JOURNAL OF VIROLOGY, Oct. 1983, p. 74–78
0022-538X/83/100074-05$02.00/0

Vol. 48, No. 1

Expression of *Campoletis sonorensis* Virus in the Parasitized Host, *Heliothis virescens*

JO-ANN G. W. FLEMING, GARY W. BLISSARD, MAX D. SUMMERS,* AND S. BRADLEIGH VINSON

Department of Entomology, Texas A & M University and Texas Agricultural Experiment Station, College Station, Texas 77843

Received 28 March 1983/Accepted 6 July 1983

Polyadenylated mRNA transcripts of the virus of *Campoletis sonorensis* were detected in *Heliothis virescens* host larvae 2 h after the larvae had been parasitized by *C. sonorensis* females and continued to be present in host larvae through 9 days postparasitization while *C. sonorensis* developed endoparasitically. cDNAs of viral transcripts present in *H. virescens* hybridized with only certain *C. sonorensis* viral DNAs.

Female *Campoletis sonorensis* [Hymenoptera: Ichneumonidae] wasps contain a DNA virus in the calyx region of their reproductive tracts and inject this virus into their habitual host, *Heliothis virescens* [Lepidoptera: Noctuidae], during oviposition (10). Within 24 h after parasitization two major effects are observed (10). First, the host cellular immune response is altered such that the parasitoid egg is not encapsulated by hemocytes. Second, host metabolism is significantly reduced, resulting in a pronounced decrease in weight gain. Biological studies indicate that the *C. sonorensis* virus (CsV) may play a significant role in the abrogation of host cellular immunity and the protection of the parasitoid egg from encapsulation (3, 11). Electron microscopic studies show that CsV nucleocapsids are present in nuclei of host hemocytes and fat body and muscle cells at 2.25 h postparasitization (p.p.) (9). However, no biochemical studies demonstrating the expression or replication of CsV in the parasitized host have been reported.

CsV is a unique virus with a complex double-stranded DNA genome (molecular weight, 135×10^6 to 170×10^6) composed of at least 25 different covalently closed circular molecules (molecular weight range, 4.0×10^6 to 13.6×10^6), which are present in nonequimolar ratios (5). The initial characterization studies also demonstrated that most of the covalently closed circular DNA molecules are largely composed of nonhomologous sequences, suggesting that the genome is multipartite (5).

In the present study, we demonstrated that CsV-specific transcripts were present in *H. virescens* larvae by 2 h after parasitization and continued to be present for 9 days while *C. sonorensis* larvae developed endoparasitically. Only part of the CsV genome was expressed in *H. virescens* since cDNAs of viral transcripts hybridized with only certain CsV superhelical DNAs.

MATERIALS AND METHODS

Insects. *C. sonorensis* (Cameron) and *H. virescens* (Fab.) were reared essentially as described previously (5). Third instar *H. virescens* larvae were parasitized for 4 h by 7-day-old *C. sonorensis* females that had mated 6 days previously. Because most oviposition occurs within the first 30 min p.p., times indicate periods after the commencement of parasitization.

Virus purification and isolation of viral DNA. Virus was purified from excised calyces by centrifugation on sucrose gradients by previously described methods (5). DNA was isolated from purified virions by digestion with proteinase K and sodium dodecyl sulfate disruption according to the method of Krell et al. (5). The viral DNA was extracted twice with buffer-saturated phenol-chloroform-isoamyl alcohol (25:24:1) and twice with chloroform-isoamyl alcohol (24:1) and ethanol precipitated in the presence of 200 mM sodium acetate (pH 5.0).

