to clinical pollenosis has not received extensive investigation. Landsteiner's work on sensitization to hapten-protein complexes demonstrated that the hapten alone was capable of inhibiting the precipitin reaction between certain hapten-proteins and antisera.[1] He also showed inhibition of anaphylaxis by preliminary injection of hapten into guinea pigs sensitized to hapten-protein complexes.[2] Heidelberger and Kendall[3] observed that partial hydrolysis products of specific polysaccharide of the pneumococcus could inhibit precipitation with homologous rabbit antisera. Tillet, Avery, and Goebel[4] also showed hapten inhibition of anaphylaxis in guinea pigs. They sensitized guinea pigs to carbohydrate-protein complexes capable of producing anaphylaxis when the whole complex was used to challenge the pigs. When the glucoside alone was used, specific desensitization for short periods was produced.

It was proposed by one of us (K.P.M.) that if ragweed hapten existed as such or could be produced by alteration of ragweed extracts, such a hapten could possibly neutralize reagin in the ragweed-sensitive patient without the release of histamine or histamine-like substances. The possibility of such a hapten existing was suggested by the work of Benjamins, Von Dishoeck, and German[5] who reported that ultrafiltrates of ragweed extracts contained low molecular weight substances which were "activated" by large protein molecules. Long and Teller reported similar results.[6] Service[7] stated that polysaccharides obtained from pollens would inhibit anaphylactic or Dale reactions in guinea pigs if they were administered prior to the pollen antigen.

Hapten in ragweed extract might exist as a free carbohydrate substance, a free polypeptide, or as a carbohydrate or polypeptide group attached to a larger protein molecule. The extensive investigations conducted by several laboratories[8-17] on ragweed antigens have demonstrated skin reactivity in fractions of extracts obtained by several different chemical procedures. These results could be explained in part by the presence of a single or limited number of haptenic groupings on protein molecules widely variant in size. Some substantiation of this concept is offered by the work of Pringle[8] whose data suggested the presence of a very limited number, if any at all, of terminal amino acid residues

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in crude ragweed extract. For this investigation, crude ragweed extracts were chosen without attempting to purify the material physically or chemically. If hapten exists as a free carbohydrate or polypeptide material of low molecular weight, dialysis through a cellophane membrane should separate a majority of it from larger molecules. If it exists attached to a larger protein molecule, its separation might be possible by hydrolysis under varying chemical or physical conditions such as proteolytic digestion, acid hydrolysis, heat, or combinations of the above methods.

Such a hapten could theoretically be demonstrated by a modification of the passive transfer nertralization reaction. This method, based on the work of Cooke and his associates[18] and Stull and Sherman,[19] and critically evaluated by Arbesman and Eagle,[20] is one of the most reliable methods presently available for assaying allergens. Ragweed extract in sufficient amount will neutralize the ragweed reagin in serum from a sensitive individual. This mixture, injected into the skin of a nonsensitive recipient, will not result in a skin reaction when challenged with ragweed extract 24 hr later.[20] Serial dilutions of ragweed extract added to aliquots of serum from a ragweed-sensitive individual, planted intradermally in a nonsensitive recipient and challenged with ragweed extract, will give a measure of the extract's neutralizing ability and thus its antigen content.

The presence of haptens capable of neutralizing reagin without the release of histamine or histamine-like substances might be demonstrated in the following manner. The neutralizing capacity of a ragweed extract is determined as just described. The eliciting ability of the same ragweed extract is also obtained by challenging passive transfer sites sensitized by the same reagin-containing serum with serial dilutions of the ragweed extract. If material containing ragweed hapten is added to the ragweed extract and the above passive transfer neutralization and elicitation reactions are then repeated, the results should show a relatively greater increment of neutralizing ability than eliciting ability. Thus the test is designed to identify the presence of haptenic substance by its ability to alter the neutralizing:eliciting ratio of ragweed extract. Accordingly, various hydrolysates of ragweed extract were tested in this manner to determine whether haptenic activity was present. An attempt was made to hydrolyze them to a degree which produced some, but not complete, loss of skin eliciting ability.

2.5.2 Experimental Method

2.5.2.1 Preparation of ragweed extract

A 1:50 solution of ragweed extract was prepared by extraction of *Ambrosia elatior* with buffered saline in the usual manner.[21] One milliliter of the 1:50 ragweed extract contained .35 mg of nitrogen as determined by the Kjeldahl method.

2.5.2.2 Acid hydrolysis

A solution with a final concentration of 10 N HCl was prepared by adding concentrated hydrochloric acid to 1:50 ragweed extract. Aliquots of this solution were hydrolyzed at 37°C for periods of 5, 10, and 24 days, evaporated to dryness to remove the hydrochloric acid, and the residue dissolved in distilled water to approximate the original concentration of 1:50. This extract was dialyzed against buffered saline, and the dialysates and dialysate residues were tested for eliciting and neutralizing activity on Prausnitz-Kustner sites. Neither eliciting nor neutralizing ability remained, and these hydrolysates were discarded.

Acid hydrolysis was next conducted in the above manner at concentrations of 3 N, 1 N, and .1 N for varying periods of time. At completion of hydrolysis, the hydrolysates were evaporated to dryness under reduced pressure at 37°C to remove the hydrochloric acid. The dried residue was dissolved in distilled water to original volume. Some samples were neutralized to pH 8 with sodium hydroxide. The dissolved residues were sterilized by passage through a Seitz filter and cultured for sterility prior to use. Periods of hydrolysis were chosen which did not appear to destroy eliciting ability completely. This was done by removing aliquots of the hydrolysate at various times, neutralizing to pH 8 with NaOH sterilizing by passage through a Swinney filter and testing for eliciting activity on P.K. sites. This was done to try to avoid complete hydrolysis which might destroy any hapten possibly produced by incomplete hydrolysis.

Some samples of ragweed extract were hydrolyzed at varying concentrations of hydrochloric acid after heating the ragweed extract to 56° for 30 min.

2.5.2.3 Enzymatic Digestion

A review of the literature concerning the ability of digestive enzymes to destroy the skin eliciting activity of ragweed extract reveals conflicting reports on this subject.[22-26] In our experiments, both tryptic and peptic digestion were carried out at the optimum pH for these enzymes.[27] Ragweed extract was digested with 3%, twice recrystalized trypsin for 3 days at pH of 8. Pepsin digestion was conducted for 3 days at pH of 2 followed by neutralization of the hydrolysate to pH 8 with NaOH. Since both pepsin and trypsin are inactivated by autolysis, additional trypsin and pepsin were added to the hydrolysates at daily intervals for the 3-day periods. Hydrolysis was discontinued at the end of this period because there was decreased eliciting activity of the ragweed extracts. Following the digestions, the hydrolysates were dialyzed against buffered saline through a Visking cellophane membrane to separate the enzymes from the ragweed antigen, and the dialysate used for testing.

2.5.2.4 Evaporation and Refreezing

1:50 ragweed extract was evaporated to dryness under reduced pressure at

37°C and redissolved to original volume in distilled water. This was repeated five times. A separate sample was frozen and thawed a total of 67 times and the resulting materials were used for testing.

2.5.2.5 Dialysis alone

1:50 ragweed extract was dialyzed against buffered saline at pH 8 though a Visking membrane. Periods of dialysis were 4, 8, 16, 24, 72, and 96 hr. The dialysates of all samples gave positive skin tests on P.K. sites, and the 4-hr dialysate was used to test for hapten activity.

The materials prepared by the above methods which were tested for haptenic activity are shown in Table 2.5.2. The method of testing the altered extracts is shown in Table 2.5.1. Using this method, the ability of an altered extract to change the neutralizing and eliciting activity of a standard ragweed extract is compared with buffered saline to provide a constant dilution factor.

2.5.3 Results

The results of measurement of the neutralizing and eliciting ability of the altered ragweed extracts are demonstrated in Table 2.5.2. Both neutralizing and eliciting ability were destroyed by acid hydrolysis at 10 N concentration and by 3 N with or without heat, except for 3 N HCl hydrolysis for 24 hr. This period of hydrolysis did not completely destroy the neutralizing or eliciting ability of the ragweed extract. Trypsin and pepsin digestion destroyed activity under the conditions used in the experiments. The 4-hr dialysate of ragweed extract showed some eliciting ability but no detectable neutralizing activity was demonstrated. Repeated freezing and thawing destroyed the neutralizing activity, but repeated evaporation did not destroy the activity of the extract.

Table 2.5.3 illustrates the results obtained with repeated evaporation of ragweed extract. Both neutralizing and eliciting activity remain, and there is no significant alteration of the neutralizing:eliciting ratio of the control ragweed extract.

Table 2.5.4 shows the results obtained with acid hydrolysis at 3N HCl for 48 hr with destruction of both eliciting and neutralizing activity of the treated ragweed extract.

The initial results obtained with certain acid hydrolysates were very encouraging. An example is shown in Table 2.5.5. The material tested in this experiment was ragweed extract treated with acid hydrolysis at a concentration of 3 N HCl for 24 hr after the extract had been heated to 56°C for 30 min. These results seemed to show definite neutralization with the hydrolysate and suggested that the extract contained neutralizing hapten. Although greater neutralizing activity was observed, there was actually a diminished eliciting reaction as compared with the control. A possible explanation for this was that the neutralizing hapten had greater affinity for reagin than the unaltered

TABLE 2.5.1

TECHNIQUE OF DETERMINING THE ABILITY OF AN ALTERED RAGWEED EXTRACT TO CHANGE THE NEUTRALIZING-ELICITING RATIO OF A CONTROL RAGWEED EXTRACT

Day One			
Neutralizing		Eliciting	
Control	Altered Extract	Control	Altered Extract
Plant P.K. sites with mixture of equal amounts of: 1. Reagin serum. 2. Serial four-fold dilutions of ragweed extract. 3. Buffered saline	Plant P.K. sites with mixture of equal amounts of: 1. Reagin serum. 2. Serial four-fold dilutions of ragweed extract. 3. Altered extract.	Plant P.K. sites with reagin serum	Plant P.K. sites with reagin serum

Day Two			
Neutralizing		Eliciting	
Control	Altered Extract	Control	Altered Extract
Challenge all sites with ragweed extract.	Challenge all sites with ragweed extract.	Challenge all sites with mixture of equal amounts of serial four-fold dilutions of ragweed extract and buffered saline.	Challenge all sites with mixture of equal amounts of serial four-fold dilutions of ragweed extract and altered extract.

TABLE 2.5.2

MEASUREMENT OF THE NEUTRALIZING AND ELICITING ABILITY OF ALTERED RAGWEED EXTRACT

Method	Days Hydrolysis	Neutralizing Activity	Eliciting Activity
Acid Hydrolysis			
Ion	5	Absent	Absent
	10	Absent	Absent
	24	Absent	Absent
3 N	2	Absent	Absent
1 N	10	Absent	Absent
.1 N	26	Present	Present
3 N + Heat	1	Present	Present
	2	Absent	Absent
	3	Absent	Absent
Trypsin	3	Absent	No End Point
Pepsin	3	Absent	Absent
Trypsin + Heat	3	Absent	Absent
Pepsin + Heat	3	Absent	Absent
Dialysis	4 hr	Absent	Present
Freezing and Thawing		Absent	No End Point
Re-evaporation		Present	Present

TABLE 2.5.3

PERSISTENT NEUTRALIZING AND ELICITING ACTIVITY OF RAGWEED EXTRACT TREATED BY REPEATED EVAPORATION

Technique used is illustrated in Table 2.5.1.

	Neutralizing Activity		Eliciting Activity	
	Control	Treated Ragweed	Control	Treated Ragweed
Increasing serial four-fold dilutions of ragweed extract	-	-	+++	++++
	-	-	++	+++
	-	-	++	+++
	±	-	+	++
	+	-	+	++
	++	+	±	++
	++	+	±	++

TABLE 2.5.4

HYDROLYSIS OF RAGWEED EXTRACT AT 3 N HCl FOR 48 HR SHOWING COMPLETE DESTRUCTION OF NEUTRALIZING AND ELICITING ACTIVITY OF THE TREATED RAGWEED EXTRACT

	Neutralizing Activity		Eliciting Activity	
	Control	Treated Ragweed	Control	Treated Ragweed
Increasing serial four-fold dilutions of ragweed extract	-	-	+++	+++
	-	±	+++	+++
	±	+	++	++
	+	+	++	++
	++	++	+	+
	++	++	±	±
	+++	+++	-	±
			skin control ±	skin control ±

TABLE 2.5.5

APPARENT INCREASE IN NEUTRALIZING ACTIVITY OF RAGWEED EXTRACT TREATED WITH 3 N HCl FOR 24 HR AFTER HEATING

	Neutralizing Activity		Eliciting Activity	
	Control	Treated Ragweed	Control	Treated Ragweed
Increasing serial four-fold dilutions of ragweed extract	±	±	++++	++++
	±	±	++++	++++
	±	±	++++	+++
	++	±	+++	±
	+++	±	+++	±
	+++	±	+	±
	++++	±	+	++

antigen in the control dilutions. Subsequent investigation, however, showed that although the hydrochloric acid had been removed by evaporation to dryness under reduced pressure, after dissolving the hydrolysate residue in distilled water, the resultant material had a pH of 2. Following neutralization of these hydrolysates to pH 8 with sodium hydroxide, all apparent neutralizing activity disappeared. Consequently, an experiment was conducted to compare the neutralizing:eliciting ratio of reagin-standard ragweed extract mixtures to which were added buffered saline acidified to pH 2 with HCl, unneutralized hydrolysate, or buffered saline at pH of 8. The results of this experiment are shown in Table 2.5.6. This showed that the apparent neutralizing ability of the hydrolyzed extracts with a final pH of 2 was not a result of haptenic effect but a function of acid concentration, since the acidified saline also "neutralized" the reagin completely. The most logical explanation for this "neutralization" seemed to be that the reagin was altered by the concentration of acid present in the hydrolysates. This hypothesis was tested by comparing the reactivity of passive transfer sites planted with serial dilutions of ragweed reagin in an acid solution with serial dilutions of ragweed reagin in a neutral solution. The results of this experiment are shown in Table 2.5.7 and illustrate the alteration of reaginic activity by the acid diluting solution. Serum which was acidified to a pH of 2 with acidified saline, incubated for 1 hr, and then neutralized to a pH of 8 with NaOH, did not sensitize a passive transfer site. To determine whether acid altered post treatment "blocking antibody" in a manner similar to the effect on reagin, both were acidified, and no significant difference was observed between the effect of an acid concentration of pH 2 on reagin or blocking antibody.

2.5.4 Discussion

A low molecular weight material existing naturally in ragweed extract or which could be produced by degradation of ragweed extract by various chemical or physical means would possibly have haptenic activity that would be of great theoretic interest and therapeutic potential. Our method of testing for haptenic activity by measuring the ability of the treated ragweed extract has proved to be a feasible modification of the passive transfer neutralization technique. An extension of this technique for use for comparative determinations of the degree of antigenicity of ragweed extract is under investigation.

We were unable to demonstrate haptenic activity in any of the materials utilized for testing. This does not exclude the possibility that such a material exists or may be produced.

Our results indicate that activity of ragweed extract is destroyed by pepsin and trypsin and by repeated freezing and thawing under the conditions of the experiments. Hydrochloric acid hydrolysis of ragweed extract can destroy the activity of ragweed extract, but this destruction can be controlled by variation of factors of time and acid concentration. Thus, the neutralizing and eliciting ability of ragweed extract is not destroyed in 24 hr but in 48 hr in in 3 N HCl.

2.5.5 Summary

A method is described which may serve to demonstrate the presence of haptenic activity in native or modified extracts. Employing a modification of the passive transfer neutralization technique, the procedure measures the ability of the material being studied to alter the ratio of neutralizing:eliciting activity of whole pollen extract.

No hapten-like material was found in unaltered ragweed extract or extracts treated by several chemical and physical methods to degrade the antigen. Degradation of ragweed extract under various conditions is described. Reaginic activity of serum is decreased by acid solutions. No difference between the effect of acidity on "blocking antibody" and reagin was observed.

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2.6 THE HISTOPATHOLOGY OF NASAL TISSUE REMOVED BY BIOPSY IN PATIENTS WITH RAGWEED POLLINOSIS DURING THE RAGWEED SEASON*

2.6.1 Procedure

Between August 23, 1957, and September 19, 1957, biopsies of tissue from the middle or inferior turbinates of 21 patients were performed. This falls within the ragweed pollination period in this part of Michigan. Counts in Ann Arbor during this time disclosed ragweed pollen in the air in significant numbers. The biopsies were taken with a small punch biopsy, usually from the edge of the middle turbinate. Sometimes local 4% cocaine in normal saline was used as a decongestant and anaesthetic; in other patients it was possible to obtain tissue without this local analgesia. The size of the biopsy varied. It was usually about 2 mm in diameter. It was fixed in Carnoy's fixative, sectioned in a paraffin block at 10-mm thicknesses, and stained with aldehyde fuchsin stain.**

The tissues were obtained from 10 ragweed-sensitive patients. These all had a characteristic history and whealing reactions (4+) on prick tests with ragweed pollen extract. The occurrence of symptoms at the time of biopsy varied from mild to severe nasal symptoms. None of these 10 patients had ever received treatment with allergenic extracts. Two were taking antihistamines. The other 11 patients who were referred for an allergic investigation during the season had no symptoms definitely suggesting ragweed pollenosis. One of these had a history suggestive of seasonal exacerbation but a negative intracutaneous test to ragweed; two had positive (4+) prick tests to ragweed.

2.6.2 Preliminary Results

Study of these tissue sections is still underway. Preliminary observation fails to reveal any pollen grains in the tissue sections. Further observations on the presence of mast cells, plasma cells, and other cellular and connective tissue elements are being carried out.

2.6.3. Discussion

Strömme[1] and Chevance[2] reported finding birch and rye pollen beneath the nasal mucosa in serial sections of tissue obtained after the inhalation or in-

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**Through the courtesy of Dr. Alexander Barry, Dept. of Anatomy, The University of Michigan.

sufflation of pollen into the nose of sensitized animals (guinea pigs and rabbits) and also (Strömme) in lesser amounts in unsensitized animals. As far as could be determined, no report of the search for pollen beneath human nasal mucosa has appeared. That pollen or pollen material is rapidly absorbed from the nasal mucosa has been demonstrated by Cohen et al.[3] It has been assumed that pollen is extracted by the nasal mucosa and the allergenic fraction absorbed. The possibility that pollen grains are incorporated in toto into the nasal mucosa has not been suspected. In the animal study mentioned, it seems apparent that the pollen is not phagocytosed by cells but enters between the cells and migrates deeper into the tissue in some unexplained manner.

Our inability to find pollen in a small section of mucosa under conditions of natural exposure does not disprove that it is incorporated into the tissue because of the conditions and the size of the tissue examined. It should be noted, however, that guinea pig nasal mucosa does not have a prominent basement membrane such as the human.

Study of the histopathology of allergic human nasal mucosa has been reported by Rappaport, et al.[4] (biopsy specimens), Vaheri[7] (biopsy specimens), and Hansel[5] (operative specimens). Hilding[6] has reported the histopathology of nasal mucosa in the common cold. Though the presence of eosinophiles, edema, and other elements is noted, no mention is made of the presence of mast cells. These cells have recently been of much interest in allergy. Our tissues were fixed and stained according to the special techniques required to demonstrate their presence. Mast cells have been found; however, exact evaluation of their numbers and relation to the condition of the patient awaits further study.

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3. METEOROLOGICAL PHASE*

3.1 INTRODUCTION

During the past year further efforts have been directed towards improving our knowledge of the release and diffusion of ragweed pollen. Since the elegance and scope of experimental designs is limited by our ability to obtain quick and accurate measures of pollen concentration, we have continued to review and improve our sampling methods. A new impinger of original design, and modifications to our gravity-slide technique and to our volumetric sampler are described in this report. Substantial progress has also been made in developing an electronic pollen counter.

The success of the first preseasonal pollen experiment in 1956 led to a second, more comprehensive, preseasonal experiment in 1957, in which 3,056 flowering ragweed plants were produced by the botanists in late June. The diffusion of ragweed pollen from this source located on the Jackson Prison Farm was studied by impaction and volumetric sampling techniques. A detailed account of the experiment appears in Section 3.3 of this report.

A limited pollen-sampling and weather-observing program was maintained at the Jackson Prison Farm tower site during the regular pollen season in support of the clinical work being undertaken with prison inmates. However, it is our feeling that experiments designed on a vastly larger scale are required during the natural pollen season if we are to learn much about the distances over which ragweed pollen ranges and the heights to which it is carried. Techniques based on sampling on 1000-ft television towers, from aircraft, along the lea side of Lake Michigan, etc. appear to be appropriate when the sources are large and widespread.

A modest beginning has been made in the study of possible reflotation of ragweed pollen that has fallen to the ground or landed on the leaves of the ragweed plants. The preliminary results, presented in Section 3.5, indicate the importance of more elaborate investigations along these lines. We have recently started an evaluation and comparison of the different pollen sampling methods which we have been using. A progress report on the test chamber, required for medical experiments, is presented in Section 3.6. Every effort is being made to expedite completion of the chamber.

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In addition to experimental studies of diffusion of ragweed pollen, theoretical studies mentioned in the first progress report have been continued. These are described briefly in Section 3.7.

3.2 DEVELOPMENTS IN SAMPLING TECHNIQUES

3.2.1 Modification of Gravity-Slide Technique

The practice of measuring pollen concentration by exposing a 1- x 3-in. glass slide, coated with petrolatum, has been more or less standard. We had used this technique along with others until June, 1957, but we had become somewhat critical of the method for two reasons: (1) the coating of the slides is a tedious and messy operation; and (2) when the slides are stored on edge in slide files, over a period of time the petrolatum runs down the slide, a serious matter for us since several weeks may elapse before we can count a slide.

Faced with the task of preparing over 6000 gravity slides for the 1957 preseasonal experiment, we looked around for an alternate method of coating them. After testing two nondrying resins, we settled on doubly-coated Scotch tape No. 666, 3/4 in. wide. Eighteen slides were laid side by side and a strip of the Scotch tape was stretched across the middle of the eighteen slides and pressed firmly against them. The protective backing was removed from the surface of the tape and the eighteen slides, now held together by the Scotch tape, were cut apart by a razor blade and stored in a slide file.

After exposure of the slides, the cover glass was held firmly by the adhesive of the Scotch tape and the slide could be stored indefinitely before counting. No effort is made to stain the pollen before counting, but the uniformity of the objective plane within which the pollen lie compensates for this disadvantage. All pollens on the Scotch tape surface 3/4 x 1 in. are counted.

3.2.2 Six-head Sequential Sampler Unit - Type II

For the preseason and in-season experiments for 1957, several additional six-head sequential sampler units were needed. Since we could no longer obtain the solenoid valves of the type used in 1955 and 1956 (see Progress Report No. 1, April, 1957, pp. 59-62), and since some weaknesses had developed in the use of this type of six-head unit, it was appropriate to consider a new design. (The millipore filters and filter holders had worked well and no change in their design was considered.)

The main weaknesses in the earlier six-head solenoid operated sequential samplers were:

1. On two or three occasions it was found that one of the six solenoid valves leaked slightly, thus permitting air to be drawn through its millipore

filter during the five periods it was supposed to be inactive. This resulted in erroneously high pollen counts for that particular filter corresponding to a particular period of the day. Since the possibility of this leaky condition would exist as long as we used this basic design, it was desirable to change it.

2. Our sequential timing switches gave some trouble. A combination of heavy solenoid currents and a dusty atmosphere (our pollen sampling operations were in an open field) caused arcing and subsequent pitting of the relay contacts. High resistance at one set of contacts could cause the respective solenoid not to operate for the given 4-hr period. Although such occurrences were very infrequent, it was desirable to eliminate their recurrence, if possible, in the new design.

