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ATMOSPHERIC POLLUTION BY AEROALLERGENS

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ABSTRACT

The research program undertaken represents a fundamental attack by specialists in allergy, botany, meteorology, and statistics on ragweed allergy, as a public health problem. The program is outlined briefly below.

The botanical phase has been concerned with the following topics: preseasonal pollination; pollen discharge in relation to meteorological factors; the structure of the pollen-producing flowers, including the tapetal fluid and its possible antigenicity; possible mechanisms of pollen discharge; the various kinds of ragweeds and their general characteristics; perennial ragweed and its distribution in Michigan; chromosomes; a new hybrid ragweed plant; the phenology of ragweed; experiments in the germination of pollen; the ultimate disposition of pollen after it has landed on soil and water surfaces; and plans for a comprehensive bibliography of published papers on ragweed.

The section on the medical phase begins with a description of clinical investigations at the State Prison of Southern Michigan at Jackson durigg the ragweed pollen seasons of 1955 and 1956. Detailed results for 1955, and a discussion of the 1956 program, are given. Presentation of more detailed results for the latter awaits analysis of IBM tabulations. Preliminary studies of the antigenicity of tapetal fluid are encouraging and further work is planned. Studies have also been started of the antigenicity of exine and intine portions of ragweed pollen. These studies will continue. Finally, the recovery of pollen from animal and human respiratory tissues is discussed.

The section on the meteorological phase opens with a chronology of activities for 1955 and 1956, and plans for 1957. The volumetric methods which have been developed to measure pollen concentration, and the meteorological instrumentation used, are described in detail. Specific dispersion experiments were conducted on the North Campus in late June of 1956. A preliminary analysis of these data is presented and discussed in terms of the dispersion models of Rombakis and Sutton as modified by Chamberlain. A Graeco-Latin experiment was designed to study the horizontal homogeniety of the pollen during the regular 1955 season. Additional studies of the horizontal and vertical distributions of pollen were conducted during the 1956 season, and preliminary analyses have subsequently been made. The design features of the pollen tests chamber are described in detail and theoretical studies of pollen removal from air by agglomeration and impaction are discussed briefly.

The statistical phase is not given separate treatment but incorporated in the other analyses.

The report concludes with a list of papers which have been or will

be presented and published and with acknowledgments to the staff of the State Prison of Southern Michigan at Jackson.

OBJECTIVE

The atmosphere may be considered to be contaminated by two groups of substances, natural and artificial, depending on their method of production and introduction into the air. Among natural contaminants are the aeroallergens, airborne 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 results 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 nature of the physiological reaction of sensitive individuals to it. The sections of the present investigation represent a fundamental attack on the problem in all its phases by specialists in botany, internal medicine, meteorology, and public health, all working in the closest cooperation.

1. BOTANICAL PHASE

by

W. H. Wagner, Jr.

1.1 INTRODUCTION

The botanists have focussed their attention primarily on the biological problems associated with ragweeds (Ambrosia). The botanical work has two important facets: (1) to assist and collaborate with the workers in the other phases in problems involving plants; and (2) to seek basic facts of a strictly botanical nature involving ragweeds. A number of problems, making collaboration of botanists desirable or even imperative, have already arisen in the researches of the meteorologists and the allergists. For example, in the study of pollen dispersal in the air, the botanists have been able to provide plant at times other than the normal season for field experiments to be described below. The botanists have similarly contributed ideas, information, and materials that directly or indirectly bear on the special problems arising in matters of pollen discharge, pollen structure, pollen development, and the general biology of the ragweed plant.

It has accordingly been desirable for the botanical group to investigate the plants themselves as separate entities to learn as much as possible about them. A number of pertinent observations have already been made on the relationships and distribution of the different types of ragweeds, on the structure of the flowers, on the mechanism of pollen discharge, and on the growth and periodicity of ragweeds. There is also in preparation at this time a comprehensive bibliography of the literature bearing on the biological aspects of ragweeds. The following report will deal with these aspects of botanical research, especially the studies of perennial ragweeds, and also with some of the contemplated future experimental and descriptive work.

1.2 EXTRA-SEASON EXPERIMENTS

To carry out more precise investigations on abundance and behavior of pollen grains in the atmosphere with relation to such meteorological factors as wind direction, wind velocity, turbulence, and time of day, it was decided to set up controlled pollen sources at times when no pollen of ragweed was present in the atmosphere. The botanists attended to the problem of obtaining flowering ragweeds at a proper time. They collected a large stock of seeds (technically, "achenes") in the fall of 1955. These were stored at 5°C in enclosed glass containers. For the first extra-seasonal season experiment, seeds were removed from storage on March 25, 1956. They were then soaked in water for 24 hours to insure uniform germination. The seeds and seedlings were planted in soil in flats in the greenhouses of The University of Michigan's Botanical Gardens. The developing plants were then grown under ideal vegetative conditions until they had reached a reasonably large size, and on May 28 they were transferred to pots and placed in a cold-frame out of doors.

The ragweeds are described as "short-day plants" and they will not flower during the latter half of summer until the day's light period has reached the proper duration. Accordingly, exaggeratedly short days of only seven hours' duration were produced by covering the cold frames with opaque dark cloth at the proper times. By June 6, numerous embryonic flower spikes were evident. By June 12, the young flowers had become conspicuous; some of the spikes reached an inch in length. Warning was given at this time to the meteorologists that the plants would soon be ready for field experimental work, and preparations were then made by them in collaboration with the botanists to accommodate the plants in the field. On June 20, most of the ragweed plants were in full bloom, some 56 days ahead of the normal time in this area. On this date, 136 of the best developed, flowering specimens were removed to the experimental area in an open field on the North Campus of the University. The plants, still rooted in their individual pots, were placed in four square soil-filled flats, and were watered and otherwise tended as necessary during the succeeding days of the tests of pollen dispersal in the atmosphere.

The results of this study in terms of the meteorological correlations will be discussed elsewhere in this report. Suffice it to say here that the method of preparation of extra-seasonal flowering ragweeds is a successful one, and the approach to problems of pollen dispersal seems to be a good one. A larger and more refined experiment based on the 1956 work is now contemplated for June, 1957, involving several thousand plants brought to flower two months out of season.

1.3 POLLEN DISCHARGE

The question of effects of various meteorological factors on dispersal of pollens is of primary concern in our work. The meteorologists have dealt especially with the behavior of the grains in the air, after release from the ragweed plants. The botanists, on the other hand, have been chiefly interested in the biological processes involved in pollen production and pollen release. Present observations indicate that the flower of ragweeds is a highly specialized mechanism which is capable of ejecting the pollen forcibly. Furthermore, the pollen apparently does not scatter at first, but instead is sent out of the flower in a mass which seems to be able to adhere to the surfaces of other, nearby plants. The studies of the flower and of the actual process of pollen discharge, then, have become especially fruitful aspects of our research, and will be described briefly below.

1.4 FLOWER STRUCTURE

The ragweeds are in the small minority of their family, the Compositae, which depend on wind for pollination. Most of the Compositae are insect-pollinated. The ragweeds have developed, therefore, certain fundamental differences in floral structure related to their dependence on wind for pollen distribution. The flowers are sharply differentiated into two kinds—the male or pollen-producing flowers, and the female or seed-producing (usually called "staminate" and "pistillate" flowers, respectively). The male flowers are tiny objects, much smaller than average for the family Compositae, and they produce much more pollen, relatively, per flower. The female flowers are drastically modified from the basic composite prototype, possessing much enlarged surfaces for catching the pollen grains brought by the wind; and they have developed other unique characters in connection with heavily walled and resistant seeds. Our attention thus far has been devoted mainly to the structure of the male or pollen-producing flowers.

The pollen flowers are borne in clusters in cup-like "involucres" arranged along a vertical axis or spike. To study the structure of the flower fully, it is necessary to section the spikes upon which they are borne and to prepare carefully stained microscope slides. In addition, to understand the parts it is essential to observe the embryonic development of the floral organs so that they can be correlated and interpreted. Consequently, numerous sections of all stages of embryonic formation were prepared during 1956 by the dehydration technique, embedding the specimens in paraffin and cutting sections in the range of 10- to 20-micron thickness. These sections were stained in safranin and fast green after being mounted serially on microscope slides. The slide collection is now quite extensive, and it is planned to prepare a detailed botanical report on the ontogeny and mature structure of the flowers.

The flowers that produce the pollen are basically small tubes open at the apex, about 2 millimeters in length at maturity. The tube is actually composed of five petals fused side by side. It encloses five anthers or pollen sacs which are arranged in a circle, and the anthers, in turn, surround a central structure called the "Pistillodium." The latter, a columnar body with a wheel-shaped apex, is part of a vestigial pistil (the female part of the flower in more primitive types), but it is almost undoubtedly a specialized functional unit connected with pollen discharge. In the insect-pollinated flowers of the same family,the pistil grows up between the mature anthers, pushing out the pollen grains as it grows; in the ragweed flower, on the contrary, the corresponding structure or pistillodium apparently has approximately the same length as the anthers at all stages and must function, therefore, in some other way. The current hypothesis held by our group is that the pistillodium in some way helps to build up pressure in the flower, associated finally with sudden and forcible ejection of the pollen.

The pollen grains begin their formation when the embryonic flower is very small, one-third or less the mature size. They develop inside the young pollen sacs by cell divisions, and they come to lie in a matrix called the tapetal fluid. As will be described shortly, the pollen grains do not seem to scatter immediately upon release, which tends to suggest that the tapetal fluid may contribute more or less to the adherence of the grains in clusters as they emerge. This is a point on which we particularly need data. The possibility exists, furthermore, that the tapetal fluid (which is generally considered by plant anatomists to represent a nutritive material for developing pollen grains) may contain an active principle in the production of ragweed hay fever, and the medical group has become interested in testing this possibility.

1.5 OBSERVATIONS ON DISCHARGE

Our current knowledge of pollen discharge in ragweeds is based largely on general observations of plants grown artificially in the greenhouse. One of the most striking facts, which we intend to examine in much greater detail, concerning the process is that the grains are found in bright yellow patches on the foliage of the same and nearby plants. We found some evidence that these clumps of grains may be ejected up to two feet, perhaps more, from the pollen-producing plant. This immediately suggests that a mechanism may be present which forcibly ejects the pollen grains. There must also be something present which prevents the pollen from flying apart as it is ejected, so that the grains remain together. As proposed above, it may be that the grains are actually "sticky" because of residual tapetal fluid or moist material on the surfaces of the pollen grain walls. It could also be due to the slightly spiny surfaces of the grains, which might hold them together in their passage in the air before landing on another surface. Another question is whether all the grains from one flower (i.e., all the grains from all five anthers) are ejected at once. It seems most likely that this will prove to be the case, although precise observations will have to be made and conditions will have to be closely controlled.

One of our problems in making observations is that the pollen seems to be discharged very early in the morning. The process may even occur before dawn. We have not yet ascertained the exact time, but the observations from our field extra-seasonal experiment indicate that pollen is more abundant in the air in the early hours of the day Also, if the process depends at all on building up water pressure in the tissues of the flower, the moist, still air of early morning may provide the ideal surround

The important point here is that we are now inclined to visualize the process of pollen dispersal as divided into four distinct phases: (1) the ejection of pollen from the flowers; (2) the temporary attachment of pollen clumps to neighboring foliage or vegetation; (3) the later reflotation of the pollen grains from vegetation by atmospheric currents; and (4) the final distribution of the grains in air masses.

1.6 THE KINDS OF RAGWEEDS

All the species and varieties of ragweeds are evidently allergenic. The differences in their importance to the allergy problem are due to inherent peculiarities of the different kinds—their periodicity, abundance, and habitats. For example, if a given species is relatively rare, it may have no general importance; on the other hand, if the uncommon species comes into flower at a different time from the common one, it can potentially have a distinctive importance locally in connection with its characteristic periodicity. As a group of plants, the ragweeds provide a rather complex system of varieties and forms which are not fully understood from the standpoint of their inter-relationships and distribution.

A study of the different kinds of ragweeds was begun in the summer of 1956, inspired in part because it was not clear from the literature whether the perennial species was native in Michigan or whether it had spread from further west into the state in recent times. The rather similar "low ragweed" or "common ragweed," Ambrosia artemisiifolia is an annual, and it includes an especially bewildering array of forms and variations. The other species, though less variable, also are problematic and possess a number of forms, some of which are due to environmental growth influences and others of which depend on fundamental hereditary differences. Plants of any of the ragweeds found growing in unusually dry and exposed habitats are much smaller and narrower than those of damp and shaded places.

The species studied by our group thus far are primarily those of the Great Lakes area—Ambrosia artimisiifolia, low or common ragweed; A. psilostachya (eastern variety), the western or perennial ragweed; and A. trifida, the tall or giant ragweed. The rare and peculiar hybrid ragweed, A. X helenae (A. artemisiifolia X trifida) has been observed in the field, but is far too sporadic in occurrence to have any importance. Our attention during the 1956 growing season was centered in large part on Michigan populations of the perennial ragweed, which, as discussed further below, resembles in many respects the common ragweed but lives for an indefinite period of years, overwintering from year to year by means of an underground stem or rhizome which is completely absent in other ragweeds. The common ragweed and the giant ragweed must replenish themselves each year by seeds and new germination each spring.

The results of our present observations suggest that a thorough monograph on the ragweed genus Ambrosia in North America, which would trace the species and varieties through their geographical ranges and their evolution and inter-relationships, would be a valuable contribution to the basic botany of these important allergenic plants.

1.7 PERENNIAL RAGWEED IN MICHIGAN

Michigan is a key state for the study of the eastward spread of perennial ragweed. Since this state is on the easternmost edge of the presumed original range of the variety, it was considered possible that the populations became naturalized here from further west. Although one authority (Gleason, 1952) treated Ambrosia psilostachya as a single species unit, another authority (Fernald, 1950) considered the eastern plants as a distinct variety (var. coronopifolia) from the western plants (var. psilostachya). Some authors have even considered the two varieties as separate species. The far western variety had earlier been reported to have over 100 chromosomes (Heiser, 1952) in contrast to the common, annual species with only 36 (Jones, 1943). The eastern variety (coronopifolia) had never been examined with respect to its chromosomes. The perennial ragweed is reported in the allergy literature to have much larger pollen grains than the common ragweed, but no one has apparently attempted to determine whether the two varieties of perennial ragweed differ in their pollen sizes.

Little has been published on the distribution, ecology, and abundance of the perennial ragweed in Michigan; it was reported to be "rare or absent" in the Upper Peninsula and "found occasionally to frequency" in the Lower Peninsula. A field survey was made in August, 1956, to cover eight counties in the Lower Peninsula; in addition, laboratory and herbarium studies were undertaken to find more evidence to bear on whether the eastern variety of A. psilostachya was native or introduced in the state, whether it forms hybrids with its annual relative, A. artemisiifolia, and to what extent its chromosome complement and pollen grains differ from those of the typical variety of A. psilostachya of the west.

Because of its underground stems, A. psilostachya var. coronopifolia, can, over a period of years, form extensive populations or clones; these may cover as much as an acre or more when conditions permit. The common ragweed, which grows in similar habitats (primarily disturbed, open places), is unable to make successful invasions into densely grassy fields. The perennial ragweed, however, can invade fields and even mowed lawns where its shoots are cut repeatedly to the level of the grass. Of Michigan collections on which habitat data were recorded, approximately one-sixth specify that the habitat was along railroads, and one-third state "along roads" or "along highways." Some two-thirds of the collections have come from in and around cities, villages, and settlements. Perennial ragweed has the capacity to become a locally serious weed in yards and gardens (for example, in the area of the Interlochen R.R. Station and the outskirts of Frederick and Gaylord, Michigan), and it could produce a local hayfever problem. The soil in which it grows is dry, well drained, and commonly sandy or gravelly. It is found in such places as cleared ground, along open trails, baseball diamonds, railroad embankments, dumps, and waste ground.

As stated earlier, there is a question whether A. psilostachya was originally native in Michigan or not. One authority (Fernald, 1950) considered it indigenous in Michigan, and another (Gleason, 1952) apparently considered it introduced. Present evidence favors the latter interpretation. Correlations made during 1956 show that the first definite record of this species in Michigan was in 1900, and there are apparently no prior collections. The herbaria of The University of Michigan, New York Botanical Gardens, U.S. National Museum, Michigan State University, Cranbrook Institute of Science, University of Wisconsin, University of Illinois, and Chicago Natural History Museum were checked. The species is now known from thirty counties in Michigan. For both of the native annual species of ragweeds (A. artemisiifolia and A. trifida), there are much earlier records for the state, going back to as early as the 1830's. Michigan was reasonably well explored botanically prior to 1900, and it is difficult to imagine that the perennial ragweed was overlooked. Also, the botanist C. K. Dodge, who was a very active observer and collector of Michigan flora from about 1880 to 1920, wrote several times that this was a "weed in waste places ... becoming frequent." Thus the conclusion is that perennial ragweed has spread into Michigan in that last half century from further west.

It has already been reported in the literature that A. psilostachya pollens precede those of A. artemisiifolia by two weeks. Our own observations confirmed this, and showed specifically that the plants of the former are on the average approximately half a month ahead of the latter developmentally. The earliest record of flowering in A. psilostachya in herbarium records is July 12, nearly a month ahead of the average for A. artemisiifolia. The perennial ragweed may therefore cause all ragweed hay fever in certain areas of Michigan during the several weeks preceding the blooming of the other species.

1.8 CHROMOSOMES AND POLLEN

Chromosomes studies of the eastern variety of the perennial ragweed revealed that the number was $2n = 72$, in contrast to the earlier reports of the western variety ($2n$ = ca. 104, probably 108), and the common ragweed ($2n = 36$). Thus the three different varieties apparently represent three different polyploid levels—diploid ($2x$), tetrapoloid, $4x$, and hexaploid ($6x$)—based on the number $x = 18$. The difference in chromosome number indicates that the eastern variety of the perennial ragweed is probably genetically isolated from the western variety, and that their hybrids would in all likelihood be sterile. Chromosomes are shown in Fig. 1-1.

The pollen grains of the eastern variety of the perennial ragweed

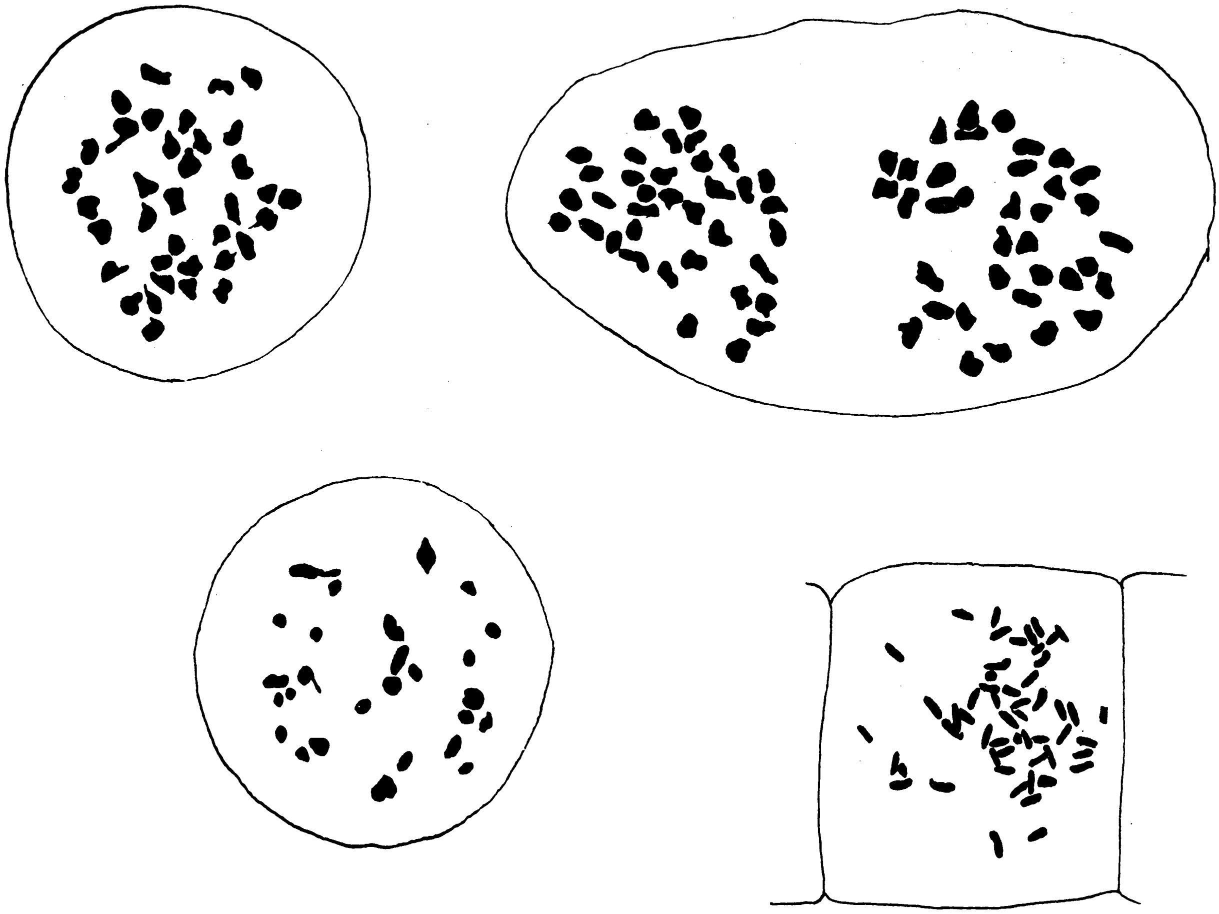


Fig. 1-1. Chromosomes of ragweeds. Upper left: Ambrosia psilostachya var. coronopifolia, first meiotic metaphase showing n = 36 pairs. Upper right: The same, second meiotic metaphase showing n = 36 (both figures from plants collected in Benzie Co., Mich.). Lower left: A. artemisiifolia X psilostachya var. coronopifolia, first meiotic metaphase showing irregularity (plant collected in Benzie Co., Mich.). Lower right: The same, somatic division of root tip showing 2n = 54 (plant collected in Crawford Co., Mich.).

turned out to average larger than those of the common ragweed, but smaller than those of the western variety of the perennial ragweed, as shown in Table 1-1. Ambrosia psilostachya var. coronopifolia has an average diameter of approximately 20.5 microns; A. psilostachya var. psiolostachya of the West, 23.0 microns; and A. artemisiifolia, 18.0 microns.

1.9 PERENNIAL HYBRID RAGWEED

While studying mixed populations of the eastern variety of perennial ragweed and common ragweed, several colonies of plants were discovered which seemed to combine their characters. The smallest of the putative hybrid clones covered only a couple of square feet along a roadside, but the largest extended

Table 1-1. 20-spore samples measured in microns. All specimens from Michigan, unless otherwise indicated.

	Average	Range	Standard Deviation
Ambrosia artemisiifolia, 2n = 36.			
Tecumseh, Lenawee Co., Wagner 8350a (MICH)	17.9	17.9-19.6	0.94
Ann Arbor, Washtenaw Co., Wagner 8351 (MICH)	19.7	18.5-20.8	0.67
Willis, Washtenaw Co., Wagner 8354b (MICH)	18.8	17.3-20.2	0.74
Willis, Washtenaw Co., Wagner 8354a (MICH)	18.8	16.6-20.2	0.89
Frederick, Crawford Co., Wagner s.n. (MICH)	17.4	16.7-19.0	0.68
Ann Arbor, Washtenaw Co., Wagner s.n. (MICH)	18.7	17.9-19.6	0.58
Beaumont, Texas, Tharp s.n. (MICH)	17.3	15.5-19.0	0.93
Polk Co., Florida, McFarlin 3214 (MICH)	16.3	14.3-17.9	0.93
Dade Co., Small et al. (MICH)	17.9	16.7-19.6	0.81
A. artemisiifolia X psilostachya var. coronopifolia, 2n = 54.			
Benzie Co., Wagner 8335 (MICH)	21.5	17.9-25.0	2.65
Benzie Co., Wagner 8335 (MICH)	21.3	17.9-25.0	2.20
Crawford Co., Wagner 8348 (MICH)	20.9	17.9-26.2	1.97
Crawford Co., Wagner 8348 (MICH)	20.2	18.4-22.6	1.42
Crawford Co., Wagner 8348 (MICH)	20.2	17.8-23.8	1.42
A. psilostachya, incl. var. coronopifolia, 2n = 72.			
Grand Traverse Co., Wagner 8336 (MICH)	19.9	17.6-21.4	0.96
Wexford Co., Wagner 8330 (MICH)	20.1	19.0-22.0	0.94
Kalkaska Co., Wagner 8337 (MICH)	20.3	19.0-21.4	0.72
Otsego Co., Wagner 8344 (MICH)	20.1	19.0-21.4	0.77
Ironwood, Gogebic Co., H.I.D. (MSC)	20.6	18.4-21.4	0.84
Newaygo Co., Mich., McVaugh 9848 (MICH)	22.2	20.2-23.8	1.09
E. of Riply, Houghton Co., Richards 2731 (MICH)	20.8	19.0-23.8	1.21
Calumet, Houghton Co., Hermann 798 (MICH)	21.5	19.0-23.5	1.32
Baraga, Baraga Co., Richards 4342 (MICH)	20.7	17.9-23.8	1.35
Port Huron, St. Clair Co., C. K. Dodge (MICH)	20.6	19.1-22.6	1.05
Port Edward, Lampton Co., Ont., C. K. Dodge (MICH)	21.4	20.8-25.0	1.37
San Diego Co., California, Palmer 161 (UC)	20.2	18.5-22.0	0.92
Lathrop, California Walker 889 (UC)	21.8	19.0-23.3	1.29
Yuba Co., California, Howell 28288 (UC)	20.5	19.0-22.6	1.14
A. psilostachya var. psilostachya, 2n = ca. 100-104.			
Solano Co., California, Heiser 1966 (UC)	23.3	20.2-25.0	1.19
San Diego Co., California, Alderson s.n. (MICH)	23.1	20.8-25.0	1.37
Colusa Co., California, Chandler s.n. (UC)	23.0	21.5-25.0	1.04
Stanislaus Co., California, Hoover 165 (UC)	22.4	20.0-25.0	1.75
Los Angeles Co., California, Wolf 4241 (UC)	23.3	22.1-25.0	0.84
San Luis Obispo Co., California, Summers s.n. (UC)	22.7	21.5-25.6	1.02

over an area of some 20 square feet and had probably been growing there for at least several years. As stated by Gleason 1952), A. psilostachya is "similar to" A. artemisiifolia. The resemblance between these two kinds of ragweeds is now emphasized by the study of their apparent hybrid. A. psilostachya differs from A. artemisiifolia in the following gross respects: (a) perennial rather than annual habit; (b) anthesis beginning from two to three weeks earlier; (c) leaves averaging slightly thicker in texture, the petioles shorter, blade outline narrower, and divisions fewer; (d) hairiness generally denser, the hairs larger and stiffer; and (e) the fruiting structure tending to be unarmed or with only blunt tubercles rather than short spines. However, apparently the species overlap in each of these characteristics. For example, Fernald (1950) gives A. psilostachya as also being "annual." Some of the forms of A. artemisiifolia (e.g., forma villosa) are as hairy as extreme individuals of A. psilostachya. In Fig. 1-2, approximately 10 leaves from each of the putative parents were traced, and a similar collection of leaves from the hybrid, to show the variation present. The most distinguishing characteristic of these leaves is the relative width of the narrow wing between the bottom pair of leaflets and the pair above it: in A. psilostachya the average width of the narrowest point on the wing is 3.1 millimeters; in the hybrid, 2.4 millimeters and in A. artemisiifolia, 1.4 millimeters. Specimens of the hybrid are illustrated in the photograph in Fig. 1-3.

The chromosomes of the hybrid revealed interesting facts. At the time of spore production, the division of the chromosomes is very irregular, as would be expected in a sterile hybrid. The ordinary cell divisions showed, however, that the chromosome number (in stained root-tip preparations) is intermediate between the parents, i.e., $2n = 54$, combining 36 from the perennial plant and 18 from the annual plant. The hybrids have inherited the perennial characteristic of the perennial parent, and could therefore be transplanted to The University of Michigan's Botanical Gardens for future studies in 1957 and following years. Overwintering plants of the hybrid were brought out early (February, 1957) and found to grow normally from the underground rhizomes.

One of the curious facts which still need investigation with respect to the new hybrid ragweed is that, in spite of the great irregularity of the nuclear divisions leading to spore formation, the pollen grains themselves appear (superficially, at least) to be perfectly normal. One would expect a high degree of abortive and distorted grains, but such is not the case. This problem is now being considered in some detail, and it is hoped that a completed report on the cytology of pollen formation in the hybrid can be prepared after further studies.

1.10 ANNUAL PHENOLOGY OF RAGWEED

A phenological comparison of ragweed over a period of successive years is contemplated to answer the question "To what extent does ragweed vary

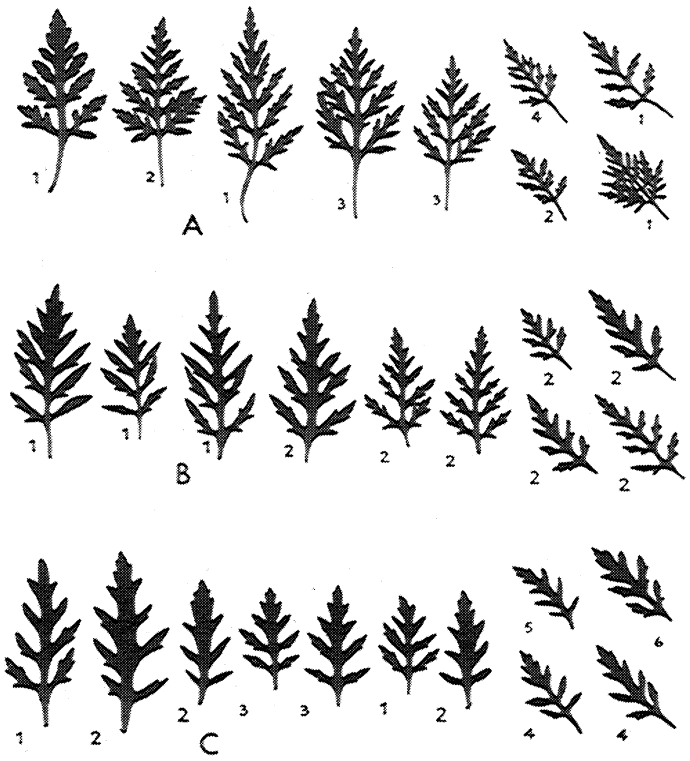


Fig. 1-2. Outlines of approximately median leaves (moderate-sized plants) from Michigan of various ragweeds. (A) Ambrosia artemisiifolia: (1) Washtenaw Co.; (2) Otsego Co.; (3) Benzie Co.; (4) Crawford Co. (B) A. artemisiifolia X psilostachya var. coronopifolia: (1) Crawford Co.; (2) Benzie Co. (C) A. psilostachya var. coronopifolia: (1) Benzie Co.; (2) Grand Traverse Co.; (3) Leelanau Co.; (4) Antrim Co.; (5) Otsego Co.; (6) Kalkaska Co.