Isolation of mRNA. Total nucleic acids were isolated from 500 adult *C. sonorensis* males or females, 600 *C. sonorensis* pupae, or 200 parasitized *H. virescens* larvae (per sampling time) by a modification of previously described methods (2, 4). Insects were homogenized in 20 volumes (vol/fresh weight) of cold (−20°C) 6.6 M guanidine hydrochloride–99 mM potassium acetate–142 mM 2-mercaptoethanol with a Polytron homogenizer (Brinkmann Instruments, Inc.) After the removal of cellular debris by two centrifugations at $1,700 \times g$ for 5 min at 4°C, the total nucleic acids were precipitated with ½ volume of ethanol. The pellets were twice resuspended in 10 to 20 volumes of cold (−20°C) 6.6 M guanidine hydrochloride–25 mM EDTA–142 mM 2-mercaptoethanol and precipitated with ½ volume of ethanol after adding potassium acetate (pH 5.9) to a final concentration of 200 mM. The final pellets were suspended in 10 volumes of 20 mM Tris-hydrochloride (pH 7.4)–10 mM EDTA–100 mM KCl and digested with proteinase K (200 μg/ml) at

37°C for 30 min. The samples were adjusted to 200 mM potassium acetate and extracted once with 1 volume of buffer-saturated phenol, once with 1 volume of phenol-chloroform-isoamyl alcohol (25:24:1), and once with 1 volume of chloroform-isoamyl alcohol (24:1). Nucleic acids were precipitated with 2 volumes of ethanol. Polyadenylated (polyA$^+$) mRNAs were isolated by oligodeoxythymidilic acid-cellulose chromatography essentially as described previously by Aviv and Leder (1) and stored at −80°C.

Isolation of *Heliothis* DNA. DNA was extracted from nonparasitized *H. virescens* larvae by the guanidine hydrochloride procedure described above, but 2 volumes of ethanol were used for all precipitations. The phenol-extracted total nucleic acids were digested twice with heat-treated RNase A (10 μg/ml) and RNase T (10 μg/ml) in the presence of 10 mM EDTA, reextracted with phenol-chloroform-isoamyl alcohol (25:24:1), and ethanol precipitated. Larval DNA was suspended in water and stored at −80°C.

cDNA systhesis. PolyA$^-$ mRNA (5 μg) was mixed with 25 nmol each of dATP, dGTP, and dTTP, 12.5 nmol of dCTP, and 31.25 pmol of [α-^{32}P]dCTP (3,200 Ci/mmol; New England Nuclear Corp.) and lyophilized. cDNAs were synthesized in 25-μl reactions containing 100 mM Tris-hydrochloride (pH 8.3), 10 mM $MgCl_2$, 10 mM dithiothreitol, 50 mM KCl, 0.5 μg of oligodeoxythymidilate$_{12\text{-}18}$, 1.25 μg of actinomycin D, and 100 U of avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories) at 37°C for 60 min. The reactions were terminated by cooling to 0°C and adding EDTA to a final concentration of 10 mM. After the addition of 100 μg of *E. coli* tRNA (Boehringer Mannheim), cDNAs were twice precipitated from reactions with 2 volumes of ethanol. The final pellet was washed with cold 67 mM NaCl–70% ethanol and suspended in 300 μl of water. cDNAs (specific activity, 1.50×10^7 cpm/μg) were heat denatured before addition to hybridization buffer to a final concentration of 20 ng/ml.

Radioactively labeled DNA. CsV DNA was labeled by nick-repair synthesis with [α-^{32}P]dCTP to high specific activity (1×10^8 to 4×10^8 cpm/μg) by the method of Rigby et al. (7). CsV DNA probes were heat denatured and diluted to 20 ng/ml with hybridization buffer.

Gel electrophoresis and blot hybridization. CsV DNA was digested with *Sal*I or *Hin*dIII under conditions specified by the manufacturer (Bethesda Research Laboratories). Undigested or digested viral DNA was electrophoresed in 0.8% agarose gels and was unidirectionally transferred to nitrocellulose as described previously (8). Denatured cDNA probes were diluted to 20 ng/ml in hybridization buffer composed of 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt reagent, 20 mM sodium phosphate (pH 6.5), and 100 μg of sonicated calf thymus DNA per ml and were hybridized to prehybridized blots at 43°C for 48 h (8).

RESULTS

Presence of CsV transcripts in parasitized larvae. To determine whether CsV is expressed in the parasitized host, we isolated polyA$^+$ mRNA from *H. virescens* larvae at various times. (1, 2, 4, 6, 12, 24, 36, and 48 h and 6 and 9 days) after the larvae had been parasitized by *C. sonorensis* females. [^{32}P]cDNAs were synthesized with avian myeloblastosis virus reverse transcriptase and hybridized to Southern transfers to CsV DNA. Because of the difficulty of resolving the 25 superhelical CsV DNAs, cDNAs also were hybridized to CsV DNA digested with *Sal*I or *Hin*dIII.

