(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 October 2012 (11.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/139004 A2

(51) International Patent Classification:
A01H 5/00 (2006.01)

(21) International Application Number:
PCT/US2012/032531

(22) International Filing Date:
6 April 2012 (06.04.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/472,865 7 April 2011 (07.04.2011) US

(71) Applicant *(for all designated States except US)*: **MONSANTO TECHNOLOGY LLC** [US/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOWEN, David J.** [US/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US). **CHAY, Catherine** [US/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US). **EVDOKIMOV, Artem** [US/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US). **SCHRODER, Megan N.** [US/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US). **SLIGHTOM, Rachael N.** [US/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US). **SUKURU, Uma R.** [IN/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US). **TAO, Nengbing** [US/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US). **WOLLACOTT, Andrew M.** [GB/US]; 800 North Lindbergh Boulevard, St. Louis, Missouri 63167 (US).

(74) Agents: **DAVIS, J. Wendy** et al.; Thompson Coburn LLP, One US Bank Plaza, St. Louis, Missouri 63101 (US).

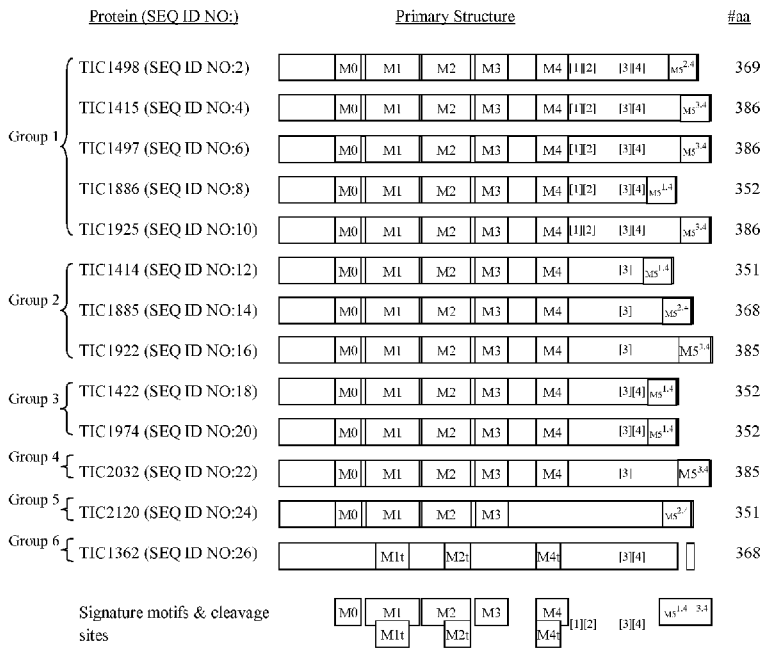
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD,

[Continued on next page]

(54) Title: INSECT INHIBITORY TOXIN FAMILY ACTIVE AGAINST HEMIPTERAN AND/OR LEPIDOPTERAN INSECTS

(57) Abstract: The present invention discloses a genus of insect inhibitory proteins that exhibit properties directed to controlling Lepidopteran and/or Hemipteran crop pests, methods of using such proteins, nucleotide sequences encoding such proteins, methods of detecting and isolating such proteins, and their use in agricultural systems.

FIGURE 1



WO 2012/139004 A2

SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

INSECT INHIBITORY TOXIN FAMILY ACTIVE AGAINST HEMIPTERAN AND/OR LEPIDOPTERAN INSECTS

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Application Serial No. 61/472,865 filed April 07, 2011, which is incorporated herein by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

[002] The file named "38-21_58195_A_PCT_SEQUENCE_LISTING_ST25.txt" contains the Sequence Listing that was created on April 6, 2012. This file is 136 kb (measured in MS Windows), is contemporaneously filed by electronic submission (using the United States Patent Office EFS-Web filing system), and is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[003] The present invention generally relates to the field of insect inhibitory proteins. In particular, the present invention relates to proteins exhibiting insect inhibitory activity against agriculturally relevant pests of crop plants and seeds, particularly Lepidopteran and/or Hemipteran species of insect pests.

BACKGROUND OF THE INVENTION

[004] Insect inhibitory proteins derived from *Bacillus thuringiensis* (*Bt*) are known in the art. These proteins are used to control agriculturally relevant pests of crop plants by spraying formulations containing these proteins onto plants/seeds or by expressing these proteins in plants and in seeds.

[005] Only a few *Bt* proteins have been developed for use in formulations or as transgenic traits for commercial use by farmers to control Coleopteran and Lepidopteran pest species, and no *Bt* proteins have been used for commercial control of Hemipteran pest species. Certain Hemipteran species, particularly *Lygus* bugs, are pests of cotton and alfalfa, and typically are only controlled using broad spectrum chemistries, *e.g.*, endosulfan, acephate, and oxamyl, which can persist and harm the environment. However, dependence on a limited number of these *Bt* proteins can result in occurrence of new pests resistant to these proteins, and reliance on broad-spectrum chemistries can harm the environment.

[006] Hence, there is a continuous need for the discovery and commercial development of new proteins active against pests of crop plants.

SUMMARY OF THE INVENTION

[007] The present invention provides a novel group, i.e. a new genus, of insect inhibitory polypeptides (toxin proteins) which are shown to exhibit inhibitory activity against one or more pests of crop plants. Each of the proteins can be used alone or in combination with each other and with other *Bt* proteins and toxic agents in formulations and *in planta*, thus providing alternatives to *Bt* proteins and insecticide chemistries currently in use in agricultural systems.

