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Viral and Insect Genes that Inhibit the Immune System and Methods of Use Thereof

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VIRAL AND INSECT GENES THAT INHIBIT
THE IMMUNE SYSTEM AND METHODS OF
USE THEREOF

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U.S. Cl. 424/186.1, 424/199.1, 424/204.1; 424/93.2; 424/93.6; 435/320.1;
536/23.72

Field of Search 435/69.1, 172.1, 435/320.1, 172.3; 536/23.1, 23.5, 23.7,
23.72; 424/186.1, 199.1, 204.1, 93.2, 93.6

References Cited


Krell et al., “Virus with a Multiparitic Superhelicidal DNA Genome from the Ichneumonid Parasitoid Campoletis sonorensis”, May 27, 1982, Department of Entomology, College Station, Texas.


ABSTRACT

Viral, endoparasitoid and/or host genes that specifically inhibit the immune response of insect pests, useful for broadening the host range of insect viruses. Symbiont viruses of insect pests are genetically modified to express immune-suppressing proteins or biologically active fragments thereof and, optionally toxins, to increase the virus host range and/or improve the efficacy of insect pathogens.

14 Claims, 8 Drawing Sheets
TGGAGACAAG ACAGTATCCG GAACAGAAAA CTGGGTACAA TCCCCAGACA CGGATTCGCC
TATTAACAAC AAACCTGTAAC GTACATCATT GTAGACACTT TGTCACACAA CCATTGGAGC
ATATGCCTGG CTGAAACTCT TTACCAGACGA ATATGTCGTA AGTGAAGTGAT TGGAGATGCC
TACTTTATAT ATGGGAATAG AATATCTATA TCTAACATAC CATGTCAAAT TCAATTAAGA
TAATATGTTC TTGTATGGTT TCGTGACTTG AGTCGAAAGA GTCCCGCTGC CGACTTGAAA
ATCGCACATT GGTCGAAATT GGACGTAAGA AGGACATCTA TGGTCGATTTC CTGGCCGGGA
TCTATGCTCC ATTAATAGTC GTGAACACGT CCACACTGTA TTTAGAATTG AGTAAAGGAA
TGAACGAAC TAAATTTGTCC AATCTCCAGCG ATGGGTATAT AGCAGCAGCT GTAATCCCCA
TGCCCCGAAATT CAAGCCAGAA TCCAAAGATAG AAGATGAGCG AAAATCCCAA GAAG CCCAG
AATCTCGATC ACATGCACAT CCAAAATTATG AACTGTTAAG TAAATAATGG ATACACCTTC
ATTATTCGAT CCGTCAAGCA TATGACTGAT GGAACCTCCC AAACAAATGT ATGTTTTATG
CCACCAATGT GAAAACCTCC GAAGATGCTG GCGACAGATG CCTTGACAGA TGAACGATTA
CAGCATATAT TGGAAATAGGC ATCGTGACT TTTCCACATG TCACGCCCTT TAAACATGTAT
TGAGTAGGAC ATCCTGCAGT TTTATCTCGT GGAGATCTCG TGAGCAATTG CACGCGGTAC
ACTCTTAGTT ATTCCTCAATC TGTCGCTCGT GACTTGTGTA ACTTGAACAC CATCAATGTC
ATTTTGTATA TTTTCCAGTG GCAGATTCCGA AGAGCCCGTG TTGCTGGGAG ATAAAGCTGT

Figure 1b: SEQ ID NO:1:
Figure 1c: SEQ ID NO:1:

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TCGGGGTTC ATCTAAAACC CCAAAATTGC TAAATTTTTC ATACCGCCGA AGCTACTGTT
CACCACTGGA TGGCTAAGCA ATGGGATATT CGTGTGGAGG TGCCACGATT AAGGGGACA
GAATGGGATCA AGAGGGAGAT TTACGGTGTC GACTCTTATC ACAAAGGAC CACTTTCCC
GGTTGCAAC TGGAGTACAC GTGCTGCTTG TTATATAGA GATAATTTTA ATGTCTTGGG
TGTTGCTAATT ACGATTTTTT GAAATTGTGT CTCTCTTAAAT ATCAATAATT TTGCGTGGAA
TTCCGTTGGA ACACTATATTA GTTATATGTA AGAGGAATA ATCTATATG TTTCAAA
TTTTCGAT ATCAGATAAT GATATTTAA
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1980
2040
2100
2160
2220
2280

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Oct. 27, 1998
Sheet 3 of 8
5,827,518
|    | Met | Lys | Phe | Leu | Trp | Phe | Ala | Leu | Val | Ala | Val | Val | Thr | Val | Ala | Ala |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| 1  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 5  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 10 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 15 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 20 | His | Pro | Val | Val | Glu | Thr | Ser | Thr | Glu | Lys | Glu | Ala | Asp | Gly | Lys | Thr |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 25 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 30 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 35 | Ser | Pro | Gln | Cys | Glu | Pro | Gly | Cys | Ile | Gly | Asn | Tyr | Gln | Pro | Cys | Ile |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 40 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 45 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 50 | Glu | Ser | Thr | Lys | Pro | Cys | Cys | Arg | Leu | Glu | Asp | Arg | Thr | Ser | Val | Gln |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 55 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 60 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 65 | Phe | Gly | Arg | Lys | Glu | Tyr | Ile | Cys | Asp | Arg | Phe | Phe | Gly | Gly | Leu | Cys |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 70 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 75 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 80 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 85 | Ala | Pro | Leu | Asp | Val | Ile | Asn | Asn | Leu | Thr | Leu | Tyr | Lys | Glu | Leu | Ser |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 90 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 95 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 100| Ala | Gln | Leu | Asn | Glu | Thr | Asn | Leu | Ala | Glu | Leu | Ser | Asn | Leu | Tyr | Phe |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 105|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 110|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 115| Gln | Gly | Ile | Lys | His | Thr | Leu | Gly | Ile | Lys | Pro | Glu | Pro | Lys | Ile | Glu |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 120|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 125|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 130| Asp | Ala | Gly | Lys | Val | Glu | Glu | Val | Val | Lys | Gln | Ser | Thr | Asp | Asn | Met |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 135|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 140|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 145| Lys | Leu | Ser | Thr | Glu | Ala | Glu | Arg | Glu | Pro | Gly | Asp | Lys | Thr | Val | Ser |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 150|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 155|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 160|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 165| Gly | Thr | Glu | Asn | Trp | Val | Gln | Ser | Pro | Asp | Thr | Asp | Ser | Ser | Pro | Ile | Asn |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 170|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 175|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |

**Figure 2a: SEQ ID NO:3:**
Asn Lys Pro Cys Ile Glu Ser Thr Glu Ser Arg Cys Arg Leu Glu Asn
180
185
190
Arg Thr Leu Val Gln Phe Gly Arg Glu Glu Asp Ile Tyr Gly Arg Phe
195
200
205
Leu Phe Phe Ile Tyr Ala Pro Leu Ile Val Val Asn Asn Ser Thr Leu
210
215
220
Tyr Leu Glu Leu Ser Lys Gly Met Asn Glu Thr Lys Leu Ser Asn Leu
225
230
235
240
Ser Asp Trp Tyr Ile Ala Ala Ala Val Ile Pro Met Pro Glu Phe Lys
245
250
255
Pro Glu Ser Lys Ile Glu Asp Glu Arg Lys Ser Pro Glu Ala Pro Glu
260
265
270
Leu Glu Ser Gln Cys Ile Pro Asn Tyr Glu Leu Cys Val Asn Ser Lys
275
280
285
Arg Pro Cys Cys Trp Glu Asn Lys Leu Phe Ala Gly Ser Ser Lys Pro
290
295
300
Arg Asn Phe Val Cys Gly Leu His Gly Arg Ser Tyr Cys Ser Pro Phe
305
310
315
320
Asp Gly

Figure 2b: SEQ ID NO:3:
Figure 3a: SEQ ID NO:4:

```plaintext
GTCGAACTGT ATCTCTAACG ATCACAGTAG CTCAACCCAA ACTTTTCAA ATTTTTGCAA 60
AAATCTGTTT TTTGGTGCTT ATGTGTTGCG TGTTGCTGTA TAAAACATC AATTTGTAAA 120
CAATTG ATG TAC AAA TTT GTT TTG GTG AGC CTT CTG AGC TGT GTG CTG
Met Tyr Lys Phe Val Leu Val Thr Leu Leu Ser Cys Val Leu
1 5 10 335
GCC CAA GCG AAT CCG CAG GTG TCG CGC CAT GGT CCC GCT GCT GTT GTA
Ala Gln Ala Asn Pro Gln Val Ser Arg His Gly Pro Ala Ala Val Val
15 20 25 30 30
TCG GAT GCG AAT CGA AGC GTT CAT CCT CCA CCA GCT CAA AAC CAC GCC
Ser Asp Ala Asn Arg Thr Val His Pro Pro Pro Ala Gln Asn His Ala
35 40 45
GAG ATG GCA CGT TTC ATC GTT AAT CAA GCC GAC TGG GCA TCT CTG GCA
Glu Met Ala Arg Phe Ile Val Asn Gln Ala Asp Trp Ala Ser Leu Ala
50 55 60
ACA ATC AGC ACT ATA GAA AAC ATC GCT TCT TAT CCA ATT GCC AGC ATA
Thr Ile Ser Thr Ile Glu Asn Ile Ala Ser Tyr Pro Ile Ala Ser Ile
65 70 75
AAA TCA ATT AGT GAC GGA CCG GCC GGC GCG AAT GGT ACC GGA GAT CCT TAT
Lys Ser Ile Ser Asp Gly Pro Gly Gly Asn Gly Thr Gly Asp Pro Tyr
80 85 90
TTG TTT ATC TCA CCG AGG ACT TTC TCT GTT AGA GAC ATA GTT GCT GAT
Leu Phe Ile Ser Pro Arg Thr Phe Ser Gly Arg Asp Ile Val Ala Asp
95 100 105 110
```
Figure 3b: SEQ ID NO:4:
Figure 4

Figure 4 illustrates the SHV (15.2 kbp) with various restriction enzyme sites labeled. The enzyme cuts at the following sites: XbaI, Sall, XhoI, PstI, XhoI, EcoRI, Sall, XhoI, and HindIII.
VIRAL AND INSECT GENES THAT INHIBIT THE IMMUNE SYSTEM AND METHODS OF USE THEREOF

GOVERNMENT LICENSE RIGHTS

The U.S. Government has a paid-up license in this invention and the right to license others on reasonable terms as provided for by the terms of Grant No. AI-33114-03 awarded by the National Institutes of Health, Department of Human Services.

FIELD OF THE INVENTION

The invention relates to the new genes, particularly viral, endoparasitic insect and insect host genes, encoding products that specifically inhibit the insect immune response. The invention further relates to methods of expanding the host range of insect viruses and methods of biological control of plant insect pests using the genes of the invention. The invention is thus useful, for example, in the biological control of insect pests and, in particular, in the protection of crops from insect damage.

BACKGROUND OF THE INVENTION

Insects, like other animals, have effective immune systems to combat both biotic and abiotic foreign invasion. It is of interest, then, that certain insect species, the endoparasitic insects, spend a part of their life cycle inside the body of other insect hosts. Considerable effort has been expended investigating the mechanism by which these endoparasitic insects avoid the host immune system in this parasitic relationship.

Mechanisms of “immune evasion” include (1) avoidance (e.g., by not coming into prolonged contact with the immune system), (2) evading the immune system, for example, by molecular mimicry, (3) blocking the immune recognition system, (4) subversion of the host immune system, and (5) suppression of the immune system. (Vinson, UCLA Symposia on Molecular and Cellular Biology, 112: 517 (1990)). For large foreign bodies, in particular, encapsulation by the granulocytes and plasmacytocytes of the hemolymph is a common immune response.

It is currently thought that encapsulation results from a first recognition of the foreign body surface by the granulocytes, which then degranulate to release one or more chemoattractant substances that are assumed to attract additional granulocytes and plasmacytocytes. The plasmacytocytes then attach to the foreign body, flatten out and form a microtubule and microfilament matrix, ultimately enclosing the foreign body in several layers of cells. In some cases, the inner layers of plasmacytocytes melanize. Encapsulation thus serves to isolate the foreign body in the insect.

One well characterized parasitoid-host system in which there is immune system evasion is that of the endoparasitic wasp Capellois sonorenensis and its host, the tobacco budworm Heliotis virescens. In investigating how immune-suppression is regulated in this system, it became apparent that a group of wasp viruses, known generically as polydnaviruses, play a role in the suppression of the host immune system. It is believed that during oviposition, the endoparasitic insect, for example C. sonorenensis, injects not only eggs but also polydnavirus and oviposition proteins. Shortly thereafter, the host insect immune system begins to show evidence of altered activity and the endoparasitoid eggs remain free from encapsulation. The precise mechanism of this immune suppression is not, however, presently known but may involve disruption of the hemocyte cytoskeleton.

Additional factors in immune system suppression may be contained in the wasp oviposition fluid and venom. It is also known that insect venom, ovarian and viral proteins share certain epitopes and that one or more ovarian proteins transiently inhibits the immune response (Webb and Luckhart, Archives of Insect Biochemistry and Physiology, 26: 147 (1994)).

