

# An appraisal of Rumex pollen as an aeroallergen

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*Particle concentrations derived from operation of a rotobar sampler at a midwestern location during 1965 to 1968 have confirmed the regular and substantial occurrence of Rumex pollen; field studies have implicated Rumex acetosella (sheep sorrel) as the primary source. Comparisons of rotobar and gravity slide data suggest that purely aerodynamic factors may decrease the recovery of Rumex pollen by the Durham sampler by as much as 50 per cent relative to grass pollen. A high frequency of positive skin reactivity to Rumex pollen extract was noted among 367 persons demonstrating some wheal and flare reactions on skin testing with extracts of common inhalant allergens. More than half of a group of positive skin reactors to Rumex extract also demonstrated positive nasal provocative tests with this material. Most, but not all, positive nasal reactors to Rumex had experienced pollinosis during the common period of Rumex and grass pollen prevalence, and at least some of these manifested negative responses on mucosal testing with grass pollen extracts. The combined aerobiological and clinical data suggest that specific sensitivity to Rumex pollen may contribute significantly to the severity of pollinosis in selected patients.*

**A**lthough major aeroallergens were recognized early in this century, the clinical importance of many airborne particles remains uncertain. Among these, pollen of the sorrels and docks (the genus *Rumex* of the family Polygonaceae) is of particular interest in view of its annual occurrence in substantial concentrations over much of the United States. At Ann Arbor, Michigan, gravity slide data from 1956 to 1966 have suggested that over two thirds of *Rumex* pollen deposition occurs between May 25 and June 20. This period is one in which late tree pollens, grass pollen, and spores of imperfect fungi are recognized as dominant aeroallergens. Considering this temporal association, the possibility that *Rumex* pollen might serve, in selected cases, to worsen or prolong symptoms induced by acknowledged seasonal offenders seemed to justify re-examination.

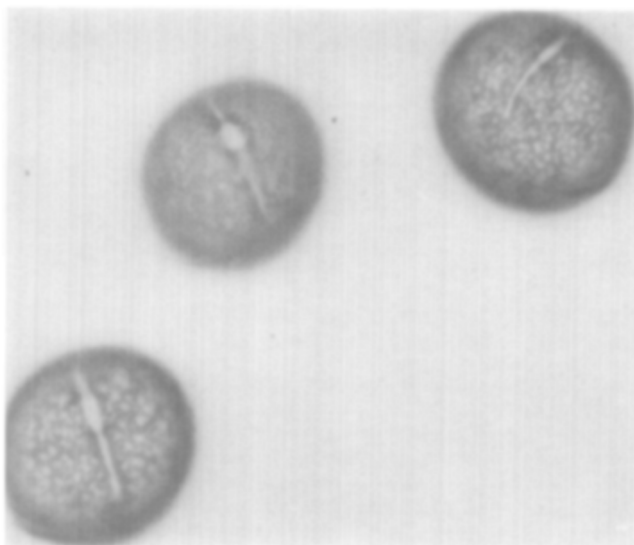
Individual *Rumex* grains (Fig. 1) demonstrate a thin wall and 3, 6, or, most

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**Fig. 1**

Pollen grains of *Rumex acetosella*. The densely packed starch granules and slit-like furrows containing elliptical pores are prominent microscopic features.

commonly, 4 slender furrows, each with an elliptical pore. Closely packed starch granules are a constant and striking microscopic feature. Although several species of the genus *Rumex* (with similar pollen morphology) are present in the midwest, only sheep or red sorrel (*Rumex acetosella*) has been considered significantly anemophilous by Wodehouse.<sup>1</sup> Field observations and the local predominance of 4 furrowed *Rumex* grains, each less than  $25\ \mu$  in diameter, further suggest *Rumex acetosella* as our major local source of these particles. This plant has become widely established in dry upland fields, waste places, and poorly kept lawns following its introduction from Europe. Sheep sorrel often grows as a perennial, spreading by underground stems to form clones of male or female plants. The leaves are usually confined to basal rosettes, and the plants are apparent only in early summer when spikes of minute red or yellow flowers reach a height of 12 to 18 inches (Fig. 2); thereafter, all but the basal leaves involute.