[008] Recombinant polypeptides are provided which exhibit insect inhibitory activity against Hemipteran and/or Lepidopteran pest species, which optionally:

(a) exhibits at least from about 47% to about 100% amino acid sequence identity, or any percentage point between 47% and 100%, to one or more of the proteins having the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24;

(b) exhibits at least from about 56% to about 100% amino acid sequence identity, or any percentage point between 56% and 100%, to one or more of the proteins having the amino acid sequence as set forth in any of SEQ ID NO:26, SEQ ID NO:136, or SEQ ID NO:138;

(c) contain in operable position within the polypeptide, at least one of each of six different motif peptide segments in consecutive order M0, M1, M2, M3, M4 and M5, each motif peptide segment exhibiting at least about 80% identity to a consensus sequence specified for the respective motif peptide segment, in which the consensus sequence for motif peptide segment M0 is set forth in SEQ ID NO:31, the consensus sequence for motif peptide segment M1 is set forth in SEQ ID NO:48, the consensus sequence for motif peptide segment M2 is set forth in SEQ ID NO:53, the consensus sequence for motif peptide segment M3 is set forth in SEQ ID NO:62, the consensus sequence for motif peptide segment M4 is set forth in SEQ ID NO:65, and the consensus sequence for motif peptide segment M5 is set forth in SEQ ID NO:139;

(d) contain in operable linkage within the polypeptide, at least one of each of three different motif peptide segments M1t, M2t, and M4t, in consecutive order, wherein each motif peptide segment exhibits at least about 80% identity to a consensus sequence specified for the respective motif peptide segment, and wherein the consensus sequence for motif peptide segment M1t is set forth at SEQ ID NO:70, the

consensus sequence for motif peptide segment M2t is set forth at SEQ ID NO:87, and the consensus sequence for motif peptide segment M4t is set forth at SEQ ID NO:120;

(e) contain an amino acid sequence exhibiting from about 195 to about 386 amino acid identities to the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:136, or SEQ ID NO:138;

(f) contain an amino acid sequence exhibiting at least from about 56 to about 100% identity to the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24;

(g) contain an amino acid sequence exhibiting at least from about 56% to about 100% identity, or any percentage point in between 56% to 100% to the amino acid sequence set forth in any of SEQ ID NO:26, SEQ ID NO:136, and SEQ ID NO:138; or

(h) are encoded by a polynucleotide segment that hybridizes under stringent hybridization conditions to one or more of the nucleotide sequences set forth in any of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:135, or SEQ ID NO:137, or the complement thereof.

[009] Insect inhibitory compositions are provided comprising the aforementioned recombinant polypeptides along with methods for controlling Lepidopteran and/or Hemipteran species using such recombinant polypeptides.

[010] Recombinant polynucleotides are provided comprising a nucleotide sequence encoding the aforementioned recombinant polypeptides. Transgenic plant cells, plants, or plant parts comprising such recombinant polynucleotides and methods of controlling a Lepidopteran and/or Hemipteran species pest using such transgenic plant cells, plants or plant parts are also provided.

[011] Processed plant products are provided that comprise a detectable amount of the recombinant polynucleotide. Such processed products include, but are not limited to, plant biomass, oil, meal, animal feed, flour, flakes, bran, lint, hulls, and processed seed.

[012] Methods of making transgenic plants are also provided. Such methods include introducing the recombinant polynucleotide into a plant cell and selecting a transgenic plant

that expresses an insect inhibitory amount of the recombinant polypeptide encoded by the recombinant polynucleotide.

[013] Other embodiments, features, and advantages of the invention will be apparent from the following detailed description, examples, and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[014] Figure 1 illustrates the primary structure of proteins exemplified in this application, showing the features and motifs characteristic for each of the proteins in this genus. Each protein is depicted by name, SEQ ID NO, structural schematic, and amino acid length (i.e., number of amino acids), and organized into groups or clusters based on amino acid identity between the various proteins. The proteins within a group generally exhibit at least about 90% amino acid identity. Generally, most proteins in the genus of novel proteins disclosed herein contain, in operable linkage within the polypeptide, at least one of each of six different signature motifs (M) or motif peptide segments, each motif being unique to this genus of proteins. The motifs are referenced herein and consecutively numbered as M0, M1, M2, M3, M4 and M5. The M0 motif is proximal to the amino terminus of each protein toxin, and the M5 motif is positioned most proximal to the carboxy terminus of each protein toxin. The M5 motif can also be present in more than one copy in each protein. Each motif contains a core amino acid segment unique to that particular motif. The presence of any of the referenced motif peptide segments in a protein derived from Bacillus thuringiensis or related species of bacilli, and the observation for such protein of toxic activity directed to one or more species of Hemiptera and/or Lepidoptera, is sufficient to provide for classification of such protein as being within the scope of the present invention, particularly if the full length of the protein amino acid sequence exhibits at least about 47% or greater identity to any of the proteins embodied herein including proteins as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24. Secondary motifs observed within each of the major motifs M0-M5 in certain of the proteins of the present invention are referenced herein as M1t, M2t, and M4t. Certain of the proteins also contain proteolytic cleavage sites KK, EH, TF, and FG, the relative positions of each being marked in applicable proteins represented in Figure 1 as [1], [2], [3], and [4], respectively (the two letters represent the two amino acid residues bracketing the cleavage site), are features of proteins within the scope of this invention.

[015] Figure 2 is a Venn diagram depicting the activity profile of the proteins of the present invention. Circle [A] represents Lepidopteran-active proteins, while Circle [B] represents Hemipteran-active proteins. Area ['c'] is an intersection of Circles [A] and [B], which represents proteins that tested positive for activity against both Lepidopteran and Hemipteran insects. Area ['a'] is [A] minus ['c'], which represents proteins that tested positive for activity against Lepidopteran insects but not positive for any activity against Hemipteran species indicated at the dose at which Lepidopteran activity was observed. Area ['b'] is [B] minus ['c'], which represents proteins that tested positive for activity against Hemipteran insects but not positive for activity against the Lepidopteran species indicated at the dose at which Hemipteran species activity was observed.

BRIEF DESCRIPTION OF THE SEQUENCES

[016] SEQ ID NO:1 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bacillus thuringiensis* (*Bt*) species having an open reading frame at nucleotide positions 1-1107 encoding a TIC1498 protein.

