Effect of the Ectoparasite *Necremnus breviramulus* (Hymenoptera: Eulophidae) and Its Venom on Natural and Factitious Hosts

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ABSTRACT Prepupae of *Hypera eximia* (LeConte) (Coleoptera: Curculionidae), a natural host, and prepupae of factitious insect hosts were tested for their physiological responses to stinging by the gregarious ectoparasite, *Necremnus breviramulus* Gahan (Hymenoptera: Eulophidae), and to injection with an extract of the venom gland tissue dissected from the lower reproductive tract of the female parasite. The arrestment of development produced by *N. breviramulus* venom was expressed in the natural host, *H. eximia,* and in all insects tested outside the natural host range of the parasite (Coleoptera: Curculionidae, Cerambycidae, and Chrysomelidae; Lepidoptera: Plutellidae and Noctuidae). Arrestment activity was found to be associated with the aqueous extract of the venom apparatus, and the response was shown to be dose-dependent. The protein composition of the venom from *N. breviramulus* differed from other eulophids tested and did not contain the molt-arresting protein found in *Euplectrus* spp. The difference in venom proteins may account for the different physiological effects and host range of these eulophid parasites.

KEY WORDS *Necremnus breviramulus, Hypera eximia,*developmental arrestment, molt inhibition, parasitism, venom

BIOACTIVE SUBSTANCES FROM the venom of arthropods are implicated in the regulation of critical physiological systems in insects and, as such, are an important source of natural insect growth regulators which have potential for controlling insect pests (Coudron 1991, Bonning and Hammock 1992, Bonning 1996,). Several paralyzing substances and two nonparalyzing substances have been structurally characterized (Soldevila and Jones 1991, Coudron and Brandt 1996). Other recent advances include the enhancement of insect mortality from baculovirus infection via the expression of insect-selective protein toxins (Jarvis et al. 1996), and the development of amphiphilic peptide mimics with greater resistance to proteolytic degradation and enhanced topical activity (Abernathy et al. 1996). Innovations such as these greatly improve the feasibility of administering these natural bioactive substances to pest insects for their control through the expression of venom genes inserted into plant and entomopathogenic vectors, or through topical application of pseudo-peptide analogs (Coudron 1991, Miller 1995, Jarvis et al. 1996).

The venoms of the ectoparasitic Hymenoptera in the family Eulophidae are of particular interest because of their ability to act alone in some species rather than in conjunction with teratocytes, polydnaviruses, or secretions of the larval parasite (Coudron 1991, Dahlman and Vinson 1993). The physiological effects caused by the venoms of wasps within this family vary depending on the host species and stage attacked. Some wasps possess venom that paralyze or kill the host, whereas others arrest or slow growth and development. Such disparate physiological effects suggest different mechanisms of action which may be of importance as insect resistance to current pesticides increases.

We examined the effect of parasitism and venom injection on both natural and factitious hosts of the eulophid *Necremnus breviramulus* Gahan (subfamily Eulophinae). *N. breviramulus* is a Nearctic gregarious ectoparasite of *Hypera eximia* (LeConte), *H. compta* (Say), and *H. paludicola* Warner (Puttler et al. 1973). Females prefer to sting and oviposit on the prepupal stage of beetle larvae enclosed within their mesh-type cocoons (Puttler et al. 1973). Previous work on the ectoparasitic eulophids of free-living lepidopteran larvae, *Euplectrus comstockii* Howard and *E. plathypenae* Howard (subfamily Euplectrini), showed that the females possess venoms that inhibit host larval development by arresting the molting process (Bouček and Ashew 1968, Coudron et al. 1993). A 66-kDa protein isolated from the venom of *E. comstockii* has been shown to arrest molting in a lepidopteran host (Coudron et al. 1990, Coudron and Brandt 1996). Although the natural host range of these *Euplectrus*

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spp. is limited to a few species within four lepidopteran families (Noctuidae, Geometridae, Sphingidae, and Pyralidae) (Peck 1963), the arrestment effect of the venom was observed in all natural noctuid hosts tested and in several other species outside of the natural host range (e.g., 11 families in Lepidoptera and four families in four additional orders) (Coudron and Puttler 1988). In this study, we tested prepupae of *H. eximia,* a natural host, and prepupae of factitious insect hosts for their physiological responses to parasitism by *N. breviramulus,* and to injection with an extract of venom gland tissue. We described the response of the beetle prepupae to the venom and also compared the protein composition of the venom from *N. breviramulus* with that from other hymenopteran parasitoids.