3. The opening and closing of the solenoids was distinctly noisy. For field operations this was inconsequential, but annoying for prison hospital use. With the 6-head units mounted just above the beds of the volunteer patients and within 4 ft of their heads, the clatter of the solenoids at odd hours of the night was very disturbing.

In designing the Type II sequential sampler, very careful consideration was given to means through which it would be virtually impossible to have the vacuum applied to more than one millipore filter at a time. Several types of multi-port valves were considered, but apparently few manufacturers make a small six-port valve that is suitable for high-vacuum use. The only one we located is the Type S-649 manufactured by the Sprague Engineering Corp. of Gardena, California. It is a precision-built valve using "O" rings as vacuum seals. The company claimed that, in the one test under high vacuum that they performed, there was no leak of the valve after 500 complete cycles of rotation at a vacuum of 27 in. of mercury. Since this number of cycles corresponds to about 15 years of normal operation in our work, and since the vacuum was higher than our requirements, samples were purchased, given utility tests, and sampler units were then assembled.

Rotation of the valve by means of a rotary solenoid was first considered but rejected because of the heavy current required for its operation, the loud snap that would occur each time that it operated, the possibility of repeated operation if there was a poor contact, and the generally low dependability of this device. The superior characteristics of a motor-driven unit resulted in our selection of a synchronous motor to actuate the valve instead of a rotary solenoid.

Figure 3.2.2.1 shows a complete 6-head sampler unit and its timer. In Fig. 3.2.2.2 the cover is removed to show the drive motor, limit switch, gear reduction, and valve body.

Referring to Fig. 3.2.2.2, a 10-rph, 110-volt, 60-cycle motor turns the shaft of the valve through a pair of spur gears having a reduction ratio of 1:6.

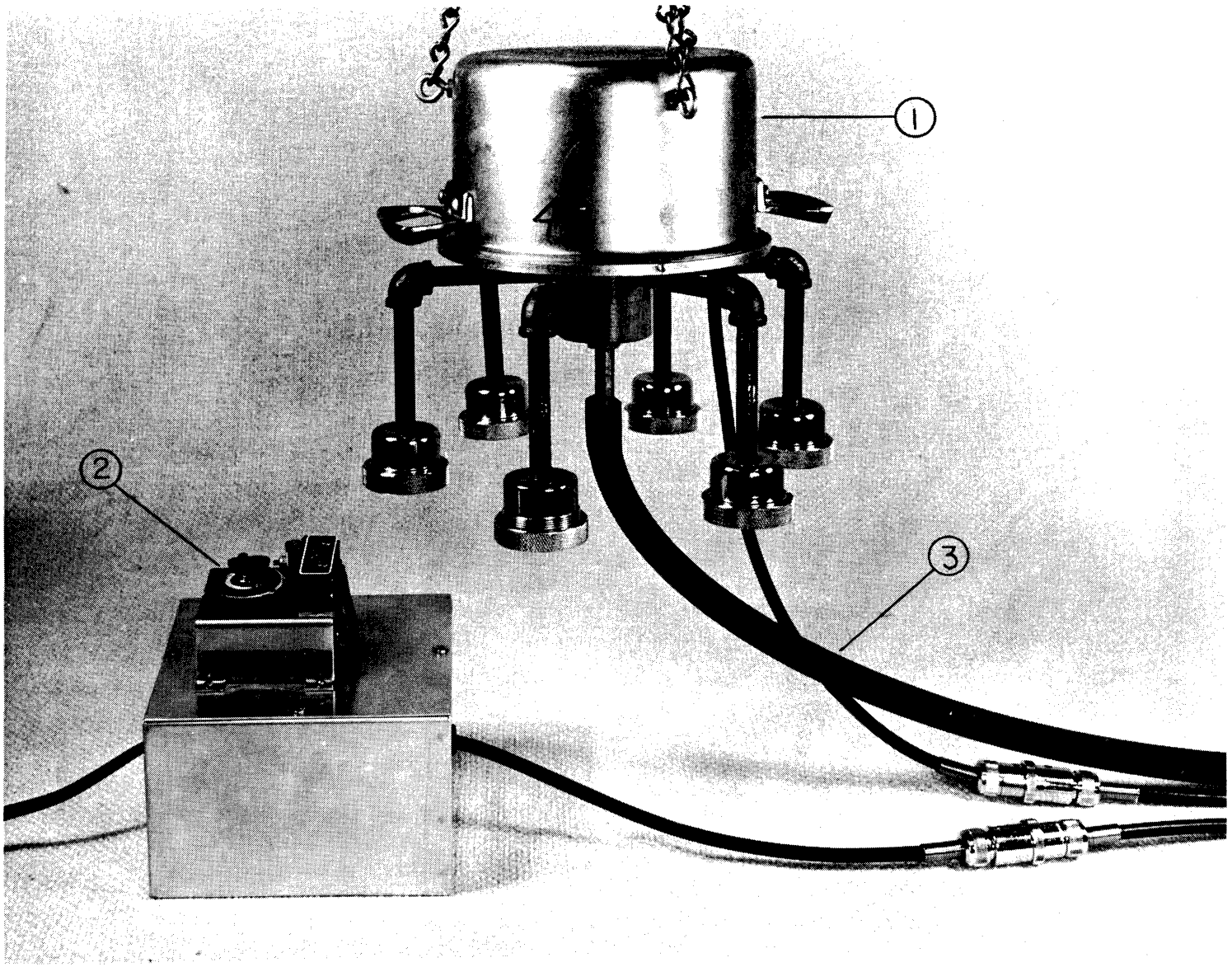

Fig. 3.2.2.1. Six-head sequential sampler unit - Type II. Each head insequence is connected for 4-hr period to vacuum line. (1) rain and dust cover for moving parts, (2) 4-hr timer, (3) vacuum hose.

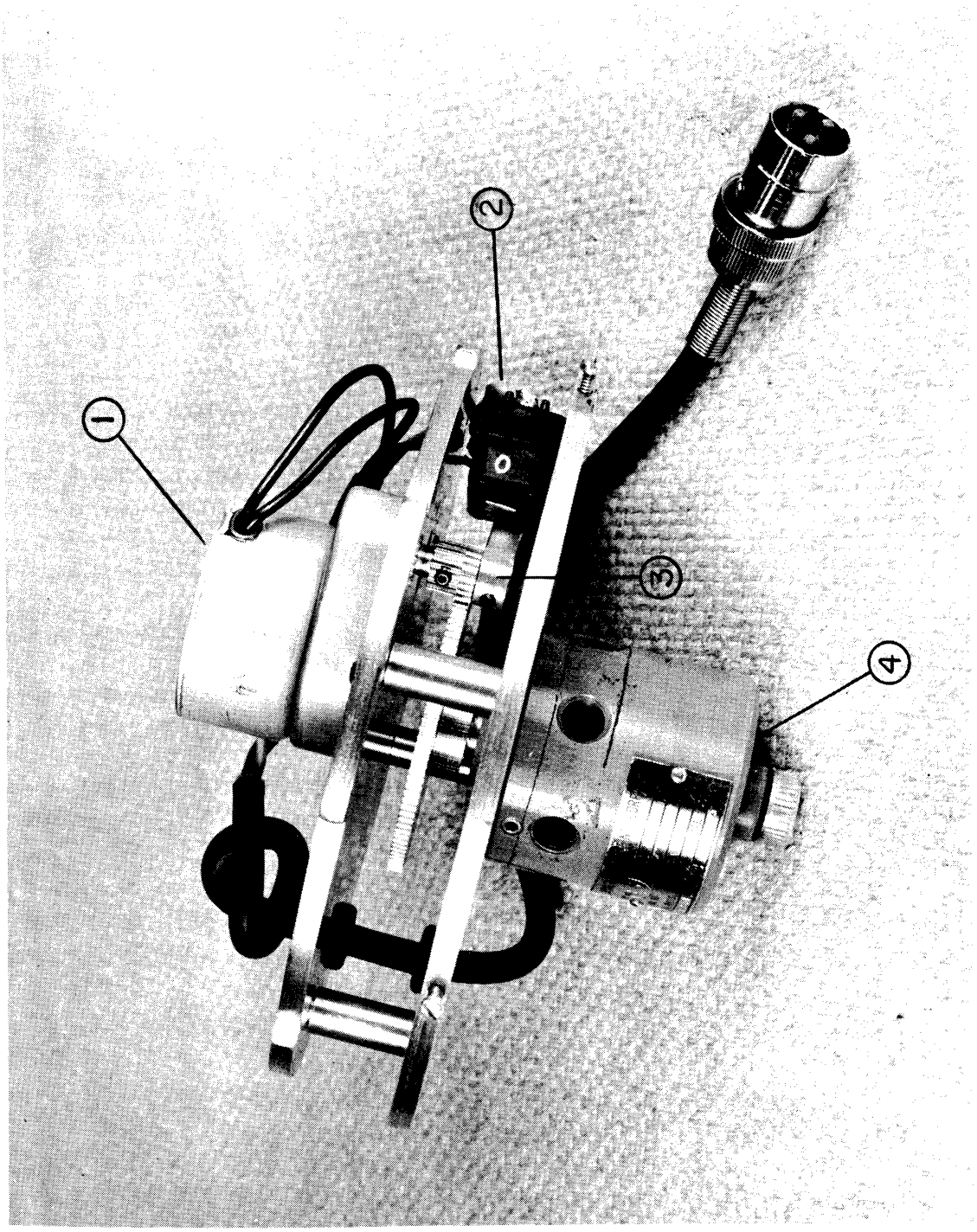

Fig. 3.2.2.2. Details of six-head sequential sampler unit - Type II. (1) 10-rph motor, (2) limit switch, (3) cam, (4) 6-port valve.

The motor shaft also carries an adjustable cam that operates a limit switch. A 3-wire cable connects this motor and switch to the timer unit of Fig. 3.2.2.2. The limit switch is in parallel (electrically) with the timer switch.

At the end of each 240 min (4 hr) the timer switch is closed for approximately 2 min, permitting power to flow to the valve motor, and causing its shaft to rotate through 120°. But after 30° of rotation its cam trips off the limit switch, closing the latter and providing a parallel electric circuit to the valve motor. Thus when the circuit through the timer switch opens, the valve motor continues to run until the cam again operates the limit switch and stops the motor. The shaft of this motor stops at exactly the same position as it was stopped prior to the closing of the timer switch. Thus the shaft of the valve is rotated through exactly 60° at each operation, and the vacuum is connected to one port after another at 4-hr intervals. With 6 heads the unit will sample a complete 24 hr between filter changes.

Two of these Type II samplers were used on the 100-ft tower during the 1957 ragweed season and two in the prison hospital. A timer unit was required at each location. Both installations worked perfectly without any maintenance, and none is currently required. There is practically no possibility of vacuum being applied to more than one port at a time. With only 3 watts per unit being consumed there is small likelihood of pitted contacts. Enclosed switches are used to reduce contact trouble further. And the quiet operation of these samplers was appreciated by our voluntary hay fever patients in the prison hospital. The new design appears to be very satisfactory for our needs.

3.2.3 The High-Volume Impingement Sampler

The use of the millipore filter in volumetric sampling has certain attractive features but also several disadvantages for the present investigation:

1. The samples are necessarily intermittent since they are collected over a time interval which may be as short as 15 min when pollen concentrations are very high, but must usually be several hours to accumulate a sufficient sample. This fact, of course, is a consequence of the low flow rate through the filter. The extended time of sampling introduces a number of additional questions and problems, for example: (a) the sample must be treated as an average over the time period, whereas wind and other effects upon pollen dispersal need to be studied in more detail than that available on the time scale of the millipore sample; and (b) preliminary tests suggest that turbulent gusts and eddies, may act to impact pollen grains at the surface of the filter exposed to the free air.

2. Evaluation of the samples is a laborious process requiring visual identification of pollen grains and manual counting by use of a microscope, usually over the entire filter area because of the typically low concentrations encountered. The average 1-in-diameter filter can be examined by a skilled microscopist in about 15 min, but when one needs thousands of samples to represent

a research situation adequately, this task becomes far too great.

3. The necessity for handling the samples in a more or less exposed situation in the process of changing filters, and the associated opportunities to make mistakes in the identification of samples, present difficulties.

As a result of these three main difficulties, some effort toward the development of different volumentric sampling systems has been exerted. One solution is the continuously operating impingement sampler which is diagrammed in Fig. 3.2.3.1. Air is drawn through the entrance slit (a) 0.2 by 9.5 mm, at a rate of about 3.0 cu ft per min. It impinges directly upon a sticky tape (b) located 2 mm above the entrance slit. The tape advances 1 in/hr in the prototype model, but this rate can be varied widely depending upon the purpose to be served (concentration of sample versus detail of record). The sample-bearing tape is covered continuously and automatically at roller (c) where it meets another tape supplied from reel (d). The sealed sample is taken up on reel (e) at the top of the assembly. Time marks are punched into the edge of the sampling tape at a predetermined frequency by means of a solenoid-activated punch. By using regular rolls of cellophane tape 3/4 in. wide and 1296 in. long in this sampler, it is possible to sample continuously for a considerable period. At 1 in/hr, 1200 in. last 50 days, although it is probably not advisable to leave the sampler that long without checking and servicing it.

This system provides for a time resolution of the sample which is directly determined by the tape speed. At the 1-in./hr rate, it is not difficult to resolve a 5-min sample. Furthermore, the time continuity of the sample supplies information about pollen dissemination patterns that is useful in the study of continuous wind-speed records for causal relationships. Further development is expected to include automatic reduction of the samples by use of densitometric technique.

The system has inherent difficulties that are under laboratory investigation. One difficulty, typical of all volumetric methods, is that of obtaining results in the naturally turbulent air of the field study which are comparable with the controlled flows obtained in wind tunnels and in laboratory-type wind currents.

3.2.4 The Electronic Counter

An additional means of sampling ragweed pollen volumetrically has been investigated since the last report on this project, namely, an electronic counting device.

The counting device is similar to a particle counter developed by Guyton[1] in 1946 with the difference that wide ranges of particle size have been ignored and sensitivity in the 18-22-micron range has been stressed.

Basically the counting device consists of a glass tube with a small orifice

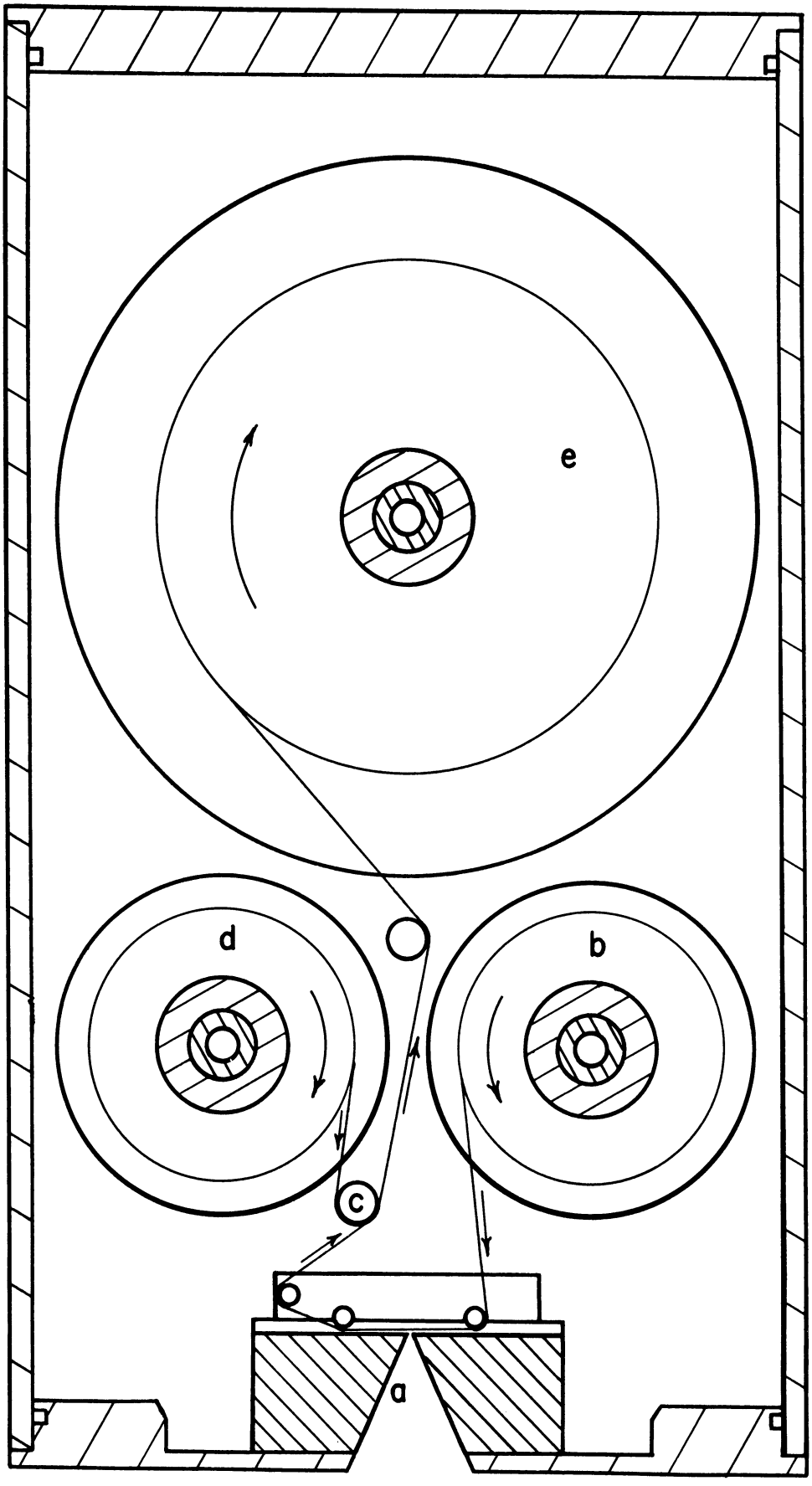

Fig. 3.2.3.1. Ragweed pollen sampler; impinger type, continuous feed.

section or capillary through which an air stream passes. As the air stream leaves the orifice or capillary section, the particles in the stream impinge on a No. 26 wire connected to the grid of a triode (cathode follower connected for a low noise level). A transfer of charge between the grid input and the particles results in a series of pulses. These pulses are amplified electronically and are counted by an electronic counter.

Calibration of this instrument is presently in progress.

3.2.4.1 Reference

Guyton, A. C., "Electronic Counting and Size Determination of Particles in Aerosols," J. Ind. Hyg. Toxicol., 28 (1946) pp. 133-41.

3.3 THE PRESEASONAL POLLEN EXPERIMENT OF 1957

3.3.1 Purpose of the Experiment

Consideration of the ragweed—hay fever problem poses a number of questions essentially meteorological in nature. Among these are the following:

1. How far is pollen carried, from its point of release, in quantities sufficient to produce symptoms of hay fever, and how is this distance influenced by weather factors?

2. Is there any relationship between the shape and orientation of a ragweed patch and the area of contamination?

3. How does the rate of release of pollen correlate with time of day, temperature, relative humidity, wind, and precipitation?

4. What is the relative importance of fallout compared to diffusion in the attenuation of airborne ragweed pollen?

5. How does the pollen concentration vary with height during the various hours of the day?

Answers to these fundamental questions are basic to any solution of the hay fever problem. Among the possible lines of attack, perhaps the simplest and most promising one is the preseasonal experiment of the type described herein.

Many diffusion problems may be solved by examining the dispersal of a substance as it is released from a point source at a known constant rate. For obvious reasons it is difficult to achieve such a simple model in the study of ragweed. In the first place the multiplicity of ragweed plots during the natural season makes it virtually impossible to find a study area which is contaminated by one and only one patch of ragweed.

Secondly, several thousand ragweed plants covering an area of several hundred square feet are required to provide a release of pollen that is large enough to be studied with available air-sampling techniques. Only at distances of a few hundred feet from such a source can it be considered a point source and it is doubtful if a substantial proportion of the pollen is carried that far. Finally, there is no satisfactory measurement of the rate of release of pollen, which is certainly not constant.

In the design of a model experiment, the first difficulty has been overcome by providing, as a source, several thousand ragweed plants that have been brought to maturity under controlled conditions several weeks before the natural season.* The second difficulty, which concerns the realization of point-source conditions, cannot be resolved. However, the use of a circular plot with the pollen samplers distributed uniformly outside the plot permits certain normalizing analytical techniques. These techniques make it possible to combine data obtained under different wind directions so as effectively to increase sample size and add to the certainty of the findings. The third difficulty, pertaining to the rate of release of pollen, has been recognized and there has been an attempt to measure, at least qualitatively, the rate of emission of pollen from the ragweed source. However, it cannot be claimed that the rate of release of pollen has been measured quantitatively.

Experiments of this kind are described as extra-seasonal or preseasonal. A pilot experiment of this type, using 136 plants, was carried out on the North Campus of The University of Michigan in June of 1956 and has already been described in the first progress report. Certainly the most important result of this first preseasonal experiment was the demonstrated feasibility of the technique. With the experience gained, another preseasonal experiment, broadened in scope and more refined in technique, was designed for 1957.

3.3.2 Design of the Experiment

On June 24, 1957, 3,056 flowering ragweed plants were transferred in flats from the Botanical Gardens at Ann Arbor to the tower site on the Prison Farm near Jackson. Figure 3.3.1 is a plan of the experimental array. The pollen plot was 13 ft in radius. Concentric with it were 90 gravity slides (dots on the figure), each supported 2 ft above the ground, on circles 20, 40, 80, 160, 320 ft in radius, and on radial lines 20° apart. For purposes of comparison, volumetric samplers of the millipore filter type (x's on the figure) were used adjacent to the gravity slides at selected locations. In addition, volumetric samplers were located at heights up to 50 ft on the meteorological tower. One gravity slide was placed at the 2-ft level and another at ground level near the center of each quadrant of the pollen plot, shown in enlarged scale in the upper left of Fig. 3.3.1. A continuous feed, cellophane tape, impinger-type volumetric

*In Section 1.2 of this report is a detailed account of the methods and techniques whereby several thousand flowering ragweed were provided late in June, almost 8 weeks before the normal time of flowering in this area.