[017] SEQ ID NO:2 is an amino acid sequence of a TIC1498 protein toxin.

[018] SEQ ID NO:3 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1158 encoding a TIC1415 protein.

[019] SEQ ID NO:4 is an amino acid sequence of a TIC1415 protein toxin.

[020] SEQ ID NO:5 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1158 encoding a TIC1497 protein.

[021] SEQ ID NO:6 is an amino acid sequence of a TIC1497 protein toxin.

[022] SEQ ID NO:7 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1056 encoding a TIC1886 protein.

[023] SEQ ID NO:8 is an amino acid sequence of a TIC1886 protein toxin.

[024] SEQ ID NO:9 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1158 encoding a TIC1925 protein.

[025] SEQ ID NO:10 is an amino acid sequence of a TIC1925 protein toxin.

[026] SEQ ID NO:11 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1053 encoding a TIC1414 protein.

[027] SEQ ID NO:12 is an amino acid sequence of a TIC1414 protein toxin.

[028] SEQ ID NO:13 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1104 encoding a TIC1885 protein.

[029] SEQ ID NO:14 is an amino acid sequence of a TIC1885 protein toxin.

[030] SEQ ID NO:15 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1155 encoding a TIC1922 protein.

[031] SEQ ID NO:16 is an amino acid sequence of a TIC1922 protein toxin.

[032] SEQ ID NO:17 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1056 encoding a TIC1422 protein.

[033] SEQ ID NO:18 is an amino acid sequence of a TIC1422 protein toxin.

[034] SEQ ID NO:19 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1056 encoding a TIC1974 protein.

[035] SEQ ID NO:20 is an amino acid sequence of a TIC1974 protein toxin.

[036] SEQ ID NO:21 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1155 encoding a TIC2032 protein.

[037] SEQ ID NO:22 is an amino acid sequence of a TIC2032 protein toxin.

[038] SEQ ID NO:23 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1104 encoding a TIC2120 protein.

[039] SEQ ID NO:24 is an amino acid sequence of a TIC2120 protein toxin.

[040] SEQ ID NO:25 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bt* species having an open reading frame at nucleotide positions 1-1053 encoding a TIC1362 protein.

[041] SEQ ID NO:26 is an amino acid sequence of a TIC1362 protein toxin.

[042] SEQ ID NO:27 is an artificial nucleotide sequence encoding a TIC1415 protein.

[043] SEQ ID NO:28 is an artificial nucleotide sequence encoding a TC1414 protein.

[044] SEQ ID NO:29 is an artificial nucleotide sequence encoding a TIC1422 protein.

[045] SEQ ID NO:30 is an artificial nucleotide sequence encoding a TIC1362 protein.

[046] SEQ ID NO:31 is a consensus amino acid sequence for the M0 motif segment.

[047] SEQ ID NOs:32-47 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:31.

[048] SEQ ID NO:48 is a consensus amino acid sequence for the M1 motif segment.

[049] SEQ ID NOs:49-52 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:48.

[050] SEQ ID NO:53 is a consensus amino acid sequence for the M2 motif segment.

[051] SEQ ID NOs:54-61 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:53.

[052] SEQ ID NO:62 is a consensus amino acid sequence for the M3 motif segment.

[053] SEQ ID NOs:63-64 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:62.

[054] SEQ ID NO:65 is a consensus amino acid sequence for the M4 motif segment.

[055] SEQ ID NOs:66-69 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:65.

[056] SEQ ID NO:70 is a consensus amino acid sequence for the M1t motif segment.

[057] SEQ ID NOs:71-86 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:70.

[058] SEQ ID NO:87 is a consensus amino acid sequence for the M2t motif segment.

[059] SEQ ID NOs:88-119 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:87.

[060] SEQ ID NO:120 is a consensus amino acid sequence for the M4t motif segment.

[061] SEQ ID NOs:121-122 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:120.

[062] SEQ ID NO:123 is an amino acid sequence representing an insect inhibitory fragment of TIC1497 and corresponds to an amino acid translation of nucleotide positions 1 through 933 of SEQ ID NO:5.

[063] SEQ ID NO:124 is an amino acid sequence representing an insect inhibitory fragment of TIC1497 and corresponds to an amino acid translation of nucleotide positions 1 through 885 of SEQ ID NO:5.

[064] SEQ ID NO:125 is an amino acid sequence representing an insect inhibitory fragment of TIC1497 and corresponds to an amino acid translation of nucleotide positions 1 through 939 of SEQ ID NO:5.

[065] SEQ ID NO:126 is an amino acid sequence representing an insect inhibitory fragment of TIC1497 and corresponds to an amino acid translation of nucleotide positions 1 through 882 of SEQ ID NO:5.

[066] SEQ ID NO:127 is an oligonucleotide sequence in a primer for hybridizing to the (+) strand of the 5' end of DNA encoding a protein of the present invention and corresponds to positions 1..29 of SEQ ID NO:3 (tic1415 forward primer).

[067] SEQ ID NO:128 is an oligonucleotide sequence in a primer for hybridizing to the (-) strand of the 3' end of DNA encoding a protein of the present invention and corresponds to positions 1131..1161 of SEQ ID NO:3 (tic1415 reverse primer).

[068] SEQ ID NO:129 is an oligonucleotide sequence in a primer for hybridizing to the (+) strand of the 5' end of DNA encoding a protein of the present invention and corresponds to positions 1..40 of SEQ ID NO:11 (tic1414 forward primer).

[069] SEQ ID NO:130 is an oligonucleotide sequence in a primer for hybridizing to the (-) strand of the 3' end of DNA encoding a protein of the present invention and corresponds to positions 1015..1056 of SEQ ID NO:11 (tic1414 reverse primer).

[070] SEQ ID NO:131 is an oligonucleotide sequence in a primer for hybridizing to the (+) strand of the 5' end of DNA encoding a protein of the present invention and corresponds to positions 1..35 of SEQ ID NO:17 (tic1422 forward primer).

