TECHNICAL BULLETIN No. 70 DECEMBER 1971

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ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Richard L. Doutt for his advice, guidance, and assistance given throughout this study. Dr. Doutt, Dr. Edward A. Steinhaus, and Dr. John W. Oswald critically read the manuscript and offered valuable criticism.

Grateful acknowledgment is also made to Dr. Kenneth Hagen, Dr. Yoshinori Tanada, Dr. Edward Sylvester, and Dr. Duco Hamasaki of the University of California and Dr. Martin Sherman and Dr. Ryoji Namba of the University of Hawaii for their helpful suggestions and words of encouragement. Special thanks are due to Mr. Alvin M. Tanabe for his assistance in rearing some of the host material used in this study. Thanks are also due to Dr. Anna R. Whiting of the University of Pennsylvania and Mr. George Angalet of the United States Department of Agriculture for providing the initial stocks of *Bracon hebetor* and the Indian *Bracon*, respectively.

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INTRODUCTION

Venomous arthropods have interested man for thousands of years. The oldest medical writings containing recommendations for prescriptions to be used on bites of various sorts date back to 1600 B.C. from Egypt (Leake, 1956). Man's interest, however, has been directed primarily at those arthropods that directly affect his health or the health of his animals. There is a wealth of information on the chemical, physiological, and pathological properties of animal venoms, as indicated by the *Bibliography of Animal Venoms*, compiled by Harmon and Pollard (1948), and the American Association for the Advancement of Science's symposium on venoms, edited by Buckley and Porges (1956).

These studies, however, deal almost exclusively with venoms that are known to be toxic to mammals. Among the insects, therefore, the studies have been primarily confined to mammalian toxicity of venoms of certain Hymenoptera which are capable of stinging mammals. An example of this type of study is the concerted effort made to investigate the venom of the fire ant, *Solenopsis saevissima* var. *richteri* Forel (Blum *et al.*, 1958; Adrouny *et al.*, 1959; Wilson, 1959).

There is, however, another large group of venomous Hymenoptera that do not affect man directly since they do not sting, or are not capable of stinging, man. These Hymenoptera, nevertheless, are of extreme importance in the natural and biological control of many insect pests.

Clausen (1940) lists 36 families of Hymenoptera which possess species that utilize venoms to paralyze their hosts either temporarily or permanently. According to this author, practically all parasitic or predaceous Hymenoptera which are able to sting utilize this weapon to overcome their hosts. There is a paucity of information, however, on the venoms of these parasitic or predaceous Hymenoptera and their effects on their insect hosts (Doutt, 1959*b*). Most of the observations on the effects of the venom have been made in conjunction with the study of the biology of the hymenopteron and are usually confined to the brief statement that the host is either temporarily or permanently paralyzed, or killed outright, by the venom of the parasite or predator.

There is a lack of quantitative observations on the host-parasite interaction in terms of the venom. Beard (1952) conducted the first toxicological study of the venom of a parasite, *Bracon hebetor* Say, that does not sting man. However, it is desirable to obtain much more toxicological information

on the relative potency of the venom of other species of parasites as well as additional biological information on the numbers of hosts paralyzed by a parasite, the rate at which the parasite stings hosts, the venom production by the parasite, the immunization of the host to the venom of its parasite, and the effect of the venom on other organisms associated with the host.

The susceptibility of the host to the venom of its parasite, in terms of the rapidity at which the host is inactivated and the permanence of the inactivation, is a characteristic that may partially determine the effectiveness of the parasite in natural and biological control. Smith (1929) stated that the inherent fecundity of the female of any species of parasite is usually in excess of that needed to enable it to oviposit on all the hosts it can find. Any factor, therefore, that can give the parasite more time for searching should aid the parasite in maintaining the host density at a lower level. Other things being equal, a host that succumbs more rapidly to the effects of the venom should be maintained at a lower density than one that takes a long time to be inactivated.

Two closely related species of gregarious ectoparasites, *Bracon hebetor* Say and a species of *Bracon* from India, were utilized in this study. Both of these parasites possess venoms that cause a flaccid paralysis in their hosts. The use of two closely related species of parasites afforded an opportunity to determine whether there were differential potencies expressed in a given host species to two apparently closely related venoms that cause identical symptoms of paralysis. This would indicate physiological differences in species that are morphologically almost identical. The venoms, however, apparently both have the same site of action in the host. Beard (1952), from electrophysiological evidence, postulated that the venom of *Bracon hebetor* affected the neuromuscular junction.

Four species of hosts, common to the two species of parasites, all in the lepidopterous family Pyralidae, were used in this study.

THE TAXONOMIC STATUS OF THE PARASITES

Two species of *Bracon* were used in this study. The first species, *Bracon hebetor* Say, was obtained from Dr. A. R. Whiting of the University of Pennsylvania, and bore the label "wild-type stock *Habrobracon juglandis*." This is the species that has been extensively studied by geneticists and is well known to them as *Habrobracon juglandis*. Since most of the genetic work on this insect has been done under the name *Habrobracon juglandis*, Martin (1947) suggested the continued use of this name. In this study, however, the insect will be referred to by its valid name, *Bracon hebetor* Say (Muesebeck, 1951).

The second species was sent from India as *Bracon brevicornis* Wesmael but appears to be a segregate intermediate between the typical *B. hebetor* of the Western hemisphere and the typical *B. brevicornis* of Europe (Doutt,

1959*a*). The separation of *B. hebetor* from *B. brevicornis* has been based primarily on the differences in the number of segments in the antennae between the two species (Cushman, 1922; Muesebeck, 1925). Schlottke (1926), however, showed that there was a positive correlation between the size of *B. hebetor* and the number of antennal segments. According to Richards and Thomson (1932), *B. hebetor* is a domestic species while *B. brevicornis* attacks many lepidopterous pests in the field.

Studies on the identity of *B. hebetor* and *B. brevicornis* in India have led to some conflicting results. Lal (1946) examined progeny from a single pair of *B. brevicornis* and arrived at some peculiar results. Although most of the offspring from this pair resembled their parents and appeared to be typical *B. brevicornis*, there were four specimens that were atypical. When these atypical specimens were sent away to experts for identification, two were identified as *B. stabilis* and two as *B. hebetor*. These determinations were based primarily on the number of antennal segments and on whether the abdominal tergites were smooth or punctate.

Puttarudriah and Channa Basavanna (1956), also working in India, found the characters used to separate *B. hebetor* from *B. brevicornis* to be so variable that they were unable to detect clear-cut differences. According to these authors, the genitalia of both sexes of the two species were identical and reciprocal crosses produced fertile offspring. They concluded, therefore, that the two insects were conspecific, but for the present, they felt that the two insects should be left as separate species. Cherian and Margabandhu (1949) also implied that, although the two species may be the same, they should be kept separate until definite proof is obtained.

Narayanan *et al.* (1958), on the other hand, after examining the male genitalia and attempting reciprocal crosses, concluded that *B. hebetor* and *B. brevicornis* were different species. They were able to detect differences in the basal rings, the volsellar plates, and the phallic organs of the two species. Moreover, reciprocal crosses of the two species did not produce any females although mating occurred. They also found that the number of antennal segments varied among the individuals and there was a considerable amount of overlap between the two species. Except for all the *B. hebetor* females examined, in many of the individuals of both species, the number of segments in the right antenna differed from that of the left antenna, although in none of the specimens was there more than a differential of one segment between the two antennae. All of the *B. hebetor* females examined had an equal number of segments in both antennae.

Although it is not known whether the Indian species utilized in this study is the same as the *B. brevicornis* studied by Narayanan *et al.* (1958), there is sufficient evidence to indicate that the *B. hebetor* obtained from Dr. Whiting and the Indian segregate are different species. Differences were observed in the behavior, coloration, and in the key criterion for the separation of closely related species according to the nondimensional

species concept (Brown, 1959), *i.e.*, reproductive isolation between the two species. Reciprocal crosses attempted in this study failed to produce any females although mating occurred.

The Indian segregate, therefore, will be considered a separate species from *B. hebetor,* but since it is not within the scope of this study to determine the exact taxonomic status of this species, it will simply be referred to in this paper as the Indian species, the Indian *Bracon,* or the Indian segregate.

Figure 1 presents the male genitalia of the two species of parasites used in this study.

REARING METHODS

Throughout this study, most of the experiments were conducted on *Corcyra cephalonica* (Stainton), although *Anagasta kühniella* (Zeller), *Plodia interpunctella* (Huebner), and *Galleria mellonella* (Linnaeus) were used in many of the experiments. These four species of pyralids are all hosts of the two species of parasites. The first three species of hosts were reared in gallon-sized jars on an oatmeal medium. Adult moths were either anesthetized with CO_2 and collected, or, in the latter stages of the study, were collected with a hand-operated insect aspirator similar to the one described by Keiser (1958). These moths were introduced into the jars and allowed to oviposit directly into the medium. Periodically more medium was added to insure sufficient food for the growing larvae. The availability of an abundant supply of food promoted the production of larvae that were relatively uniform in size.

G. mellonella was reared on a medium consisting of 350 grams of Pablum mixed cereal, 100 ml. of honey, 100 ml. of glycerin, and 25 ml. of water. The honey, glycerin, and water were completely mixed and folded into the cereal. Paper toweling was crumpled and placed on top of the medium to provide ovipositional sites for the adults.

All species of host larvae to be used in tests were extracted from the medium by carefully tearing open the silken webbing of the tunnels. Only last instar larvae were used in the tests.

The parasites were reared on hosts that had been removed from the host-rearing media. Twenty to 30 host larvae were placed in mason jars or ice cream carton tops and approximately five pairs of parasites were introduced. Although these parasites host-feed, a small amount of honey was smeared on the walls of the jar to provide additional food. The parasites were removed after a 24- or 48-hour ovipositional period. After emergence of the adults started, collections were made daily so that each day's collection contained parasites that had emerged within the previous 24 hours.

Throughout most of this study, the hosts completed their life cycles in approximately 2 months and the parasites in 10 to 15 days.

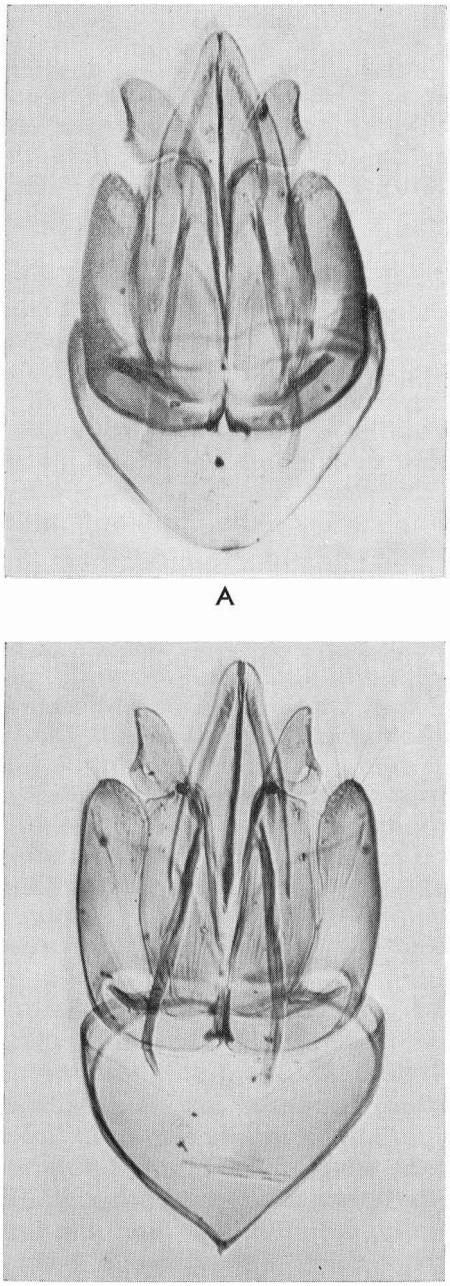

FIGURE 1. The male genitalia of (*A*) *B. hebetor* Say and (*B*) the Indian *Bracon.*

BIOLOGY

B. hebetor is important both to geneticists as an experimental animal and to entomologists as a biological and natural control agent for many lepidopterous pests. The biology of *B. hebetor,* therefore, has been studied in detail by many authors, in various parts of the world, including Doten (1911), Whiting (1918), DeOng (1923), Hase (1924), Showell (1928), Knowlton (1931), Payne (1931), Morrill (1942), Silva (1947), Appanna (1952), and Rao (1956).

