

THE APHID—AN INSECT ALLERGEN*

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THE POTENTIAL of insects as inhalant allergens has been well established. Parlato¹⁻³ has shown that the emanations from the wing scales and hairs of the caddis fly were capable of producing hay fever and asthma in 5 per cent of his cases in the Lake Erie area. Parlato⁴ and, later, Figley⁵ concluded that hay fever and asthma were caused by the May fly in over 7 per cent of cases in the western Lake Erie section. Additional data⁶ also include moths and butterflies in the list of inhalant allergens.

REASONS FOR THE INVESTIGATION

During the late summer and early fall of 1948 in New York City, Westchester County, and surrounding areas, there were an exceptional number of patients with asthma and coryza. While a number of these patients were ragweed problems, others were insensitive to this pollen by previous history and skin testing.

It has been observed for many years that many patients with summer and fall asthma and coryza fail to obtain relief from the usual pollen hyposensitizing therapy and continue with unabated symptoms during and beyond the period when grass and ragweed pollens are found in the air. While it is unquestionably true that some of these people are inadequately treated or are not responsive to treatment, there is also a likelihood that some hitherto unsuspected allergen is responsible for their discomfort.

During the fall of 1948 it was also noted that swarms of winged aphids were migrating in the Westchester area. These were so profuse that the following item appeared in the *New York Herald Tribune*⁷:

"The annual mating flight of aphids has brought such clouds of the tiny green insects to Westchester County that the residents are faced with the choice of being driven into their homes or out of their minds."

Believing these insects might in some fashion cause difficulty during the pollen seasons, an investigation into their life cycle was started. A number of the insects were caught and an extract was prepared. Skin testing with this extract produced definite positive reactions in many of the patients who were faring poorly on pollen therapy alone. Many of these patients' bloods were capable of producing positive passive transfer reactions. One patient had a systemic reaction from a skin test dose. All of these findings led to a continuation of the investigation.

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PERTINENT FACTS RELATING TO THE CHARACTER, DISTRIBUTION, AND
LIFE CYCLE OF THE APHID

The aphid, or plant louse, is one of the most widely distributed members of the insect family. Geographically, it is found in all areas and in all climates in which vegetation is found. It is known to infest well over 7,000 food plants^{8, 9} throughout the world and all of its food plants probably have not yet been catalogued.

Its economic significance is tremendous since the aphid destructively infests all varieties of cereals and grains, garden vegetables and flowers, fruit, hard wood and soft wood trees, in short, all manner of succulent vegetation including grasses, plantain, and weeds. The destructive green pea louse alone is estimated to have caused a loss of over \$7,000,000 in two seasons just along the Atlantic Coast states.^{10, 11}

An attempt to epitomize the life cycle of the aphid is like trying to draw an orderly sketch of chaos.¹² Simply stated, the aphid of the north, after having wintered as a gamogenetic (fertilized) egg upon its winter host, comes forth as a stem mother in the spring of the year. This stem mother reproduces a series of parthenogenetic (agamic) viviparous generations of female plant lice which feed upon the succulent spring foliage of their winter host. Later, because of a diminution of food supply, or by the dictates of reasons unknown, a generation of winged lice is formed which flies to the summer host of its species. Here, still by parthenogenetic viviparous reproduction, it reverts again to the wingless louse form and as such multiplies and feeds through the summer. With the onset of cold or unfavorable weather or with failure of its food supply, it again generates winged forms and flies to its winter host. These fall migrants, however, are bisexual and give rise to the gamogenetic egg which is the overwintering form of the insect. Actually, wide variations from the above life cycle take place, but are of little interest in a monograph of this nature. In the warmer climates, the Aphididae may retain their louse form and may continue parthenogenetic reproduction on a limited scale during periods of scant vegetative growth or they may hibernate during the period of their first instar. From this life cycle, it is obvious that the greatest numbers of aphids are present at the time of year the host is at its maximum vegetative vigor. Thus, different varieties of the aphids would be present in profuse numbers at different times of the year. The rosy apple aphid is at its numerical peak on the apple blossoms in late May and again on narrow and broad leaf plantain in September,¹³ the potato aphid during August and early¹⁴ September, etc. The forms taken during its life cycle are polymorphic. It is reproductively prolific. (Fig. 1.)