[071] SEQ ID NO:132 is an oligonucleotide sequence in a primer for hybridizing to the (-) strand of the 3' end of DNA encoding a protein of the present invention and corresponds to positions 1021-1059 of SEQ ID NO:17 (tic1422 reverse primer).

[072] SEQ ID NO:133 is an oligonucleotide sequence in a primer for hybridizing to the (+) strand of the 5' end of DNA encoding a protein of the present invention and corresponds to positions 1..28 of SEQ ID NO:25 (tic1362 forward primer).

[073] SEQ ID NO:134 is an oligonucleotide sequence in a primer for hybridizing to the (-) strand of the 3' end of DNA encoding a protein of the present invention and corresponds to positions 1025-1056 of SEQ ID NO:25 (tic1362 reverse primer).

[074] SEQ ID NO:135 is a nucleotide sequence representing a recombinant polynucleotide derived from a *Bacillus thuringiensis* (*Bt*) species having an open reading frame at nucleotide positions 1-1008 encoding a TIC2335 protein.

[075] SEQ ID NO:136 is an amino acid sequence of a TIC2335 protein toxin.

[076] SEQ ID NO:137 is a nucleotide sequence representing a polynucleotide derived from a *Bacillus thuringiensis* (*Bt*) species having an open reading frame at nucleotide positions 1-1014 encoding a TIC2334 protein.

[077] SEQ ID NO:138 is an amino acid sequence of a TIC2334 protein toxin.

[078] SEQ ID NO:139 is a consensus amino acid sequence for the M5 motif segment.

[079] SEQ ID NOs:140-141 are each individual amino acid sequences from each of the various toxin proteins disclosed herein which were used in formulating the consensus sequence as set forth in SEQ ID NO:139.

[080] SEQ ID NO:142 is an N-terminal consensus sequence shared by proteins of the present invention.

[081] SEQ ID NO:143 is a C-terminal consensus sequence shared by proteins of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[082] *Bacillus thuringiensis* (*Bt*) proteins are a rich source of diverse toxin proteins; however, many problems exist in the process of identifying new *Bt* toxins. Screening methods that involve morphological typing of *Bt* strains, (*e.g.* structural analysis of parasporal inclusion bodies, cell coat morphology, visible color, and morphology under different growing conditions), do not provide a good correlation with the presence of novel toxic proteins. Additionally, screening methods that involve highly matrixed bioassay processes for identifying proteins with toxic properties yield inconsistent results. Such processes include but may not be limited to testing proteins expressed at various stages of *Bt* growth and development, testing different *Bt* protein preparations, testing *Bt* proteins activated by various proteolytic treatments, testing *Bt* proteins with other ancillary proteins, and testing *Bt* proteins under various induction conditions. Some screening methods rely on structural and functional design, which require very labor and skill intensive procedures to elucidate structure/function relationships, and often these protocols can only be effective

when carried out on fully elucidated toxins. In view of the inherent problems in finding new *Bt* toxin proteins, screening for genes encoding *Bt* toxin proteins has changed due to recent improvements in bioinformatics and genome sequence capabilities.

[083] The inventors herein have taken advantage of high throughput sequencing and improvements in bioinformatics capabilities to screen *Bt* genomes for novel protein-encoding *Bt* toxin genes, which are then cloned and expressed in acrystalliferous Bt strains to produce protein samples for insect inhibitory activity screening. As described herein and using this method, a novel protein genus has been discovered and exemplary proteins exhibiting insecticidal activity against Hemipteran and/or Lepidopteran species. Those skilled in the art will appreciate that the teaching of the present invention enables related gene/protein members to be identified or engineered that exhibit the properties and features of the proteins of the present invention.

[084] The polypeptides/proteins of the present invention are related by source or origin (from *B.t.* strains of bacteria), by biological toxin activity against insect pests within the orders Hemiptera and/or Lepidoptera, by primary structure (conserved amino acid sequences), and by length (from about 300 to about 400 amino acids).

[085] Proteins of the present invention, and proteins that resemble the proteins of the present invention, can be identified by comparison to each other using various computer based algorithms known in the art. Amino acid identities reported herein are a result of a Clustal W alignment using these default parameters: Weight matrix: blosum, Gap opening penalty: 10.0, Gap extension penalty: 0.05, Hydrophilic gaps: On, Hydrophilic residues: GPSNDQERK, Residue-specific gap penalties: On (Thompsonet al (1994) Nucleic Acids Research, 22:4673-4680).

[086] It is intended that a recombinant polypeptide exhibiting insect inhibitory activity against a Lepidopteran and/or Hemipteran insect species is within the scope of the present invention if an alignment of the polypeptide with any of SEQ ID NO:2 (TIC1498), SEQ ID NO:4 (TIC1415), SEQ ID NO:6 (TIC1497), SEQ ID NO:8 (TIC1886), SEQ ID NO:10 (TIC1925), SEQ ID NO:12 (TIC1414), SEQ ID NO:14 (TIC1885), SEQ ID NO:16 (TIC1922), SEQ ID NO:18 (TIC1422), SEQ ID NO:20 (TIC1974), SEQ ID NO:22 (TIC2032), SEQ ID NO:24 (TIC2120), SEQ ID NO:26 (TIC1362), SEQ ID NO:136 (TIC2335), and SEQ ID NO:138 (TIC2334) results in at least 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254,

255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, or 386 amino acid identities. *See* Table 1. That is, in certain embodiments, the recombinant polypeptide of the present invention comprises an amino acid sequence exhibiting 195-386 amino acid identities when compared to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:136, and SEQ ID NO:138.

Table 1. Pair-wise matrix display of toxin proteins of the present invention.