B. hebetor is primarily a gregarious ectoparasite of lepidopterous larvae. Thompson (1953), in his parasite catalogue, lists 27 hosts for *B. hebetor* of which 25 are Lepidoptera. The great majority of its hosts are stored-product pests. The female is able to sting and paralyze hosts almost immediately after emergence. After being stung, a complete flaccid paralysis of the host results in 10 minutes to 1 hour, depending on the species of host. Both males and females feed on host fluids (Grosch *et al.,* 1955). Mating and oviposition occur shortly after emergence. The female usually lays the white cigar-shaped eggs on the host, although occasionally eggs are laid on the container in the immediate vicinity of the hosts. The number of eggs laid per host depends on host size and development of progeny (Ullyett, 1945).

On *Plodia interpunctella,* the parasite laid from 1 to 11 eggs per day when individual pairs of parasites were given one host per day, and from 2 to 358 eggs per female were laid with a mean of 90 per parasite (Morrill, 1942). The eggs hatch in 1 to 2 days and the larvae feed on host fluids through punctures chewed into the integument. The larvae pass through four instars, greatly increasing in size. According to Grosch (1948), however, the main increase in larval size is due to the enlargement of the midgut and not to multiplication of living tissue. Pupation takes place in a silken cocoon in the immediate vicinity of the paralyzed host. The entire life cycle, from egg to adult, can be completed in 7 days at 32° C. On a diet of honey and water, the males lived 24 days and the females 29 days, at constant conditions of 30° C. and 79.5 percent relative humidity (Georgiana, 1949).

The Indian *Bracon* goes through a life cycle that is almost identical to that of *B. hebetor.* It also undergoes three molts and four instars. There were, however, biological differences noted between the two species of parasites. Doutt (1959*a*) found that the mean number of eggs laid per host was greater in the Indian *Bracon* than in *B. hebetor.* He also found that the Indian *Bracon* stings and oviposits before seeking a new host. Genieys (1925) observed the same type of behavior in *Bracon brevicornis. B. hebetor,* on the other hand, stings a host and leaves. Oviposition is accomplished later after the paralyzed host is found again. The Indian *Bracon* also is able to exercise restraint in oviposition at low host densities (Doutt, 1959*a*) while *B. hebetor* does not (Ullyett, 1945).

Another difference in behavior was the amount of stimulation needed to force the parasites to take to flight. It was difficult to force *B. hebetor* to fly. Disturbing this parasite merely resulted in its walking away. Even when the parasite was pushed around with the tip of a pencil, it normally did not take to flight. The Indian species, however, flew very readily. Just a slight jarring of the container that held the parasite was usually sufficient to start flight.

B. hebetor did not even attempt to sting larvae of *Spodoptera mauritia* (Boisduval), *Trichoplusia ni* (Huebner), *Hedylepta blackburni* (Butler), *Pieris rapae* (Linnaeus), *Otosema odora* (Linnaeus), and *Achaea janata* (Linnaeus), although it usually stung hosts removed from cocoons or silken webbing more readily than did the Indian *Bracon*. The Indian segregate stung all of the species listed and venom caused paralysis in all of these species. Oviposition, however, did not occur on these larvae.

When reared on the same host species at the same temperature, the Indian *Bracon* usually completed its life cycle a full day ahead of *B. hebetor* and was generally lighter in color. When reared at 32° C., however, both species of parasites were equally light in color.

There were no apparent differences in the size of the adults of the two species. Longevity, however, was greater in the Indian *Bracon*. The adult females of the Indian species when fed only honey or a mixture of honey and water were able to subsist for more than 3 months at an average temperature of 29.5° C. Longevity was greatly reduced, however, when the female was actively paralyzing and ovipositing on hosts. The males all died within a month.

The larvae of either species of parasites were able to develop on hosts paralyzed by the other species. The transfer of *B. hebetor* eggs and larvae to hosts paralyzed by the Indian *Bracon* or the reverse transfer of eggs and larvae of the Indian *Bracon* to hosts paralyzed by *B. hebetor* produced normal, fertile adults. The larvae of either species of *Bracon*, therefore, were able to develop on hosts paralyzed by the other species.

Specific differences may also be reflected as differences in host preference even in common hosts. Myers (1929) found that *B. hebetor* preferred *Plodia interpunctella* for oviposition although *Ephestia cautella* (Walker) was just as abundant and just as readily stung.

A short experiment was conducted to determine whether there were any behavioral differences in the two species of parasites as evidenced by differences in host-stinging preference. *Galleria mellonella* and *Anagasta kühniella*, both normal hosts of the two species of parasites, were utilized in this experiment. The experiment was arranged so that there was complete intermingling of the two hosts within a single container. Mature larvae of the two host species were removed from their culture media and placed in the containers. The stinging preference, therefore, had to be strong

TABLE 1. Host-stinging preference of *B. hebetor* Say and the Indian *Bracon* when confronted with *G. mellonella* (L.) and *A. kühniella* (Zeller) at different host-density combinations. Each combination was replicated four times.

NO. OF HOSTS		TOTAL NO. PARALYZED IN 5 DAYS, ALL REPLICATES		
G. mellonella	*A. kühniella*	*G. mellonella*	*A. kühniella*	Total
		B. hebetor		
20	0	215	—	215
15	5	199	62	261
10	10	155	153	308
5	15	55	234	289
0	20	—	244	244
Total		624	693	1317
		Indian *Bracon*		
20	0	211	—	211
15	5	192	53	245
10	10	130	137	267
5	15	53	229	282
0	20	—	277	277
Total		586	696	1282

enough to be exhibited in a situation where there was a complete mixing of host odors.

Petri dishes were set up with five different host-density combinations. The five combinations were as follows: (1) 20 *G. mellonella* larvae; (2) 15 *G. mellonella* and 5 *A. kühniella;* (3) 10 *G. mellonella* and 10 *A. kühniella;* (4) 5 *G. mellonella* and 15 *A. kühniella;* and (5) 20 *A. kühniella.* One newly emerged *B. hebetor* was placed in each of the containers. A duplicate series was set up with the Indian *Bracon.* Both species of parasites were reared from larvae of *A. kühniella.* Each host-density combination was replicated four times for each parasite species.

Counts of the number of hosts paralyzed were made at 12-hour intervals. All paralyzed larvae were removed and replaced with new hosts when the counts were made. Therefore, during any one 24-hour period, each parasite had access to 20 plus the number of larvae it had paralyzed during the preceding 12-hour period. The experiment was terminated after 5 days.

Table 1 summarizes the results of this experiment. There were no significant differences in the host-stinging preference of the two parasite species under the conditions of this experiment. Neither *B. hebetor* nor the Indian *Bracon* significantly stung more of one species in preference to the other, although in actual numbers, both parasites paralyzed a few more *A. kühniella* than *G. mellonella.*

DESCRIPTION OF STINGING AND SYMPTOMS

The mode of attack of the parasites and the symptoms elicited in hosts by the venom of both species of *Bracon* were very similar.

Murr (1930) found that the female *B. hebetor* was able to find the host with its olfactory sense alone. Both species were definitely attracted by the silk secreted by their hosts. The silk alone sometimes was sufficient to cause the parasite to start probing with its ovipositor. In the actual stinging process, the abdomen was bent forward between the legs and the ovipositor was thrust out anteriorly. At maximum extension, even the tip of the abdomen was pushed forward, anterior to the head. In this position with the ovipositor fully extended, the parasite was able to reach larvae farther away than its own body length.

If this stinging position was assumed and the host was still out of reach, the parasite often remained in this position without moving for as long as 5 minutes. The stinging position often was held even after the host moved away from the immediate area. This behavior was especially true of the Indian *Bracon.*

Because of the extremely minute tips of the stylets and the violent reaction of the host when stung, the actual penetration of the stylets into the host was not observed. Noble (1932) stated that drops of fluid could be seen passing down the ovipositor of *Habrocytus cerealellae* (Ashmead) when stinging the Angoumois grain moth. Such was not the case in the two species of *Bracon,* however. Penetration by the stylets was a very short and momentary phenomenon. At most, only the extreme tip of the stylets was involved since penetration any deeper than that would have surely been observed under the binocular microscope. The venom, therefore, must have started down the stylets when the ovipositor was extruded since it would have been difficult if not impossible for the venom to be forced out of the reservoir, down the stylets, and into the host on such a short contact with the host. Sufficient venom was injected with one sting to cause complete paralysis of the host. The situation, of course, was slightly different in those cases where the parasite was attacking a host that had already been attacked once and was already partially paralyzed. The stylets remained in contact with the host for a much longer time in these situations. In these cases, however, the parasite usually inserted its ovipositor in the ventral region of the host, hiding most of the ovipositor from view.

The host was stung on any part of its body. There was no preference exhibited by the parasites and there was no special part of the host that had to be stung before paralysis could occur. The reaction of the host to the sting was generally quite violent. If the stylets contacted the anterior part of the body, the head was jerked back. If the posterior part was stung, the host either jerked forward or immediately swung its head back and attempted to bite the stung area.

After the host was stung, one of the first symptoms of the effect of the venom was the loss of coordination in the host. Activity gradually slowed down and the host seemed to be moving in "slow motion." Movement often stopped unless it was stimulated. There was a general appearance of

lethargy. It seemed to lose the ability to locomote and though it still made walking movements, it was not able to move from one place to another. This phenomenon, in appearance, generally resembled an insect attempting to walk on a surface on which it has no traction, although in the semi-paralyzed larva the movements were much slower than in a normal insect.

Even these slow walking motions gradually ceased and the larva, when stimulated, was able to make only slow lateral movements of the head. In the final stages, the larva was immobile except for occasional twitching or slow movement of the mouth parts. The larva was completely flaccid.

These same general symptoms prevailed no matter where the host was stung. The paralysis generally seemed to start from the posterior region of the body and to progress anteriorly. The muscles that aid in maintaining the turgidity of the body seemed to be affected later than the locomotor muscles.

The heart and gut in a paralyzed larva still functioned for many days after the larva was completely paralyzed. Pulsation of the heart could easily be seen through the integument although, with time, the rate and strength of these heartbeats gradually diminished until the larva died.

The larva continued to defecate but the excreta came out in one long connected piece instead of the normal little pellets. The anal sphincter apparently was paralyzed. Figure 2 shows a typical paralyzed larva with the excreta attached.

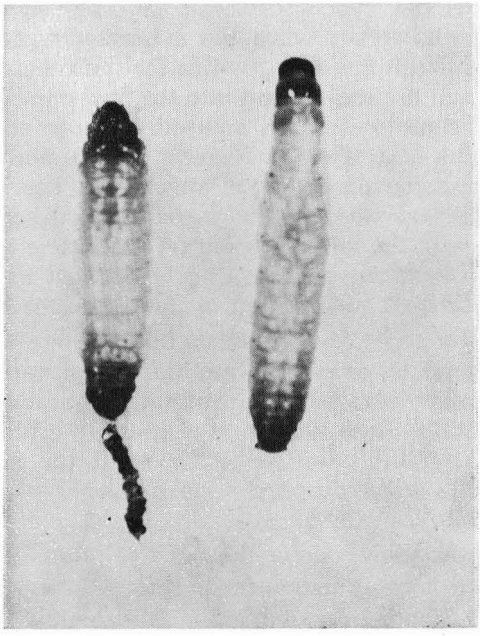

FIGURE 2. *C. cephalonica* paralyzed by the Indian *Bracon*. Note long excreta.

A larva that had been paralyzed for a protracted period of time shrank and flattened dorsoventrally as it desiccated. The rate at which desiccation occurred depended on the host species. The last few segments of the abdomen were laterally compressed, and usually darker than the rest of the body (Figure 2). The anterior limits of this lateral compression were marked by the beginning of the hindgut. The rest of the larva usually retained its natural color. Death was apparently due to dehydration and not to the venom itself. After death, the larva generally dried into a hard, light-brown scale.

In a very few instances, a larva recovered sufficiently from the effects of the venom to start pupating but died before pupation was completed (Figure 3).

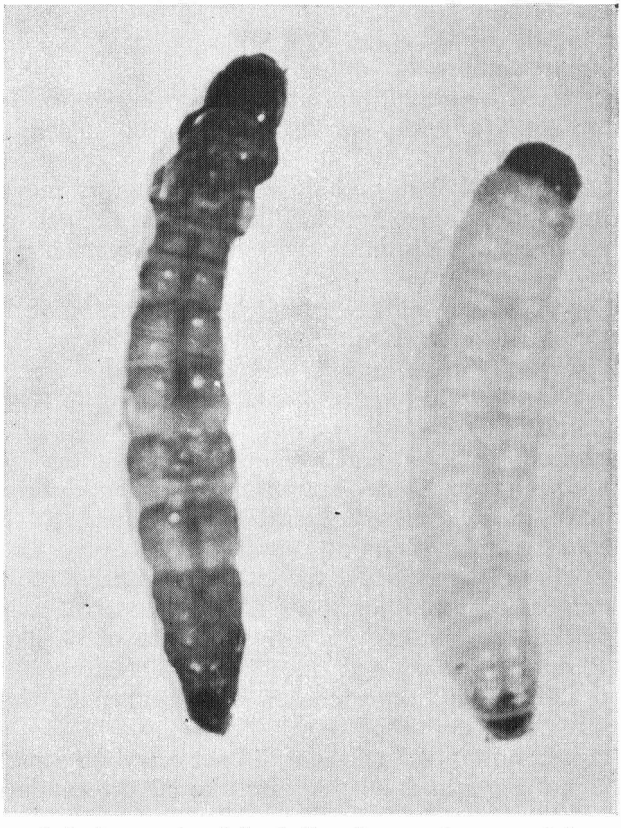

FIGURE 3. *C. cephalonica* paralyzed by Indian *Bracon*. Insect on left started pupating but died before the process could be completed. Larva on right just paralyzed.