QUALIFICATIONS AS AN INHALANT ALLERGEN

The aphid is a most prolific insect. Eight to 10 days¹⁵ following its birth, it begins its agamic reproduction. Each aphid will produce from 1 to 4 young daily for a total of 10 to 28 during its life. In the temperate zone there will be from 8 to 21 generations during a season. Allowing 10 young per generation and 10 generations a year 1 egg would produce 1,111,111,111 aphids were it not for enormous destruction by predators, disease, and unfavorable weather con-

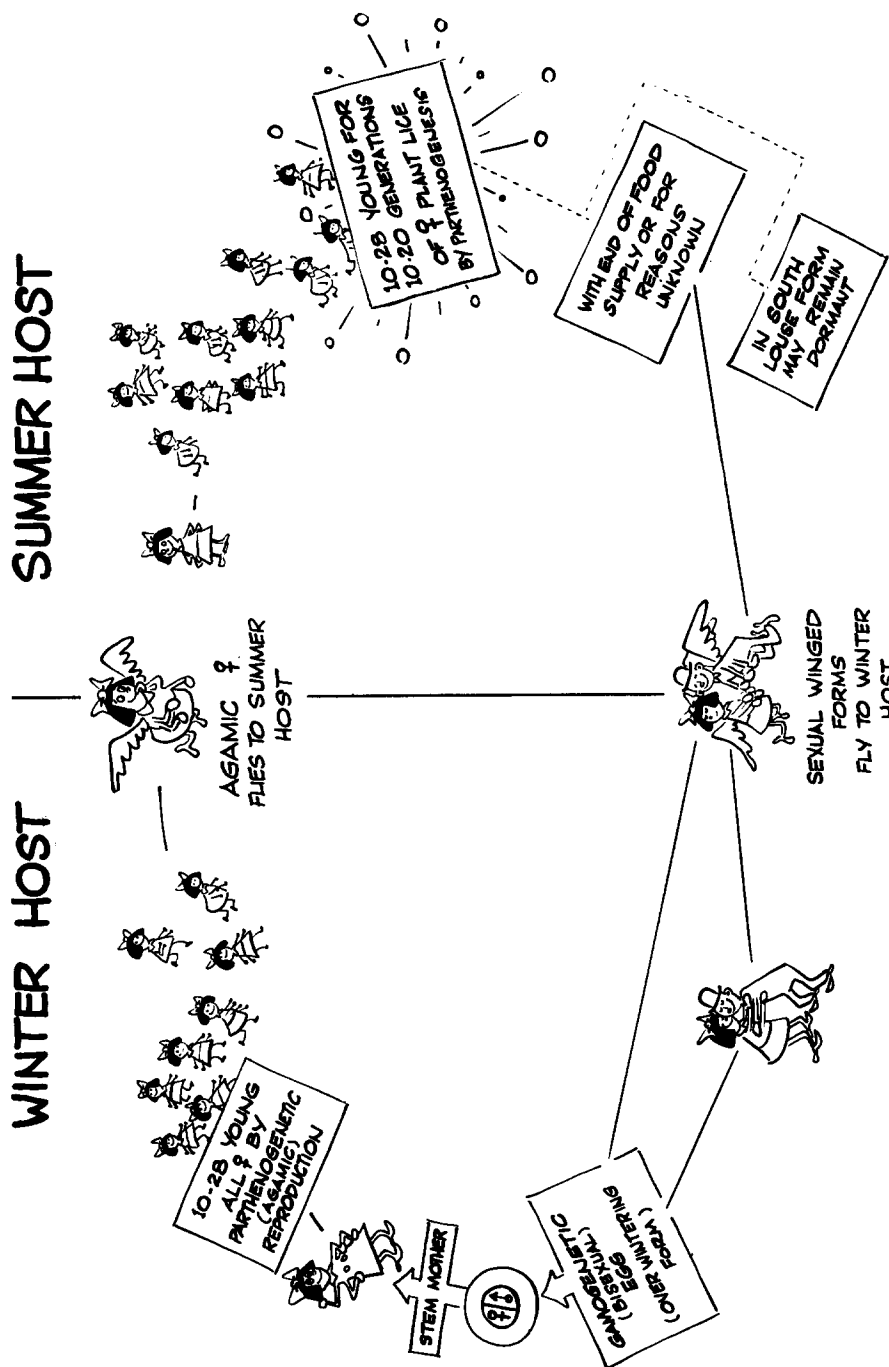


Fig. 1.—Cartoonist's version of the life cycle of the aphid. The gamogenetic egg develops to a stem mother who parthenogenetically reproduces female lice. These, in turn, parthenogenetically reproduce winged forms who fly to the summer host and revert to lice. These lice, all females, multiply prolifically, each producing 10 to 28 young for 10 to 20 generations during the summer. With the end of the food supply, sexual winged forms are developed who fly to the winter host and deposit the gamogenetic egg, the overwintering form. (Drawing courtesy Mr. James C. Raymond.)