(M)	(N)														
	2	4	6	8	10	12	14	16	18	20	22	24	26	136	138
SEQ ID NO:2 (TIC1498)		99.2	98.9	94.6	99.2	75.9	79.9	83.5	85.1	84.6	91.3	80.2	46.1	37.1	37.4
	-	366	365	349	366	280	295	308	314	312	337	296	170	137	138
SEQ ID NO:4 (TIC1415)	94.8		99.5	90.4	99.7	75.9	79.8	83.9	81.3	80.8	92.5	79.8	45.3	35.5	35.8
	366	-	384	349	385	293	308	324	314	312	357	308	175	137	138
SEQ ID NO:6 (TIC1497)	94.6	99.5		90.2	99.7	75.9	79.8	83.9	81.1	80.6	92	79.8	45.1	35.5	35.8
	365	384	-	348	385	293	308	324	313	311	355	308	174	137	138
SEQ ID NO:8 (TIC1886)	99.1	99.1	98.9		99.1	79.8	83	83.2	88.9	88.4	90.9	83.2	48	38.9	39.5
	349	349	348	-	349	281	292	293	313	311	320	293	169	137	139
SEQ ID NO:10 (TIC1925)	94.8	99.7	99.7	90.4		76.2	80.1	84.2	81.3	80.8	92.2	80.1	45.3	35.5	35.8
	366	385	385	349	-	294	309	325	314	312	356	309	175	137	138
SEQ ID NO:12 (TIC1414)	79.8	83.5	83.5	80.1	83.8		99.7	100	77.5	76.9	90.9	81.8	45	39	38.7
	280	293	293	281	294	-	350	351	272	270	319	287	158	137	136
SEQ ID NO:14 (TIC1885)	80.2	83.7	83.7	79.3	84	95.1		99.7	77.2	76.6	90.8	82.1	45.7	37.2	36.7
	295	308	308	292	309	350	-	367	284	282	334	302	168	137	135
SEQ ID NO:16 (TIC1922)	80	84.2	84.2	76.1	84.4	91.2	95.3		74	73.5	90.9	78.7	43.6	35.6	35.3
	308	324	324	293	325	351	367	-	285	283	350	303	168	137	136
SEQ ID NO:18 (TIC1422)	89.2	89.2	88.9	88.9	89.2	77.3	80.7	81		99.4	85.2	79.3	47.2	38.1	39.5
	314	314	313	313	314	272	284	285	-	350	300	279	166	134	139
SEQ ID NO:20 (TIC1974)	88.6	88.6	88.4	88.4	88.6	76.7	80.1	80.4	99.4		84.7	78.7	47.2	38.1	39.5
	312	312	311	311	312	270	282	283	350	-	298	277	166	134	139
SEQ ID NO:22 (TIC2032)	87.5	92.7	92.2	83.1	92.5	82.9	86.8	90.9	77.9	77.4		80.3	45.5	35.3	35.3
	337	357	355	320	356	319	334	350	300	298	-	309	175	136	136
SEQ ID NO:24 (TIC2120)	80.4	83.7	83.7	79.6	84	78	82.1	82.3	75.8	75.3	84		45.9	35.6	37.8
	296	308	308	293	309	287	302	303	279	277	309	-	169	131	139
SEQ ID NO:26 (TIC1362)	48.4	49.9	49.6	48.1	49.9	45	47.9	47.9	47.3	47.3	49.9	48.1		41.6	37.9
	170	175	174	169	175	158	168	168	166	166	175	169	-	146	133
SEQ ID NO:136 (TIC2335)	40.8	40.8	40.8	40.8	40.8	40.8	40.8	40.8	39.9	39.9	40.5	39	43.5	-	39
	137	137	137	137	137	137	137	137	134	134	136	131	146		131
SEQ ID NO:138 (TIC2334)	40.8	40.8	40.8	41.1	40.8	40.2	39.9	40.2	41.1	41.1	40.2	41.1	39.3	38.8	-
	138	138	138	139	138	136	135	136	139	139	136	139	133	131	

(M) is SEQ ID NO and protein name (TIC#)
(N) is SEQ ID NO.
The percent amino acid identity between all pairs is calculated relative to (M) and is represented by the upper numbers.
The lower number in each box in the matrix represents the numbers of identical amino acids between the pair.
The gray shaded boxes represent closely related toxin proteins which are more distantly related to the other proteins in the table.

[087] It is also intended that a first protein exhibiting insect inhibitory activity is within the scope of the present invention if a Clustal W alignment of such protein with any of the following second proteins set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, results in at least about 47% amino acid sequence identity between the first and the second proteins; or specifically, at least 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97,

98, 99, 99.2, 99.5, 99.8, or 100% amino acid sequence identity between the first and the second proteins; or optionally a first protein exhibiting insect inhibitory activity is within the scope of the present invention if a Clustal W alignment of such protein with any of the following second proteins set forth in any of SEQ ID NO:26, SEQ ID NO:136, or SEQ ID NO:138, results in at least about 56% amino acid sequence identity between the first and the second proteins; or specifically, at least 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.2, 99.5, 99.8, or 100% amino acid sequence identity between the first and the second proteins.

[088] Polypeptides/proteins of the present invention are observed to be related by the presence of six signature amino acid sequence motif segments known to exist only in members of this particular insect inhibitory protein family. The relative position of each of the signature motif segments is illustrated in Figure 1 as "M0", "M1", "M2", "M3", "M4", and "M5". SEQ ID NO:31 represents the M0 motif consensus sequence, in which X_1 is N or T, X_2 is D or A, X_3 is I or T, and X_4 is R or S. Each M0 motif segment is represented by the corresponding amino acid sequences set forth in SEQ ID NOs:32-47. The M0 motif segment corresponds to amino acid sequence positions 48 through 70 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and SEQ ID NO:20, positions 47 through 69 of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:22, and SEQ ID NO:24. A core amino acid sequence $QX_2FQTX_3PX_4L$ is embedded within the M0 motif segment. The presence of this core sequence (or the M0 motif segment), or of a peptide segment exhibiting at least about 80% amino acid sequence identity to this core sequence (or the M0 motif segment) in a particular toxin protein derived from *Bt*, alone or in combination with other motif segments described herein and operably positioned within the primary sequence of any such toxin protein, is determinative that the toxin protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties.