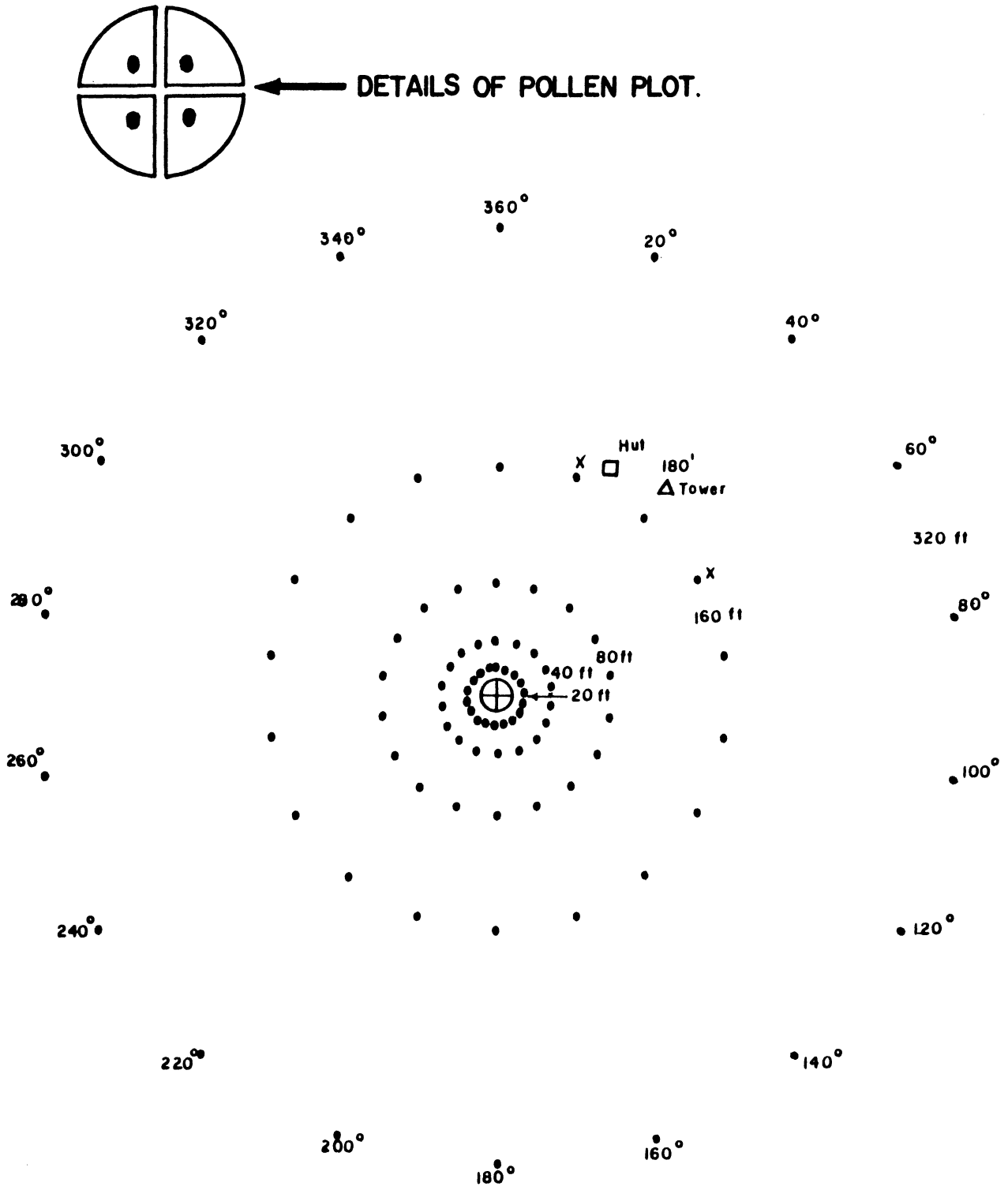

Fig. 3.3.1. Plan of the experimental array for the 1957 preseasonal experiment.

sampler of original design was placed at the 2-ft level in the center of the pollen plot. (See Section 3.2.3 for description of this sampler.)

The pollen plot as viewed from the top of the meteorological tower is shown in Fig. 3.3.2. The field of the Prison Farm in which the experimental array stood was planted with tomatoes, which are the lines of dots shown in the photograph. A close-up view of the ragweed plot showing some of the samplers mentioned above is presented in Fig. 3.3.3.

The gravity slides in the circular array were changed at 0030 (12:30 a.m.), 0430, 0830, 1230, 1630, and 2030 E.S.T. Slide changing thus coincided with the approximate times of sunrise (0430) and sunset (2030), and required only one change during darkness (0030), yet provided samples at 4-hr intervals throughout the day. Gravity slides in the plot were changed twice as frequently—at the above hours, and at intermediate times, 0230, 0630, etc. Volumetric samples were obtained for the same 4-hr periods as the gravity slides in the circular array. In all, over 6,000 slides were obtained in the 10-day period of the experiment.

Complete weather instrumentation was used near the source and on the tower. This included, in addition to the instruments listed on page 65 of the first progress report, a Beckman-Whitley wind-speed and direction system at the 2-ft level adjacent to the source, recording wind vanes at 8 ft over the source and at the 25-ft station on the tower, and a net radiometer to measure the net flux of radiant energy. Rockwell "150" gas meters were used to measure the air drawn through all volumetric samplers, including the new impingers. A Duvdevani dew gauge was also used in the source.

3.3.3 Analysis and Results

The design of the 1957 preseasonal experiment allows solutions of 2 distinctly different problems. The first concerns emission of pollen by the ragweed plant, and the second concerns dissemination of pollen by the wind.

3.3.3.1 Emission of pollen.

3.3.3.1.1 Experimental Procedure.—As mentioned previously, 2 gravity slides were placed in the center of each quadrant of the plot, one on the ground, and the other 2 ft above the ground. The slides at the 2-ft level were above the tops of the highest plant. The slides were changed every 2 hours, starting at 0030 E.S.T. The slides were completely unshielded to avoid interference with normal convection currents and mechanical turbulence.

The total number of so-called source slides during the 10-day period of the experiment was 960. Slides for June 28 were spoiled owing to rain (Table 3.3.1). The pollen counts varied from zero to over 30,000 grains on 3/4 sq in. of slide. The ground-level counts in particular were very high. As a result, it became necessary to adopt a statistical procedure for estimating those counts

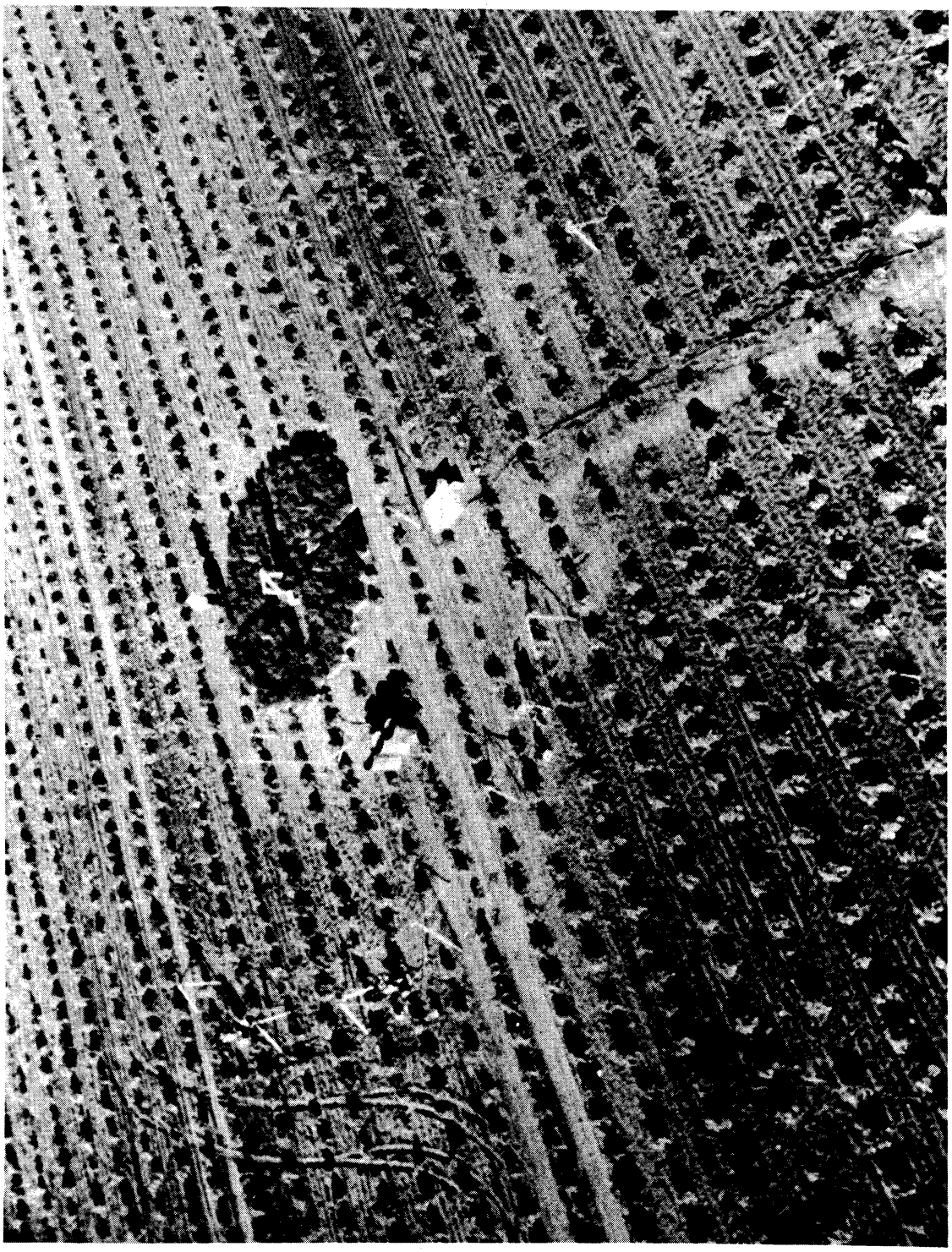

Fig. 3.3.2. Pollen plot as viewed from top of 100-ft meteorological tower.

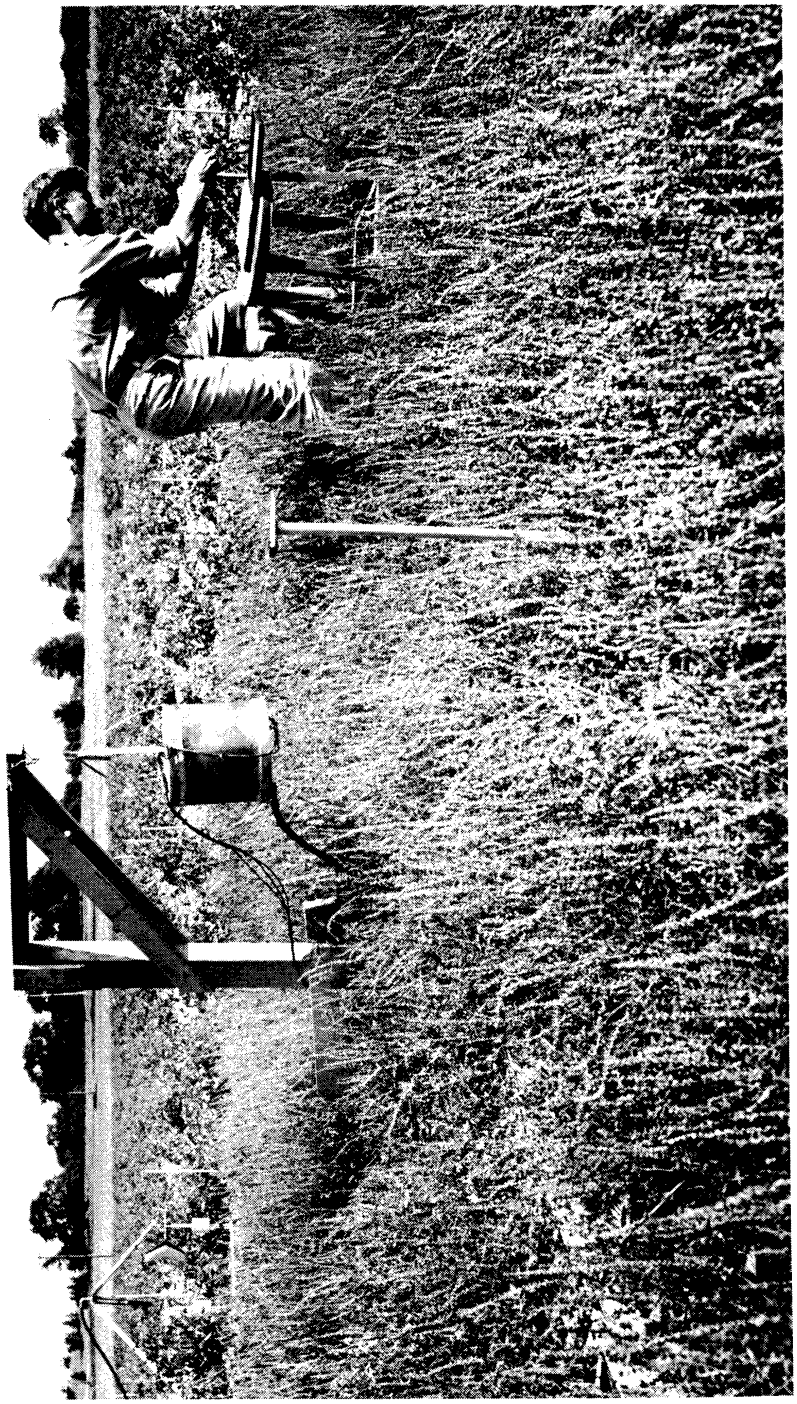

Fig. 3.3.3. Close-up of ragweed plot showing volumetric impinger supported over the plot, Beckman and Whitley wind system in left background, and graduate student changing gravity slide in source.

TABLE 3.3.1

WEATHER CONDITIONS DURING THE 1957 PRESEASONAL EXPERIMENT

DATE		0030 TO 0230	0230 TO 0430	0430 TO 0630	0630 TO 0830	0830 TO 1030	1030 TO 1230	1230 TO 1430	1430 TO 1630	1630 TO 1830	1830 TO 2030	2030 TO 2230	2230 TO 0030	MAXIMUM MINIMUM TEMPERAT.
JUNE 25	WEATHER			⊕ 3FR--	◍ 7	◍	◍	◍	◍	◍	◍	⊕	⊕ R-	
	WIND			187-3-10	236-4-11	257-6-14	256-5-14	251-5-18	246-6-15	252-4-8	240-1-9	221-0-4	181-1-4	MAX 70
	STABILITY			44	39	12	-3	16	26	15	-18	-16	-11	
JUNE 26	WEATHER	⊕ 7R-	◍ 7	◍7	◍7	◍7	◍	◍7	◍	⊕ RW	⊕	⊕	◍ 7	MIN 61
	WIND	148-2-9	175-4-10	195-6-13	207-7-14	214-8-18	226-9-18	206-8-17	210-9-20	209-7-17	208-2-16	168-2-10	179-4-11	MAX 75
	STABILITY	-8	-8	-1	2	4	4	7	9	7	-3	-9	-2	
JUNE 27	WEATHER	⊕ R-INTMT	⊕ 5R	⊕R-	⊕R-	⊕R-	⊕ R-	⊕R-	⊕ R-	⊕R-	⊕ R-	⊕ R-	⊕ R-INTMT	MIN 56
	WIND	200-4-10	223-3-10	203-2-8	191-3-8	170-3-9	196-4-10	175-4-10	160-4-8	102-1-6	138-1-6	190-1-4	156-2-9	MAX 76
	STABILITY	-2	0	-8	2	5	26	4	4	3	3	-2	-7	
JUNE 28	WEATHER	⊕	⊕ L-INTMT	⊕L-R-INTM	⊕R-	⊕R-	⊕ R-INTMT	⊕RW-OCNL	⊕RW-OCNL	⊕RW+OCNL	◍	◍ RW--	◍	MIN 66
	WIND	140-3-8	170-4-11	160-3-12	150-5-11	150-3-9	170-4-10	170-5-11		257-6-17	270-6-14	270-6-17	257-6-14	MAX 70
	STABILITY	3	1	3	-1	-4	2	3	5	5		2	9	
JUNE 29	WEATHER	◍	◯	◯	◯	◯	◍	◍	◍	◍	◍	◯	◯	MIN 60
	WIND	247-6-13	247-6-14	252-6-14	254-7-18	259-8-22	269-10-23	266-11-24	266-10-23	274-9-20	269-5-17	239-2-6	231-2-5	MAX 76
	STABILITY	1	0	0	2	7	5	17	4	-1	-8	-20	-24	
JUNE 30	WEATHER	◯	◯	◯	◯	◯	◍	◍	◍	◍	◍	◯	◯	MIN 58
	WIND	239-2-7	239-2-7	242-3-7	266-4-11	281-5-13	279-7-19	279-7-18	274-7-17	280-6-17	280-4-14	277-1-5	328-1-6	MAX 77
	STABILITY	-16	-18	-12	-1	0	6	8	6	0	5	-8	-8	
JULY 1	WEATHER	◯7	◯	◯7F	◯7	◯7	◯	◯	◯	◯	◯	◯	◯	MIN 50
	WIND	339-1-5	146-1-3	217-1-4	292-2-7	342-4-11	297-4-11	291-4-12	285-4-11	269-3-9	308-1-6	274-0-3	271-0-2	MAX 75
	STABILITY	-8	-19	-38	-9	2	12	12	2	7	-6	-40	-58	
JULY 2	WEATHER	◯7	◯ 2-¼F	◯¼-3F	◍	◍	◯	◯	◯	◯	◯	◯	◯	MIN 52
	WIND	260-0-2	256-0-2	104-0-0	219-1-3	199-3-9	191-4-11	222-5-12	250-4-12	221-4-9	181-3-9	185-2-6	207-3-10	MAX 83
	STABILITY	-51	-42	-31	-2	5	11	13	1	5	-12	-20	-16	
JULY 3	WEATHER	◯7	◯ 7	◯7	◯7	◯7	◍ 7	◍7	◍5RW--	◍5	◍4	◍4	◍ 4	MIN 68
	WIND	214-3-9	224-4-10	231-6-14	235-7-14	241-8-17	245-8-17	240-9-16	256-6-16	255-5-15	195-1-4	230-2-7	238-1-3	MAX 83.5
	STABILITY	-13	-12	-6	9	20	23	16	12	7	-2	-20	-25	
JULY 4	WEATHER	◯5	◍ ¼F	◯¼-½ F	◍5	◍5	⊕ 5	⊕5 L--	◍	◍RW--	⊕RW-INTMT	⊕	⊕	MIN 62.1
	WIND	212-0-2	85-1-8	205-0-3	184-2-8	218-4-11	215-6-12	188-8-17	214-7-17	216-8-17	252-5-18	253-5-12	259-4-11	MAX 82.2
	STABILITY	-22	-24	-5	-8	4	6	7	5	5	-9	-4	-5	
JULY 5	WEATHER	◯	◯7	◯	◯7	◯	◍	◍	◍	◍	◯			MIN 61
	WIND	251-4-11	241-5-13	246-5-12	264-7-18	269-8-20	274-9-21	275-10-23	274-8-22	277-7-19	261-3-16			MAX 81
	STABILITY	-6	-5	-5	-1	5	7	8	7	3	10	-32		

LEGEND: WEATHER: Sky Condition: ◯ Clear, ◍ 1/10-5/10 Cloudiness, ◍ 6/10-9/10 Cloudiness, ⊕ Overcast.
Visibility: No remark means a visibility of more than 10 miles, otherwise as noted in miles.
Weather: F fog, R rain, RW rain showers, L drizzle, minus indicates light intensity.
Temperature: Minimum and maximum temperature for the day in degrees Fahrenheit.

WIND: Mean direction, average speed, maximum speed. (2' level)

STABILITY: Temperature drop from 25' to 50' expressed in Celsius degrees per thousand feet (negative numbers indicate an inversion).

which were known to exceed 500 grains. The entire 3/4-sq-in. area was examined on all slides at the 2-ft level and on those slides on the ground with less than 500 grains. Counting was completed by December 25, 1957.

3.3.3.1.2 Frequency Distribution of Cluster Size.—The botanists, as noted in Section 1.3 of this report, had discovered that, in still air, pollen is emitted from the ragweed plant in clumps of many hundreds of grains. In a natural environment the air is seldom still and obstacles such as foliage intercept the grains as they fall. A study of the ground-level-source slides revealed a power-law distribution: $f = f_1 N^{\alpha}$ (Fig. 3.3.4), where f is the frequency of occurrence of clusters containing N grains, f_1 is the frequency of occurrence of single grains, and α is a constant. Had the pollen been released as single grains and fallen randomly onto the slides, a Poisson distribution $f = f_1 e^{N\beta}$ would have been expected, where β is a constant. Our results corroborate those of the botanists in indicating a much larger number of multiple grain clusters than would have been expected through chance alone. The slides at the 2-ft level show the same type of distribution, although with fewer clusters.

3.3.3.1.3 Wastage of Pollen Within the Plot.—The total number of pollen grains collected at ground level was 16 times the number of grains collected at the 2-ft level. (See Table 3.3.2.) This gives some measure of the wastage of pollen by plants within the plot. If we can assume that the ground-level slides were representative of conditions in the plot, then during a 10-day period 34 billion pollen grains were deposited within the plot itself.

3.3.3.1.4 Daily Cycle of Pollen Emission.—The total pollen count at the 2-ft level when plotted against time of day (Fig. 3.3.5) reveals a striking peak emission during the early morning. (The ground level slides show the same distribution.) Emission is very steady during the night with roughly 2% of the total catch in each 2-hr period. Between 0430 and 0630 the count rises abruptly, with a further large increase in the 0630-to-0830 period. By the 1230-to-1430 period the count has declined to near the evening minimum. It should be noted that 58% of the total emission occurs during a 4-hr period in the morning.

3.3.3.1.5 Meteorological Parameters Affecting Emission.—The peak emission of pollen follows sunrise, at 0430, by about 2 hours. Since several meteorological parameters are changing rapidly at this time, it is of interest to speculate about their relative importance. Parameters which change most rapidly at this time of day are solar radiation, ambient air temperature, relative humidity and wind speed.

Changes in these 4 weather elements have a high degree of correlation. Solar radiation affects the temperature which in turn influences the relative humidity. Diurnal changes in wind speed are governed to a large extent by instability in the atmosphere, which in turn is controlled in part by changes in surface temperature. As an example of the complexity of the problem, the maximum efflux of pollen occurs during the early morning when solar radiation is increasing rapidly, air temperature is increasing rapidly, relative humidity is

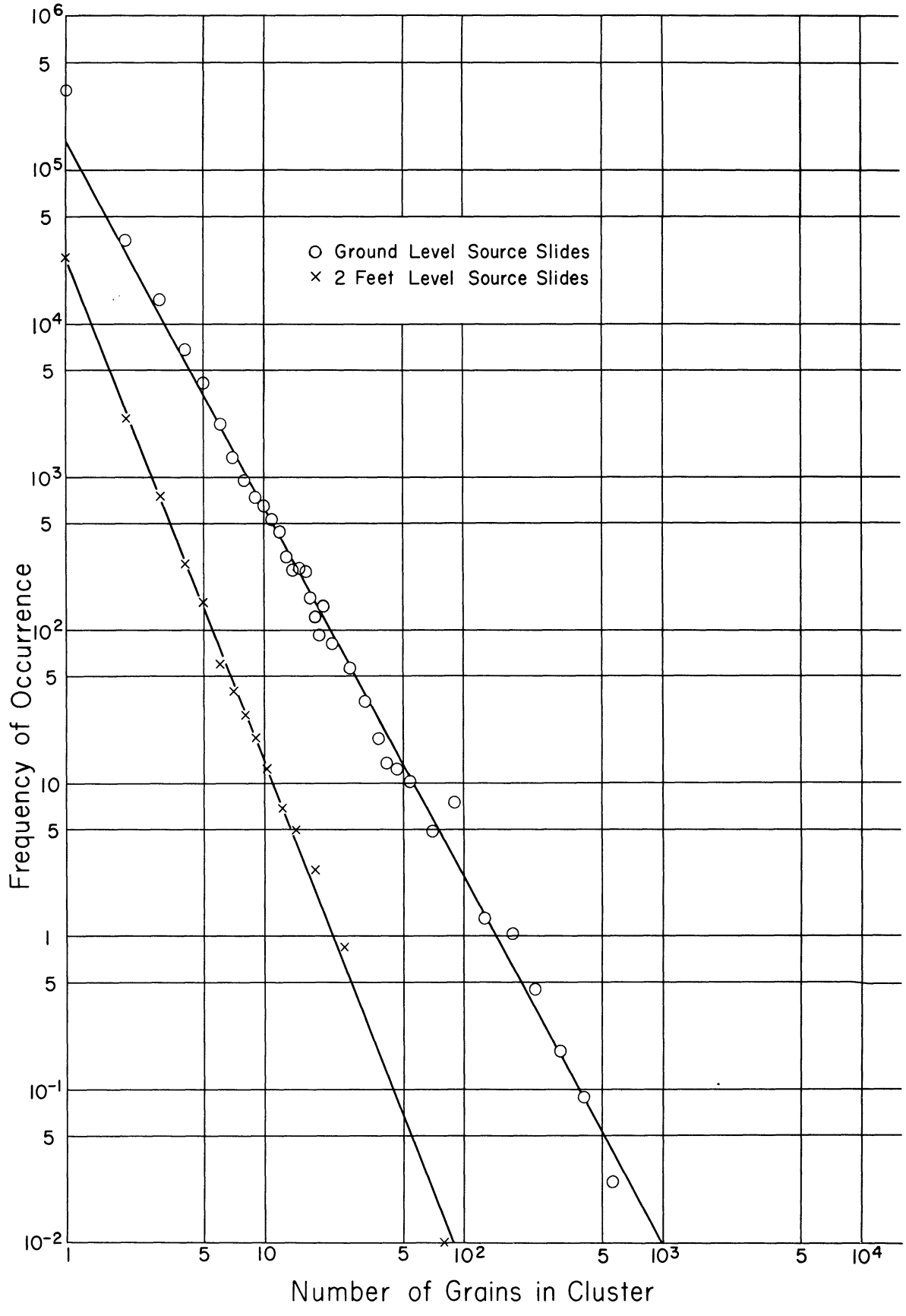

Fig. 3.3.4. Relationship between number of pollen grains per cluster and frequency of occurrence.