DETERMINATION OF LEVELS OF PARALYSIS

Paralysis in a host stung by either species of *Bracon* was a smooth continuous process from the initial onset of symptoms until the larva was completely paralyzed. It is a dosage-response situation and the rapidity and degree of paralysis of the host is a function of time and dosage. In dosage-mortality determinations, however, there usually are only two alternatives. The treated insect is either alive or dead, and the percentage mortality is determined from the number dead out of the total treated.

The analogous method of calculating the percentage paralysis was not adequate, since there were many hosts at the low dosages when the venom was artificially injected that were not completely paralyzed. These larvae, however, showed definite effects of the venom and sometimes did not recover. Therefore, a system had to be evolved which included more than two alternatives. A scoring system was established so that the data could be treated quantitatively.

The system devised placed the larvae in five categories or stages or phases according to the following symptoms:

I. Unaffected larvae.

II. Affected larvae. Movements start slowing down. Can move but normally do not. When stimulated, move but movements uncoordinated and slow.

III. Cannot walk but capable of making gross movements. Can make anterior-posterior contractions as is done in walking but seem to have no traction. Can make gross lateral movements of head and cauda.

IV. Capable of making only slight movements of head. When turned on their side, not able to right themselves. Body retains turgidity.

V. Paralyzed. No movements visible to naked eye although under the microscope there may be involuntary twitching in the mouthparts. The body is flaccid.

The larvae were observed and assigned to the various categories according to their symptoms. Since paralysis moved smoothly from one phase to the next without any clear-cut break, the assignment of larvae either ending or beginning a new phase was necessarily arbitrary. The percentage paralysis was obtained by equating the maximum paralysis to 100 percent. Since 10 larvae per concentration were used in the great majority of the experiments, each larva in stage V was given a value of 10, stage IV = 7.5, stage III = 5, stage II = 2.5, and stage I = 0. Therefore, if all 10 of the larvae treated with a given concentration were at stage V, the percentage paralysis was 10×10, or 100 percent.

A percentage paralysis of 50 percent did not carry the same connotation as 50 percent mortality. In the latter case, the 50 percent level indicated that one-half of the treated insects died. Fifty percent paralysis, however, meant that most or all of the larvae were half-paralyzed, or in stage III.

THE VENOM

Although this study was concerned primarily with the biological properties of the venoms, some observations were made on the physical and chemical properties. There were no visually detectable differences in the venom of the two parasites. The venom was a clear waterlike liquid that seemed to be about the same viscosity as water. The minute droplet of venom that sometimes collected at the tip of the ovipositor of a parasite held by its wings, dried up within a couple of minutes after ejection. Under the microscope this dried venom, which was only a fraction of the size of the original droplet, was an opaque, amorphous solid. It was neither hard nor brittle and could easily be crushed under a cover slip. It could be taken up in water again and cause paralysis in hosts.

The venom of the Indian *Bracon* was still active in parasites dead for more than 6 months. These dead parasites had been stored at room temperature in mason jars covered with muslin. No special precautions had been taken for preservation of the material. Since it was not possible to remove the venom glands and reservoirs from the dead and dried parasites, the entire abdomens of the dead females were triturated in a mortar with 1 milliliter of distilled water. The resultant mixture was filtered through a Swinney hypodermic adaptor containing a HA (Hydrosol Assay) type millipore filter (Millipore Filter Corporation, Watertown, Massachusetts). The filter effectively removed all debris and bacteria from the venom solution. This sterile venom solution was injected into *Corcyra cephalonica.* Although the venom had lost most of its potency, it was still active enough to cause almost complete paralysis in most of the hosts injected.

Sterile venom solutions held in the refrigerator at approximately 4° C. still retained a slight amount of activity even after a year. The venom, however, was heat-labile. Venom solutions heated to 65° or 70° C. quickly lost their potency. Inactivation was probably by hydrolysis, since even at room temperature (27° C.), sterile venom solutions lost most of their activity after 3 to 4 weeks. Massive dosages of 4-week-old venom solutions were required to show any visible effect when injected into *C. cephalonica.* The venom of *B. hebetor* apparently was more susceptible to hydrolysis than that of the Indian *Bracon.* This loss of potency was greatly accelerated if the bacteria were not removed from the venom solution. Inactivation in solutions where the microorganisms were not removed occurred in 3 to 5 days.

The chemical nature of the venom is not known, but Beard (1952) postulated that the venom of *B. hebetor* was a proteinlike material since it was heat-labile and did not pass through a dialyzing membrane, although the minute amounts involved, according to Beard, may lay the latter test open to question.

To add further evidence to this plausible hypothesis, paper chromatography was attempted but because of the minute amounts involved,

satisfactory results were not obtained. It was then decided to attempt to hydrolyze the venom with proteolytic enzymes. These enzymes are generally specific to proteins, and deactivation of the venom by these enzymes would lend substantial strength to the hypothesis that the venom is a protein.

Pancreatin, a pancreatic extract, was utilized in these tests. This extract was once thought to contain a single protease, trypsin, but was later found to be a complex of several enzymes (Mitchell, 1956).

A sterile venom solution containing the venom from glands and reservoirs of 64 newly emerged Indian *Bracon* females per milliliter of distilled water was divided into two parts. A drop of 2 percent pancreatin solution was added to the first part, and the second was held as an untreated venom control. A third vial containing sterile distilled water with a drop of 2 percent pancreatin was included in the experiment as the pancreatin control. All three solutions were incubated at 32° C., and samples were withdrawn with a sterile syringe at ½, 1, 4, and 8 hours after initiation of incubation. Each sample was injected into 10 larvae of *C. cephalonica* at a dosage of 0.002 ml. per larva. Table 2 presents the results of this experiment.

TABLE 2. Percent paralysis in *C. cephalonica* larvae injected with Indian *Bracon* venom which was incubated with 2 percent pancreatin for different lengths of time. Ten larvae per trial were used.

TREATMENT	INCUBATION TIME (HOURS)			
	0.5	1.0	4.0	8.0
Venom	100	100	100	100
Venom + 2% pancreatin	100	90	25	0
2% pancreatin	0	0	0	0

All aliquots of the untreated venom solution caused total paralysis in the injected larvae. None of the larvae injected with the pancreatin control showed any evidence of paralysis or toxicity.

There was, however, a definite change in the potency of the venom treated with the pancreatin. The ½-hour sample still retained most of its activity and there was no detectable difference in the speed of paralysis when compared to the untreated venom solution. The 1-hour sample was slower acting than the control venom solution, but was still potent enough to completely paralyze 6 of the 10 larvae injected. The 4-hour sample had lost most of its activity and the 8-hour sample was completely inactive.

These results support the hypothesis that the venom is proteinaceous in nature. The evidence accumulated thus far, however, is still not conclusive and cannot be considered so, until a microchemical analysis of the venom is successfully completed.

PRODUCTION OF THE VENOM

The adult females of both species of parasites have a full complement of venom when they emerge. Therefore, they are able to sting and paralyze hosts shortly after emergence. Venom production was continuous throughout life, and since the venom was present in an active state even in parasites that had been dead for 6 months or more, this indicated that a considerable amount of venom was still present in the parasite when it died. The venom supply was not exhausted even when the parasites had access to all the hosts they could sting.

In order to determine whether parasite larvae were able to secrete venom, third and fourth instar Indian *Bracon* larvae were triturated and the sterile filtrate was injected into larvae of *C. cephalonica* and *G. mellonella.* Sufficient numbers of parasite larvae, 100 per test, were used so that even a small amount of venom could be detected by the method used. The sex of the parasite larvae was not known but the usual sex ratio of the parasites was approximately 1:1, so it was assumed that approximately 50 of the *Bracon* larvae were females. Theoretically, however, if the *Bracon* larvae were able to produce venom, all the larvae regardless of sex should possess this ability. In all tests, a sterile distilled water control was included.

The results of the first tests indicated that there was some venom present in the Indian *Bracon* larval filtrates. *C. cephalonica* larvae showed very slight symptoms of paralysis at a dosage of 0.002 ml. of filtrate per host, but *G. mellonella* were definitely affected.

There was still a question, however, of whether the parasite larva produced the venom or whether the venom was just ingested with the host fluids when feeding and was stored unchanged in the midgut. The midgut in *Bracon* larvae is a blind sac which may account for 89 percent of the body weight of *B. hebetor* larvae (Grosch, 1948). Therefore, if the venom ingested along with the host fluids remained unchanged in the parasite's midgut, there would be a sufficient amount of venom to cause paralysis in a sensitive host.

To overcome this shortcoming in the previous tests, parasite larvae that had been reared on hosts that were inactivated by some mechanical method had to be used. Two inactivation techniques were used. In the first, a hot needle was jabbed into the anterior part of the host. In the second, hot water was used to inactivate the hosts. Neither method was very satisfactory but enough larvae were obtained to conduct a test. Eggs of the Indian *Bracon* were transferred to these mechanically inactivated hosts.

The filtrates from parasite larvae reared under these conditions apparently did not contain any venom. Injected hosts were unaffected even at high dosages. The parasite larva, therefore, did not produce any venom that could be detected by the bioassay methods used. However, the venom

ingested by the parasite larva apparently was not immediately inactivated by the digestive enzymes of the midgut.

Venom production in the females, however, must start during the pupal period, since emerging adults have their full complement of venom. To determine the approximate age at which venom can be detected, pupae were ground up at 24-hour intervals commencing with the initiation of pupation. The duration of the pupal period at the temperatures at which these tests were conducted was slightly over 6 days for the Indian *Bracon.* Since the sex of the pupae could easily be ascertained, only females were used. The abdomens of 25 female pupae were used to make up the solutions. A single test of filtrates from 50 adult males was conducted to determine whether they were able to produce the venom. Ten *G. mellonella* and 10 *C. cephalonica* were used to test each day's filtrate.

The venom could be detected 4 days after pupation. Although venom production may have started slightly sooner in the Indian *Bracon,* the 24-hour period between tests precluded a more accurate determination. The lack of venom in young pupae, although reared on paralyzed hosts, shows that the digestive processes eventually do inactivate the venom. Filtrates of the meconium also do not possess paralytic properties.

By the time pupal filtrates showed the presence of venom, the head and thorax of the parasite already were darkened, and parts of the abdominal terga were sclerotized. The ovipositor, however, was still soft and transparent. The venom reservoir and the glands were not fully developed and hardened, so that they could not be removed from the parasite without tearing.

Venom production, therefore, started before the venom glands were sclerotized, but apparently did not begin until the parasite was more than halfway through the pupal period. Neither filtrates of the adult males nor those of the larvae reared on unparalyzed hosts indicated the presence of venom.

SPEED OF HOST PARALYSIS

The speed at which a host succumbs to the venom of a hymenopterous parasite that requires an immobile host for oviposition is of importance in determining the degree of effectiveness of a parasite in natural and biological control. Prolonged activity by the host after stinging may enable it to move out of the immediate area in which the stinging occurred. The host, therefore, although paralyzed, would not be available for oviposition. Moreover, with a parasite that remains with its host after stinging, such as the Indian *Bracon,* prolonged host activity may reduce the total number of hosts the parasite can attack during its lifetime.

The speed of paralysis for hosts stung by various species of parasites varies considerably. Fulton (1932) reported that Angoumois grain moths stung by *Habrocytus cerealellae* became quiescent in just a few seconds.

On the other extreme, *Hylobius abietus* Linnaeus stung by *Bracon hylobii* Ratzeburg continued feeding for 4 to 6 days before the host was paralyzed (Munro, 1917). Genieys (1925) found that *Pyrausta nubilalis* Huebner may move for several days after being stung by *Bracon brevicornis*, although under normal conditions, repeated stingings by the parasite immobilizes the host in 1 to several hours.

The speed of host paralysis is indicative of venom potency and host susceptibility. With two closely related species of parasites whose stings produce identical symptoms of paralysis, such as *B. hebetor* and the Indian *Bracon*, a chemical difference in their venoms may be expressed as differential speeds of paralysis in a given host species.