Low levels of hybridization between CsV DNA and cDNA probes synthesized from polyA$^+$ mRNA isolated from *H. virescens* larvae at 2 h p.p. were detected in autoradiograms exposed for 96 h or longer (Fig. 1, lane B, and Fig. 2, lanes B and H). In contrast, no hybridization between CsV DNA and cDNA synthesized from 1 h p.p. mRNA fractions was detected in autoradiograms exposed for 4 weeks (data not shown). The level of expression of CsV appeared to increase significantly by 4 h p.p. (Fig. 1, lane C, and Fig. 2, lanes C and I) and remained readily detectable through 9 days p.p. (Fig. 1, lane E, and Fig. 2, lanes E and K). Both the number and the intensity of DNA bands which hybridized with cDNAs from ≥4 h p.p. samples increased, even though the concentrations (19.4 ± 1.2 ng/ml) and specific activities ($1.46 \times 10^7 \pm 0.22 \times 10^7$ cpm/μg) of all cDNA probes (1 h to 9 days p.p.) did not differ significantly. cDNAs synthesized from polyA$^+$ mRNA isolated from nonparasitized control *H. virescens* larvae did not hybridize with CsV DNA (data not shown). In other preliminary control experiments, no hybridization of ^{32}P-labeled CsV DNA with Southern transfers of undigested or *Hin*dIII-digested *H. virescens* DNA was detected (data not shown). The transcripts detected with cDNAs synthesized from mRNAs of parasitized larvae, therefore, were virus specific.

Only certain supercoiled or relaxed circular CsV DNA bands hybridized with cDNAs synthesized from mRNAs of parasitized larvae (Fig. 1, lanes B through E). DNA bands migrating faster than the 9.6-kilobase-pair (kbp) lambda *Hin*dIII fragment are mostly superhelical DNAs (Fig. 1, SH [superhelical] region; [5]), whereas the more slowly migrating bands are either relaxed circular or linear forms of the DNAs (Fig. 1, RC [relaxed circular] region; [5]). Most of the cDNA-positive bands in the RC (upper) region therefore represent relaxed circular forms of corresponding cDNA-positive superhelical DNAs in the SH (lower) region. Approximate sizes of cDNA-positive superhelical bands were extrapolated from the published sizes of the six major CsV superhelical DNAs (5). Only 3 (15.3, 12.3, and 9.5 kbp) of the 23 superhelical CsV DNAs in the SH region of the gel hybridized with 2 h p.p. cDNAs (Fig. 1, lane B). Seven