## MATERIALS AND METHODS

### Atmospheric studies

During the years 1965, 1966 and 1967, a roto-bar sampler<sup>2</sup> was operated at a rooftop site approximately 55 feet above grade in Ann Arbor, Michigan. Each weekday, individual samples were obtained for the periods: 8 A.M. to 10 A.M., 10 A.M. to 3 P.M., 3 P.M. to 5 P.M., and 1 A.M. to 3 A.M. The device operated continuously during the designated intervals, and a timer permitted automation of the early morning collections. In addition, glycerin jelly-coated gravity slides were exposed in a standard Durham sampler<sup>3</sup> nearby, and, in 1965, a Hirst

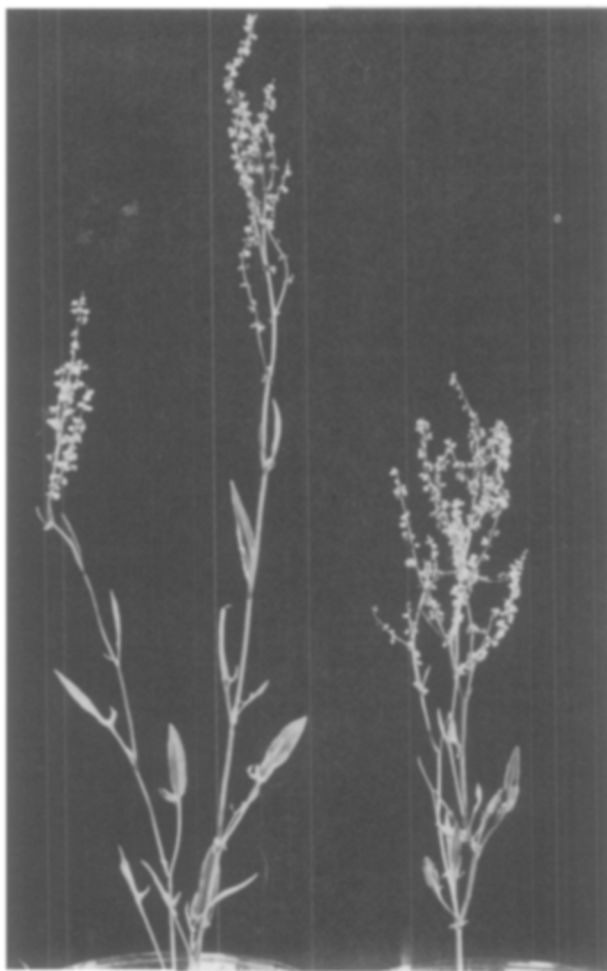


**Fig. 2**

Sheep sorrel (*Rumex acetosella*) in flower. Close inspection is required to distinguish individual male (*left*) from female (*right*) plants. When viewed from a distance, groups of flowering male plants appear red; by contrast, the females are tinged with yellow. After the fertile shoots involute, even large clones of both sexes are easily overlooked.

spore trap<sup>4</sup> was used at this station as well. During 1965 and 1966, gravity slide sampling was confined to the interval from 8 A.M. to 5 P.M., while in 1968, more conventional 24 hour exposures were employed for comparison with a "fly-shield" roto-bar sampler<sup>5</sup> which operated intermittently (for one minute out of every 12) throughout each 24 hour period.