Differences in the amount of venom available to the parasite also can be reflected as differences in the speed of host paralysis. If a parasite stings a series of hosts within a relatively short period of time, its venom supply may be exhausted or greatly reduced. This decrease in venom supply may be expressed as a decrease in the rate of paralysis for each successive host stung.

Materials and Methods

Individual female parasites of all ages were introduced into ice cream carton tops containing the host to be tested. The Indian *Bracon* was tested with *G. mellonella*, *A. kühniella*, *P. interpunctella*, and *C. cephalonica*, while *B. hebetor* was tested with *G. mellonella* and *A. kühniella*. Each of the cartons contained an ample supply of hosts. The females in these cartons were kept under constant surveillance and as soon as a host was stung, it was removed and the time of stinging recorded. These stung hosts were kept individually in containers and the time required to pass through each of the categories of paralysis, as explained in the previous section, was noted and recorded. Because of the necessity for careful and relatively constant observation of both the parasites and the hosts already stung, no more than four parasites at a time could be utilized.

Stinging was recorded from indirect evidence. The hosts reacted characteristically to contact by the ovipositor. This reaction, however, was not always indicative of penetration by the stylets, since a tactile stimulation of setae sometimes elicited the same reaction. Positive evidence that the stylets had penetrated the host integument was, therefore, not obtained until the ostensibly stung hosts started to show signs of paralysis. Because of the extreme potency of the venom or the extreme susceptibility of the host, it was safe to conclude that if the female was able to produce the venom, penetration of the integument of the host by the stylets would have caused symptoms of paralysis. Topical applications of *Bracon* venom were found to be ineffective.

Therefore, in those cases where the host reacted as if stung, but was not paralyzed within a reasonable length of time, it was assumed that penetration of the host integument did not occur. There was a remote possibility that a given host may have been completely immune to the effects of the venom, but if such an individual existed it would have been an extremely rare and aberrant individual, since the thousands of larvae injected with known amounts of venom at levels far below what the parasite would inject all showed effects of the venom.

Results and Discussion

The conditions of the experiments were not the same as those that the parasites would normally encounter in nature. Normally, the host would have been enclosed in a silken cocoon or tunnel, but in these experiments the parasites were confronted with exposed hosts in the cartons. Moreover, since the hosts were removed immediately after being stung, any additional stinging of the same host which could have occurred after the host had slowed down was prevented.

Table 3 summarizes the mean times elapsing from stinging to complete paralysis along with the mean times required for the hosts to pass through each of the levels of paralysis. The times listed under stage I are the mean times required from stinging to the onset of the first symptoms, and stage IV includes the period from the end of stage III until the hosts were completely paralyzed.

Although paralysis of a stung host was generally a smooth and continuous phenomenon, the rates at which the hosts passed through the categories of paralysis were not equal. The stung hosts generally took longer to pass through stages I and IV. The data also show that the time the hosts remained in these two stages was more variable than in stages II and III. Some of the variation in stages I and IV can no doubt be ascribed to the difficulty in determining exactly when the first symptoms appeared and when the host was no longer capable of any movement.

However, over and above the variations due to the reasons mentioned above, there were indications that differences in the amount of venom injected or individual susceptibility were expressed most disparately in stages I and IV. A proportionately longer time was required for the host to show the first symptoms and to become completely paralyzed after passing stage III. The time required to pass through stages II and III did not change appreciably. With changes in dosage and host susceptibility, therefore, most of the increase or decrease in paralysis times occurred in stages I and IV. These observations were confirmed in the experiment where known amounts of venom were injected.

If the rate of paralysis is taken as a criterion of susceptibility, *G. mellonella,* although the largest of the host species tested, was by far the most susceptible to the venom of both species of parasites. Table 4 presents

TABLE 3. The mean times and standard errors, in minutes, required for *B. hebetor*- and the Indian *Bracon*-stung hosts to pass through the various stages of paralysis

HOST	NO. OBSERVED	TIME FOR STAGE				
		I	II	III	IV	Total
			B. hebetor			
A. kühniella	20	7.6 ± 1.01	8.0 ± 0.91	9.0 ± 1.91	18.4 ± 2.18	43.1 ± 3.10
G. mellonella	34	2.9 ± 0.30	1.8 ± 0.15	2.3 ± 0.32	5.8 ± 0.86	12.8 ± 1.25
			Indian *Bracon*			
A. kühniella	20	7.2 ± 0.44	7.5 ± 0.39	7.0 ± 0.56	9.8 ± 1.23	31.5 ± 1.63
G. mellonella	26	5.0 ± 0.23	1.9 ± 0.21	1.6 ± 0.14	5.8 ± 0.42	14.3 ± 0.72
P. interpunctella	25	11.4 ± 0.72	3.4 ± 0.35	4.7 ± 0.67	10.7 ± 1.88	30.2 ± 2.59
C. cephalonica	21	10.8 ± 1.30	5.0 ± 0.43	6.6 ± 0.48	13.0 ± 2.44	35.3 ± 3.52

TABLE 4. Mean weights and the standard errors, in grams, of the last instar larvae of the host species used

SPECIES	NO. WEIGHED	MEAN WEIGHT	STANDARD ERROR
A. kühniella	50	0.0242	0.0018
G. mellonella	40	0.1628	0.0065
P. interpunctella	50	0.0211	0.0004
C. cephalonica	40	0.0471	0.0003

the mean weights of the larvae of the four host species tested. In spite of the relatively large within-sample variations, statistical tests (Student's) indicated that *G. mellonella* was completely paralyzed in a significantly shorter time than any of the other hosts stung by the same species of parasite at odds of 99 to 1. This was a within-parasite species comparison, *i.e.*, *B. hebetor*-stung *G. mellonella* versus *B. hebetor*-stung *A. kühniella*, and Indian *Bracon*-stung *G. mellonella* versus Indian *Bracon*-stung *A. kühniella*, *P. interpunctella*, and *C. cephalonica*. The differences in the rates of paralysis between *G. mellonella* and the rest of the host species may have been due in part to differences in the rate of diffusion and/or transport of the venom to its sites of action. Since the venom is transported by the blood (Beard, 1952), the rate at which the blood is circulated would affect the speed of paralysis.

There were no significant differences among the speeds of paralysis of Indian *Bracon*-stung *A. kühniella*, *P. interpunctella*, and *C. cephalonica*. They were all completely paralyzed in slightly over ½ hour.

Although there were also no significant differences between *B. hebetor*- and Indian *Bracon*-stung *G. mellonella*, *i.e.*, between parasite species comparison, Indian *Bracon*-stung *A. kühniella* was completely paralyzed in a significantly shorter time than *B. hebetor*-stung *A. kühniella*. This differential potency of the two venoms indicates that there probably are chemical differences in the two venoms, although the similarity of symptoms elicited by the two venoms indicates that their modes of actions were similar. If the differential potency of the venoms to *A. kühniella* had been due merely to differences in concentration rather than chemical differences, *G. mellonella* should also have reacted differentially to the two venoms. Other things being equal, therefore, the Indian *Bracon* would be a more effective parasite of *A. kühniella* than *B. hebetor*.

There was no decrease in the rate of paralysis between the first and the last host stung, in those few cases where the parasite stung a series of hosts in a short span of time. Usually the parasite just stung one or two hosts before stopping for ½ hour or longer. This was especially true of the Indian *Bracon*. Many attempts were made to devise a method to force

the parasites to successively sting hosts until the venom supply was exhausted, but none of the attempts were successful.

In one instance where a *B. hebetor* female ostensibly stung 14 *A. kühniella* in 62 minutes, only 6 were paralyzed but these paralyzed individuals did not come in any orderly sequence. The first, fourth, eighth, tenth, eleventh, and thirteenth larvae stung were paralyzed. Moreover, there were no differences in the rates of paralysis of the first or the last larva paralyzed. The ovipositor apparently did not penetrate the integument in the other 8 unparalyzed larvae.

NUMBER OF HOSTS PARALYZED PER FEMALE

Although there have been studies conducted on the number of eggs laid by *B. hebetor* on various hosts (DeOng, 1923; Showell, 1928; Knowlton, 1931; Payne, 1933; Morrill, 1942; Silva, 1947), there has been no quantitative study made of the total number of hosts that a female is capable of paralyzing in her lifetime. Ullyett (1945) stated that *B. hebetor* paralyzed up to 30 *Ephestia* per day. Beard (1952) stated that *B. hebetor* can sting only a few hosts per day although as many as 175 *G. mellonella* were paralyzed in the lifetime of the parasite. According to some of these authors, *B. hebetor* paralyzes more hosts than it utilizes for oviposition. This phenomenon, however, normally would not occur with the Indian *Bracon* since it paralyzes and oviposits on a host before seeking a new host (Doutt, 1959*a*). This behavior of the Indian *Bracon* obtains only in those cases where the parasite attacks cocoon larvae which are unable to move away from the parasite. In this study, the host larvae were exposed, and were prevented from spinning cocoons.

Since density is known to play an important role in the rate of attack by density-dependent agents such as parasites (Smith, 1935), it was desirable to determine whether the number of hosts stung could be correlated with density. According to the data from the tests conducted by Doutt (1959*a*), the mean number of eggs laid per female per day remains relatively constant when 4 to 10 hosts are exposed to the parasites. If this is assumed to be the maximum number of eggs that the parasite can produce each day, and if stinging was correlated with egg production, then the maximum number of hosts stung per day should be no greater than the maximum daily egg production by the parasite. However, if stinging was independent of the ovipositional instinct, *i.e.*, the parasite stings because there are hosts available to sting and it has sufficient venom, then the number of hosts stung would be correlated with availability of hosts and venom.

In addition, the recovery of hosts stung by the two species of parasites was determined. A host stung by *B. hebetor* or the Indian *Bracon* is usually completely and irreversibly paralyzed. However, there are a few larvae that seem to recover from the effects of the venom. Observations were

made, therefore, to determine the number recovering, differential recovery among the host species, and the recovery of a given host species from the venoms of the two parasites.

Materials and Methods

Newly emerged parasites were used to initiate all of the experiments, and periodic counts were made to determine the number of hosts paralyzed. At the time that the counts were made, paralyzed hosts were removed and replaced. Since more than one count was made during a 24-hour period, the total number of hosts available to the parasite during a given 24-hour period was the number of hosts originally in the container plus the number of hosts that was added when the counts were made. Therefore, in some cases, the table shows that there were more hosts paralyzed in a given 24-hour period than were initially available in the container. This was especially true of the containers with 10 hosts. Since the parasites were able to paralyze more than 10 hosts a day, the number of larvae paralyzed per 24 hours generally exceeded 10.

In the first series of experiments, 20 mature larvae of *C. cephalonica* were exposed to individual pairs of the Indian *Bracon* in ice cream carton tops. Counts were made at hourly intervals from 0700 to 2300 hours for 6 consecutive days to determine whether attack by the parasite was sporadic or uniform. No hourly counts were made from 2300 to 0700 hours the following day. The larvae paralyzed during this period were lumped together in a single group. There were 10 replicates in this experiment.

In the next series of tests, four host densities (10, 20, 30, and 40) in two different types of containers (petri dishes and ½-pint ice cream carton tops) were utilized. Again, the host was *C. cephalonica* and the Indian *Bracon*, the parasite. Counts in this series were made at 12-hour intervals and continued until the death of the parasite. As in all of the tests conducted in this study, no food was provided for the hosts and they were prevented from spinning cocoons. All of the silk secreted was removed when the counts were made.

All of the *C. cephalonica*-Indian *Bracon* experiments were carried out at room temperature, which averaged 27° C., with a mean minimum temperature of 23.5° ± 1.8° C. and a mean maximum temperature of 30.6° ± 1.8° C.

For the recovery study, hosts paralyzed in other experiments usually were used. Five containers were set up for each host species stung by a given species of parasite. These were labelled I to V for the five stages of paralysis. As the hosts recovered from paralysis, they were shifted into the correct container for their respective levels of paralyses. Observations were made periodically until all of the hosts died or pupated.

TABLE 5. Mean number of *C. cephalonica* paralyzed per hour by the Indian *Bracon*. Each figure represents the mean of 10 replicates.