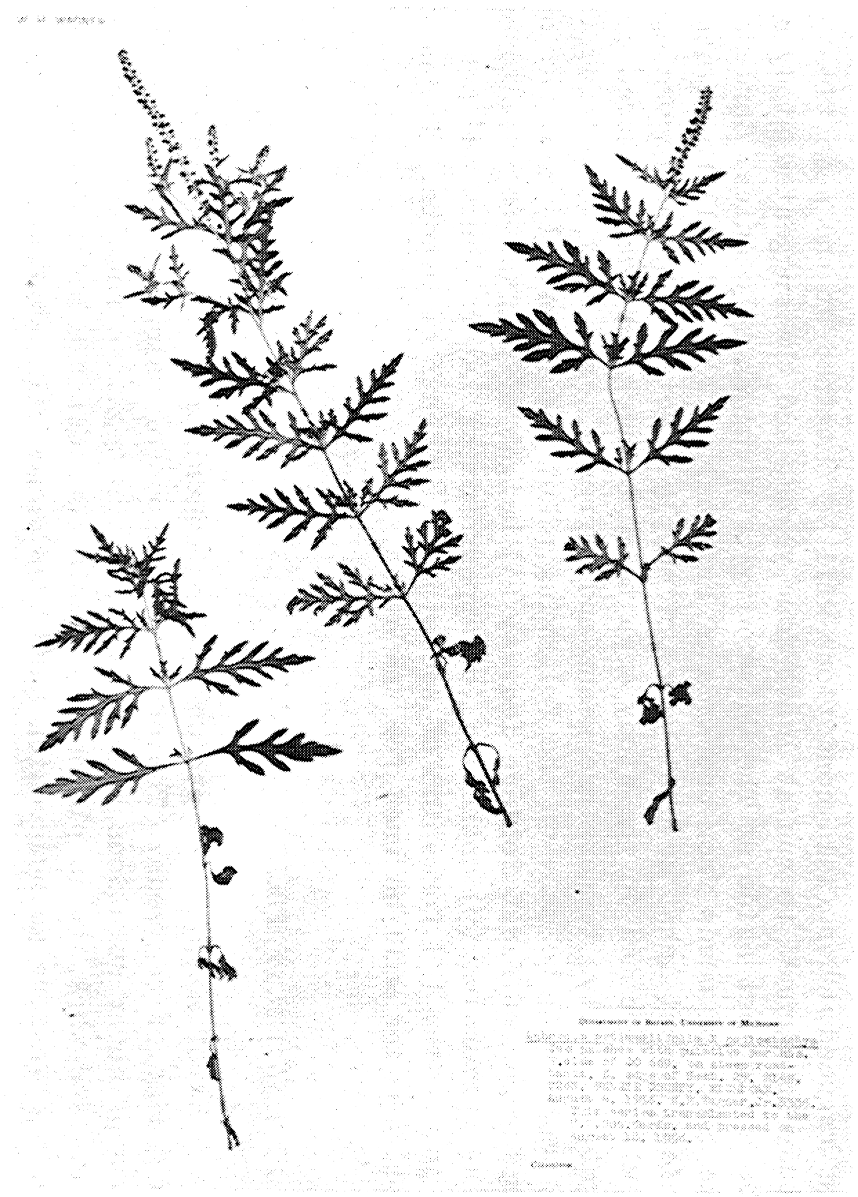


Fig. 1-3. Perennial hybrid ragweed, Ambrosia artemisiifolia X psilostachya. Benzie Co., Michigan.

in its yearly growth and pollen production in relation to annual weather conditions?" It is expected that precise data on the normal course of development of ragweed under field conditions will be obtained at the same time. The study is planned to run at least four years, and the suggested study area will be a plot set aside at The University of Michigan's Botanical Gardens. A large stock of seeds from one area of original collection will be maintained, sufficient for at least several years of supply. Meteorological recording devices will be located in the study area to make checks on such features as sunlight, humidity, temperature means and extremes, and wind velocities. Weekly notes will be made on the following plant reactions: (1) germination; (2) appearance of first foliage leaves; (3) height of plants; (4) development of spike primordia; (5) anthesis; (6) appearance of lateral buds and new spike primordia; (7) formation of seeds; and (8) death of plants. The common (annual) species will be used for the study.

1.11 GERMINATION OF POLLEN

Experimental attempts carried out thus far have failed to germinate pollen grains of ragweeds. The ordinary methods of using different concentrations of sugar solutions have thus far given negative results. During this summer's work, it is planned to devote considerable attention to this matter, and various methods which have been especially adapted for other species of plants will be tried. One of the botanists who will participate during the coming season is especially competent in microtechnical research and he will endeavor to make a systematic attack on this problem.

1.12 FATE OF WIND-BORNE POLLEN

A repeated query at the meetings of the Aeroallergen Research Group has been "What becomes of the millions of wind-borne pollen grains which settle to the surface of the earth?" Why are the grains not accumulated on the surface of the soil? Are they destroyed in some manner? By chemical action? By biolgical action? One may similarly ask what becomes of the pollen which falls on the surfaces of bodies of water. The problem may be essentially the same in both cases. By coincidence, it was learned that one of the doctoral candidates in the Department of Botany, Mr. Solomon Goldstein, is contemplating an investigation of the action of microorganisms, especially fungi, in the destruction of pollen grains in nature. He was invited to attend our meetings, and it is now planned by the Aeroallergen Project to support his research for this summer. We expect that considerable new information will result from Mr. Goldstein's investigations, bearing on the problems of pollen destruction on water and on land. Already there is good evidence from his trial experiments that the fungi are highly active in this respect. For this reason, and also because he will undoubtedly learn much with the microscope concerning the disappearance of the living contents of the pollen grains and the durability of pollen grains walls, Mr. Goldstein's research seems most pertinent to the broad and fundamental approach we are endeavoring to take.

1.13 BIBLIOGRAPHY ON BIOLOGY OF RAGWEED

Not only as background to our own researches, but also as a publication of potential value to other workers elsewhere, we are in the process of compiling a large bibliography on the biology of ragweed. This will include all aspects of ragweed biology, including the botany of the plant and its allergenic effects. It will also include references not specifically devoted to ragweed but which bear on the biological problems of these plants more or less directly. The references will be classified under the various headings determined by the several groups in the Aeroallergen Project. For each reference a brief annotation will be given, summarizing in a sentence or a paragraph the contents of the article, as necessary. Examples of references are given below:

Chamberlain, J. S. 1891. A comparative study of the styles of the Compositae. B. T. B. C. 18: 175-186, 199.

The function of the style is "to push up through the anther tube and the brush hairs brush out and collect the pollen as the anthers dehisce" and this is described generally for the whole family. In Xanthium, which is anemophilous, the brush hairs are not needed, and consequently they are more or less abortive. The variation in styles of the Helianthoideae is described and classified into groups. Iva, Xanthium, Berlandiera, Polymnia, and Silphium, are grouped together. No mention is made of Ambrosia.

Cocke, Elton C. 1938. Atmospheric pollen survey of Charlottesville, Virginia, for 1936. Jour. Elisha Mitchell Sci. Soc. 54 (1): 143-153.

Ragweed began to shed its pollen on August 12 and continued until October.

Eagler, Harry, Carl E. Arbesman, and Walter Windenwerder, 1939. The production in experimental animals of antibodies to short-ragweed pollen (precipitation, complement-fixation, and anaphylaxis), Jour. Immunol. 36 (5): 425-434.

Rabbits injected subcutaneously, intraperitoneally, intradermally, or intravenously, with a suspension of ground short-ragweed pollen, with saline extracts of ground pollen, or with buffered extracts of defatted whole pollen, regularly developed preciptins and complement-fixing antibodies in high titre.

At this time, well over 500 references have been collected and annoted from the various botanical journals and elsewhere in biological literature, and these are being typed and organized alphabetically. The final work will be published as a report of the project, and will be made generally available.

1.14 SUMMARY OF THE BOTANICAL PHASE

The botanists serve partly as assistants and collaborators to other members of the Aeroallergen Project. More strictly in the realm of botany are several problems involving plants of ragweed to which the group has assigned itself, as follows:

1.14.1. For experimental field studies of pollen dispersal, the botanists have utilized techniques for mass production of ragweeds in flower at times when these plants do not normally flower.

1.14.2. The peculiar form of the flowers of ragweed is being studied because of its association with the mechanism of pollen release. Clear understanding of the embryonic development and mature structure should enable us to interpret the release process.

1.14.3. The tapetal fluid (believed to be a nutritive matrix in which pollen grains form) may bear also on pollen release, especially in view of the apparent tendency of the grains not to scatter immediately.

1.14.4. The pollen-discharge process in ragweed evidently involves forcible ejection of clumps of pollen which adhere to nearby vegetation. Thus the process of dispersal may not be simple; tentatively we consider four steps to be involved—pollen ejection, temporary attachment to vegation, reflotation, and air-mass distribution. All four need investigation.

1.14.5. The kinds of ragweeds are important in ragweed allergy according to their abundance and periodicity. The different kinds are not fully understood in terms of their inter-relationships and geographical distribution. A thorough monograph on the ragweed genus Ambrosia in North America would be a valuable contribution.

1.14.6. Our attention has been focussed on the eastern variety of perennial ragweed (_Ambrosia psilostachya_ var. _coronopifolia_) in Michigan. Field, herbarium, and laboratory studies reveal that the variety was probably introduced in the state from further west over the last half century; that the perennial ragweed can potentially form a local allergy problem because it becomes abundant in certain places, and flowers several weeks before the common ragweed (_A. artemisiifolia_); and that the chromosomes and the pollen of the eastern variety of the perennial ragweed differ not only from the western variety of the same species, var. _psilostachya_, but from the common ragweed as well.

1.14.7. A new perennial hybrid ragweed, discovered in 1956, combines the characteristics of two species, common ragweed and perennial. Its chromosomes behave irregularly in pollen production, but apparently the pollen grains are nevertheless regularly formed, and the pollen development in the hybrid plant needs further study.

1.14.8. A several-year phenological experiment is planned to answer the question "To what extent does ragweed vary in its yearly growth and pollen production in relation to annual weather conditions?" This will entail uniform growth conditions for the plants over successive years and periodic observations at The University of Michigan's Botanical Gardens.

1.14.9. Ragweed pollen has not been successfully germinated, and the botanists will endeavor to discover proper techniques for accomplishing this.

1.14.10. To determine what becomes of the great excess of wind-borne pollen that falls on the ground and on the water, a member of the Botany Group is carrying out experimental researches (a doctoral thesis).

1.14.11. An annotated biological bibliography on ragweeds, to contain all pertinent references to the subject, is in preparation. It is hoped that it will eventually be published as a separate contribution from the project.

2. MEDICAL PHASE

by

J.M. Sheldon, K.P. Mathews, F.M. Hemphill,
R. Lewis, J.E. Goodwin, N.K. Cohen, and J.A. McLean

2.1 CLINICAL STUDIES

The medical aspect of the study of atmospheric pollution by aeroallergens has progressed along four lines. The first concerns the longitudinal study of hay fever and asthma caused solely by ragweed sensitivity. This work has been primarily clinical to date and has had a dual purpose: 1) to understand better the relationships between pollen counts and the patient's specific allergic disorder (i.e., to correlate allergic status with specific fractional volumetric pollen counts); and 2) to evaluate more specific studies to be done on human subjects in "chamber" experiments.

The studies conducted during the 1955 ragweed season are described in Section 2.1.1.

2.1.1 Clinical studies in 1955 (Clifford W. Gurney, M.D. and Sydenham Cryst, M.D.)

2.1.1.1 Introduction.—Many of the basic problems in allergic disease are unsolved. The existence of a dose-response relationship to aeroallergens is not established. Investigation is hampered by failure to produce in experimental animals an allergic disease akin to the extrinsic airborne protein sensitization in man. Study of the course of allergic rhinitis and bronchial asthma in man is complicated by variability of environment and a myriad of factors such as diet, fatigue, emotions, temperature, and humidity which contribute to the "total allergic load"[1] but are not defined quantitatively. In addition, the laws governing pollen distribution are unknown. A five-year study at The University of Michigan by physicians, botanists, meteorologists, and engineers is underway in an effort to contribute to a better understanding of some of the factors involved in allergic responses to airborne allergens. The initial clinical phase of this investigation is the subject of this report.

In a previous investigation into the course of pollenosis[2], inability to control the environment of subjects was thought to contribute to the inconclusiveness of the results. To achieve better environmental control, and at the same time to observe the patients more frequently, this study was pursued during August and September, 1955, at the State Prison of Southern Michigan. Thirteen inmates with ragweed sensitivity of clinical significance were observed and examined daily. The availability of the subjects and the similarity of environment of these individuals, confined in one small area, eating the same food, and rigorously adhering to similar schedules of activity each day, afforded opportunity to follow the course of allergic disease more closely than is usually possible. Further, none of the subjects was receiving hyposensitization, and the manifestations of allergic disease were, therefore, not masked by the presence of artificially induced blocking antibodies.

2.1.1.2 Methods.—From volunteer inmates with allergic rhinitis or bronchial asthma, a group of individuals who experienced symptons primarily or exclusively in August and September was selected. Only those with a positive skin test to ragweed antigen were considered. The final selection of subjects was made after complete allergic and general medical histories had been obtained and after thorough physical examinations, inspection of prison health records and x-rays, and skin testing to extract of mixed fungus, house dust, and pollen of mixed weeds, mixed grasses, and mixed trees[3], and on consideration of all information obtained. Of particular importance was evidence of rhinitis or asthma during August and September of previous years as recorded on the prison health records.

After the subjects were selected, a schedule was arranged whereby each would be seen at the same time each day. Table 2.1.1.1 shows the pertinent data. At each daily visit, vital signs were noted, the conjunctivae, nasal membranes, pharynx, and chest were examined, and the symptoms experienced over the previous 24 hours were reviewed with each subject. These symptoms were recorded on a card carried by each subject and exchanged for a new card at each examination. An index of symptoms was calculated for each subject and collectively for the group as previously described[2]. One-second and total vital capacities were determined, employing a Gaensler-Collins Vitalometer. In addition to these daily data, periodic eosinophil counts were obtained. Daily gravity counts were determined for ragweed pollen[4], the sampler being located on the roof of a high building within the prison walls, and not far from the center of activity. A volumetric apparatus was also employed to obtain the concentration of ragweed pollen six times in each 24-hour period. The construction of this apparatus and the method of expressing results has been described in detail elsewhere[5].

A survey of the prison gounds and adjacent areas was made for distribution and concentration of dwarf and giant ragweed plants. Cell blocks were inspected and found to be tidy and clean, in accordance with routine prison regulations. A minimum of simple wood furniture was present in the cells. No rugs or curtains were permitted. To minimize exposure to antigenic dust, plastic pillow and mattress covers were used.

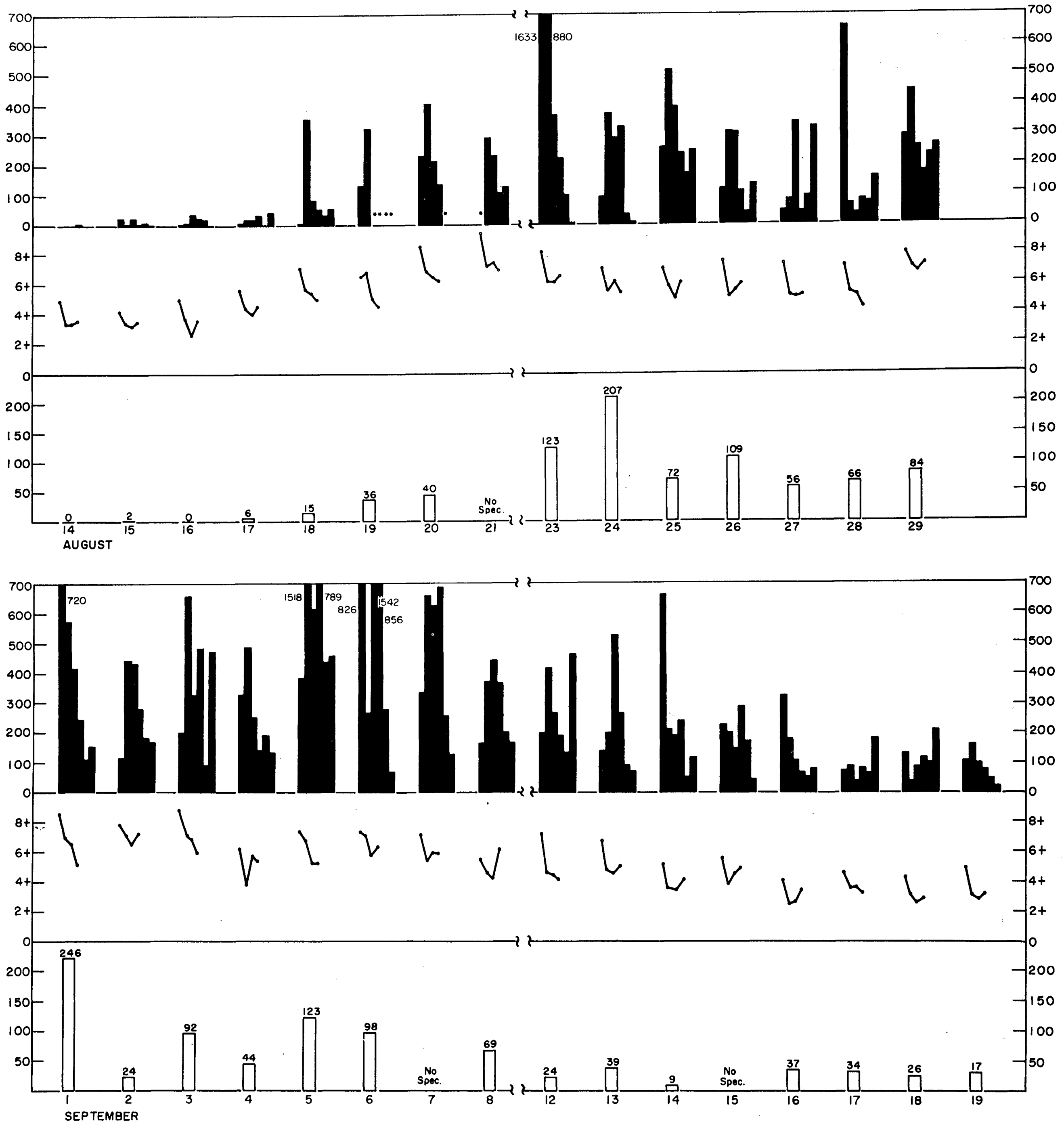
2.1.1.3 Results.—One patient (not included in Table 2.1.1.1 was excluded from the study because of pleuritis, which was thought to interfere with vital capacity determinations. The remainder of the group, 13 subjects in all (see Table 2.1.1.1), was free from illness other than allergic disease during the period studied. No significant temperature or pulse changes were noted.

Figure 2.1.1.1 shows the daily gravity count of ragweed pollen, the daily pollen profiles, and the fluctuation of index of symptoms of the entire group during the waking hours. To some degree, the severity of symptoms may have been reduced by antihistamines or Quadrinal which were taken daily throughout the season by some individuals, for long periods of time by others, and not at all by others. Except for subcutaneous epinephrine, administered rarely when asthma was severe, patients did not receive casual medication. Either they were on a roster to receive the medication three times daily regardless of symptoms, or they did not receive medications. Consequently it was felt that fluctuations in symptoms were not likely to be attributable in any large degree to medications received.

Some patients experienced mild allergic symptoms prior to the appearance of ragweed pollen in the air, possibly due to dust and other aeroallergens. As ragweed pollen appeared in the air and its concentration increased from 8/14 to 8/21 as measured by both gravity slides and volumetric sampling, there was a steady increase in severity of symptoms. As has been reported previously[2], there does not appear to be a temporal relationship between fluctuations of pollen concentration and fluctuations in subjective evaluation of symptoms. On numerous occasions, high concentrations of ragweed pollen failed to produce the expected increase in severity of symptoms in our group of ragweed-sensitive individuals.

Seven patients gave a history of both allergic rhinitis and bronchial asthma, and objective evidence for both conditions was present during the course of the investigation. One subject (R.H.) denied asthma, but on two occasions, 8/22/55 and 8/23/55, moderately loud musical rales were heard on expiration. Five subjects who denied asthma in their initial history either reported definite chest symptoms such as cough, unusual shortness of breath, wheezing, or tightness of the chest, or they were found to have wheezing or rhonchi on physical examination. The pulmonary findings in allergic rhinitis patients are summarized in Table 2.1.1.2.

The range of fluctuation of daily one-second and total vital capacities during the interval from 8/15 to 9/22 for each patient is shown in Table 2.1.1.3. With only slight overlap, it appears that the fluctuations of vital capacity in patients with asthma are, as expected, greater than the fluctuations in allergic rhinitis. We wish to call attention to the wide range over which measurements fluctuate in the group whose illness was originally thought to be confined to the upper respiratory tract. In neither group of patients did a consistent pattern appear; severe reduction of vital capacity might be found in some individuals, while others would show little or no reduction on any single day. In the case of one asthmatic patient, as will be described in more



• *PUMP FAILURE*

Fig. 2.1.1.1 Solid bar graphs record ragweed pollen grains per 1000 liters of air sampled during 30-minute periods. The initial value each day is the 8:00 A.M. determination and subsequent counts represent values obtained at 4 hour intervals. Data is expressed in this manner so each daily profile corresponds to a gravity count (expressed as open bar graph). Severity of symptoms, expressed on an arbitrary scale from 0 to 10+, is also recorded, the four points daily representing 8:00 A.M., noon, 4:00 P.M., and 8:00 P.M. estimations.

detail below, continuous bronchodilator and expectorant therapy until 9/6 may have prevented the development of symptoms. This was not true for other patients on maintenance therapy, who, nevertheless, experienced moderate to severe symptoms during the course of the study.

The eosinophil counts are plotted in Fig. 2.1.1.2 and are expressed in eosinophils per cubic millimeter. The values are plotted above the dates when counts were obtained. Values from allergic rhinitis patients are plotted as crosses, and values from bronchial asthma patients are plotted as closed circles. If 450 eosinophils per cubic millimeter are accepted as the upper limit of normal[7], six of seven asthmatic and one of six allergic rhinitis patients demonstrated a significant eosinophilia on one or more occasions. The one individual (R.H.) in the allergic rhinitis group who had an eosinophilia on four occasions, reaching 621 per cubic millimeter on 9/14, is the individual who was observed to have musical rales on examination, although he denied having asthma.

Since the prison yard and adjacent grounds are well cultivated, it was anticipated that little ragweed would be found within or in close proximity to the prison compound. A botanical survey, however, disclosed patches of ragweed within the prison, primarily in recreation areas, and extensive growth in several locations just outside the prison wall.

The vital capacity fluctuations, medications, eosinophil counts, and subjective symptoms of two asthmatic subjects are shown in Fig. 2.1.1.3. The course of these individuals is described in detail because of the interesting effect of medication on the course of their illness and because we wish to discuss the eosinophilia in relation to the asthma.

A.B. had experienced severe asthma in previous years and had obtained relief with an aerosolized bronchodilator. Because this medication was not permitted in prison, and because the patient was fearful and apprehensive, he was maintained on one tablet of Quadrinal® three times daily prior to and during the initial weeks of the ragweed season. E.J. received no medication prior to the development of severe asthma. With the rise of ragweed pollen concentration in mid-August, A.B. experienced only minimal nasal symptoms, and no subjective or objective evidence of asthma. E.J., however, reported nasal symptoms which increased with rise in the ragweed concentration. He experienced a slight cough on 8/21, and on 8/22 the vital capacity had fallen to 20/28. At this time the eosinophil count was in the normal range. The following day, bilateral high-pitched musical rales were heard. Except for 0.5 milliliter of 1:1000 aqueous epinephrine after the examination of 8/22, no medication was taken prior to 8/25. Although A.B. was almost completely free of both subjective and objective symptoms, and E.J. experienced little asthma between 8/29 and 9/5, both had elevated blood eosinophil counts during this period.

Medication was discontinued by E.J. on 9/8, and although a decrease of one-second vital capacity for six days and total vital capacity for five

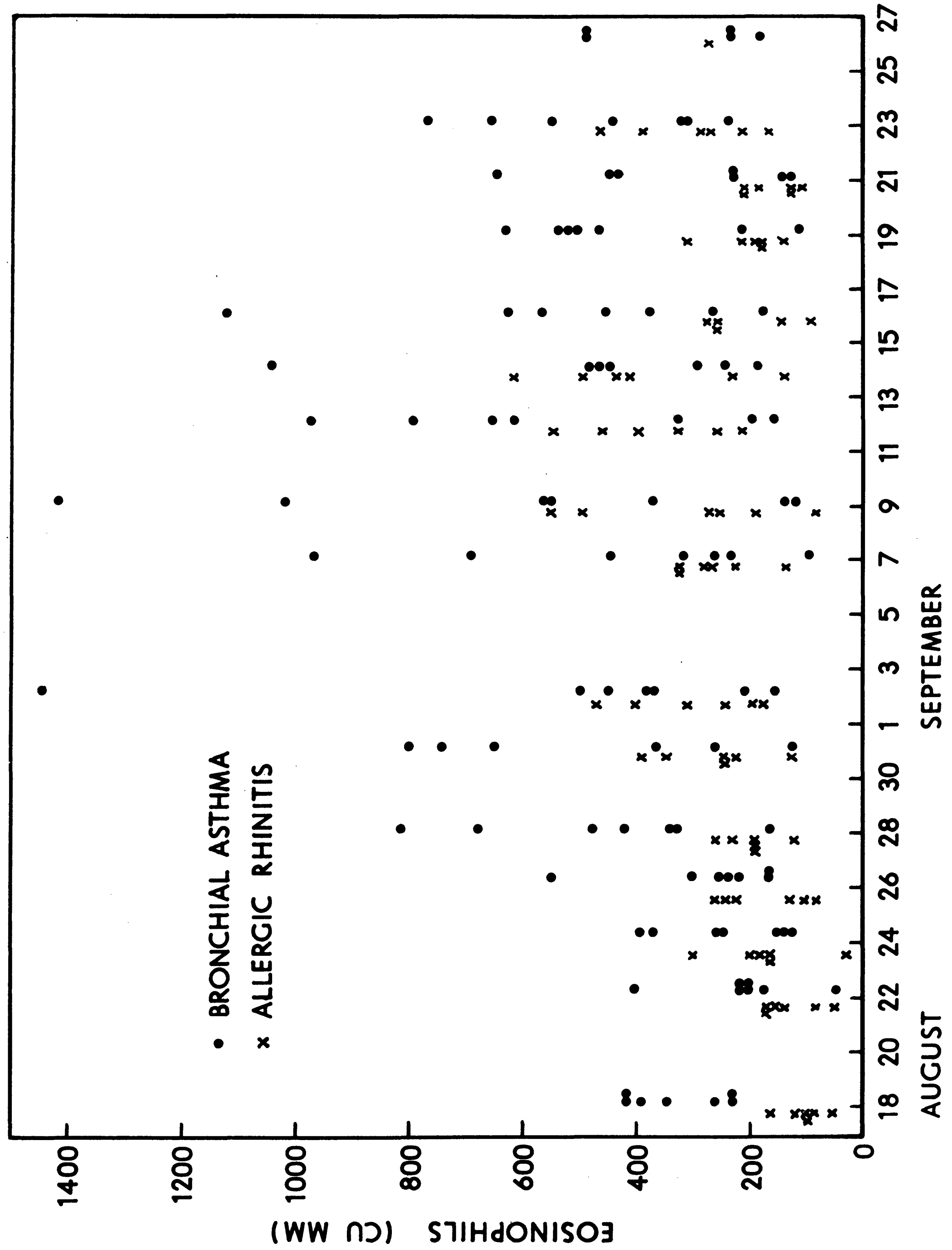


Fig. 2.1.1.2 Eosinophil counts, expressed as eosinophils per cubic millimeter, from August 18 to September 27, 1955.

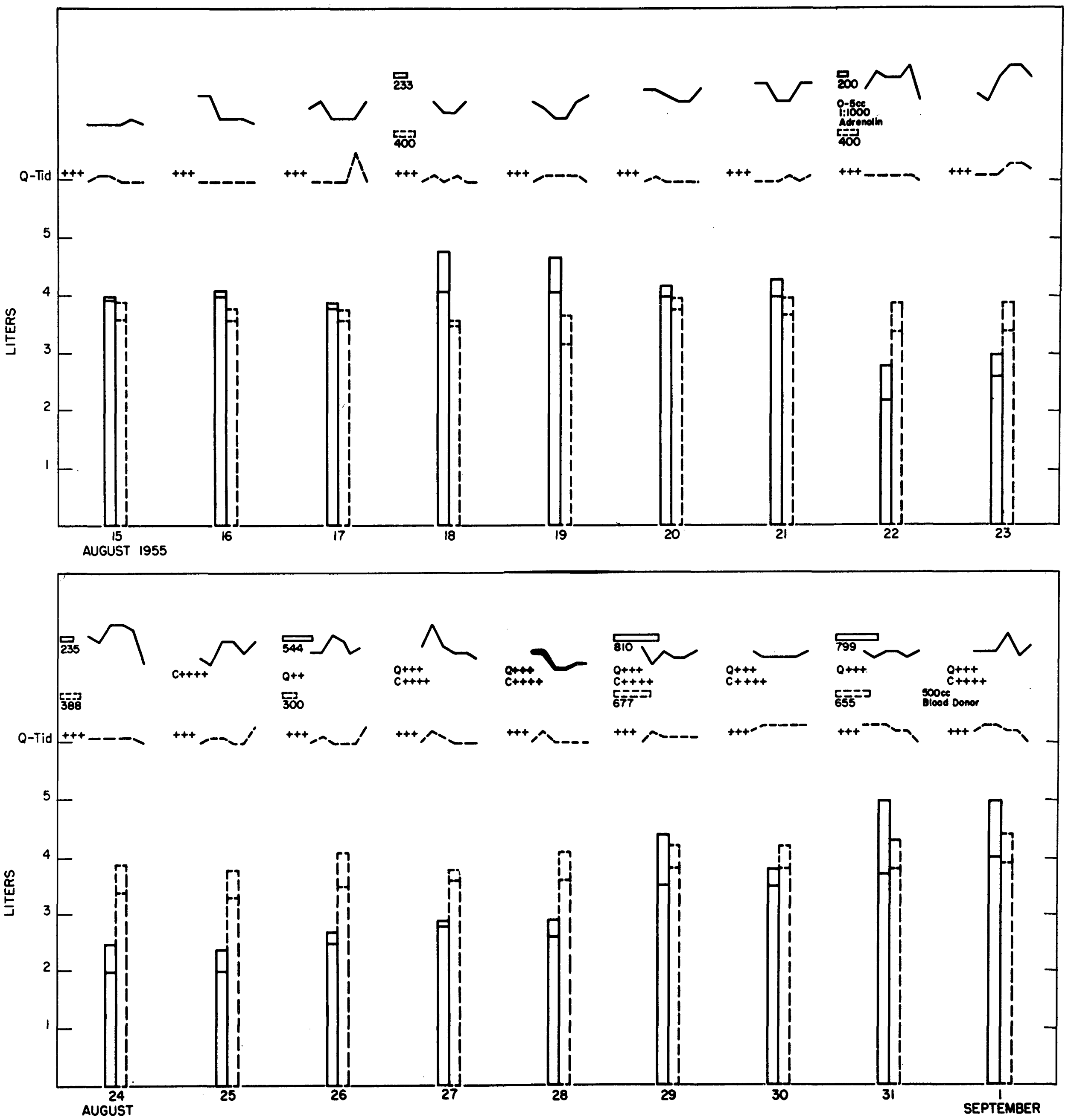


Fig. 2.1.1.3 Solid lines refer to subject E. J., dotted lines to A.B. One-second and total vital capacities are plotted as vertical bar graphs. Horizontal line segments represent fluctuation of index of symptoms. Horizontal bar-graph segments and accompanying numerical values record eosinophils per cubic millimeter. Medications are also recorded (Q = Quadrinal®, C = chlorprophenpyradine maleate).

