

Antigen E content of pollen from individual plants of short ragweed (*Ambrosia artemisiifolia*)

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A study was conducted to ascertain how much variation in allergenic potency of ragweed pollen occurs among individual plants and different plant populations in a restricted geographic area. Seeds of the short ragweed (Ambrosia artemisiifolia) were collected from 38 different sites in Champaign County, Ill. Plants from these seeds were grown under similar conditions, and antigen E (AgE) was determined on pollen samples harvested from individual plants. The populations from various sites in Champaign County differed by as much as severalfold with respect to mean AgE values. There were statistically significant differences among population means, although large variations occurred among plants within populations. Further research was conducted to learn whether the level of AgE was determined by genetic factors within the plants. Genetic regulation was indicated by the finding that 3 plants which had unusually low levels of pollen AgE all produced offspring with significantly lower mean AgE levels than those of control plants.

The samples of ragweed pollen extracts available to allergists are known to exhibit great variations in allergenic potency and antigen E (AgE) content, so there is research on improved extraction and extract storage procedures.¹⁻⁸ The cause of variation in allergen content among samples of short ragweed (*Ambrosia artemisiifolia* L.) pollen is not known.⁸ Variation in pollen allergens might be expected because the short ragweed is an extremely variable species, and it may be an amalgamation of several subspecies.⁹ Various ragweed species do exhibit intraspecific variability in seed proteins,¹⁰ sesquiterpene lactones,¹¹ and volatile oils.¹²

While little is known about the nature and extent of intraspecific variation in ragweed allergens, differences among species are recognized. AgE, the major ragweed allergen,¹³ exhibited species-dependent differences in binding to rabbit antibodies raised against

short ragweed AgE.² Whole pollen extracts from various species also differed in ability to compete for binding sites on immunoglobulin E antibodies from ragweed hay fever sufferers.^{14, 15}

The present work was undertaken to establish the extent of variation in pollen AgE content of individual short ragweed plants and to learn whether various local populations differed in AgE content. Several plants giving relatively low values for AgE were identified. Their progeny were grown and tested to see if the trait was inherited.

MATERIALS AND METHODS

Plant material

Seeds of *A. artemisiifolia* were collected in 1975 at various sites in Champaign County, Ill. The sites are referred to by number, and information concerning their geographical location is available upon request. Plants from this seed collection were grown on the Horticulture Farm of the University of Illinois during the summers of 1976 and 1977. Ten plants from each site were grown in 1976, and various numbers of plants from selected sites were grown in 1977. Seedlings were transplanted to the field in June. The plants were spaced 1 m apart in rows which were 1 m (1976) or 2 m (1977) apart. No chemical pesticides were applied, and all volunteer weeds were removed by mechanical cultivation. Pollen was harvested from late August to early September, and seeds were harvested in October. Plants were

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numbered and records kept as to which plant was the source of each sample of pollen and seed.

Pollen

Flowering branches were covered with paper bags. Pollen shed each morning was removed later the same day, dried overnight at room temperature, and stored desiccated at 2° C until use. Pollen of Fig. 1 and Table I (series A) was stored 4 months prior to determination of AgE, and pollen of Table I (series B) was stored 9 months. Each sample represented a single plant and contained pollen from at least two collection dates. Pollen samples were not obtained from all plants due to death of occasional seedlings or presence of the pistillate condition described by Jones.¹⁶ A composite pollen sample was obtained in 1977 from a plot containing 10 plants from each of 14 sites (site numbers 4, 5, 17, 20, 36, 41, 45, 53, 56, 60, 63, 90, 117, 126). The pollen moisture content was 5% or less when AgE was determined, so no correction for moisture was made.

Reagents

All reagents were prepared with glass-distilled water. Agarose was from Bio-Rad Laboratories (Richmond, Calif.). Worthington Biochemical Corp., Freehold, N.J., was the source of highly purified AgE (Lot No. 56H585P) and sheep antiserum (Lot No. 05K25) for highly purified AgE. An aqueous AgE stock solution (1 mg/ml) was prepared and stored frozen. The dry sheep antiserum was reconstituted with water according to the manufacturer's instructions. The antiserum was tested and was specific for the AgE in crude pollen extracts. The barbital buffer used throughout the study was at pH 8.6 and contained (per liter) 1.0 gm sodium azide, 1.8 gm barbital, and 10.3 gm sodium barbital.