The volume of air swept out by a single roto-bar may be calculated as the product of the sampling area (0.254 cm.<sup>2</sup>), the circumference of rotation ( $\pi \times 11.0$  cm.), the rotational speed of approximately 1,700 r.p.m. determined stroboscopically for the samplers used, and the duration of the period of active collection in minutes. Particles were captured on 1 inch wide No. 666 Scotch tape

(Minnesota Mining and Manufacturing, St. Paul, Minn.) applied to the rotobars. For the 2 to 5 hour sampling periods of 1965 to 1967, this was coated, in turn, with a mixture of one part of Carter's rubber cement and 9 parts of a commercial thinner (Bestine, Union Rubber & Asbestos Co., Trenton, N. J.) with a paint sprayer. During 1968, a thin coating of Lubriseal (Arthur H. Thomas Co., Philadelphia, Pa.) provided the adhesive surface for the intermittent collections of the fly-shield device. Following exposure, the tapes were transferred to  $1 \times 3$  inch glass slides and mounted with either Calberla's solution or hard glycerin jelly<sup>6</sup> and  $22 \times 30$  mm. cover slips. Two hundred-fold magnification was used routinely for examining these preparations, with higher power being used when necessary.

Data for the period 1 A.M. to 3 A.M. were derived by modifying the gross counts to allow for particle deposition that might have been expected to occur before and after the sampling period. This was necessary because the sampling surfaces were unshielded during the intervals from 5 P.M. to 1 A.M. and from 3 A.M. to 8 A.M. Since it is known that particle deposition on the trailing edge of a rotobar is negligible, one of each pair of the 1 to 3 A.M. sampling surfaces was reversed with reference to the direction of rotation. All particles on the surface so oriented could be assumed to represent adventitious deposition, and their numbers were subtracted from those of a normally positioned sampling bar.

To estimate actual particle concentrations more closely, an attempt was made to correct the derived rotobar data for the collection efficiency of the sampler employed. Accordingly, based upon its characteristic mean diameter, crude counts of each particle type were divided by a factor (from 0.66 to 0.74). Calibration data for both the basic rotobar sampler and the intermittent fly-shield modification had previously suggested an efficiency of capture for short ragweed pollen of approximately 70 per cent.<sup>5</sup> These estimates were obtained with a pollen monodisperse in a unidirectional flow situation by comparison with an array of four Millipore filters sampling isokinetically. Over-all efficiency can be regarded as the product of an adhesive factor (describing the probability that intercepted particles will stick to the sampling surface) and an impaction efficiency factor (expressing the probability that a given particle in the volume of air swept out by the sampler will strike the sampling surface). For non-defatted short ragweed pollen, the theoretical impaction efficiency is approximately 93 per cent,<sup>7</sup> suggesting a value of 73 per cent for the adhesive efficiency of our sampling surfaces. This latter figure has been assumed as the adhesive efficiency governing the collection of other pollens as well. For each pollen type, the theoretical collection efficiency of a 1 mm. cylinder has been derived for the situation in which the carrier gas moves past the sampling surface at 21 miles per hour (the relative speed of the rotobar); this value  $\times 0.73$  has yielded the correction factor applied. All particle concentrations have been expressed as particles per cubic meter of air.

### **Clinical studies**

Commercially obtained sheep sorrel pollen (Greer Drug & Chemical Corp., Lenoir, N. C.) was examined microscopically to exclude significant contamina-

tion with foreign particle types. This material was extracted with equal parts of glycerin and phosphate-buffered saline (pH 7.4) containing 0.25 per cent phenol to yield a 1:5 (weight by volume) extract. Concurrently, after defatting with ether, portions of the dry pollen were extracted with phenolated phosphate-buffered saline (PBS) to produce a 1:10 extract; further dilutions were made with both phenolated and nonphenolated normal saline. The 1:10 material contained 238.8  $\mu\text{g}$  of phosphotungstic acid-precipitable nitrogen (PTA N) per milliliter.

Epidermal (prick) tests were performed on 527 consecutive new patients to the Allergy Clinic of the University of Michigan Medical Center, above age 5, whose clinical evaluation dictated immediate-type skin tests. Reactions were read at 15 minutes with the use of the following criteria: 2+, erythema greater than 21 mm.; 3+, a wheal of at least 3 mm. with erythema; 4+, a wheal with pseudopods and erythema. Intracutaneous tests were done with 0.02 ml. portions of 1:500 extract and reactions, read after 15 minutes, were graded according to wheal size by comparison with a PBS control.<sup>8</sup>