Materials and Methods

Parasite Rearing. The collection and rearing of *E. comstockii* and *E. plathypenae* has been previously recorded (Coudron et al. 1993). The pteromalid *Erixestus winnemena* Crawford, an egg parasite of several chrysomelid beetles (Krombein et al. 1979), was reared from the elm calligrapha, *Calligrapha scalaris* (LeConte). The braconid *Bracon mellitor* Say, a larval ectoparasite of several curculionids (Surles et al. 1975, Puttler et al. 1978, Krombein et al. 1979), was reared from musk thistle flower head weevils, *Rhinocyllus conicus* Froelich, collected in central Missouri.

Several dense populations of *N. breviramulus* have been found within a 100-mi radius of our laboratory (Puttler et al. 1973, B.P., unpublished data). Typically, collections of parasites have been made from April to June during 1993–1998 from one of its natural hosts, the weevil *H. eximia* which is monophagous on pale dock, *Rumex altissimus* Wood. In the field, *N. breviramulus* is multivoltine (B.P., unpublished data). Several generations were maintained in the laboratory on *H. eximia* reared on pale dock, at 26°C. Ovipositional chambers for the parasite consisted of 0.35-liter paper cartons, held at 26°C, 55% RH, and a 12:12 (L:D) h photoperiod, that contained honey drops on a plastic petri dish lid, 10–15 prepupae of *H. eximia* within pupal cocoons, and ≈20 *N. breviramulus* adults at a 1:1 ♀:♂ ratio. Prepupae were exposed to parasitism for 24 h, removed from the ovipositional chamber, and held in acrylic cages (30 by 30 by 30 cm) until the parasites emerged. A preovipositional period of 4 d was allotted for parasite mating and egg maturation before the adults were used in parasitism studies.

Voucher specimens of *N. breviramulus, E. comstockii, E. plathypenae, Er. winnemena,* and *B. mellitor* have been deposited in the W. R. Enns Entomological Museum at the University of Missouri, Columbia.

Venom Isolation. Venom extraction was done over a 1–2 mo period from adult females held at 26°C and a 14:10 L::D) h photoperiod. The venom produced by these parasites was isolated from the female parasite after adult eclosion as an aqueous extract of the reservoir of the venom glands attached to the lower reproductive tract (Coudron and Brandt 1996). One reservoir equivalent (RE) is equal to the gland-reservoir material obtained from one insect and is equivalent to gland-equivalents (GE) and parasite equivalent units (PEU) used in previous publications. The venom extract was stored at −70°C before host injection and electrophoretic analysis.

Host Insect Rearing. Larvae and prepupae of *H. eximia* were collected from pale dock in central Missouri. Eggs and larvae of the boll weevil, *Anthonomus grandis grandis* Boheman, were obtained from a laboratory colony maintained at Starkville, MS. Larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, were obtained from a laboratory colony that was maintained on potato plants at our laboratory. Larvae of the cereal leaf beetle, *Oulema melanopus* (L.), were collected from oat and wheat fields in Boone County, MO. Eggs, larvae, and adults of the asparagus beetle, *Crioceris asparagi* (L.) and the spotted asparagus beetle, *Crioceris duodecimpunctata* (L.), were collected from asparagus ferns within Columbia, MO. Prepupae of the pine sawyer, *Monochamus carolinensis* (Olivier), were obtained from a laboratory colony maintained at the University of Missouri on precut logs of jack pine. Larvae of the leaf beetle *Chrysomela knabi* Brown were collected from willow trees in Boone and Callaway counties in Missouri. Larvae of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) were obtained from our laboratory colonies and maintained on a modified formulation of the Berger (1963) and Wilkinson et al. (1972) wheat germ-based diet.

Natural and factitious hosts were held in 0.35-liter paper cartons at 24–26°C, 40–60% RH, and a 12:12 (L:D) h photoperiod, and reared on bouquets of the host plant from which they had been collected, or on artificial diets, as indicated in Tables 1 and 2. Larvae and prepupae of those insects that normally pupate in soil were transferred as late larvae to 0.5-liter plastic cartons containing moistened medium consisting of either a 1:1:1 sand:soil:potting mixture sieved through a #16 screen, or a 1:10 Perlite:peat moss mixture. For each host, subsets of last instar larvae or prepupae were observed every 12 h. Only individuals that demonstrated synchronous growth (i.e., those that developed prepupal characteristics within the 6-h time period) were used for subsequent bioassays (Coudron et al. 1990). Prepupal characteristics include transformation from a flattened to a cylindrical shape, cessation of feeding, and contraction and swelling of the thorax.