TABLE 3.3.2

TOTAL NUMBER OF POLLEN GRAINS FOUND ON 4 GRAVITY SLIDES, 3/4 BY 1 IN., EXPOSED AT GROUND LEVEL AND AT HEIGHT OF 2 FT, FOR 2-HR PERIODS AS NOTED

	0030 TO 0230	0230 TO 0430	0430 TO 0630	0630 TO 0830	0830 TO 1030	1030 TO 1230	1230 TO 1430	1430 TO 1630	1630 TO 1830	1830 TO 2030	2030 TO 2230	2230 TO 0030	TOTAL	No. Obs.
June 25 Ground level			32	8338	41518	2473	1379	962	1022	306	5636	4906	66572	10
2' level			21	1000	1550	179	72	113	43	50	69	471	3568	
June 26 Ground level	230	4285	18399	16012	4625	4581	621	713	5607	2010	4054	541	61678	12
2' level	103	1036	2165	1048	275	233	100	113	56	49	123	120	5421	
June 27 Ground level	6659	9983*	38335	7579	2685**	76509	43919	8505					194174	8
2' level	839	221*	299	87	1054	2826	640	161					6127	
June 28 Ground level														0
2' level														
June 29 Ground level			13729	46661	27000	13290	4598	2716	3021	2259	1329	666	115269	10
2' level			2545	2988	811	300	115	76	32	33	712	593	8305	
June 30 Ground level	869	2928	6679	61216	19258	12796	3607	2102	3819	3282	1453	3913	121922	12
2' level	72	189	1059	4412	866	358	108	63	26	32	27	64	7296	
July 1 Ground level	392	572	1652	44267	38843	16178	7015	3693	5754	759	1170	405	120910	12
2' level	36	13	48	3494	2303	698	110	74	344	73	33	18	7246	
July 2 Ground level	526	288	296	53982	112909	26935	4604	2128	5831	3698	5978	10549	227728	12
2' level	14	17	40	2689	5593	1346	540	143	78	1325#	40	716	12571	
July 3 Ground level	4296	6366	29720	36974	18108	4606	4406	3040	3732	762	1672	1244	114926	12
2' level	151	578	2730	2493	1070	254	178	58	173	809	726	147**	9367	
July 4 Ground level	193	606	603	90265	45432	15024	11449	3637	12987	53516	1392	270	235374	12
2' level	60	80	79	7300	4590	778	532	169	239	1080	37	54	14998	
July 5 Ground level	2931	2120	12796	30493	12290	3851	12833	1324	1005	579			80222	10
2' level	105	535	2032	3077	678	224	159	71	41	38			6960	
No. Obs.	8	8	10	10	10	10	10	10	9	9	8	8		110
Total Ground level	16696	27148	122451	395787	322668	176247	94431	26820	42778	67171	22684	22494	1338775	
Total 2' level	1382	2669	11118	28588	18810	7196	2554	1041	1032	3489	1767	2213	81859	

No cover slips used from July 4 0430 on; many slides very dirty.

* 1 Slide only

** 3 Slides

\# 1 Slide probably in error (best approximation, 187

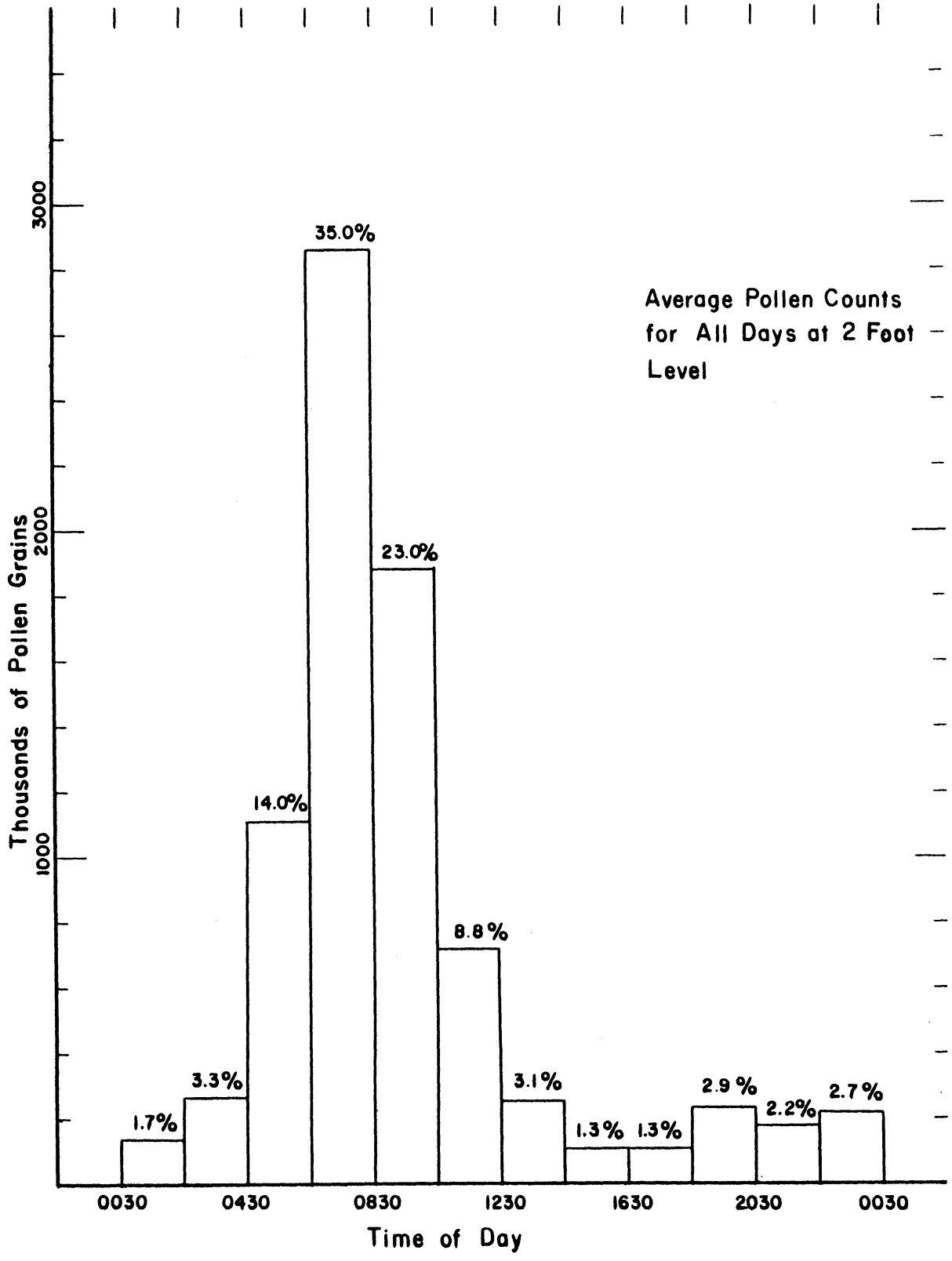

Fig. 3.3.5. Diurnal variation of pollen counts observed at 2-ft height within pollen source (all days).

decreasing rapidly, and low-level wind speed is increasing.

To separate the 4 elements mentioned above, we may observe the rate of emission of pollen during anomalous situations in which one or two of the elements are changing while the remainder are constant. For example, in the early morning of June 26 (Fig. 3.3.6), the relative humidity dropped suddenly at about 0200 with no corresponding rise in temperature. (It should be noted that relative humidity is very dependent on temperature, falling as the temperature rises and vice versa. A drop in relative humidity with no corresponding rise in temperature marks a change to a drier air mass.) A glance at the graph shows that the peak emission occurred between 0430 and 0630, one period earlier than normal, with considerable emission between 0230 and 0430, before sunrise.

The anomalous change of relative humidity on June 26, followed by an earlier-than-usual emission of pollen, points to drying action as a possible initiator of pollen release by the ragweed plant.

To test this hypothesis further, we observed the time of pollen emission on 4 clear days—June 30 to July 3 inclusive. A plot of the average emission for these days (Fig. 3.3.6) closely resembles that for the 10-day average (Fig. 3.3.5) with 65% of the pollen being emitted between 0630 and 1030; on July 1 80% was emitted between those hours. Although the days appeared similar, a closer look reveals striking differences during the early morning. June 30 and July 3 had wind speeds of 2-4 mph at the 2-ft level and no fog. The sun rose at 0430 and the temperature began rising shortly afterward. In both cases the 4-hr peak in pollen emission occurred between 0430 and 0830. On July 1 and 2 the winds were calm to 1 mph at the 2-ft level and fog occurred. The early morning rays of the sun were reflected from fog and as a result the temperature rise was delayed until 0530-0630. In both cases peak emission occurred between 0630 and 1030.

Other cases may be cited which point to the same conclusion. June 25 was a cloudy morning with a few light sprinkles of rain (Fig. 3.3.6). No temperature or humidity measurements were taken that morning, the first of the experiment, but it may be assumed that temperatures rose slowly and later than on a clear day. Peak emission occurred between 0830 and 1030, 2 hours later than normal. July 4 was a sunny day with convective showers during the afternoon (Fig. 3.3.6). Although our graph indicates rain from 1430 onward, showers prior to 1830 were extremely light. The relative humidity graph shows marked deviations as would be expected during a showery afternoon. In particular, there was a period of marked drying between 1530 and 1730, and a corresponding peak in emission between 1830 and 2030.

It is felt that the results are not conclusive. In particular, only one drop in relative humidity occurred not accompanied by a corresponding temperature rise. However, the indications are very strong that drying of the ragweed plant initiates pollen emission.

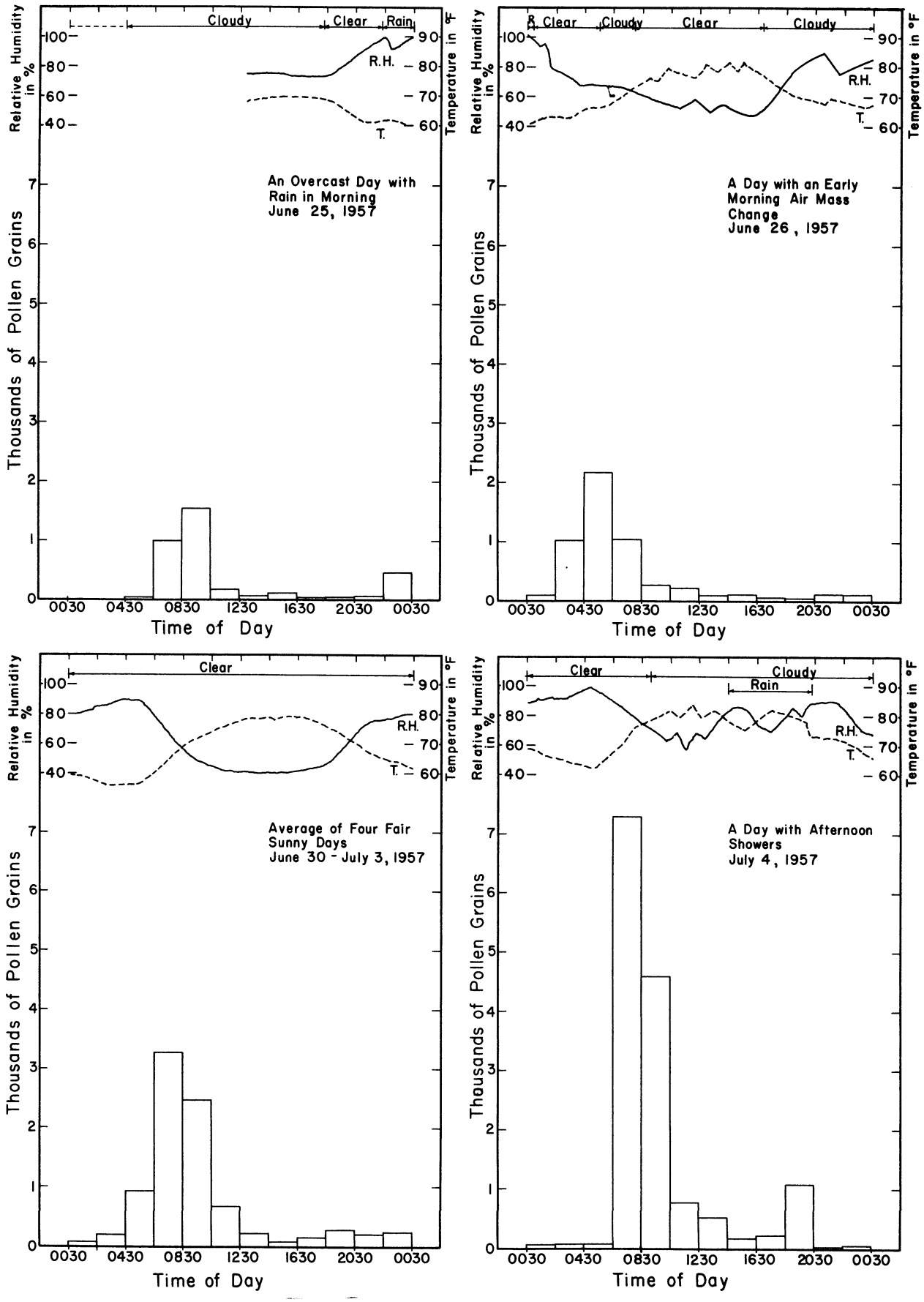

Fig. 3.3.6. Variations of pollen count and weather factors on selected days.

3.3.3.1.6 Influence of Wind Speed on Pollen Concentration.—When one is dealing with a steady source of pollution such as smoke from a chimney, the concentration in the air downstream will be inversely proportional to the wind speed. We are not sure that the ragweed plant is such a source. Some evidence, however, does suggest that extremely light winds remove all available pollen from the plants and if this is true, we can consider the emission as being independent of wind speed. Let us assume that such is the case in the following discussion.

The concentration of pollen in the air downstream from a source, all other things being equal, will be a function of time of day and wind speed. As noted earlier, from 50 to 80% of all pollen emission occurs during a 4-hr period in the early morning. The pollen which remains air-borne will be carried into a half cone whose length in miles is four times the wind speed in mph and in which the average concentration is inversely proportional to the wind speed if deposition on the ground is neglected, as of course it cannot be in a rigorous analysis. At points downstream, arrival of this cone may be marked by a small peak in the pollen count if attenuation by deposition and diffusion is not too great.

For example, suppose we are located 20 miles downwind from a large pollen source where maximum emission is at 0600. On a day when winds average 10 mph, we may expect a concentration peak at 0800. On the other hand, had the wind averaged 20 mph, we could have expected a peak with half the concentration 1 hr earlier. Of course local pollution will complicate the pattern. A graph of pollen concentration measured at frequent intervals versus time might be expected to appear as in Fig. 3.3.7. In this case the peaks at 1000 and 1300 could be caused by two additional strong sources upwind. Such a hypothesis needs testing since few in-season pollen measurements have been made at sufficiently frequent time intervals to give clear results. If the hypothesis proves to be valid, such a graph may be of real value to a community which wishes to determine the sources of ragweed pollution in connection with a program of ragweed eradication.

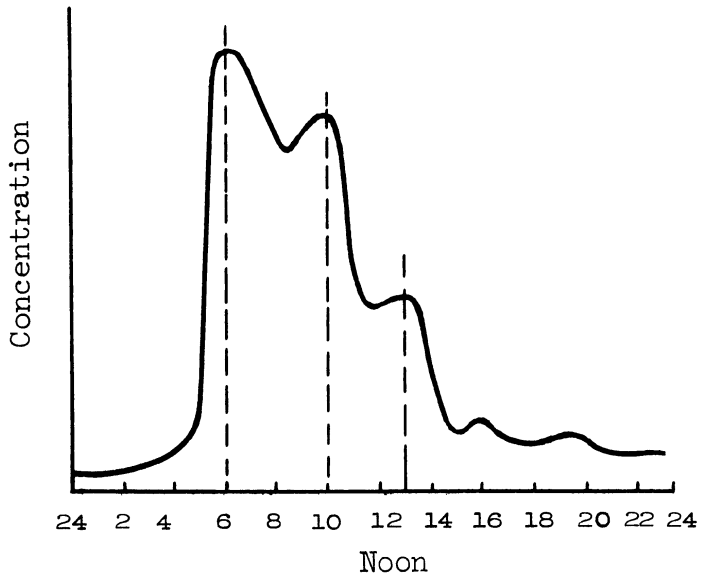

Fig. 3.3.7. Suggested explanation for irregular nature of diurnal variation of ragweed pollen concentration.

Atmospheric turbulence and hence diffusion are also related to wind speed, which has thus a two-fold importance in determining the concentration of pollen downwind from a finite source. Because of the significance of wind speed, graphs showing the diurnal variation of wind speed at the 2-ft level were drawn and are presented in Fig. 3.3.8. Three distinct weather types were selected since the

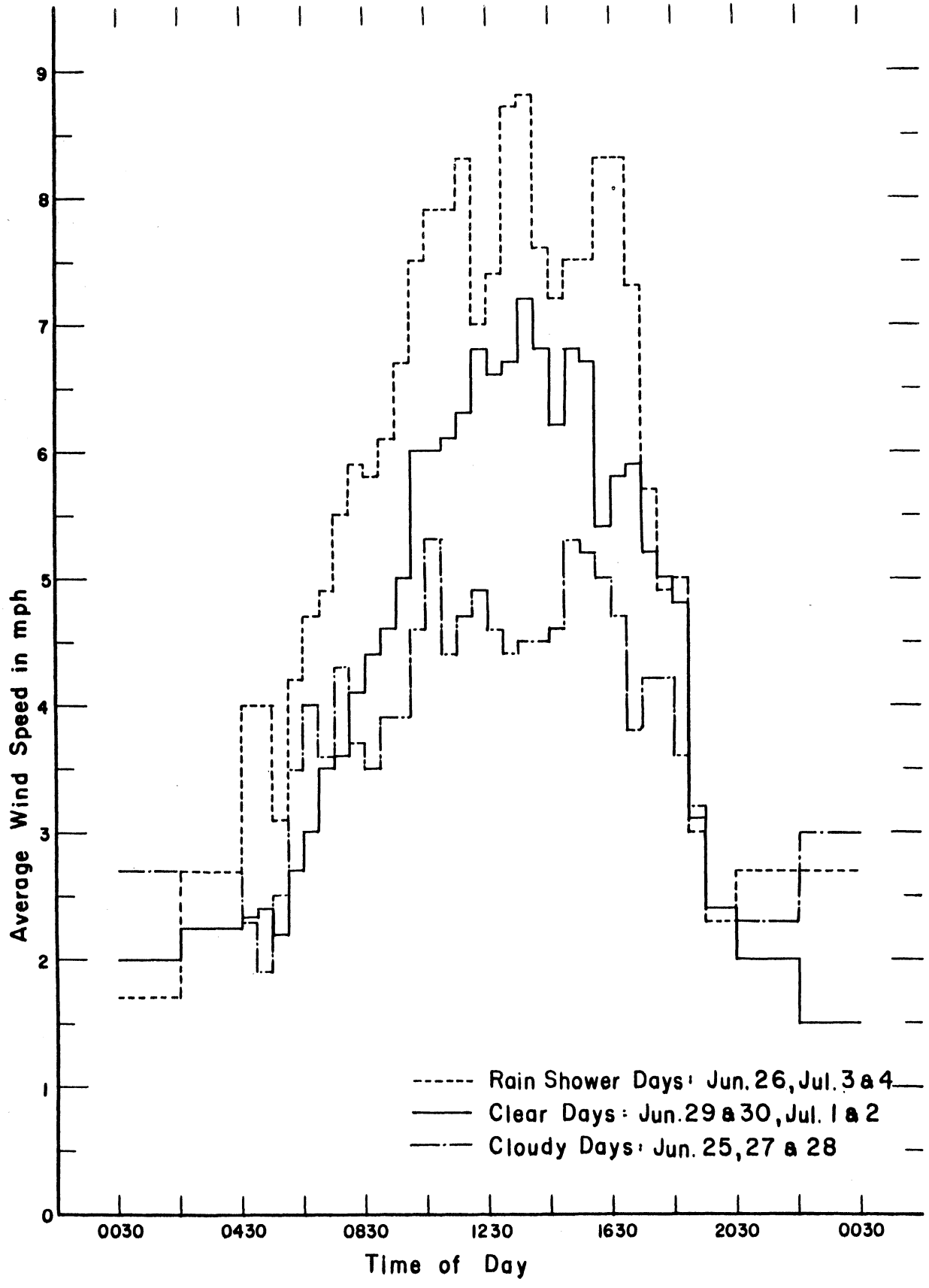

Fig. 3.3.8. The diurnal variation of wind speed averaged at half-hourly increments for three types of weather situations.

pattern of wind change varies markedly with the type of day. In all cases wind speeds during the time of peak pollen emission were at about the average speed for the day and on their upward march toward the early afternoon maximum.

3.3.3.2 Dissemination of pollen by the wind

3.3.3.2.1 Experimental Procedure.—The second phase of the 1957 preseasonal experiment concerned dissemination of pollen from the plot. For this phase an array of 90 slides was placed on 2-ft stakes at 20, 40, 80, 160, and 320 ft from the center of the ragweed plot along radii at 20° intervals (see Fig. 3.3.1). The slides were changed every 4 hr starting at 0030 E.S.T. The total number of array slides exposed during the 10-day period numbered about 5,000. The entire 3/4-sq-in. area of scotch tape was examined for pollen grains.

3.3.3.2.2 Elimination of Errors Due to Artificial Contamination of Sampler.—To determine the degree of artificial contamination from various possible causes, all slides were counted for the first 3 days of the experiment. It was found that artificial contamination was sizable in the area where slides were changed by the man who had first changed slides in the ragweed plot. Unfortunately, during the first day and a half he had changed source slides and then changed the 320- and 160-ft array slides. At these distances counts were normally low so the relative contamination was high. After noon of June 26 he changed the 20- and 40-ft slides after finishing those in the source. The relative contamination here was considerably smaller.

A simple method of correcting for artifical contamination was employed. From measurements of the maximum angle of deviation of the wind from its mean direction during each 4-hr period, the total area upwind from the source was determined. Counts per slide on each upwind arc were computed and subtracted from the counts on the downwind side of the arc. After noon of June 26 the routine was changed and it was possible to forego counting upwind slides except for the 20- and 40-ft arcs where contamination was still sizable. It should be noted that any contamination occurring on slides from the outer 3 arcs after noon of June 26 would result in counts at 80, 160, and 320 ft being slightly high.

3.3.3.2.3 Diffusion models.—The diffusion of a smoke plume downwind from a source has been studied extensively and satisfactory mathematical models for the prediction of concentration downstream have been devised. In general, to simplify the mathematics, either a point source or an infinite line source has been specified. Other simplifying assumptions are usually made, such as: gravitational settling may be neglected for very fine particles of, say, less than 5 μ in diameter, and the surface of the earth may be consideredas a perfect reflector.