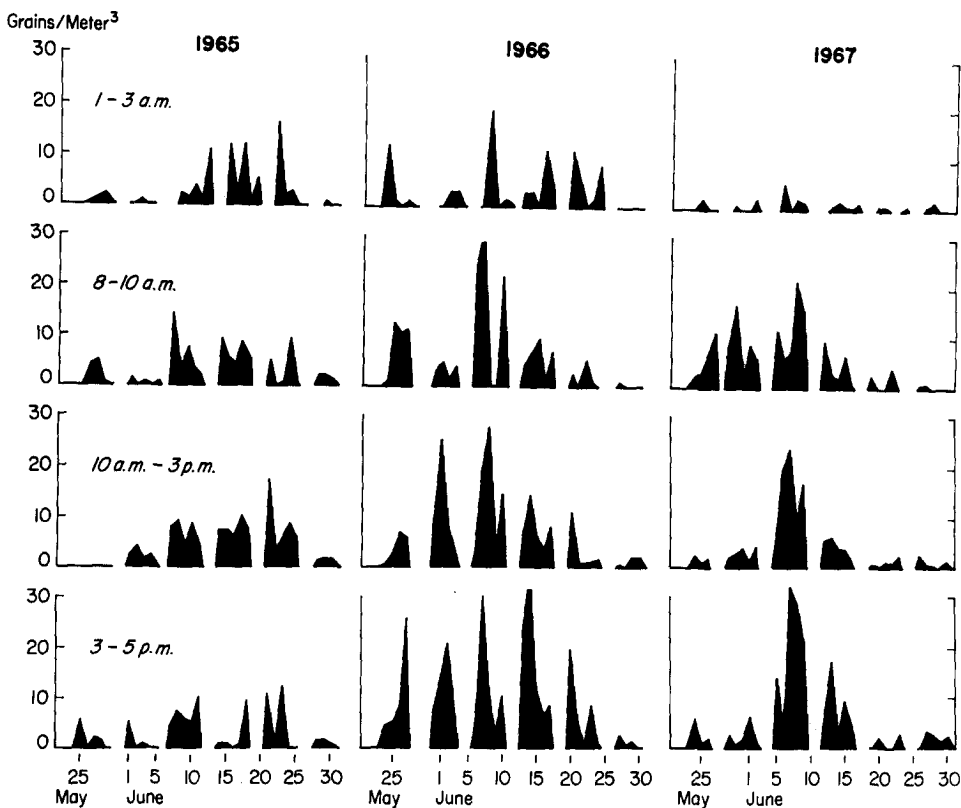
Nasal provocative tests were performed with 1:500 *Rumex* pollen extract (4.8  $\mu\text{g}$  PTA N per milliliter) and 1:500 mixed grass pollen extract (21.8  $\mu\text{g}$  PTA N per milliliter), the latter comprising equal parts of timothy, red top, June grass, and orchard grass extracts; material containing less than 0.005 per cent phenol was used exclusively. Nasal airway resistance (NAR) was directly measured with a method previously described,<sup>9</sup> and readings in centimeters of water per liter per second were taken at a flow rate of 30 L. per minute during the second half of expiration. Challenge was begun when NAR had been stable during 3 consecutive tests done at 3 minute intervals; 0.2 ml. portions of nonphenolated materials were delivered by passing oil-free compressed air through a DeVilbiss atomizer,\* the olive tip of which was positioned just within the nares. The majority of the particles emitted by this device are larger than 10  $\mu$ , assuring predominant deposition upon the nasal mucous membranes. Initially, 0.2 c.c. portions of nonphenolated PBS were atomized as a control, followed by provocative exposure to 1:500 *Rumex* and, last, to 1:500 mixed grass pollen extract. NAR was monitored 5 and 15 minutes after each challenge and then followed at 5 minute intervals until a stable base-line value was regained; a 50 per cent increase in NAR at 15 minutes constituted a positive test result.