Parasitism Procedure. Host ranges of *N. breviramulus,* and its venom, were determined by natural parasitization of hosts. This was accomplished by inserting individual larvae or prepupae into an empty cocoon (i.e., an open weave mesh) of *H. eximia,* sealing the opening in the cocoon with Elmer's Glue and acid-free insect label cardstock, and then exposing ≈10 test insects within the cocoons to ≈10–15 *N. breviramulus* female adults for 4–36 h in a 0.35-liter paper carton with a petri dish lid, held under continuous light at 26°C, and 55% RH. Oviposition was used as a sign of a successful envenomation in coleopteran hosts. Insertion of the parasite ovipositor-stinging apparatus into the host was used as a sign of envenoma-

Table 1. Incidence of arrested development in insects parasitized in the laboratory by *N. breviramulus*

Species [origin]	Recorded host[a]	Stage treated	Parasitized[b]		Control[d]		Parasite development[e]	Stage attained[f]
			No. parasitized/exposed	% arrested[c]	No.	% pupated		
Coleoptera								
Curculionidae								
Hypera exima [-/pale dock]	+	Prepupa	25/25	100	20	100	25/25	Adult
Anthonomus grandis grandis [boll weevil/lab]	−	Prepupa	19/38	100	15	80	19/19	Adult
Chrysomelidae								
Crioceris spp.[g] [asparagus beetle/asparagus]	−	Late larva	7/18	100	10	100	5/7	Adult
Oulema melanopus [cereal leaf beetle/wheat]	−	Late larva-prepupa	17/44	100	26	77	14/17	Adult
Lepidoptera								
Plutellidae								
Plutella xylostella [diamondback moth/laboratory]	−	Prepupa	10[h]/16	100	2	100	N/A	N/A

[a] +, previously recorded; −, not previously recorded in the literature.
[b] Studies done with insects parasitized under controlled laboratory conditions.
[c] Percentage of parasitized hosts showing arrested development following treatment.
[d] Insects were held under the same conditions as the treated insects, except there was no exposure to the parasite.
[e] Number of hosts supporting some larval development of the parasite/number parasitized; N/A, not applicable (no eggs oviposited).
[f] Oldest developmental stage attained by the F_1 generation of the parasite on the host specified.
[g] Mixture of the common asparagus beetle, *Crioceris asparigi*, and the spotted asparagus beetle *Crioceris duodecimpunctata.*
[h] Parasite observed probing (i.e., stinging with the ovipositor), but no deposition of eggs.

tion in lepidopteran hosts because eggs were not laid, yet the host did not continue to develop.

Parasitized larvae and prepupae were transferred to inverted petri dishes lined with moistened filter paper or to 0.35-liter paper cartons containing plant material. Larvae and prepupae of those insects that normally pupate within the soil were transferred to a moistened medium (previously described). Control insects were handled in the same manner as treated insects, but were not exposed to parasites.

Injection Procedure. Insects that could be handled and injected during the late larval or prepupa stages were selected from available species and subjected to injection tests. Venom extract was injected into the test hosts 4–8 h after ecdysis into the last larval instar or after the development of prepupal characteristics to test for alterations from normal development. For injections, test insects were removed from the rearing cartons and kept on moist filter paper in a 0.2-liter paper carton until injected. If necessary, these insects were briefly anesthetized with CO_2 to aid the injection process, with no detectable adverse effects. An aliquot of 1–5 μl was injected into the abdominal hemocoel of the test insects using a 250-μl syringe with a 33-gauge needle. Control insects were injected with 1–5 μl of a 25 mM phosphate buffer. Control and treated insects were continuously observed for 30 min to ensure the absence of bleeding postinjection. Subsequently, insects were observed every 24–48 h.

Interpretation of Arrestment Results. Arrested development of treated insects was determined by comparing the time of molting with that of control insects held under identical conditions. Responses to injections were quantified and correlated with venom concentration (Coudron and Puttler 1988). Arrested development in parasitized and injected insects was measured in larvae or prepupae by the absence of ecdysis. Parasitized or injected hosts that molted at the same time as their controls were scored "no arrestment of development"; those that had not molted for 7 d after controls had molted, were scored "developmentally arrested." Also, insects that remained healthy (i.e., retained normal color and were able to move in response to stimuli) for 48 h after treatment, and subsequently died, were scored "developmentally arrested." In the lepidopteran host tested, insects that molted incompletely and subsequently failed to develop into adults were also scored "developmentally arrested." In contrast, insects that turned black (necrotic) or became flaccid were removed from the data set. These were considered to have died from the physical trauma associated with the injection process because these effects were not observed in naturally parasitized hosts. This usually occurred within 24 h after treatment. Insects that showed signs of microbial contamination (developed mycelia on the cuticle or liquified) or developed nonrelated parasites were also removed from the data set.