[089] SEQ ID NO:48 represents the M1 motif consensus sequence, in which X_1 is V or I, and X_2 is R or K. Each M1 motif is represented by the corresponding amino acid sequences set forth in SEQ ID NOs:49-52. The M1 motif corresponds to amino acid sequence positions 76 through 118 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and SEQ ID NO:20, and positions 75 through 117 of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:22, and SEQ ID NO:24, or any shorter segment comprising the core amino acid sequence QTX_1SFNEX_2TT of this M1 motif. The

presence of this core sequence (or the M1 motif), or of a peptide segment exhibiting at least 80% amino acid sequence identity to this core sequence (or the M1 motif), in a particular protein derived from *Bt*, alone or in combination with other motifs described herein, is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties. Certain proteins within the genus of proteins exemplified herein include the M1 motif as well as a secondary core motif segment Mt1 represented by the consensus amino acid sequence as set forth in SEQ ID NO:70, in which X_1 is V or F, X_2 is S or T, X_3 is H or T, and X_4 is V or T. Each M1t secondary core motif segment is represented by the amino acid sequences set forth in SEQ ID NOs:71-86. M1t corresponds to amino acid positions 94 through 112 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and SEQ ID NO:20, positions 93 through 111 of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:22, and SEQ ID NO:24, and positions 83 through 101 of SEQ ID NO:26. The presence of this secondary core motif M1t, or of a peptide segment exhibiting at least 80% amino acid sequence identity to this secondary core motif, in a particular protein derived from *Bt*, alone or in combination with other motifs described herein, is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties.

[090] SEQ ID NO:53 represents the M2 motif consensus sequence, in which X_1 is S or A, X_2 is V or T, and X_3 is T or S. Each M2 motif is represented by the corresponding amino acid sequences set forth in SEQ ID NOs:54-61. The M2 motif corresponds to amino acid positions 134 through 170 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and SEQ ID NO:20, and positions 133 through 169 of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:22, and SEQ ID NO:24. The presence of this M2 motif, or of a peptide segment exhibiting at least 80% amino acid sequence identity to this M2 motif, in a particular protein derived from *Bt*, alone or in combination with other motifs described herein, is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties. Certain proteins within the genus of proteins exemplified herein include as a part of the M2 motif a secondary motif M2t as set forth in SEQ ID NO:87, in which X_1 is E or A, X_2 is G or S, X_3 is V or T, X_4 is T or S, X_5 is L or I. Each M2t motif is represented by amino acid sequences set forth in SEQ ID NOs:88 – 119. M2t corresponds to amino acid positions 153 through 168 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and SEQ ID NO:20, positions 152 through 167 of

SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:22, and SEQ ID NO:24, and positions 139 through 154 of SEQ ID NO:26. The presence of this motif M2t or of a peptide segment exhibiting at least 80% amino acid sequence identity to this motif M2t in a particular protein derived from *Bt*, alone or in combination with other motifs described herein, is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties.

[091] SEQ ID NO:62 represents the M3 motif consensus sequence, in which X_1 is D or N. Each M3 motif is represented by amino acid sequences set forth in SEQ ID NOs:63-64. M3 corresponds to amino acid positions 172 through 200 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and SEQ ID NO:20, and positions 171 through 199 of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:22, and SEQ ID NO:24, or any shorter segment comprising the core amino acid sequence $AGSVX_1VPID$ of this M3 motif. The presence of this M3 motif or its core, or of a peptide segment exhibiting at least 80% amino acid sequence identity to this M3 motif or to its core sequence, alone or in combination with other motifs described herein, in a particular protein derived from *Bt* is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties.

[092] SEQ ID NO:65 represents the M4 motif consensus sequence , in which X_1 is P or T, and X_2 is D or N. Each M4 motif is represented by amino acid sequences set forth in SEQ ID NOs:66-69. M4 corresponds to amino acid positions 267 through 294 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and SEQ ID NO:20, and positions 266 through 293 of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, and SEQ ID NO:22, or any shorter segment comprising the core amino acid sequence $SLATX_1X_2QILS$ of this M4 motif. The presence of this M4 motif or its core, or of a peptide segment exhibiting at least 80% amino acid sequence identity to this M4 motif or to its core sequence, alone or in combination with other motifs described herein, in a particular protein derived from *Bt*, is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties. Certain proteins within the genus of proteins exemplified herein include as a part of the M4 motif a secondary motif M4t as set forth in SEQ ID NO:120, in which X_1 is A or T. Each M4t motif is represented by amino acid sequences SEQ ID NO:121 and SEQ ID NO:122. The M4t motif corresponds to amino acid positions 267 through 281 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:18, and

SEQ ID NO:20, positions 266 through 280 of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, and SEQ ID NO:22, and positions 254 through 268 of SEQ ID NO:26, or any shorter segment comprising the core amino acid sequence PGFTGETR. The presence of this M4t motif, or of a peptide segment exhibiting at least 80% amino acid sequence identity to this M4t motif, alone or in combination with other motifs described herein, in a particular protein derived from *Bt*, is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties.