## RESULTS

Calculated levels of airborne *Rumex* pollen for 1965, 1966, and 1967 are shown graphically in Fig. 3; a well-defined season of prevalence lasting 4 to 5 weeks is evident. Concentrations encountered before May 25 are typically low, and the morphology of the deposited grains often suggests other *Rumex* species, particularly *R. crispus*, as their source. Thereafter, *R. acetosella* is the major contributor of airborne *Rumex* pollen.

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\*Four-tenths milliliter volumes of extract were measured into test tubes of 8 mm. internal diameter specially cut to a length of 45 mm. and held in the reservoir of the atomizer. Extract was drawn from these tubes directly into the atomizing assembly from which approximately 0.2 ml. was released, the balance remaining within the "dead space" of the device.

**Fig. 3**

Concentrations of airborne *Rumex* pollen calculated from rotobars exposed during 4 daily sampling periods for the years 1965, 1966, and 1967.

Fig. 3 illustrates the year-to-year variation in mean pollen prevalence that may be observed. For each year, *Rumex* pollen concentrations during the 3 diurnal periods studied were roughly equivalent, while between 1 and 3 A.M. distinctly lower levels, averaging 40 per cent of the daytime values, were recorded. This circadian pattern was roughly confirmed when Hirst spore trap deposits from 1965 were studied and the results corrected for ambient wind speed.

Fig. 4 depicts the relative contribution made by *Rumex* pollen to the total pollen load during the late spring of 1968 at our Ann Arbor sampling station. These data were obtained during successive 24 hour periods with the use of an intermittent fly-shield rotobar sampler. Average concentrations of *Rumex* pollen and total pollen may be read directly from the ordinate. Levels of grass pollen and of pollen other than grass and *Rumex* are plotted with the same units but with *Rumex* and grass pollen, respectively, as their base lines to emphasize the proportion of the total pollen load attributable to each type. Late tree pollens form the major part of the nongrass, non-*Rumex* category, with smaller contributions from genera including *Vitis* (wild grape), *Thalictrum* (meadow

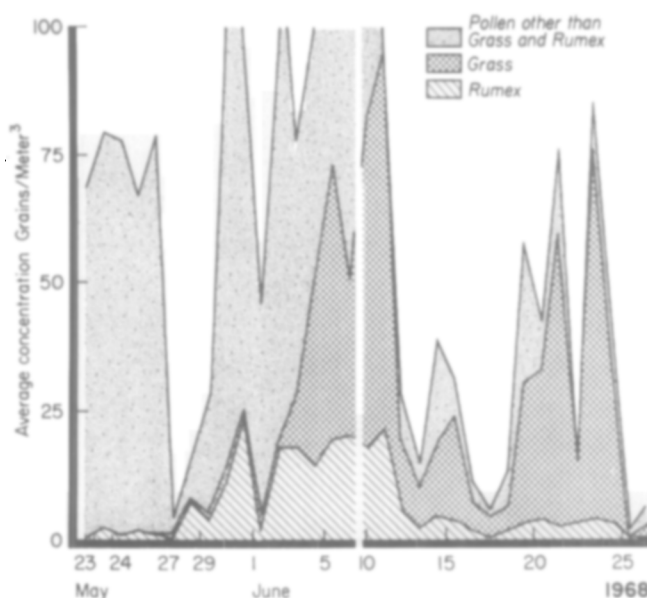


**Fig. 4**

Concentrations of Rumex, grass, and other pollens for the period from May 23 to June 26, 1968, calculated from 24 hour intermittent fly-shield rotobar samples exposed at Ann Arbor, Michigan. Concentrations of Rumex and of total pollen (represented by the topmost line) may be read directly from the scale on the ordinate. For grass pollen and pollen other than grass and Rumex, concentrations are depicted as height above the underlying curve with units of the same value as those for Rumex.

rue), and Typha (cat-tail), as well as representatives of the rose family (Rosaceae). The prominent biphasic pattern of grass pollen prevalence has been frequently observed, the latter peak occurring concurrently with the anthesis of timothy (*Phleum pratense*). It has been uncommon to encounter Rumex as the dominant pollen type for more than a few days of each year. However, as shown in Fig. 4, its prevalence regularly exceeds that of grass pollen during the initial 10 days of June, a period in which the latter is often incriminated as the sole cause of emerging pollinosis. Subsequently, the airborne load of grass pollen progressively overshadows that of other types, although rarely exceeding concentrations of 200 grains per cubic meter at Ann Arbor or at other Michigan stations recently studied.<sup>10</sup>