ditions.¹⁶ It has been estimated that the cabbage aphid¹⁷ from one stem mother under perfect conditions would reproduce a sufficient quantity of progeny to cover the entire earth in one season.



Fig. 2.—Showing aphids and their cast skins upon a potato plant tip. The darker figures on the plant are the living lice and the lighter figures, the cast skins. (Courtesy Maine Agricultural Experiment Station, Orono, Me.)

During the course of its development from birth to adult form, the aphid progresses through 4 instars if it is to remain in the louse form and 5 instars if it is to become a winged form. At each instar it leaves a cast skin which is similar in type, although not in antigenic qualities, to that of the May fly.

Thus, the billions of aphids produce over four times their numbers of cast skins. These cast skins either remain for a period of time upon the food plant—being adhered by the action of honeydew—or fall upon the ground. It is logical to assume that these skins eventually dry and by breakage enter into the air as minute particles similar to the exuvia of the May fly. Because of the



Fig. 3.—Enlargement of the central portion of Fig. 2. It amply demonstrates the dark living aphids, the white newly cast skins, and the small white shrunk cast skins.

abundance of the insects, it is also logical to assume that these exuvia are present in the air in not inconsiderable quantity. In our opinion, this material is the major aphid antigen, rather than the winged form of the insect which originally induced interest in this allergen.

Thus it is established that we have from the aphid a profuse quantity of light exuvia in minute particles. This is spread over a wide geographic area and is capable of contacting many allergic individuals. (Figs. 2 to 5.)