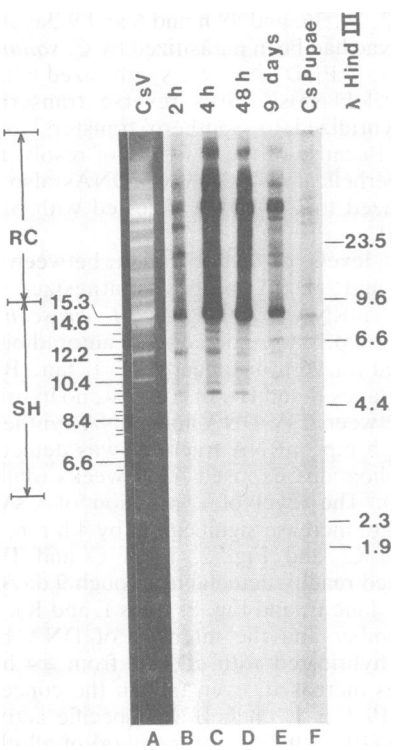

FIG. 1. Hybridization of cDNAs of polyA$^+$ mRNA from parasitized *H. virescens* larvae and *C. sonorensis* pupae to CsV DNA. CsV DNA was electrophoresed in 0.8% agarose gels, transferred to nitrocellulose, and hybridized to [^{32}P]cDNAs synthesized from polyA$^+$ mRNA isolated from *H. virescens* larvae 2 h (lane B), 4 h (lane C), 48 h (lane D), and 9 days (lane E) after parasitization by *C. sonorensis* or from *C. sonorensis* pupae (lane F). Autoradiograms were exposed at −80°C with Dupont Cronex Lighting-Plus intensifying screens for 4 days for the 4-h, 48-h, and 9-day treatments, for 14 days for the 2-h treatment, and for 48 days for the *C. sonorensis* pupae treatment. Regions containing mostly relaxed circular (RC) or superhelical (SH) forms of CsV DNAs are indicated to the left of lane A. Superhelical CsV DNAs in ethidium bromide-stained gels (lane A) and the sizes in kbp of six major superhelical CsV DNAs are indicated to the left of lane A. The migration and sizes in kbp of *Hin*dIII fragments of lambda DNA are indicated to the right of lane F.

superhelical DNAs hybridized with labeled cDNA made with 4 or 48 h p.p. mRNA as a template (Fig. 1, lanes C and D, respectively). Labeled cDNA synthesized from 48 h p.p. mRNA hybridized to two additional SH bands (10.4 and 10.9 kbp), which were detected in autoradiograms exposed for a longer period (data not shown). By 9 days p.p., the number of positive SH bands detected decreased to five (Fig. 1, lane E). Except for a weakly hybridizing 7.0-kbp SH band observed between 4 and 48 h p.p., SH bands which hybridized with cDNAs ranged in size from 9.5 to 15.3 kbp. A 15.3-kbp band was the most strongly hybridizing band at all time periods.

The observed hybridization of the probes with CsV *Hin*dIII or *Sal*I fragments (Fig. 2) more clearly showed that the CsV DNA sequences were unequally represented in the cDNAs synthesized from mRNA of parasitized larvae. Only about 25% of the CsV *Hin*dIII or *Sal*I fragments hybridized with 4 or 48 h p.p. cDNAs (Fig. 2, lanes C, I, and D, J, respectively) and a slightly smaller proportion hybridized with 2 h or 9 day p.p. cDNAs (lanes B, H, and E, K, respectively). Positive bands observed at 4 or 48 h p.p. ranged in size from approximately 1.6 to 24.5 kbp for *Hin*dIII fragments and 0.6 to 11.5 kbp for *Sal*I fragments. The 6.2-, 5.3-, and 3.2-kbp strongly hybridizing *Hin*dIII fragments (Fig. 2, lanes C through E) appeared to correspond to the *Hin*dIII M, O, and Z fragments, respectively, which contain sequences found on approximately two to four of the superhelical molecules (5).

Superhelical CsV DNA molecules or CsV restriction fragments were not present in equimolar ratios (Fig. 1, lane A; Fig. 2, lanes A and G; [5]). Fragments of both major and minor intensity hybridized with cDNA probes (for example, Fig. 2, lane A versus lane D).

Presence of CsV mRNA in wasps. cDNA probes synthesized from polyA$^+$ mRNA from adult male or female *C. sonorensis* wasps did not hybridize with CsV to detectable levels (data not shown). Probes synthesized from polyA$^+$ mRNA isolated from developing *C. sonorensis* pupae when their heads, thoraces, and abdomens were pigmented hybridized to the 13.3- and 15.3-kbp superhelical CsV DNAs, as well as to several relaxed circular or linear forms or both (Fig. 1, lane F). The probe hybridized with several *Hin*dIII fragments (1.8 to 9.1 kbp) (Fig. 2, lane F) or *Sal*I fragments (1.2 to 8.9 kbp) (Fig. 2, lane L). Most of the restriction fragments which hybridized with cDNA prepared from wasp pupae mRNA also hybridized with cDNA probes synthesized from mRNA of parasitized larvae. However, the 1.8-kbp *Hin*dIII and the 1.2- and 3.6-kbp *Sal*I fragments hybridized only with cDNA of *C. sonorensis* pupae mRNA.