Considering the disparity in size between pollen grains of *Rumex acetosella* and the larger grains of the common grasses, preferential deposition of grass pollen upon gravity slides is expected. To evaluate this potential source of bias, gravity slide counts (in grains per square centimeter of slide area) were compared with concentrations (in grains per cubic meter of ambient air) calculated from rotobar samples obtained concurrently. Data for grass and Rumex pollen were studied during the years 1965 and 1966 with a series of continuous samples obtained daily between 9 A.M. and 5 P.M. and for 1968 when a fly-shield rotobar sampler operated intermittently during 24 hour periods; in each case, a standard Durham sampler was exposed during the corresponding period.

**Table I**

| Year | Period          | Mean concentration in grains/M. <sup>3</sup> |       | Grass concentration/Rumex concentration (Ratio A) | Average count in grains/cm. <sup>2</sup> of gravity slide |       | Grass/count Rumex count (Ratio B) | Ratio B/Ratio A |
|------|-----------------|----------------------------------------------|-------|---------------------------------------------------|-----------------------------------------------------------|-------|-----------------------------------|-----------------|
|      |                 | Grass                                        | Rumex |                                                   | Grass                                                     | Rumex |                                   |                 |
| 1965 | 8 A.M. - 5 P.M. | 7.50                                         | 3.65  | 2.06                                              | 5.60                                                      | 1.42  | 3.94                              | 1.91            |
| 1966 | 8 A.M. - 5 P.M. | 10.90                                        | 5.56  | 1.96                                              | 7.27                                                      | 1.90  | 3.82                              | 1.95            |
| 1968 | 8 A.M. - 8 A.M. | 20.72                                        | 7.59  | 2.73                                              | 5.22                                                      | 1.11  | 4.70                              | 1.72            |

Table I summarizes mean values from these studies for May 25 to June 24 of each of the 3 years. Ratio B/Ratio A expresses the extent to which gravity slide sampling favors the recovery of grass pollen from an atmosphere in which defined concentrations of grass and Rumex pollen coexist. The derived values suggest that, in comparison with grass pollen, gravity slide counts tend to underestimate the relative prevalence of Rumex pollen by a factor approaching 50 per cent.

The pattern of skin reactivity among 527 patients tested at the Allergy Clinic of the University of Michigan Medical Center is shown in Fig. 5. This group was comprised of persons in whom atopic respiratory disease relating in part, at least, to seasonal factors was seriously considered. Of 160 patients presenting no epidermal test reactions of 2+ or greater to appropriate tree, grass, or weed pollen allergens, no one subsequently demonstrated a significant positive reaction to 1:5 Rumex pollen extract. Among the 367 patients with at least one epidermal test reaction of 2+ or greater, 135 (36.7 per cent) developed appreciable positive reactions to Rumex pollen; over half of these showed pseudopodial wheals. Seventy per cent of the group manifesting less than 2+ Rumex reaction epidermally showed 3+ or greater reactions when intracutaneous testing with Rumex pollen extract was later carried out. Over-all, among the pollen-extract skin-reactive group, therefore, approximately 91 per cent had skin sensitivity to Rumex pollen extract elicitable by conventional techniques of epidermal or intracutaneous testing.

All patients studied were also skin tested to grass and weed pollen extracts. Of the 135 persons with positive epidermal reactions to Rumex, only 7 had less than a 2+ prick-test reaction to dwarf or giant ragweed pollen extracts. When test reactions to extracts of the four most common grass pollens were considered, 7 persons showed less than 2+ reactions to these coupled with 2+ or greater epidermal reactions to Rumex; 6 additional patients had pseudopodial wheals to Rumex pollen extract with significant, but lesser, reactions to grass pollen preparations.