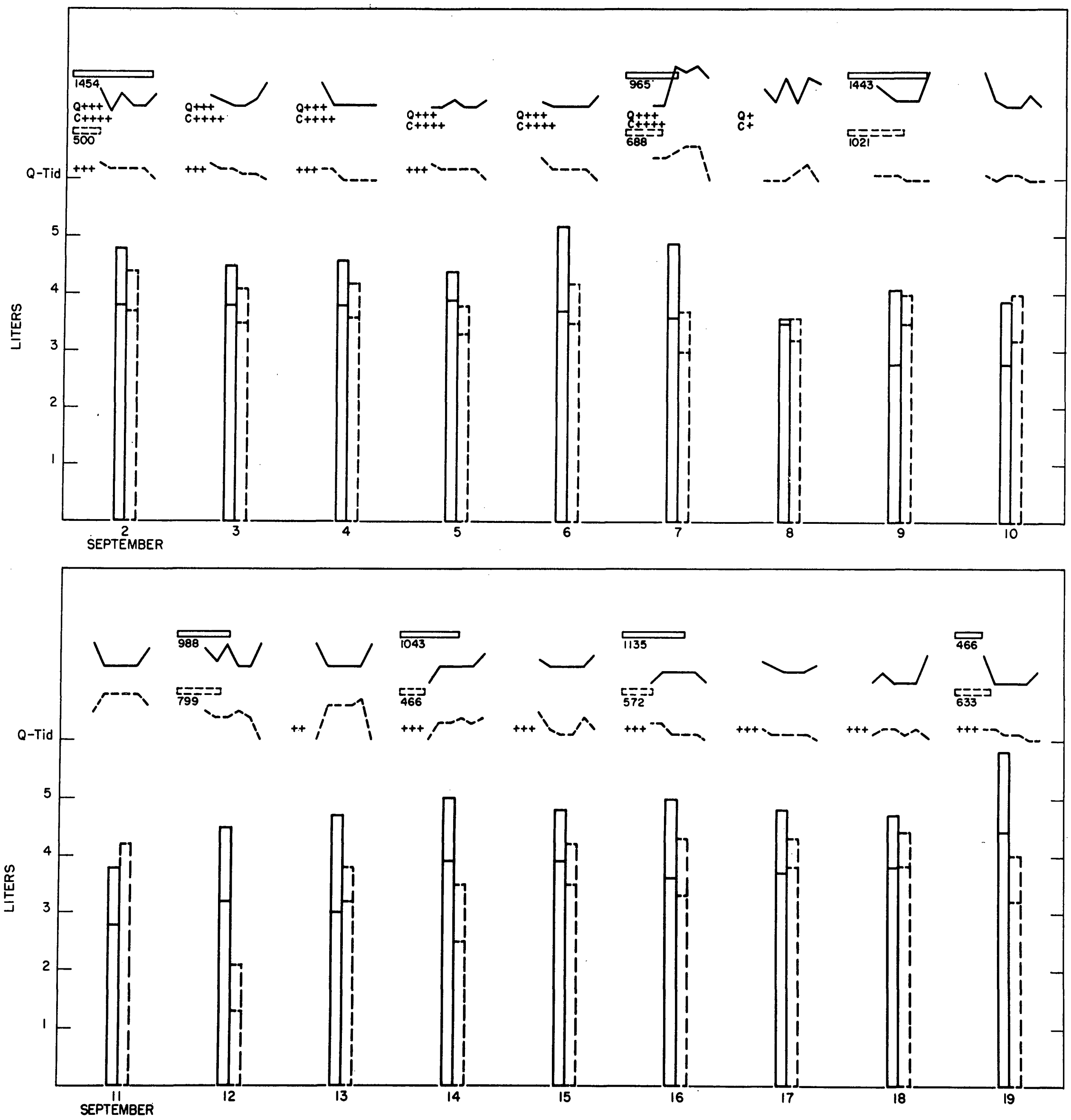


Fig. 2.1.1.3 (Concluded)

days was recorded, and eosinophilia persisted, the patient experienced only a daily mild cough and occasional mild dyspnea and wheezing for the remainder of the season.

A.B. experienced only minimal nasal symptoms and a cough prior to 9/6, and was free of objective evidence of asthma except for a localized area of musical rales at the right base posteriorally during forced expiration with the aid of external compression on 8/31. However, eosinophilia was found on and after 8/29. The patient willingly discontinued medication on 9/6, and the following day coughing, wheezing, and dyspnea were reported and bilateral inspiratory and expiratory rales were heard. A precipitous fall in vital capacity did not occur until 9/12, however, and the patient resumed medication on the following day. After 9/14, only occasional mild wheezing and coughing were reported and no objective evidence of asthma was recorded.

2.1.1.4 Discussion.—This investigation was designed as a pilot study and therefore conclusions are only suggested. Nevertheless, we believe they are of enough interest to warrant a preliminary report at this time. We believe much is to be learned by daily observation of a limited number of subjects under environmental control as rigorous as possible. Only a small number of intensive investigations over a prolonged period has been reported. Even among the most outstanding of these,[6,7,8,9] daily objective evaluation was not possible or thought essential.

The increase of severity of symptoms in the first week of the season roughly paralleling the rise in concentration of ragweed pollen would suggest the existence of some kind of a dose-response relationship. The absence of any consistent similarity of fluctuation of symptoms and pollen concentration on any given day suggests that the method of sampling is unreliable, symptoms do not follow rapid fluctuations of pollen concentration, or perhaps fluctuations of pollen concentration at one location are in no way representative of fluctuations over a small area. Studies at the Engineering Research Institute of The University of Michigan in the last year [10] so far indicate that the apparatus and procedure yield reliable results. These studies will continue, and in future investigation, continuous sampling over each four-hour period rather than 30-minute sampling every four hours may yield results that correlate better with subjective evidence of severity of illness. Even more rigorous environmental control is contemplated in future investigations, with several patients and a volumetric sampler confined to a small homogeneously ventilated environment. It became apparent that the environmental uniformity desired was not present when patches of ragweed plants were found in the areas to which the subjects had free access on several occasions daily. The existence and nature of a dose-response relationship between ragweed pollen and allergic rhinitis or bronchial asthma remains to be defined.

An eosinophilia above 450 per cubic millimeter in five of seven asthmatics and only one of six allergic rhinitis patients suggests that systemic manifestations of asthma are more severe than in allergic rhinitis. The eosinophilia failed to correlate with other manifestations of the disease. One patient developed severe asthma and demonstrated reduction of the vital capacity prior to the appearance of significant eosinophilia, and later in the season his eosinophil count exceeded 1400, although his symptoms at that time were only mild and he was not receiving any medication. The minor degree of symptoms experienced by A.B. while on Quadrinal®, and the simultaneous development of an eosinophilia to a high level constitutes further evidence that underlying pathological processes are in action, in spite of symptomatic relief or control obtained with this medication. It will be noted that most asthmatic patients had eosinophil counts in the morning, and most allergic rhinitis patients had determinations in the afternoon. Although diurnal variations in eosinophil count have been reported,[11,12] they are probably not sufficient to account for the differences observed in our subjects. Daily eosinophil counts in the fasting state in both groups might yield interesting results.

Since Plesch first used the vital capacity in asthma in 1913[13], this test has been employed as an objective measure of the degree of respiratory embarrassment in bronchial asthma. The timed vital capacity constitutes a further modification thought to increase the significance of the results obtained [14,15]. In this study both one-second and total values are recorded. The one-second value does not consistently appear to be any more indicative of subjective or objective impairment of respiration than does the total vital capacity. We agree with Lowell, Schiller, and Lynch[6] that there is no consistent relationship between physical findings and changes in pulmonary function.

It is of interest to note a greater fluctuation of vital capacity in allergic rhinitis patients than we would expect, and this observation is in accord with the findings of Brown, et al[9], who conclude that patients with uncomplicated hay fever and no history of bronchial asthma may frequently demonstrate a diminished vital capacity during their pollen seasons. It has also been demonstrated by Tuft[8] that some allergic rhinitis patients experience a decrease in breathing capacity during the pollen season.

We agree with Lowell, Schiller, and Lynch[6], that "... no single criterion can serve as an adequate measure of the severity of bronchial asthma." It is our hope that as a result of intensive study of volunteer subjects in an environment as completely controlled as possible, additional information relating to the pathogenesis of allergic rhinitis and bronchial asthma can be obtained. To this end, additional investigation is in progress.

2.1.1.5 Summary and conclusions.—Careful frequent observations were made of 6 allergic rhinitis and 7 bronchial asthma subjects. The following conclusions are suggested:

1. Early in the ragweed season, as the pollen concentration increases, the patient's symptoms also increase. A relationship appears to exist between symptoms and the ragweed concentration as measured by the gravity counts. A relationship between symptoms and frequent volumetric counts over each 24-hour period cannot be demonstrated.

2. Pulmonary symptoms or signs, sufficiently mild or infrequent to escape detection unless searched for carefully, can be uncovered or observed frequently in patients with allergic rhinitis.

3. Eosinophilia is common and reaches higher levels in patients with bronchial asthma than in patients with allergic rhinitis. The level of eosinophil concentration is not related to severity of symptoms. Development or persistence of eosinophilia can be observed while the subject is rendered or main-

tained asymptomatic with conservative drug therapy. This suggests that abnormal systemic processes may continue although their manifestations are not apparent.

2.1.1.6 Acknowledgement.—We wish to acknowledge our indebtedness to John M. Sheldon, M.D., University Hospital, Ann Arbor, Michigan and to E. Wendell Hewson, Ph.D., A. Nelson Dingle, Sc.D., and Gerald C. Gill, M.A., of the Department of Civil Engineering and Engineering Research Institute, The University of Michigan, for their assistance and advice. The generous assistance and cooperation of the staff of the State Prison of Southern Michigan, and particularly of Warden Bannan, and Mr. White, Hospital Director, made this investigation possible.

TABLE 2.1.1.1

Subject	Age	Manifestation of disease (by history)	Skin test to ragweed antigen* Scratch	Intracutaneous	Time of Daily visit
1. G.R.	39	Rhinitis	0	2	8:30
2. J.R.	32	Rhinitis and asthma	3+		8:55
3. C.S.	64	Rhinitis and asthma	4+		9:20
4. A.B.	34	Rhinitis and asthma	0	3+	9:45
5. J.S.	42	Rhinitis and asthma	2+		10:35
6. E.J.	29	Rhinitis and asthma	3+		11:00
7. L.B.	35	Rhinitis and asthma	3+		1:00
8. L.C.	27	Rhinitis	3+		1:25
9. R.H.	36	Rhinitis	4+		1:50
10. S.R.	50	Rhinitis	4+		2:40
11. E.M.	26	Rhinitis and asthma	2+	4+	3:05
12. L.F.	27	Rhinitis	3+		3:30
13. W.W.	41	Rhinitis	3+		3:55

*Intracutaneous tests performed only where results of scratch testing were negative or questionable.

TABLE 2.1.1.2

LOWER RESPIRATORY TRACT SYMPTOMS AND SIGNS
IN 6 SUBJECTS WITH ALLERGIC RHINITIS

(Number refers to date symptom was recorded or sign was observed)

Subject	Month	Symptoms				Signs
		Wheezing	Cough	Shortness of breath	Chest tightness	
L.C.	Aug.	14	-	19,20,21, 22,23,24	14	-
	Sept.	-	9	-	-	6 Scattered rales at lung bases
G.R.	Aug.	-	-	-	-	-
	Sept.	12,13	-	-	12	9 Expiratory wheeze
S.R.	Aug.	-	13,15,19-25, 29,30,31	-	17,18,21, 27,29,31	-
	Sept.	8,11	1,2,4,5,8-12, 17,19-22	-	1,6	-
W.W.	Aug.	-	-	-	-	-
	Sept.	-	2,3,13-15	-	-	2,13 Coarse rhonchi
R.H.	Aug.	-	-	-	-	22,23 Musical rales
	Sept.	-	-	-	10	-
L.F.	Aug.	-	21,22	18-22,26, 29,30	-	-
	Sept.	-	6	-	-	-

TABLE 2.1.1.3

VITAL CAPACITY FLUCTUATIONS

Bronchial asthma			Allergic rhinitis		
J.R.	$\frac{31-43}{37-48}$	$= \frac{12}{11}$	L.C.	$\frac{38-51}{40-55}$	$= \frac{13}{15}$
C.S.	$\frac{12-35}{32-44}$	$= \frac{23}{12}$	R.H.	$\frac{33-44}{39-52}$	$= \frac{11}{13}$
A.B.	$\frac{13-39}{21-45}$	$= \frac{26}{24}$	S.R.	$\frac{30-40}{34-44}$	$= \frac{10}{10}$
J.S.	$\frac{24-35}{25-41}$	$= \frac{11}{16}$	L.F.	$\frac{39-48}{40-50}$	$= \frac{9}{10}$
E.J.	$\frac{20-44}{24-58}$	$= \frac{24}{34}$	W.W.	$\frac{32-36}{35-38}$	$= \frac{4}{3}$
L.B.	$\frac{34-40}{37-48}$	$= \frac{6}{11}$	G.R.	$\frac{37-42}{40-46}$	$= \frac{5}{6}$
E.M.	$\frac{30-44}{36-48}$	$= \frac{14}{12}$			

The numerator records the lowest and highest values of the one-second vital capacity in hundreds of cubic centimeters. The denominator expresses the lowest and highest values of the total vital capacity in hundreds of cubic centimeters. The range over which these values fluctuate is indicated as the difference between the highest and lowest value.

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2.1.2 Clinical studies in 1956

During the ragweed pollen season of 1956, studies were again carried out on volunteers at the State Prison for Southern Michigan at Jackson. This was done to allow studies on patients whose activities were confined to definite geographic limits. Volunteers were screened by a complete evaluation of allergy, and skin tests were done on appropriate individuals.

During the second season, a total of eleven men was selected as the most suitable. Three of these men (two with asthma and one with hay-fever) were confined in the prison hospital for the major part of the ragweed season, while the others continued their daily chores within the prison confines. One of the three men is shown in his room in Fig. 2.1.2.4.

All patients were seen daily; the in-patients were seen twice daily and their objective signs of hay fever and asthma were checked. Details of the procedures and equipment used are illustrated in Figs. 2.1.2.1, 2.1.2.2, 2.1.2.3, and 2.1.2.5. Each patient also kept a numerical record of his subjective signs,

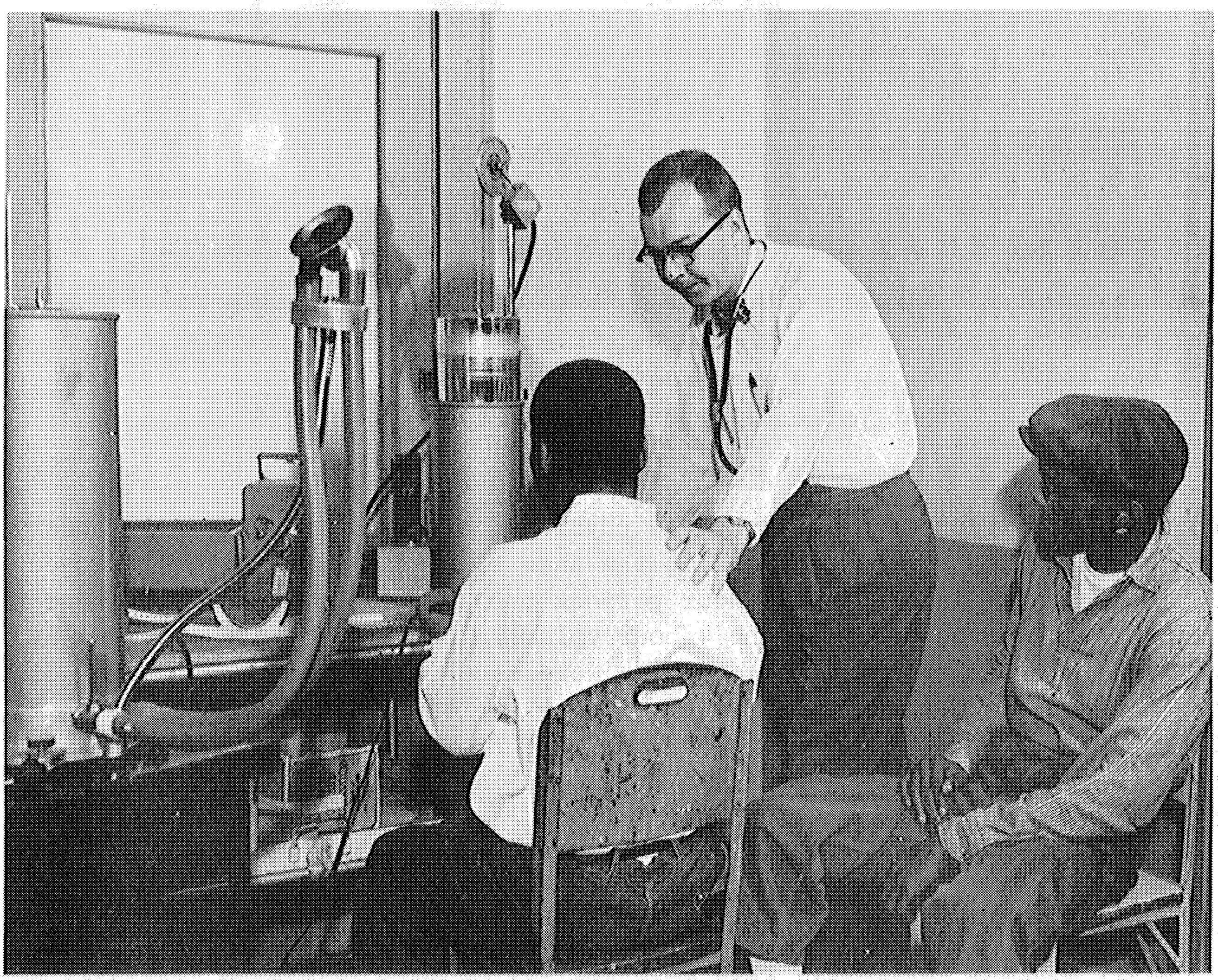

Fig. 2.1.2.1 Physician instructing patient on performing timed vital capacity correctly.

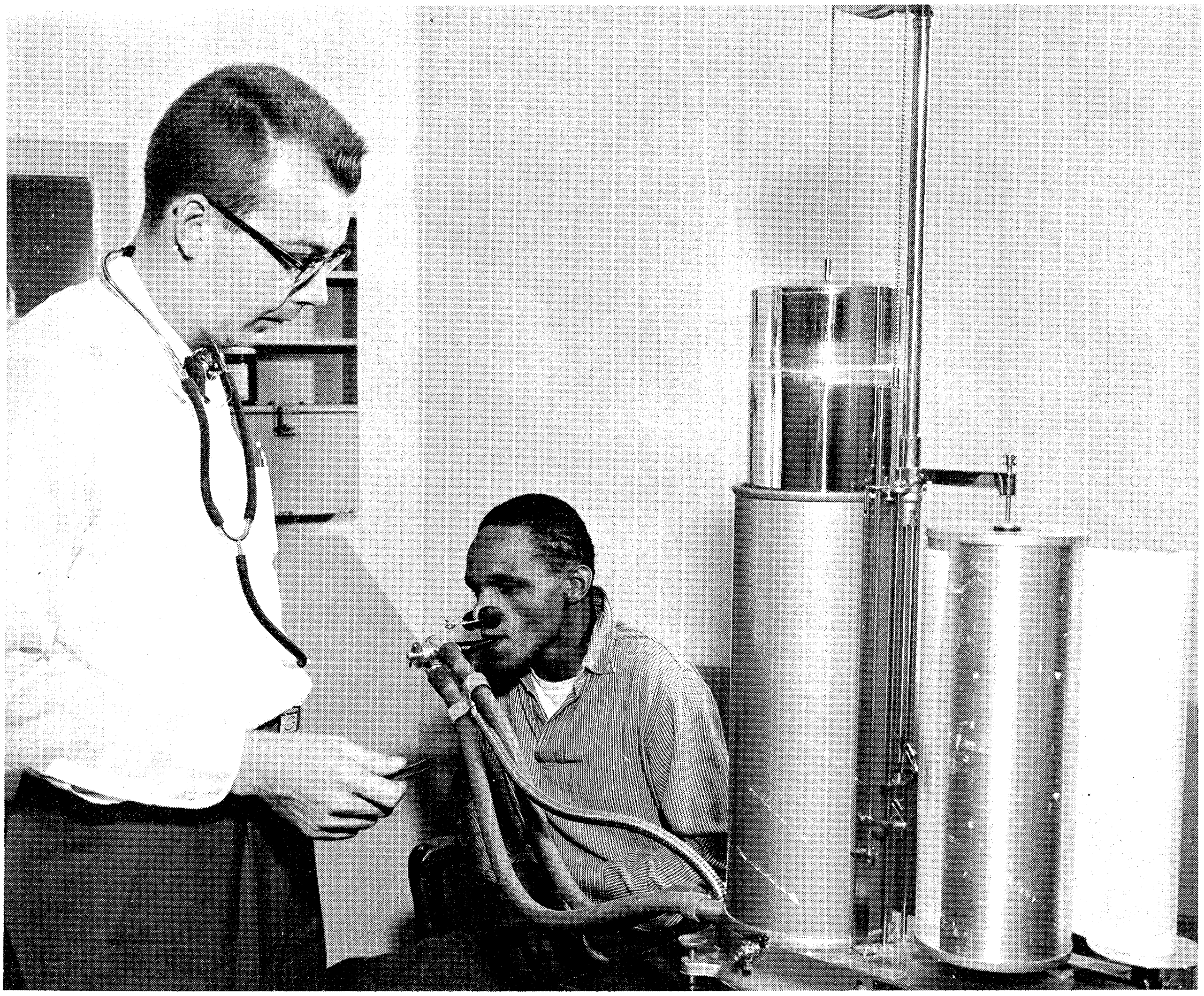

Fig. 2.1.2.2 Physician checking to see that patient is performing MBC adequately.

and these were rechecked daily by the physician after making objective observation. The patients were examined at the same time each day and their subjective sign cards were divided into 4-hour periods throughout the 24 hours. These time periods also corresponded to the 4-hour volumetric pollen counts obtained by the meteorological group. These counts were made in three separate areas of the hospital ward, which was partitioned off for the three in-patients, and on a 100-foot tower located approximately one mile from the prison, as well as on the prison hospital roof. The standard gravity-slide method was also used for daily pollen counts on the hospital roof.

Daily laboratory examinations of the hospitalized patients included quantitative blood eosinophil counts, nasal smears for eosinophils, timed vital capacity, maximum expiratory flow-rate curves, maximum breathing capacity, and pneumotachographic tracings. Eosinophil counts, maximum expiratory flow-rate curves, and spirograms were made every other day on the out-patients. Salivary

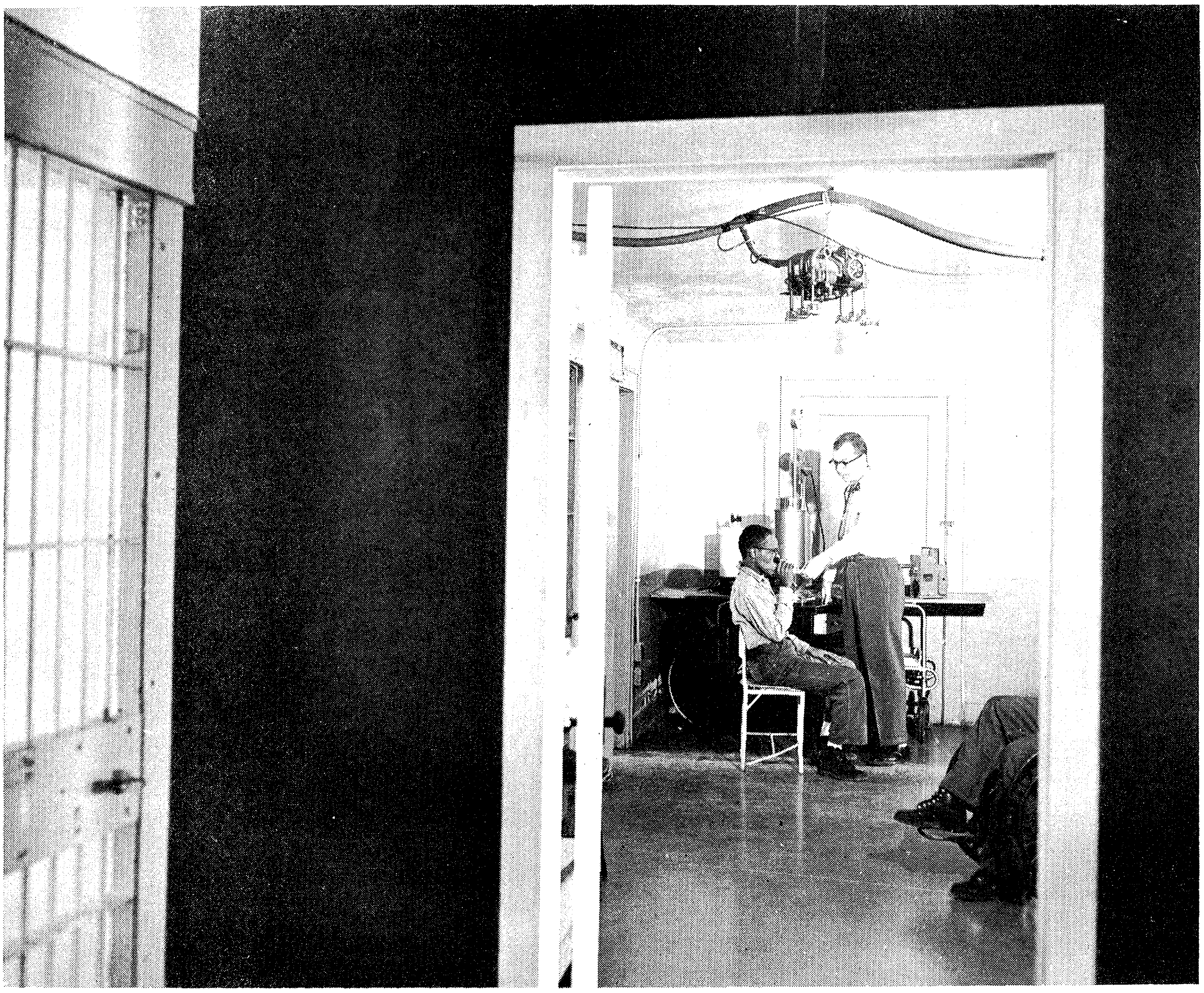

Fig. 2.1.2.3 Patient and physician in enclosed cell block in prison hospital performing MBC. Photo taken through the door in cell block and showing the multiple-head millipore-filter volumetric pollen sampler.

smears for eosinophils were made for a limited time until they proved unsatisfactory. Residual lung volumes and N_2 washout studies were planned; however, the necessary equipment did not arrive as ordered and promised, and the substitute measures that were attempted did not prove satisfactory.

Unfortunately for present purposes, the 1956 pollen season was a relatively mild one, and only five of the patients had definite, spontaneous asthma. Only one had moderately severe asthma. All patients had unequivocal hay fever. Some patients, mainly late in the pollen season, had definite changes in their pulmonary function tests, with no objective evidence of asthma. A total of 20 MEFR's, 14 MBC's, 63 vital capacities, and 64 eosinophil counts were abnormal in the asthmatics. In the hay-fever patients, 1 MEFR, 4 MBC's, 32 vital capacities, and 2 high eosinophil counts were abnormal. The greater number of these abnormal tests were found during the peak pollen counts and during

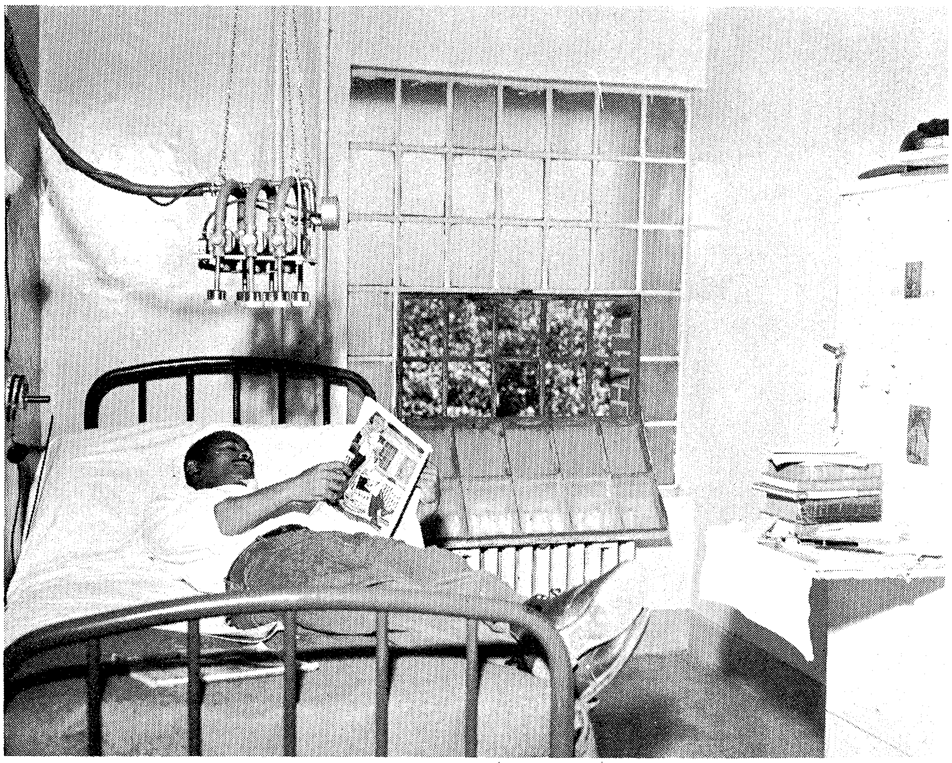

Fig. 2.1.2.4 Patient relaxing; note multiple-head volumetric pollen sampler suspended directly over bed.

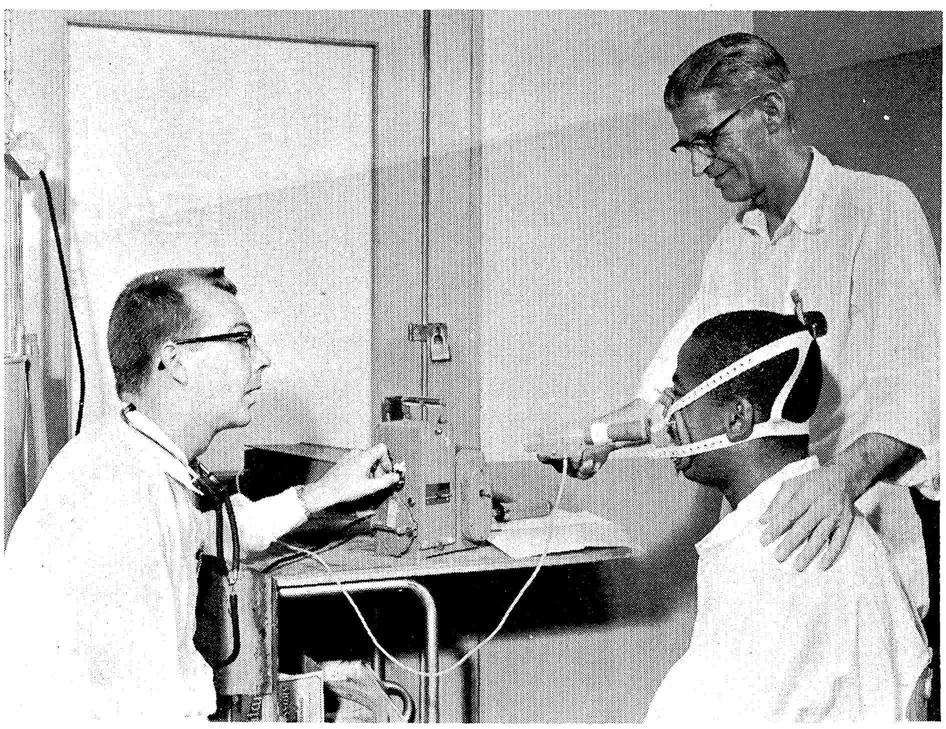

Fig. 2.1.2.5 Physician and assistant directing patient in proper performance in making a pneumatachographic record of respirations.

the latter part of the pollen season (see Figs. 2.1.2.6—2.1.2.17).

Although we could not rely on a given V.C. level, we noted that our patients fit into Feinberg's classification, i.e., a V.C. of 73% with tightness in the chest, 60% in a moderate attack, and 41% of the expected value in severe asthma. Three of our patients having the most days of moderate asthma had V.C.'s in the range of 61, 62, and 67%; those with minimal asthma had their lowest V.C. in the range of 73, 77 and 78%. One asthmatic patient's V.C. never was lower than 82%. One hay-fever patient with no subjective sensations of asthma—although he did have objective evidence of minimal wheezing—had a V.C. in the 77% range.

An interesting observation is that the one-second timed vital capacity appears to correlate better with the patient's own subjective wheezing rating than with our objective wheezing evaluation. In addition, the one-second timed vital capacity appeared to correlate well with the size of the nasal airway, nasal color and discharge, conjunctival color and discharge—all being objective data. Subjectively this same correlation seemed to appear with tightness in the chest, the pulse rate, choking sensation, and breathlessness while at rest and while walking. No correlation was noted with the respiratory rate, tightness in the chest, or cough. All these data, however, are on a limited number of patients.

It should be noted that the asthmatic symptoms built up more slowly than the hay-fever symptoms, and also tapered off gradually at the end of the season while the hay-fever symptoms stayed higher throughout most of the usual season (See Fig. 2.1.2.17). The average hay-fever-symptom severity count for the asthmatic patients was higher than in those with hay fever alone, or the patients with more severe hay fever were more likely to have asthma. Asthma patients had 17% more hay fever by subjective rating than did patients only experiencing hay fever. Objective asthma symptoms started after objective hay-fever symptoms in three patients, on the same day in one patient, and actually one week before in another patient.