The number of insects treated (parasitized or injected) was low for some test species. This reflects a limited number of host insects available that did not succumb to pathogens or parasites acquired in the field, or a low rate of parasitism by *N. breviramulus.* Sufficient control insects of each species were retained to estimate the time of molting. Egg deposition, parasite development, and survival to adult stage were recorded.

Protein Analyses. The chemical reagents used include methanol 99.9%, glacial acetic acid, 2-mercaptoethanol, sodium chloride, sodium dodecyl sulfate (SDS), polyacrylamide gel electrophoresis (PAGE) molecular weight standards, and bovine serum albu-

Table 2. Development and molting of insects tested with injection of an extract from *N. breviramulus*

Species [origin]	Stage treated	RE[b]	Treated[a] No. treated	Treated[a] % arrested[c]	Control[d] No. treated	Control[d] % arrested[e]	Control[d] Death from injection[f]
Coleoptera							
Curculionidae							
Hypera eximia [-/pale dock]	Prepupa	0.5	15	40	10	0	5
		1	18	94			
		2	21	90			
		4	10	100			
		6	5	100			
Anthonomus grandis grandis	Late larva	1	18	27	8	0	3
[boll weevil/lab]		2	10	33			
	Prepupa	1	15	73			
		2	8	100			
Chrysomelidae							
Crioceris spp.[g]	Prepupa	1	15	7	6	0	1
[asparagus beetle/asparagus]		2	26	27			
		4	10	100			
Leptinotarsa decemlineata	Prepupa	5	15	7	23	0	3
[Colorado potato beetle/potato]		10	12	42			
Chrysomela knabi	Late larva	2	3	0	29	0	0
[-/willow leaves]		4	5	0			
		9	3	100			
		12	5	100			
Cerambycidae							
Monochamus carolinensis	Prepupa	15	3	33	2	0	1
[pine sawyer/jack pine]		20	3	66			
Lepidoptera							
Noctuidae							
Trichoplusia ni	Prepupa	1	14	0	38	0	7
[cabbage looper/lab]		2	15	0			
		4	46	54			
		8	23	96			

[a] Insects were mechanically injected in the abdominal hemocoel with 1–5 μl of an extract from *N. breviramulus.*
[b] The venom reservoir was excised from the female *N. breviramulus,* washed, centrifuged, and the supernatant used for injections. One RE represents the amount of parasite material extracted from one venom reservoir.
[c] Percentage of injected hosts that exhibited arrestment in development attributed to the injection of the parasite material.
[d] Insects were injected with 1–5 μl of a 25 mM phosphate buffer and held at the same conditions as the treated.
[e] Percentage of the control insects that exhibited arrestment in development attributed to the injection of buffer.
[f] The host died as a result of the injection and before the development of the host could be measured.
[g] Mixture of the common asparagus beetle, *Crioceris asparigi,* and the spotted asparagus beetle *Crioceris duodecimpunctata.*

min (Sigma, St. Louis, MO); TRIS, bromophenol blue, acrylamide, bis-acrylamide, TEMED, ammonium persulfate, glycine, nonfat dry milk, and Tween 20 (Bio-Rad, Richmond, CA); goat antirabbit IgG horseradish peroxidase conjugate and SuperSignal Substrate (Pierce, Rockford, IL).

Venom samples were analyzed by PAGE using the Bio-Rad Protean II system. Gels contained 10% polyacrylamide. For SDS-PAGE, venom concentrations of 10–80 RE for Coomassie staining or 0.1–2.5 RE for western blotting were diluted 3:1 with sample buffer (0.125 M TRIS pH 6.8; glycerol, 12.5%, vol:vol; bromophenol blue, 0.005%, wt:vol; SDS, 6%, wt:vol; 2-mercaptoethanol, 12.5%, vol:vol), boiled for 10 min, and run for 1.5 h at 150 volts. The procedure for native PAGE was identical with the exception of no boiling of samples and no SDS in the buffers. Broad-range SDS-PAGE molecular weight markers or bovine serum albumin were used as standards. The gels were stained with Coomassie Blue R-250 to visualize the protein bands.