3.3.3.2.4 Diffusion Problems and the 1957 Preseason Experiment.—The conditions under which the 1957 preseasonal experiment was conducted varied significantly from the simple models usually considered. The slides were exposed

4 hr during which time the emission of pollen was highly variable. The source itself was an area rather than a point. The ground cannot be considered as a reflector; it is much more likely that the ground acts as an absorber of pollen. Gravitational settling cannot always be neglected.

A simple diffusion model was designed for use with the data from the pre-seasonal experiment. Observed pollen counts have been compared with the theoretical counts suggested by the model. In the light of these comparisons, work is now progressing on the design of a more satisfactory model.

3.3.3.2.5 Angular Distribution of Pollen Downwind from an Area Source.-- The problem of an area source may be considered from the point of view of an array slide lying on the mean wind vector. If ρ is the radius of the plot, a wind blowing from the mean direction will move a distance 2ρ across the plot as it approaches the slide. When the wind is blowing from some other direction, it will have a shorter traverse over the plot and, presumably, will pick up less pollen. The pollen count at the slide will depend, all other things being equal, on the frequency distribution of the wind direction about the mean and on the distance of the slide from the source.

To illustrate, suppose we have a point source and 3 slides distributed 0°, 20°, and 40° from the mean wind direction along an arc downstream as indicated in Fig. 3.3.9. If the wind were to blow 40% of the time down the mean wind direction, 20% 20° off the mean, and 10% 40° off the mean, we could expect the pollen counts to be in the ratio 40:20:10, respectively. These ratios should be independent of wind, instability, and all other meteorological factors since these factors affect pollen deposition on each of the slides equally.

In carrying out the numerical calculations several assumptions have been made:

(1) The wind direction over a 4-hr period is normally distributed about the mean, i.e., wind direction is proportional to

$$f(\phi) = \frac{1}{\sqrt{2\pi}\,\sigma} \exp \frac{-\phi^2}{2\sigma^2}$$

where ϕ is the deviation of the wind direction from the mean, and σ is the standard deviation of the wind.

(2) Pollen emission is uniform within the plot.

(3) Gravitational settling is negligible.

The percentage of time that the wind, if normally distributed, will blow within a given small arc defined by $\Delta\phi$ will depend upon the total range of angular deflection ω for the time period considered. Thus a larger percentage of winds will blow within, say, 5° of the mean when the total range of angular

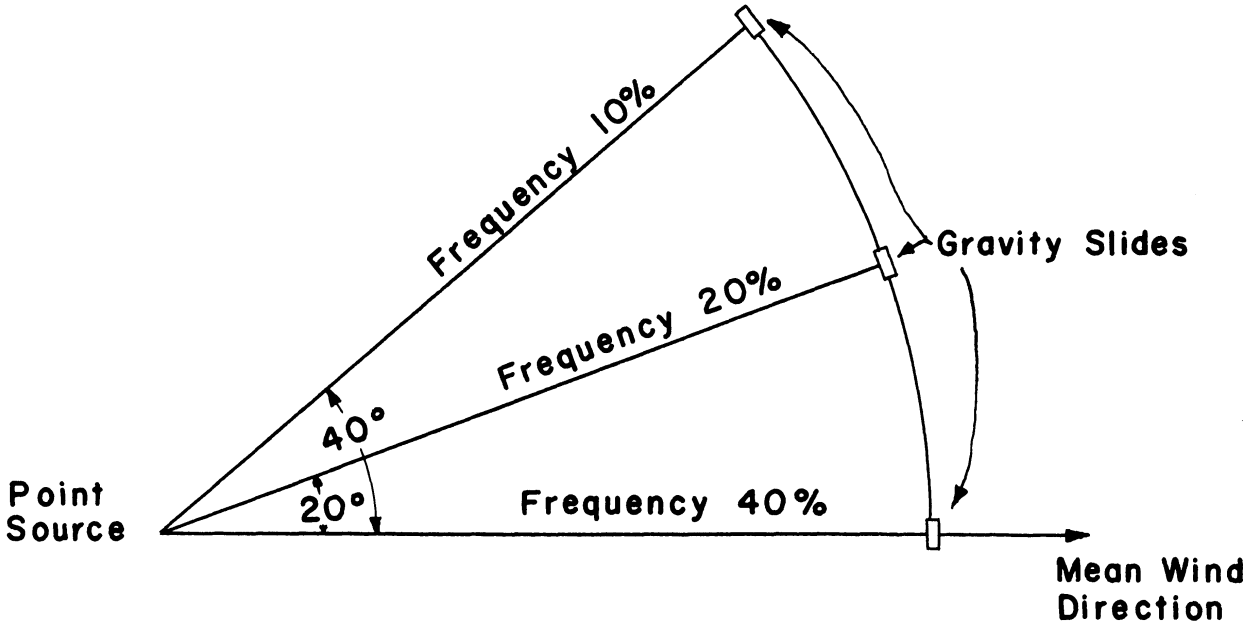

Fig. 3.3.9. Schematic diagram of gravity slides on different bearings from a point source.

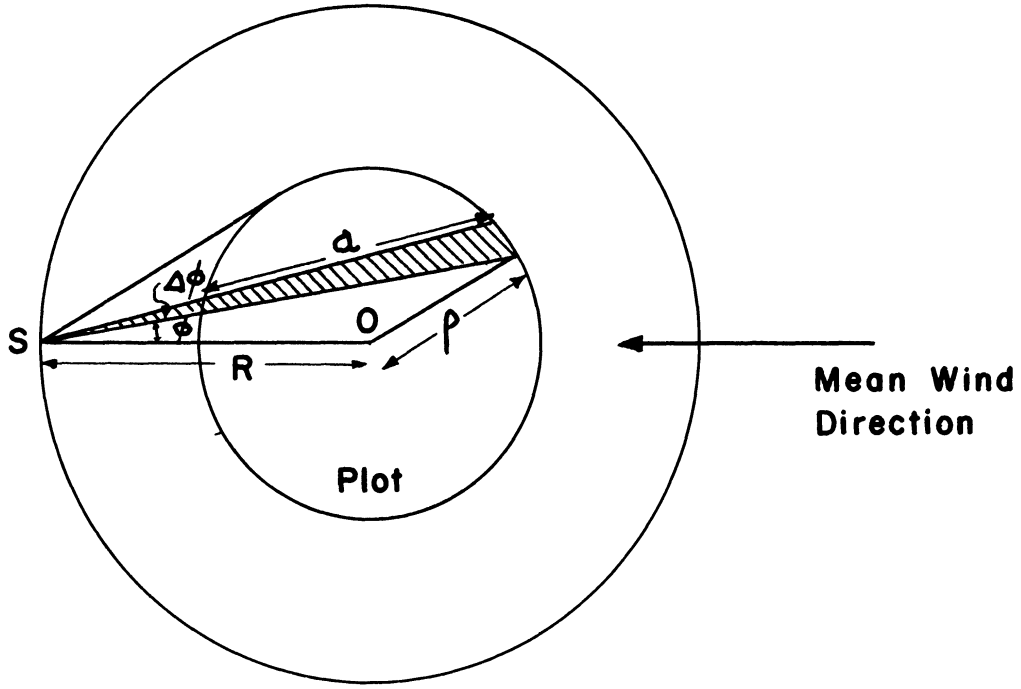

Fig. 3.3.10. Representation of a circular source centered at O, and a slide at S.

deflection for the period is 60° than when it is 120°. For computational purposes, $\Delta\phi$ was chosen to be 5° and frequency distributions were calculated for total angular deflections, ω, of 80, 100, 120, 140, and 160°.

The following discussion outlines the method used to calculate the effect of an area source (see Fig. 3.3.10). Let the radius of the plot be ρ, the distance of slide from the center of the plot be R, the angular deviation of the wind from the mean direction be ϕ. Air approaching the slide S at an angle ϕ from the mean direction will travel a distance a across the plot, where a is given by the expression

$$a = 2\sqrt{\rho^2 - R^2 \sin^2 \phi} \quad .$$

Now if we let r be the distance from S to some point in the circle, then an element of area inside the circle subtended by the angle $\Delta\phi$ will be

$$dA = \Delta\phi \, r dr \quad ,$$

or, when integrated from one boundary of the source to the other, say, r_1 to r_2:

$$\Delta A = \Delta\phi \int_{r_1}^{r_2} r dr \quad ,$$

where

$$r_1 = R \cos \phi - \sqrt{\rho^2 - R^2 \sin^2 \phi}$$

$$r_2 = R \cos \phi + \sqrt{\rho^2 - R^2 \sin^2 \phi} \quad .$$

Integration yields

$$\Delta A = 2R \cos \phi \sqrt{\rho^2 - R^2 \sin^2 \phi} \, \Delta\phi \quad .$$

The total count on a slide on the mean wind vector will be proportional to the sum of the products of each element of area ΔA by the frequency of the wind from the direction of ΔA. The relative counts on other slides on the same arc are computed by a simple manipulation of the figures for ΔA and will not be discussed in detail here. Relative counts on the various arcs have to be computed separately because ΔA, as may have been noted above, is a function of R as well as ϕ.

The use of an angle $\Delta\phi$ of finite size in our computations means that at some distance downstream from the source the angle subtended by the source will be less than $\Delta\phi$. Here the area source begins to behave as a point source. For a $\Delta\phi$ of 5° the 26-ft diameter source of the 1957 preseasonal experiment begins to behave as a point source about 150 ft downstream as indicated by Table 3.3.3.

TABLE 3.3.3

THE ANGLE ψ SUBTENDED BY THE RAGWEED PLOT AT A DISTANCE R FROM THE CENTER OF THE PLOT

R (ft)	20	40	80	160	320
ρ/R	.650	.325	.162	.081	.041
ψ	40° 40'	18° 30'	9° 20'	4° 40'	2° 20'

The theoretical counts downstream from an area source are tabulated (Table 3.3.4) and curves drawn (Fig. 3.3.11) for arcs 20, 40 and 80 ft downstream from the source. A fourth section of the table and a fourth set of curves marked "N.D." ("Normal Distribution") are included for arcs beyond 150 ft where the source may be considered a point.

No assumptions have been made regarding actual dimensions and distances except for the choice of 5° for $\Delta\phi$. If it were desired to apply the results to an actual field of ragweed, the radius of the field may be substituted for ρ and the distance downstream for R. For a constant ratio of ρ/R the same set of curves may be used. The only other measurement needed is the total angular deflection of the wind for the period in question.

Several factors make a comparison between the actual and theoretical curves difficult. First, the highest counts do not coincide with the mean wind direction in most cases. This effect is caused primarily by the variability of pollen emission, particularly in the morning. The wind direction during a short interval near 0600 may have a greater influence on slide counts than the wind for the entire preceding 2 hr. To overcome this difficulty, the mean wind direction at the 2-ft level was assumed to lie in the direction of the highest pollen counts on the 20-ft arc.

The second factor is that, owing to the 20° spacing between the slides, no actual measure of the peak count was available. An estimate of the peak count was made in the following way. Since we know that the location assigned to the peak is within 10° of its true location, the mean location obtained by averaging many cases should lie within 5° of the true mean location. The error involved in this assumption should be small since the curves are relatively flat near the peak. Hence we can estimate the true peak by plotting our observed peak values at ±5° and drawing, freehand, the portion of the curve from -5° to +5°.

The third difficulty involves the wind direction, which over a 4-hr period was seldom distributed normally about the mean. Both synoptic and diurnal changes tended to skew the curve. Furthermore, the 4-hr wind-direction frequency distribution may tend to be either more or less peaked than normal.

TABLE 3.3.4

TOTAL RELATIVE POLLEN COUNT AT AN ANGLE θ FROM THE MEAN WIND DIRECTION FOR TOTAL WIND DEVIATION ω

θ	Selected Values of ω																			
	R = 20 ft			ρ/R = .650		R = 40 ft			ρ/R = .325		R = 80 ft			ρ/R = .162		N.D.* 160 and 180			ρ/R <.100	
	80	100	120	140	160	80	100	120	140	160	80	100	120	140	160	80	100	120	140	160
0	1000	916	833	756	689	1000	839	717	626	552	1000	808	678	591	512	1000	801	671	576	502
5	985	904	824	749	684	969	819	705	616	547	955	790	666	580	508	909	758	641	558	494
10	942	870	797	729	668	880	764	671	595	531	845	733	638	554	494	770	680	597	528	472
15	874	814	754	696	642	718	683	616	557	505	697	639	575	515	467	597	576	532	481	442
20	784	744	697	652	608	595	583	549	509	471	533	534	507	471	431	429	463	459	437	407
25	679	654	629	599	567	442	474	472	436	432	371	424	428	417	394	277	355	377	377	364
30	564	560	553	540	519	304	370	393	397	388	241	316	353	356	352	169	255	303	320	320
35	448	464	474	476	469	191	273	316	337	341	137	228	280	300	306	91	173	234	264	277
40	338	371	396	411	417	109	188	246	277	293	59	154	210	248	270		113	169	212	234
45	240	286	321	347	363	54	120	184	224	247	16	90	151	195	223		69	121	165	195
50	161	211	254	286	310	21	70	128	177	205		44	112	149	181			87	121	156
55	100	149	193	229	260	5	37	85	133	167		13	69	114	150			52	95	121
60	56	100	143	182	214		16	52	95	133			29	83	116				65	95
65	30	63	101	139	173		4	28	65	104			10	52	87				43	74
70	12	37	69	103	136			11	40	76				27	67					52
75	3	19	44	74	105			3	22	51				8	42					39
80		8	26	51	78				10	32					23					
85		2	14	33	57				2	18					7					
90			6	20	40					8										
95			2	11	25					2										
100				5	16															
105				2	9															
110					4															
115					1															
Total	7216	7172	7130	7090	7055	5288	5240	5176	5118	5103	4854	4773	4706	4660	4630	4242	4243	4243	4242	4244

*Normal Distribution.

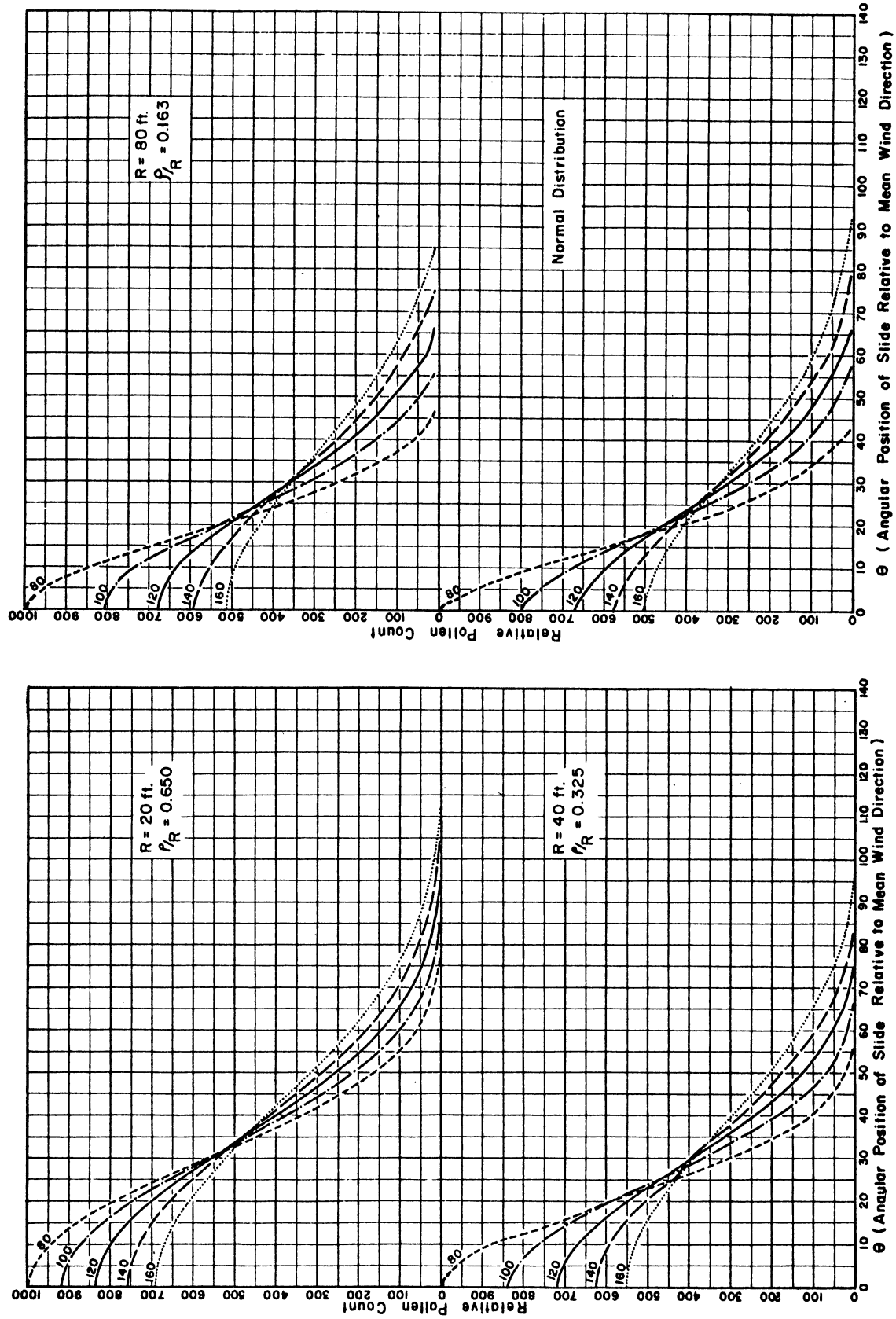

Fig. 3.3.11. Theoretical pollen counts on arcs downwind from an area source for total wind deviation of from 80° to 160° from the mean. The set of curves marked "Normal Distribution" are for arcs beyond 150 ft where the source may be considered as a point.

An analysis of the 2-ft wind data revealed that in most 4-hr periods the total range of wind deviation varied from 110 to 160°. During periods of very light winds, however, deviations of more than 160° were common. Periods for which the array slides have been counted were divided into groups having total deviational ranges of 110-120, 130-140, and more than 150°, respectively. A comparison of the actual angular distribution of pollen with the theoretical was made for each of these groups and for the 20-, 40-, and 80-ft arcs. Counts on the 160- and 320-ft arcs were too low to yield significant results.

Some cases showed reasonable agreement, but in general the actual distribution was more peaked than expected. The curves for the 110-120° total deflection, in particular, showed poorer agreement than the others (Fig. 3.3.12). This example includes 11 4-hr periods, 5 of which occurred during the peak emission time, 0400-0800. In the figure the actual counts on either side of the mean are compared with the theoretical curves for an area and for a point source.

The marked peaking of the actual counts may be due to a peaked distribution of the 2-ft wind direction about the mean. On the other hand, the total wind deflection may define too wide an angle. Short-period variations near the outer margins of the total deflection may transport little or no pollen, and a good fit may result using the angular deflection of the short period mean of the wind trace.

It should be noted that this phase of the work and that described in the following section are preliminary in nature owing to the recent availability of the data. It is thought worthwhile, however, to include one or two paragraphs to indicate in which directions the work is progressing.

3.3.3.2.6 Attenuation of the Pollen Plume.--The concentration in a plume of pollen will decrease with distance from the source owing to 2 effects. The first is attenuation due to diffusion, or spreading, of the plume. The second is deposition.

The diffusion of a substance in the atmosphere is due to eddies which act to spread the pollutant both horizontally and vertically. If the horizontal and vertical components are equal, the pollutant, pollen in our case, spreads downwind from a source in a right circular cone. If the ground were not present, the axis of the cone would remain horizontal and the concentration at a point downwind on the axis could be considered, to a first approximation, inversely proportional to the square of the distance from the source. A reflecting ground surface would result in a smaller rate of decrease, while an absorbing ground would result not only in a greater rate of decrease but also in a gradual lifting of the axis of the plume.

The question of whether pollen is absorbed or reflected by the ground is of considerable importance. The contribution of pollen from distant sources to local pollution will depend to a large extent upon the degree of deposition

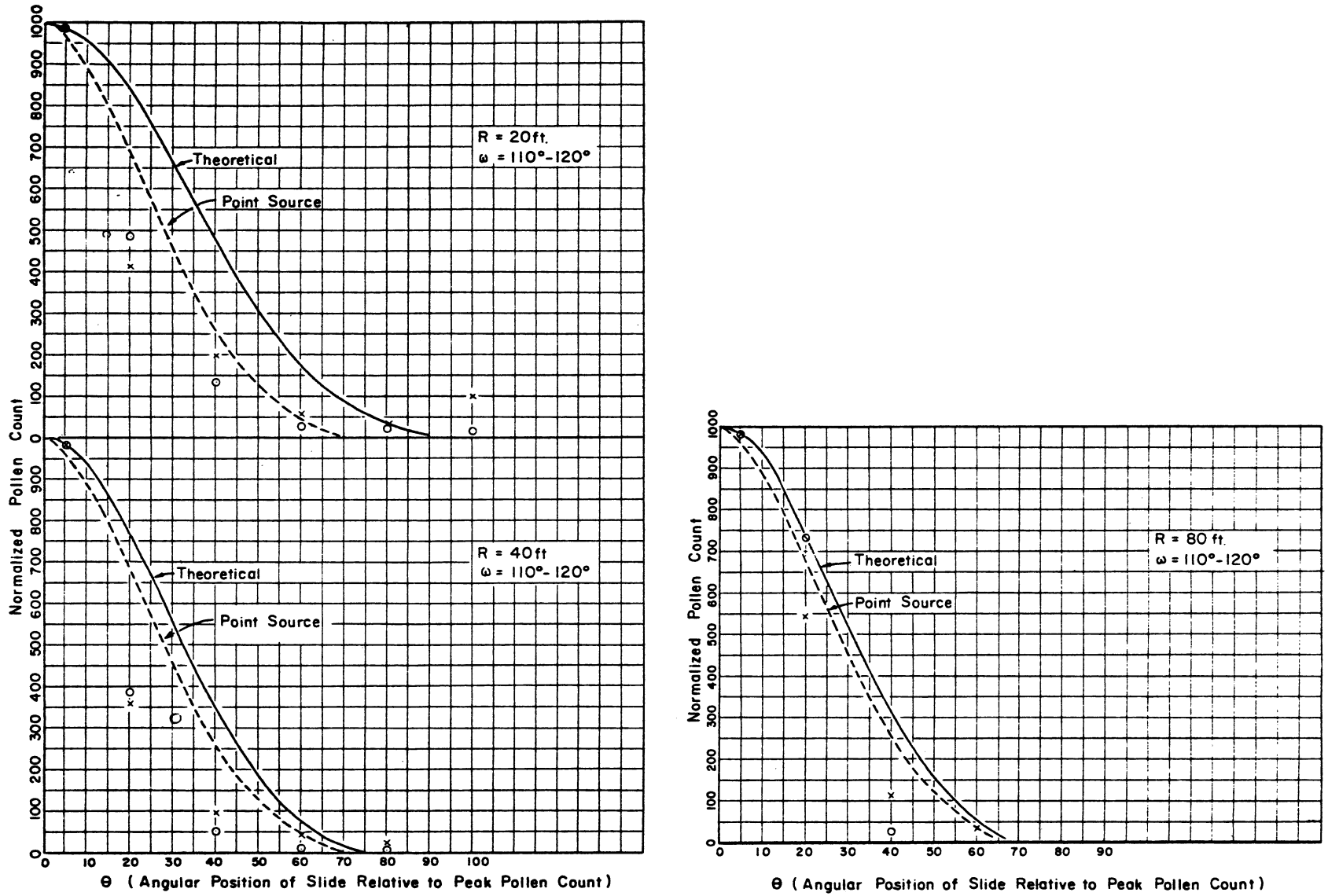

Fig. 3.3.12. The pollen counts on three arcs 20, 40, and 80 ft from the source at angles 20, 40, 60, 80, and 100° relative to the mean wind direction are compared to the theoretical distributions for an area and point source. Total angular deflection in wind direction is 110-120°. Positive angular displacements are indicated by x, negative displacements by o.

on grass, trees, buildings, etc. Preliminary results of wind-tunnel studies, conducted here to test various pollen sampling devices, indicate that obstacles normal to the wind flow have a high collection efficiency. If these results prove to be true, vegetation projecting into the wind may act like a giant broom sweeping pollution of pollen size from the lower layers of the atmosphere.