Previous attempts to carry out nasal provocative challenge with phenolated materials had been complicated by prolonged irritant reactions. Fortunately, the use of nonphenolated extracts in the present study appeared to eliminate this problem, and, in all subjects tested, nasal airway resistance had decreased

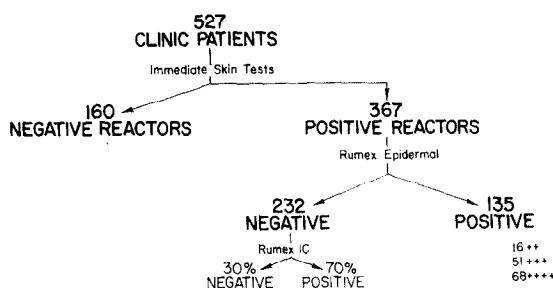


**Fig. 5**

Results of skin testing with *Rumex acetosella* pollen extracts.

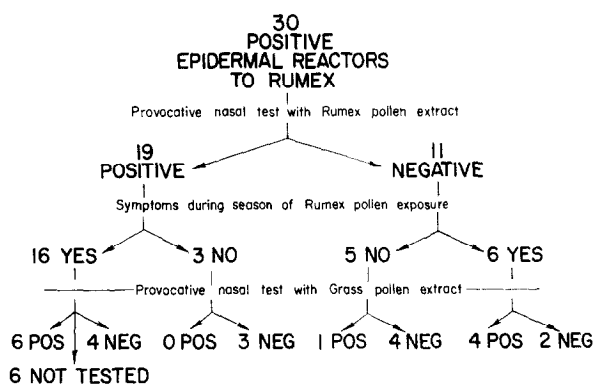


**Fig. 6**

Results of nasal provocative testing with *Rumex* and grass pollen extracts in 30 patients with strong skin-test reactions to *Rumex*.

to within 20 per cent of the control value within 15 minutes following atomization of phosphate-buffered saline. Nineteen of 30 skin test-positive patients tested had positive reactions on nasal provocative testing with *Rumex* pollen extract as shown in Fig. 6; 16 of these had a definite history of nasal symptoms in the grass-*Rumex* pollen season and 3 did not. On further provocative testing with grass-pollen extract, 6 of those with a corresponding seasonal history were positive and 4 were negative, as were the 3 who denied appropriate seasonal symptoms. Six patients could not undergo testing with grass-pollen extract because of prolonged nasal obstruction following the initial *Rumex* pollen extract challenge.

Eleven skin test-positive patients had negative responses on nasal exposure to *Rumex* pollen extract; of 5 who denied suggestive symptoms in the month of June, one had a positive inhalant test to grass pollen; while 4 of the remaining 6 who reported seasonal symptoms were positive when challenged with grass pollen extract. In addition, 8 patients without evidence of skin sensitivity on intracutaneous testing with *Rumex* pollen extract were also uniformly negative on nasal challenge with this material.

## DISCUSSION

These data suggest that airborne *Rumex* pollen may be present annually in noteworthy concentrations and that its comparative prevalence has been understated previously by gravity slide surveys. That very much higher concentrations can occur locally has been shown by exposing rotobars in more rural situations near pastures and old fields. Furthermore, levels of *Rumex* pollen reaching several hundred grains per cubic meter have been encountered during the mowing of lawns heavily invaded by *Rumex acetosella*<sup>10</sup> in Michigan. Obviously, conclusions regarding aerobiological conditions elsewhere must await volumetric sampling in other localities.

It is apparent also that, among the group of exposed atopic patients studied, a high percentage manifested skin and mucosal sensitivity to components of *Rumex* pollen extracts. Positive nasal provocative tests were more common among persons with late spring and early summer symptoms but were not present exclusively in this clinical group. Collateral studies have also shown that among 65 patients with persistent respiratory symptoms in June despite optimal hyposensitization programs (with extracts other than *Rumex*) 74 per cent showed significant positive epidermal test reactions to *Rumex* pollen. However, since this refractory group may be especially prone to demonstrate a broad spectrum of strong clinical and skin sensitivities, the observation must be interpreted with caution.