Measurement of AgE

Radial immunodiffusion (RID), a procedure used by others^{3, 5, 6} to determine AgE in pollen extracts, was used in the work reported here. The single plant samples of pollen contained enough AgE that the extremely sensitive radio-immunoassay² was not needed. The RID plates contained barbital buffer and 1% agarose. Sheep antibody was present at a final dilution of 42-fold compared to the stock solution. Pollen extract or AgE standard (30 µl) was placed in 4.0-mm-diameter wells. The plates were incubated 24 or 36 hr at ~22° C, and precipitin rings were measured to the nearest 0.05 mm with a calibrated viewer (Kallestad Co., Chaska, Minn.). Each RID plate contained a series of standards (50-1,000 µg/ml) and a good fit to a straight line was obtained by plotting log of AgE concentration vs log of ring diameter. A programmable calculator (Hewlett-Packard Model 9815A) was used to determine the best-fitting straight line for the AgE standards on each RID plate and to compute the concentrations of AgE in the pollen extracts. Pollen (2.5 rng) was extracted 24 hr at 1 to 3° C with 0.1 ml barbital buffer, and then duplicate portions were pipetted into wells of the RID plates as described above. The duplicates generally agreed well. Greater than 95% of duplicates

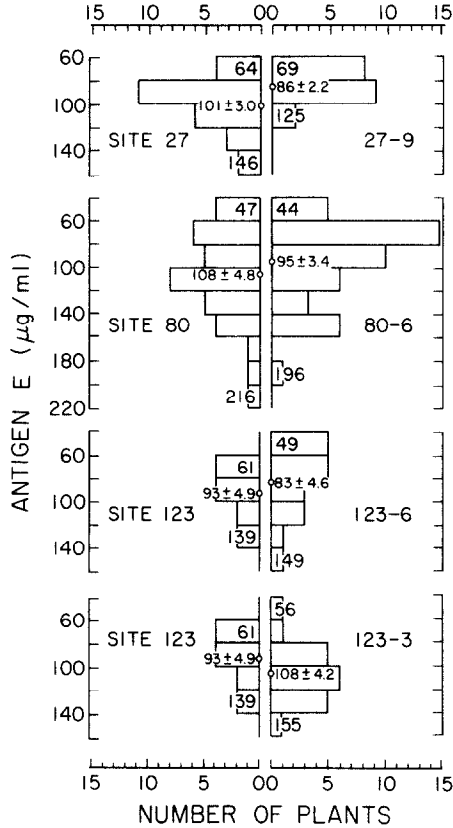


FIG. 1. Antigen E content of extracts from short ragweed pollen samples harvested from individual plants in 1977. A frequency histogram for each population is given. The populations designated Sites 27, 80, and 123 were grown from the seed lots collected at those sites. The populations designated 27-9, 80-6, 123-6, and 123-3 were grown from open-pollinated seeds of the 4 plants assigned these numbers. The antigen E contents of these 4 parent plants appear in Table I as the highest (No. 123-3) or lowest (other 3 plants) values of their respective populations. The mean and standard error are given for each population as well as the lowest and highest values. Methods of extracting and determining antigen E were the same as for Table I.

of Table I (Series A) differed from each other by less than 10%. Differences between duplicates were taken into account in all statistical analyses (see below).

The validity of procedures for extraction and measurement of AgE were established by preliminary experiments. *A. artemisiifolia* pollen purchased from Sharp & Sharp Co. (Everett, Wash.) was used. Precipitin ring diameter was not altered by removal of pollen grains from the extract immediately prior to starting the RID, so pollen and fluid were not separated in the work reported here. AgE was not affected by the 24-hr extraction and 36-hr diffusion in agar because the expected increase in precipitin ring diameter was observed when pollen was extracted with barbital buffer that contained 100 µg purified AgE/ml. The proportionality between number of pollen grains extracted and amount of AgE recovered was judged adequate for the pres-

TABLE I. Antigen E content of extracts from short ragweed pollen harvested in 1976*

Site no.†	No. of plants	Antigen E content (μg/ml)		
		Range	Mean ± SD	
Series A				
123	7	89-213	153 ± 44	a
80	6	76-202	154 ± 46	ab
63	4	106-202	155 ± 34	abc
99	4	141-180	158 ± 15	abc
57	8	117-222	160 ± 28	abc
102	6	111-207	160 ± 30	abc
27	6	59-221	162 ± 49	abc
35	6	119-211	164 ± 31	abc
17	8	97-196	165 ± 27	abc
20	8	112-203	166 ± 26	abc
46	4	131-201	167 ± 24	abcd
59	7	141-205	168 ± 15	abcd
56	9	122-237	173 ± 32	abcd
73	6	187-239	178 ± 40	abcde
23	10	122-230	178 ± 29	bcde
83	10	140-221	181 ± 20	cde
110	7	151-223	183 ± 22	cde
60	9	149-220	185 ± 21	cde
107	7	145-231	186 ± 32	cde
74	7	157-225	194 ± 21	de
90	8	182-224	202 ± 13	e
Series B				
36	2	142-170	158 ± 12	a
3	4	157-203	174 ± 18	a
116	4	86-250	198 ± 68	ab
4	8	125-338	201 ± 64	ab
104	4	181-220	206 ± 16	abc
112	6	192-250	209 ± 22	abc
5	6	157-301	215 ± 45	abc
93	4	187-245	221 ± 21	abcd
63	2	220-234	227 ± 8	abcd
114	8	137-397	241 ± 91	bcd
52	5	231-271	250 ± 12	bcd
41	9	160-364	255 ± 61	cd
53	8	146-360	260 ± 67	cd
117	10	152-349	262 ± 54	cd
126	8	203-364	269 ± 47	d
103	9	106-364	272 ± 68	d
40	9	280-468	350 ± 71	e