[093] SEQ ID NO:139 represents the M5 motif consensus sequence segment, in which X_1 is C, Y or R, X2 is H or R, X3 is N, D or H, X4 is Y or H, X5 is R or G, and X6 is D or N. SEQ ID NOs:142-143 are two exemplary M5 motifs. M5 corresponds to amino acid positions 327 through 343 of SEQ ID NOs: 12, 14, 16, 22, and 24, positions 328 through 344 of SEQ ID NOs: 2, 4, 6, 8, 10, 18, and 20, positions 344 through 360 of SEQ ID NOs: 14, 16, 22, and 24, positions 345 through 361 of SEQ ID NOs: 2, 4, 6, and 10, positions 361 through 377 of SEQ ID NOs: 12, 16, and 22, positions 362 through 378 of SEQ ID NOs: 4, 6, and 10, or any shorter segment comprising the core amino acid sequence (C/Y)EHNYDE of this M5 motif. The core amino acid sequence (C/Y)EHNYDE of this M5 motif corresponds to seven of the last 8 N-terminal amino acid residues in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24. The presence of this M5 motif or its core, or of a peptide segment exhibiting at least 80% amino acid sequence identity to this M5 motif, alone or in combination with other motifs described herein, in a particular protein derived from *Bt* is determinative that the protein is a member of the genus of proteins described herein, particularly when the protein is also shown to exhibit insect inhibitory properties. The polypeptides/proteins of the present invention are related by this M5 motif which can occur once as exemplified by SEQ ID NOs: 8, 12, 18, and 20; as a double repeat as exemplified by SEQ ID NOs: 2, 14, and 24; and as a triple repeat as exemplified by SEQ ID NOs: 4, 6, 10, 12, 16, and 22. Interestingly, TIC1414 and TIC1922 can be aligned with 100% identity with reference to TIC1414 (Table 1), which is possible because the difference between TIC1414 and TIC1922 are two repeats of the M5 motif (gaps allowed in the pair-wise alignment).

[094] The present invention provides a recombinant polypeptide comprising a peptide segment exhibiting at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:31 (motif M0), SEQ ID NO:48 (motif M1), SEQ ID NO:53 (motif M2), SEQ ID NO:62 (motif M3), SEQ ID NO:65 (motif M4), SEQ ID NO:141

(motif M5), SEQ ID NO:70 (motif M1t), SEQ ID NO:87 (motif M2t), SEQ ID NO:120 (motif M4t), and any combination thereof. Such polypeptide exhibits insect inhibitory activity against Lepidopteran and/or Hemipteran species. As used herein, the term "insect inhibitory activity" refers to activity of a protein, or a fragment thereof, effective in inhibiting a pest, preferably a pest of one or more crop plants, when provided in the diet of the pest and ingested by the target (intended) pest. Pests of crop plants include nematodes and arthropods, including insects. Proteins of the present invention are effective in inhibiting the growth, development, viability or fecundity of a particular target pest, particularly an insect pest, including but not limited to insects of the orders Lepidoptera and Hemiptera.

[095] In certain embodiments, the insect inhibitory polypeptides/proteins contain amino acid segments exhibiting at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity to any one or more of the signature motifs (SEQ ID NOs:31-122, 142 and 143) of the proteins of the present invention.

[096] In certain embodiments, the recombinant polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:136, or SEQ ID NO:138, or an insect inhibitory fragment thereof. Exemplary insect inhibitory fragments include, but not limited to, those comprising the amino acid sequence as set forth in SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, and SEQ ID NO:126.

[097] Additional signature motifs include proteolytic cleavage sites "KK", "EH", "TF", and "FG", the relative positions of each shown as [1], [2], [3], and [4], respectively in Figure 1.

[098] An additional signature motif includes an N-terminal consensus sequence as set forth in SEQ ID NO:142, where X_1 is A or E, X_2 is N or D, X_3 is Q or E, and X_4 is S or L, shared by proteins that are members of the genus of proteins exemplified herein, with the exception of the protein having the amino acid sequence as set forth in SEQ ID NO:26. Forward oligonucleotide primers, *e. g.*, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, and SEQ ID NO:133 can be designed to hybridize to the plus strand of the DNA sequence encoding for the N-terminal consensus sequence of proteins of the present invention. The C-terminal consensus sequence as set forth in SEQ ID NO:143, where X_1 is H or E, and X_2 is N or Y, is a signature motif shared by proteins of the present invention. Reverse oligonucleotide primers, *e. g.*, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, and SEQ ID NO:134, can be designed to hybridize to the minus strand of the DNA sequence encoding for the C-terminus consensus sequence of proteins of the present invention. Oligonucleotide primers can be

designed to hybridize to plus or minus strands of any one or more of the signature motifs (SEQ ID NOs:31-122, 140 and 141) of the proteins of the present invention.

[099] When combined, forward and reserve primers can be used to amplify nucleotide sequences encoding proteins (or fragments thereof) of the present invention.

[0100] Using a Venn diagram (Figure 2) together with bioassay evidence demonstrating toxin activity, the relationships of the proteins of the present invention are illustrated by common function and insecticidal activity towards Hemipteran and/or Lepidopteran insect species. Table 2 correlates the proteins illustrated in the Venn diagram of Figure 2 to insect inhibitory activity by insect species. The results from which Table 2 was assembled are described in more detail in the examples.

Table 2. Activity profiles of exemplary proteins of the present invention

Insect Order	Insect Species	['a']	['c']					['b']	
		TIC1422	TIC1886	TIC1498	TIC1497	TIC1415	TIC1414	TIC1362	TIC1922	TIC1974
Lepidoptera	*H. zea*		M							
	O. nubilalis	M/S		M/S	M/S	S				
	D. saccharalis	M		M/S	M/S			M/S		
	D. grandiosella	M		M/S	M/S			M		
	A. gemmatalis	M/S		M/S	M/S	M/S	M/S			
Hemiptera	*L. lineolaris*		M	M/S	M/S*	M/S	M	M	M/S	S
	L. Hesperus		M	M/S	M/S*	M		M/S		

M = observed Mortality (compared to buffer control)
S = observed Stunting of survivors (compared to buffer control)
* = N-terminal segment that has been truncated at its C-terminus also demonstrated insect inhibitory activity
['a'], ['b'], and ['c'] are Venn diagram regions depicted in Figure 2.

