

specific effects at very low concentrations, anthopleurine clearly meets the criteria for a pheromone (11). It is the second pheromone from a marine invertebrate animal to be isolated and fully characterized chemically.

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## Suppression of a Field Population of Houseflies with *Spalangia endius*

**Abstract.** Sustained releases of the microhymenopteran pupal parasite *Spalangia endius*, at a commercial poultry installation in north Florida, completely suppressed a population of houseflies within 35 days.

Control of houseflies, *Musca domestica*, by chemical or physical measures is often inadequate (1), and resort to other methods is frequently necessary. The use of predators and parasites of pest insects as an adjunct or replacement measure is a promising alternative (2). We found that *Spalangia endius*, a parasitic wasp, originally obtained from housefly pupae collected from an isolated dairy in Alachua County, Florida, could be an effective agent for housefly control (3), although it does parasitize other species of muscoid flies (4). We report here the successful suppression of a population of houseflies at a commercial poultry installation in north Florida as the result of the release

of this parasite during the summer of 1974.

The test area consisted of three open-sided poultry houses containing a total of 6700 caged layers. Our observations indicated that most of the housefly breeding occurred at the larger structure (3 by 114 m), which was separated by a distance of 33 m from the two smaller houses (7 by 25 m). The density of adult flies at the test area and at the check site, a similar poultry installation 6 miles (9.6 km) away where no releases were made, was determined before (test site only) and during the test by using a modification of the Scudder grid (5). Three times a week samples of housefly pupae were collected from the breeding area at the test site and at the check site and returned to the laboratory, where they were held for 10 days at 27.8°C and 60 percent relative humidity to allow for emergence of adult flies or pupal parasites. Housefly pupae that did not eclose in that time were examined microscopically for evidence of parasitism or natural mortality. The natural parasitism of pupae collected in the samples at the test site before the test was 24 percent (Fig. 1); at the check site, it averaged 22 percent throughout the 10 weeks of the test.

The *Spalangia endius* released at the test site were reared in the laboratory. The adult wasps were held in plastic cages (61 by 61 by 48 cm) at 26.7°C and 60 percent

relative humidity. Also, because the parasites are attracted to the nearest light source, rearing was conducted in total darkness except when adjustments or manipulations were necessary. Three times a week 160,000 2-day-old housefly pupae were introduced into a cage containing 24,000 parasites. After 18 to 24 hours, the pupae were removed, placed in 4-liter paper containers, and held at 60 percent relative humidity and 27.8°C until the next generation of parasites began emerging 18 to 20 days later. Three to 5 days later, the empty fly puparia and emerged adults were removed. The remaining (parasitized) fly pupae were divided into groups of 6000 and held in 2-liter containers.

The test began the week of 23 June. Three times a week for 10 weeks, 14 of the 2-liter containers of parasitized housefly pupae were placed at six release sites adjacent to the larger poultry house.

Four weeks after the initial release (Fig. 1), all housefly pupae collected from the test area were parasitized. Thereafter, for another month, parasitism ranged from a low of 93 percent to a high of 100 percent. However, on 25 August it dropped to 14 percent, a result of the removal of a majority of the manure during the week of 4 August. Most of the existing population of adult *Spalangia* was removed with the manure, as was most of the larval breeding medium. Thus, for several weeks it was difficult to find housefly pupae, and the few that were collected from an isolated group during the week of 25 August were young pupae that were not parasitized at the time they were collected. It is assumed that if the pupae had been collected when they were older, the rate of parasitism would have been higher. Thus, the removal of the adult wasps, combined with the reduced numbers of emerging parasites, which resulted from the removal of many pupal sites, so decimated the parasite population that immigrating houseflies were able to reinitiate a fly population in the larval medium that had a low number of parasites.

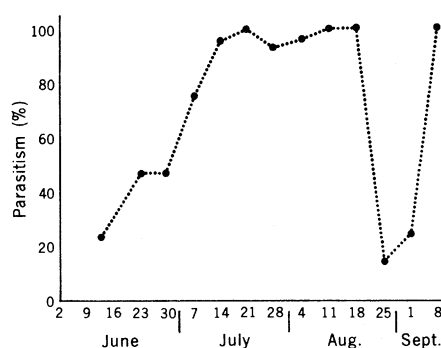


Fig. 1. Parasitism of pupae collected at the release site from 23 June through 25 August 1974.

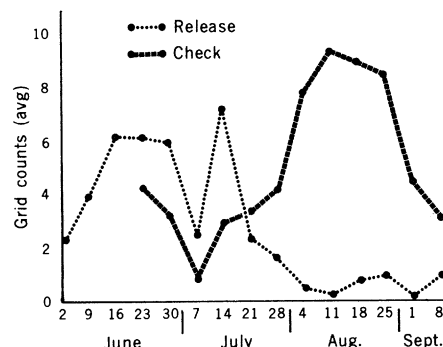


Fig. 2. Effect of releases (from 23 June through 25 August 1974) of *Spalangia endius* on a field population of houseflies.

However, the released parasites again dispersed throughout the larval breeding area, and 2 weeks later all housefly pupae that were collected were parasitized.

The grid counts made at the test area (Fig. 2) showed that the population of houseflies increased from the prerelease count of 2.3 flies per grid to about 6.2 flies per grid, dropped because of the releases, and then on 14 July, perhaps because of immigration, increased to 7.2 flies per grid. However, by 21 July—that is, within 35 days after the first release—the count had decreased to the lowest level before the treatment and continued to decline throughout the remainder of the study. Indeed the counts never exceeded 1 fly per grid during the remainder of the test, although adequate larval breeding medium was always present except during a short period in August just after the manure was removed.