The rate of attenuation of the pollen plume with distance from the source was measured indirectly by comparing counts on the 20-,40-,80-,160- and 320-ft circles. The method used was to add the counts on all slides on a particular arc and then sum up for the same time period on all 10 days. The time period was kept constant so that attenuation during stable and unstable periods could be compared. Figure 3.3.13 shows the counts for the peak emission period 0400-0800 plotted on log-log scales. The points lie very close to a straight line. The regression equation for the line is

$$C_r = C_{20}\left(\frac{r}{20}\right)^{-2.7} = \text{const} \times r^{-2.7} \quad ,$$

where C_r = pollen count a distance r from the source. Had the attenuation been proportional to the inverse square law, the equation would have been

$$C_r = \text{const} \times r^{-2} \quad .$$

This line is also drawn in Fig. 3.3.13 for comparison. The greater slope of the observed line may indicate that pollen is being absorbed by the ground. It should be noted that, for other times of the day when pollen concentrations were lower, the deviations of the points from a straight line were considerably higher. These curves have not been reproduced in this report pending further investigation.

To summarize, it appears likely that pollen concentration decreases exponentially with distance downstream from a source. Sizable absorption by the ground is indicated. In the example discussed above, the count at 320 ft was only 15% of that which would have been expected had we used the inverse square law.

3.4 1957 IN-SEASON OPERATIONS

During the natural pollen season, a rather limited program was carried out at Jackson Prison. As pointed out in Section 3.1, it is our feeling that experimentation on a different scale is required during the natural season. In addition to the pollen samples collected within the prison as detailed in Section 2.2, samples were collected at the meteorological tower site on the Prison Farm. During this period the meteorological instrumentation described in the first progress report was in operation. The task of counting the 6000 gravity slides collected during the 1957 preseasonal experiment was given priority over the in-season samples, but counting of these latter will begin shortly.

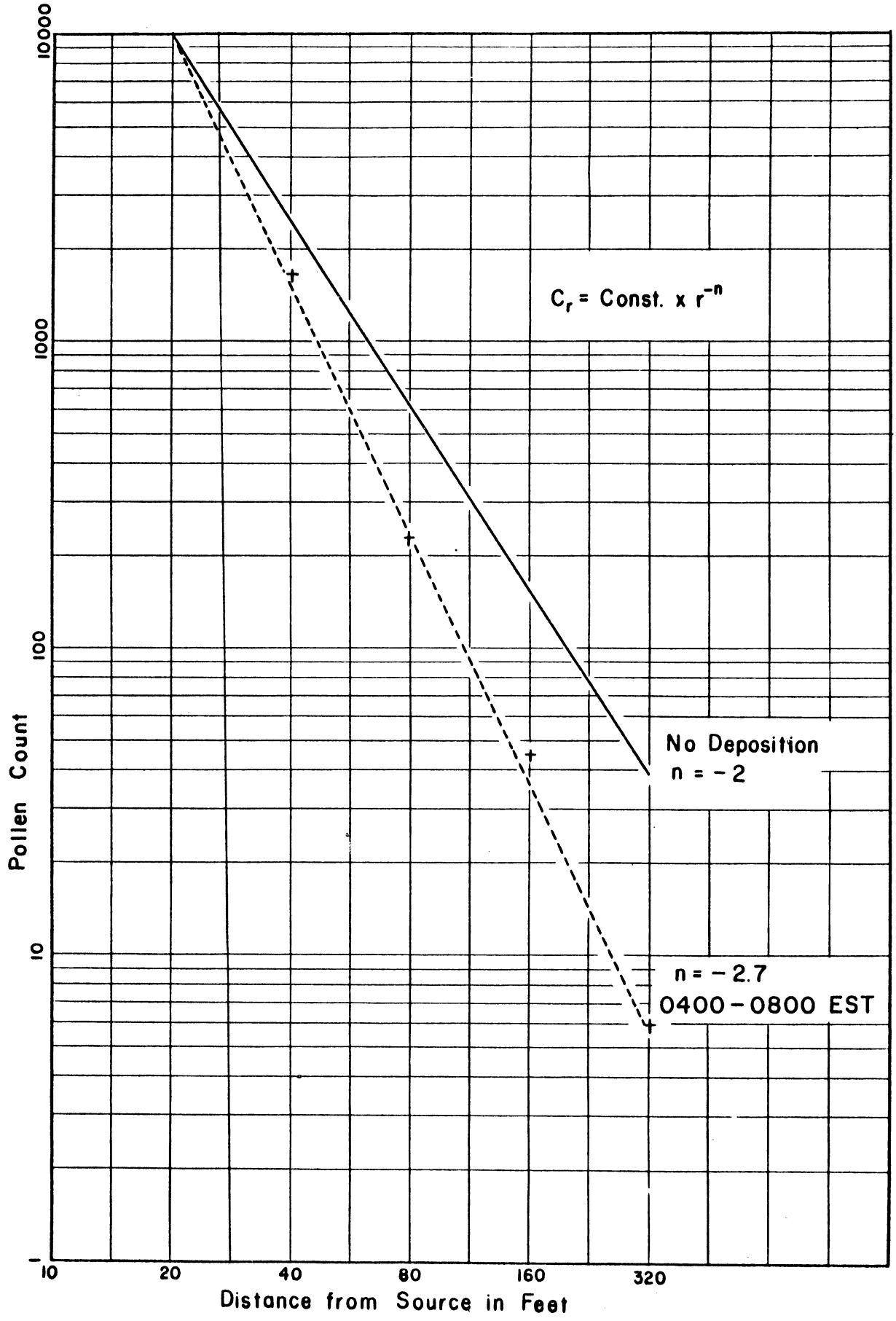

Fig. 3.3.13. The dashed line shows the actual decrease in pollen concentration with distance from a source for the period 0400 to 0800 E.S.T. The solid line indicates the expected counts using the inverse square law.

3.5 REFLOTATION OF RAGWEED POLLEN

The results of the 1956 preseasonal experiment had suggested that reflotation of pollen from the ground or from leaf surfaces might result in a vast enlargement of the original source of pollen. If this were the case, much of the advantage of having a limited plot of pollinating ragweed in a preseasonal experiment would be lost. Some preliminary work has been done to attempt to determine the degree of reflotation. This work is outlined below.

A small square the size of a microscope field of view at 100X magnification was outlined on a microscope slide. The slide was carefully cleaned and pollen puffed from a dispenser onto it. The slide was held to the side of the dispenser opening so that mostly single grains were deposited on it. The slide was then carefully transported to a microscope and the pollen within the square counted and its distribution noted. The slide was then subjected to various treatments, the results of which were noted:

1. It was gently waved in the air; no change resulted.

2. It was moved at approximately 4 mph; no change resulted.

3. It was inverted and waved gently; no change resulted.

4. Using a small wind tunnel and a Hastings Air Meter, slides were subjected to wind speeds starting at 2 mph and increasing at 2 mph increments to 25 mph. The slides were observed under the microscope after each increase in wind speed. Multiple grains lying in more than one level were observed to disappear at about 10 mph. Single grains and grains lying in one plane began to move at about 14 mph. A few grains remained on the slide to 25 mph.

5. The effect of tilting the slide into the wind and away from the wind was studied. No change was noted.

6. The effect of increased exposure time was studied. While the range of critical speeds remained the same, a few more particles moved in the longer periods.

7. It was noted that those grains which remained even at 25-mph speeds could be blown off with a sharp puff from the mouth.

8. The region of critical speeds from 8-14 mph was studied more closely with the wind speed being increased at 1-mph increments and using 1- and 5-min periods. A cluster of 7 grains in two layers blew off at 10 mph. The grains in a single layer moved at 14 mph.

9. Clusters of pollen grains were studied. A cluster of 10 grains in

three layers was reduced to a cluster of 7 grains in two layers at 9 mph and disappeared altogether at 10 mph. In a second trial the focus at 430X magnification was used to determine the height of the clustered grains above the plate. One cluster of 19 grains lay in three planes and extended a total distance of 51μ above the slide. At 8 mph the grains on the third tier blew off, leaving the grains extending 37μ above the slide. At 10 mph the second tier was removed.

The ultimate objective of these experiments is to place pollen on vegetation in winds of various strengths and observe the degree of reflotation, and then to apply the findings to wind speeds observed at the level of vegetation in the open.

The use of a glass slide as a surface is not ideal. First, the glass is easily charged and may hold the pollen through electrostatic force, and secondly, the edge of the glass slide splits the air flow, producing a relatively deep boundary layer. Slides have been prepared with aerodynamic leading edges and various surfaces, but tests have not as yet been conducted.

These preliminary results suggest that, where pollen is deposited in multiple layers, the higher grains may extend through the laminar boundary layer and be refloated by the wind. On the other hand, pollen grains deposited in a single layer are difficult to remove.

3.6 STATUS OF THE TEST CHAMBER

The basic concept of the pollen-exposure chamber remains unchanged from that presented in the first progress report. Certain modifications have been made, however, to permit the locating of the chamber in the Kresge Medical Research Building where a number of the allergy research activities are centered. Some revisions were necessary to fit the chamber into the available building floor space and ceiling height. In addition, the mechanical equipment specifications were changed to take advantage of stock items.

The cooling unit has been purchased and delivered. It is a standard, Frigidaire, nominal 2-ton unit with a cooling capacity of 20,000 to 24,000 Btu/hr. Heating coils of 5 kw (17,500 Btu/hr) capacity have also been purchased. The humidifier has a 650-w heater and should evaporate slightly over 2 lb of water per hour at maximum output.

The air-cleaning unit which was purchased is a Trion electrostatic precipitator. This unit has a high air-cleaning efficiency with low maintenance costs.

The chamber size is now specified at 9 ft by 8 ft with a ceiling height of 7 ft 6 in. The University Plant Department is currently investigating material costs to develop the most practical construction details.

A subcommittee of four members of the research team is already engaged in formulating plans for the calibration of the chamber and the design of experiments.

3.7 THEORETICAL INVESTIGATION OF THE COLLISION PROCESS OF AEROSOLS IN A TURBULENT ATMOSPHERE

The object of this study is to find the relation between the collision frequency of aerosols and the turbulence of the atmosphere in which aerosols are in suspension. The present research constitutes a continuation of earlier work which dealt with the dispersion of aerosols in the turbulent atmosphere (V. C. Liu, J. Met., August, 1956).

It has been established that the turbulent motion of the air tends to enhance the rate of collision of aerosols by the following mechanisms: (1) diffusional transport of aerosols with the eddies; and (2) the creation of differences of mobility of aerosols in the turbulent air. Eddy diffusion is the main cause of the individual particles coming together in a monodisperse (uniform-sized) aerosol. Many aerosol suspensions are polydisperse. The mobilities of the aerosols depend upon their resistance and inertia. A relative velocity between two particles of different size and inertia is thus developed whereby the larger ones overtake and probably collide with the smaller ones.

The ultimate aim of the analysis is to arrive at a collision rate of aerosols expressed as a function of the turbulence and other relevant parameters such as the physical size of the aerosols, etc.

4. STATISTICAL PHASE*

Progress in statistical analysis over the year has been limited primarily to studies in specific areas of the overall investigation. Studies were conducted of relationships between selected variables from the climatological observations and counts of ragweed pollen from the 1957 preseasonal experiments. Limited comparisons were made between the results of these studies and results from comparable studies carried out during 1956. Likewise, statistical studies of selected medical observations of the 1957 studies of allergic persons were made and their results were compared with results from the 1956 experience. These medical observations pertain to volunteers at the Jackson State Prison during the annual ragweed season. Reports on these limited statistical studies are included in papers and reports by the special groups in this progress report and in that of 1957.

The need for coordination of statistical processing and synchronization of analytical approaches in our research became apparent during the year and provision for such a service was included in the request for supplemental financial support for the research. The volume of observations made within each experiment each year constitutes a mass of data which cannot be economically analyzed through hand methods and desk calculator techniques. Orderly and timely scheduling, planning for processing of data, and standardization of analytical steps have not been accomplished in our research to date. There is no opportunity to accomplish these ends within the limitations of the original budget.

During the year limited statistical assistance was made available to the project by students of public health statistics who were on fellowships made possible through training grants for biostatisticians to the School of Public Health. These services were of real value, but they do not provide the continuous, coordinated statistical effort which is needed for the continuing research and analytical efforts for interdisciplinary and inter-seasonal investigations. This phase of our work is recognized to be in urgent need of improvement.

IBM equipment, including a 650 computer, is available for processing data from the studies, but our present budget does not provide for the skilled personnel and machine time required to process the data and program the studies on these efficient machines. The mass of observations now accumulated, as well as those planned for accumulation during the 1958-59 experience, preclude exhaustive statistical studies until a statistical program utilizing these efficient methods is established.

*By F. M. Hemphill.

5. PUBLICATION OF RESULTS

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

1. Gurney, C. W., and Cryst, S., "Observations on the Course of Allergic Rhinitis and Bronchial Asthma in Ragweed-Sensitive Subjects," Ann. Allergy, 15 (July-August, 1957), 367-378.

2. Dingle, A. N., "Meteorological Considerations in Ragweed Hay Fever Research," Fed. Proc., Federation of American Societies for Experimental Biology, 16 (July, 1957), 615-627.

3. Goodwin, J. E., McLean, J. A., Hemphill, F. M., and Sheldon, J. M., "Air Pollution by Ragweed: Medical Aspects," Fed. Proc., Federation of American Societies for Experimental Biology, 16 (July, 1957), 628-631.

4. Dingle, A. N., "Ragweed Pollen Concentrations in Relation to Meteorological Factors," J. Air Pollution Control Assoc. In press.

5. Wagner, W. H., Jr., and Beals, T. F., "Perennial Ragweeds (Ambrosia) in Michigan with the Description of a New Intermediate Taxon." In press, Rhodora.

6. Gill, G. C., and Hewson, E. W., "Air Pollution by Ragweed Pollen," submitted for publication.

7. Patterson, R., Correa, J. N., and Mathews, K. P., "Studies on the Neutralizing and Eliciting Activity of Altered Ragweed Antigen," submitted for publication.

6. ACKNOWLEDGMENTS

The research group wishes to express its deep appreciation, collectively and individually, of the cooperation and assistance willingly offered by many staff members of the State Prison of Southern Michigan at Jackson in the course of the investigations. The contributions of the following merit special mention: Mr. William H. Bannan, Warden; Dr. David B. Sher, Hospital Physician; Mr. John A. White, Hospital Director; Mr. L. D. Johnson, Plant Engineer; and Mr. C. Rossman, Farm Superintendent.

APPENDIX

MINUTES OF REGULAR MEETINGS OF THE UNIVERSITY OF MICHIGAN RESEARCH TEAM ON ATMOSPHERIC POLLUTION BY AEROALLERGENS

MEETING OF 3 APRIL 1957

The following were present: Cohen, Cook, Elder, Dingle, Gill, Goodwin, D. L. Jones, Lewis, Hewson, Mathews, McLean, Ruffner, Sheldon, and Wagner.

Medical Phase

Dr. Sheldon opened the discussion of the program for the 1957 regular ragweed season by commenting on several aspects of the problem: (1) the inability to determine at the present time, since the results of the statistical data are not yet available, which laboratory and pulmonary functions studies would be most useful from the medical standpoint for further work at Jackson Prison; (2) that the chamber experimentation may prove more fruitful than a continuation of the medical study as carried out during the previous two seasons at the Jackson Prison. Drs. Goodwin and McLean were of the opinion that, if the Jackson Prison medical phase were to be continued, a "battery" of studies would be necessary, (as the studies to date seem to indicate), since most of the studies measure different phases of the problem, and one test alone, or just a few, would not furnish the desired information. They favored not cutting down on the number of observations and tests performed on the patients in spite of the vast amount of statistical work required. In addition, they felt that it would also be desirable to do some of the studies more frequently, and some earlier in the day, nearer the patient's awakening period. Thus more personnel might be needed. They concluded that, consequently, the chamber experiment might furnish more information for the same investment of time and medical personnel.

The question concerning the date for completion of the experimental chamber was raised; it was thought that the chamber would probably be in a usable form by the coming ragweed season. A dispersion unit for pollen utilizing a fluidized bed of pollen was demonstrated.

Botanical Phase

Professor Wagner discussed the necessity of acquiring additional facilities at the Botanical Gardens to grow the 3000 ragweed plants necessary for the forthcoming pollen studies before the natural ragweed season. This would require financial assistance to the Botanical Gardens. He also commented that the Botany Department was appointing a plant cytologist, who might be of considerable aid to this project in the pollen germination and tapetal fluid studies.

Meteorological Phase

A continuous impinger was demonstrated by Dr. Dingle. This instrument could be used during the June pollen studies as well as during the regular ragweed season for collecting continuous pollen samples through the entire season on one continuous strip of tape, which could then be counted later. The possibility of using the tower near Northland outside Detroit for additional meteorological data was brought up by Professor Hewson; this would be in addition to the tower near the Jackson Prison.

During the discussion of further research, the addition of an immunochemist was felt to be desirable. A full-time statistical analyst was also considered necessary. Mr. Lewis also discussed other statistical problems now arising due to the ever-increasing amount of data being acquired, and the need for further personnel.

Dr. Mathews summarized the medical problems relative to the Jackson Prison program, and wondered if more definitive work would be accomplished by the chamber experiments. The meteorological problems along this same line, however, should still be correlated with the medical work at the prison in relationship to the usual ragweed season. It was suggested that the card system for collecting the patients' subjective symptomatology could be continued at Jackson Prison this summer and that these data be used in correlation with the meteorological findings.

Medical Phase

Dr. Sheldon introduced the topic of ionization of the air in relation to hay fever symptoms. He had been approached by the Research Director of the Whirlpool Corp., a subsidiary of RCA, concerning objective testing of an air-conditioning unit emitting negative ions for ionization of room air. Such a unit had been used by a medical group in Philadelphia, and patients had reported temporary subjective improvement of their hay fever. The entire group was asked for its opinion on this matter and if this type of problem could be studied by means of the proposed chamber. The group also wondered if ions had an effect on pollen and if pollen particles might be precipitated out or coagulated by ionization.

MEETING OF 1 MAY 1957

The following were present: Baynton, Cohen, Cook, Elder, Evans, Dingle, Gill, Goldstein, Goodwin, Hemphill, Hewson, D. L. Jones, Liu, McLean, Remington, Rubin, Sheldon, and Wagner.

The meeting was held at the Botanical Gardens and the superintendent, Mr. Kleinschmidt, escorted the group through the greenhouses and demonstrated the various phases of artificially growing young ragweed plants.

Meteorological Phase

Dr. Hewson began the discussion of the coming June artificial ragweed season. This year the experiment will be carried out near the tower at the Jackson Prison rather than on the North Campus. He summarized the suggestions of previous meetings: the use of the 100-foot tower for obtaining patterns of vertical distribution of pollen; the desirability of the ragweed plot being approximately 550 feet away, since this is the calculated distance from the source at which the plume height reaches 100 ft; the advisability of slides being placed in radial arcs at definite spacings and the slides to be again placed on stakes at grass-top level; the question of having slides also in the plot of ragweed itself; the appropriate spacing of volumetric sampling devices in the area; the question of using the scotch-tape continuous pollen sampling method in addition to the other methods; the advisability of having a portable pollen bed for use when the wind changes direction; the use of a dew-point recorder.

In the ensuing discussion, the question was raised as to whether the scotch-tape radial arcs would flutter in the wind and thereby have an effect on the pollen count. In locating the portable samplers, it was thought advisable to run them also along arcs, but, for purposes of statistical analysis, still at constant distances from the tower. The question of placing samplers all around the pollen source was brought out, since some pollens last year were found "up wind." It was decided to have 360-degree coverage at least close to the pollen source, and to place the samplers proportionally on a logarithmic basis rather than on a linear basis. It was also suggested that the portable samplers have fixed stations, so that the results could be interpreted with the other findings. The question of wind gustiness being different for various elevations was raised.

Since the number of ragweed plants this year will be more than 20 times that of last year's experiment, it was decided to put out slides in all quadrants and then count only those in the major wind directions. If discrepancies occur or if more help is available, then all the slides can be counted at a

later time. Four impingement samplers will be utilized; in addition to the same slide holders that were used last year, the question was considered of also having a few vertical slide holders.

The suggestion of counting the pollen grains with an automatic counter was again proposed. The advisability of using a fluorescent dye and photographing the grains for counting was considered. By this method, used elsewhere, all objects taking the dye are counted. However, there was speculation that the ragweed pollens might have optical properties different from other pollens owing to their spines.

Medical Phase

Dr. Sheldon commented again on the forthcoming natural ragweed season and the proposed Jackson Prison phase from the medical standpoint. It was again suggested that a larger group of volunteers keep the same daily symptomatic log cards as used previously, since these correlated as well as any other of the many tests performed last year. In addition, these cards would be used for a longer period than last year, i.e., beginning earlier in August and perhaps extending into late September or early October. Also the patients might be examined for a short interval beginning at 6:00 a.m. The advisability of also having a volumetric pollen sampler within the prison grounds as well as the 24-hour gravity slide was brought out. The medical activities would also be aligned in preparing for the chamber experiments.

Chamber Phase

Mr. Jacob Rubin was introduced to the group, since he is taking over the designing of the test chamber in place of Seymour Calvert, who is leaving the campus. Mr. Rubin has been reviewing plans in detail with Dr. Calvert, and the acquiring of approval for the air conditioning equipment appears to be the main feature holding up this phase of the project at present. As designed, the chamber will be a vertical duct system, i.e., the pollen and air will move up from the floor and out through the ceiling. This design was used to insure an even pollen concentration in the air, which would not be possible with a horizontal flow, e.g., in from a window or door, due to settling out.

The upward draft will be from 4-10 feet per minute and will not be noticed by the subjects; this is necessary to counteract the normal downward fall of the pollen grains. These definite limits were thought to be necessary to insure an even and accurate pollen distribution at all times. The unit is being constructed so that the top and bottom sections will stay assembled and the sides can be taken apart to permit transport and reassembly of the whole chamber.

The unphysiologic aspect of this chamber experiment as far as natural exposure to ragweed pollen is concerned was recognized and discussed. Professor

Cook mentioned experimental work done by others on pollen dispersion on the mucous membranes in living experimental animals and artificially constructed head models.

Botany Phase

Professor Wagner commented concerning the late artificial germination of ragweed grains this year and the various manipulative processes carried out by Mr. Kleinschmidt to make the grains germinate. The soggy spring weather was also a factor this year. He introduced Mr. Theodore Beals from the Botany Department who will assist in the micro-technique procedures necessary to study pollen germination. One of the difficulties in this microdissection is that apparently sticky substances make the pollen grains adhere together in clumps during germination.

The manpower problem for the June ragweed experiment was mentioned, since this year the summer vacation will occur earlier than last year, i.e., June 12, as compared to June 25 last year. Therefore the artificially grown ragweed plants should be ready by then in order to obtain student help for this phase of the project.

MEETING OF 5 JUNE 1957

The following were present: Baynton, Cohen, Cook, Dingle, Gill, Goldstein, Goodwin, Hemphill, D. L. Jones, Lewis, Liu, Hewson, McLean, Mathews, Patterson, Remington, and Wagner.