For Western blot analyses, the gels were equilibrated in transfer buffer (25 mM TRIS, 192 mM glycine, 20% methanol) and transferred onto 0.45 μm nitrocellulose (BAS-NC, Schleicher and Schuell, Keene, NH) using the Bio-Rad *trans*-Blot Electrophoretic Transfer Cell at 100 volts for 1.5 h. Membranes were blocked in blocking buffer TBS (100 mM TRIS pH 7.5, 0.9% sodium chloride, 5% nonfat dry milk) for 30 min at room temperature and then incubated in antiserum to the N-terminal sequence of the 36 kDa subunit of the 66 kDa protein from *E. comstockii* diluted 1:50 in TTBS (TBS plus 0.05% Tween 20) for 1 h at room temperature. Following three 10-min washes in TTBS, the membranes were incubated for 30 min at room temperature in goat antirabbit horseradish peroxidase conjugate diluted 1:20,000 in TTBS. Following three 15-min washes in TTBS, the membranes were incubated for 5 min in SuperSignal Substrate (Pierce) and exposed to X-OMAT AR film (Kodak) for 1–5 s (Burnette 1981).

Antisera to the 66-kDa protein from *E. comstockii* was produced by using a synthetic peptide generated with the N-terminus sequence of a 36-kDa subunit (T.A.C., unpublished data). Polyclonal antibodies against the synthetic peptide were generated using standard procedures. The synthetic peptide was emulsified by mixing with equal volumes of complete

Freund's Adjuvans (Sigma) for primary immunization, and emulsion was injected into subcutaneous dorsal sites of New Zealand white rabbits. Four booster injections were given at 2-wk intervals using incomplete Freund's Adjuvans (Sigma). Antipeptide antibody titer was tested between boosters using an enzyme-linked immunosorbent assay (Research Genetics). Three weeks after the fourth immunization, the blood was collected, allowed to coagulate for 4 h at 26[deg]C, centrifuged for 10 min at 5,000 × *g* at 4°C, and the supernatant stored in 100 μl aliquots at −70°C until used in the Western blot procedures.

Results

Effect of Parasitism by *N. breviramulus*. Parasitism by *N. breviramulus* on its natural host, *H. eximia,* was closely observed under laboratory conditions. Before oviposition, female parasites probed hosts with their ovipositors within 30 min of presentation. This continued intermittently for ≈2 h. Probes consisted of two main types. The first was a brief (<30 s) touching or penetration of the host integument. The second was accompanied by a "drilling" action of the abdomen and usually lasted longer than 30 s and sometimes as long as 3 min. Most prepupae were still responsive to touch immediately after being stung by the female parasites. However, when oviposition occurred (i.e., between 2 and 4 h after probing) probed larvae were significantly less responsive to touch than controls. Successful parasitism, including both stinging and oviposition, occurred only when the host was in its cocoon. Interaction of the female parasite with the cocoon appeared to be a critical part of the preoviposition activity. The long length and the central positioning of the stinging apparatus made stinging difficult from anywhere except the outside of the cocoon. Consequently, the parasitism studies were limited to available species that could be placed within the *H. eximia* pupal cocoon. Additionally, attempts were made to parasitize alfalfa weevils, *Hypera postica* (Gyllenhal), and clover leaf weevils, *Hypera punctata* (F.), that were obtained through field collections (data not shown). Unfortunately, the majority of these individuals had been parasitized in the field or developed a fungal infection, thereby precluding meaningful results.

Development was arrested after probing by *N. breviramulus* in the natural host, *H. eximia,* in three coleopteran factitious hosts (i.e., *A. g. grandis*, *Crioceris* sp., and *O. melanoplus)*, and in one lepidopteran factitious host (i.e., *P. xylostella*) (Table 1).Because immatures of the two species of asparagus beetles are not distinguishable, yet we achieved arrestment in all individuals, it is likely that parasitism arrested development in both species. In most instances, prepupae on which *Necremnus* eggs were laid supported parasite larval development. Adult parasites were reared from all five coleopteran host species that were tested; four of which had not been recorded as natural hosts. No eggs were oviposited on the lepidopteran species.