There was fairly good agreement between objective and subjective asthma and hay-fever ratings with the pollen counts when the total pollen season was considered, although there were many individual variations (see Fig. 2.1.2.17). Actually, subjective ratings appeared to follow the general pollen season the best. We had hoped to determine if a lesser pollen count would cause symptoms once the pollen peak was reached as compared to earlier in the season, but our data did not lend themselves to this type of interpretation.

The specific pollen count patterns are of interest. The mean and median pollen counts showed their greatest increase and rose to the daily pollen peak in the time period from 8:00 A.M. to 12:00 noon while the mean and median subjective symptom increase for both asthma and hay fever occurred prior to this, namely, between 4:00 and 8:00 A.M. (see Fig. 2.1.2.18). The rise in pollen counts as reflected by the 100-foot-tower readings also reflected this

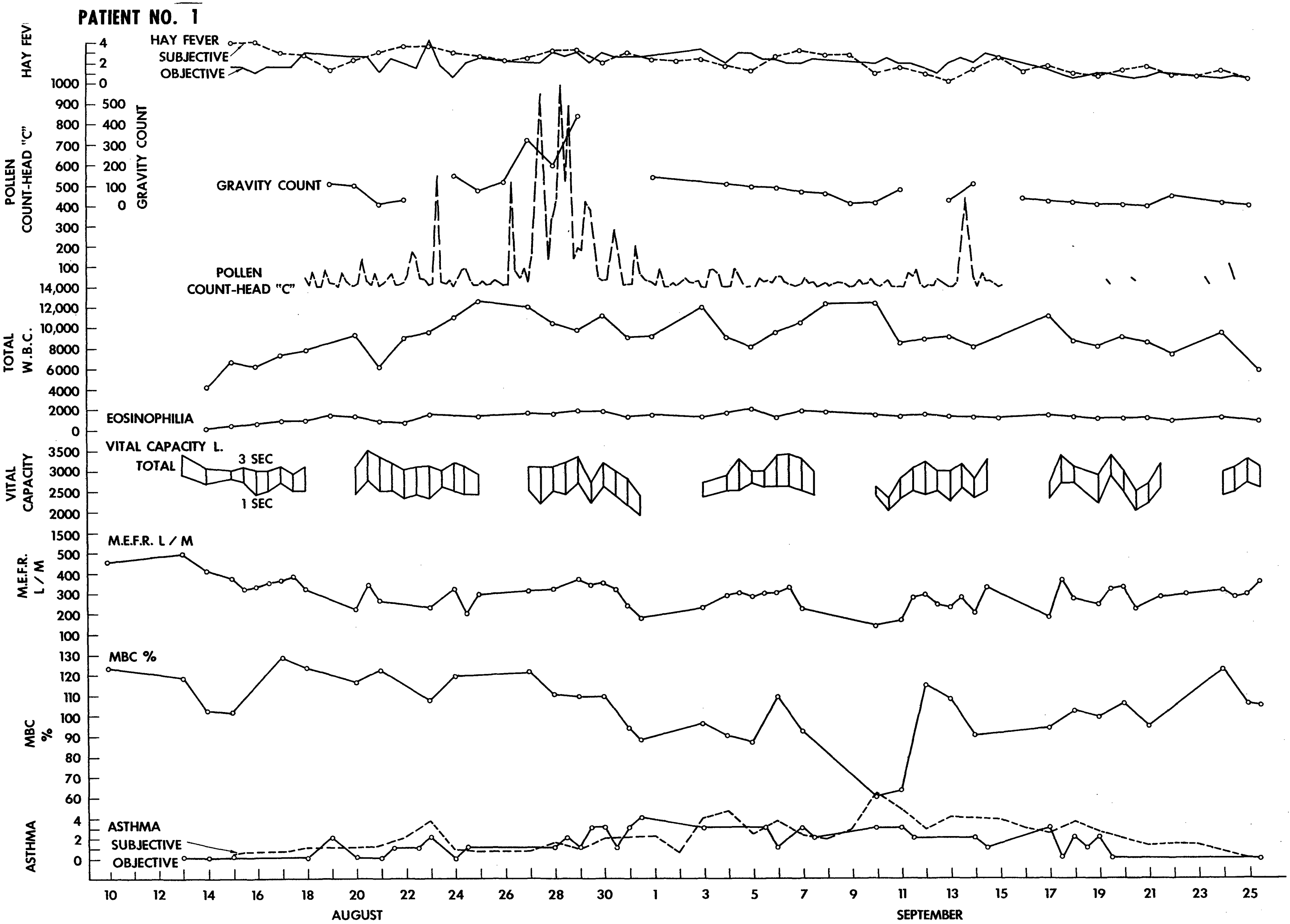

Fig. 2.1.2.6 Graphic presentation of patient No. 1. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophils, and white blood counts, and the pollen count (volumetric and standard 24-hour gravity slide).

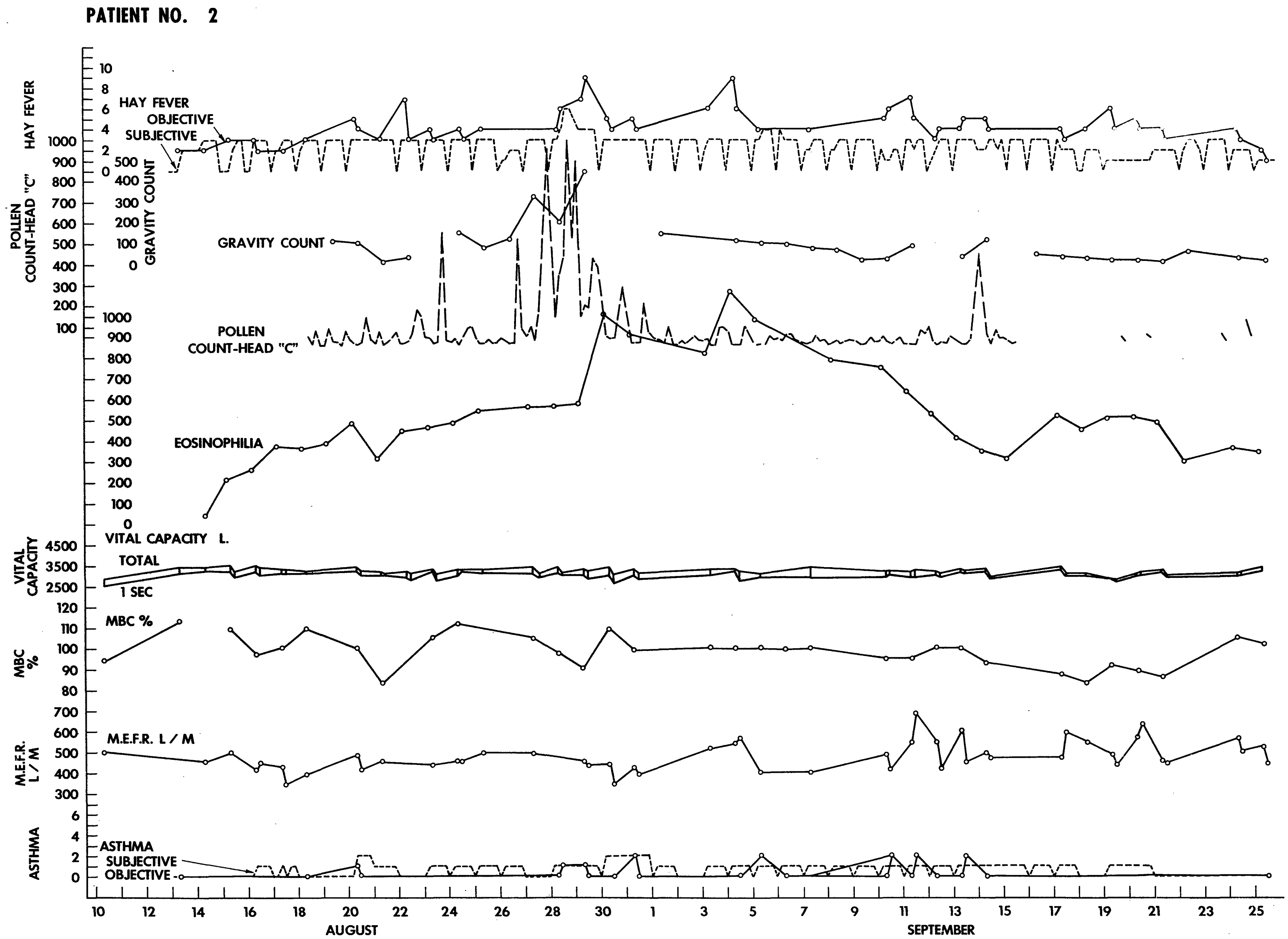

Fig. 2.1.2.7 Graphic presentation of patient No. 2. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

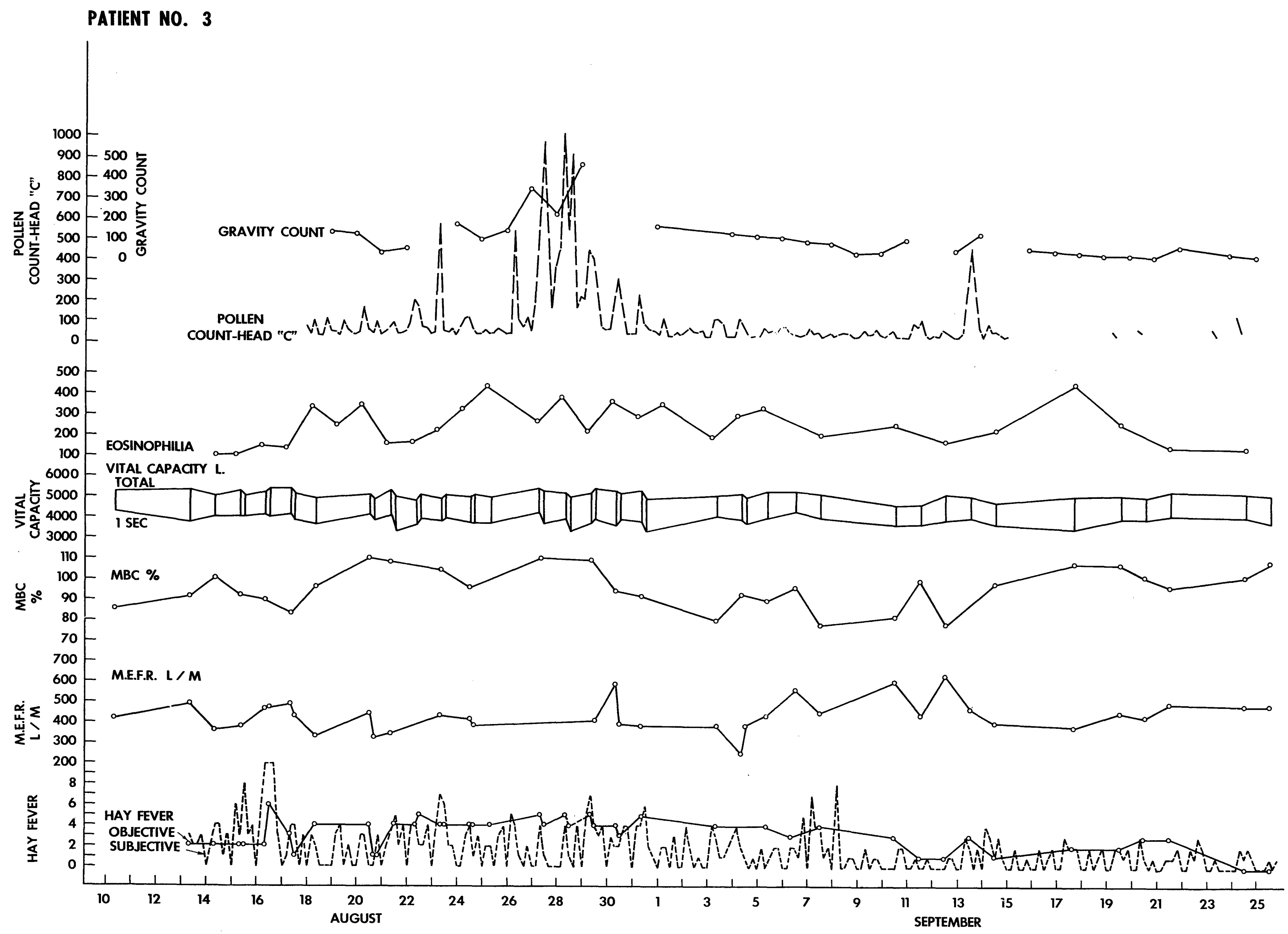

Fig. 2.1.2.8 Graphic presentation of patient No. 3. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

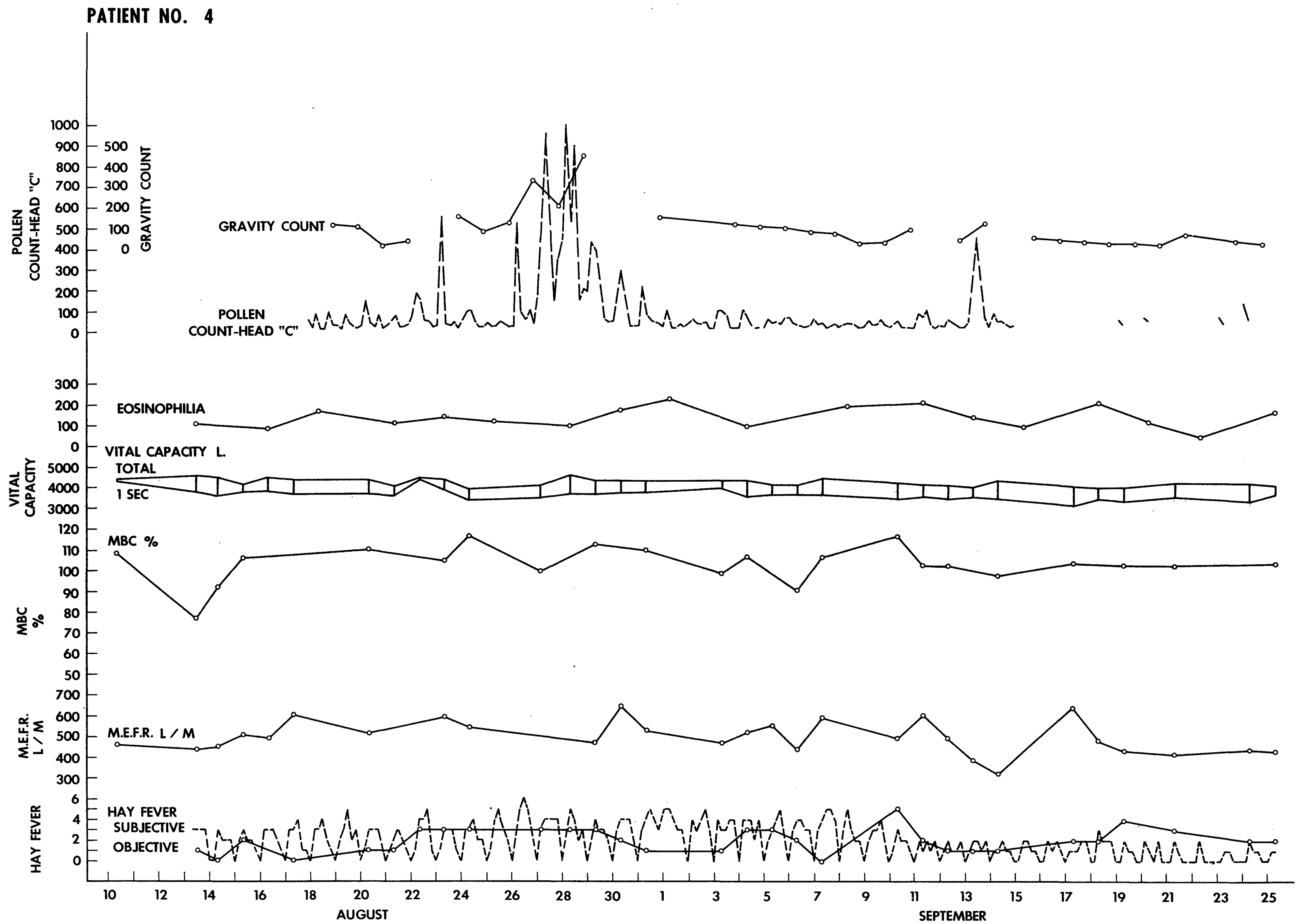

Fig. 2.1.2.9 Graphic presentation of patient No. 4. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

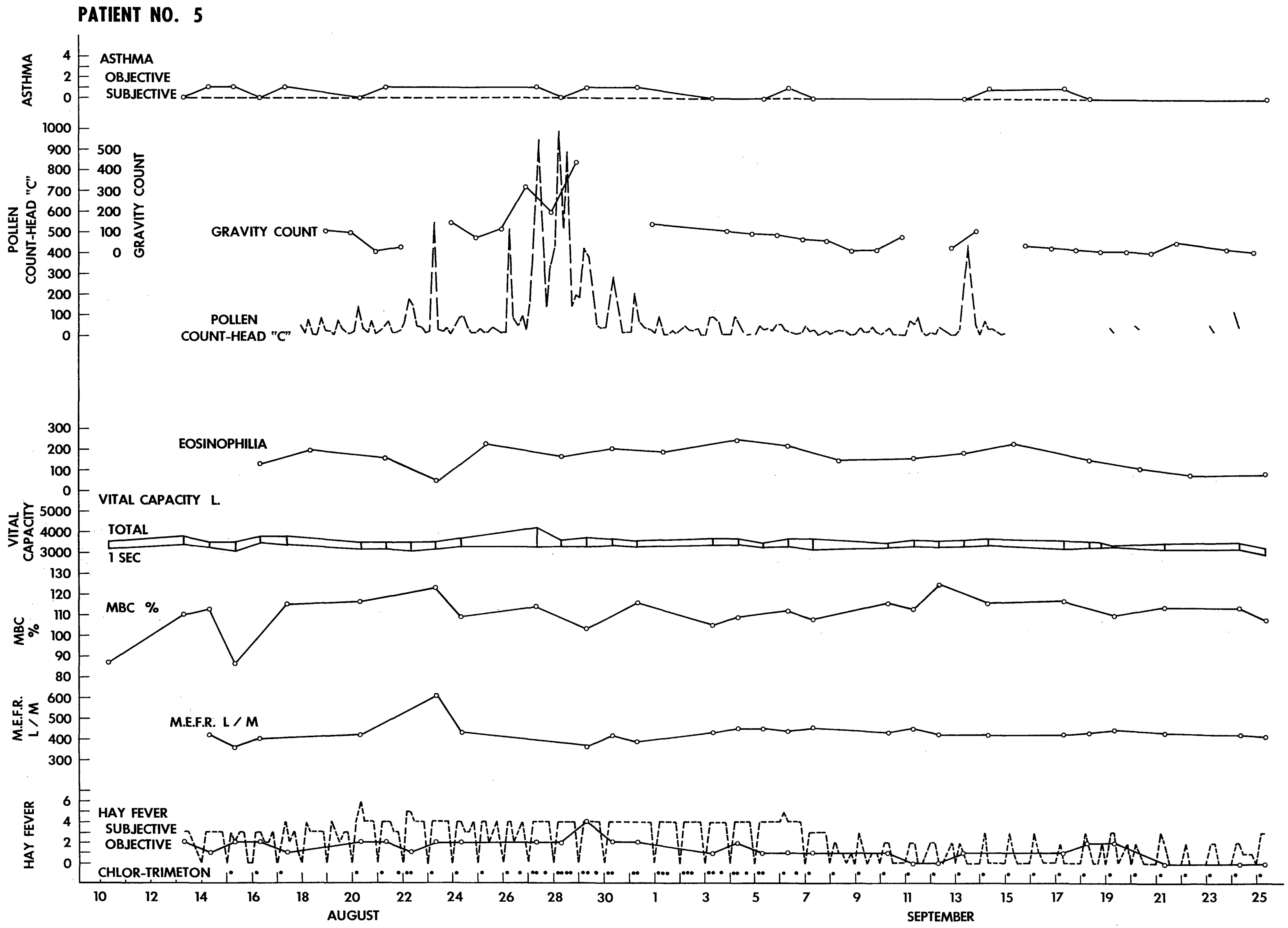

Fig. 2.1.2.10 Graphic presentation of patient No. 5. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

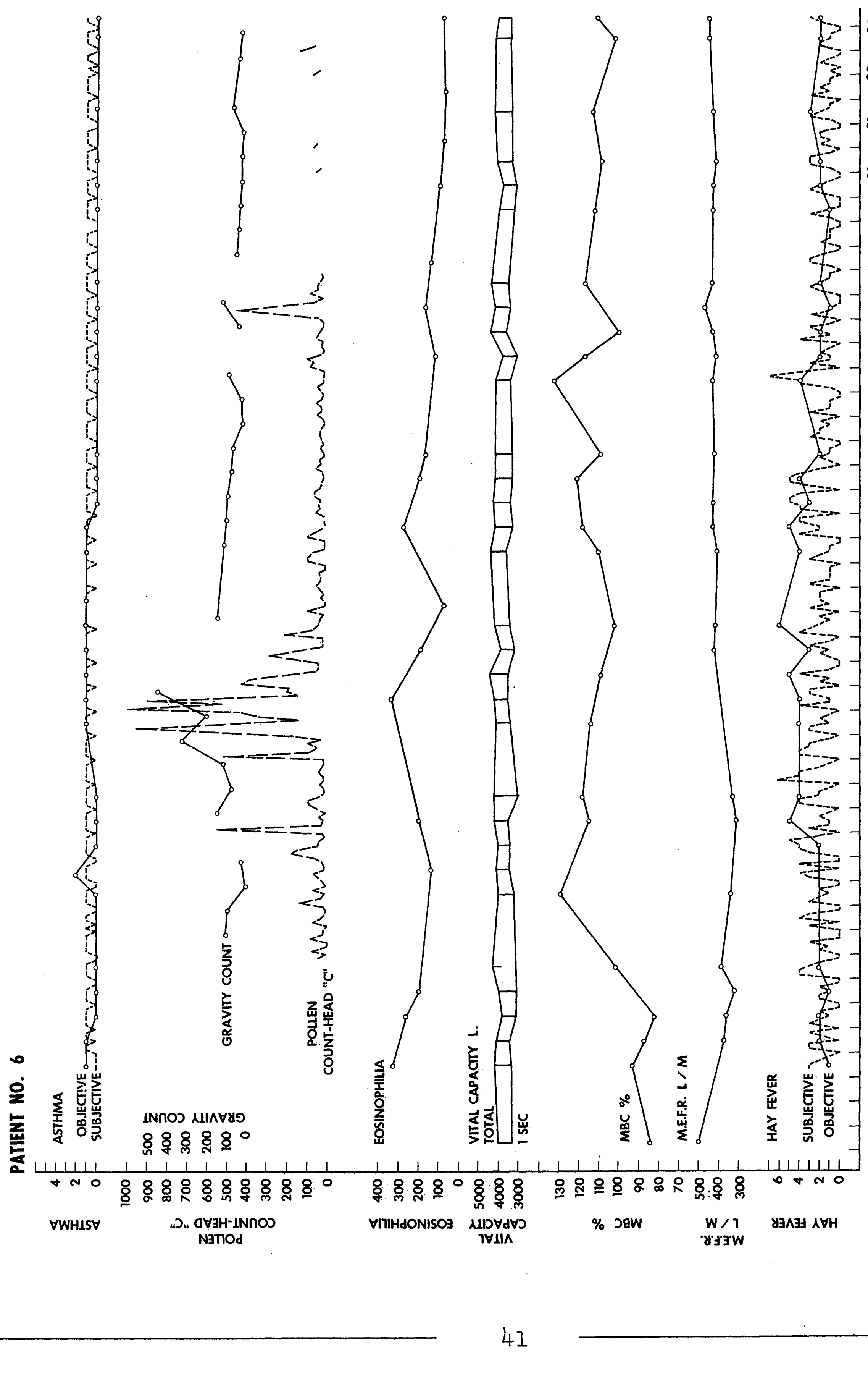

Fig. 2.1.2.11 Graphic presentation of patient No. 6. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

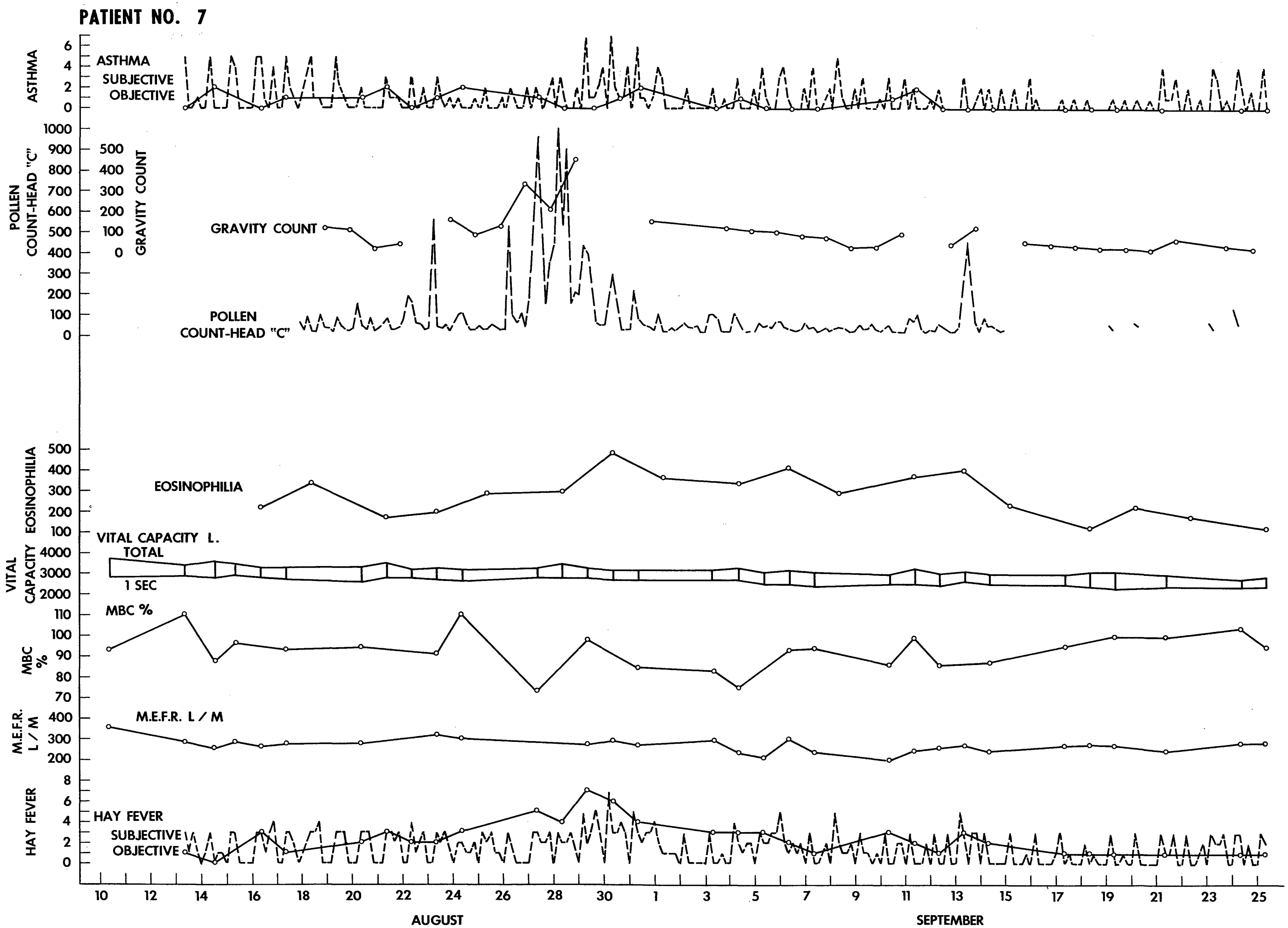

Fig. 2.1.2.12 Graphic presentation of patient No. 7. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

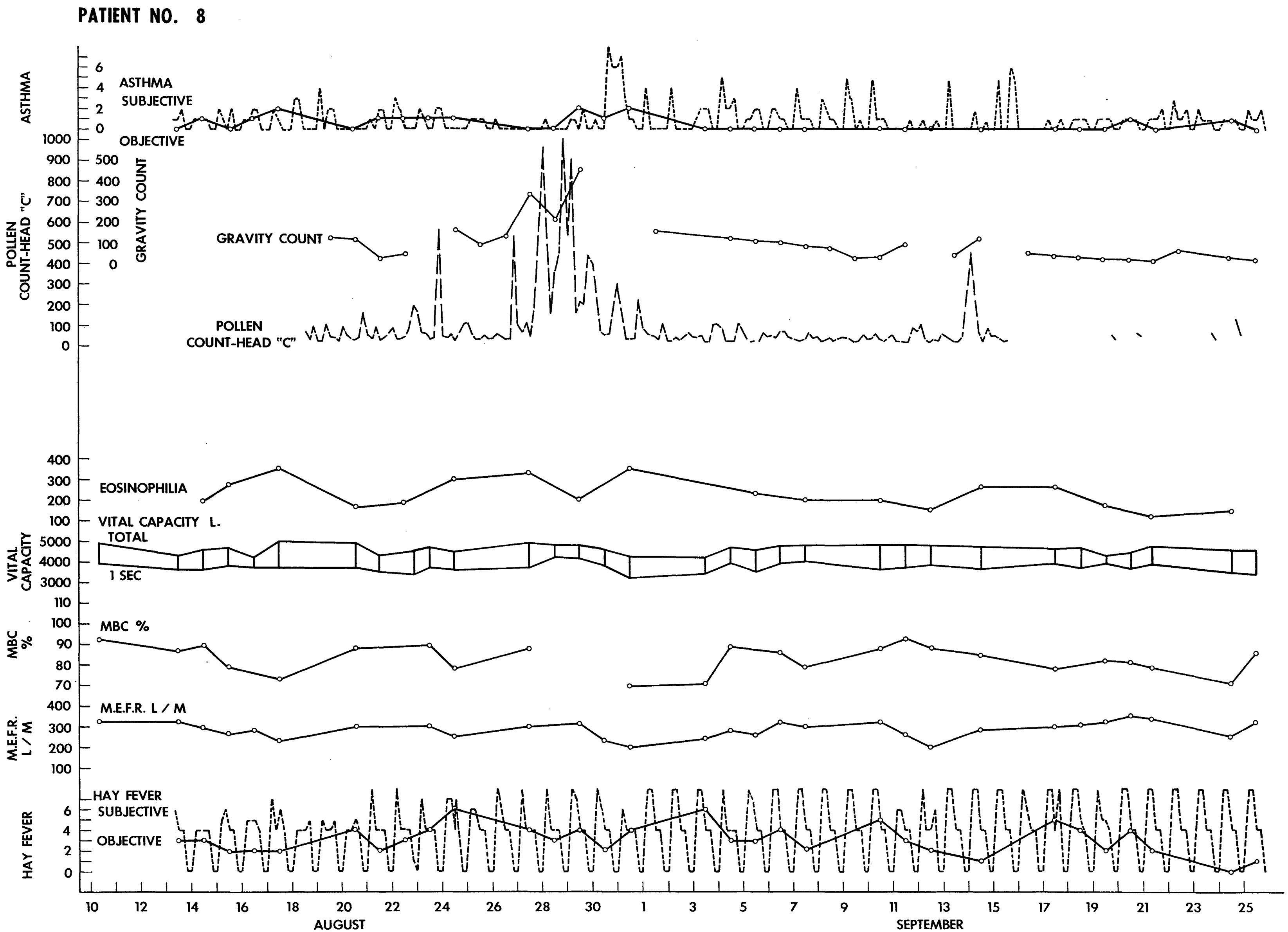

Fig. 2.1.2.13 Graphic presentation of patient No. 8. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