It has been shown that oviposition proteins may, at least in part, mediate the immunosuppressive effect observed in some systems. Additionally, the effect can be generated by the injection of purified polydnavirus particles and virus-like particles (VLPs). For example, VLPs (which are devoid of nucleic acids) are thought to be involved in the suppression of the immune response in Venturia canecceae. (Schmidt et al., Subcell. Biochem., 15: 91 (1989)). Certain VLP proteins may be related to a host protein, designated p42, in this system. Another report indicates interference of plasmacytocyte-dependent immune phenomena by polydnavirus-rich calyx fluid. (Davies et al., Cell and Tissue Research, 251: 467 (1988)) It is conjectured that successful parasitism may require immunosuppression of the host to a level that interferes with other cellular immune reactions in addition to encapsulation.

Host cellular factors may also be involved in the immune suppression in some cases. For example, a cellular immunosuppressive protein factor, ISP, has been isolated from the larval plasma of the armyworm Pseudaelia separata parasitized with the wasp Cotesia kariyai. The factor, which suppresses encapsulation of foreign bodies, is suggested to be a 470 kDa hexamer composed of identical 82 kDa subunits. (Hayakawa, J. Biol. Chem., 269:14536 (1994)).

Thus, depending upon the particular system studied, host, parasite and/or virus factors may be involved in the suppression of the host immune system. (Vinson, Archives of Insect Biochemistry and Physiology, 13: 1 (1990)). It is therefore believed that each of these sources may play a role in host insect immune system suppression generally, and that there may be a cooperative effect between factors which allow the immune system to be compromised sufficiently for parasitization.

The WHV1.0, WHV1.6 and WHV1.1 genes of Campeoloeis sonorenensis polydnavirus (CSPDV) have recently been cloned and sequenced. These genes are described as members of a polydnavirus “cysteine-rich” gene family. (Dib-Hajj et al., Proc. Natl. Acad. Sci. (USA) 90: 3765 (1993)). It has been conjectured that these genes may play a role in preventing the recognition of foreign objects and/or the normal response of components of the immune system. (Summers et al., Proc. Natl. Acad. Sci. (USA) 92: 29 (1995)). Indeed, the WHV1.1 gene product of the C. sonorenensis polydnavirus has been implicated in the inhibition of the cellular immune response. This 30 kDa protein is shown by indirect immunofluorescence to bind both granulocytes and plasmacytocytes and is thought to inhibit encapsulation. (Li et al., J. Virol., 68: 7482 (1994)).

As parasitoid insects eventually kill their insect hosts, the parasitoids represent a natural biological means for controlling insect pests, in particular those pests responsible for crop damage. Traditionally, such parasitoids have not provided a highly effective strategy for insect control, in large part because the host range of the parasitoids is limited.

It would therefore be advantageous to provide methods whereby the host range of parasitoid insects could be
broadened, such that the parasitoids would provide effective biological control for a larger number of insect hosts.

It would be a further advantage to identify specific virus, parasitoid and/or host genes involved in the successful suppression of the insect immune system by the endoparasitoid. Such genes could then be used, through recombinant DNA techniques, to generate genetically modified insects, viruses and/or plants, that express one or more immune suppressing factors.

The invention provides these and other advantages, as will be apparent to those skilled in the art based on the disclosure hereunder.

SUMMARY OF THE INVENTION

The invention first provides a DNA useful for the suppression of an insect host immune system. In particular, the invention provides the VHv1.4 genomic DNA, derived from the C. sonorensis polydnavirus, and the sequence of which is set forth in SEQ ID NO: 1. The invention further provides a VHv1.4 cDNA (SEQ ID NO: 2), which encodes the VHv1.4 protein product (SEQ ID NO: 3) involved in insect immune system suppression. The invention also provides the SOPS cDNA (SEQ ID NO: 4) of Campeolites sonorensis and the protein encoded thereby (SEQ ID NO: 5), also useful in suppressing the insect immune system of the methods of the invention. Each of these DNAs and protein is useful for the expansion of viral host range.

The invention further provides methods for expanding parasitoid insect host range comprising:

- providing one or more DNAs encoding an insect immune suppressing factor, or a biologically active fragment thereof, operably linked to one or more expression signals,
- inserting said DNA into the genome of an endoparasitic insect virus, an endoparasitoid or a plant,
- and expressing said DNA to provide for immune suppression of one or more insect hosts,

wherein the insect hosts are not a natural host for said endoparasitic insect.

The invention additionally provides for genetically modified viruses, particularly polydnaviruses, endoparasitoid insects and/or plants capable of expressing a DNA encoding an immunosuppressive protein or polypeptide.

The invention also provides plasmids, vectors and, especially, expression vectors operably linked to the DNA of the invention.

The invention yet further provides a recombinant protein encoded by a DNA, or biologically active fragments thereof, wherein said protein or fragment suppresses the immune system of one or more insect hosts, as well as methods of broadening the host range of insect viruses and parasitoids comprising applying the protein or fragment to plants, whereby said protein is ingested by said pests.

The invention further provides methods of protecting crops, particularly commercially important crops, from damage by one or more insect pests.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the genomic DNA sequence of the C. sonorensis polydnavirus VHv1.4 DNA (SEQ ID NO: 1).

FIG. 2 depicts the predicted amino acid sequence (SEQ ID NO: 3) encoded by the VHv1.4 cDNA sequence (SEQ ID NO: 2).

FIG. 3 depicts the C. sonorensis SOPS cDNA SEQ ID NO: 4 and the protein product (SEQ ID NO: 5) encoded thereby.

FIG. 4 schematically depicts the plasmid pSH V.

DETAILED DESCRIPTION OF THE INVENTION

Genes that disrupt the insect immune system are of practical importance in the area of biological pest control. Specifically, the insect immune system is thought to determine the host range of the group of insect viruses known as baculoviruses. Additionally, virus host range is a major factor in determining whether a particular virus will be of commercial importance. Genes that suppress the immune system and thereby expand the virus host range would significantly improve the commercial prospects and performance of insect viruses as a biological pest control means. Immune suppressive genes under the invention provide for methods in which viruses, for example baculoviruses, can be used for expression, with the concomitant immune suppression and expansion of the virus host range.

The endoparasitic wasp Campeolites sonorensis injects a polydnavirus into its host Heliotis virescens during oviposition. Viral gene expression protects the wasp egg and larva from encapsulation by host hemocytes. The invention relates to the isolation and purification of genes involved in escaping the host immune response. As exemplary of the invention, the VHv1.4 genomic and cDNA have been isolated from C. sonorensis polydnavirus. As shown below, the VHv1.4 protein is involved in suppressing the immune system of the host insect. This protein is further capable, by means of its immune system targeting function, to broaden the host range of the endoparasite and thereby provide an efficient means of pest control.