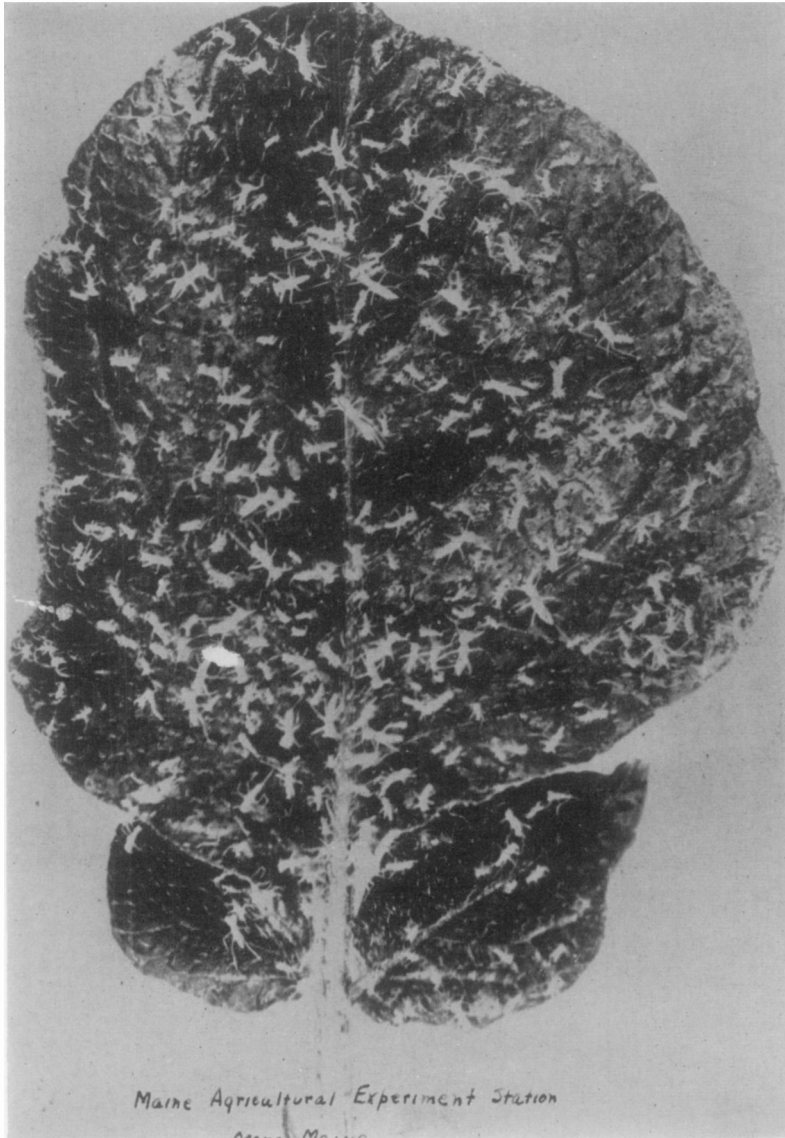


Fig. 4.—Photograph of a potato plant leaf, a large portion of which area is covered with the cast skins.

CHARACTER OF CAST SKINS (EXUVIA)

One of the anatomical characteristics of the insect is its exoskeleton. This is a relatively rigid structure giving the animal form as well as protection. During the process of growth the insect moults this structure at intervals.

Each ecdysis produces a cast skin. The number of moults or instars is constant for each species. The cast skins or exuvia is the discarded portion of exoskeleton. (Fig. 4.)