In persons with flares of pollinosis in June, a few demonstrated stronger skin reactivity to *Rumex* than to grass pollen extracts; far more provocative was the observation of 4 such patients in whom mucosal tests were positive with *Rumex* but not with grass pollen challenge. Generally however, skin test reactivity to *Rumex* pollen extract was accompanied by comparable skin sensitivity to other pollen allergens. To evaluate the possibility that shared allergens might underlie these correlations, passive-transfer neutralization studies were performed with the use of serum of a single untreated patient who demonstrated intense 4+ skin reactivity on epidermal testing with extracts of *Rumex*, dwarf ragweed, timothy, and English plantain pollens. Titrations of passively sensitized sites were initially done to determine the concentration of each allergen that, in 0.02 ml. portions, would elicit a 3+ P-K reaction. An equal volume of the test serum and of a 1:100 concentration of one of each of the four allergens was then combined and the four mixtures incubated in syringes at 20° C. for one-half hour. Duplicate skin sites in a suitable recipient were planted with these individual combinations and, 24 hours later, 0.02 ml. of each allergen in a concentration previously shown to produce a 3+ reaction was used to challenge the sites. Although areas sensitized with serum plus ragweed, timothy, or plantain pollen extracts no longer reacted to the corresponding allergens, definite positive responses to *Rumex* pollen extract could still be elicited. The latter reactions, however, were generally smaller than those observed on challenging control sites planted with serum alone, and this decrement was most marked where serum plus ragweed pollen extract had been used for sensitization. It is uncertain whether these findings show a nonspecific loss of reaginic activity as described by Pruzansky and associates<sup>11</sup> or, in fact, reflect allergens common

to *Rumex* and other pollens. However, the inability of other pollen preparations to completely neutralize P-K reactivity to *Rumex* extract does suggest the existence of allergens peculiar to this material. This impression was supported by the finding that sites planted with serum plus *Rumex* pollen extract retained strong wheal and flare reactivity to each of the pollen allergens except *Rumex*.

These data may well redirect attention to this neglected aeroallergen, but unfortunately they cannot unequivocally define the role of *Rumex* pollen exposure in the clinical setting of any symptomatic patient. The postulates emphasized previously by Thommen<sup>12</sup> provide neither guidance nor confidence in relating these findings to clinical practice. More recently, many authors have stressed the value of mucosal test reactions as indicators of clinical sensitivity, and an association between these seems well established. However, the intensity and duration of allergen dosage imposed by mucosal testing with extracts differ sufficiently from those encountered during natural exposure to particulate aeroallergens that conservatism in the interpretation of these tests seems mandatory. It may be significant, in this regard, that 3 of 19 subjects having positive nasal provocative reactions to *Rumex* pollen denied symptoms during previous seasonal periods of *Rumex* pollen exposure; these patients were also quite normal when examined during early June of the following year. Equally intense positive nasal provocative tests were elicited in 2 normal medical students who, despite intense skin reactivity to *Rumex* and other pollen extracts, had never experienced clinical atopic disease.

Whether exposure to *Rumex* pollen can serve alone as a significant source of pollinosis symptoms remains unclear. Final resolution of this problem almost surely will require study with the use of test procedures in which the form, duration, and strength of the normal particulate exposure are simulated. Of greater practical significance is the extent to which these particles may contribute to the constellation of factors promoting seasonal respiratory disease. Although, once again, this cannot be stated with assurance, the data presented seem to make such a role highly likely. Considering its relative prevalence, *Rumex* pollen might be expected to add to the allergenic load imposed by grass pollen upon patients with multiple sensitivities or might alone be responsible for grass-season pollinosis in a few of these. Furthermore, in a fashion recently re-emphasized by Connell,<sup>13</sup> these particles could well condition the mucous membranes for a more florid inflammatory response upon later grass pollen exposure. In each instance, considering the apparently unique allergenic groups involved, there would result pollinosis refractory to all specific treatment measures that overlooked the potential of *Rumex* pollen as an aeroallergen.

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