The current data indicates that the binding of immune-suppressing proteins to granulocytes and plasmacytocyte surfaces is involved in immune suppression and disruption of the hemocyte cytoskeleton. The binding of hemocytes has been shown to occur in permissive and semi-permissive hosts. Moreover, the binding additionally occurs in some, but not all, non-permissive hosts. These results suggest that the host range of endoparasitic insects is related to the effectiveness by which these insects suppress the immune system of the potential host.

EXPERIMENTAL

A. Isolation of the VHv1.4 cDNA

Insect rearing and viral DNA purification from calyx fluid were done as described (Krell et al., J. Virol. 43: 859 (1982)). For RNA analysis requiring parasitized H. virescens, 15 to 20 third-instar larvae were parasitized by 8 to 10 female wasps within about 30 minutes. At the end of this period, larvae were designated as 0 hr p.p (post parasitization).

A lambda gtl1 cDNA library was constructed from mRNA of parasitized H. virescens and screened by colony hybridization using the VHv1.4 cDNA as a probe (Sambrook et al. 1989). Positive hybridization plaque DNA was amplified by polymerase chain reaction (PCR) using lambda gtl1 forward (5'-GGTGGCAGCCGACTCTGGGAGC-3') (SEQ ID NO: 6) and reverse (5'-GACACCAAAGTGTGAATG-3') (SEQ ID NO: 7) primers (Tung et al. 1989). Amplification reactions were carried out in 1x PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl), with 50 μM each dNTPs, 1.25 mM MgCl₂, 0.5 μg of each of the primers, 5 μL phase suspension and 2.5 units of Taq DNA polymerase. Phase DNA was denatured at 94° C. for 2 min. and 35 amplification cycles were performed (94° C., 2 min., 55° C., 2 min., 72° C., 3 min.) in a Model 480 DNA thermocycler (Perkin Elmer Cetus). The amplified DNA fragments were digested with EcoRI and
cloned in “BLUESCRIPT II KS(−) (Stratagene) for sequence analysis.

To clone the 3′ end of VHv1.4 cDNA, 0.5 μg of total RNA from parasitized H. virescens larvae at 24 hr. p.p. was reverse transcribed using oligo(dT) primer (GCAGT(T)AACTG(−)) (SEQ ID NO: 8). Reverse transcription was performed at 42°C for 30 min. in 20 μL of reaction mix containing 1x cDNA synthesis buffer (50 mM Tris-Cl, pH 8.9, 20 mM KCl, 2.5 mM MgCl2, 200 μM of each dNTP, 10 mM DTT, 0.2 μg primer and 200 units MMLV reverse transcriptase (Promega)). The reaction mixture was digested with 2 units of RNase H at 32°C for 10 min. One microliter of the reverse transcription mixture was removed for PCR with oligo(dT) and 1.4 kb cDNA-specific primers, using 35 cycles of 94°C, 1 min., 48°C, 1 min., 72°C, 2 min. The PCR product was cloned in the pCR-TRAP vector (GeneHunter) for sequence analysis.

B. Isolation of the VHv1.4 Genomic DNA

The viral genomic copy of the VHv1.4 gene was cloned from a 12.9 kb EcoRI fragment of SH V, contained in pVE12.9 cloned in pBS (Dib-Hajj et al. 1993). This clone was used to screen a CsPDV PstI library in “BLUESCRIPT II KS(−) to select an overlapping 7.9 kb clone (pVP7.9) that hybridized to both termini of pVE12.9. Another CsPDV Sau3A library made from partially digested viral DNA was probed with the 0.8 kb fragment of the 1.4 kb cDNA. Clones hybridizing to the probe were re-screened by PCR with 1.4 kb-specific primers. Amplification was performed with the GeneAmp 6000 system (Perkin Elmer Cetus) using the following protocol: 94°C, 2 min., 94°C, 30 sec., 55°C, 30 sec., 72°C, 1 min., for 35 cycles. Overlapping SH V genomic clones were mapped with restriction enzymes.

E. DNA Sequencing and Analysis

DNA sequences were determined by the dideoxy chain termination method (Sanger et al. 1977) using “SEQUENASE” 2.0 kit (United States Biochemical). Sequence data were analyzed using the University of Wisconsin Genetics Computer Group DNA analysis software for the VAX computer (release 7.2).

The predicted amino acid sequence of the longest ORF of the VHv1.4 cDNA insert is given (SEQ ID NO: 3). The cDNA sequence is 1338 bp long, and the longest ORF identified is 966 nt from nucleotide 57 (relative to the 5′ end of the cDNA clone) to nucleotide 1022. The sequence surrounding the first methionine codon in the cDNA is consistent with the translation initiation consensus sequence (Kozak 1983). A putative polyadenylation signal is located 15 nt upstream from the poly(A) tail.

The longest ORF in the 1.4 kb cDNA encodes a protein of 322 amino acids with a predicted molecular mass of 42 kDa. The N-terminal amino acid sequence is very hydrophobic and encodes a signal peptide according to the rules of von Heijne, indicating that this protein is destined either for insertion into the membrane or secretion. There are six potential N-glycosylation sites in the protein and, similar to the VHv1.1 cDNA, there are two complete cysteine motifs (amino acids 40 to 80 and 277 to 317).

The genomic clone was determined by Southern hybridization to reside on a 2.7 kb XhoI genomic fragment. This 2.7 kb fragment was sequenced, confirming that the cDNA is encoded by this genomic DNA fragment. A putative TATA box is located in the genomic DNA 42 bp upstream of the 5′ end of the cDNA clone. Four introns 124, 186, 187 and 342 bp in length were identified in the genomic DNA. Splicing signals are consistent with the consensus for eukaryotic genes. Intron 1 is found in the 5′ leader region, 27 bp upstream of the translation initiation ATG. The three other introns lie within the coding sequence.

F. Inhibition of the Immune Response by the VHv1.4 Gene Product

To demonstrate that the VHv1.4 gene product inhibits host immune response, the cDNA and genomic clone are inserted into bacterial expression vectors operably linked to transcription control signals. A suitable vector for such expression is PET22b(+), which allows the fusion with a 6x histidine tag to facilitate purification. Such construction is suitably made by attachment of, for example, EcoRI linkers to the VHv1.4 DNA and insertion into EcoRI site of the vector by standard procedures. The expressed product from the vector is thus a fusion that facilitates purification.

After transformation of a suitable bacterial host, (e.g. E. coli), expression of the fusion protein is induced with IPTG and purified from the bacterial lysates. The fusion protein can be engineered, where desired, to contain a unique protease cleavage site at the fusion junction. For example, a Factor Xa cleavage site may be used, allowing isolation of intact or nearly intact VHv1.4 protein. As is known to the skilled artisan, this purified protein can also be used for immunization to raise antibodies against one or more anti- genic determinants.