Statistical Phase

Dr. Hemphill started the discussion on the contemplated statistical operations for the next three years. He read a report to the group, and the following is a summary of this frank, critical, and highly instructive analysis of the present problems of this project by the statistical group. He reported that in general, the project will be two years old this coming August, and at the present time the following collection of data has been assembled: one season of pollen count from the East Engineering Building roof; one season's data on results of the air samplers; two seasons' data on the special pollen counts at the Jackson Prison and the medical data at the Jackson Prison; one season's data from the out-of-season North Campus experiment; and various botanical observations (no statistical approach has been contemplated on this last group). At the present time only the pollen counts and the clinical and medical data from the Jackson Prison experiment have been processed into I.B.M. cards. It was Dr. Hemphill's impression that other data had been collected but that they had not yet been made available to the statisticians and may not even be available to the various units now participating in this project.

In the future he foresees data from the extra-seasonal pollen experiment done at Jackson Prison this spring of 1957, as well as the regular ragweed seasonal data at Jackson Prison this year and also the human chamber experiments that are in the planning stage. He thought that in the future it would be well for all the various phases of this project to make a clear statement of the purposes of the studies to be undertaken prior to the experiments. This would then help in the statistical analysis of the data collected. It was also hoped that the data could be made available to the statisticians on a more even-flow basis rather than on an irregular basis, or as an emergency to be analyzed due to an oncoming paper presentation. Many of these suggestions were alluded to by Dr. Hemphill in his report to the group in May of 1956.

As far as the next three years of the study are concerned, he recommended that future reports be scheduled in advance, and also reintroduced the concept, suggested by Bob Lewis in a previous meeting, of a plan for fundamental statistical analysis on this project. He proposed that a coordinator be added to the group; this person should be a liaison man between the different scientific

disciplines within the group and could make recommendations to the group in advance to help with the statistical planning and analysis of data. He thought that the budget should allow for this individual, and it would be best to have this person on a twelve-month basis rather than part time. He thought that approximately 10 percent of the budget could be used for statistical procedure, and there is a danger that valuable time may be lost by scientific personnel handling routine records and statistical work. He thought that so far the progress has been satisfactory considering the problems involved, and would continue to be better in the future.

Dr. Hewson said that the present budget did not allow for statistical help on the required scale, but that additional funds were being requested to pay for adequate statistical services.

Dr. Hemphill showed that one computation had required 30 hours of calculation and that over 101 different results were required for this one calculation. Dr. Dingle suggested that the present statisticians be maintained as advisors and personnel be obtained for the actual machine work and computations on a lower level than a graduate student. He also brought out the fact that this project was still in the initial investigative stages and it was difficult to predict what statistical data would be necessary and what the definite purpose of each phase of these experiments was.

Botanical Phase

Dr. Wagner reported on the germination of the ragweed plants and reported that 3,600 plants would germinate within 2 to 4 weeks from the present time. This would amount to 241 flats of ragweed plants. There was considerable discussion about the exact time of the ragweed germination and the variables involved.

Meteorological Phase

Gerald Gill started the discussion on the extra-seasonal experimental pollen dispersal studies, and then Harold Baynton passed out a detailed proposal for the experiment to all members of the group of which only a brief summary will be included here. In general, samples of pollen will be collected at 20 degree radii, at 5 distances from the pollen source and there will thus be a total of 90 gravity slides for each sampling period. That is, we will have twice the number of slides per period we had last year. In addition, six volumetric samplers will be included; four will be on the tower at 2-, 12-, 25-, and 50-foot heights, and the two others will be at 2-foot heights on the 20-degree and 60-degree radii. Therefore, we hope to compare the counts on the gravity slides with those obtained with the volumetric samplers. It is also proposed that a change be made so that counts will be started at 8:30 p.m. and done every four hours from then on, to insure that samples will be collected directly at sundown and at dawn. The cellophane-tape impinger is merely to be

used as an experimental instrument during this year's pollen survey. In addition the meteorologists will have other instrumentation in the field so that certain other basic information can be obtained; this information then may or may not apply to this specific experiment. It was also thought that it would be best for a botanist to be present at least some of the time during this phase of the experiment.

Another suggestion brought forth included the third dimensional slide-collection of pollen, i.e., collecting the data at other levels than those specified on the tower. It was thought that it would be pertinent to add slides at the six-foot level on 20- and 40- degree arcs.

Dr. Hewson reported that Mr. Rubin thought that the chamber model would be ready for preliminary testing sometime in July.

Medical Phase

Dr. Cohen reported on the experiments used in studying the location of antigenicity of ragweed pollen. So far no attempts had been made previously on this, and this experiment was carried out to find some answers. At first it was not possible to break the ragweed pollen using sonic and ultrasonic energy. Finally it was found that using a Waring blendor with glass beads would macerate pollen. After repeated differential configuration, two fractions were obtained, one consisting of almost 99% protoplasm while the other material contained approximately 50% protoplasm and 50% cell walls. The two materials were extracted and then passive transfer neutralization tests were done, using these extracts on three recipients; 36 series were run with 3 sera. Sixteen satisfactory end points were obtained. In 14, the protoplasm extract neutralized better than the cell wall. These studies show that the protoplasm was more potent than the cell wall, although the cell wall appeared to contain some antigen not present in the protoplasm.

In the ensuing discussion Dr. Mathews commented that the results were as expected but the question had been raised as to the antigenicity of tapetal fluid, and therefore there was some possibility that the pollen cell walls might be the main locus of antigen. However, this was not borne out by this experiment. It is planned, however, in the future to continue with the studies on the antigenicity of the tapetal fluid itself. Dr. Wagner suggested that living grains might be different from the dead pollen grains, and this might influence antigenicity. He wondered about the possibility of sensitizing the recipients in passive transfer testing, and Dr. Mathews brought out that is was not seen in clinical practice although the possibility of infectious hepatitis was always to be kept in mind. The recipients were not likely to become allergic because none were allergic initially and none had a positive family history of allergy.

Meteorological Phase

Gerald Gill then reported on newer types of equipment for collecting pollen. He visited Camp Detrick in the latter part of May and found that they had devised a wire "U" bracket which was 6 centimeters long on the two arms and had a 4-centimeter radius. The wire-shaped U bracket turned on a shaft at 25 revolutions per minute and at approximately the linear speed of 25 miles per hour. They found that they had a 100% pickup of the pollen grains in the range of 5 - 50 microns. Unfortunately they had no new methods of counting, and they were also interested in the Coulter counter. They had an automatic spore counter, but there were two disadvantages; mainly, the cost, which was $24,000, and the fact that the spore colonies could not be counted until they were of one millimeter in size or larger.

MEETING OF 30 OCTOBER 1957

The following were present: Akerman, Baynton, Beals, Cook, Dingle, Gill, Goldstein, Goodwin, Hemphill, McLean, Patterson, Sheldon, and Wagner.

Botanical Phase

The first part of the agenda was a Botany Report, by Mr. Solomon Goldstein, on the destruction of pollen by aquatic fungi.

Mr. Goldstein divided four soil collections into 33 subsamples each, and used 33 species of pollen to "bait" one subsample each of the four soil specimens. Slides were made periodically to determine the incidence of infection on each species of pollen over a period of time. The incidence of infection was tabulated on the basis of the number of grains infected per 100 grains counted. The growth curve was similar to that obtained when one grows microorganisms in culture: there is a lag, then a phase of rapid growth (in this case up to approximately two weeks), and then there is a tendency for the number of infections to decrease. A possible explanation of this may be lysing of the infected bodies, or perhaps a tendency of infected grains to sink to the bottom of the petrie dish, where they are no longer encountered.

Also noted was a disparity between the frequency of infections and the types of pollen grains. Pseudotsuga mucronata was the most susceptible (virtually 100%), and pines were found to be only slightly less susceptible. On the other hand, Ambrosia grains showed little or no infection. This may be due to the thick wall of the Ambrosia pollens. However, since in Ambrosia as well as in a number of other species of pollen, the aquatic fungus Nowakowskiella was frequently noted in intimate association with the exine of the grain, the possibility therefore exists that this fungus may be capable of degrading the external coating of these pollen grains. Physiological studies are now being made to determine the nutritional requirements and the metabolic capacities of this organism.

It was found that cinnamon-fern spores that had germinated showed little or no evidence of infection, but this was true of nongerminated spores. To determine whether the attacking fungus inhibited germination or whether nongerminated spores are more susceptible to infection, boiled fern spores were used as "bait," and the incidence of infection was found to rise. This would seem to indicate that the latter hypothesis is more likely to be correct.

The question was raised whether the temperature and moisture content

could be varied, using the same soil and ragweed pollen. Also, it was pointed out that there may be a difference in reactivity between the infective and non-infective ragweed grains as far as allergic sensitivity is concerned. These latter ideas were discussed following Mr. Goldstein's presentation.

Medical Phase

The second part of the agenda consisted of the medical report, by Dr. Jack Goodwin, concerning the medical studies at Jackson Prison during the 1957 season. He first reported on further evaluation of the data from the 1956 ragweed season at the prison. The WBC was not particularly helpful, since there appeared to be no correlation with the quantitative eosinophil counts, and the subjective and objective symptomatology. The nasal smears have not been evaluated further than on a tentative 1 to 4+ basis. Analysis of the pneumotachograph records by calculation of the expiration-duration/inspiration-duration ratio revealed correlation clinically in about one-half the cases.

During the medical phase of the 1957 ragweed season at Jackson Prison, 19 volunteers were used. Operations were started on August 2, and were carried on till September 29; the longer period this year was chosen to insure proper base-line studies before and after the usual ragweed season.

In addition, the main objectives of this study were to check on the second, third, and fourth time periods, when the patients were having an increase of symptomatality subjectively. Therefore, objective tests were done and the following results were found: as far as hay fever was concerned, approximately 78% of the time the patients had a peak of their subjective symptomatality during time period 2, and 88% of the time they had a subjective peak in asthmatic symptomatality in time period 2. However, objectively their asthama was worse in time period 5 in approximately 70% of the patients. The results with objective hay fever increase in time period 2 were approximately the same as the subjective results. Therefore, one would tend to imply that hay fever patients knew when they were worse, whereas the asthmatic patients did not. It was noted again that the pollen peak occurred after their increase in hay fever symptoms during the day time, but before their increase in asthma symptoms during the day. Again there was speculation about the change in body position, activity, etc., causing this apparent paradox.

Dr. Goodwin then described the nitrogen meter. Using the one-breath nitrogen dilution test, he found that one or two hay fever patients had one abnormal test, whereas general abnormality of this pulmonary function study occurred in the patients who were asthmatic. This was merely a preliminary report on the findings at the Jackson Prison, and further analysis will have to be carried out on all of the work mentioned.

During the ensuing discussion the limitations of tests carried out at Jackson Prison were again outlined. Also, the possibility of a different antigenicity of pollen grains during the day and night was suggested.

Meteorological Phase

Gerald Gill then briefly commented on the meteorological studies and showed slides on the setup at the Jackson Prison during the ragweed dispersion test, which was run in June of this year. Color 35-mm slides were shown covering most phases of the tower experiment as well as the equipment setup for the dispersion studies.

Professor Akerman then briefly talked about the plans for the future chamber experiments. Under the present plans the chamber requires approximately an 11-foot height, while it was noted that the Allergy Lab in the Kresge Building had a ceiling of only 10 feet three inches. A further complication is that the pipes in the ceiling reduce the effective height to 8 feet 9-1/2 inches. Both Gerald Gill and Professor Akerman were going to study the problem further.

The meeting was concluded with the motion that future meetings would be held monthly, usually on the second Wednesday of each month.

MEETING OF 19 NOVEMBER 1957

The following were present: Baynton, Cook, Elder, Dingle, Gill, Goodwin, Harrington, Hemphill, Hewson, Lewis, Liu, McLean, Mathews, Patterson, Remington, Ruffner, Sheldon, and Wagner.

Botany Phase

The report on the botany studies was begun by Professor Wagner, who read a report on perennial ragweed which had been prepared by Mr. Richard Hanlin on the problem of how the ragweed plant discharges its pollen. This study was done last summer. It was found that, if the plants dry out, ragweed pollen will not be discharged, whereas if the plants are in water and hydrated, some pollen will be discharged, although less than in the natural state. This therefore suggested that internal water relationships are very important. Two plants were studied in a cubicle, closed so that air currents were cut to the minimum, and the temperature and humidity were recorded. The ragweed pollen discharge was then observed beginning at 4:30 a.m. It was found that the pollen did not shoot out as had previously been thought. Instead, it seemed to fall on the wax paper on the floor and the pattern of the branches was dimly outlined on the floor. If the windows were opened to create a breeze, there was a larger drift of the pollens. When the temperature rose and the humidity decreased, it seemed that there were fewer and larger clumps of pollens.

Counts were made approximately every one-half hour and it was found that the pollen discharge began at 7:00 a.m., increased until 8:00 a.m., and then dropped off considerably. One plant was shown ro reach its peak pollen discharge for the day at 9:00 a.m. In one instance, the humidity was lowered by accident and the pollen discharge increased. It was thought that the relative humidity was important but this was only one factor; temperature also played a part and it was wondered if light intensity was also important.

It was noted that some pollen was discharged before 4:30 a.m. Professor Wagner then described the mechanism of the pollen actually being pushed out along the anthers to the edge and then dropping off rather than being actually propelled. It was thought that some internal mechanism aided pollen discharge because some discharge occurs during the night.

During the discussion the question of reflotation afterwards was again raised, and it was thought that in humid weather pollen was actually held together.

Professor Wagner then said that the ragweed bibliography was about ready

and would be reported on at the next meeting. Also, it was wondered if observations in the natural habitat of ragweed pollen will be different from observations in the laboratory and if multiple-picture photography could be used as an aid in this study. The previous part of the report was summarized by saying that appendages on the anther act as a cage and that this could be considered a passive act of pollen dispersion through meteorological factors, while the rest of the plant is living and an actual turgor movement pushes the pollen out.

Professor Wagner then reported on the distribution of the perennial ragweed. It has now been found in 42 counties in Michigan, an increase of more than one-half of the total number of counties previously known. There is no evidence that it was present in Michigan before 1900; at least it was very rare; and now it appears to be quite common in both the Upper Peninsula and the lower western part of the state. It spreads by underground roots and reproduces by root proliferation producing new plants. Its habitat appears to be sandy, exposed soil or gravel along highways on the edges of towns and railroad tracks. It appears to flower as early as July 12 and it is wondered whether hay fever may not occur at that time due to this ragweed plant.

This new species appears to be a hybrid of the common ragweed and the usual perennial ragweed. Professor Wagner showed three colored slides of these three types of ragweeds. The common ragweed had 77% of its fruit developing; the perennial has approximately 55% of its fruit developing and going on to reproduction, while the hybrid has only 16% and does not reproduce as readily as the other forms of ragweed.

Mr. Ted Beals then talked on the cytology of the ragweed plant. He first described the technique of staining the material and then making drawings from photographs. He reported that, in the perennial ragweed, meiosis (the early sexual phase) is completely regular with 36 pairs of chromosomes and all appear approximately the same size. The annual ragweed also has regular meiosis and has 18 pairs of chromosomes, whereas the new hybrid plant has very irregular chromosomes and may have clumps of three chromosomes together. The three forms also had different numbers in the mitotic figures in the root tips.

After looking at the pollen grains to see how many grains could be stained for protoplasm and comparing their size, Mr. Beals noted the characteristics tabulated below.

Ragweed:	Common	Perennial	Hybrid
	Ambrosia Artemesiifolia	Ambrosia Coronopifolia	Ambrosia Intergradiens
Percent of total grains with no Protoplasm	16.0	22.6	54.9
Average size, microns*	17.6	20.5	21.8

*Normally the size of the pollen grain is a function of the number of chromosomes

The table shows, from the size of the pollen grains, that there is an unexpectedly high percentage of normal looking pollen grains with the hybrid.

Mr. Beals showed slides of the different types of chromosomes and stated that studies on germination of pollen had been started but satisfactory media had not been developed as yet.

In the discussion it was shown that the hybrid plants originally were formed by sexual means but now were reproducing by means of root systems. The possibility that there may be a gene exchange between the species was discussed and it appeared that the "grandparent" of the ragweed may have been in the southwestern part of the United States. It was mentioned that the hybrid plant invades a plowed field or a plot of ground on the prairie or a field of grass. Since these plants appear only in western Michigan, it is rather difficult to explain their spread further west by railroads because most railroads are in the southeastern area; the possibility of boat transportation exists.

Meteorological Phase

As far as the meteorological studies were concerned, James Ruffner first commented on further analysis of the data during the regular ragweed season of 1956.

To simplify the analysis, he discussed six days with no large-scale weather changes or precipitation. Counting the previous four days that had been reported earlier, a total of ten days has been extensively studied with regard to wind speed, pollen counts, and solar radiation.

The second part of the meteorological report concerning the 1957 preseasonal experiment at Jackson consisted of a talk by James B. Harrington about pollen pick-up and the gravity slide counts. He questioned the reliability of counts on slides at ground level because of pollen falling directly on to the slide rather than being air-borne. However, it was noted that relative pollen counts at ground level and 2-ft height correlated well from period to period. He commented on the fact that the counting technique was modified for those slides with more than 500 pollen grains while the rest of the slides were counted *in toto*. He next demonstrated cluster distribution on a graph where the number of grains in a cluster was plotted against the frequency of the clusters. The distribution on the slides was different from that obtained by the botanists in their controlled laboratory study, in that many single pollens were found. No Poisson distribution curve was found as would have occurred had the pollen begun to fall as single grains. It appeared that the pollen initially began to fall as pollen clusters rather than single grains. When the log of the frequency was plotted against the log of the number of grains in a cluster, the resulting curve was a straight line.

A second graph, furnished to members present, demonstrated the variation

of the gravity slide count with time of day. It showed that there was a strong emission of pollen at about 6:00 a.m. with a dropping off at 10:00 a.m. In addition, it was mentioned that reflotation was still of interest in these counts and Mr. Harrington reported on the experiment he was attempting to do on this aspect. Slides were fixed so that single pollen grains on the slide were exposed to winds varying from zero to 24 miles per hour. Microscopic techniques were used to view a selected area of the slide before and after exposure to the wind from a miniature wind tunnel. It was noted that single grains began to be moved at 14 miles per hour but that a few grains remained on the slide to speeds of 25 miles per hour. Some doubt was cast on the probability of single pollen grains being refloated from leaves or ground during the ragweed season. The problems inherent in this type of experiment were brought out and further analysis was planned.

During the discussion period it became apparent that the findings of the botany group in their controlled laboratory experiments and these so-called field studies in the natural ragweed season resulted in divergent data. It was noted that there was some discrepancy between the botanical, meteorological, and medical pollen counts. The botany group still felt that most of the clumps fall to the leaves and then down to the ground in smaller clusters.

MEETING OF 11 DECEMBER 1957

The following were present: Akerman, Baynton, Cook, Dingle, Gill, Goodwin, Harrington, Hemphill, Hewson, Lewis, Mathews, Patterson, Ruffner, Sheldon, and Wagner.

Medical Phase

Dr. Jack Goodwin opened the agenda on the medical phase of this project by reporting on work in the recovery of pollen from lung tissue.

The portal of entry of the ragweed pollen is not entirely known. It is known that nasal insufflation will cause symptoms in an allergic individual in a few minutes, while oral ingestion will cause symptoms only in some people. Dr. Mathews had suggested that the tissues be searched for the pollen grains. In reviewing the literature it was shown that pollen had been found in nasal secretions, sputum, and in stools. It also has been found in nasal biopsies and in casual pathological slides; however, no one has actually searched for it in the lungs. The ragweed pollen diameter is approximately 18 to 24 microns and this size of pollen grain could penetrate only to certain distances in the lungs. Several authors have given dimensions of the smallest bronchi as being 0.15 mm in the dog. Bone meal with fragments 75 to 100 microns have been found in animal alveoli. There is known to be a rapid loss of dust particles in the lungs and this is probably due to ciliary action.

It is thought that approximately 150 pollen grains are the average daily intake of an adult in the early part of the ragweed season (this has been estimated by one author). The question is where the pollen goes. Various workers have found pollen in sweeping dust from the floors. During the ragweed season, Dr. Goodwin found there is a "background count"; i.e., one to two pollen grains per slide during the season will be found. Glassware collects several grains of pollen and actually five pollen grains were found on the inside of glassware which had been suspended upside down for a three-week period.

In 1955 secretions obtained at autopsy from various portions of the respiratory tract were examined, and there were fewer than two pollen grains except in the nares. The pollen count of fewer than two, however, was no more than the "background count"; the bronchial washings in a few patients also showed fewer than two pollen grains.

A method was set up in 1956 by Mr. Kurt Mikat to digest lung tissue without destroying the ragweed pollen. It was attempted to digest portions in

boiling KOH for 20 minutes and then to centrifuge, decant, and resuspend in NaCl solution. It was then filtered with millipore filters. It was found that if more than 10 grams of tissue were used, too much debris remained. With smaller amounts of lung tissue, satisfactory recoveries of known numbers of ragweed pollen grains added to guinea pig lung tissue were obtained.

In 1956, tissue was obtained from 6 autopsies at the height of the ragweed season; muscle from the cest wall was used as controls. The results are shown below.

RECOVERY OF POLLEN FROM LUNG TISSUE*

Autopsy	Nose		Trachea		Bronchus		Lung		Control	
	Count	gr/gm	Count	gr/gm	Count	gr/gm	Count	gr/gm	Count	gr/gm
1	20	400	13	11			15	25	0	0
2	15	300	34	30	9	6	7	4		
							143	292		
3	6	171	10	6			8	23	3	2
4	18	277			33	41	21	150		
							21	191		
							20	182		
							12	172		
5	14	127	3	27			9	90	0	0
	4	80					9	100		
6	1	8					0	0		
	1	8					8	100		
							1	17		

*Count = actual no. of pollen grains; gr/gm = calculated no. of grains per gram tissue.

It seems that the ragweed pollen actually gets into the lung tissues; perhaps some people get more pollen further down than do others. As far as Dr. Goodwin could determine, the method was reliable for positive results. When pollen was added to tissue and this control was then run, there was a large percentage of loss; pollen grains were often obscured by the tissue debris. It is thought that they are actually present when the pollen grains are really seen.

These studies suggest the following questions: (1) How long are the pollen

grains found after the ragweed season? (2) What method will fix tissues without losing ragweed by washing or destroying it? (3) If it can be confirmed that pollen gets into the alveoli, then are they in contact with capillaries and thus in a so-called storage bin, probably for absorption?

In the discussion that followed, Dr. Wagner suggested that polyvinyl alcohol could be useful as a fixing solution since this will fix material and remain transparent; it is thus easy to focus through it. Dr. Hemphill thought that further animal experiments were indicated. Dr. Mathews said that this had been tried with guinea pigs and that there was the problem of contamination by the hair of the animal. He also mentioned that the number of pollen grains recovered was unexpectedly high, considering that pollen are not usually found by the pathologist, and considering the estimated intake of 150 pollen grains per day, as mentioned previously. In view of this, these results should be confirmed by further experience. Professor Cook noted that mineral particles were rarely found greater than ten microns in size in the alveoli except for asbestos fibers of 10 to 20 microns in size. Dr. Mathews brought out that aerosols must be less than 5 microns in size to reach the lower lung areas. It was wondered if the larger mineral particles go beyond the larger terminal bronchi. Professor Cook stated that NaCl aerosols were used to mix with fine dust dispersions, and then iron particles were blown into animals. Sections then made reveal that very few particles actually reach the alveoli, but appear to remain in the bronchioles.

Botanical Phase

Professor Wagner then reported on the progress of the ragweed bibliography. He first discussed the new ragweed hybrid again; and then stated that in the new bibliography there was a total of 535 references. Of these a number had been contributed by the medical group. The medical part of the bibliography has been proofread. The question was raised whether the bibliography should be turned over to the meteorologists for proofreading and for addition of references particularly in their field, and it was decided to do this.