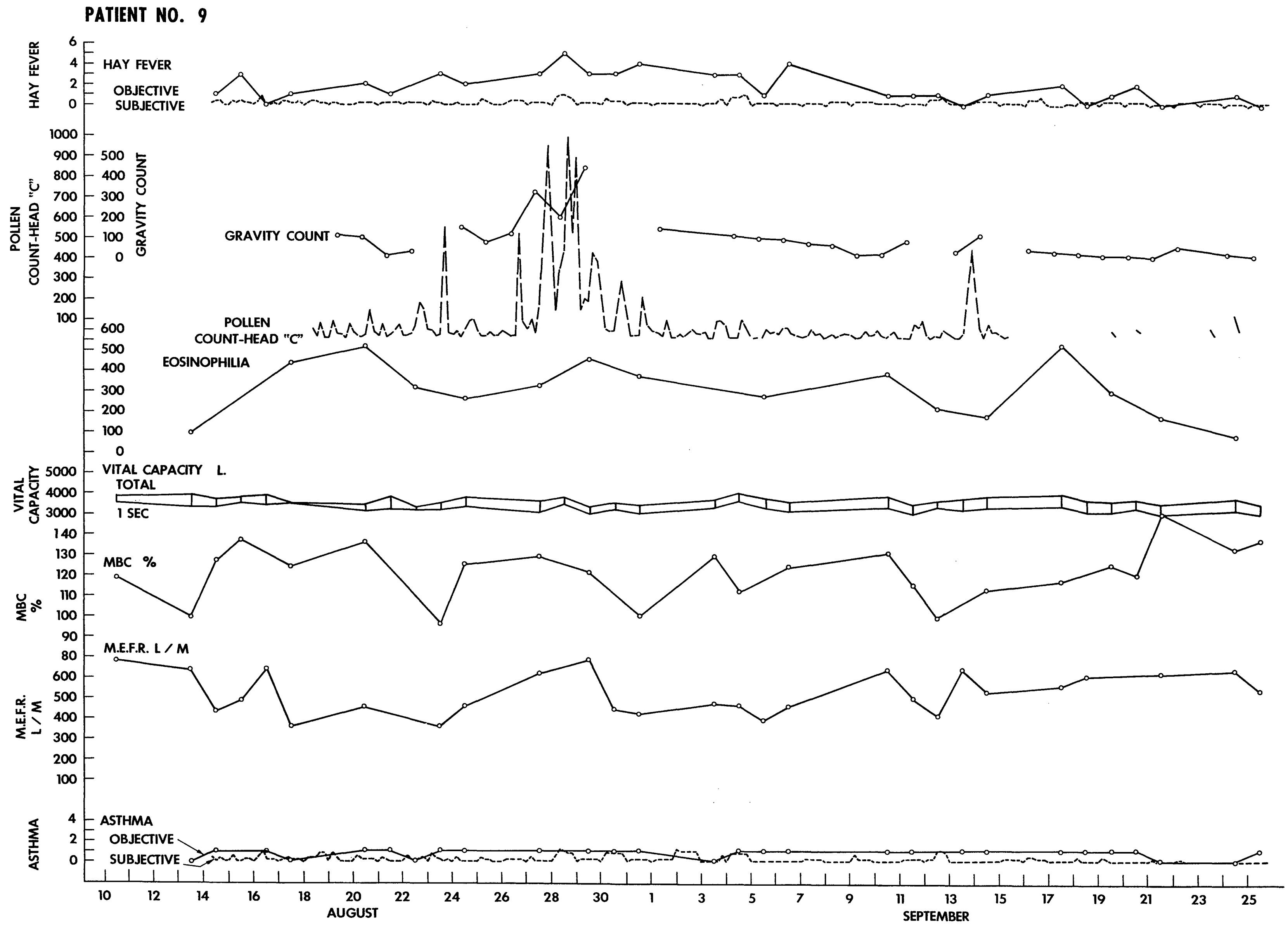

Fig. 2.1.2.14 Graphic presentation of patient No. 9. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

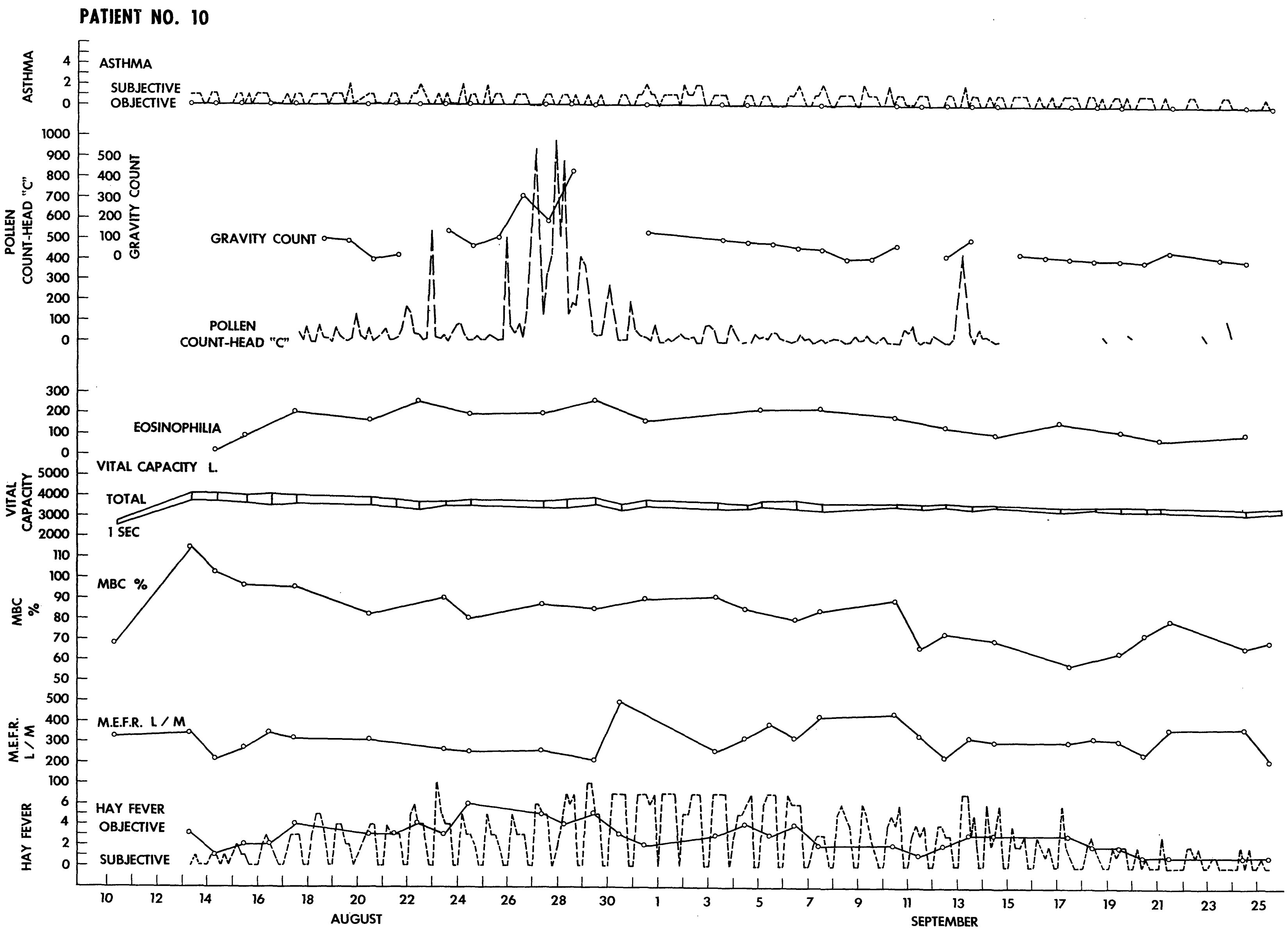

Fig. 2.1.2.15 Graphic presentation of patient No. 10. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

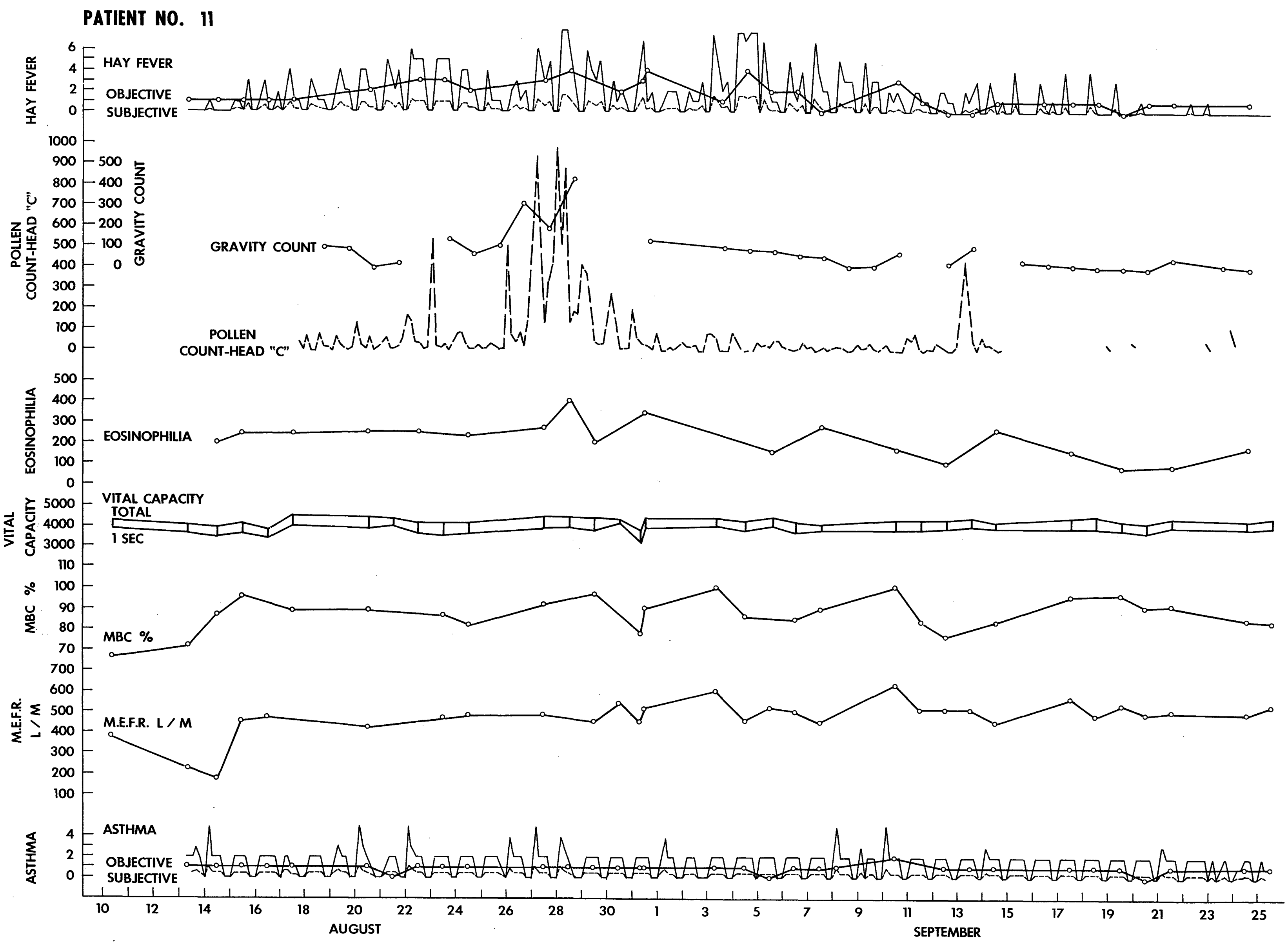

Fig. 2.1.2.16 Graphic presentation of patient No. 11. Daily fluctuations in subjective and objective findings, MEFR, MBC, timed vital capacity, total blood eosinophil counts, and the pollen count (volumetric and standard 24-hour gravity slide).

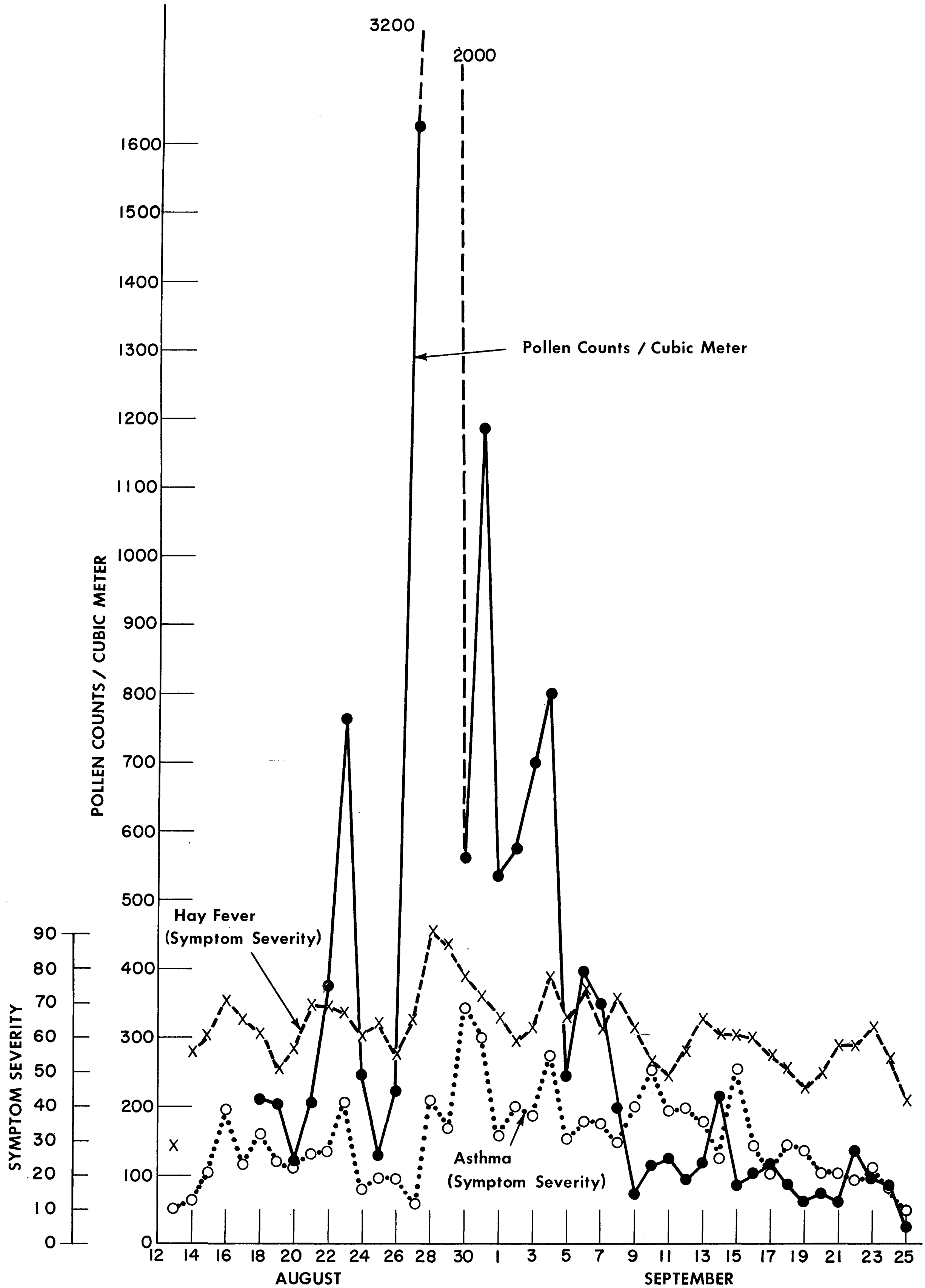

Fig. 2.1.2.17 Severity of asthma and hay-fever symptoms and volumetric pollen counts.

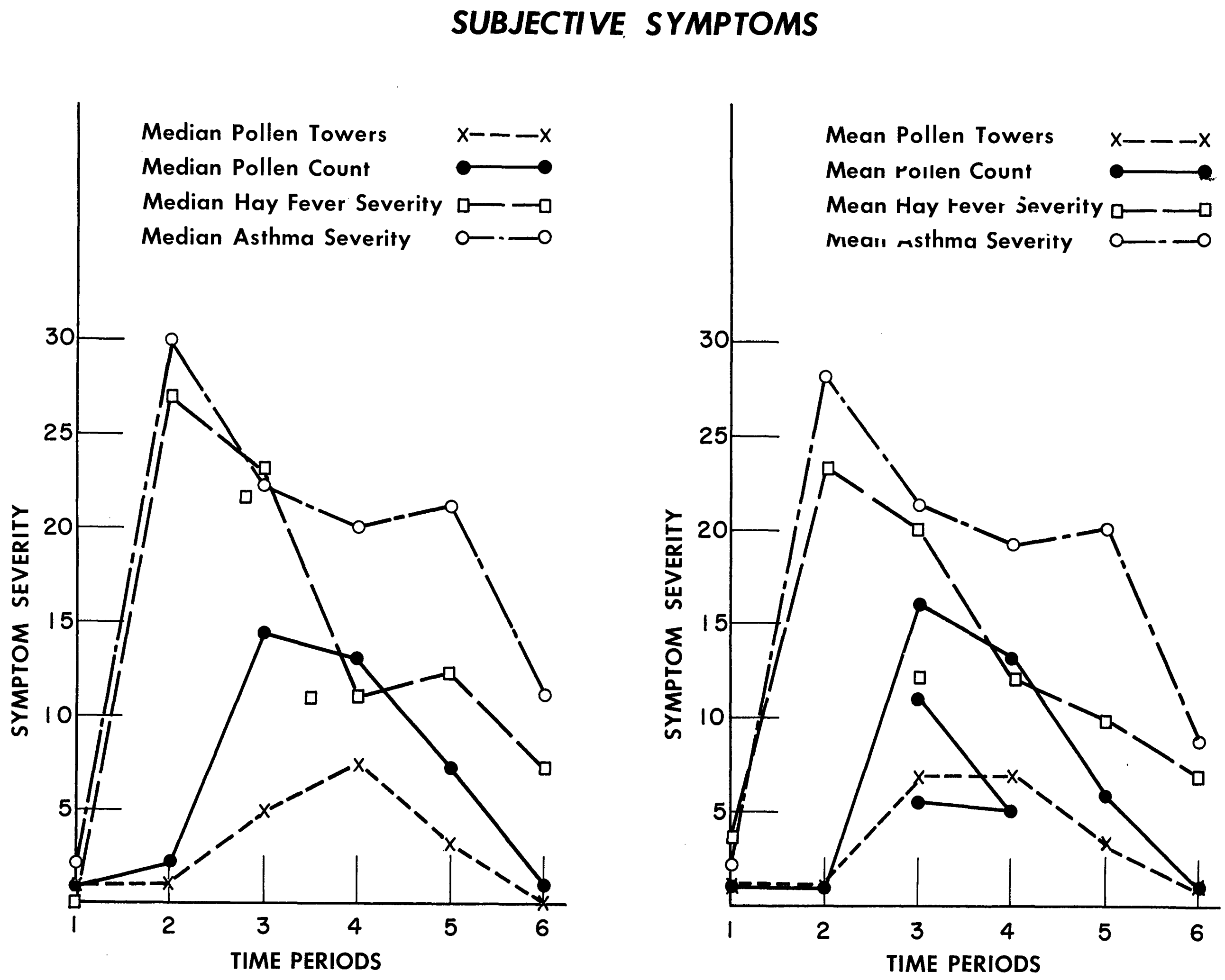

Fig. 2.1.2.18 Severity of subjective asthma and hay-fever symptoms by 4-hour time periods versus volumetric pollen counts for same time periods (average).

same lag, and it was even further delayed to the period from 12:00 noon to 4:00 P.M. This apparent discrepancy has been noted by others; a diurnal variation in symptoms with an increase upon arising in the morning (6:00 A.M. at the prison) may partially explain these findings. Other experiments conducted by the meteorological group (ragweed plants were made to mature earlier in the season and the pollen dispersal was studied in this "non-seasonal" interval) revealed that pollen was collected in highest concentration between 5:00 A.M. and 8:00 A.M. on slides exposed at the 2-foot-level. Thus, pollen emission occurs early in the morning and air turbulence carries this pollen to the higher elevations.

All these data are now being coded for statistical analysis and no conclusions have as yet been drawn.

2.2 STUDIES OF TAPETAL FLUID ANTIGENICITY

Another phase of medical activity has been concerned with the study of "tapetal fluid" antigenicity. Since pollen grains are bathed in this fluid in the anthers during their course of development, it seemed appropriate to try to ascertain the antigenicity of tapetal fluid. Obtaining this fluid in the absence of pollen grains presented a major technical problem. The botanists assisted by preparing sections of the floral parts of developing ragweed plants. The potential volume of tapetal fluid from one anther obviously is very small, since the anthers have a diameter of only about 360 microns, and are largely filled by cells. A microforge was borrowed to make glass capillaries having a tip 10 microns in diameter. With the aid of a micromanipulator, it was possible to insert the capillaries into anther sacs. However, even with rather strong suction it was practically impossible to withdraw a microscopically visible amount of tapetal fluid. High viscosity of the fluid, the small diameter of the capillary tubes, and blockage of the orifice by pollen grains during suction may have accounted for the lack of success. It is of interest however, that when the tip of one capillary, which had been inserted into another sac without visible recovery of tapetal fluid, was placed in a drop of saline covering a prick in the arm of a ragweed-sensitive patient, a four-plus skin reaction resulted. Since immature anthers appeared to contain much more tapetal fluid than mature ones, a large number of ragweed flowers were dissected, and the mature and immature anthers placed in separate containers. These anthers have been preserved in the deep freeze for further study.

2.3 STUDIES OF THE ANTIGENICITY OF EXINE AND INTINE PORTIONS OF RAGWEED POLLEN

The third medical phase of activity has been the disruption of the ragweed-pollen unit through fragmentation for the testing of antigenic specificity of exine and intine portions. The procedures and methods used are as follows:

2.3.1 Tissue homogenizer. This method failed with dry, whole pollen and whole pollen in ether.

2.3.2 Ultrasonic vibrations. Numerous methods were tried with units ranging from 10 to 55 kilocycles per second. At 55 kilocycles per second, we were able to distort the unit shape of the whole pollen unit, although no disruption of the integrity of the exine wall occurred.

2.3.3 Blender homogenization. A Standard Waring Blender motor base coupled with a semi-micro water-cooled container head was used as a homogenizing unit. With this basic unit, fragmentation was accomplished when the whole pollen was suspended in acetone and mixed with Super-Brite glass beads in a ratio of one gram of pollen to 43.5 grams of glass beads and 20 cubic centimeters of acetone. With the mixture temperature at 7 - 10°C, the disruption would be approximately 99% complete in 45 minutes of continuous running of the unit.

After fragmentation, the resultant solution was repeatedly washed with acetone to obtain exine-intine fractions in acetone without the glass beads and then final separation was accomplished after centrifugation with the intine fractions staying in the upper and exine in the lower portion of the centrifuged specimen. After multiple separations, a relatively pure intine fraction was obtained. The exine fraction could be concentrated only with the production of a solution containing approximately 50% exine and 50% intine portions.

The antigen fractions are currently being extracted from the two resultant products, and a projected method of determining specific antigenicity of the two fractions by a passive transfer neutralization procedure is being formulated.

2.4 POLLEN RECOVERY FROM RESPIRATORY TISSUES

Recovery of pollen from respiratory tissues has been the fourth area of medical activity. It is often assumed that, in the pathogenesis of bronchial asthma due to ragweed pollen, the pollen grains actually reach the bronchiolar mucosa and there elicit a local antigen-antibody reaction. However, a review of the literature revealed no data showing recovery of pollen grains from the lower respiratory tract of man, and the size of these particles is such that one would not expect them to reach the bronchioles at least under physiologic conditions.

During the 1955 ragweed season, a preliminary attempt was made to recover pollen grains from the nasal mucosa, pharynx, and major bronchi simply by making smears of secretions from these areas taken from autopsy material of patients dying during the ragweed season. In general, no pollen could be recovered from the lower respiratory tract by this method (beyond rare grains which did not exceed the "background" count due to contamination of the specimens from the air during handling and counting).

In 1956 it was hoped to work out a reliable method for recovering pollen quantitatively from tissues, before the ragweed season. Initial observations indicated that pepsin digestion of animal lung tissue was not adequate for this purpose. Boiling in 10% KOH was then evaluated. Ragweed pollen could survive this treatment under some conditions, and guinea-pig lungs were digested quite well after boiling for 15 minutes in 10% KOH. However, as the digest cooled, considerable precipitate formed. The pollen was recovered by filtration (with negative pressure) through Millipore filters. The filters tore if strong suction was used, but allowing the digest to drip from a separatory funnel on to the center of the filter proved satisfactory. The filters were dried, mounted, and stained by the method of Cryst and Gurney, and the pollen counted right on the filter. However, the Millipore filters were dissolved by hot 10% KOH, and the precipitate which formed on cooling caused the filters to clog. Attempts to remove the debris with organic solvents were unsuccessful. The clear, hot KOH digests could be filtered through ordinary filter paper, but finding the pollen under these conditions was unsatisfactory due to the rough texture of the paper. Three attempts were made to utilize the cooled KOH digests for Millipore filtering by centrifuging off the pollen and debris, redigesting in fresh 10% KOH, repeating the centrifugation and resuspension once or twice more, and then filtering through the Millipore filter. Many variations in technique were tried. Special 100-solution centrifuge flasks with a tapered bottom leading into a long stem proved to be of help. Rapid cooling of the KOH digests also reduced the amount of precipitate. The final filtration was more rapid when the grains were suspended in water rather than in 10% KOH, but this also caused many of the grains to appear as "ghost" forms on the filter paper. Ten-percent NaCl solution was found to be most satisfactory for the final suspension and filtration. An attempt was also made to reflux the tissue with ethylene-diamine, since this has been reported to be useful in removing the organic matrix of bone. Guinea-pig lung refluxed with this solvent in a Soxhlet apparatus was quite thoroughly digested, but this approach was abandoned when it was found that ethylene-diamine dissolves ragweed pollen.

The most satisfactory technic which could be evolved from this experience was as follows:

1. one guinea-pig lung or trachea boiled for 15 min in 90 ml of 10% KOH;

2. centrifuge in special flask 10 min at 2000 rpm;

3. resuspend in fresh 10% NaCl;

4. same as 2;

5. resuspend in 10% NaCl and filter through one-in. Millipore filter (together with washings); and

6. dry, mount, and stain filter and count pollen.

Of 134 ragweed pollen grains added to one guinea-pig trachea, 128 were recovered by this technic.

The initial application of this technic was in studies in which guinea pigs were exposed in a chamber to high concentrations of ragweed pollen in the air. Diminishing pollen concentrations due to fallout were reduced by blowing a jet of air into a beaker containing pollen within the chamber every five minutes. To avoid misleading results from possible rapid removal of pollen from the lungs by ciliary action, some of the animals were killed as rapidly as possible by a blow on the head, and the lungs and trachea were removed immediately.

Possible contamination of pulmonary tissues by pollen grains on the animal's body hair was minimized by careful technic in dissection and washing the external surface of the removed tissue under running water. Under these conditions several hundred pollen grains were recovered from guinea-pig lung and trachea preparations. However, by this time (August 21-31) significant amounts of pollen were present in the air, and a control animal not placed in the chamber had several hundred pollen grains in its trachea when sacrificed on August 31. It was concluded that these experiments would need to be repeated after the pollen season to get valid results.

Human autopsy material was collected during the peak of the 1956 ragweed season. One entire lung, a segment of lower trachea, a piece of nasal mucosa, and a piece of exposed muscle (as a control) were collected from five cases with notations made as to the length of time the patient had been in the hospital before death, his home town, and time of death. Initial attempts to recover pollen from this human tissue at that time revealed such high counts in controls due to contamination from extraneous sources that this work was abandoned for the time being. The material has been covered and stored in the deep freeze. Unfortunately, the technic for recovering pollen described above is applicable for use only with small amounts of tissue (on the order of 2 grams). However, a number of representative portions of the human autopsy material will be examined by this technic.

3. METEOROLOGICAL PHASE

by

E. W. Hewson, H. W. Baynton, S. Calvert, W. A. Cook
A. N. Dingle, G. C. Gill, F. M. Hemphill, R. Lewis,
V. C. Liu, A. H. Pierson, and J. Ruffner

3.1 INTRODUCTION

This section of the report describes the investigations into the dispersion of ragweed pollen, which is the principal task of the meteorological group. In addition, there is an account of the engineering activities of the group which has served as consultant in all engineering matters and especially those pertaining to instrumentation. The study of dispersion in its broadest sense includes analyses of the dependence of the annual ragweed crop on weather, of the dependence of daily pollen releases, within the ragweed season, on weather, and of the manner of dispersal of pollen once it has become airborne. To date most effort has been expended on the last phase. However, present plans call for a beginning this spring on the other two important phases.

The attack on the problem of dispersal of airborne pollen has led to extensive developmental work in pollen sampling methods. A complete account of this work to date is included in this report, as well as a discussion of new avenues still being explored. Specifically, since the best available methods ultimately depend on a laborious and time-consuming counting of pollen grains under a microscope, there is an urgent need for a suitable automatic counting device.

A great deal of work has also been done in designing and installing meteorological instrumentation that is appropriate for a study of diffusion of airborne particles. A full description of this instrumentation is included later in this report. Further work along these lines is anticipated as studies are begun on the ecology of the ragweed plant.

Data are now available for analysis from four separate field experiments, three of which were conducted during the two natural ragweed seasons since the project began, and one of which, using artificially grown ragweed plants as a pollen source, was conducted in June of 1956. An account of the preliminary analyses of these data is presented in some detail. These experiments have also served to improve the techniques and design of succeeding experiments with a resulting improvement in the quality of the data. As a consequence, more rigorous analysis of future field experiments will be possible than has seemed advisable up to the present.

3.2 CHRONOLOGY OF ACTIVITIES

In the spring of 1955, when the possibilities of this study were being explored, the present project supervisors negotiated with the authorities of the State Prison of Southern Michigan near Jackson, located 30 miles west of Ann Arbor, and were offered prison facilities and cooperation for a research project of this type. The meteorological group was also engaged in another project at the time, which had led to considerable developmental work in the problems of sampling for pollen. As a result of these earlier activities, approval of the grant in July of 1955 permitted some measurements of a preliminary nature during the natural ragweed season.

Pollen samples were collected in Ann Arbor on a tower on the East Engineering Building (Fig. 3.1) and at a point 4 feet above the ground on the adjacent street. Temperatures and winds were also observed at selected heights above the ground. At the same time pollen samples were collected at Jackson Prison where the medical team was observing the allergic response of a group of patients. All the samples referred to above were volumetric, i.e., the number of pollen grains in a measured volume of air was measured. Samples of one-half-hour duration were collected every 4 hours at each of the above locations.

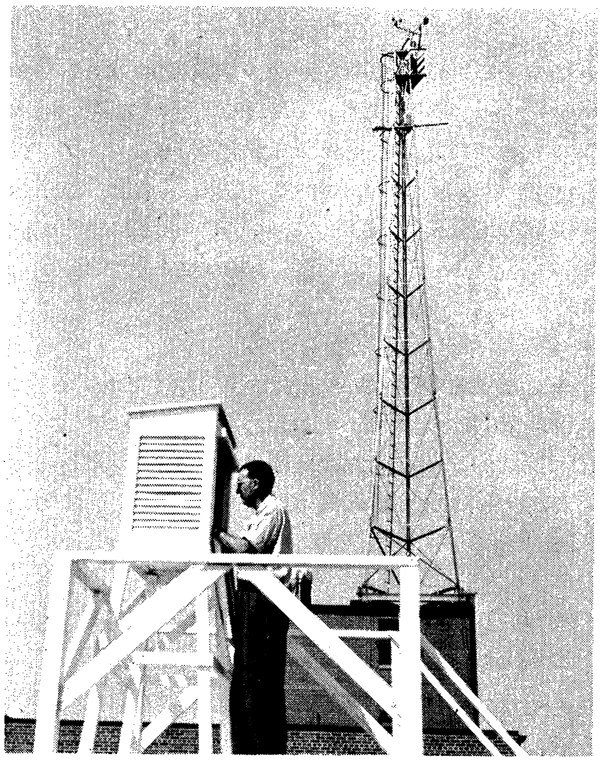

Fig. 3.1. Meteorological tower on top of the East Engineering Building, Ann Arbor.

During part of this period an experimental-statistical study of pollen behavior was undertaken on the roof of the East Engineering Building. For this study, the Graeco-Latin square experiment described in detail later, pollen counts were based on gravity slides rather than on volumetric samples.