The VHv1.4 cDNA or genomic DNA may also suitably be expressed in a baculovirus system. The recombinant DNA is cloned into a suitable vector, for example phiL1393, and cotransfected with the E2 strain of Autographa californica nuclear polyhedrosis virus into Spodoptera frugiperda (SF9) cells to produce a recombinant virus. This virus may then be assayed according to standard procedures (Webb et al. 1990).

To demonstrate the immunosuppressive function of the VHv1.4 protein, washed eggs are prepared from 20 chilid C. sonorensis female wasps. The eggs are suitably dissected from wasp ovaries about 5 days after mating. Eggs are suspended in 1 mL of Pringle’s saline and collected by centrifugation (1500g, 7 min.). Eggs are then resuspended with Pringle’s saline and pelleted about five times to remove CsPDV and ovarian proteins. Recombinant virus (10⁴ PFU) in a volume of about 1 μL is injected into chilled fourth-instar H. virescens larvae with a 10 μL Hamilton microsyringe. The E2 stain of wild-type virus or a saline solution can be injected into additional larval as controls to which the activity of the recombinant virus can be compared. Naturally parasitized insects may also be used as a control.

Twenty four hours post injection, pretreated larvae are injected with washed wasp eggs (8 to 12 eggs/larva) with a finely drawn glass capillary. The encapsulation response to the eggs is then determined. In the absence of virus and ovarian proteins, a strong encapsulation response to parasite eggs is seen at about 24 hours post injection. If, at 24 hours post parasitization, one or more of the eggs had 100 or more adherent hemocytes, covering at least one third of the egg, then the host is scored as immunoresponsive. If fewer than 100 hemocytes are adhered on all the eggs recovered, then the insect is scored as immunosuppressed. These data can then be analyzed by chi-square statistical analysis.

The above-described experiment demonstrates that the VHv1.4 protein inhibits the host immune response. Thus, vectors expressing the VHv1.4 protein, or biologically active fragments thereof, and other toxic proteins may be useful in biological pest control. For example, inclusion in the vector of an expression cassette for scorpion toxin and/or other toxins, coupled with the immune suppression of the pest, allows for a faster and more efficient kill of the pests.
It is thus another embodiment of the invention to have a vector, preferably a baculovirus vector, containing a DNA which encodes an immune-suppressing protein or fragment thereof, preferably the VHv1.4 CDNA, genomic DNA, or a biologically active fragment thereof, and one or more genes encoding a polypeptide or protein possessing a toxic activity. A suitable vector for use in this embodiment of the invention is the above-mentioned pVL1393 baculovirus vector. Expression of the immune-suppressing sequences in a recombinant virus, a parasitoid, a host or a plant is used to affect biological control of the insect pest.

In yet another embodiment of the invention, the VHv1.4 cDNA, genomic DNA or biologically active fragment thereof, is introduced into plants to create transgenic plant varieties. Such plants, when producing the VHv1.4 protein or biologically active fragment thereof, become resistant to insect pests. According to this embodiment of the invention, a transgenic plant capable of expressing an immune suppressing protein or polypeptide, is made by any of the known techniques using the DNA of the invention. Insect larva feeding on such transgenic plants become immune suppressed and thus susceptible to a large variety of diseases.

A further embodiment of the invention is directed to use of the VHv1.4 protein, or a biologically active fragment thereof, for direct application onto plants. In this embodiment, the VHv1.4 protein is overexpressed, for example, in bacteria, yeast, plants, insect cells, etc., isolated and purified. In one preferred embodiment, the protein or fragment thereof is produced as a fusion with an amino acid sequence that assists in purification. Preferably, a polyl-histidine linker is used for a N- or C-terminal fusion product, thereby allowing rapid isolation and purification of the fusion protein. Most preferably, the polyl-histidine linker comprises about 7 contiguous histidine residues and may be removed by endoproteolytic enzymatic cleavage.

The recombinant protein so produced may be conveniently lyophilized to increase storage life or, as one alternative, may be kept in a buffered solution, for example, phosphate buffered saline. The product so produced is then applied to plants, preferably after reconstitution of the lyophilized product in water or buffered saline. In practice, insect larva ingest the recombinant protein and become immune suppressed, thereafter being susceptible to lethal infections.

As examples of other genes useful in the practice of the invention, mention is made to the C. sonorensis OPs 33 genes, in particular the SoPs gene (SEQ ID NO: 4), and the C. sonorensis polydnavirus WHv1.0, WHv1.6, VHv1.1 and 2.6 kb RNA genes, as well as functionally related genes from virus, endoparasitoids and hosts. Following the disclosure herein, other genes related to the above-mentioned genes can be isolated by, for example, library hybridization, PCR and reverse transcription technologies. Such genes are therefore meant to be embraced within the scope of the present invention.

The invention further is useful for increasing the host range of endoparasitic pests. In this embodiment, a recombinantly engineered virus, preferably a baculovirus, is constructed to express an immune suppressing protein, preferably VHv1.4 protein, or a biologically active fragment thereof. A variety of endoparasitic pests are then produced which are capable of expressing the recombinant virus. Upon oviposition, the immune suppressing protein (e.g., VHv1.4 protein) is expressed, leading to the suppressing of the host immune system. As above, such a recombinant virus may also encode one or more toxic substances, to increase the speed and efficacy of the insect kill.

In these and other embodiments of the invention, the parasitoid host range is broadened to include non-natural host insects. By “non-natural” it is meant that the host insect is not the naturally-occurring host for the particular endoparasitoid insect.

Additionally, vectors under the invention may also encode a marker for the rapid identification of recombinant virus, endoparasitic, host or plant. Such a marker may provide a visible result (e.g., β-galactosidase, luciferase, etc.) or may be either a positive or a negative selectable marker.

The invention is exemplified by the VHv1.4 gene of the C. sonorensis/H. virescens parasitic system. It is by no means meant, however, to be restricted to this system. For example, there are tens of thousands or insect species, many if not all of which are expected to contain viruses that function analogously to the C. sonorensis polydnavirus in suppressing the host immune response. Moreover, certain toxins and oviduct proteins are also believed to have immune suppressing function. Each of these immune suppressing proteins, which may be related by a common cysteine motif (Dib-Hajj et al., Proc. Natl. Acad. Sci. (USA) 90: 3765 (1993)), are within the scope of the invention.