HOUR	DAY							
	1	2	3	4	5	6	Total	$\bar{x}$/hr
0800	0.3	1.2	1.6	0.8	2.0	1.5	7.4	1.2
0900	0.3	1.8	3.1	0.3	4.1	3.5	13.1	2.2
1000	0.6	2.3	2.0	1.7	2.0	1.9	10.5	1.8
1100	0.9	2.1	3.5	2.3	1.7	1.0	11.5	1.9
1200	0.2	2.2	2.5	1.7	3.3	2.6	12.5	2.1
1300	1.2	2.6	3.4	4.5	3.7	1.5	16.9	2.8
1400	1.1	2.5	2.8	2.7	3.7	1.3	14.1	2.3
1500	1.6	2.4	2.1	3.3	3.2	2.1	14.7	2.5
1600	1.8	2.8	2.4	2.2	1.9	1.4	12.5	2.1
1700	1.8	3.0	2.7	2.2	2.0	2.3	14.0	2.3
1800	2.2	3.0	3.9	4.8	4.4	3.5	21.8	3.6
1900	1.4	2.2	1.9	1.2	2.3	3.6	12.6	2.1
2000	1.8	4.1	2.4	3.2	2.0	0.6	14.1	2.3
2100	1.7	3.2	2.4	2.3	2.2	1.8	13.6	2.3
2200	1.8	1.9	2.0	1.3	2.1	1.4	10.5	1.8
2300	1.8	1.8	1.6	2.7	1.7	0.9	10.5	1.8
Total/ 16 hours	20.5	39.1	40.3	37.2	42.3	30.9	210.3	35.0
2400– 0700	10.9	13.4	12.4	11.2	11.7	7.5	67.1	11.2
Total/ 24 hours	31.4	52.5	52.7	48.4	54.0	38.4	277.4	46.2

Duncan's multiple range test:*

0800	1000	2200	2300	1100	1200	1600	1900	0900	1400	1700	2000	2100	1500	1300	1800
1.2	1.8	1.8	1.8	1.9	2.1	2.1	2.1	2.2	2.3	2.3	2.3	2.3	2.5	2.8	3.6

*All means included in a single subset as indicated by the underlining are not significantly different from one another.

Results and Discussion

Number Paralyzed Per Hour

Table 5 summarizes the mean number of *C. cephalonica* paralyzed each hour by the Indian *Bracon* during a 16-hour period from 0700 to 2300 hours. There were 10 replicates and the counts were made for 6 consecutive days after the newly emerged parasites were introduced into the cartons.

The data obtained from the 16 hourly counts were subjected to analysis of variance (Snedecor, 1956). There were significant differences among the hourly counts and among the totals for the 16-hour periods at a level of $P = 0.01$. Using Duncan's multiple range tests for mean separation (Duncan, 1955), comparison at odds of 99:1 shows that there were signifi-

cantly more larvae stung at 1800 hours than at any other time except 1300 hours.

The activity of the parasite was quite low during the period from 2400 to 0700 hours. When the totals for this period are reduced to hourly figures, although this is not truly valid since no hourly counts were made during this period, the mean number paralyzed per hour was found to be 1.4, with a high of 1.7 on the second day and a low of 1.1 on the sixth day. The temperatures during this period remained quite constant, at 25° C. There is another consideration, however, in analyzing the data on the number stung in the 8-hour period during which hourly counts were not not made. Since the paralyzed larvae were not removed until the end of the 8-hour period, the parasites had more than sufficient time to oviposit on these hosts. This, along with the slightly lower temperatures, may partially have been responsible for the lower number of hosts paralyzed during this period.

The maximum number of hosts paralyzed by any one parasite in 1 hour was 8, but out of a total of 960 individual observations this large a number paralyzed occurred in only 3 instances. There were more than 4 larvae paralyzed per hour in only 73 instances, or 7.6 percent of the readings made. Figure 5 is a histogram of the frequency distribution of the number paralyzed per hour. It appears to have a normal distribution but exhibits a slight expected skewness.

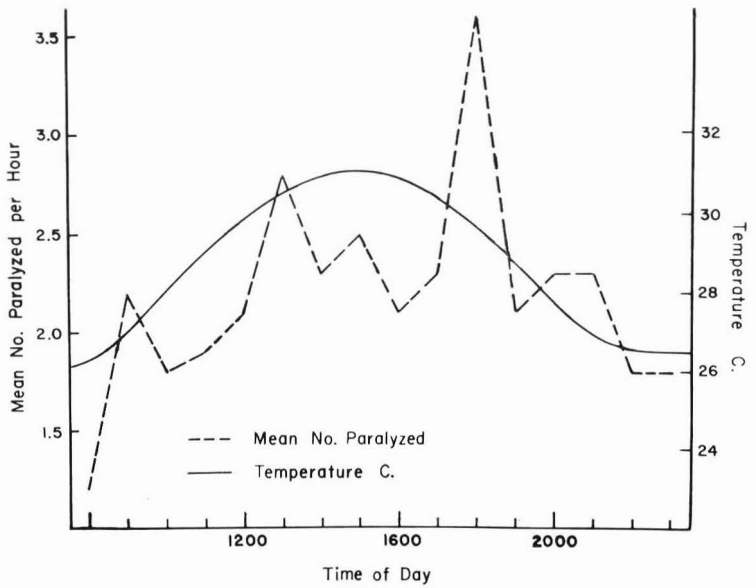

FIGURE 4. Mean number of *C. cephalonica* stung per hour by the Indian *Bracon*. The temperature is superimposed.

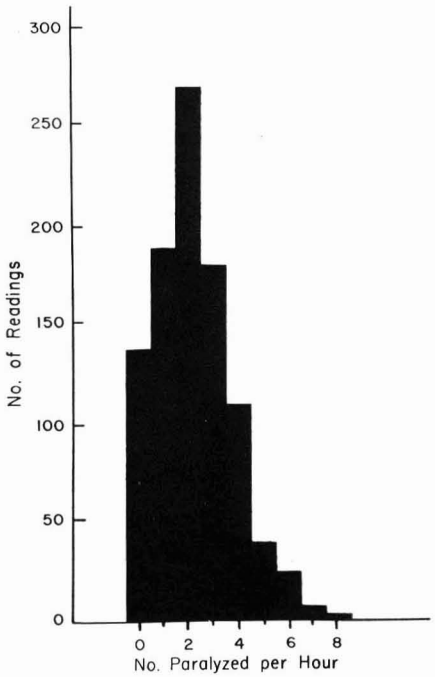

FIGURE 5. Histogram of the number of readings at which the various number of *C. cephalonica* were paralyzed by the Indian *Bracon*. A total of 960 readings was made.

The great majority of the means, 14 out of the 16, fell within the same subset. Except for the two extremes at 0800 and at 1800 hours, the mean number paralyzed did not vary significantly.

Figure 4 shows the grand means of the number of hosts paralyzed each hour for the 6 days during which the experiment was conducted. The average temperatures for these 6 days are superimposed. In general, the number of hosts stung by the parasite increased as the temperature increased but this relationship did not hold throughout the range of normal room temperature. Temperatures over 31° C. seemed to be above the optimum for adult parasite activity since the number of hosts paralyzed started to drop when the temperatures went over 31° C. Temperatures below 27° C. also seemed to decrease adult activity. The optimum temperature for stinging activity, therefore, appears to be within the narrow range from 27° to 31°C.

The definite peak in number paralyzed at 1800 hours, when the temperature was dropping, indicates that the parasite may have crepuscular tendencies. The parasite, however, is not a true crepuscular species since stinging, oviposition, and mating occur at any time of the day.

TABLE 6. Mean number of *C. cephalonica* paralyzed by the Indian *Bracon* on the first 8 days after emergence of the parasite. Each figure represents the mean for all the replicates.

TYPE OF CONTAINER	HOST DENSITY	AGE OF PARASITES (DAYS)								
		1	2	3	4	5	6	7	8	Total
Petri dish	10	13.6	17.7	17.1	18.0	18.2	18.6	17.4	16.3	136.9
(100 × 100 mm)	20	9.4	13.1	15.5	16.1	17.4	15.6	15.6	19.5	122.2
	30	20.1	19.9	21.8	21.7	24.9	24.1	24.6	21.0	178.1
	40	18.9	23.6	22.7	24.1	25.0	25.2	21.0	28.5	189.0
Total/day		62.0	74.3	77.1	79.9	85.5	83.5	78.6	85.3	626.2
Carton	10	13.8	17.1	17.8	17.2	16.4	17.8	16.6	16.9	133.6
(12 × 90 mm)	20	9.5	17.2	15.9	14.4	20.7	17.7	18.0	11.5	124.9
	30	16.7	24.5	31.3	28.2	19.6	24.8	21.7	26.0	192.8
	40	15.7	20.4	23.0	28.3	26.0	26.1	32.5	28.4	200.4
Total/day		55.7	79.2	88.0	88.1	82.7	86.4	88.8	82.8	651.7

The Indian *Bracon*, therefore, did not sting a large number of hosts consecutively in a relatively short space of time and then stop its activity. The rate of attack seemed to be relatively constant with no tremendous increase or decrease in the number stung for any one hour, with the exception of the two extremes at 0800 and 1800 hours.

Number Paralyzed Per Day

The results of the experiment to determine the number of *C. cephalonica* stung per day by the Indian *Bracon* at various host densities, in two types of containers, are summarized in Table 6. The data for the first 8 days' observations from Table 6 were subjected to analysis of variance for factorial arrangement of treatments (Snedecor, 1956).

Effect of host density. There were highly significant differences ($P = 0.01$ or less) among the means of the numbers of hosts paralyzed per day at the various densities. Each additional increment in density, however, did not automatically result in a significant increase in the number of hosts paralyzed. There were no differences in the number paralyzed between 10 and 20 and between 30 and 40 hosts per container. The highly significant increase in number of hosts paralyzed was between 20 and 30 hosts per container or between the two groups of host densities; 10 and 20 on the one hand and 30 and 40 on the other.

That the Indian *Bracon* did not sting a significantly larger number of *C. cephalonica* when the host density was increased from 10 to 20 per container was surprising. The parasites were responsive to changes in density, since significantly more hosts were paralyzed at 30 than at 20 hosts per container. In each case, the host density was increased by an increment of 10 *C. cephalonica.*

The explanation of this phenomenon may lie in the differences in the activity of the hosts at the two densities.

The sizes of the container, both petri dish and ice cream carton top, were large enough to easily absorb the increase from 10 to 20 hosts without too much evidence of crowding. The hosts were able to settle in a relatively short time. The Indian *Bracon*, thus, could paralyze and oviposit before seeking a new host. Increasing the host density from 10 to 20 per container therefore did not materially increase the number of contacts between host and parasite.

At densities of 30 and 40 hosts per container, however, there was definite evidence that the containers were crowded enough so that both the parasite and the hosts were periodically disturbed by other larvae. Although individual *C. cephalonica* larvae settled down long enough to be stung by the parasite, either the parasite or the host often was disturbed by another larva so that one or both moved out of the area.

This was especially true during the first few hours following a count of the number paralyzed. When the counts were made, every host was jabbed with the tip of a pencil to determine whether it was paralyzed. In doing so, all of the hosts were activated.

An interesting comparison can be made with the data from the previous experiment where the hourly rate of stinging was determined. This comparison partially substantiates the explanation that periodic disturbance of the parasite or hosts leads to an increase in the number of hosts paralyzed.

In the hourly-rate-of-stinging experiment, the density of the container was fixed at 20 hosts per carton. However, even at that host density, the mean number stung per parasite per day was significantly larger than any of the host densities utilized in the present experiment. A day-to-day comparison of the number paralyzed each day in the two experiments indicates that there were almost twice as many hosts stung in the hourly-rate-of-stinging experiment. This differential holds even when the comparisons were made with the highest host density (40 per container) used in the present experiment.

It is apparent, therefore, that under the conditions of this experiment, increasing the host density from 20 to 30 hosts per container was not in itself the reason for the significant increase in the number of hosts stung. The parasite apparently reacted to the consequences of the increase in density, *i.e.*, greater host activity, rather than the increase in density itself.

The experiment as conducted did not determine the total effects of density since even at the lowest host density tested, 10 per container, there was little or no necessity for the parasites to search for their hosts. They were all confined within a relatively small universe. The rate at which hosts were attacked in the 10- and 20-host containers, therefore, would probably approximate the maximum rate of attack by a parasite under conditions where there is an abundance of hosts. Since most of the hosts would be cocoons, the parasite is less apt to be disturbed in the process of stinging or oviposition, and therefore could operate at its optimum speed for stinging and oviposition.

Effect of the age of the parasite. The age of the Indian *Bracon* had a highly significant effect ($P = 0.01$) on the number of hosts paralyzed. Fewer hosts were paralyzed on the first day after emergence than on any other day. There were no significant differences in the numbers paralyzed from the second day on, however. The number of hosts stung from the day after emergence until the parasite died remained at a relatively constant level.

The fewer number of hosts paralyzed on the first day after emergence was in part correlated with egg production in the Indian *Bracon*. Doutt (1959*a*) found that egg production in the Indian *Bracon* was low for the first two days after emergence and did not reach its normal level until the

third day, at a constant temperature of 27° C. At an equivalent temperature although not constant (27° C., mean maximum 30.6° ± 1.8° C., mean minimum 23.5° ± 1.8° C.), the Indian *Bracon* apparently stung more hosts on the second day after emergence than it required for oviposition.