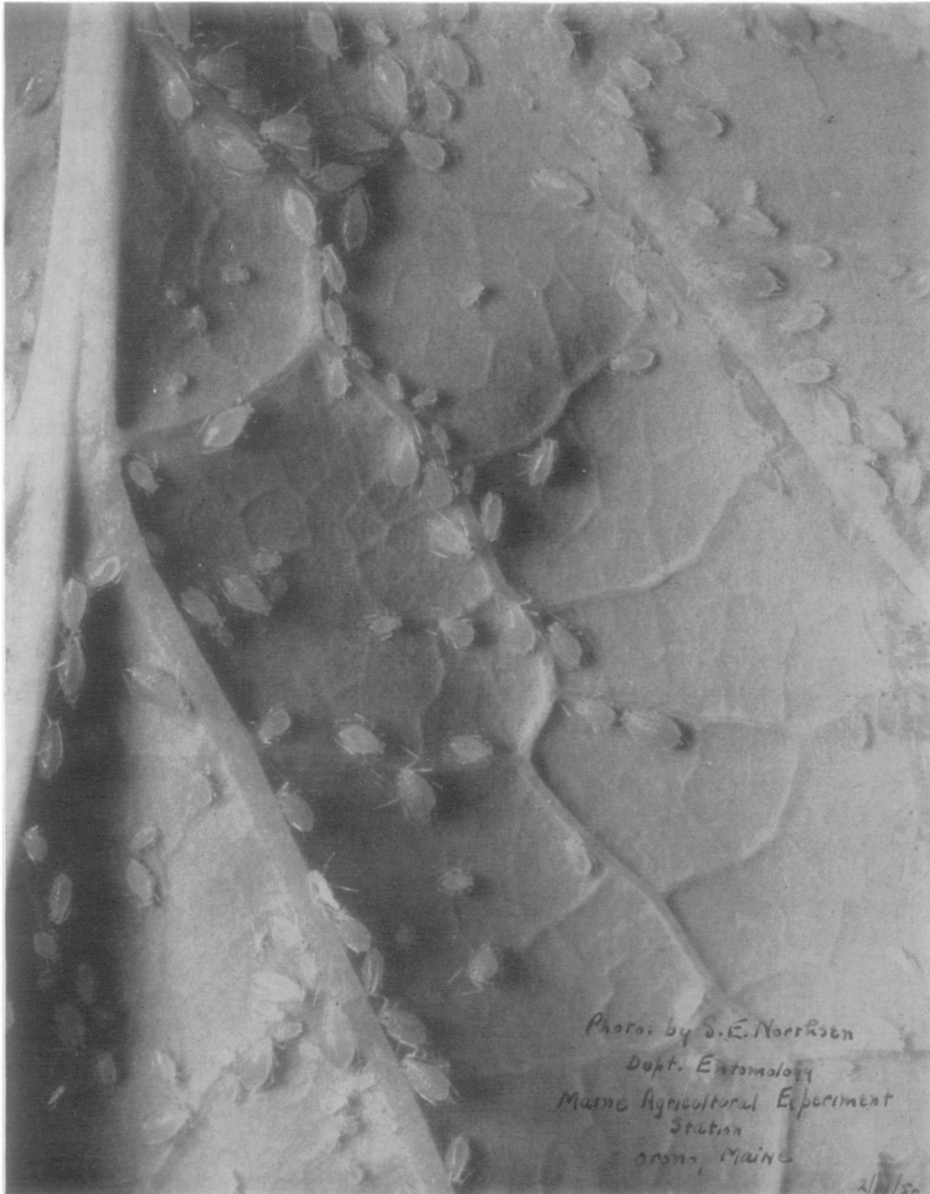


Fig. 5.—Close-up photograph showing the plant lice upon a peach tree leaf. There are 4 cast skins in the picture.

The exoskeleton or integument consists of cuticle. The cuticle¹⁸ is a non-living material, the product of a living matrix. This material is a plasticized protein sheet with waxes externally and chitin internally.¹⁹ Insect cuticle

contains chitin and a high proportion of other substances including pigment, fatty material, albuminous substance, and mineral components.²⁰

Chitin is a nitrogenous polysaccharide, whose chemical composition is $C_{15}H_{26}O_{10}$. Of this, about 8 per cent is nitrogen, about one-half the nitrogen content of protein. The chitin of all insects is similar both chemically and by crystallography.²⁰ Chitin always occurs associated with protein. The proteins in the exoskeleton vary with each insect species.²⁰ The cuticle proteins are in some respects similar to silk, but are a different product. Since the proteins in the exoskeleton cuticle vary with insect species and since the proteins are probably the antigenic elements, we may be sure the insect antigens are species specific.

SOURCE OF MATERIAL

The subjects used in this investigation were all from private practice. The patients were under treatment for hay fever, nonseasonal coryza, and/or asthma. All had been thoroughly investigated medically and for hypersensitivities. Most of the cases were pollen sensitive, usually to ragweed.

The extract was prepared by utilizing all of the collected aphid material. Both winged and louse forms were present. The insects were defatted with toluene and extracted in Coca's solution. The extract thus obtained was dialyzed, filtered, and standardized by a total nitrogen determination. The total material available was 3 Gm., which was extracted in 100 ml. of Coca's solution. This formula produced an extract containing 0.16 mg. total nitrogen per ml. (T. N./ml.). The completed extract was clear and dark brown in color. The dilutions for skin testing were made with buffered saline.

METHOD OF INVESTIGATION

The patients were tested with aphid extract in the usual manner with the intracutaneous technique. Initial tests were performed with a testing strength of 0.001 mg. T. N. and 0.01 mg. T. N. After a number of patients had been tested, it was felt the higher dilution was unnecessary. This judgment proved correct in all but one case, in whom a systemic reaction occurred from an injection of 0.01 mg. T. N. of the extract given intracutaneously. The volume of extract used for skin testing varied from 0.001 to 0.005 ml. The patients were or had been tested with ragweed, silk, and mould extracts in the strengths of 100 and/or 1000 Cooke-Stull protein nitrogen (P.N.) units. Silk was used as a testing material because of its possible antigenic relationship with other insect emanations. The moulds were tested routinely and were ruled out as probable allergens in most cases.

The patients selected were problem cases; they had what appeared to be typically allergic symptoms during the fall, both during and well beyond the period that ragweed pollen was found in the air, and they had received adequate pollen and/or inhalant therapy. Generally, these patients had received more than average attention since their treatment results were unsatisfactory.

The passive transfer tests were performed with the usual technique. Patients who had positive skin tests to the aphid and in whom the clinical story suggested sensitivity were bled. This blood was separated, and serum filtered,