Representative insect species that may be biologically controlled by the process of the invention include: Autographica californica, Heliothis virescens, Heliothis zea, Spodoptera frugiperda, Peridroma saucia, Prodenia eridonia, Prodenia ornithogalli, Pseudaleuthera unipuncta, Spodoptera exigua, Trichoplusia ni, Agrotis ipsilon, Estigmena acrea, Malacosoma plumulut, Nomophila noctuella, Pieris rapae, Prodenia pratetia, Crambidea picta, Dargida procnicta, Felidia sp., Gra- pholithya molesta, Heliothis armigera, Heliothis assulta, Hymenopterus, Lacinipolia stricta, Miselis sp, Morrisonia confusa, Neoleucania sp., Neouris sp., Plutia sp., Ostrinia nubilalis, Vanessa atalanta and the like.

Similarly, representative endoparasitoids which may be genetically modified or which may carry genetically modified virus under the invention include those of the order Hymenoptera, particularly of the families Braconidae and Ichneumonidae.

As will be appreciated by those skilled in the art, the invention provides for protection of one or more crops. Most notable as the commercially important crops protected are corn (maize), sorghum, beet, cotton, tomato, tobacco, sunflower, soybean, rapeseed, groundnuts, chick pea, safflower, beets, cabbage, broccoli and cauliflower and the like.

As previously noted, the invention as presently disclosed is exemplified by the C. sonorensis polydnavirus VHv1.4 gene and protein product, as well as biologically active fragments thereof. Such a biologically active fragment preferably contains at least one of the above-mentioned cysteine motifs. Another biologically active fragment, for example, is the protein product produced devoid of the hydrophobic N-terminal sequences and DNA encoding such a protein. Additionally, a homolog of the DNAs and proteins of the invention are within the scope of the claims appended hereto. Such a DNA homolog, for example, is one that makes use of the degeneracy of the genetic code to provide a DNA of differing sequence from that disclosed herein by that at the same time encodes the same, or substantially the same, protein product.

As discussed above, the VHv1.4 gene encodes two cysteine motifs. Based upon these motifs, one of ordinary skill
in the art can design probes to search for other members of this gene family in other viruses, insect hosts and other species (e.g. arachnids). Such genes can be screened for immune suppressing activity, for example as detailed above, and used in the methods under the invention in a fashion analogous to the use of the VHv1.4 gene exemplified herein. Such immune suppressing genes may also be used in combination under the practice of the invention, to create numerous immune suppressing products for the control of insect pests.

It is a further aspect of the invention, then, to use the immune suppressing VHv1.4 gene in combination with other genes that affect an immune-suppressing response. When used in such a combination, the host range for a given parasitoid may be even more greatly expanded. Examples of such other genes are given above and include those analogous immune-suppressing genes from other parasitoid/host systems and other species under the invention. Such combinations of genes may be encoded, for example, in a single vector, on separate vectors, or incorporated into the virus, parasitoid and/or plant genome.

The above examples are meant to be exemplary of the invention, and should not be construed as a limitation to the claims appended hereto. Moreover, the scope and spirit of the invention as defined in the claims is meant to encompass those variants thereof which are obvious to those of ordinary skill in the art in light of the disclosure contained herein.

Publications and patents cited above are each incorporated herein in their entirety by reference thereto.

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**SEQUENCE LISTING**

(1) GENERAL INFORMATION:

( i ) NUMBER OF SEQUENCES: 8

(2) INFORMATION FOR SEQ ID NO:1:

( i ) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2310 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

( i i ) MOLECULE TYPE: DNA (genomic)

( i i i ) HYPOTHETICAL: NO

( i v ) ANTI-SENSE: NO

( v ) ORIGIN: Campotetis sonorensis virus

( v i ) IMMEDIATE SOURCE:

(A) ORGANISM: Campoletis sonorensis virus

(B) CLONE: VHv1.4

( x ) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTATCGCGA TACAATTTCC AGCTAATTTA TCGTTAGGTC GTCGCCGAGA GCTCAACG

GAC GCCAGCAGT ACTGGGGTGC TACATGATA AAAACACAGT CCCCCTCCCA ACAAATCAGA

GCTATTTGA TCGGGGACT TATGGAGAGA ATATTAAAAC CCAATGACTG CAGAATGGC

GCCATTTCAT ATTTAATAC AATGTATTAT TTTAATTTAT TCGCAGATCA TAAATCATAG

CCTACCTAG TTTGTGGTT TGCACTGATG GCATGGTGA AGCTGCTGAG

GCTATGGGAG TGTCTACTTT ATATATGGGA ATAGAATATC TATACCCATCC ATACATGTCG

AAATCTCTCTT GGAGTGCAGC CTGTCTCGAA AATCTTACGG AGTAATGCTG ATGTGAAGAG

CTGCCCCCATG GACATGCTG TATGATCAGA CTGATGCTG AGCGAATGGA

TATAAGGCTA ACGCTGGGAA TCAAGCGAGA ACCCAAGGTA GAAGACGGCG GAAAAGTGC
| GGG AAA ACT TCG | CCC CAA TGC GAG CCA GGG TGC ATC GGC AAT TAC CAA |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gly Lys Thr Ser Pro Glu Cys Glu Pro Gly Cys Ile Gly Asn Tyr Glu | 30 | 35 | 40 | 45 |
| CTT TGC ATT GAG | TCG ACG AAG CCC TGC TGC CGA CTG GAA GAT CGC ACA |
| Pro Cys Ile Glu Ser Thr Lys Pro Cys Arg Leu Glu Asp Arg Thr | 50 | 55 | 60 |
| TCG GTGCAA TTT GGA CGT AAA GAG TAC ATC TGT GAT CGA TGC TGC GCC |
| Ser Val Glu Phe Gly Arg Lys Thr Ile Cys Asp Arg Phe Gly | 65 | 70 | 75 |
| GGA CTC TGT GCC CCA TTA GAC GTT ATA AAC AAC CTG ACA CTG TAT AAA |
| Gly Leu Cys Ala Pro Leu Asp Val Ile Asn Asn Leu Thr Leu Tyr Lys | 85 | 90 |
| GAA TTG AGT GCA CAA TTT AAC GAA ACT AAT TTT GCG GAA CTG TCC AAT |
| Glu Leu Ser Ala Glu Leu Asn Glu Thr Asn Leu Ala Glu Ser Asn | 95 | 100 | 105 |
| CTG TAT TTC CAA GGT | ATA AAG CAC ACG CTG GGA ATC AAC GCA GAA CCC |
| Leu Tyr Phe Glu Ile Glu His Thr Gly Ile Gly Pro Glu Pro | 110 | 115 | 120 | 125 |
| AAG ATA GAA GAC GCG GGA AAA GTC GAG GAA GTG TGT AAA CAG AGT AGC |
| Lys Ile Glu Asp Ala Gly Lys Val Glu Val Val Lys Glu Ser Thr | 130 | 135 | 140 |
| GAC AAC AAGG AAA TGG AGT ACC GAA GCC GAA CGT GAA CCT GGA GAC AAG |
| Asp Asn Met Lys Thr Val Glu Arg Gly Asp Gly | 145 | 150 | 155 |
| ACA GTA TCC GGA ACA GGA AAC TGG GTA CAA TCC CCA GAC AGC GAT TCG |
| Thr Val Ser Gly Thr Glu Asn Trp Val Glu Ser Pro Asp Thr Asp Ser | 160 | 165 | 170 |
| CCT ATT AAC AAA CAA CCC TCG ATT GAG TCG ACG GAG TCC CGC TGC CGA |
| Pro Ile Asn Asn Lys Pro Cys Ile Asn Ser Arg Cys Arg | 175 | 180 | 185 |
| CTT GAA AAT CGC ACA TTT GTG CAA TTT GGA CGT GAA GAG GAC ATC TAT |
| Leu Glu Asn Arg Thr Leu Val Glu Phe Gly Arg Glu Asp Ile Tyr | 190 | 195 | 200 | 205 |
| GGT CGA TTC TTT TTC GCT TTT ATC TAT GCT CCA AAT GCA TTC ACG AAT |
| Gly Arg Phe Leu Phe Ile Tyr Ala Pro Leu Ile Val Asn Asp | 210 | 215 | 220 |
| TCC ACA CTG TAT TTA GAA TTT AGT AAA GGA ATG AAC GAA ACT AAA TGT |
| Ser Thr Leu Tyr Leu Leu Ser Lys Met Asn Glu Thr Lys Leu | 225 | 230 | 235 |
| TCG AAT CTC AGC GAT TGG TAT ATA GCA GCA GCT ATC CCC ATG CCG |
| Ser Asn Leu Ser Asp Trp Tyr Ile Ala Ala Val Ile Pro Met Pro | 240 | 245 | 250 |
| GAA TTT AAG CCA GAA TCC AAG ATG GAA GAT GAG CGA AAA TCC CCA GAA |
| Glu Phe Lys Pro Glu Asp Glu Arg Lys Ser Pro Glu | 255 | 260 | 265 |
| GCC CCA GAA CTC GAG TCA CAG TGC ATC CCA AAT TAT GAA CTG TGC GTG |
| Ala Pro Glu Leu Glu Ser Glu Cys Ile Pro Asn Tyr Glu Leu Cys Val | 270 | 275 | 280 | 285 |
| AAT TCG AAG CCG TGT TGC TGG GAG AAT CTG TCC GCT GGT TCA |
| Asn Ser Lys Arg Pro Cys Cys Trp Glu Asa Lys Leu Phe Ala Gly Ser | 290 | 295 | 300 |
| TCT AAA CCC CGT ATT TCC GTG CTG CTA GAC CCC GCA AGC TAC TGT |
| Ser Lys Pro Arg Asn Phe Val Cys Gly Leu His Gly Ser Tyr Cys | 305 | 310 | 315 |
| TCA CCC TTC GAT GGC TAAACCATG GATATTGCG GGAAGGTCG AAGTTAAGG |
| Ser Pro Phe Asp Gly | 320 |
| GAAACAAAGT CGATCAAGAAA GGAGGTTCAC GCTGTCGACT TTTCATCAAC AAGGACCACT |
| 1255 |
| TTTTCCGTT TTTTCACTGGA GCTAGAGGTTG GTCCCTGGTTTT TATACGAATA TTTTTATAATG |
| 1315 |
(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 322 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:3:

His Pro Val Val Glu Thr Ser Thr Glu Lys Glu Ala Asp Gly Lys Thr 20 30
Ser Pro Gin Cys Glu Pro Gly Cys Ile Gly Asn Tyr Glu Pro Cys Ile 35 45
Glu Ser Thr Lys Pro Cys Cys Arg Leu Glu Asp Arg Thr Ser Val Glu 50 60
Phe Gly Arg Lys Glu Tyr Ile Cys Asp Arg Phe Phe Gly Gly Leu Cys 65 80
Ala Pro Leu Asp Val Ile Asn Asn Leu Thr Leu Tyr Lys Glu Leu Ser 85 95
Ala Gin Leu Asn Glu Thr Asn Leu Ala Glu Leu Ser Asn Leu Tyr Phe 105 110
Gin Gly Ile Lys His Thr Leu Gly Ile Lys Pro Glu Pro Lys Ile Gin 120 125
Asp Ala Gly Lys Val Glu Val Val Lys Gin Ser Thr Asp Asn Met 130 140
Lys Leu Ser Thr Gin Ala Glu Arg Gin Pro Gly Asp Lys Thr Val Ser 145 160
Gly Thr Glu Asn Try Val Gin Ser Pro Asp Thr Asp Ser Pro Ile Asn 165 175
Asn Lys Pro Cys Ile Gin Ser Thr Glu Ser Arg Cys Arg Leu Glu Asa 180 190
Arg Thr Leu Val Gin Phe Gly Arg Glu Asp Ile Tyr Gly Arg Phe 195 205
Leu Phe Phe Ile Tyr Ala Pro Leu Ile Val Val Asn Ser Thr Leu 210 220
Tyr Leu Gin Leu Ser Lys Gly Met Asa Gin Thr Lys Leu Ser Asn Leu 225 240
Ser Asp Tryr Ile Ala Ala Ala Ile Gin Pro Met Pro Gin Phe Lys 245 255
Pro Gin Ser Lys Ile Gin Asp Glu Arg Lys Ser Pro Gin Ala Pro Glu 260 270
Leu Ger Gin Cys Ile Pro Asn Tyr Gin Leu Cys Val Asn Ser Lys 275 285
Arg Pro Cys Cys Tryr Gin Asa Lys Leu Phe Ala Gin Ser Ser Gin Pro 290 300
Arg Asa Phe Val Gin Leu His Gly Arg Ser Tyr Cys Ser Pro Phe 305 315
Asp Gly
(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 882 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) ORIGINAL SOURCE:
(A) ORGANISM: Camperellis sonorensis virus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 127,819

(x) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GTGCAACCTG ATCTCTACACG ATCAACATG TCTCAACCCAA ACTTTTTCAA ATTTTGCGAA
AAATCTGTCT TTGGTGGTCT ATGGTGTCGG TTGGTTGCTAA TAAAAACATC AATTGATAAA

CAATTG ATG TAC AAA TTT GTT TTG GTG AGC CTT GTG AGC TGG GTG CTG
Met Tyr Lys Phe Val Leu Val Thr Leu Leu Ser Cys Val Leu
15 5 10 335

GCC CAA GCC AAT CCG CAG GTG TCG GCC CAT GGT CCC GCT GCT GTT GTA
Ala Gln Ala Asn Pro Gln Val Ser Arg His Gly Pro Ala Ala Val Val
20 25 30