Statistically nonsignificant effects. There were no significant differences in the number of hosts paralyzed, attributable to the two types of containers. The Indian *Bracon* was not materially affected whether the experiment was conducted in a petri dish with its smooth glass surface or in a cardboard ice cream carton. The differential amount of light penetrating these two containers also did not affect the parasite.

None of the first order interactions, (1) Age × Density, (2) Age × Container, and (3) Density × Container, had a significant F value. This means that (1) the age of the parasite had the same effect at all densities, *i.e.*, fewer hosts were paralyzed on the first day at all densities, (2) the age of the parasite had the same effect in both types of containers, *i.e.*, the parasite did not sting more hosts in one type of container at different ages, and (3) the effect of density was the same in the two types of containers.

Total Number of Hosts Paralyzed

There was a tremendous variation in the total numbers of hosts paralyzed by the Indian *Bracon*. This variation was due in great part to the variations in longevity of the parasites, and to the aggressiveness of the host, *C. cephalonica*. Many of the parasites were killed or injured by the host. If a parasite, in stinging, was not quick enough, it was caught and bitten. Antennae, legs, or entire parts of the body were snipped off. Although the obviously injured or host-killed parasites were eliminated from the summation, there probably were some that sustained minute injuries that reduced the total efficiency of the parasite.

Table 7 summarizes the observations on the mean and maximum numbers of hosts paralyzed.

The variations in individual parasites made it difficult to extract any meaningful information. In general, the total number of hosts paralyzed apparently was not correlated with density. Ability to sting a given number of hosts was an attribute of the individual, and under these conditions where a sufficient number of hosts were provided, was independent of density. The total number of hosts available at any one time in a container did not have a measurable effect on the total number stung. It did, however, have an effect on the rate at which the total number was stung. A parasite that stung more hosts per day usually died in a shorter time. There were many exceptions of course.

The maximum number of hosts paralyzed in a single day by the Indian *Bracon*, 52, was much greater than the 30 reported for *B. hebetor* by Ullyett (1945). The maximum paralyzed by a single female was 314.

TABLE 7. Mean and maximum numbers of *C. cephalonica* paralyzed by the Indian *Bracon* at various host densities

CONTAINER	HOST DENSITY	NO. OF REPLICATES	MEAN LONGEVITY (DAYS)	STANDARD DEVIATION	MAXIMUM NO. PARALYZED	MEAN NO. PARALYZED /♀/ LIFETIME	STANDARD DEVIATION	MAXIMUM NO. PARALYZED/DAY	MEAN NO. PARALYZED /DAY/♀	STANDARD DEVIATION
Petri dish	10	17	7.3	2.8	228	116.4	49.6	20	15.9	1.4
	20	14	9.5	3.6	193	136.7	49.6	34	14.7	3.7
	30	10	6.0	1.8	251	129.8	58.6	37	21.6	5.2
	40	10	6.6	2.1	279	156.5	64.2	46	23.7	4.0
Carton	10	16	9.9	4.0	314	153.1	68.3	20	15.4	1.8
	20	10	8.1	3.4	234	120.9	45.6	31	15.5	3.6
	30	10	6.6	2.3	280	162.3	58.5	52	24.6	7.2
	40	8	7.0	2.1	268	172.8	40.6	51	24.7	7.1

TABLE 8. Degree of recovery from paralysis of parasite-stung host

HOST	NO. OF LARVAE OBSERVED	PERCENT DEAD IN STAGE*				PERCENT PUPATED
		II	III	IV	V	
		Indian *Bracon*				
A. kühniella	200	7.0	9.5	34.5	47.5	3.5
G. mellonella	100	0.0	1.0	47.0	52.0	0.0
P. interpunctella	100	0.0	4.0	58.0	38.0	1.0
C. cephalonica	300	0.7	9.0	50.0	42.3	0.7
		B. hebetor				
A. kühniella	200	6.0	32.0	41.0	11.5	9.5
G. mellonella	100	0.0	3.0	52.0	38.0	1.0
P. interpunctella	100	5.0	4.0	61.0	30.0	0.0

*See text for explanation of stages.

Recovery from Paralysis

Donohoe (1938) reported that 12 to 29 percent of *Ephestia figulilella* Gregson paralyzed by *B. hebetor* completely recovered from the effects of the venom. However, observations made on the four host species used in this study indicate that recovery, although occurring to some extent in most of the completely paralyzed larvae, is rarely complete.

Table 8 summarizes the observations on recovery. The same rating system as previously described was used to determine levels of paralysis. Larvae were considered to have completely recovered when pupation occurred.

The degree of recovery was dependent on both the host species and the parasite. Complete recovery was very uncommon in Indian *Bracon*-paralyzed *C. cephalonica*. Extremely large numbers of this host species were available for observation since hundreds of larvae were paralyzed each day in the series of tests where the total number of hosts paralyzed per female at various densities was being determined. Although only 300 larvae of *C. cephalonica* were carefully observed for recovery, more than 2,000 paralyzed larvae of this species were cursorily examined. Of these, only 7, or 0.3 percent, recovered sufficiently to pupate. The rest of the larvae all died in various stages of paralysis. Most of the larvae, however, showed some degree of recovery before dying from desiccation.

Partial recovery of most of the paralyzed larvae also occurred in the other host species tested, with the exception of Indian *Bracon*-stung *G. mellonella*. The extreme susceptibility of *G. mellonella* to the venom, coupled with the fact that it was more susceptible to desiccation than any of the other host species, prevented further recovery.

A. kühniella showed the greatest degree of recovery from the effects of the venom of both species of parasites. Moreover, this recovery was not

entirely attributable to the ability of this host to withstand desiccation. Although *A. kühniella* lived as long as 3 weeks after being stung, other host species, notably *P. interpunctella,* lived for more than a month after being paralyzed but did not recover from paralysis to the degree that was shown in *A. kühniella.*

Recovery among hosts stung by *B. hebetor* was more extensive than among those stung by Indian *Bracon.* This same situation also obtained in the experiment where venom extracted from the parasites was artificially injected into these hosts. Recovery was common in this latter experiment since the amount of venom injected was not as large as the amounts injected by the parasites. At the lower dosages, many of the larvae recovered sufficiently to pupate, although pupation usually occurred without a cocoon, or even an attempt at spinning a cocoon. *G. mellonella* larvae, however, usually attempted cocoon spinning, but were almost never successful. This cocoon-spinning activity started before the larva recovered sufficiently to walk. Spinning, therefore, was uncoordinated and erratic and resulted in a relatively heavy layer of silk being laid in the immediate vicinity of the head of the larva. The larva then pupated without cocoon construction proceeding any further.

Recovery, therefore, cannot be stated in terms of just two alternatives, *i.e.,* complete recovery or no recovery. Complete recovery was rare in those individuals that were stung by the parasites. There was usually a partial recovery, however, especially with *A. kühniella, C. cephalonica,* and *P. interpunctella.*

Recovery was a function of species of venom, dosage, and time. The longer the paralyzed larva stayed alive, all other things being equal, the better was its chance of recovery. A greater degree of recovery occurred when the host was stung by *B. hebetor* than when the Indian *Bracon* was involved.

ARTIFICIAL INJECTION OF THE VENOM

That the venoms of both *B. hebetor* and the Indian *Bracon* are very potent can be surmised from the obviously minute quantities of venom injected by the parasites into comparatively large hosts. These minute quantities of venom, especially in *G. mellonella,* are sufficient to cause a complete and irreversible paralysis in a relatively short time. Beard (1952), from estimations of the amount of venom injected by the parasite and the volume of blood in *G. mellonella,* calculated that 1 part *B. hebetor* venom in 200,000,000 parts *G. mellonella* blood was sufficient to cause complete and permanent paralysis. Beard based his estimation on measurements of the minute droplet of venom that is sometimes ejected by the parasite when it is held by its wings. He assumed that this was the amount of venom injected by the parasite when stinging its host.

However, a relatively accurate and replicable determination of potency of the venoms must have, as its basis, artificial injections of known amounts

of venoms at various dosages, to establish a valid dosage-paralysis curve. This would place the venoms of the two species on a comparable basis so that the host susceptibility to the venom of the two species of parasites and differential potency of the two venoms could be determined.

There was evidence from the experiment in which the speed of host paralysis after stinging was determined that the venoms of the parasites were differentially effective against *A. kühniella*, but equal in potency to *G. mellonella*. This would indicate that there were slight differences in the chemical make-up of the two venoms. Confirmation of this differential potency, however, depends on results from injection of known amounts of venom.

Attempted Immunization Against the Venom

The early investigations on the phenomenon of immunity in insects have been reviewed by Briggs (1958) and Stephens (1959) in their respective studies on immunity. The results, in general, indicate that lepidopterous larvae can be immunized against some bacterial antigens. Briggs (1958) demonstrated a bacterial inhibitory principle in the sera of larvae inoculated with bacteria. The inhibitory principle was nonspecific within groups of bacteria. Although Chorine (1929) was able to immunize *G. mellonella* against diphtheria toxin, Briggs (1958) was not able to duplicate the results in *Bombyx mori* (Linnaeus) and *Junonia coenia* Hübner.

Stephens (1959) immunized *G. mellonella* against *Pseudomonas aeruginosa*. Immunity was evident after a single vaccination, was of short duration, and was more specific than nonspecific.

Immunization of a host against the venom of its parasite, however, has never been attempted. If the host is able to engender an immunity to the venom of its parasite, then a host accidentally receiving an extremely minute amount of venom may either not be paralyzed when stung again or recover from the second sting before it succumbs to the effects of dehydration. The immunity, if engendered, probably would not be complete but rather one in which the degree of resistance of the host to the venom is slightly increased.

The determination of the degree of immunity engendered in vaccinated hosts, the relative potency of the venoms, the differential susceptibility and recovery of the hosts from the venom, and the effect of temperature on the venom-host interaction all depended upon a replicable and relatively accurate method of injecting known amounts of venom into the hosts. Therefore, techniques and methods to obtain the venom, to measure the quantity, and to inject it into hosts had to be evolved. These techniques and methods have been separated into several sections to facilitate presentation.

Materials and Methods

Method of Venom Collection

The venom was collected from newly emerged females that had not had an opportunity to come in contact with hosts. The parasites were allowed to feed on honey which was smeared on the walls of the jars.

Several methods of venom collection and sterilization were tried until a satisfactory method was evolved.

The method used to obtain the venom involved removal of the entire venom apparatus of the parasite. The parasite was held by the thorax and the ovipositor gently pulled out with a pair of Swiss watchmaker forceps. The entire venom apparatus was easily removed by this method. The venom glands and the reservoir were placed in distilled water and completely shredded by tearing and crushing these structures under a dissecting microscope. This venom-containing solution was then centrifuged to remove all cellular debris and bacteria. The supernatant was siphoned off with a syringe. Although this method of sterilization was generally satisfactory, there were occasional collections where the bacteria were either incompletely centrifuged out or were resuspended after centrifugation by the currents created when the supernatant was siphoned out.

Subsequently, all debris and bacteria were filtered out of the venom solution by passing it through a Swinney hypodermic adaptor containing a HA (hydrosol assay) type millipore filter. The filter effectively removed all debris and bacteria from the venom solution.

Method for Determining Dosages

The volume of venom available in a newly emerged *B. hebetor* and Indian *Bracon* was determined by measuring the venom-containing organs and calculating their volumes.

The anatomy and histology of the poison glands and reservoir of *B. hebetor* were described by Bender (1943), while those of two closely related species, *B. brevicornis* Wesmael and *B. gelechiae* Ashmead, were described by Genieys (1925) and Narayanan and Rao (1955), respectively. In all respects, the glands and reservoirs of these three species are almost identical. There are eight venom glands in all three species. The glands are cylindrical in shape and consist of a single layer of cuboidal cells surrounding a central lumen. Each gland is connected by a stalk to a cuticular bulb which is connected to the reservoir. The reservoir is surrounded by a muscular coat of longitudinal striated muscles. These muscles are attached to a cuticular helix in the reservoir, which, according to the authors, acts like a spring to return the reservoir to its normal shape after the muscles contract to eject the venom.

The Indian species of *Bracon* used in this study possessed the same type of venom-secreting and -storing organs as the three species mentioned above.

TABLE 9. Mean dimensions of venom reservoir and venom glands from 20 newly emerged females of *B. hebetor* and the Indian *Bracon*. All measurements are in millimeters. The volumes are in milliliters. One gland of each pair was measured.