TABLE I. RESULTS OF DIRECT SKIN (INTRACUTANEOUS) TESTS

CASE NUMBER	APHID 0.01 T.N. UNITS PER C.C.	ALTERNARIA 1000 P.N. UNITS	RAGWEED 1000 P.N. UNITS	SILK 1000 P.N. UNITS
1	neg	mkd	neg	neg
2	neg	neg	neg	mkd
3	neg	mod	mod	sl
4	neg	mkd	slmod	slmod
5	neg	neg	sl	slmod
6	neg	mkd	mkd	mkd
7	mod	mod	mkd	mod
8	neg	slmod	mkd	modmkd
9	modmkd	mkd	mod	mod
10	neg	mkd	mkd	mod
11	neg	mkd	neg	mkd
12	mod	mod	neg	mod
13	slmod	mod	mkd	slmod
14	neg	mkd	mkd	mod
15	slmod	sl	mkd	slmod
16	mkd	mod	mod	mkd
17	neg	neg	neg	neg
18	neg	neg	neg	neg
19	neg	neg	mod	neg
20	neg	mkd	sl	mkd
21	neg	mkd	mkd (a)	mod
22	neg	sl	mkd (a)	mkd
23	sl	mkd	mkd (a)	sl
24	neg	neg	sl	neg
25	sl	mkd	mkd	mod
26	mkd	---	neg	neg
27	mod	---	mkd	---
28	mod	---	mkd	---
29	neg	sl	mkd	neg
30	neg	neg	neg	neg
31	mkd	neg	neg	mkd
32	mod	neg	neg	neg
33	mkd	neg	mkd	sl
34	mkd	sl	mkd	mod
35	neg	neg	mkd	neg
36	sl	mkd	mkd	mkd
37	neg	mod	mkd	neg
38	mkd	sl	mkd	neg
39	sl	neg	mkd	neg
40	mod	neg	mkd	neg
41	mod	mkd	mod	sl
42	mod	neg	mkd	mod
43	neg	neg	mkd	neg
44	mkd	sl	mkd	neg
45	mod	neg	mod	neg
46	neg	neg	mkd	sl
47	mkd	neg	mkd	mkd
48	neg	neg	neg	neg
49	mkd	---	mkd	---
50	sl	mod	mod	neg
51	mkd	mod	mkd	mkd
52	mkd	neg	neg	neg
53	mkd	mkd	mkd	neg
54	mkd	neg	mod	sl
55	mkd	mkd	mkd	mod
56	neg	mod	mkd	mkd
57	mod	neg	mkd	sl
58	sl	neg	neg	neg
59	mod	neg	mod	mod
60	mkd	mkd	mod	slmod
61	mod	neg	neg	neg
62	mkd (a)	mkd	mkd	neg
63	mkd	neg	sl	mod

TABLE I—CONT'D

CASE NUMBER	APHID 0.01 T.N. UNITS PER C.C.	ALTERNARIA 1000 P.N. UNITS	RAGWEED 1000 P.N. UNITS	SILK 1000 P.N. UNITS
64	mkd	mkd	mod	slmod
65	modmkd	neg	mod	neg
66	mkd	mod	mkd (a)	neg
67	neg	neg	mod	neg
68	neg	neg	neg	neg
69	mkd	neg	neg	mod
70	mod	sl	mkd (a)	mod
71	mkd	mod	mod	mkd
72	neg	mkd (a)	mkd	neg
73	mkd	neg	mkd	neg
74	mod	mod	mkd	mod
75	mkd	neg	mod	neg
76	mod	neg	mkd	sl
77	mod	neg	mkd	neg
78	sl	mkd	mkd (a)	sl
79	neg	neg	mod	neg
80	mkd	mkd	mkd	neg
81	mkd (a)	mkd	mod	mod
82	neg	mod	mod	mod
83	slmod	neg	neg	mod
84	slmod	mod	mkd	neg
85	neg	mod	mkd	mod
86	mkd	sl	neg	mkd
87	mkd	neg	mkd	neg
88	mod	mkd	mkd	mkd
89	mkd	mod	mkd	mod
90	neg	mod	mkd	mkd
91	mod	mkd	mkd	neg
92	slmod	mkd (a)	mkd	neg
93	mkd	mod	mod	mod
94	neg	sl	mkd	neg
95	neg	mod	neg	neg
96	mkd (a)		mkd	mod
97	mkd		sl	mod
98	mod		mkd	sl
99	mod		mod	mod
100	(systemic reac- tion)		neg	mkd
	mkd			
	aphid 0.001 mod			
101	mkd		mod	modmkd
102	mod		modmkd	sl
103	mkd		mkd	neg
104	mkd		mod	neg
105	mkd		mkd	sl
106	mkd		mod	sl
107	mkd		mod	neg
108	modmkd		mkd	mod
109	mod		sl	neg
110	slmod		slmod	neg
111	mod		mkd (a)	sl
112	modmkd		mkd	mod
113	mkd		mkd	mkd
114	mkd		mkd	sl
115	mkd		mkd	
116	mkd		modmkd	mod
117	modmkd		mkd	modmkd
118	mkd		modmkd	sl
119	mkd		mkd	mod
120	mod		neg	neg
121	mkd		mkd	sl

All cases had asthma and/or coryza with symptoms aggravated during the summer and fall.