The work of evaluating the results, the techniques, and the equipment used in this first set of experiments occupied the late months of 1955 and the early months of 1956. Preliminary analysis of pollen counts and careful testing of the various components of the sampling facility indicated occasional failure of the solenoid valves used to rotate the air suction among the six filters at each sampling point. In due course, methods were devised to improve the performance of the valves. In addition, the program of sampling for one-half hour out of each four-hour period, necessitated by the inability of the vacuum pumps to perform under continuous operation, came under review. A complete review of the instrumentation was undertaken in January of 1956 when Mr. G. C. Gill joined the project on a full-time basis. As a result, new heavy duty pumps capable of continuous operation were purchased, and new methods of sampling air and measuring air volumes were developed.

The processes by which pollen is removed from air, either in the open atmosphere or in the human nasal passages and lungs, are of fundamental importance. Theoretical studies of the processes of removal by agglomeration and impaction were begun at this time.

During this period plans were made to erect a 100-foot guyed tower in an open field on the prison farm at Jackson, and details of the meteorological and pollen sampling instrumentation on the tower were worked out. Quotations on this equipment were obtained and orders placed. Arrangements were again made with the authorities of the prison at Jackson to conduct a more detailed type of experiment during the 1956 ragweed season.

At the same time another experiment was planned and designed to study the dispersion of ragweed pollen from a point source. The plan was to force a number of ragweed plants to maturity in a greenhouse, place them outdoors in June when there was no natural ragweed pollen present, and observe the concentration of ragweed pollen at various distances from the plants under varying weather conditions. This procedure achieves the same results as tagging ragweed pollen with radioactive isotopes as tracers, but without the complications inherent in handling and dispersing radioactive materials. This experiment was carried on between June 26 and 29 on the North Campus and is described later in this report.

The mid-summer of 1956 was devoted to counting over 1000 pollen slides exposed in the North Campus experiment, to the erection and instrumentation of the tower at Jackson, to the testing and calibration of new equipment, and to the formulation of final operational plans for the cooperative medical-meteorological experiment at Jackson Prison. Figure 3.2 shows the relative locations of the prison and tower. Certain items of equipment, ordered many weeks in advance, did not arrive in time, and some modification of the original plans was required.

Volumetric pollen samples were taken in the prison hospital from August 17 to September 25, 1956. The tower instrumentation, including pollen sampling, was operative from August 30 to September 17. Again, over 1000 pollen samples were collected, each of which had to be counted under a microscope. Since an experienced counter completes one pollen slide in 10 to 20 minutes, depending on the number of grains, this constitutes a major undertaking.

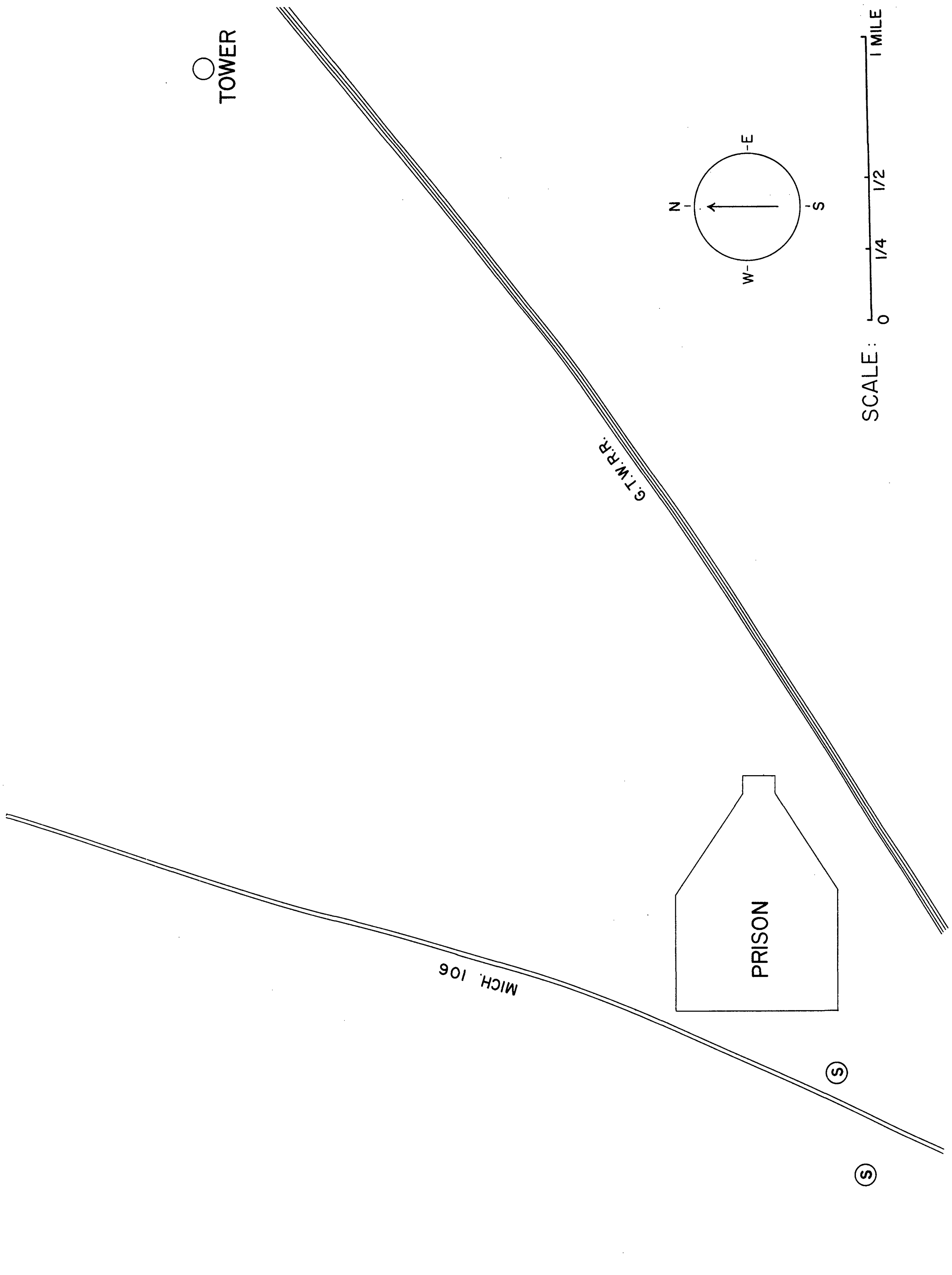

Fig. 3.2. Location of the meteorological tower and portable sampler sites, S, in relation to Jackson Prison.

Evaluation of the results obtained at Jackson began at the conclusion of the experiment and has continued since. For each pollen sample collected, not only were the grains per sample counted, but also the volume of air sampled was evaluated. Weather records were also compiled as detailed later, and the work of relating pollen concentrations to weather was begun. In addition, late in 1956 a first report on the North Campus experiment was prepared for the internal use of the research group. Highlights from this report are presented later.

Considerable attention has been devoted to the design of a test chamber within which subjects may be exposed to known concentrations of ragweed pollen under varying conditions of temperature and humidity. The important features of the design are described later in this report. This developmental work has stimulated interest in the search for an automatic device for counting pollen grains. Further refinements are being sought in our sampling techniques so that spatial and temporal fluctuations of pollen concentration may be documented more accurately. An impinger of original design, to provide a continuous record of pollen concentration rather than the present six discrete four-hour samples per day, is nearing completion. Defects in the design and technique of earlier experiments which become evident on analysis of the data are being noted and continue to lead to improved design of equipment and to more adequate techniques.

3.3. STUDIES PLANNED FOR 1957

Plans for the coming months include the following major enterprises:

1. Fabrication, calibration, and operation of the test chamber.

2. Another dispersion study of pollen released by ragweed plants which have been forced in the greenhouse. Once again the experiment will be in June, but this time it will be at the tower site in Jackson to take advantage of the special instrumentation available there. Attempts will be made to observe the rate of release of pollen by the plants and relate this to weather factors.

3. Continued theoretical studies of pollen removal from air by agglomeration and impaction processes.

4. The collection of pollen samples during the ragweed season over a larger area to permit more adequate statistical analyses of the horizontal homogeneity of pollen concentrations.

5. The taking of pollen samples at still greater heights from an aircraft, to further examine trends in vertical pollen distributions found from the tower samples in 1956.

6. Another complete study at Jackson during the ragweed season with increased meteorological instrumentation.

7. Cooperation with the botanical group in beginning the study of the ecology of the ragweed.

3.4 MEASURING CONCENTRATIONS OF POLLEN

3.4.1 Historical

Ever since the importance of pollen to the victim of hay fever first became clear, the amount of ragweed in the air has been measured. Traditionally this has been done by horizontally exposing for 24 hours a glass slide greased with petrolatum and counting the number of grains deposited thereon. Such a procedure has for many years provided a rough basis for classifying days as good, bad, or indifferent, and areas of the country as good, bad, or indifferent from the standpoint of the hay-fever patient. But this procedure does not express the relative incidence of the pollen as a volumetric concentration. For this reason it neither documents for the allergist the amount of pollen a patient may breathe, nor does it provide the meteorologist with the explicit concentration data he must have to study pollen dispersal adequately.

Volumetric sampling for ragweed pollen, being more complicated, has generally been limited to research studies. Durham,[1] after making simultaneous gravity and volumetric measurements of pollen, tried to correlate the measurements, but found very great inconsistencies. However, two volumetric samples from devices of totally different design, one of which sampled more than ten times as much air as the other, were approximately equal. His results indicated that there is no practical way of converting gravity-slide figures into accurate volumetric equivalents.

Experience to date indicates that gravity slides give a relative measurement of the amount of pollen in the air over local areas and may be used to compare amounts over nearby areas. However, when studying the dispersion of pollen, a quantitative measurement of concentration is required. This led to the development of the volumetric sampling procedure described below. In some experiments volumetric samples and gravity slides may both be advantageously used.

3.4.2 Volumetric sampling of ragweed pollen

The following is an account of the present experimental procedure followed in volumetric sampling. The procedure will be discussed in four parts:

1. the millipore filters;

2. the filter head and mounting techniques;
3. the sampling techniques (described in Section 3.5.1); and
4. the mounting and counting of samples.

3.4.2.1 The millipore filters.—The filters used in this study are made by the Millipore Filter Corporation located in Watertown, Massachusetts. They are available in boxes of 100 in either black, white, or white with a black grid. After trying all three styles, the one-inch-diameter, white aerosol filter was chosen because it provided a better background for the microscopic counting of the pollens.

A millipore filter is a membrane made from cellulose esters of approximately 150-micron thickness (0.150 millimeter). Even though the pores cannot be seen at 100-power magnification, the volume of the pores in this filter is approximately 85 percent of the total volume of the filter. The pores have a calculated size of 0.8 micron. Since the pollens have a diameter of approximately 20 microns, this filter is ideal for our application.

3.4.2.2 The filter head and mounting techniques.—The millipore filter holder, commonly called a filter head, is made up of three parts: the cap, the body, and the filter support. The cap and the body screw together, holding the filter support and the filter between them (see Fig. 3.3).

The body resembles a hollow hemisphere with an outside diameter of approximately one inch and an inside diameter of approximately one-half inch. On the outside of the hemisphere, at the open end, are threads by which the cap of the filter head screws on to the body.

Within the open face of the hollow hemisphere, a disk of porous bearing material supports the millipore filter so that it will not tear when air is passed through it. Suction is applied at the bottom of the hemisphere through a hole tapped for a short length of one-eighth-inch brass pipe.

The filter support is a thin metal disk with a hole in the middle. It has approximately the same outside and inside dimensions as the body of the filter head. The filter support, which clamps the millipore filter to the body, is mounted over the disk of bearing material by means of two positioning pegs. A small trough is cut into the filter support on the side that goes next to the body. A round rubber ring, fitting in this trough, insures a vacuum-tight seal when the filter head is assembled and operating.

The cap resembles a wide-mouth jar cap, but also has a one-inch-diameter hole in the middle. The cap is screwed onto the body of the filter head, clamping the filter and the filter support between them. When assembled in this way, all the pollen grains contained in a volume of air drawn through the filter will remain on the surface of the filter. A detailed account of the measuring of the air volume sampled is included in Section 3.5.1.

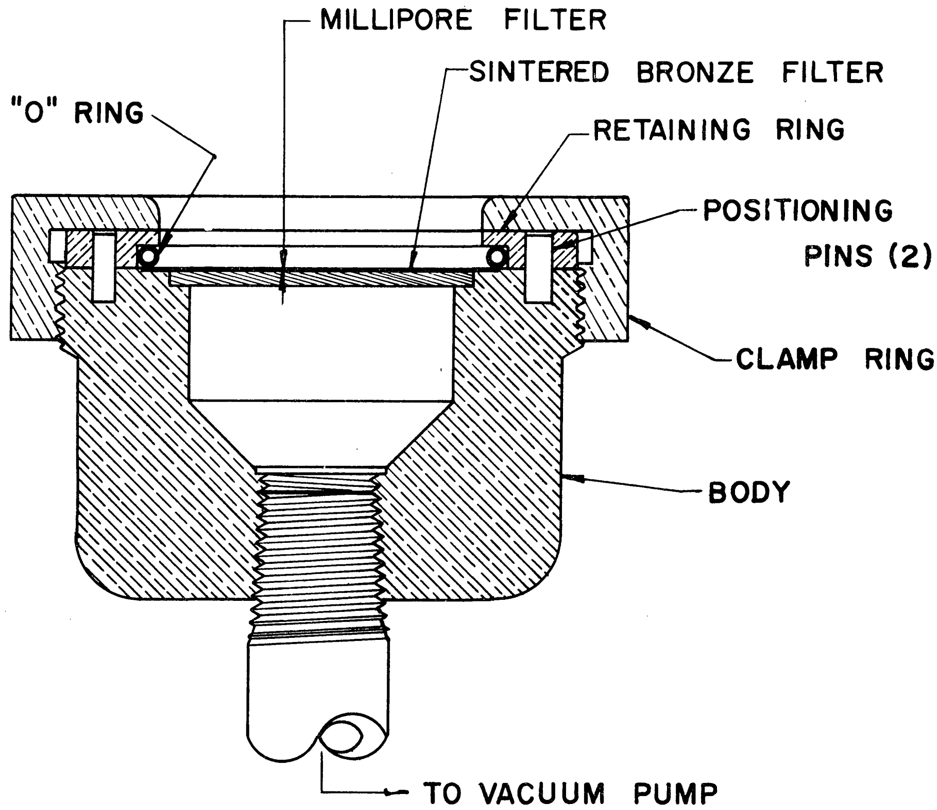

Fig. 3.3. Cross section of sampling head used with millipore filters.

3.4.2.3 The mounting and counting of samples.—The round millipore filters are removed from the sampling head and mounted on standard-size (1 by 3 inches) microscope slides in the following way. After unscrewing the cap from the rest of the filter head, the millipore filter is lifted from the sampling head by touching it with a two-inch strip of one-inch-wide Scotch-brand cellophane tape. The tape with the filter stuck to the bottom side is then fastened securely to one of the microscope slides. Two filters may be mounted per slide. The slides are then stored in this way until counted.

It has been the experience of this group that counting pollen grains on the filters is facilitated by staining the filters. When the red dye, Calberla's solution, is used, the pollens are dyed a deep red while the filters themselves are dyed pink. Dirt and other foreign matter remain undyed. The filters are dyed by simply lifting up the cellophane tape, painting the back of the filters with the dye, and then retaping the filters to the slides.

The counting procedure consists of noting each single pollen and each cluster of pollen grains that occur on the filter. A record is kept of the number of single grains, and the number of clusters of 1, 2, 3, 4, etc., pollens that occur. The entire area of the filter must be examined by moving the field of the microscope. An experienced counter may take from 10 to 20 minutes per filter, depending on the number of grains present. Figure 3.4 shows the kind of permanent record that is made for each slide counted.

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RECORDS OF POLLEN COUNTS

IDENTIFYING NO.__________ DATE COUNTED__________
Mo. Day Yr.

NUMBER OF	NAME OF COUNTER________
SINGLE GRAINS________	TOTAL PARTICLES________
CLUSTERS OF 2 ________	TOTAL GRAINS________
" " 3 ________	REMARKS: ________
" " 4 ________	
" " 5 ________	
LARGER CLUSTERS________ (Specify).	

Fig. 3.4. Card used to record pollen counts of each sample.

3.5 INSTRUMENTATION

3.5.1 Volumetric pollen sampling

3.5.1.1 Pollen sampling in 1955.—Details of the basic equipment for making volumetric determinations of pollen concentration are as follows. A vacuum pump driven by an electric motor supplied the necessary vacuum. A regulator was adjusted to maintain a vacuum in the system of about 30 centimeters of mercury, as indicated by a vacuum gage. A flow-limiting orifice largely determined the rate of the flow of air drawn through the millipore filter. Any ragweed pollen in the air drawn through the system was collected on the outer surface of this filter.

To permit sampling of the air at regular periods each day without frequent monitoring of the equipment, this single head was replaced by a unit (Fig. 3.5) containing six filter heads, each with its own solenoid value, all connected to a common vacuum line. By electrically connecting these solenoid valves to a two-component repeat cycle timer (Fig. 3.6), these filter heads were connected one at a time in rotating sequence to the vacuum line. In routine use it was found that the vacuum pumps overheated and produced an unsteady vacuum if operated for periods exceeding forty minutes. Accordingly,

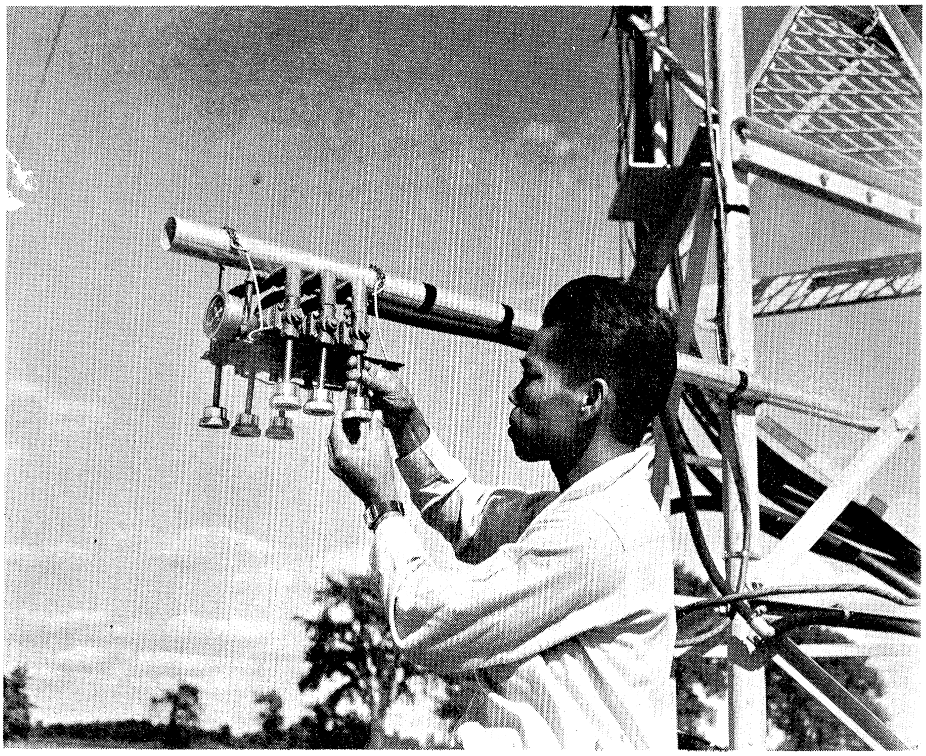

Fig. 3.5. Six-head volumetric pollen sampling unit.

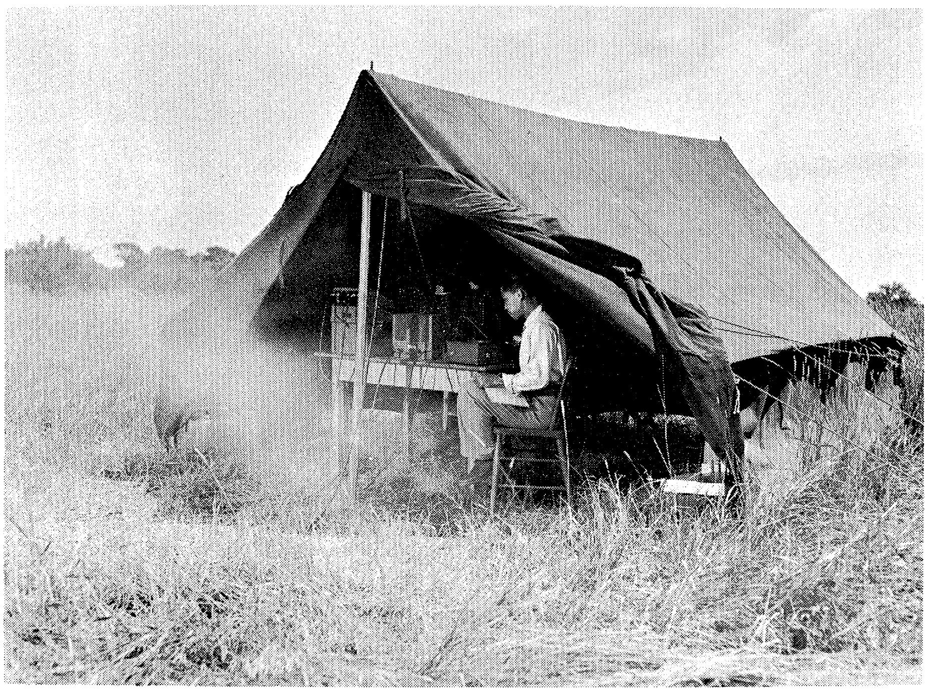

Fig. 3.6. North Campus experiment. Tent housing recorders.

one timer was adjusted to operate the pump for a period of thirty minutes every four hours, and the second timer was set to switch in simultaneously a different filter head for each successive four-hour period. Thus individual pollen samples were obtained for the following periods each day: 00.00 (midnight) - 00.30; 04.00 - 04.30; 08.00 - 08.30; 12.00 - 12.30; 16.00 - 16.30; and 20.00 - 20.30 hours.

3.5.1.2 Pollen sampling in 1956.—Since there were only about four weeks to select, obtain, and assemble the pollen-sampling equipment for the 1955 season, there had been no time to test it properly before installation in the field. Some parts worked very well, others, fairly well, and some, poorly. The millipore filters and the filter heads (shown in detail in Fig. 3.3) worked very well. The characteristic of the flow-limiting orifices, whose function is to maintain constant flow rates, had not been determined. The variation in rates of flow of air through different millipore filters for a fixed vacuum was also unknown. If the pumps were operated for more than 40 minutes at a time, consequent overheating produced large variations in vacuum. The only possible solution with this equipment was to sample for pollen for a period of 30 minutes, then shut down for 3 hours and 30 minutes.

From November, 1955, to August, 1956, many tests were conducted to evaluate the performance of the equipment and improve it for the 1956 season.

Tests were made on flow-limiting orifices, both the commercially available screw-in type, and the equally effective length of capillary glass tubing, ground square at both ends. Two main conclusions may be drawn from these tests: (1) both types act as flow-limiting orifices; and (2) the rate of flow of air through any particular orifice remains constant regardless of the vacuum, provided the pressure drop across the orifice does not fall below 45 centimeters of mercury. For the 1956 season it was decided to try to maintain a vacuum of 50 centimeters of mercury at all times to insure constant air flow. The vacuum of 30 centimeters of mercury used in 1955 was insufficient.

Two sizes of flow-limiting orifices (capillary type) were selected for the 1956 season. The larger flow rate was used early in the season and again near the end of the season, when ragweed pollen counts were relatively low; the smaller flow rate was used during the peak of the season. This arrangement worked out quite well in providing pollen counts in the range of 0 - 400 per filter most of the time. The two flow rates were approximately 6.5 and 1.6 liters per minute, respectively.

With absorber periods of only 30 minutes per sample in 1955, relatively high rates of air flow through the filters were used. Laboratory tests showed that variation in rates of flow from filter to filter of ± 5 percent could occur at these high rates owing to density variations in the individual filters. By reducing the rates of flow to one-quarter or less of the former value, and by using the flow-limiting orifices at a higher vacuum, the variations in rates of flow through different millipore filters was reduced to less than one percent.

A study was made of vacuum pumps suitable for several weeks of continuous operation, at vacuums in excess of 50 centimeters of mercury, and with a capacity of at least 30 liters per minute. A Lieman pump Model No. 26—1-1/2 was purchased and tested. Since it appeared to meet our needs, several pumps of this type were purchased. These gave greatly improved service, and will be used again in 1957.

In connection with the portable samplers used near the prison in 1956, unless special precautions were taken, there might be some doubt as to the volume drawn through each millipore filter; errors in recording the times of starting and stopping the small gasoline engines, and occasional engine failures, were possible. To provide a reliable measure of the volume of air drawn through each filter, special dry-test meters were purchased. These Rockwell No. 150 meters are light and portable, but do not collapse if a high vacuum should accidentally be applied. One was installed in each portable sampling unit. In addition, for check purposes, one meter was installed in series with one of the six-head sampling units in the prison hospital, and two others were used in the same way at the tower installation. Their use will be expanded to every volumetric sampling unit in 1957.

3.5.2 Meteorological instrumentation

3.5.2.1 1955 Season.—Time did not permit any meteorological instrumentation at the prison. However, thermometer shelters were constructed and installed at three levels in the University area—at 4 feet and 30 feet above the ground adjacent to Dr. Hewson's office at 321 West Engineering Building, and across the street, at 144 feet above the ground on a 69-foot tower installed on top of the elevator shaft of the East Engineering Building (see Fig. 3.1). Thermographs recorded the temperatures at these three levels from August to October, 1955. Volumetric pollen samplers were located at heights of 4 and 144 feet.

3.5.2.2 1956 Season.—

Instrumentation for pollen dispersal studies at the North Campus, June 25-29, 1956

To record the meteorological variables associated with pollen dispersal, the following equipment was installed: a thermometer shelter with a recording hygrothermograph; a Beckman and Whitley three-cup anemometer; and a Gill bivane. These were placed near the point source of ragweed pollen, and, excepting for the thermometer shelter, may be seen in Fig. 3.18.

The recording hygrothermograph was a standard commercial instrument. The Beckman and Whitley anemometer is a very sensitive instrument, responding quickly to small changes in wind speed. The Gill bivane is a similar fast-response special-purpose instrument, able to follow slight fluctuations of wind direction both vertically and horizontally. It provides records of wind direction and turbulence. The sensitive elements of both instruments were 2 meters above ground. The recorders for both these instruments were located in the tent (see Fig. 3.6).

Instrumentation in and near the State Prison of Southern Michigan

In March, 1956, a 100-foot Type 3500-H Trylon Steel Tower was ordered from the Wind Turbine Company of West Chester, Pa. In May the meteorologists in consultation with Messrs. L. D. Johnson, Plant Engineer, and Clare Rossman, Farm Superintendent of the prison, selected a suitable site for the meteorological tower in a grain field northeast of the prison (see Fig. 3.2). The tower was erected in early July, and installation of meteorological equipment was begun immediately. The erection of a power line was completed in early July, but delays in obtaining permission for the power line to cross a railroad prevented operation of equipment until mid-August.

Various views of the tower, the instrument hut, and the meteorological equipment are shown in Figs. 3.7, 3.8, and 3.9. The tower is three-legged and has an internal ladder for climbing. Each of the three sides measures 35 inches. Prefabricated booms, each 8 feet in length, were provided for mounting anemometers at approximately 102, 50, 25, and 12 feet above the ground.

The instrument hut was located approximately 100 feet west northwest of the tower. The thermometer shelter (Fig. 3.7) was located about 12 feet to the south of the tower. The lower shelter immediately adjacent to the tower provided protection from the weather for the pollen-sampling vacuum pump and the Spencer Midget Turbo Exhauster that provided artificial ventilation for the temperature-measuring thermocouples.

The complete instrumentation on the tower was designed to provide profiles of temperature, wind speed, and pollen concentration. To obtain temperature profiles, thermocouples were installed at heights of 1, 25, 51, and 102 feet, with a reference junction in a standard thermometer shelter at a height of 4 feet. Ventilation and shielding of the thermocouples was provided by two concentric sleeves connected by 1-1/4-inch inside-diameter heavy-duty rubber tubing to a centrifugal exhauster located at the base of the tower. The temperature indications were registered on a Bristol Dynamaster electronic strip-chart recorder operating at a chart speed of 7-1/2 inches per hour. The one-millivolt full-scale deflection feature of this recorder provided a calibrated range of plus or minus 10°C from the reference junction, and a sensitivity of plus or minus 0.02°C.

Profiles of wind speed were provided by cup anemometers located at heights of 12, 25, 50, and 102 feet. The passage of each 1/6 mile of wind was registered on a ten-channel Esterline Angus operations recorder. With this type of equipment, instantaneous wind speeds are not registered. Wind passage in miles during each hour is totaled for each height, thus providing mean hourly speeds at four heights, and hourly data on wind profiles. Profiles of pollen concentration were obtained by collecting samples at heights of 4, 50,

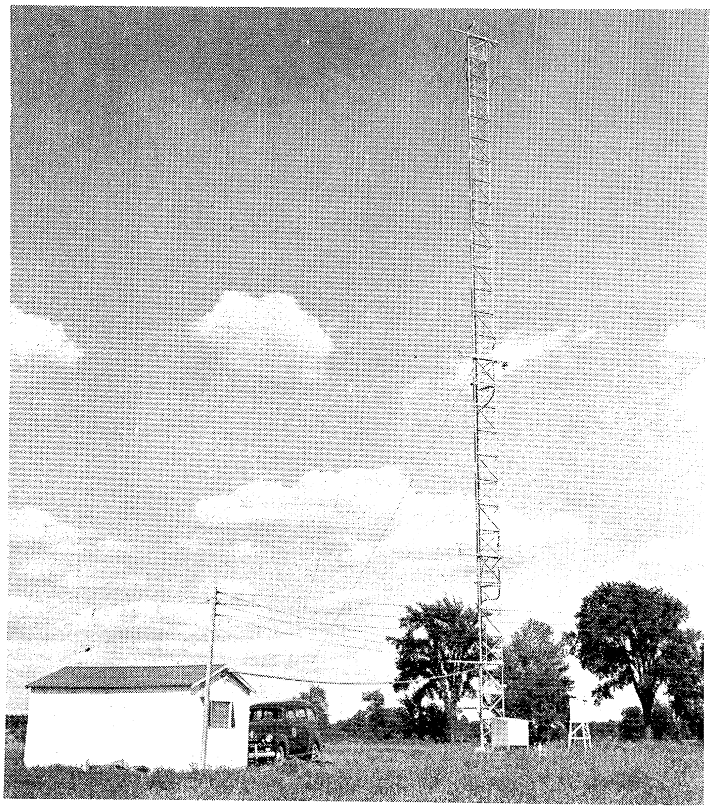

Fig. 3.7. Meteorological tower at Jackson, showing prefabricated hut, thermometer screen, and shelter for vacuum pumps.

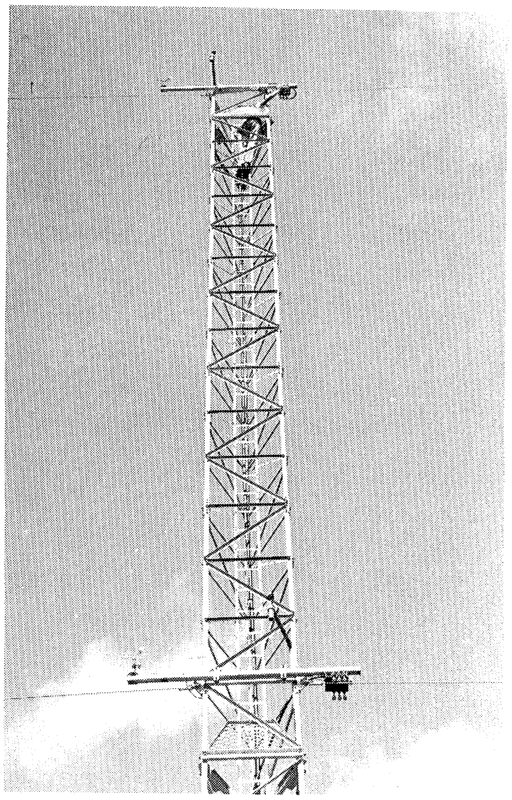

Fig. 3.8. Tower at Jackson showing booms supporting anemometers, 6-head sampling units for pollen, and temperature element at top.