	WIDTH	STANDARD DEVIATION	LENGTH	STANDARD DEVIATION	VOLUME
			B. hebetor		
Reservoir	0.182	0.015	0.288	0.032	49.9×10^{-7}
Sting glands	0.107	0.014	0.717	0.023	65.7×10^{-7}
Gland II	0.092	0.011	0.588	0.080	39.1×10^{-7}
Gland III	0.102	0.017	0.537	0.063	43.9×10^{-7}
Gland IV	0.099	0.008	0.517	0.081	40.6×10^{-7}
			Indian *Bracon*		
Reservoir	0.225	0.064	0.354	0.052	93.9×10^{-7}
Sting glands	0.099	0.021	0.591	0.045	46.4×10^{-7}
Gland II	0.093	0.015	0.525	0.069	34.9×10^{-7}
Gland III	0.102	0.011	0.516	0.059	42.2×10^{-7}
Gland IV	0.093	0.037	0.519	0.060	34.5×10^{-7}

The poison glands and reservoirs were carefully excised from newly emerged females. Immediately after extraction, the glands and reservoirs were mounted on a slide in a drop of water and measured under a compound microscope with an ocular micrometer. The mean lengths and widths and the standard deviations of these structures from 20 newly emerged females from each species are given in Table 9. Using these mean dimensions, the volumes of these structures were calculated.

In calculating the volume of venom present in these glands and reservoirs, two assumptions were made:

1. That the entire structure measured was completely full of venom. The volume of the organ was calculated from the outside diameter, and the thickness of the walls was not considered. This led to a slight overestimation of the total amount of venom but the magnitude of the dilutions in the tests minimizes this overestimation.
2. That all of the venom in the glands and reservoirs went into solution in the water in which the organs were triturated. The extremely minute volumes of venom involved, the high solubility of the venom in water, and the care that was taken to insure complete trituration of all the organs gave this assumption validity.

In the actual mathematical calculations of the volume, the glands were assumed to be cylinders. The volumes, therefore, were calculated by the formula: Volume $= r^2h$, where r is one-half the width and h is the length of the gland. The volume of the reservoir was calculated by assuming that the reservoir was a prolate spheroid. This is a structure that is formed when an ellipse is rotated around its major axis. The formula for the volume of this type of structure is: Volume $= 4/3\ ab^2$, where a and b are the major and the minor semiaxes, respectively. Using these formulae, it was found that the total volumes of venom present in newly emerged females of the two species, *B. hebetor* and the Indian species, were almost equal. These volumes were 4.1×10^{-5} ml. for the Indian species and 4.3×10^{-5} ml. for *B. hebetor*. The Indian species had slightly larger venom reservoirs than *B. hebetor* but this was more than compensated for by the larger glands in *B. hebetor*.

For the injection tests, stock solutions containing the venom from glands and reservoirs of 64 newly emerged females in 1 ml. of distilled water were made for each species. The concentration of venom per milliliter of stock solution was 68.8×10^{-7} ml. for the Indian species and 75.7×10^{-7} ml. for *B. hebetor*.

Injection of the Venom

The intrahemocoelic injections of the venom of the two species of *Bracon* were carried out by the same method. A microinjector similar to the one described by Roan and Maeda (1953) or a Dutky-Fest microinjector was used in all tests. The needle consisted of an extremely fine-pointed glass capillary tube attached to a 27-gauge steel hypodermic needle with sealing wax. A ¼-ml. tuberculin syringe was used with this needle. Since the needle could not withstand high temperatures, chemical sterilization methods were used.

The larvae to be injected were kept under continuous CO_2 anesthesia by the method described by Williams (1946). The larvae were inactivated in less than a minute after introduction into the CO_2 atmosphere, and recovered in 2 to 5 minutes after they were removed. The larvae were injected in the left member of the second pair of abdominal prolegs. The prolegs provided an ideal location for the injections since they were endowed with sphincter muscles which close the puncture when the needle is removed, thus preventing any loss of the material injected. However, if the larvae were kept under continuous anesthesia for over ½ hour, the proleg sphincter failed to contract and the material injected oozed out of the puncture along with the hemolymph. For the tests, therefore, no larva was subjected to CO_2 anesthesia for more than 10 minutes.

In all treatments, the volume of material injected was kept constant and the desired dosages were obtained by changing the concentration.

Unless otherwise noted, 10 larvae per dosage were used and each dosage was replicated a minimum of seven times for *C. cephalonica* injected with Indian *Bracon* venom and four times for all other hosts, whether injected with *B. hebetor* venom or Indian *Bracon* venom. Control larvae were injected with sterile distilled water.

For the series of tests to determine whether the hosts could be immunized to the venom, *C. cephalonica* larvae were injected with a low concentration of Indian *Bracon* venom 2 days before injecting the dosages to be tested. A 2-day period between injections was necessitated since the larvae did not completely recover from the effects of the venom in the immunizing inoculation before 2 days. The concentrations injected caused minor symptoms of paralysis. The immunizing inoculations were made in the left member of the second pair of abdominal prolegs and the injection of the dosage to be tested was made in the right member. Control insects were injected both times with sterile distilled water.

After treatment, the larvae were held in ½-pint ice cream carton tops, either at room temperature (27° C.) or at 32° C. All 10 larvae from a replicate treated with the same dosage were held in the same container. In the tests using *C. cephalonica* and the venom from the Indian *Bracon,* the degree of paralysis was ascertained at 5- or 10-minute intervals for an hour after injection. After the first hour, periodic observations were made until the maximum level of paralysis was achieved. Subsequently, observations were made at 12-hour intervals. Twice a day, observations to determine paralysis were made for all other hosts.

The degree of paralysis was scored according to the method previously described.

Results and Discussion

Potency of Indian Bracon *Venom to* C. cephalonica

Figures 6 and 7 depict the paralysis curves for *C. cephalonica* injected with Indian *Bracon* venom and held at room temperature and at 32° C., respectively. Figure 8 shows the paralysis curves for *C. cephalonica* that had been vaccinated with a low concentration of venom 2 days prior to injecting the regular series of dosages of Indian *Bracon* venom.

The curves from all three test series resemble each other in general outline. There was a sharp initial rise that gradually tapered off and was followed by a gradual decline in the percent paralysis. The steepness of the initial rise and the flatness of the subsequent recovery portion of the curve was directly correlated with the concentration of venom. The rate of paralysis, depth of paralysis, and degree of recovery therefore were proportional to dosage. The higher the concentration of venom injected, the faster and deeper the paralysis and the poorer the degree of recovery.

The venom was very potent. Dosages as low as 17.2×10^{-10} ml. of venom per host were sufficient to cause complete paralysis in both series

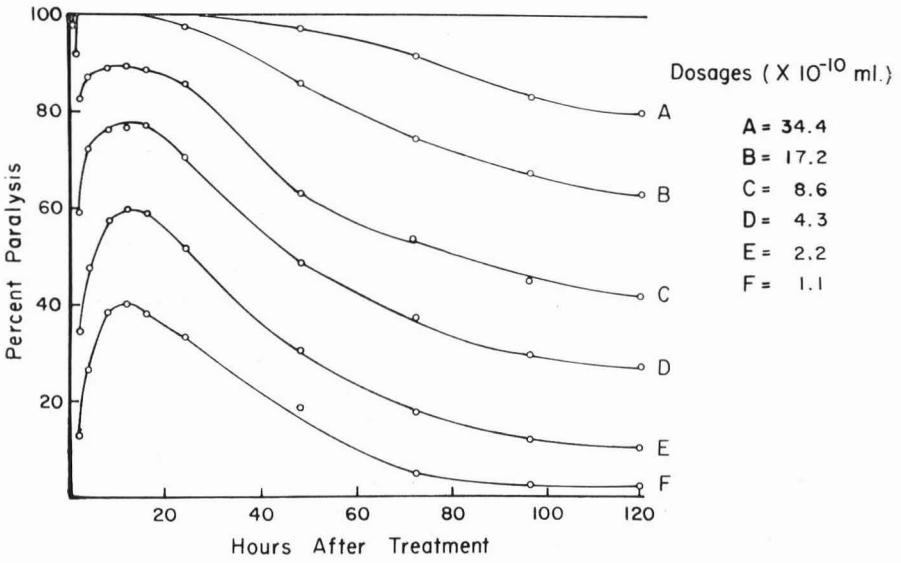

FIGURE 6. Paralysis curves for *C. cephalonica* injected with various dosages of the Indian *Bracon* venom and held at room temperature (27° C.).

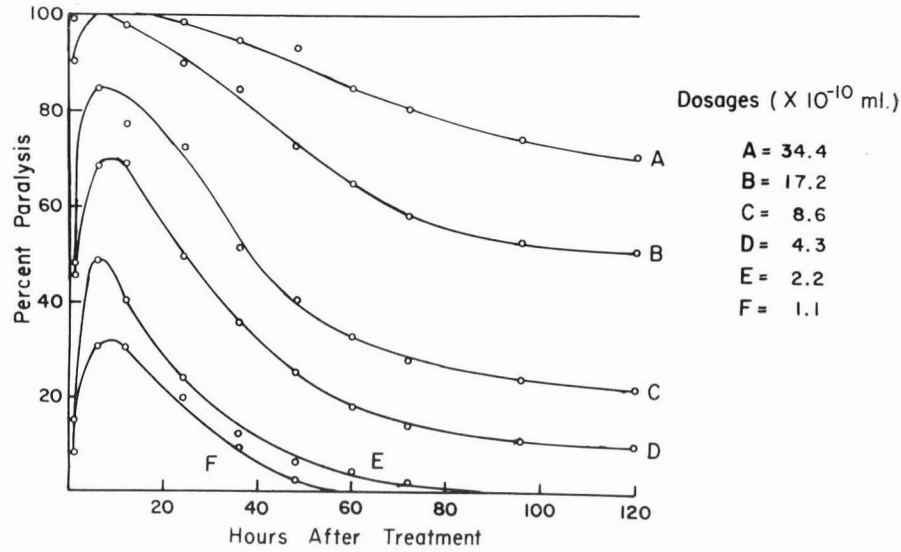

FIGURE 7. Paralysis curves for *C. cephalonica* injected with various dosages of the Indian *Bracon* venom and held at 32° C. after treatment.

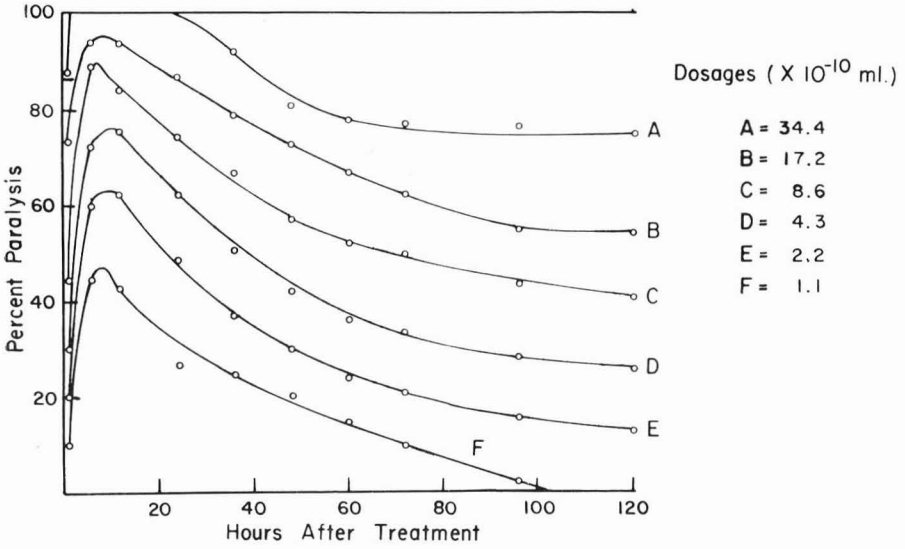

FIGURE 8. Paralysis curves for *C. cephalonica* vaccinated with a low dosage of venom 2 days before injecting the regular dosages.

of tests where normal rice grain moth larvae were used. Even at the lowest dosages tested, 1.1 $\times$ 10^{-10} ml. per larva, the venom was potent enough to cause a loss of coordination in all of the larvae treated. Within a given treatment, the response of the larvae to the venom was surprisingly uniform. Although there were some variations among the larvae, especially among the series held at 32° C. and the vaccinated series, the variations did not reach the magnitude that sometimes accompanies insecticide treatments. The potency of the Indian *Bracon* venom compares favorably with the botulinal toxins considered by Lamanna (1959) as "the most poisonous poison." These toxins kill mice at dosages as low as 0.1 $\times$ 10^{-9} grams per mouse.

Since the paralysis curves all have the same general shape and it was difficult to measure the differences in the curves, four points on the curves were selected to compare the three series of treatments. These points were (1) the time in minutes to reach 50 percent paralysis, (2) the time in minutes to reach maximum percent paralysis (The maximum percent paralysis is defined as the highest level of paralysis achieved at any given dosage.), (3) the maximum level of paralysis, and (4) the percent paralysis at 120 hours after treatment. Table 10 summarizes these observations.