The abbreviation method used by Chemical Abstracts was suggested as the best bibliographical method and it was recommended that this be accepted. The question of publication and the type of journal was raised, and it was wondered if the Cumulative Medical Index would be suitable. The University of Michigan Medical Bulletin and the Library of Congress were also suggested. A table of contents was indicated. The final details of publication were undecided, but left to be discussed further with the Chief of the Medical Library.

Chamber Experiments

A short discussion ensued concerning chamber experiments, and the dimensions were changed to 8 ft x 8 ft x 9 in.

The next meeting will be on January 8, 1958, in the Meteorological Laboratory.

MEETING OF 8 JANUARY 1958

The following were present: Akerman, Baynton, Cook, Elder, Dingle, Gill, Harrington, Hemphill, Hewson, D. L. Jones, McLean, Mathews, Patterson, Ruffner, Sheldon, and Wagner.

Botanical Phase

Professor Wagner started the discussion of the botanical phase by stating that Professor Jones of the Botany Department was pleased with the ragweed bibliography. He then read a letter from another botanist commenting on the work which the Botany Department had done on the ragweed plant.

As far as the plans for the coming year were concerned, he thought that the primary problem from the botanical standpoint was studying the pollen discharge and flowering of the plant, and correlating these with meteorological data rather than strictly ecological studies. Last year, because of the wet season and the ideal growing conditions afforded to the ragweed plant, the out-of-season experiment produced abnormally large ragweed plants not comparable with those of other phenology studies. Professor Wagner commented that Professor Norman, a plant physiologist in the Botany Department, believes that basic research can be fone on the ragweed plant as far as the photoperiodic studies of plants are concerned.

Gerald Gill observed that there is actually not much shortening of daylight hours between June 22 (15 hours 19 minutes) and mid-July (15 hours) when the flowering process is initiated in ragweed, presumably in response to a shortening day. The usual definition of a short day is one with less than 12 hours daylight, but this does not occur until September 26 at this latitude. The flowering of the plant, Professor Wagner said, is thought to be controlled by the photoperiod; French workers have actually modified plant growth (other species than ragweed) by brief introduction of light during their dark period.

It was mentioned that even in the artificial conditions used at the Botanical Gardens there was extreme variation among the ragweed plants. It was hoped that when the new Botanical Gardens were constructed, strictly controlled photoperiodic experiments could be carried out on the ragweed plant. Dr. Wagner suggested some questions for the study of pollen behavior: Can pollen production be studied? Can the pollen tube fluid be studied? And can the pollen tube fluid be separated from the true shells? He also asked whether the extra-seasonal experiments should be repeated again this year.

Dr. Sheldon suggested that a large store of pollen should be set aside so that a portion could be used at one time and another portion at a later date in medical experiments. The discussion revealed that the pollen collection method was still not very successful, although it has been tried under adverse conditions in the past. Dr. Dingle and Dr. Hewson thought that the extra-seasonal experiments should be carried out again this year, but on a smaller scale and with more precise experimental methods. Phenology studies were stressed as the most practical because, with limited funds and equipment, results could still be obtained and analyzed.

Meteorological Phase

Jim Harrington then described the present status of the meteorological analysis of the 1957 pre-seasonal and in-season experiments. He said that all 960 slides used at the source of the pollen plot had been completely counted. There were approximately 5,400 slides in the "array slides" which consisted of those on the various radii at 20° arcs at distances out of 20, 40, 80, 160, and 320 feet. One and one-half days of these slides had been counted; there was a total of ten days in all. In addition, three impingers had been used which sampled with scotch tape at the rate of one inch per hour; all these have yet to be counted and analyzed. It was mentioned that there might be some question of validity in the impingement count because they had not been used extensively as yet. Beside the "source slides," "array slides," and impinger counts, there were also 540 millipore filters to be counted and analyzed.

As far as the specific meteorological data for the preseasonal experiment are concerned, the wind data, lapse rate data, radiometer measurements, soil-temperature measurements, and two hygrothermograph recordings have been abstracted. The group is to start on the in-season data in the near future.

In tentatively discussing the results of the pre-season experiment, Mr. Harrington said that the group did find some pollen up-wind from the ragweed plot but that this could have been due to contamination from the workers carrying pollen out of the source when they change slides or to pollen contamination actually in the slide boxes themselves. This contamination was extremely small when compared to the pollen counts at the down-wind side of the ragweed source. Fungus growth has been present on only one slide to date.

Mr. Harrington then distributed some graphs for discussion. The first graph showed pollen counts plotted in thousands-of-grains and average value for four clear days. It was noted that there was a very strong pollen emission between 6:30 and 8:30 a.m. and also a small pollen emission around 4:30 a.m. on other days, although this was not present on clear days.

The pollen count graph for June 25, 1957, showed the pollen count to be far less than on clear days; there was a peak between 8:00 and 10:30 in the morning, possibly due to cloudy weather delaying the normal time of emission,

but it was found that this pollen peak was approximately only one-half of that on the clear days. The next graph consisted of the counts on June 26, 1957. Correlating the weather with the pollen counts showed that an increased pollen emission both at the ground and two-foot levels had occurred after rain showers. The last graph, for July 4, 1957, again showed a pollen peak occurring during the time after a rain shower. The question was raised if rain could actually cause this; it was brought out that the exact time of the rain was not certain, and that there might be a leeway of an hour.

Mr. Harrington mentioned that relative humidity is the actual moisture in air divided by the moisture content of saturated air at the same temperature. As the temperature increases, the relative humidity decreases; if these functions are plotted, their curves form a mirror image of each other in the absence of water-vapor and air-mass changes.

On studying the charts of relative humidity, it was noted that an unusual occurrence had taken place on June 26, 1957; there was a sudden drop of humidity around 2:30 a.m. and no change in temperature occurred, due to a change in the air mass. Shortly thereafter a pollen peak occurred; thus pollen emission appeared to depend on the sharp drop in the relative humidity. When relative humidity was plotted against the pollen count, it was noted that a sharp drop in relative humidity was usually followed after a lag by an emission peak.

In the discussion, questions were raised as to whether pollen clumps on the slides would occur with moisture and rain, and if this would influence the actual pollen count and distort the true pollen count. It was thought that under the square-inch of scotch tape used, there was no significant change with the rain and that the impingement counts should corroborate this. Impact of the rain drops on the plants might shake pollen off rather than merely washing it off; it was wondered if this were a mechanical emission rather than a biological one. It was suggested that the drop in the relative humidity was more important than the rain in the increases in pollen count because there was no real increase in the production of pollen by the plant. Professor Wagner said that there is probably a steady plant production of pollen and that we were actually talking about pollen emission. Dr. Dingle said that we were studying only what is on the slide, not the amount of pollen in the rest of the air. This discussion concerned the counts of the "pollen source" and the results from the "array slides" were not included.

Professor Hemphill wondered if sampling certain of the counts could be done to reduce the tremendous work involved in counting slides. This was on the assumption that most of the pollen was produced before noon on clear days, which seems to be evident from the work to date. It was thought that, once the pollen counts were normalized, the "array counts" in cones rather than all counts could then be done. It was also suggested to make the counts every other day rather than every day.

Chamber Experiments

Professor Akerman opened the discussion on the experimental design for calibration and use of the test chamber. He showed the new set of plans which had been modified from the previous design because of the change in size of the area now available for the test chamber. The chamber would be constructed so that the floor would have a wood grating; the pollen would be pumped into ducts under the floor and then come up through small holes in these ducts with a jet velocity of 145 feet per minute to ensure turbulent mixing. Then it would be drawn on up through the chamber via six outlet grilles in the ceiling. Since the fall of ragweed pollen is approximately three feet per minute, this suction at the roof would be great enough to have the pollen rise at approximately four feet per minute; it was thought that single pollen grains would thus rise and some clumps might fall out.

It was mentioned that the floor and duct system for the pollen dispersion unit would be in sections and movable so that all could be taken apart and cleaned thoroughly between experiments, thus permitting the exact amount of pollen to be ascertained and preventing large accumulations. It was also shown that the velocity within the different areas could be changed throughout the chamber depending on the distance from the fans so that approximately equal pollen distribution would occur throughout the chamber regardless of the distance from the pump.

Gerald Gill said that the proposed construction permitted modification as the experiment progressed and improvements could be made without great cost. It was suggested that flush-type lights be used inside the chamber to allow the subjects to read. A bed and chair are to be in the chamber, which might have the effect of modifying the pollen distribution around the patient. It was suggested that the bed be the type which could be lowered from the wall when in use and raised up when not in use, thus insuring more room for the subject in this test chamber.

Dr. Mathews mentioned a definite temperature and humidity range; the test chamber can be regulated so that a temperature drop will occur in the range of 20° F from the outside air. The temperature can also be raised to above that of room air. The humidity can be increased to practically any extent, but no figures were given on the extent to which humidity can be decreased. Others are working on the system to check the pollen counts inside the chamber.

It was decided that the next meeting would be on Wednesday, February 12, 1958.

MEETING OF 12 FEBRUARY 1958

The following were present: Akerman, Baynton, Beals, Cook, Elder, Dingle, Goldstein, Harrington, Hewson, D. L Jones, McLean, Mathews, Patterson, Ruffner, and Wagner.

Professor Hewson introduced two new members to the group: Paul Giever from the School of Public Health who will assist Professor Cook, and Arvy Wagner who will aid the meteorologists in instrumentation of the meteorological equipment for the chamber experiment.

Meteorological Phase

The first topic on the agenda was a discussion of the design for the 1958 preseasonal ragweed experiment by Dr. Dingle. He first went into the background of the preseasonal experiments and said that there still was a large reservoir of data requiring analysis. Because of the limited budget, most of the money at present will go for the construction of the chamber and its experiments and therefore this year's preseasonal experiment will be limited in scope. The following were considered to be the objectives of the coming preseasonal experiment: (1) to improve our knowledge of the time of the release of the pollen from the anther and to correlate the time relationships with its dissemination in the air; and (2) to investigate the vertical diffusion of pollen from a known source and its relationship to the meteorological data, the present data being mainly on horizontal distribution. Dr. Dingle outlined the various procedures for this year's experiment and thought that the source of ragweed plants should be about the same as last year. The same three sampling techniques, gravity slides, millipore filters, and the impinger, would be used to establish continuity with the work done in the past. There would be a gravity slide at the 2-foot level and the complete array of them around the source as last year. However, if meteorological predictions could be made accurately, it might only be necessary to put out slides in a certain sector of the circle when the wind direction was known; the work of counting the slides would thus be reduced. The millipore filters have the limitations of being a nonisokinetic type of design of giving only a low-volume sample because large volumes of air cannot be brought through, and of requiring frequent handling; but they do have the advantages of being available in quantity. Our group has had experience with this type of sampler. The impinger has not yet been fully evaluated but it has the advantage of being continuous and uninterrupted in character and has a high-volume rate of flow.

As far as the tower work and distribution of the ragweed pollen is concerned, Dr. Dingle suggested that 20-foot-high masts could be used in addition

to the tower already constructed at the Jackson Prison. Again, if meteorological predictions could be accurately made, the location of these masts could be shifted to suitable areas. As far as the meteorological studies are concerned, the same as those of last year would be carried out; bivane apparatus for vertical measurement of pollen distribution would, however, also be introduced.

The duration would probably be limited to 12 hours a day (4:00 a.m. to 4:00 p.m.). If the weather were unfavorable on certain days or if there were an extended rainfall, the time periods might be shorter. Work would probably be started sometime around June 15. It was also thought that there should be closer contact with the weather stations at Willow Run, Wayne County Airport, and Jackson Airport. Varying the vertical sampling technique, depending on the weather conditions, was also considered.

Professor Hewson then once again reminded the group that Dr. P. H. Gregory, an eminent English botanist, has said that under normal atmospheric conditions 99.9 percent of the pollen falls to the ground within a hundred meters of the plant, and discussed the significance of this conclusion for the project. Professor Wagner mentioned the possibility of using female ragweed plants at known distances from the male ragweed plant plot and ascertaining how many became pollinated. The question was raised whether these female plants would be susceptible at this out-of-season time of the year.

Professor Hewson brought up a point which was raised at the conclusion of his presentation on February 4 at the Second Annual Research Planning Seminar of the Air Pollution Medical Program, held at the Taft Sanitary Engineering Center, Cincinnati. Dr. George R. Meneely, Professor of Medicine at the Vanderbilt University School of Medicine, indicated that pollen which landed on mucous membrane sent out tubes into this tissue, and asked about the possible allergic significance of this action. Our group discussed whether the pollen actually pushes out tubes and germinates or becomes active in human tissues. Most pollen of the Compositae family, when placed in sugar solutions of too great a concentration, will exude from its confines and undergo a loss of organization. A study in Norway did not seem to indicate that these "pollen tubes" were found in the mucous membrane. Further investigation on this point was recommended.

It was asked whether it is necessary to have 3000 plants in a clump or if 1000 ragweed plants in a larger plot could be used. It was mentioned that only about 80% of the plants germinate from the seeds used; these seeds are already prepicked and so there is a tremendous loss of plants. It was the concensus that we still needed as many ragweed plants as we could get since some slide counts even with 3000 plants were still very low. Mr. Harrington stated that they would like to test the three types of samplers in some wind-tunnel experiments. The overall costs of the gravity-slide method was reviewed since the labor is considerable in counting the slides; and since it was not thought that their counts were as informative as the volumetric sampling, it was wondered if they could be discontinued. However, although the gravity slides require more

work in placing the slides and counting the pollen, it is the only method we have at present for giving a good ground-distribution pattern. The possibility of studying the actual pollen on the plant leaf was again discussed, and it was asked if pollen distribution could be seen on leaves pressed between slides coated with petrolatum. It was stated that plant leaves could be pressed between plastic sheets (pressure- but not thermo-sensitive) for preservation. It was not known if one could then focus a microscope through these plastic sheets. Professor Wagner said that we needed to study smooth-versus-hairy-surface leaves and smooth-versus-spiny pollen grains.

It was decided not to eliminate the ground-level slides at the source because they were useful in rating the source quantity of ragweed pollen. Professor Cook wondered about the arrangement of the five masts; Dr. Dingle thought that one would be in the center of the source and the others probably spread out along the meteorologically predicted best wind direction. Dr. Mathews wondered about setting up the impinger and volumetric sampling devices at the same angle as found when a patient is breathing, instead of trying to maintain isokinetic conditions which probably would be unobtainable. Professor Cook again brought up the use of dog-head models with the mineral dust studies.

Chamber Calibration

Professor Akerman then talked about the status of the test chamber. He passed out blueprints of the test chamber as it is presently planned. He explained the modifications made because of the working space available and said that he was ready to turn the plans over to the University Plant Department for an estimate of the cost of construction.

Professor Hewson than appointed a sub-committee consisting of Akerman, Dingle, Hemphill, and McLean to work out calibration of the chamber.

Botanical Phase

The ragweed bibliography was discussed. It was decided that it would be best to have it published in a journal whose contents are listed in the Cumulative Index Medicus. Such journals are The University of Michigan Hospital Medical Bulletin, The Quarterly Review of Allergy and Applied Immunology, perhaps the Annals of Allergy, and, least likely, the Journal of Allergy. It was also agreed that scientific journals other than medical ones should be able to publish extracts from the bibliography. It was thought that there would probably be fifty pages of printed copy. After considerable discussion it was decided that Dr. Mathews would contact the U. of M. Hospital Medical Bulletin and the Quarterly Review of Allergy and Applied Immunology to see whether either of these journals would publish it. The meteorological group under Dr. Dingle and Mr. Ruffner have added about forty more items. It was finally decided that it would be best to list all the articles alphabetically; some type of symbol or

number is to be placed beside each item to identify its interest to a medical, botanical, or meteorological group. It was hoped that the bibliography would be ready by the next meeting and that it could be published soon while it was up-to-date.

Professor Hewson appointed a sub-committee on phenology consisting of Drs. Wagner and Dingle.

Mr. Beals wondered if the problem of gathering pollen for the chamber experiment had been fully discussed. It was decided that a commercial source for the pollen would be acceptable for the immediate future although for chamber studies it would certainly be well to know what year the pollen was collected, its condition, etc. The question was raised whether the pollen needed to be dried or defatted to maintain its antigenicity, and Dr. Patterson said that it definitely had to be dried.

Dr. Mathews then reported relevant matters from the recent meeting of the American Academy of Allergy. The State of Oregon has instituted a ragweed control program to be conducted on an area rather than on a city basis. Apparently the City of Portland had a drop in its ragweed count last year following such a program. Dr. Ogden, the botanist, is also working on a ragweed project. At the exhibits at the meeting it was noticed that there is now a commercial ionizer available, apparently based on the work done by the Philadelphia group previously discussed in these meetings. Also in Philadelphia, a plastic-type horizontal plate has been used as an impinger counting device for pollen counting.

MEETING OF 13 MARCH 1958

The following were present: Akerman, Baynton, Dingle, Giever, Harrington, Hemphill, Hewson, Jones, Mathews, Norman, Patterson, Sheldon, and Wagner.

Medical Phase

Dr. Patterson reported on studies on the neutralizing and eliciting activity of altered ragweed antigen. The object of the study was to find or produce a material from ragweed antigen which would have the properties of a hapten, capable of neutralizing reagin without the release of histamine. Studies have been reported suggesting that such a material might exist. A method for demonstration of a hapten in ragweed antigen was devised as a modification of the passive transfer neutralization method of pollen antigen assay. Ragweed antigen was altered by various methods including acid hydrolysis under different conditions of concentration and time, proteolytic digestion, freezing, and evaporation. An attempt to recover hapten, if it existed in the free state, was made by sinple dialysis.

Results showed that the method of testing for alteration of the neutralizing-eliciting ratio of an altered ragweed is feasible and suitable for experimentation along this line. No evidence of a haptenic effect was found. The ragweed antigen can be destroyed by acid hydrolysis, but this destruction can be controlled by limitation of concentration of acid and duration of hydrolysis. Proteolytic digestion destroyed activity under the conditions of the experiment. Repeated freezing resulted in destruction of the activity of the antigen, but both neutralizing and eliciting activity remained after repeated evaporation. Initial results with certain acid hydrolysates of the ragweed antigen were very encouraging as they showed marked neutralizing activity, as compared with the control, but no eliciting ability. Careful evaluation of these extracts showed that they had a low pH although the hydrochloric acid had been removed by evaporation. The neutralizing activity was then found to be a function of the acid concentration rather than the result of any included hapten. Additional experiments showed that reaginic serum is altered at a pH of 2 and there is no difference observed between the effect of acid concentration at pH 2 on post-treatment "blocking antibody" and reagin.

In the discussion, Dr. Hemphill asked what further studies would be indicated had haptenic activity been demonstrated, and whether there would be any use for a ragweed hapten. In reply, further studies suggested were chemical analysis of the hapten-containing material, animal experimentation, and consideration of methods of application to ragweed pollenosis. The practical applica-

tion of a hapten capable of neutralizing reagin without histamine release would be the possible therapeutic application to clinical ragweed sensitivity. It might theoretically be possible to desensitize completely a ragweed-sensitive patient with a hapten for periods of time.

Chamber Experiments

In the next discussion, Dr. Hemphill raised definite questions regarding the plan of experiments to be conducted in the human pollen test chamber. These questions concerned problems which might best be considered carefully prior to initiation of any experiments so that the results might more likely be statistically sound. The questions raised were in relation to the following:

1. The criteria for the selection of test subjects.
2. The length of recovery period after an allergic response.
3. Can the allergic reaction be repeated?
4. Is repetition of the allergic reaction detrimental to the patient?
5. After recovery from reaction, will the reaction reoccur in the chamber without the presence of pollen?
6. Signs and symptoms to be evaluated in the chamber.
7. What diagnostic tests are pertinent to the response in the chamber?
8. What environmental regimen is present in the chamber prior to the allergic response?

Dr. Hemphill then included suggestions to keep the information collected as unbiased and objective as possible. The primary measure would be a team of people to select subjects, attend subject, evaluate data, and operate the chamber with each team member doing these separate portions of the experiment according to a pre-arranged plan. Each team member would be as unfamiliar with the activities and findings of the others as possible within the confines of the experimental procedures.

Dr. Mathews commented on these suggestions and pointed out that it was planned that the largest portion of data obtained will be of an objective type. Major conclusions would be based on such determinations as the vital capacity, maximal breathing capacity, pneumotachograph studies, and maximal expiratory flow rate. Consequently, it is expected that bias of the attending physician will be a negligible factor since interpretation of subjective symptoms and signs by the physician will be a small part of the study. Also, it probably will not be advisable for the physician to enter the chamber when it is in use so that the pollen distribution will not be interrupted, making evaluation of signs and symptoms even less feasible. The effect of the chamber and other environmental factors on symptoms and findings prior to addition of pollen can be determined. A patient may be stabilized by being in the chamber several times to that re-use of the same patient is possible. It does seem advisable to have the attending physician as unaware of changes occurring in the chamber as reasonably possible.

Dr. Dingle commented that the chamber operator should have a complete pre-planned schedule of operations to follow, and some or all changes could possibly be made without observation by the attending physician. The patient should be calibrated as far as his responses to temperature and humidity are concerned.

Mr. Gill said that manual control of the temperature and humidity in the chamber using a hydrothermograph had been planned. These two variables could also be controlled by a Brown recorder and controller which would enable a pre-planned sequence of temperature and humidity changes to be conducted and record-ed in a repeatable cycle. Temperature variation with this mechanism would be only one degree from that planned. The cost of such appartus would be about three thousand dollars.

Dr. Hemphill concluded by suggesting that the whole regimen of the experi-ment, insofar as possible, be worked out prior to initiation of the work.

Botanical Phase

Professor Wagner next brought up progress on the ragweed bibliography. Dr. Mathews reported that The University of Michigan Medical Bulletin seemed disin-clined to publish the material but that the editor of The Quarterly Review of Allergy and Applied Immunology had advised that the bibliography would be ac-cepted for publication divided into two or three issues. Reprints would be available bound in one unit or possibly more than one unit.

Professor Wagner then made further comments on the botanical phase of the ragweed study including the general problem of pollen viability, the problem of compatability, and photosensitivity of ragweed strains. Ragweed is a short-day or a long-night plant. It blooms in August because days shorten and nights lengthen. Ragweed plants in various parts of the continent may have different photosensitive periods. The possibility of alteration or elimination of the blooming period of ragweed by nocturnal illumination directed at the plants was mentioned; adverse effects might result. Further investigation of the photo-period will be very interesting.

Professor Norman made further comments on the photoperiod of plants. Rag-weed is clearly a short-day plant with induction of flowering induced by dura-tion of darkness; cucumber is an example of a plant which is day-length neutral. A plant must be at a certain stage of development before the response to dura-tion of darkness occurs. Light or darkness requirements vary for different va-rieties of plants to produce flowering: one night of proper conditions induces flowering in the cocklebur; thirty nights are required for the chrysanthemum.

Professor Wagner thought that similar studies on ragweed plants might be suitable for a doctoral thesis.

Dr. Dingle made suggestions for the study of the phenology of ragweed

plants and thought that certain areas could be carefully studied for several successive years; characteristics of the plants and environment could be documented in these limited areas.

Professor Wagner commented that a problem would arise since ragweed is a two-year plant with an affinity for newly exposed surfaces. The new botanical gardens might be a suitable place for such a study as Dr. Dingle suggested.

Professor Hewson led a discussion of the annual progress report.

Dr. Sheldon stated that minutes of all meetings should be included, in addition to meteorological, botanical, statistical, and medical reports, on present work and plans for the future. Reprints of published papers should be included.

Professor Hewson commented that a statistical report should indicate that statistical analysis and guidance is available and is utilized by the group.

It was suggested that the minutes of all meetings be checked for errors and suitable corrections made prior to inclusion in the annual progress report.

The next meeting is planned for Thursday, April 24, in the Kresge Medical Research Building.