Injection Test. Arrested development was recorded in all seven coleopteran species tested and in one species of Lepidoptera following injection of an aqueous extract of the venom gland-reservoir complex from *N. breviramulus* (Table 2). Again, because we did not separate the two species of asparagus beetles yet achieved 100% arrestment at the higher dose levels, it is likely that the venom arrested development in both species. Additionally, all attempts to inject *P. xylostella* failed because of injury resulting from the injection process.

The arrestment response was dose- and developmental stage- dependent. A greater percentage arrestment was observed at higher doses of the venom extract in the natural host, *H. eximia,* and in all the factitious hosts that we tested. The greatest response within the coleopteran species tested was observed with the natural host, *H. eximia,* yielding 40% and 100% of the treated insects arrested at the dose levels of 0.5 and four RE, respectively. The least responsive coleopteran species was *M. carolinenis,* yielding only 33% and 66% of the treated insects arrested at dose levels of 15 and 20 RE, respectively. In comparison, the lepidopteran species, *T. ni,* was somewhat less responsive than the natural host, yielding 0% and 96% of the treated insects arrested at dose levels of 2 and 8 RE, respectively. Also, the prepupal stage appeared to be more sensitive to the venom than the terminal larval stage in the one insect, *A. g. grandis*, that was tested at both the prepupa and late larval stages (Table 2).

The response of the coleopteran hosts to parasitism or venom injection was similar, although there was a greater incidence of partial molts in the injected group. In contrast, there was a significant difference in response to external stimuli between the lepidopteran host, *T. ni,* and the other coleopteran hosts. Most parasitized or injected coleopteran hosts showed decreased movement in response to external stimuli within 2–4 h after parasitism or injection with *N. breviramulus* venom. Often, live hosts were differentiated from dead hosts by signs of apolysis (but not ecdysis), maintenance of turgidity, and maintenance of a color similar to that before injection. Flaccid, necrotic hosts were considered to be dead. In the case of *T. ni,* however, there was no decreased movement in response to the venom for at least 12–24 h after envenomation because larvae resumed their normal movement and >50% moved to the top of the holding container and respun cocoons (Fig. 1A). After respinning their cocoons, the response of *T. ni* was very similar to that of the coleopteran hosts, however, the extent was more striking. Instead of molting, the larva underwent apolysis but not ecdysis, resulting in a pupa trapped in its larval integument (Fig. 1B). In some instances, the abdominal larval integument burst, resulting in a partial molt, although those insects did not survive to adulthood.

Venom Analyses. When the protein profile of *N. breviramulus* venom is compared with that of two other coleopteran parasites, *B. mellitor,* a larval ectoparasite which paralyzes its host, and *E. winnemena,* an egg parasite which arrests eclosion, differences are