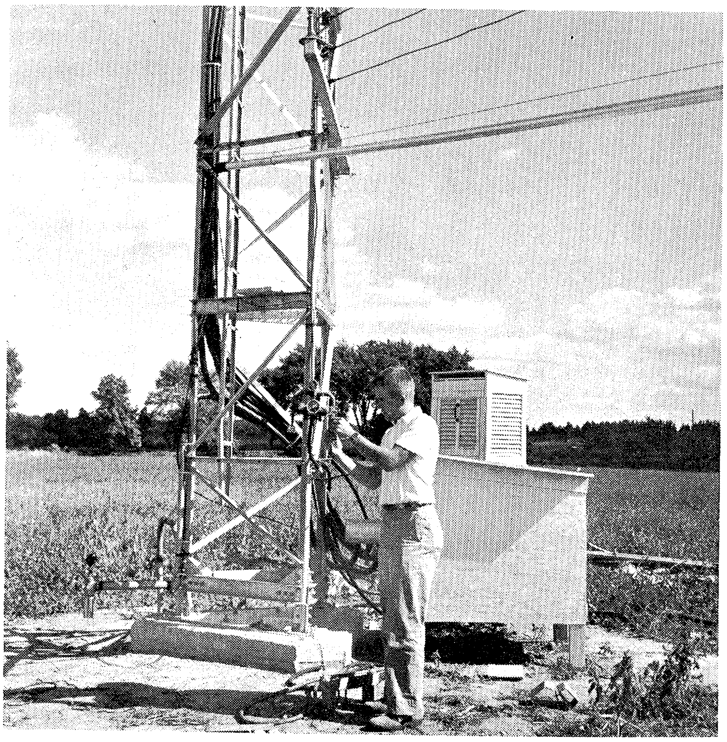

Fig. 3.9. Technician changing filters in pollen sampling head at base of tower.

and 102 feet. A view of some of the recording instruments is shown in Fig. 3.10.

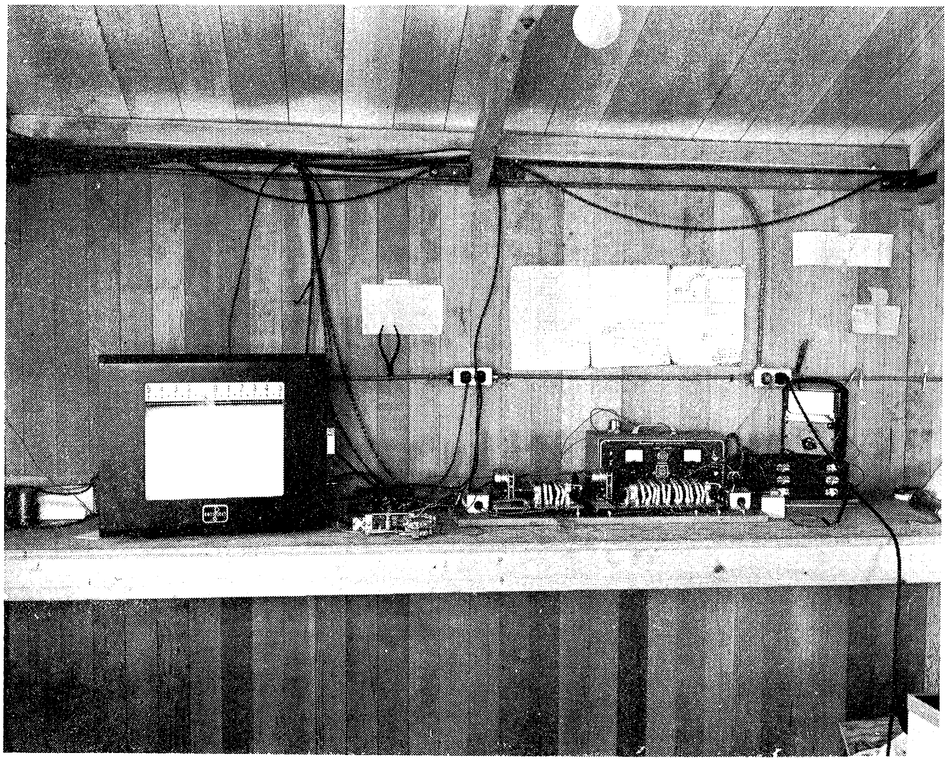

Fig. 3.10. Jackson tower installation. A view of recording equipment and timers in the hut.

Six pollen samples per day were collected at each of the three heights in the following way. A vacuum pump located in a shelter at the base of the tower applied suction to a distributing manifold. From there separate lengths of 3/4-inch inside-diameter polyethylene tubing led directly to the three sampling locations. Connections to the sampling head were made through a limiting orifice and a dry test gas meter. There were six sampling heads at each location so that six discrete samples of approximately four hours duration could be taken each day. Millipore filters were installed once daily in each of the sampling heads. A solenoid valve controlled by two time switches applied the suction in turn to the six filters, changing filters every four hours. The arrangement of the time switches provided for 3 hours and 47 minutes sampling on one filter and 13 minutes for changeover to the next sampler.

The tower instrumentation as described in the foregoing remarks was in operation from August 30 to September 17. During the same period additional pollen samples were taken at various points of interest with the portable sampling device (Fig. 3.11). This consisted of a vacuum pump driven by a 1.5 horsepower gasoline motor, both components being mounted on a small plywood platform. The suction was applied to a T pipe fitting, and thence through two lengths of rubber hose to separate millipore filters facing downward in standard sampling heads. The sampling heads were supported about 4 feet above the ground on tripods (Fig. 3.12), which were located about 100 feet apart. Since both sampling lines contained identical limiting orifices, air flow in the two lines

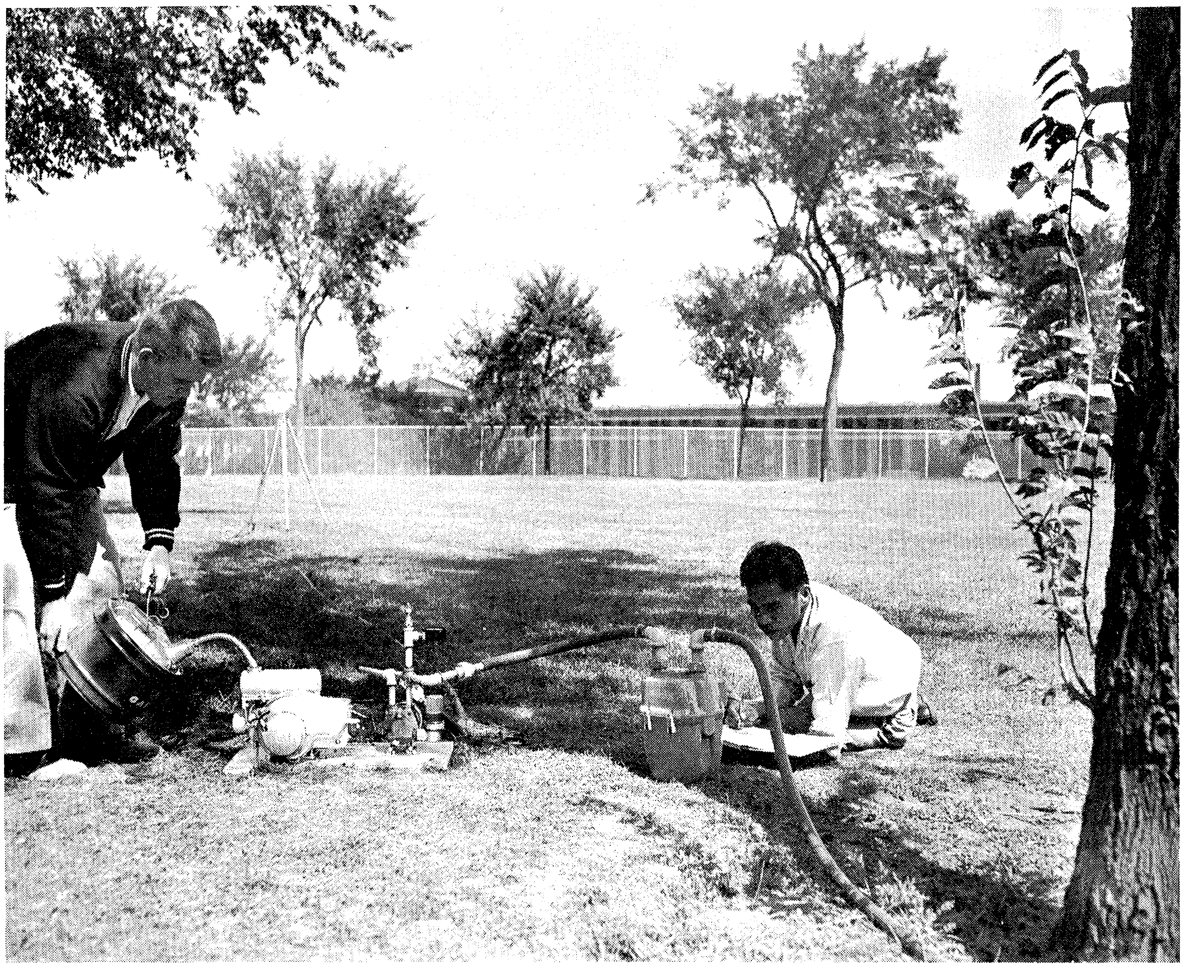

Fig. 3.11. Portable volumetric pollen sampler showing gasoline motor, dry test meter, and in the background tripod supporting sampler.

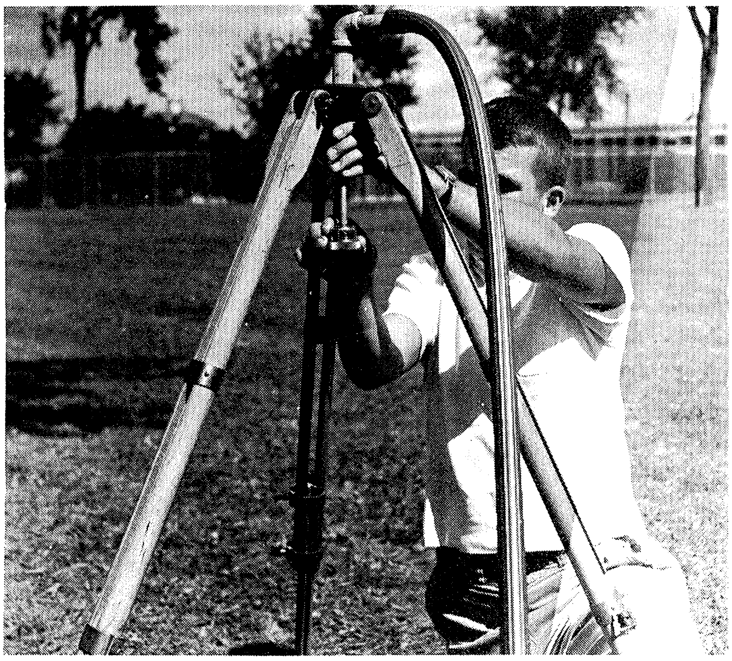

Fig. 3.12. Portable sampling head supported by tripod.

was assumed to be identical. Therefore a gas meter was provided in only one of the lines. In practice, samples were taken from noon to 4:00 P.M. at a given location; the average of the concentrations observed on the two samples was considered representative of the site. Three such units, each using two sampling heads 100 feet apart, were in operation during the period.

As stated earlier, pollen sampling within the prison was also the responsibility of the meteorological group. A section of the prison hospital on the fourth floor had been closed off. Three inmates remained confined, voluntarily, within this area from August 17 to September 25 to act as subjects for the special study described in the medical report. Arrangements for collecting six 4-hour samples per day were identical to those on the tower. Samples were collected directly above the beds of two of the subjects and also in the center hall of this section (Fig. 3.13).

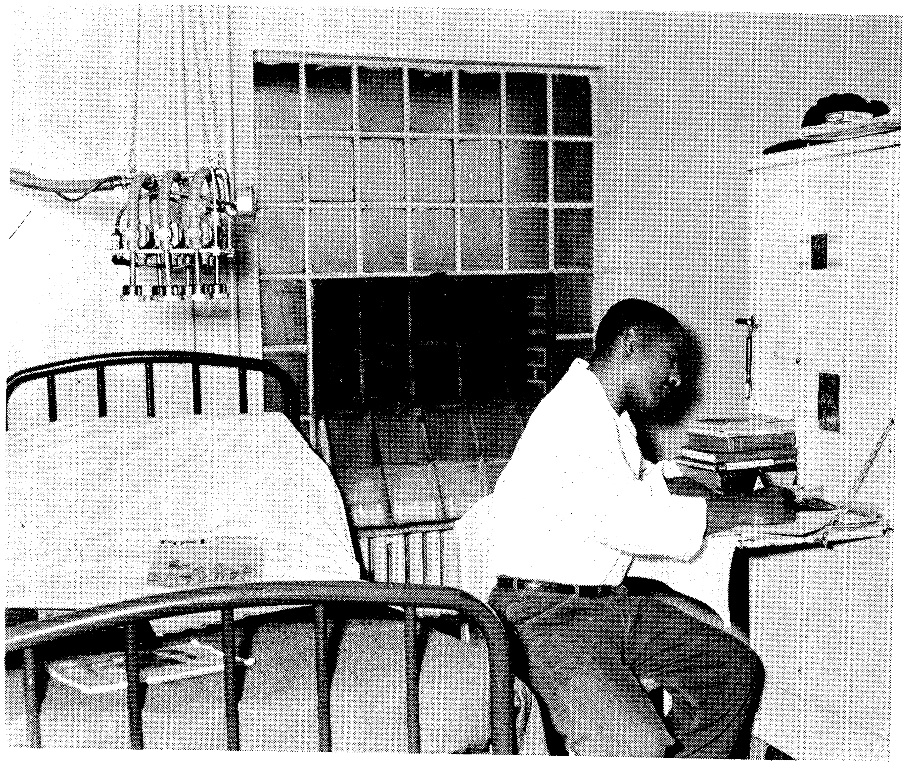

Fig. 3.13. Six-head pollen sampling unit in hospital room, Jackson Prison, 1956.

Unfortunately, delivery of the dry test meters was delayed until September 7. Up until this time, the volume of air sampled was estimated from the known relationship between observed inches of vacuum and rate of flow. After the meter was inserted in the line, it became evident that the flow rates were essentially the same as had been estimated.

A microbarograph and hygrothermograph, located within the prison hospital, completed the meteorological instrumentation (Fig. 3.14). These pro-

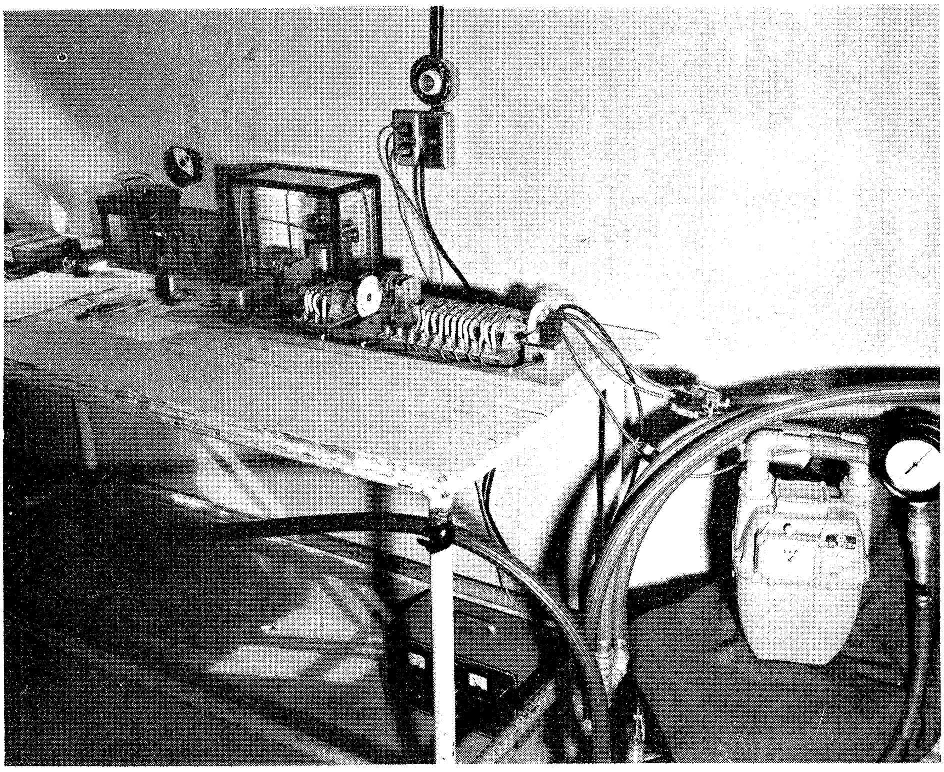

Fig. 3.14. Meteorological recorders and air sampling unit in prison hospital, 1956.

vided continuous records of barometric pressure, and temperature and humidity within that part of the hospital where the subjects were confined. Thus records of possible environmental physiological stresses other than ragweed pollen were available.

3.6 INTERPRETING THE INFLUENCE OF METEOROLOGY ON POLLEN

3.6.1 Introduction

A number of theoretical discussions of diffusion exist in the literature. These may be looked upon as models with which observed diffusion patterns might be compared by statistical means, leading to acceptance, modification, or rejection of the models. Unfortunately the available models are usually based on simple sources such as points or infinite lines, whereas the natural source of pollen is more complex. This is likely to lead to some difficulty in fitting observed pollen concentrations during the natural ragweed season to any of the available models. However, one may still expect to express in an empirical way the dependence of pollen concentration on certain meteorological and other elements. This is a problem in multiple regression that can be solved once the independent variables are identified and accurately measured. The experiments carried on at the prison during the natural ragweed season are being handled in this way.

Nevertheless, attempts to gain basic knowledge about the dispersal of pollen depend on comparisons of observed dispersion with available theoretical models. The experiments, conducted out of season with artificially grown ragweed, are designed to gain this knowledge.

Even in the case of the experiments carried on in the regular ragweed season the diffusion theories are helpful. One may write a general schematic equation of the form

$$\chi = f(Q, \bar{u}, \alpha, x, y, z)$$

to express the dependence of pollen concentration χ on a number of variables. These are:

Q, the amount of pollen emitted in unit time;
$\bar{u}$, the mean wind speed;
α, the lapse rate or rate of decrease of temperature with height;
x, the distance downwind from the source;
y, the distance crosswind from the source; and
z, the height above the source.

One is then led to conclude that the interpretation of observed pollen concentrations will require the measurement of the independent variables in the schematic equation and perhaps other variables. Accordingly, the instrumentation on the Jackson tower, described in Section 3.5 provides some of the needed basic information. The probability that the emission of pollen by the plants is a discontinuous process has emphasized the importance of studying the strength of the source. Samplers located directly over plots of ragweed will be tried this year as a means of observing this variability.

The experiments conducted to date at Jackson have been attempts to learn more about the broad integrated effect of weather upon pollen concentration during the ragweed season. The Graeco-Latin square experiment and the North Campus experiment were attempts to gain basic knowledge about the behavior of ragweed pollen. Preliminary findings of these experiments are now presented.

3.6.2 The Graeco-Latin square experiment

This experiment was carried out on the roof of the East Engineering Building on September 13 and 14, 1955, in an attempt to answer certain specific questions: "How uniformly are pollen grains distributed in the air? How long must one sample the air in order for short period fluctuations of pollen count to average out? How are the counts obtained on millipore filters facing downward affected by grains dropping off, and how by natural impaction of grains upon the filter at those times when no air is being drawn through the filter?" The Graeco-Latin square design was chosen because, by its use, three factors might be varied systematically and their effects evaluated in the ensuing analysis.

The variable factors chosen were: (1) the sampling time or duration, (2) the sampling rate, and (3) the distances between identical pairs of samplers. Eight samplers were connected in identical pairs by rubber tubes to the inlet sides of four solenoid valves which led to the vacuum tank. The spacing of the samplers and general physical setup are pictured in Figs. 3.15 and 3.16. Samples of 5, 10, 20, and 30-minute duration were collected and the rate of sampling was varied from one pair of samples to another. Available combinations of horizontal spacing were 1, 2, 4, and 8 feet. Four complete tests, each consisting of four successive 30-minute runs were carried out.

Analysis of the data led to the following conclusions.

1) Significant variations in pollen count as a result of spatial differences were not observed.

2) Pollen counts expressed as grains per unit volume did vary significantly for different sampling rates and for different durations of sampling. Lower flow rates and shorter sampling periods seemed to be more efficient at sampling pollen.

In volumetric sampling carried on since this experiment, additional care has been taken to achieve uniform sampling rates and durations.

3.6.3 Pollen dispersal at the North Campus, June, 1956

As mentioned previously, the North Campus experiment was an attempt to gain basic knowledge about the behavior of ragweed pollen. The plan was to measure the distribution of pollen at various distances from a point source, and compare the observations with some of the available models.

A primary problem of studying ragweed pollen dispersal from a known source during normal flowering periods is that the atmosphere is already filled with ragweed pollen from other sources. Therefore the plants for such an experiment must be made to flower prior to normal anthesis. Premature pollen discharge from 136 plants was accomplished by forcing them at the University's Botanical Gardens, bringing them into flower 50 days earlier than normal.

The plants were placed at the center of an area in which pollen collection slides were mounted two feet above the ground along nine radii running out 40 degrees of arc apart, and 10, 20, 40, 80, and 160 feet distant along each radius. Although volumetric pollen samplers were preferred, there were too few available to cover the cover the complete network. The tests on June 26, 27, 28, and 29 were made with full meteorological instrumentation as described in Section 3.5.2. Figures 3.17 and 3.18 illustrate the experimental arrangements. Pollen slides were set out for four periods each day: from 5 P. M. to 5 A. M., 5 A. M. to 8 A. M., 8 A. M. to 12 noon, and 12 noon to 5 P. M. Actual operation of the experiment was carried out by a crew of three technicians who worked shifts around the clock, changing the slides, refueling the portable generator

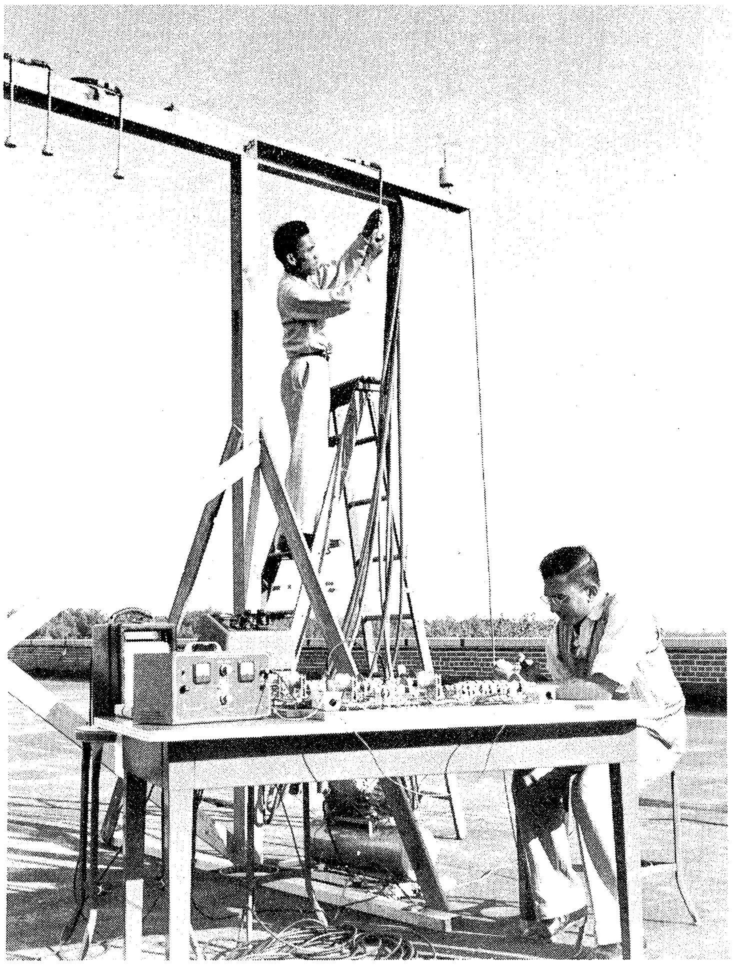

Fig. 3.15. Arrangement of sampling heads and anemometer for the Graeco-Latin square experiment.

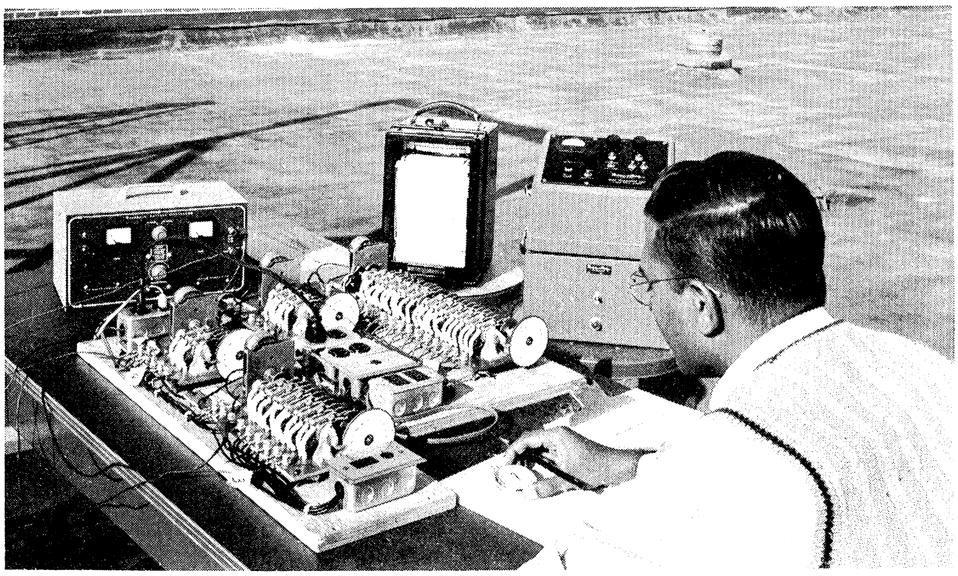

Fig. 3.16. Technician monitoring performance of timers and recording data during Graeco-Latin square experiment.

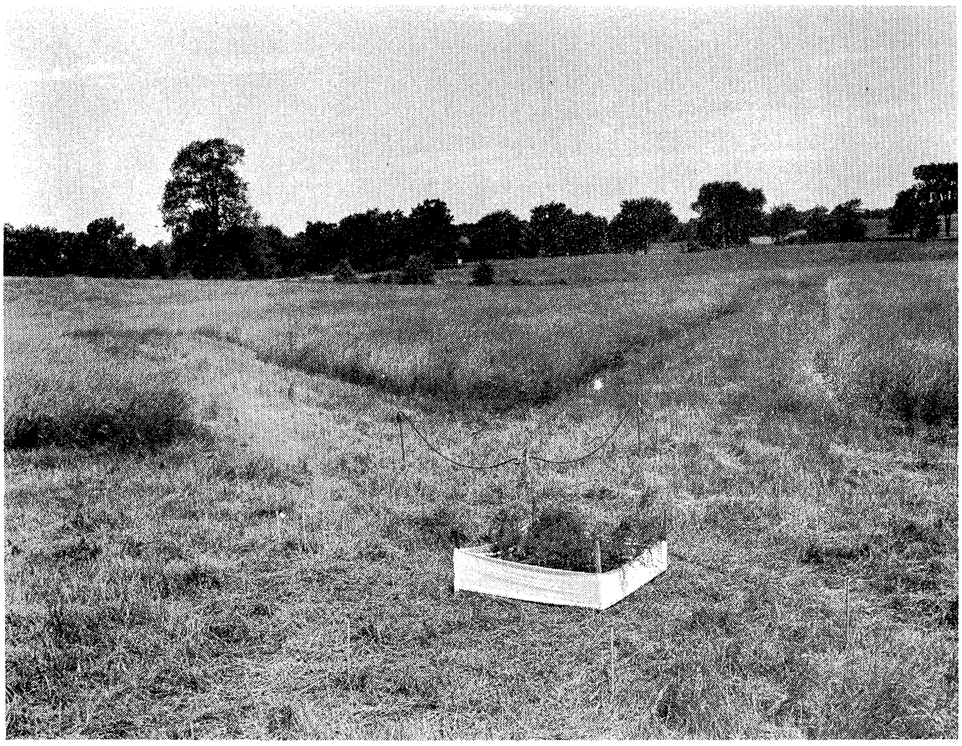

Fig. 3.17. North Campus experiment showing ragweed plants center foreground, gravity-slide samplers mounted on short stakes, and 2 volumetric samplers with connecting hose.

Fig. 3.18. Meteorological instrumentation, North Campus experiment showing the Beckman and Whitley cup anemometer and Gill bivane for wind direction and turbulence measurement.

and monitoring the performance of the various meteorological recorders.

The observed relationship between pollen counts and distance from the source is presented in Table 3.1. Relative pollen counts are computed by setting the observed count at 160 feet equal to unity.

TABLE 3.1

Distance from Source	Total Observed Pollen	Relative Pollen Counts
10 ft	2456	68
20	382	10.6
40	86	2.4
80	78	2.2
160	36	1.0

As expected, on the average the pollen counts decreased with increasing distance from the source. The observations were also compared by means of an F test with a Poisson decay of pollen counts with increasing distance from source. The theory of dispersion of aerosols as proposed by S. Rombakis[2] would predict a Poisson decay, but the observations did not provide a good fit to the model. It is not suggested at this stage that the Rombakis model should be rejected on this evidence alone, since the experimental design was preliminary in nature and did not provide very great accuracy. In the meantime the analysis of the data is continuing in an attempt to compare the observations with another model, namely, Chamberlain's modification of the Sutton equation for a point source of a particulate rather than a gaseous contaminant.[3]

Pollen counts from this experiment have been totaled in Fig. 3.19. To construct this figure, the counts for each sampling period have been oriented according to the rules that the sampling ray nearest the total wind vector for the period must be directed along the +x direction, and that the wind speed must average at least 5 miles per hour. As a result, Fig. 3.19 presents a composite average picture, over a period of 31 hours, of the deposition from the pollen "plume."

The figures represent pollen deposition reduced to the average number of pollen grains caught in 6 hours on one square centimeter of coated slide surface. The symmetry of the 0.5 line, which forms the outer limit of shaded area, is remarkable, especially when viewed in contrast to the asymmetry of the progressively denser areas near the source. The occurrence of the high counts far to the left of the average wind direction serves to emphasize the fact that the deposition patterns are produced by two factors, the wind, and pollen availability. The interaction of these two is probably of great importance near the source where extremely high concentrations of pollen are encountered. Very small scale, short-lived gusts or eddies occurring at the time of maximum pollen release rate may contribute heavily to the deposition pattern without contributing substantially to the average wind vector. On the other hand, the more

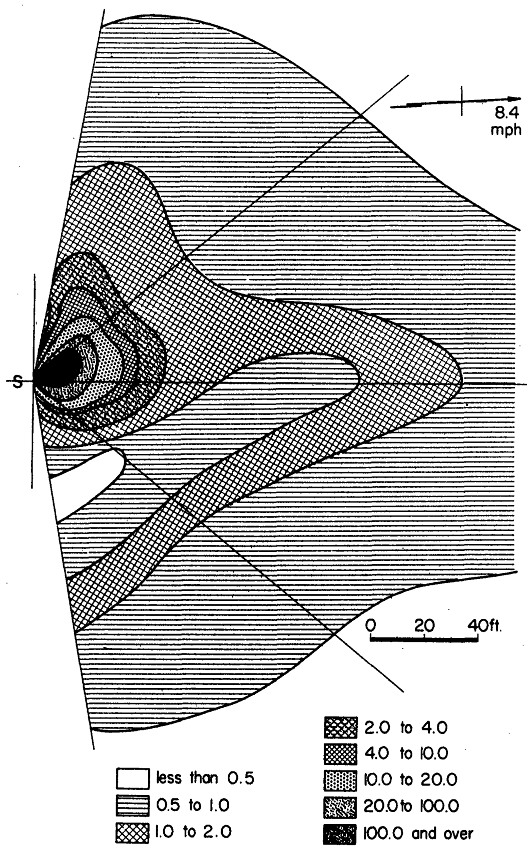

Fig. 3.19. Composite of ragweed pollen at wind speeds greater than 5 mph as constructed from impaction samples taken 2 ft above the ground. Source S was a 4-ft square bed of hot-house-forced Ambrosia elatior set out in a mown meadow. Deposition values are grains per cm^2 in 6 hr.

distant deposition pattern depends most heavily upon the average wind vector, and only slightly upon the wind details at the time of pollen release. Hence it would appear that such equations as that of Chamberlain[3] are probably best applied to the more distant portions of the "plume" to which generalizations such as the average wind speed can in fact apply. Reflotation of pollen grains is an additional factor which would tend to respond to the average wind vector and to improve the symmetry of the deposition patterns with increasing distance. It is not clear at this juncture whether the reflotation effect can be isolated to improve the applicability of Chamberlain's equation, but additional experimentation designed to evaluate reflotation directly is contemplated.