DISCUSSION

These data are the first evidence that genes of CsV, or any parasitoid virus, are expressed in the parasitized host. Virus-specific transcripts were detectable in *H. virescens* larvae by 2 h after parasitization and reached readily detectable levels by 4 h p.p. Biological studies have shown that *C. sonorensis* eggs are encapsulated

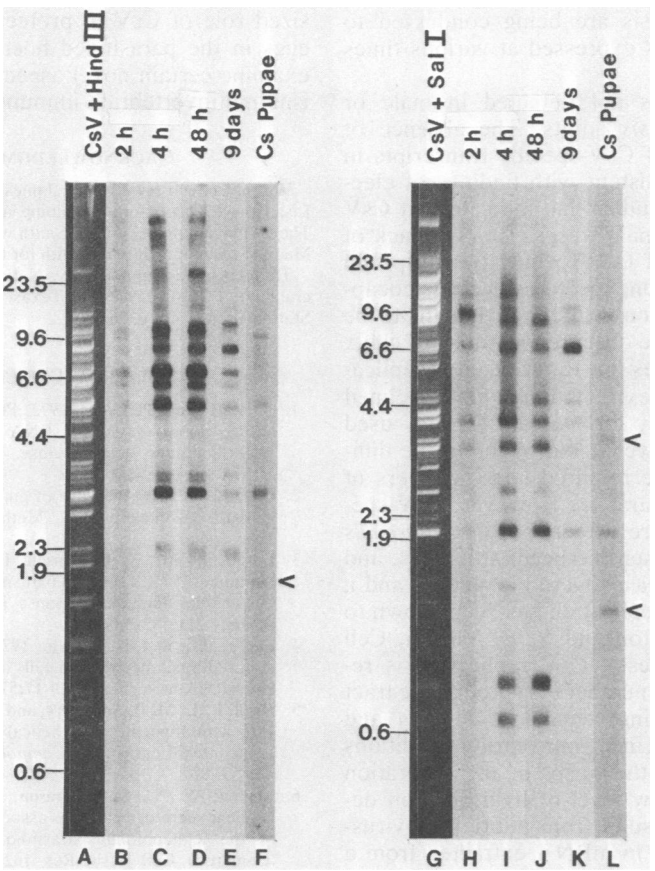

FIG. 2. Hybridization of cDNAs of polyA$^+$ mRNA from parasitized *H. virescens* larvae and *C. sonorensis* pupae to CsV DNA digested with *Hin*dIII or *Sal*I. CsV DNA was digested with *Hin*dIII (lanes A–F) or *Sal*I (lanes G–L), electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose. Digested CsV DNA was hybridized to [^{32}P]cDNAs of polyA$^+$ mRNA isolated from *H. virescens* larvae at 2 h (lanes B and H), 4 h (lanes C and I), 48 h (lanes D and J), or 9 days (lanes E and K) after parasitization by *C. sonorensis* or from *C. sonorensis* pupae (lanes F and L). Autoradiograms were exposed for the periods indicated in the legend to Fig. 1. Migrations and sizes in kbp of *Hin*dIII fragments of lambda DNA are indicated to the left of ethidium bromide-stained gels (lanes A and G). CsV DNAs that appeared to hybridize only to *C. sonorensis* pupae cDNA are indicated by arrowheads to the right of lanes F and L.

by host hemocytes within 24 h if CsV is absent but are not encapsulated if CsV is present (3). Our results suggest that CsV is expressed in the parasitized host early enough to possibly play a significant role in the abrogation of host cellular immune mechanisms. The continued presence of CsV mRNA through 9 days p.p., when fifth instar *C. sonorensis* larvae emerge from their hosts, suggests that CsV gene expression also may be important in the protection of the developing endoparasite. Most of the CsV mRNA sequences detected in parasitized larvae were not present at detectable levels in *C. sonorensis* pupae, although some of the viral mRNAs were present in both species. It is tempting to speculate that some CsV genes may be expressed only in the parasitized host and be involved in the alteration of host physiology after parasitization.

Hybridization of cDNAs with CsV restriction fragments indicated that a relatively small portion of the CsV genome was represented to detectable levels in the virus-specific mRNAs in parasitized *H. virescens* and that the most abundant CsV mRNAs sequences were similar at all times examined at ≥4 h p.p. The observed hybridization of cDNAs with only certain CsV superhelical DNAs is consistent with the hypothesis that the genome is multipartite (5). The hybridization of cDNAs with some of the superhelices may have resulted from the transcription of some of the cross-hybridizing sequences (e.g., the 6.2-, 5.3-, and 3.2-kbp *Hin*dIII fragments) present in the CsV genome. In vitro translation of selected viral transcripts and

northern gel analysis are being conducted to map the CsV genes expressed at various times p.p.