TCG GAT GCC AAT CGA AGC GTT CAT CCT CCA CCA GCT CAA AAC CAC GCC
Ser Asp Ala Asn Arg Thr Val His Pro Pro Pro Ala Ala His Ala
35 40 45

GAG ATG GCA CGT TAC ATC GTT AAT CAA GCC GAC TGG GCA TCT CTG GCA
Glu Met Ala Arg Phe Ile Val Asn Pro Gly Asp Ala Arg Ile Ser Leu Ala
50 55 60

ACA ATC AGC ACT ATA GAA AAC ATC GCT TCT TAT CCA ATT GCC AGC ATA
Thr Ile Ser Thr Ile Gln Asn Ile Ala Ser Tyr Pro Ile Ala Ser Ile
70 75

AAA TCA ATT AGT GAC GGA CCG GCC GGC GCC AAT GGT ACC GGA GAT CCT TAT
Lys Ser Ile Ala Arg Pro Pro Gly Gly Asp Leu Pro Gly Asp Pro Tyr
80 85 90

TTG TTT ATC TCA CCG AGT ACT TCT TCT GTT GAG CAT ATA GAT GTT GAT
Leu Phe Ile Ser Pro Arg Thr Phe Ser Gly Arg Asp Ile Val Ala Asp
95 100 105 110

TCG GCA GCG AGT TTC ATC TTC TCC GGT GCT CCC TAC TGC AAG
Ser Arg Ala Ser Leu Ser Leu Ala Gln Gly Asp Ser Leu
115 120 125

GAA AAT AAT TAT GAT CCA ATG GAC CCG CGA TGG GGA ATC CAG CCT CCG AAT ACC GCA
Glu Asn Asn Tyr Asp Pro Met Asp Pro Arg Cys Gly Arg Val Ile
130 135

ACC GGG CCG AGC CGA AAA AAT TGG GGA ATC CAG CCT CCG AAT ACC GCA
Thr Gly Pro Ser Arg Asp Tyr Asp Tyr Asp Ile Gln Pro Thr Ala
145 150 155

AGA GCC AGG ACT TCC TCC GTG GAT CAT CCC GCG ATG GCC TAT
Arg Ala Arg Thr Ala Phe Phe Gly Arg His Pro Ala Met Xaa Tyr Met
160 165 170

CCT AGA GAT CAT GGT TTC TTC GGC AAA ATA AAC ATT GAA AAT CTT
Pro Arg Asp His Phe Tyr Phe Ala Lys Ile Asn Ile Gln Asn Leu
175 180 185 190

CTG GCT CTT GCA TCA TTT GGT CCA TTC CAC GTG GTC TCC GCT CAA GAT
Arg Val Leu Ala Ser Phe Gly Pro Phe His Val Val Ser Ala Gln Asp
195 200 205
```
(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 231 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Tyr Lys Phe Val Leu Val Thr Leu Leu Ser Cys Val Leu Ala Gln 1 5 10 15
Ala Asn Pro Gin Val Ser Arg His Gly Pro Ala Ala Val Val Ser Asp 20 25 30
Ala Asn Arg Thr Val His Pro Pro Pro Ala Gin Asn His Ala Gln Met 40 45
Ala Arg Phe Ile Val Asn Gin Ala Asp Trp Ala Ser Leu Ala Thr Ile 50 55 60
Ser Thr Ile Glu Asa Ile Ala Ser Tyr Pro Ile Ala Ser Ile Lys Ser 65 70 75 80
Ile Ser Asp Gly Pro Gly Gly Asn Gly Thr Gly Asp Pro Tyr Leu Phe 85 90 95
Ile Ser Pro Arg Thr Phe Ser Gly Arg Asp Ile Val Ala Asp Ser Arg 100 105 110
Ala Ser Leu Val Ile Ser Leu Ala Gln Gly Ala Tyr Cys Lys Gln Asa 115 120 125
Asn Tyr Asp Pro Met Asp Pro Arg Cys Gly Arg Val Val Ile Thr Gly 130 135 140
Pro Ser Arg Lys Asa Trp Gly Ile Gin Pro Pro Asa Thr Ala Arg Ala 145 150 155 160
Arg Thr Ala Phe Phe Gly Arg His Pro Ala Met Xaa Tyr Met Pro Arg 165 170 175
Asp His Gly Phe Tyr Phe Ala Lys Ile Asa Ile Gin Asa Leu Arg Val 180 185 190
Leu Ala Ser Phe Gly Pro Phe His Val Val Ser Ala Glu Asp Tyr Tyr 195 200 205
Ser Ala Ser Val Gly Gln Arg Gin Asp Xaa Met Tyr Ser Leu Tyr Thr 210 215 220
Ser Val Gin Ile Ala Leu Arg 225 230

(2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

PRIIVEER'7
We claim:
1. A recombinant DNA comprising SEQ ID NO: 1 or a biologically active fragment or homolog thereof, wherein the encoded product suppresses the immune system of one or more insect species.
2. The recombinant DNA according to claim 1, wherein said DNA is isolated from the C. sonorensis polydnavirus.
3. A method of inhibiting the immune response of a host insect comprising:
   providing a bacterial expression vector or recombinant baculovirus containing the DNA according to claim 1 operably linked to one or more expression signals; and introducing the bacterial expression vector or recombinant baculovirus and one or more endoparasitoid insect eggs into said host insect, thereby said DNA is expressed thereby inhibiting the immune response of said host insect.
4. A vector comprising the recombinant DNA according to claim 1.
5. A recombinant DNA encoding SEQ ID NO: 3 or a biologically active fragment thereof, wherein the encoded product suppresses the immune system of one or more insect species.
6. The recombinant DNA according to claim 5, wherein said DNA is isolated from the C. sonorensis polydnavirus.
7. A method of inhibiting the immune response of a host insect comprising:
   providing a bacterial expression vector or recombinant baculovirus containing the DNA according to claim 5 operably linked to one or more expression signals; and introducing the bacterial expression vector or recombinant virus baculovirus and one or more endoparasitoid insect eggs into said host insect, thereby said DNA is expressed thereby inhibiting the immune response of said host insect.
8. A vector comprising the recombinant DNA according to claim 5.
9. The vector according to claim 8, further comprising at least one DNA sequence encoding a toxin.
10. The method of claim 7, wherein said DNA is isolated from the C. sonorensis polydnavirus.
11. The method of claim 7, wherein said insect eggs are from C. sonorensis.
12. The method of claim 3, wherein said DNA is isolated from the C. sonorensis polydnavirus.
13. The method of claim 3, wherein said insect eggs are from C. sonorensis.
14. The vector according to claim 4, further comprising at least one DNA sequence encoding a toxin.

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