In the North Campus experiment the time of day proved to be a significant factor in the pollen harvest. Although one would expect a certain amount of this variation to be related to weather factors, the variations noted may also be evidence that there are large variations of pollen emissions with the time of day. As a result, the controlled experiment planned for June of 1957, again using forced ragweed plants, will include measurements directly over and under the plants in an attempt to document the variations in rates of emission.

3.6.4 Pollen dispersal studies at Jackson, August-September, 1956

3.6.4.1 Statement of problem.—The problem of pollen dispersal by the atmosphere is more readily analyzed when there is a known and limited source of pollen, as was the case during the studies on the North Campus in June of 1956. The problem during the regular pollen season is to analyze and interpret pollen concentration measurements when there are thousands of pollen sources at various distances away from the samplers. The concentration data must then be interpreted in terms not only of local meteorological factors but also of larger scale phenomena such as frontal passages, convective activity, etc.

The sampling equipment and meteorological instrumentation employed in the August-September field program on the farm lands of the State Prison of Southern Michigan at Jackson have been described in detail in Section 3.5. Briefly restated, the various items were as follows: on the 100-foot tower, volumetric samplers at 4, 50, and 102 feet, thermocouples at 1, 25, 51, and 102 feet, and anemometers at 12, 25, 50, and 102 feet; six volumetric samplers on the ground; a hygrothermograph in an instrument shelter; and a sling psychrometer. In addition, wind directions were estimated and visual observations of cloud cover and type were made.

All pollen samples taken, both in the field and in the prison hospital, were for six consecutive 4-hour periods each day. The meteorological data were abstracted so as to permit direct comparison with the measured pollen concentrations.

Given this program of pollen sampling and meteorological measurements, the problem thus becomes one of analyzing and interpreting the pollen concentrations in the light of the accompanying meteorological conditions.

3.6.4.2 Outline of procedures used following field program.—During the period that has elapsed since the August-September field program at the prison at Jackson, the data collected have been abstracted and put into form for IBM tabulation. Certain preliminary analyses, to be described below, were also carried out. Detailed procedures used in preparing and abstracting the data are next indicated.

The anemometers on the 100-foot tower were calibrated under wind-tunnel conditions. The proper constants for the conversion of data from contacts per minute to wind speed in miles per hour were determined. The anemometer data for each of the four tower levels were analyzed and mean wind speeds were calculated for each sampling period and each sampling level. Temperatures and relative humidities were determined for each sampling period from the instrument shelter and sling psychrometer measurements in the field. Other visual and instrumental observations were used in conjunction with the appropriate surface and upper-air weather-map analyses to determine the time of frontal passage or other changes in the circulation pattern in the area.

A weather code similar to the standard U.S. Weather Bureau synoptic code was devised to encode the appropriate weather observations in a manner suitable for IBM tabulation. Flow-rate data and the total volume of air sampled were calculated for each sample period. These data together with the pollen counts and the meteorological information have been prepared for IBM analysis and are awaiting the availability of IBM computer time.

3.6.4.3 Preliminary analysis of tower observations.—Since the pollen source is at the ground, it would be logical to anticipate a distribution of pollen in the vertical dimension with the largest pollen concentration in the air near the ground, and with the concentration decreasing with increasing height. The pollen counts on the tower show a large number of deviations from this expected distribution. Only 39 out of 102, or 38 percent of the total number of sample periods, show the expected decrease of pollen concentration with height. Seven out of 102, or 7 percent of the total, were completely inverted with the pollen concentrations decreasing downward from a maximum at the 100-foot level. Five of the seven inverted distributions occurred in periods following the passage of a cold front. The significance of this fact is discussed below. No diurnal influence on the type of vertical distribution was found. This suggests a random occurrence of the controlling mechanism rather than a direct diurnal pattern of influence.

It may be that a tower height of 100 feet represents too short a vertical distance for an adequate study of the vertical distribution of pollen. Satisfactory interpretation of these results will have to await a more complete understanding of the processes, derived from additional experimentation and analysis and from observations made at higher elevations.

A portion of the pollen concentration data obtained from the tower is shown in Fig. 3.20. The first item of interest is the relatively high pollen concentration found for the sample from the 100-foot tower level for Period V on August 31. When this particular sample was counted, it was noted that the majority of the pollen collected was deposited in a diagonal streak across the filter, indicating that it was deposited at one time. This is one of several incidents which suggest that there are unusual features in atmospheric dispersion processes which need further investigation.

In Period III at 09.15* on September 1 a strong, well-defined cold front passed the station and was accompanied by heavy rain showers lasting intermittently for about 2-1/2 hours. The winds for the period, however, were not unusually high: the period means at the 12-, 50-, and 102-foot levels were, respectively, 7, 9, and 11 miles per hour. These values are equal to the average of all mean winds for Period III. The pollen concentration for this period at the 50-foot tower level, 2000 grains per cubic meter, is the highest measured during the entire test period. A significantly lower concentration, 1415 grains per cubic meter, was measured at the 4-foot level and a much lower concentration, 360 grains per cubic meter, was found at the 102-foot level.

*A 24-hour clock, with zero at midnight, is used here.

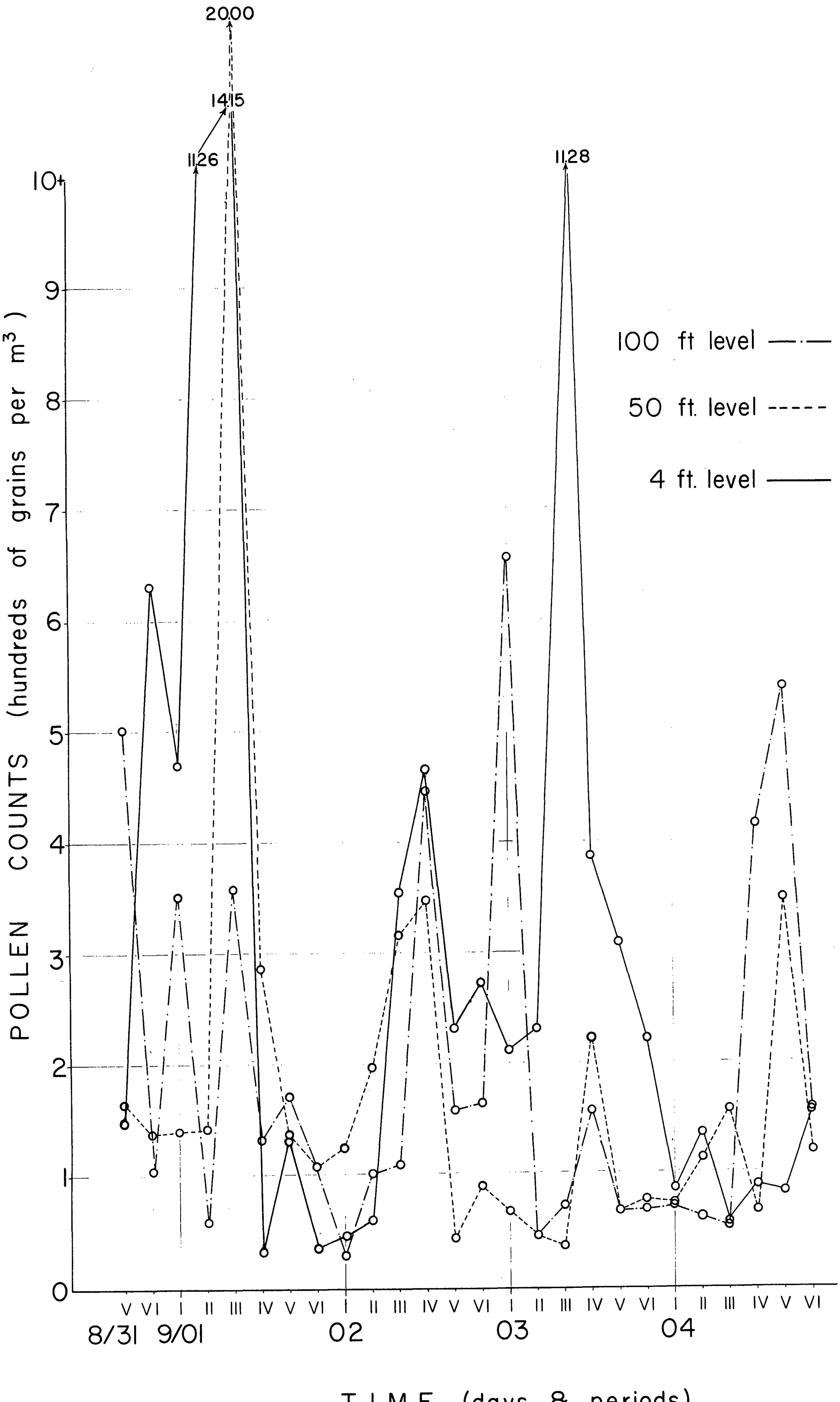

Fig. 3.20. Observed pollen counts at heights of 4, 50, and 100 (102) ft on the tower at Jackson, August 31 - September 4, 1956.

This high count and unexpected distribution suggest a local source for this pollen.

The next three periods (IV, V, VI) on September 1 show inverted conditions with relatively higher concentrations of pollen found at the upper two levels. In fact, it is interesting to note that a complete inversion occurred during the second period (Period V) following the frontal passage. A possible mechanism for this type of occurrence is one of transportation of pollen to considerable heights by the turbulent activity associated with a cold front, followed by a downward diffusion of pollen "clouds" to lower and lower levels on the tower. Future field experimentation and airplane sampling in periods following cold frontal passages may be expected to give more information on such a mechanism. No immediately obvious explanation occurs for the relatively high concentration at the 102-foot sampler for Period I on September 3.

The next significant meteorological event is the passage of another cold front in Period IV on September 4 at 15.00. This front was weak and dry with relatively little visible evidence of the passage. It is known, however, that strong vertical currents are often associated with such fronts. Again, the inverted distribution with the high concentrations at the upper two levels is found during the period following the frontal passage. This is one period earlier than in the previous case, on September 1, but if the proposed mechanism is valid, this may be accounted for by the weaker character of the front. This same type of occurrence was found on two other occasions of cold frontal passages, although it must be said that two cold frontal passages did not demonstrate such inversions of pollen distribution. Future experimentation and analysis must test these findings. It is expected that the forthcoming IBM analysis will aid us in picking out the subtle but important details of a somewhat less pronounced nature. More refined instrumentation, especially regarding vertical motion and turbulence, and aircraft sampling will be used to explore these questions during the regular 1957 ragweed pollen season.

3.6.4.4 <u>Preliminary analysis of ground concentrations</u>.—Six portable sampler heads operated by three portable gasoline engines were placed at distances of approximately 700, 800, 1600, 1700, 2000, and 2100 feet southwest of the prison hospital area. The positions of the three centers of sampling activity are shown in Fig. 3.2. The units were placed in this manner to take advantage of two relatively isolated ragweed patches nearby. Winds from the southwest quadrant would presumably carry pollen from these to the samplers. This experiment was designed to give information on the distribution of pollen downwind from relatively small sources under natural seasonal conditions.

As shown by the data in Fig. 3.21, no clear pattern is evident for situations with winds blowing from the southwest quadrant. Analysis of this information is continuing, and it is expected that future study with the aid of IBM techniques will enable us to draw meaningful conclusions from these measurements.

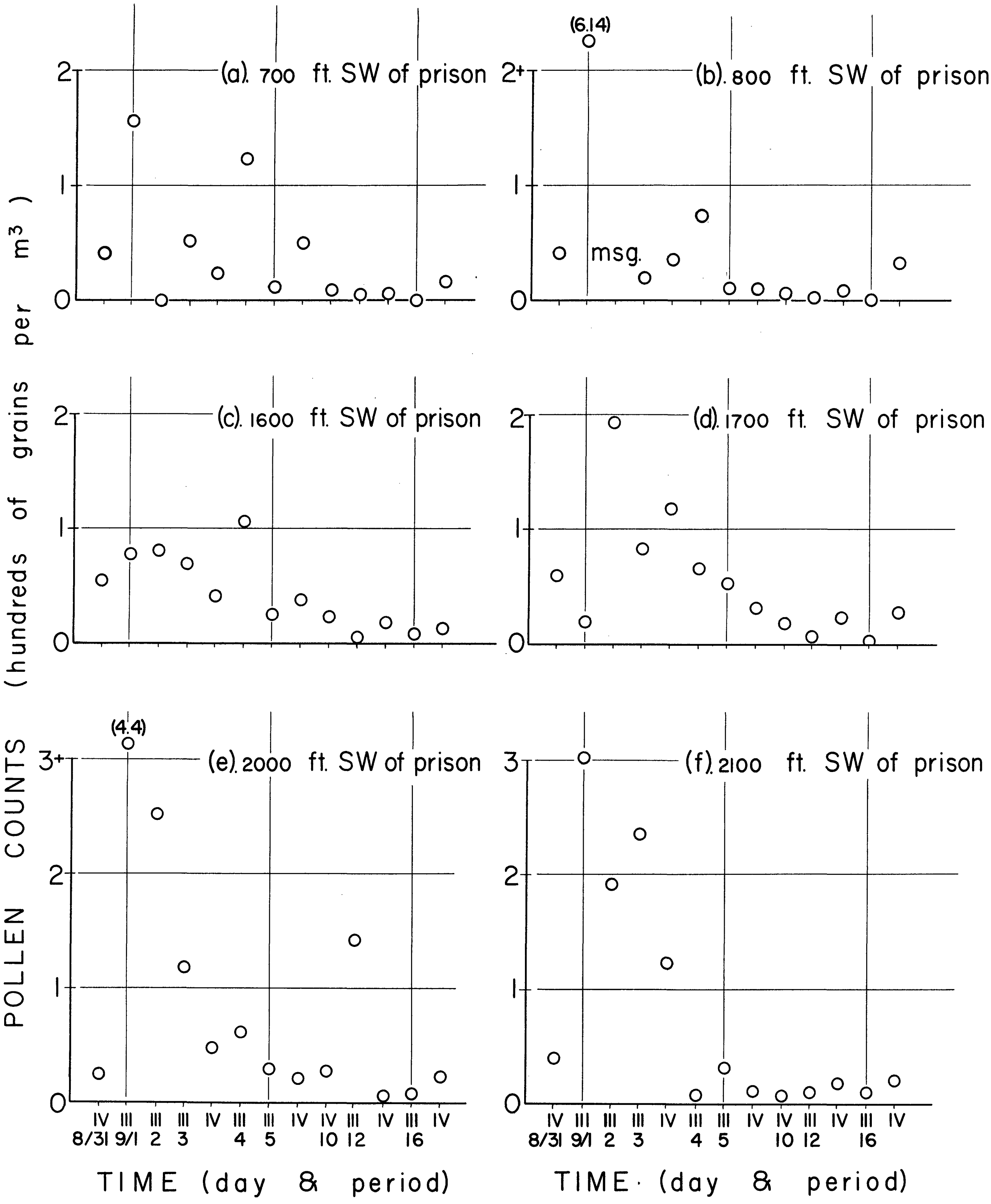

Fig. 3.21. Measured pollen concentrations near the ground at the locations designated by S in Fig. 3.2 when the wind was blowing from the southwest quadrant.

The techniques developed and the experience gained by the team in the field and by the analysis in the laboratory are invaluable for future experiments similar to those of the 1956 program at Jackson. Plans for the 1957 season at Jackson have been formalized and the necessary preparations are being made.

3.7 DESIGN OF POLLEN EXPOSURE CHAMBER

It is required for one phase of the research program on aeroallergens that human subjects be exposed to a controlled environment with a known airborne pollen concentration. For this purpose a pollen exposure chamber has been designed. Its functions are as follows:

1. To provide air with a known and controllable pollen content ranging from 50 to 5000 pollen grains per cubic meter.

2. To maintain the air at controllable values of temperature and humidity.

3. To provide a comfortable and easily accessible enclosure for the controlled environment and two subjects.

3.7.1 Components required

To achieve its functions, the exposure chamber must have the following components:

1. A device for dispersing pollen into air at a controlled rate.

2. A device for mixing the dispersed pollen with the air passing through the chamber.

3. A device for measuring the pollen content of the air.

4. An air-conditioning unit for controlling air temperatures and humidity.

5. Filters for removing all particulates from the incoming air and pollen from the air leaving the chamber.

6. Fans for moving the main air stream and the fresh air makeup stream.

7. A meter for measuring the rate of flow of the main air stream.

8. By-pass ducts and dampers for controlling air flow rates.

9. An 8- by 12- by 8-foot chamber.

A schematic layout of the components is presented in Fig. 3.22.

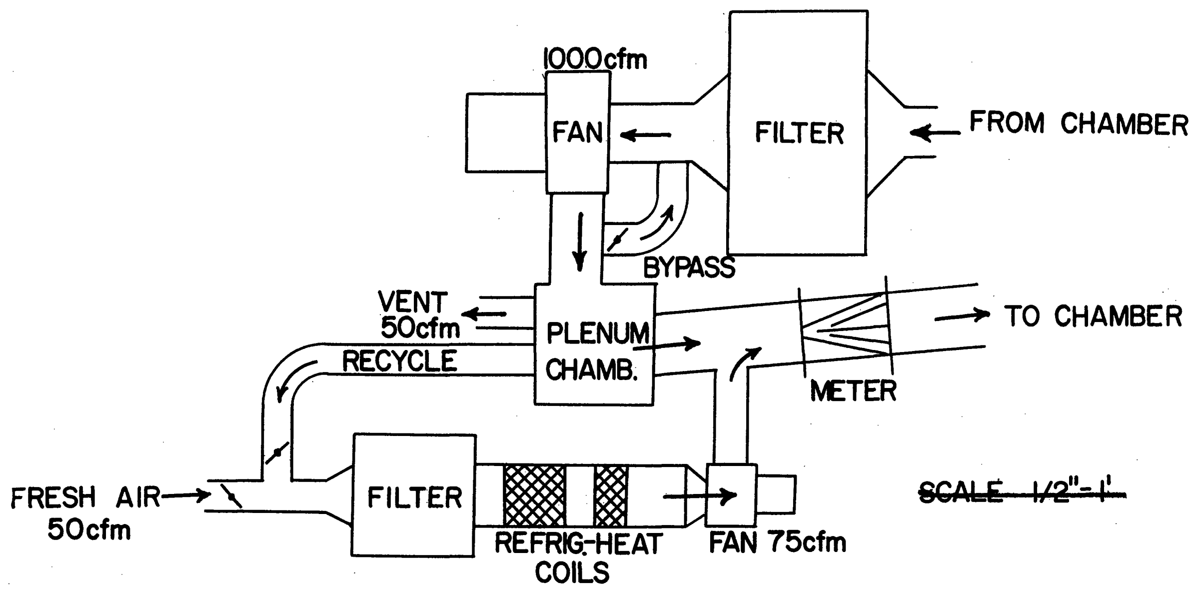

Fig. 3.22. Schematic layout of the components required for the operation of the pollen exposure test chamber.

3.7.2 Pollen disperser

The major problem involved in building the exposure chamber is that of dispersing pollen at the very low rate which is required. If the air travels upward through the chamber at a velocity of 10 feet per minute, the pollen feed rate required to maintain a count of 500 grains per cubic meter is 1.133×10^4 grains per minute, or about 6.17×10^{-5} grams per minute. It should be noted that the pollen concentration in the chamber is determined by the pollen feed rate and the difference between air velocity and the settling velocity of the pollen. In other terms:

$$R_p = (V_a - V_p)\, A\, (C_p)$$

where

R_p = pollen feed rate (grains/min),
V_a = air velocity (ft/min),
V_p = settling velocity of pollen (ft/min),
A = cross-sectional area of chamber (ft^2), and
C_p = pollen concentration ($grains/ft^3$).

The device chosen to disperse the pollen is a fluidized bed with an expanded section above the bed. The method of operation is to pass enough air up through the bed of pollen to fluidize it and to have a large enough cross-sectional area in the expanded section so that the average air velocity above the bed is lower than the terminal settling velocity of the pollen grains.

Thus no pollen grains should be carried up to the exit tube if the air velocity is uniform above the bed. Actually the air flow pattern is not uniform but contains random eddies, and these carry a few pollen grains up to the exit tube. By operating in this fashion it has been possible to obtain pollen output of the proper order of magnitude. At present it is not possible to say how constant and controllable the pollen output rate is, but data will be taken to establish operating characteristics.

3.7.3 Pollen counter

The major obstacle to obtaining data on the adequacy of the pollen disperser is the present lack of a rapid analytical technique. The filtration of pollen from an air sample and the subsequent counting of pollen grains on the filter is an exasperatingly slow technique for determining pollen concentration. It was hoped that measurement of the pressure-drop increase due to pollen buildup on a small filter would provide a means of measuring pollen concentration but the rate of pressure rise proved too small at the concentrations used.

Another approach was to collect pollen grains on a charged plate and to count the resulting current pulses by means of an electronic counter. Using the counter available, it was found that the pulse size was below the noise level of the electronic circuit.

The approach presently being followed is the use of light scattering in conjunction with a counter. This method has a fairly good change of success, and, if feasible, could be used for monitoring the air in the chamber as well as for calibrating the pollen disperser.

3.7.4 Pollen mixing

Once the pollen has been dispersed into a small air stream, it is necessary to mix this small stream with the main air stream so that the resulting pollen concentration will be uniform throughout the exposure chamber. The ideal way to accomplish this would be first to mix the two streams in a small diameter tube with baffles to increase turbulence. Then the stream would be gradually expanded in a vertical duct until the air velocity reaches that in the chamber. This would require a very high expansion duct.

The method which will be employed is an approach to the ideal. The clean air and pollen suspension streams are mixed in a 6 by 12-inch rectangular duct and then expanded through two "horns" mounted on the side of the chamber. The air flows out of these horns into a horizontal duct below the chamber, and then upward into the chamber through slots between wooden strips (1 by 4-inch boards with half-round edges). The purpose of these slots is to provide enough resistance to air flow so that the effect of horizontal velocity is minimized and the upward flow will be more uniform.

3.7.5 Air conditioning

To maintain the temperature and humidity of the air at desired values, it is necessary to provide an air cooler and an air heater. Assuming that the chamber will take in a maximum of 50 cubic feet per minute of fresh air at a high of 95°F and 50 percent relative humidity and a low of 0°F and 80 percent relative humidity, the loads are as follows:

1. Cooling load = 5,000 Btu/hr
   (to take air from 95° to 50.5°F)

2. Reheating load = 1,090 Btu/hr
   (to take air from 50.5°F, 95 percent RH to 70°F, 50 percent RH)

3. Heating load = 5,900 Btu/hr
   (to take air from 0°F, 80 percent RH to 70°F, 50 percent RH)

Thus the cooling can be handled by a 1/2-ton refrigeration unit and the reheating by a 0.32-kilowatt-hour electric heater. The heating can be handled with a 2-kilowatt-hour electric heater. Humidification can be provided by means of a water-fog nozzle.

Control of air-conditioning equipment will be by a combination of automatic and manual settings. The temperature and humidity will be determined with wet- and dry-bulb thermometers.

3.7.6 Filters

Incoming air must be filtered clean of any particulates and the outlet air must be cleaned of pollen grains. The most convenient way to accomplish this is to use Cambridge-type "absolute filters," which are capable of 99.5 percent efficiency on 0.3-micron particles. To decrease the loading on the absolute filters, Fiberglas filters are used as precleaners.

3.7.7 Fans

The fans required are as follows:

1. A fan capable of moving 1000 cubic feet per minute against a resistance of 3 inches of water.

2. A fan (fresh air) capable of moving 75 cubic feet per minute against 2 inches of water.

Fans are controlled by means of bypass ducts and dampers. The smaller fan is required to eliminate throttling of the main circulating stream to compensate for pressure drop through the fresh-air filter and air-conditioner.

3.7.8 Meter and bypass ducts

A venturi meter is required to measure the main air-stream rate.

Bypass ducts are provided to enable bypassing of the two fans and to enable movement of circulating air from the chamber through the air-conditioning system.

3.7.9 Chamber

The chamber as shown in Fig. 3.23, is an 8- by 12- by 8-foot-high room with a subway-grating floor and a perforated ceiling. The main type of construction is plywood or plastic panels bolted to an angle-iron frame. Air moves upward through the floor and out through the perforated ceiling to a plenum divided into four segments. Each segment discharges into a 6-inch-diameter duct and these are manifolded to a 10-inch-diameter duct leading to the filters and fan.

Windows are provided in the panels on three sides and a door is provided in one panel. The fourth wall of the chamber forms one side of the air-expansion horns. The outlines of these horns are formed by 1-inch-wide wooden strips and the outer sides are formed by 1/8-inch plastic sheets screwed to wooden outline strips.

Plastic panels are provided on the subfloor duct space so that pollen build-up may be observed. This area, as well as the rest of the chamber, is built so that it can be dismantled and cleaned.

3.8 THEORETICAL STUDIES OF POLLEN REMOVED FROM AIR BY AGGLOMERATION AND IMPACTION

The settling velocity of ragweed pollen has been found to be about 3 feet per minute. If agglomeration of pollen particles occurs, perhaps induced by air turbulence, the falling speed will be increased and pollen removal accelerated.

Perhaps a more significant means by which pollen is removed from air is by impaction of the pollen on surfaces. Such impaction is of importance in a number of aspects of the present investigation: in the volumetric samplers in which the pollen is collected by impingement on a surface rather than by collection on a filter; in the process of removal from the atmosphere by impingement on natural surfaces such as vegetation; and in the process of removal

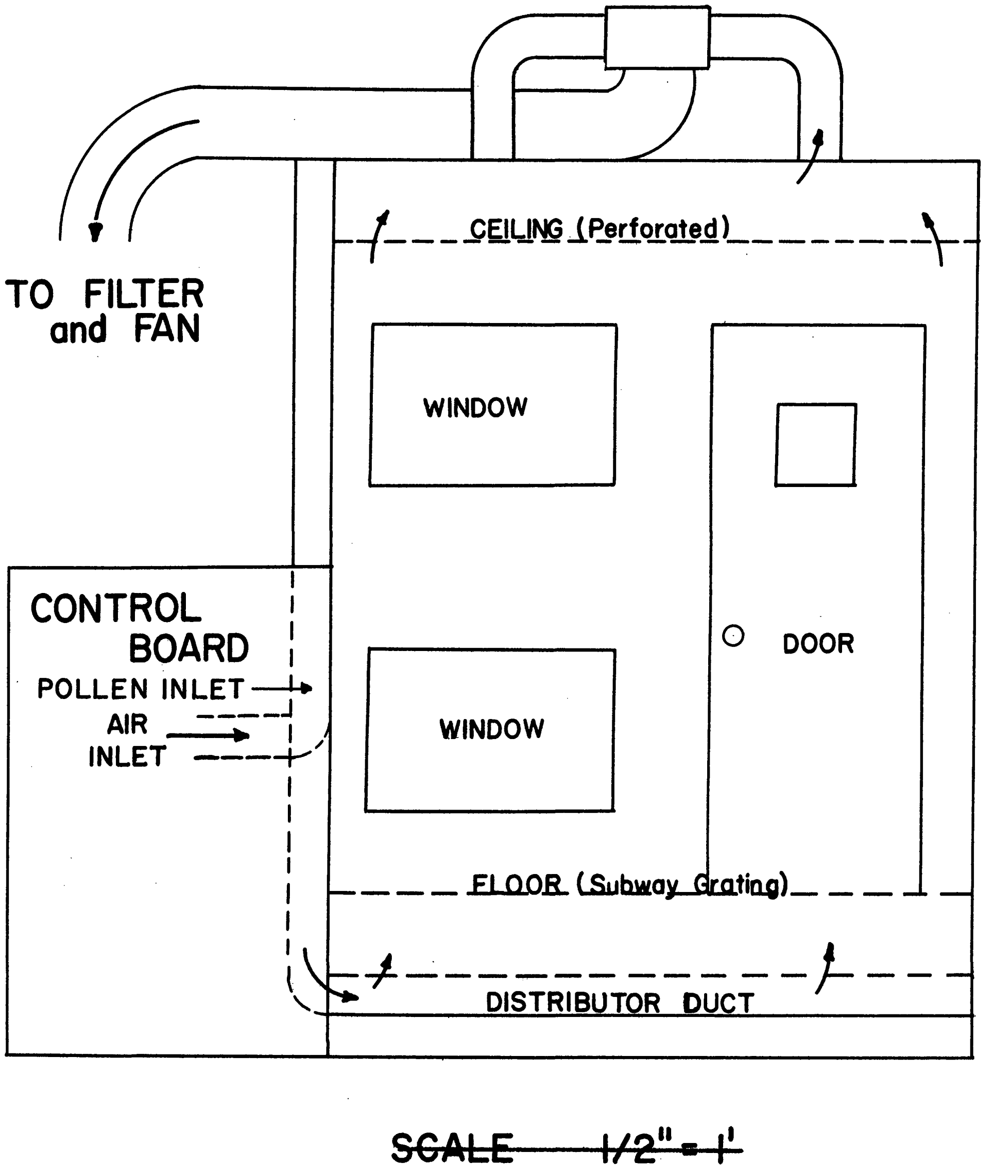

Fig. 3.23. Sketch of the pollen exposure test chamber.

of pollen from the air by impingement in the nasal passages and lungs.

Because of the significance of particle agglomeration and impaction processes in the present investigations, a theoretical study of these processes has been undertaken. The analysis represents a further development of methods used to study the motions of fine particles in turbulent air streams.[4] In these, the turbulent field is assumed to be stationary, homogeneous, and isotropic. Stationary, extraneous force fields for the particles may exist. The method of generalized harmonic analysis developed by Wiener is used to determine the statistical particle-dispersion parameters in terms of the spectrum density of the turbulent field and the physical characteristics of the particles. The discussion has been restricted to one-dimensional problems; it includes consideration of the relation between turbulent dispersion and Brownian motion of the particles due to molecular impacts.

The analysis of pollen agglomeration by turbulent action is nearing completion. When that phase has been finished, the analysis will be extended to cover the processes of impaction of pollen on surfaces in turbulent air flow.

3.8.1 Reference

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3. Chamberlain, A. C., Aspects of Travel and Deposition of Aerosol and Vapor Clouds, Atomic Energy Research Establishment, Harwell, Berks., HP/R 1261, 1953.

4. Liu, V. C., "Turbulent Dispersion of Dynamic Particles," J. Meteorology, 13, (1956), 399-405.

4. STATISTICAL PHASES

Since the contributions of the public-health statisticians in the group are integral parts of the other phases, notably the medical and meteorological phases, their work is covered, both explicitly and implicitly, in Sections 2 and 3, where they have been shown as coauthors.

5. PUBLICATION OF RESULTS

One paper has been accepted for publication in the Annals of Allergy, and is scheduled to appear in the issue of March-April, 1957. The paper is as follows:

Gurney, C. W. and Cryst, S., "Observations on the Course of Allergic Rhinitis and Bronchial Asthma in Ragweed-Sensitive Subjects."

The following two papers were presented at the Symposium on Bioclimatology, sponsored jointly by the American Physiological Society and the American Meteorological Society, April 19, 1957, at Chicago, Illinois:

Dingle, A. N., "Meteorological Considerations in Ragweed Hay Fever Research."

Goodwin, J. E., McLean, J. A., Hemphill, F. M., and Sheldon, J. M., "Air Pollution by Ragweed: Medical Aspects."

These two papers will be published subsequently.

6. ACKNOWLEDGMENTS

The research group wishes to express its deep appreciation 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. White, Hospital Director; Mr. L. D. Johnson, Plant Engineer; and Mr. C. Rossman, Farm Superintendent.