*Each pollen sample represented an individual plant. Extracts contained 25 mg pollen/ml extraction buffer, and duplicate determinations were done on each extract. Means followed by different letters differed at the 5% level of significance according to Duncan's multiple range test.

†Seeds were obtained from sites in Champaign County, Ill.

ent work because addition of 2.5, 5.0, and 7.5 mg pollen to 0.1 ml buffer gave estimates of 11, 11, and 12 μg AgE/mg pollen, respectively. In another experiment, pollen samples (2.5 mg) from 5 plants (sites 4 and 40) were extracted with diethyl ether and recovery of AgE was not improved.

Analysis of data

Statistical analysis was carried out using University of Illinois computer facilities and the SPSS computer package¹⁷ maintained by the Computer Services office. Data of Table I were subjected to univariate analysis of variance and Duncan's multiple range test, with Series A and B analyzed separately. A *t* test was used to assess the significance of differences between the pairs of means given in Fig. 1.

RESULTS

Pollen samples from 253 plants originating from 38 sites in Champaign County were analyzed, and all contained AgE (Table I). The extracts of four plants from sites 27, 80, 116, and 123 gave especially low values for AgE. The AgE values of these plants appear in the table as the lowest value for their respective populations. Three of these plants were studied further (see 1977 study below).

There were statistically significant differences among sites with respect to mean AgE content (Table I). However, the variation among plants within sites was sufficiently large compared to variation among sites that numerous population means were not significantly different from each other. Sites in the highest and lowest groups were all distributed throughout Champaign County.

Studies concerned with inheritance of AgE level were conducted in 1977. The plants from site 123 which produced the lowest (No. 123-6) and highest (No. 123-3) AgE levels in 1976 were progeny-tested to learn whether variation in AgE level within a site was under genetic control. Several plants (No. 27-9, No. 80-6, No. 123-6) which gave especially low AgE values in 1976 were also progeny-tested. These populations were compared to control populations grown from the same seed lots as plants of Table I. Antigen E was determined on pollen extracts, and results are given in Fig. 1. In all 3 cases, progeny from the low AgE plants gave mean AgE levels that were significantly lower ($p < 0.05$) than the means for the corresponding control populations. The mean AgE value of progeny from plant No. 123-3 was 15% higher than that of the control population, but the difference was not statistically significant at the 5% level ($p = 0.125$).

Progeny from single plants were about as variable as the corresponding control populations, judging from the frequency histograms and ranges given in Fig. 1. Such a result was anticipated because of cross-pollination from neighboring plants.

A comparison of results for the two different seasons does not reveal a clear-cut trend. The mean AgE values for sites 27, 80, and 123 were 30% to 39% lower for the 1977 than for the 1976 harvest (Table I and Fig. 1). This may be an environmental effect

because reagents and procedures were the same for analysis of both crops. However, the 1977 composite sample gave 201 μg AgE/ml when analyzed at the same time as the inheritance study of Fig. 1. A quite similar value (209 μg /ml) is obtained for 1976 by averaging the values of appropriate sites in Table I. The seasonal differences in AgE are small considering that 1976 was dry during the period of pollen development, whereas moisture was much above normal in 1977. Precipitation was 55.9 mm in August, 1976, and 254.0 mm in 1977 (Champaign-Urbana weather summary of the Illinois State Water Survey).

DISCUSSION

There was considerable variation in mean AgE levels of short ragweed populations from different sites in Champaign County, Ill. The range was approximately fourfold, considering data from two different growing seasons. The inheritance study indicated that pollen AgE level was at least partially controlled by genetic factors within the plants, so even greater variation might be encountered with short ragweeds from widely different geographic areas.

Some progress was made in genetic selection for plants with reduced levels of pollen AgE. However, the work was probably impeded by extensive hybridization of the desirable genotypes by airborne pollen from other plants. Recent research (D. Dickinson, unpublished 1977 field experiments) indicated that short ragweeds readily produced viable, self-pollinated seeds if parchment bags were stapled over branches bearing immature flowers. Therefore, it is feasible to continue this study by selecting among self-pollinated progeny of the desired parents.

The major objective in this study was to learn whether there is intraspecific variation in the level of a ragweed pollen allergen. Now that such variation has been established for AgE from *A. artemisiifolia*, it is important to consider the other ragweed allergens.¹⁸⁻²¹

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