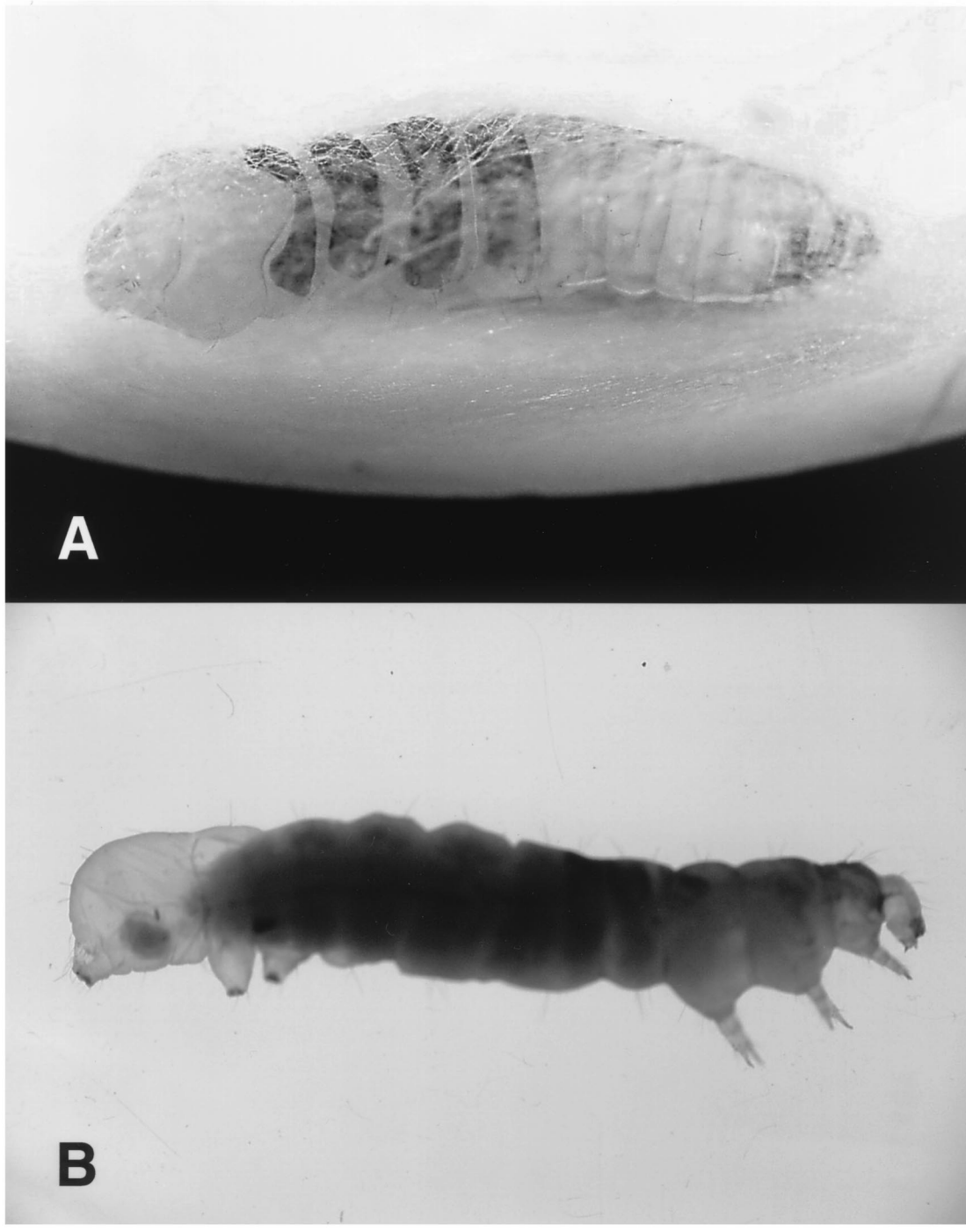

Fig. 1. Prepupae of *Trichoplusia ni* (Noctuidae) after injection of eight reservoir equivalents of venom of *N. breviramulus.* (A) Arrested prepupae within its silken cocoon. (B) Arrested prepupae excised from its cocoon showing the typical symptoms of arrestment—pupae trapped within the larval integument.

observed in banding patterns within the molecular weight range of 20 kDa to 200 kDa in both native (Fig. 2A) and denatured (SDS) (Fig. 2B) gels. Similarly, when the protein composition of *N. breviramulus* venom is compared with that of other eulophid parasites which arrest development in larval hosts, *E. plathypenae* and *E. comstockii,* differences are also seen in the banding patterns in both native and de-