Units: Cooke-Stull protein nitrogen.

Key: neg negative
slmod slight to moderate
mod moderate
modmkd moderate to marked
mkd marked
mkd (a) marked active

and 0.1 c.c. of the sterile serum was injected in selected sites into the skin of the donor. These prepared transfer sites were tested at the end of 24 hours and were exhausted as rapidly as possible. Following exhaustion of the sites with either ragweed or aphid, retests were performed with the alternate material.

The initial investigation was performed on patients in whom the symptoms were related to a large degree to the ragweed season. For this reason, it was felt it would be well to prove an absence of antigenic relationship between this pollen and the aphid. To impress this fact, transfer sites were tested and exhausted with aphid or ragweed; then retested with ragweed or aphid. (Tables II and III.)

The ophthalmic tests were performed upon only a few patients by placing a drop of the extract in the conjunctival sac. These tests were read within 15 minutes. The characteristic conjunctival vascular dilatation was interpreted as a positive reaction.

RESULTS

In the falls of 1948 and 1949, a total of 121 patients were tested with the aphid extract. Of these, 75 gave reactions interpreted as positive. The results of these tests and of tests in the same patients with ragweed, alternaria, and silk may be seen in Table I.

Among the 75 who gave positive reactions, 59 had clinical symptoms which were worse in the summertime, usually much worse in the fall, and the remainder had symptoms of equal intensity throughout the year. Most patients were prone to have difficulty during the so-called pollen-free period from mid-July until mid-August and were especially bad during September and October. In many cases evidence of upper respiratory infection was evident. This, however, was not greater than might be found in the same patient at other times of the year with milder manifestations of coryza and asthma, or with none at all. One patient, following an intracutaneous test dose, had a severe systemic reaction, which occurred within 10 minutes of the test. This consisted of angioneurotic edema of the face and eyes and asthma. Epinephrine was necessary to control the reaction.

The remaining 46 cases failed to give positive aphid skin tests. The possible reasons for this are too obvious for discussion.

Case 96, in whom a marked active aphid test was elicited, was extremely sensitive to yellow jacket. On one occasion following a sting from this insect, the patient became unconscious, asthmatic, and cyanotic with evidence of edema of the larynx. This may account in part for the activity of the aphid test. The patient, however, also is a ragweed, fall, and winter asthmatic.

Several of these cases were able to obtain some relief of their symptoms by residence in New York City during September and October. It is interesting to note that the ragweed pollen count in New York City has consistently been higher than in the White Plains area. This apparent phenomenon is readily explained. The Westchester area is, to a large extent, well tended and the unpopulated areas are well covered with perennial vegetation, leaving little area for ragweed. The many open lots in the boroughs surrounding Man-

hattan and in New Jersey lead to the propagation of large areas of ragweed from which pollen is carried to the city, regardless of the wind direction. The aphid, on the other hand, finds vegetation in more profuse quantity in the suburbs; hence, it is present in greater numbers. This may explain the symptomatic relief obtained in some patients by metropolitan residence.

The figures in Table I reveal, also, that 44 cases gave positive skin reactions to alternaria, 90 to ragweed, and 46 to silk. Among the 75 cases positive to aphid, 41 were negative to silk. Among the 46 cases positive to silk, 19 were negative to aphid. These figures would indicate an absence of antigenic relationship between the two antigens. Among the 75 cases positive to aphid, 14 were negative to ragweed. Among the 90 cases positive to ragweed, 31 were negative to aphid. These figures would indicate a probable absence of relationship between these two antigens. This was substantiated by the passive transfer tests. (Tables II and III.) Among the 75 cases positive to aphid, 29 were negative to alternaria. Among the 44 cases positive to alternaria, 21 were negative to aphid. Here again, an absence of antigenic relationship is probable. All of this data would indicate an absence of common antigenic qualities among the four materials tested.