CsV mRNA was not detected in male or female *C. sonorensis* adults. The absence of detectable levels of CsV-specific transcripts in adult males is consistent with findings of electron microscopic studies that indicate that CsV is found only in female wasps (10). The lack of detectable levels of CsV-specific transcripts in adult females was unexpected since nucleocapsids and electron-dense material thought to be virogenic stroma are observed in nuclei of calyx cells in adult females (6). Rates of virus replication in the females extracted may have declined since approximately 50% of the females used were more than 2 weeks old owing to the difficulty of rearing the required large numbers of the obligate endoparasite. However, CsV-specific transcripts were detected in *C. sonorensis* pupae extracted when the heads, thoraces, and abdomens of the wasps were pigmented, and a high level of CsV replication has been shown to occur (W. N. Norton and S. B. Vinson, Cell Tissue Res., in press). CsV replication is restricted to a limited number of reproductive tract cells in the pupating female (6; Norton and Vinson, in press). Under our rearing conditions only about 35% of the wasps in any generation are female. The low level of hybridization detected probably results from dilution of virus-specific transcripts in mRNA extracted from a population of intact *C. sonorensis* pupae, since the specific activity and concentration of the cDNA probe were comparable to those of cDNAs synthesized from mRNAs of parasitized larvae. Most of the CsV mRNA sequences detected in wasp pupae were similar to some of the viral transcripts detected in parasitized *H. virescens* larvae, but a few minor differences were noted. It is not known whether CsV replicates in the parasitized host.

Our demonstration that CsV is expressed in *H. virescens* larvae soon after parasitization by *C. sonorensis* is consistent with the hypothesized role of CsV in protecting endoparasitoid eggs in the parasitized host and permits us to examine certain novel aspects of insect parasitism and invertebrate immunity more critically.

ACKNOWLEDGMENTS

We thank Jean-Hwa Hsiao, James Tracy, Brian Blum, and Lisa Harvill Turner for maintaining laboratory insect colonies. The technical advice of Gale Smith was invaluable. We thank Mac J. Fraser and Gale E. Smith for their editorial comments.

This work was supported by National Science Foundation grant no. PCM-8021992 and Texas Agricultural Experiment Station Project no. 6316.

LITERATURE CITED

1. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. **69**:1408–1412.
2. **Cox, R. A.** 1968. The use of guanidinium chloride in the isolation of nucleic acids. Methods Enzymol. **12B**:120–129.
3. **Edson, K. M., S. B. Vinson, D. B. Stoltz, and M. D. Summers.** 1981. Virus in a parasitoid wasp: suppression of the cellular immune response in the parasitoid's host. Science **211**:582–583.
4. **Fuchs, E., and H. Green.** 1979. Multiple keratins of cultured human epidermal cells are translated from different mRNA molecules. Cell **17**:573–582.
5. **Krell, P. J., M. D. Summers, and S. B. Vinson.** 1982. Virus with a multipartite superhelical DNA genome from the ichneumonid parasitoid, *Campoletis sonorensis*. J. Virol. **43**:859–870.
6. **Norton, W. N., S. B. Vinson, and D. B. Stoltz.** 1975. Nuclear secretory particles associated with the calyx cells of the ichneumonid parasitoid *Campoletis sonorensis* (Cameron). Cell Tissue Res. **162**:195–208.
7. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
8. **Smith, G. E., and M. D. Summers.** 1980. The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. Anal. Biochem. **109**:123–129.
9. **Stoltz, D. B., and S. B. Vinson.** 1979. Penetration into caterpillar cells of virus-like particles injected during oviposition by parasitoid ichneumonid wasps. Can. J. Microbiol. **25**:207–216.
10. **Stoltz, D. B., and S. B. Vinson.** 1979. Viruses and parasitism in insects. Adv. Virus Res. **24**:125–171.
11. **Vinson, S. B.** 1977. *Microplitis croceipes:* inhibitions of the *Heliothis zea* defense reaction to *Cardiochiles nigriceps*. Exp. Parasitol. **41**:112–117.