At the check farm, grid counts were begun 24 June. The weekly larvicide treatments reduced the fly population to less than 1 fly per grid by 7 July, but subsequent intermittent larvicide treatments failed to check the increase in the housefly population. On 18 August, weekly insecticidal treatments were started again and the housefly population dropped.

Therefore, in this study parasites were as effective as the recommended insecticidal treatments in the control of houseflies (6). The costs of producing and releasing the wasps were relatively low and slightly less than those for pesticides. This approach is highly pest-specific, does not adversely affect the quality of the environment, and eliminates many of the problems that are normally associated with pesticides.

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## Peptide Inhibition of the Prausnitz-Küstner Reaction

**Abstract.** A pentapeptide was synthesized with the same amino acid sequence that occurs in a unique region of the  $\epsilon$  chain of immunoglobulin E near the cysteine residue participating in the linkage between the two heavy chains. This pentapeptide has the capacity to block a standard Prausnitz-Küstner reaction as well as to inhibit a known positive skin test reaction. Other similar synthetic polypeptides had less or no inhibitory activity. The likelihood that this pentapeptide amino acid sequence does in fact represent the structure of the specific immunoglobulin E binding site for the mast cell and basophil is considered.

Immunoglobulin E (IgE) binds to mast cells and basophils (1). The cell binding site has been localized in the Fc portion of the molecule, and as such is thought to reside in the second, third, or the fourth domain (or all of these) of the constant region of the  $\epsilon$  heavy chain (2). Efforts to isolate a smaller fragment containing the binding site by enzymatic digestion of the Fc portion of IgE have not been successful. Thus, although the IgE Fc inhibits the Prausnitz-Küstner (P-K) reaction in man, no other smaller fragments of Fc examined retain activity (3). This observation has given rise to the notion that intrachain disulfide bonds play a crucial role in the conformation essential for skin binding, and thus inhibitory polypeptides might not be readily synthesized (3). We have reasoned that a relatively short polypeptide, representing a small region of the Fc fragment of the  $\epsilon$  chain, is responsible for fixation of IgE molecules on the sterically complementary mast-cell receptor (4).

By comparing the amino acid sequence of a fragment of  $\epsilon$  chain of IgE (from PS myeloma) believed to include the hinge region, and that of fragment III of IgE (from ND myeloma) with the homologous regions of the  $\gamma$ ,  $\alpha$ , and  $\mu$  chains, five small regions (one decapeptide, one hexapeptide, and three pentapeptides) unique to the second and third constant domains of the  $\epsilon$  chain were located (5) (Table 1). Of these the pentapeptide Asp-Ser-Asp-Pro-Arg was synthesized (6). This peptide was found to have the capacity to inhibit the binding of IgE to the mast cells of the skin as measured by the inhibition of the P-K reaction (7). With the publication (5) of the complete sequence of the  $\epsilon$  chain of human IgE (ND), this pentapeptide was located as amino acids 320 through 324. The hex-

apeptide Ala-Asp-Ser-Asp-Pro-Arg was also prepared, as well as a tripeptide and a tetrapeptide contained within this sequence: Asp-Pro-Arg and Ser-Asp-Pro-Arg. In addition another synthetic peptide, the methyl ester of tosyl-L-argininylsarcosine (TASMe) and another pentapeptide Asp-Thr-Glu-Ala-Arg were synthesized for comparison testing (8).

The P-K reaction was utilized to measure the capacity of each peptide to inhibit the wheal and flare (immediate hypersensitivity) response. This classic method involves the intradermal injection of allergic serum (containing IgE specific for a known antigen or allergen), waiting 20 or more hours, and then challenging at the same sites with a prick or intradermal injection of a solution of the specific antigen. The extent of the positive reaction can be ascertained by measurement (in millimeters) of the diameter of the wheal (and flare) that develops over the following 10 to 30 minutes. All these studies were performed with a single, proven, P-K donor serum (B) with which we have had considerable experience (9). The typical sequence of events was intradermal injection of 0.1 ml of the peptide solution or control (buffered saline diluent) solution, followed in 1 to 24 hours by intradermal injection of 0.05 ml of the P-K serum into each of the previously injected sites. After 20 to 24 hours, each site was prick-punctured with the antigen solution, blotted dry in 5 minutes, and the wheal and flare were measured in both their narrowest and widest diameters at 15, 20, and 25 minutes. (Flares were measured only for confirmation of the occasionally difficult to measure wheal).

The average percentage inhibition of the standard P-K reaction with the use of six different peptides in six individuals is shown in Table 2. Results are the average of duplicate measurements on each individual at three different times, subtracted from the average control wheal measurements, and divided by the average measurement of each individual's control wheal. Control wheals in different individuals varied from 8 to 40 mm<sup>2</sup>, with a mean of 17 mm<sup>2</sup>. In this set of experiments, each peptide was used at a dilution of approximately 6  $\mu$ g/ml and 0.1 ml was injected

Table 1. Formulas of five amino acid sequences unique to the  $\epsilon$  chain near the hinge region.

Residue numbers (5)	Sequence
266–275	Asp-Val-Asp-Leu-Ser-Thr-Ala-Ser-Thr-Glu
289–293	Leu-Ser-Gln-Lys-His
320–324	Asp-Ser-Asp-Pro-Arg
354–359	Ala-Pro-Ser-Lys-Gly-Thr
367–371	Ala-Ser-Gly-Lys-Pro