The time required for 50 percent paralysis for the series held at 32° C. was not obtained since it would have entailed removing the insects from the constant temperature cabinet every 5 or 10 minutes to determine the

TABLE 10. The time, in minutes, required for larvae of *C. cephalonica* to reach various levels of paralysis when injected with Indian *Bracon* venom. (A) Larvae held at room temperature (27° C.) after treatment. (B) Larvae held at 32° C. after treatment. (C) Vaccinated larvae held at room temperature (27° C.). (D) Parasite-stung larvae.

DOSAGE $\times 10^{-10}$ ML.	TIME TO REACH 50 PERCENT PARALYSIS (MINUTES)	TIME TO REACH MAXIMUM PARALYSIS (MINUTES)	MAXIMUM PERCENT PARALYSIS	PERCENT PARALYSIS AT 120 HOURS
		A.		
34.4	22	65	100	80
17.2	36	120	100	63
8.6	51	480	91	42
4.3	89	960	79	27
2.2	184	720	58	10
1.1	—	720	39	2
		B.		
34.4	—	76	100	72
17.2	—	135	100	51
8.6	—	480	85	22
4.3	—	600	70	10
2.2	—	480	54	0
1.1	—	540	32	0
		C.		
34.4	30	100	100	75
17.2	41	480	94	55
8.6	63	480	89	41
4.3	72	720	76	26
2.2	194	600	62	14
1.1	—	480	44	0
		D.		
Parasite-stung larvae	16	36	100	98

progress of paralysis. Removing the insects from the cabinet at such frequent intervals would have made it impossible to maintain the temperature at the desired level.

The unvaccinated series held at room temperature seemed to be the most susceptible to the venom. The larvae in this series generally were 50 percent paralyzed in a shorter period of time than the larvae in the vaccinated series. The maximum percent paralysis at the two top dosages was also expressed faster than in the series held at 32° C. or the vaccinated series.

However, a comparison of the maximum percent paralysis by the probit analysis method (Finney, 1952) indicated that there were no significant differences among the three series of tests.

Therefore, although vaccinating the larvae or holding the larvae at 32° C. subsequent to treatment apparently delayed the onset of paralysis, it did not have a significant effect on the magnitude of the maximum percent paralysis at any given dosage. (The maximum percent paralysis is defined as the maximum level of paralysis achieved at any given dosage.)

The generalization made of the dosage to time relationship, *i.e.*, the higher the dosage, the faster the paralysis, did not hold in all cases. It was true for the time required to achieve any given level of paralysis below the maximum, but did not hold for the time required to achieve maximum percent paralysis at low dosages.

In all three series of tests, the most time required to achieve maximum percent paralysis was at a dosage of 4.3×10^{-10} ml. of venom per host. Above or below this dosage, the maximum percent paralysis was expressed in a shorter time.

There was no obvious explanation for this phenomenon. It was apparently correlated with magnitude of paralysis in the host. At the dosage of 4.3×10^{-10} ml. of venom per host, the maximum percent paralysis ranged from 70 to 79 percent in the three series of tests.

There apparently was some sort of "resistance" to deeper paralysis when the paralysis levels reached 70 to 80 percent. Once beyond this level of paralysis, the speed of host reaction to the venom was increased.

Although a proportionately larger amount of venom was required to increase the percent paralysis at the high levels, this alone was not sufficient to explain the phenomenon. If dosage alone was the sole cause of this phenomenon, then, the dosage above 7.3×10^{-10} ml., *i.e.*, 8.6×10^{-10} ml. of venom per host should have taken even longer to reach maximum percent paralysis. With this line of reasoning, a dosage that is just sufficient to cause 100 percent paralysis would theoretically take the longest time of all to exert its full effects. This, however, did not occur. When a high dosage of venom was injected, paralysis of the larvae was smooth and continuous. There was no evidence that the host was more "resistant" at levels of paralysis between 70 and 80 percent.

Although there were no significant differences in the maximum percent paralysis, there were differences in the percent paralysis at 120 hours. There was a greater degree of recovery in the series held at 32° C. The higher temperature apparently enabled the larvae to neutralize more venom or to produce more substrate to replace the substrate tied up by the venom.

C. cephalonica was not detectably immunized to the effects of the venom of the Indian *Bracon* with a single vaccination of venom. The degree of paralysis at equivalent dosages of venom was slightly less in

the vaccinated larvae than in the unvaccinated larvae. This difference, however, was not significant. There is a possibility, however, that if the hosts were given a series of immunizing injections, a detectable immunity may be engendered.

Since the potency of known amounts of Indian *Bracon* venom was determined in this experiment, the approximate amount of venom injected by the Indian *Bracon* when stinging *C. cephalonica* was extrapolated from the data obtained in this experiment. The reciprocal of the times required to achieve 50 percent paralysis in the series held at room temperature was plotted against the log dosage. This resulted in a straight line. By extrapolating the line and reading the dosage correlated with the reciprocal of time for 50 percent paralysis in parasite-stung larvae, a dosage of 151 $\times 10^{-10}$ ml. of venom was derived. This was the amount of venom injected by the Indian *Bracon* when stinging *C. cephalonica.* If this figure is divided into the amount of venom available in the parasite at emergence, it is found that the parasite can sting and paralyze over 2,000 *C. cephalonica* even if no further venom production occurs during the lifetime of the parasite. Moreover, if the Indian *Bracon* could sting and inject the minimum amount of venom required for 100 percent paralysis in *C. cephalonica,* then a single female has a sufficient amount of venom to completely paralyze 23,800 hosts.

TABLE 11. The maximum percent paralysis and the percent paralysis at 120 hours after hosts were injected with the venom of *B. hebetor*

HOST	DOSAGE $\times 10^{-10}$ ML.	MAXIMUM PERCENT PARALYSIS	PERCENT PARALYSIS AT 120 HOURS
G. mellonella	44.2	100	85
	30.0	100	65
	14.7	90	35
	7.3	70	25
	3.7	55	0
A. kühniella	44.2	100	52
(Room temperature	30.0	95	27
27° C.)	14.7	45	35
	7.3	20	0
	3.7	5	0
A. kühniella	44.2	100	45
(32° C.)	30.0	68	35
	14.7	73	10
	7.3	25	0
	3.7	10	0
P. interpunctella	44.2	100	100
	30.0	100	100
	14.7	95	90
	7.3	45	10
	3.7	0	0

TABLE 12. The maximum percent paralysis and the percent paralysis at 120 hours after injection with the venom of the Indian *Bracon*

HOST	DOSAGE $\times 10^{-10}$ ML.	MAXIMUM PERCENT PARALYSIS	PERCENT PARALYSIS AT 120 HOURS
G. mellonella	16.6	100	95
	13.4	100	85
	10.1	100	35
	6.7	92	5
	3.4	75	5
	1.7	38	0
A. kühniella	26.8	100	84
(Room temperature 27° C.)	16.6	100	72
	13.4	100	59
	6.7	58	16
	3.4	55	2
	1.7	25	0
A. kühniella	26.8	100	75
(32° C.)	13.4	100	52
	6.7	75	24
	3.4	30	0
	1.7	41	0
P. interpunctella	16.6	100	100
	13.4	100	100
	10.1	100	90
	6.7	85	58
	3.4	5	0
G. opercullela	26.8	70	All died in Pupation Process
	13.4	65	"
	6.7	0	0

Potency of Indian Bracon *and* B. hebetor *Venom to* G. mellonella, A. kühniella, *and* P. interpunctella

The maximum percent paralysis and the percent paralysis at 120 hours for larvae of *G. mellonella*, *A. kühniella*, and *P. interpunctella* treated with various dosages of venom of *B. hebetor* and the Indian *Bracon*, respectively, are presented in Tables 11 and 12. The paralysis curves for these species were in general similar to the curves for *C. cephalonica*.

The same order of susceptibility to the venoms of the two species of parasites occurred in this experiment, as was found in the experiment on the speed of paralysis of parasite-stung hosts. The sequence was, in decreasing order of susceptibility, *G. mellonella*, *P. interpunctella*, and *A. kühniella*. *C. cephalonica* and *A. kühniella* were equally susceptible to the venom of the Indian *Bracon*.

There were no significant differences in the degree of paralysis between *A. kühniella* held at room temperature or at 32° C. although the larvae held at 32° C. were not as deeply paralyzed as those held at room tem-

perature. This nonsignificance was probably due in part to an inadequate number of usable points at 120 hours to determine the dosage-paralysis curve.

The degree of host recovery from the venom of both species of parasites was approximately equal when dosages that resulted in equal maximum percent paralysis were compared. However, comparison of equivalent dosages of venom showed that the venom of the Indian *Bracon* was more potent than the venom of *B. hebetor.*

At equivalent dosages, the venom of the Indian *Bracon* caused more than twice the degree of paralysis than that caused by *B. hebetor* venom. The degree of host recovery was also decreased.

This differential potency of the venoms was expressed in all three host species although it was not as pronounced in *G. mellonella* as it was in *A. kühniella.*

The differential rate of paralysis observed in parasite-stung *A. kühniella,* therefore, was not due to differences in the volumes of venom injected by the two species of parasites. Rather, the difference was due to the differential susceptibility of *A. kühniella* to the two venoms. That there may have been a slight chemical difference in the two venoms was indicated by the fact that the venom of *B. hebetor* was hydrolyzed more readily than the venom of the Indian *Bracon. B. hebetor*-venom solutions held at room temperatures quickly lost their potency.

Potency of Indian Bracon *Venom to* Gnorimoschema opercullela *(Zeller)*

Table 12 summarizes the potency of Indian *Bracon* venom to *Gnorimoschema opercullela.* Although this insect was the smallest species tested, it was more resistant to the paralytic effects of venom than the larger species. A dosage of 26.8×10^{-10} ml. of venom per host caused only 70 percent paralysis in this species. This same dosage resulted in 100 percent paralysis in all of the hosts of the Bracon, *i.e., A. kühniella, G. mellonella, P. interpunctella,* and *C. cephalonica.*

There was a peculiarity in the reaction of *G. opercullela* to the venoms. Although the degree of paralysis never reached the levels of the normal host species of Indian *Bracon,* mortality was much higher in the potato tuber worm. All of the larvae injected with the two highest dosages tested died within 2 days of treatment.

The larvae, however, all had started pupating before death occurred. Death invariably occurred after the abdomen was sclerotized. Although this same phenomenon was also observed in the other species tested, it did not occur to the degree that it did in *G. opercullela.* Since the mortality occurred within 48 hours, and the insects died in the act of pupation, it would seem that desiccation was not the chief cause of death. The venom apparently did not prevent the onset of pupation but was either directly or indirectly involved in preventing the completion of pupation.

SUMMARY

A biological study of the venoms of two species of gregarious ectoparasites, *Bracon hebetor* Say and a species of *Bracon* from India, was conducted. Four hosts of the two species of parasites, *Corcyra cephalonica* (Stainton), *Anagasta kühniella* (Zeller), *Galleria mellonella* (Linnaeus), and *Plodia interpunctella* (Huebner), were utilized in this study.

The venoms of the two parasites were clear liquids that withstood desiccation without being inactivated. The Indian *Bracon* venom was still active in females that had been dead for 6 months. The venoms were inactivated in a few weeks in solution in water and in 8 hours when treated with pancreatin. The venoms apparently are proteinaceous in nature.

Secretion of venom in the Indian *Bracon* began when the parasite was about two-thirds through the pupal period. The emerging adults had a full complement of venom and were able to sting and paralyze hosts soon after emergence. The speed at which a host was paralyzed depended on the species of host and parasite. *G. mellonella*, the largest host species, was paralyzed in the shortest time when stung by either species of parasite. *A. kühniella* was paralyzed in a significantly shorter time by the Indian *Bracon* than by *B. hebetor*. Paralysis of the host was usually permanent, *i.e.*, complete recovery was rare, but there was partial recovery in most of the hosts. The degree of recovery was greater in *B. hebetor*-stung hosts.

The total number of hosts stung by the Indian *Bracon* was independent of the host density when there were more than 10 hosts per container. The maximum stung by a parasite was 314. Host density did have a significant effect on the rate of stinging. More hosts were stung per day at high densities. Fewer hosts were stung on the first day after parasite emergence than on any other day. The rate of stinging by any one parasite was relatively uniform.

Artificial injections of known amounts of venom solution indicated that the venoms of both species of parasites were very potent. The Indian *Bracon* venom was more effective against all host species. *G. mellonella* was paralyzed at dosages as low as 10.1×10^{-10} ml. of venom per host. *C. cephalonica* was not immunized to the venom of the Indian *Bracon* by a single immunizing injection of a minute amount of venom.

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