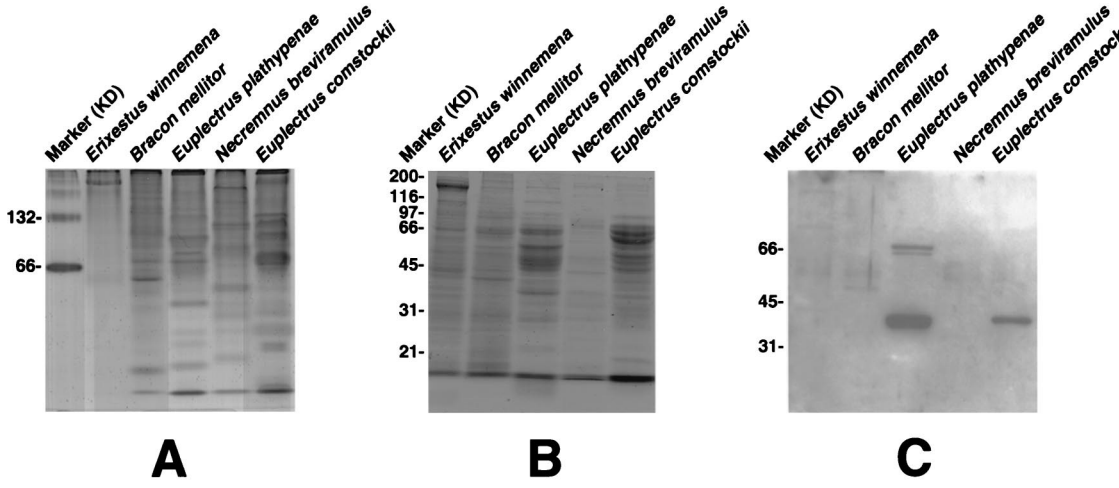

Fig. 2. Electrophoretic patterns of the proteins in extracts of the venom gland-reservoir complexes dissected from several parasitic hymenopteran species. (A) Electrophoretic pattern of the parasite proteins separated under native conditions. (B) Electrophoretic pattern of the parasite proteins separated under denaturing conditions. (C) Western blot of the electrophoretic pattern of the parasite proteins separated under denaturing conditions and then probed with an antibody to a 33kDa subunit of the molting arrestment protein isolated from the venom of *Euplectrus comstockii.* Cross reaction was detected to a 33-kDa protein (the intense band in the *E. comstockii* and *E. plathypenae* lanes between the 31 and 45 kDa markers) and with two higher molecular weight proteins in *E. plathypenae* (bands at the 66 kDa marker).

natured gels at the same molecular weight range (Fig. 2 A and B). Furthermore, a western blot analysis of these venom proteins probed with the antisera to the molting arrestment protein from *E. comstockii* shows that this developmental arrestant is found only in the two *Euplectrus* species and not in the others (Fig. 2C). Consequently, it is unlikely that a protein like the molting arrestment protein, or a protein containing the same N-terminal sequence as the 36-kDa subunit of this protein exists in the venom of *N. breviramulus* or the other species. This suggests that the substance in *N. breviramulus* venom that is responsible for developmentally arresting prepupal molting in coleopteran and lepidopteran hosts may be a novel substance.

Discussion

Previous study has shown that the ectoparasite *N. breviramulus* oviposited and developed successfully on its natural curculionid hosts, *H. eximia, H. compta,* and *H. paludicola* and on other species of *Hypera—H. postica, H. punctata, H. meles* (F.), and *H. nigrirostris* (F.) (Puttler et al. 1973). This study provides the first report of the results of parasitism by *N. breviramulus* of *A. g. grandis, Crioceris* spp., *O. melanopus,* and *P. xylostella.* Also, these studies have shown that *N. breviramulus* venom alone is capable of arresting the development of its natural host and five additional species, including coleopteran insects (i.e., *L. decemlineata, A. g. grandis,* and *M. carolinensis*) and a lepidopteran insect (i.e., *T. ni*). Most importantly, the venom of *N. breviramulus,* a parasite of insects associated with a weed of little economic importance, was found to arrest through injection or parasitization a number of important crop pests, including *A. g. grandis, O. melanopus, L. decemlineata, M. carolinensis, P. xylostella,* and *T. ni.* Consequently, there is a significant value to the venom from *N. breviramulus* because it is active on coleopteran species beyond the normal host range of the parasite, and is also active on lepidopteran species; a phenomenon previously exemplified by the activity of the venom from *Euplectrus* spp. (Coudron and Puttler 1988, Coudron 1991).

The venom from *E. comstockii* is similar to that of *N. breviramulus* in that both include an aqueous soluble active substance, both arrest host development without killing the host, both exhibit a dose–response effect, and both act independent of other substances generated by the parasite. Differences between the venom of these two eulophid species include the protein composition, the host range, and the developmental stage of the host where greatest sensitivity is found.

Because the molting arrestment substance from *E. comstockii* is composed of protein, it is reasonable to speculate that the active substance(s) from the venom of *N. breviramulus* that arrests development in host insects will also be proteinaceous in composition. However, the molting arrestment protein found in the venom of *E. comstockii* was shown to be absent from the venom of *N. breviramulus.* If insect venoms have evolved in a similar manner as the parasites themselves, we can assume that there is a greater chance that closely related taxa will have a greater similarity in their venoms than more distantly related taxons. Conversely, venoms of taxa that target the same host may be more similar than venoms between taxa that are more closely related taxonomically. Both of the eulophids, *E. comstockii* and *E. plathypenae,* are closely

related taxonomically and also attack the same type of host (free-living lepidopteran larvae). The more distantly related eulophid, *N. breviramulus,* however, attacks a different host, occluded coleopteran prepupae; therefore the fact that its venom contains a different developmental arrestant is also not surprising. It is intriguing that these venoms have a far broader host range than the parasites themselves. The venom from *N. breviramulus,* which has evolved to arrest the development of a hidden occluded host (i.e., *H. eximia*), also works on free-living lepidopterans (i.e., *T. ni* and *P. xylostella*). If this phenomenon is similar across other hymenopteran taxa, then we can expect there to be many more novel developmental arrestants which have yet to be discovered.

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