TABLE II. RESULTS OF PASSIVE TRANSFER TESTS

CASE NUMBER	RESULT OF DIRECT	PASSIVE TRANSFER TEST		RAGWEED
	SKIN TEST WITH APHID 0.01 T.N. UNITS PER C.C.	APHID 0.1 T.N. UNITS PER C.C.		2000 P.N. UNITS
1	mkd	mkd	s.e.a.	mod
2	mkd	modmkd	s.e.a.	sl
3	mkd	mod	s.e.a.	mod
4	mkd	mkd (a)	s.e.a.	sl
5	mkd	mkd	s.e.a.	mkd
6	mkd	mod	s.e.a.	sl
7	mkd	mkd	s.e.a.	mkd
8	mkd	mod	s.e.a.	sl
9	mkd	mkd	s.e.a.	sl
10	modmkd	mod	s.e.a.	mkd
11	mkd	mod	s.e.a.	neg
12	mkd	mod	s.e.a.	sl
13	mkd	mod	s.e.a.	neg
14	modmkd	slmod	s.e.a.	mod
15	mkd	mod	s.e.a.	sl
16	mkd	slmod	s.e.a.	mkd
17	mkd	mod	s.e.a.	mkd

s.e.a. - site exhausted to aphid.

Each test was controlled.

All controls were negative.

For key to table, see footnote to Table I.

In general, it may be logically deduced that such a large number of positive skin reactions to aphid extract in the cases described above would indicate the possibility of sensitivity to this material. (Table I.)

The results of the passive transfer tests may be found in Tables II and III. Of 17 bloods tested, 15 gave positive transfer reactions to aphid extract; 6 of these reactions were marked. In this group of 17 cases, 14 were clinically sensitive to ragweed. Of the 14 ragweed cases tested, 12 gave positive transfer reactions to the ragweed.

TABLE III. RESULTS OF PASSIVE TRANSFER TESTS

CASE NUMBER	RESULT OF DIRECT SKIN TEST IN RAGWEED	PASSIVE TRANSFER TEST RAGWEED 2000 P.N. UNITS	APHID 0.1 T.N. UNITS PER C.C.
1	mod	mod	s.e.r.
2	mkd	mod	s.e.r.
3	mkd	mod	s.e.r.
4	mkd	mod	s.e.r.
5	mkd	mkd	s.e.r.
6	mkd	sl	s.e.r.
7	mkd	mkd	s.e.r.
8	mod	mod	s.e.r.
9	mod	mod	s.e.r.
10	mkd	mkd	s.e.r.
11	modmkd	neg	s.e.r.
12	mod	neg	s.e.r.
13	neg	neg	s.e.r.
14	mod	slmod	s.e.r.
15	mod	neg	s.e.r.
16	mkd	mkd	s.e.r.
17	mkd	mkd	s.e.r.

s.e.r. - site exhausted to ragweed.

All controls were negative.

For key to table, see footnote to Table I.

All of the passive transfer test sites were exhausted, then retested with the alternate material. Of the 15 cases who had successfully transferred with the aphid extract, 10 still had skin-sensitizing antibody to the aphid after site exhaustion by ragweed. (Table I.) Of the 12 cases who had successfully transferred with ragweed extract, 8 were capable of demonstrating skin-sensitizing antibody to ragweed after site exhaustion with aphid.

These results would indicate that the patients with positive skin tests to the aphid extract have a skin-sensitizing antibody. They would indicate, also, that the aphid antigen is in no manner related to ragweed. (Table II and III.)

This work is presented as a preliminary report, with the hope of stimulating other workers. Much remains to be done to further prove the validity of these contentions. Efforts now in progress should produce some interesting data within the next few months.

SUMMARY

1. The life cycle of the aphid is explained. The aphid is one of the most prolific insects known.
2. An explanation of the qualifications of the aphid as an air-borne inhalant allergen is offered.
3. A number of patients with seasonal allergic manifestations have symptoms which are not explained by the known seasonal allergens.
4. Seventy-five of 121 patients, whose symptoms were unexplained by known seasonal inhalant allergens, gave positive reactions to aphid extract.
5. One patient had a systemic reaction of marked intensity following a skin test with aphid extract.
6. Passive transfer tests indicate that transferable skin-sensitizing antibody is present in the blood of skin-sensitive individuals and that the antibody is not antigenically related to ragweed.

7. It is felt that the aphid is an important insect inhalant allergen heretofore unnoticed.

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