[0101] In certain embodiments, the pest is specifically an insect pest. Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Blattodea, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, and Trichoptera. (Note: nematode has been removed per TK's comments)

[0102] The insects can include larvae of the order Lepidoptera, such as but not limited to, armyworms, cutworms, loopers, and heliothines in the Family Noctuidae (*e.g.* fall armyworm (*Spodoptera frugiperda*), beet armyworm (*Spodoptera exigua*), bertha armyworm (*Mamestra configurata*), black cutworm (*Agrotis ipsilon*), cabbage looper (*Trichoplusia ni*), soybean looper (*Pseudoplusia includens*), velvetbean caterpillar (*Anticarsia gemmatalis*), green cloverworm (*Hypena scabra*), tobacco budworm (*Heliothis virescens*), granulate cutworm (*Agrotis subterranea*), armyworm (*Pseudaletia unipuncta*), western cutworm (*Agrotis orthogonia*); borers, casebearers, webworms, coneworms, cabbageworms and skeletonizers from the Family Pyralidae (*e. g.*, European corn borer (*Ostrinia nubilalis*), navel orangeworm (*Amyelois transitella*), corn root webworm (*Crambus caliginosellus*), sod webworm (*Herpetogramma licarsisalis*), sunflower moth (*Homoeosoma electellum*), lesser cornstalk borer (*Elasmopalpus lignosellus*); leafrollers, budworms, seed worms, and fruit worms in the Family Tortricidae (*e.g.* codling moth (*Cydia pomonella*), grape berry moth (*Endopiza viteana*), oriental fruit moth (*Grapholita molesta*), sunflower bud moth (*Suleima helianthana*); and many other economically important Lepidopteran insects (*e. g.*, diamondback moth (*Plutella xylostella*), pink bollworm (*Pectinophora gossypiella*), gypsy moth (*Lymantria dispar*). Other insect pests of order Lepidoptera include, *e. g.*, *Alabama argillacea* (cotton leaf worm), *Archips argyrospila* (fruit tree leaf roller), *A. rosana* (European leaf roller) and other Archips species, *Chilo suppressalis* (Asiatic rice borer, or rice stem borer), *Cnaphalocrocis medinalis* (rice leaf roller), *Crambus caliginosellus* (corn root webworm), *C. teterrellus* (bluegrass webworm), *Diatraea grandiosella* (southwestern corn borer), *D. saccharalis* (surgarcane borer), *Earias insulana* (spiny bollworm), *E. vittella* (spotted bollworm), *Helicoverpa armigera* (American bollworm), *H. zea* (corn earworm or cotton bollworm), *Heliothis virescens* (tobacco budworm), *Herpetogramma licarsisalis* (sod webworm), *Lobesia botrana* (European grape vine moth), *Pectinophora gossypiella* (pink bollworm), *Phyllocnistis citrella* (citrus leafminer), *Pieris brassicae* (large white butterfly), *P. rapae* (imported cabbageworm, or small white butterfly), *Plutella xylostella* (diamondback moth), *Spodoptera exigua* (beet armyworm), *S. litura* (tobacco cutworm, cluster caterpillar), *S. frugiperda* (fall armyworm), and *Tuta absoluta* (tomato leafminer).

[0103] The insects can include adults and nymphs of the orders Hemiptera and Homoptera, such as but not limited to, plant bugs from the Family Miridae, cicadas from the Family Cicadidae, leafhoppers (*e. g.*, *Empoasca spp.*) from the Family Cicadellidae, planthoppers from the families Fulgoroidea and Delphacidae, treehoppers from the Family Membracidae, psyllids from the Family Psyllidae, whiteflies from the Family Aleyrodidae, aphids from the Family Aphididae, phylloxera from the Family Phylloxeridae, mealybugs from the Family Pseudococcidae, scales from the families Coccidae, Diaspididae and Margarodidae, lace bugs from the Family Tingidae, stink bugs from the Family Pentatomidae, cinch bugs (*e. g.*, *Blissus spp.*) and other seed bugs from the Family Lygaeidae, spittlebugs from the Family Cercopidae squash bugs from the Family Coreidae, and red bugs and cotton stainers from the Family Pyrrhocoridae. Other pests from the order Hemiptera include *Acrosternum hilare* (green stink bug), *Anasa tristis* (squash bug), *Blissus leucopterus leucopterus* (chinch bug), *Corythuca gossypii* (cotton lace bug), *Cyrtopeltis modesta* (tomato bug), *Dysdercus suturellus* (cotton stainer), *Euschistus servus* (brown stink bug), *Euschistus variolarius* (one-spotted stink bug), *Graptostethus spp.* (complex of seed bugs), *Leptoglossus corculus* (leaf-footed pine seed bug), *Lygus lineolaris* (tarnished plant bug), *Lygus hesperus* (Western tarnish plant bug), *Nezara viridula* (southern green stink bug), *Oebalus pugnax* (rice stink bug), *Oncopeltus fasciatus* (large milkweed bug), and *Pseudatomoscelis seriatus* (cotton fleahopper).

[0104] In certain embodiments, the recombinant polypeptide of the present invention exhibits insect inhibitory activity against Lepidopteran species selected from the group consisting of *H. zea, O. nubilalis, D. saccharalis, D. grandiosella, A. gemmatalis, S. frugiperda, S. exigua, A. ipsilon, T. ni, P. includens, H. virescens, P. xylostella, P. gossypiella, H. armigera, E. lignosellus,* and *P. citrella*, and/or against Hemipteran species selected from the group consisting of *L. hesperus, L. lineolaris, A. hilare, E. servus, N. viridula, M. persicae, A. glycines, and A. gossypii.*

[0105] The proteins of the present invention represent a new category and class of Cry protein, exhibiting no greater than 56% amino acid identity to any other *Bt* protein known in the art. The protein exhibiting the nearest identity to any of the proteins of the present invention is Cry15Aa1 (GI: 142726, ACCESSION: AAA22333) (Brown and Whiteley, Journal of Bacteriology, Jan. 1992, p. 549-557, Vol. 174, No. 2). Cry15Aa1 was aligned using Clustal W to each protein exemplified in the present invention and the results are shown in Table 3.

Table 3. Alignment of proteins to Cry15Aa1.

Protein	Amino acid identities* with Cry15Aa1	Percent amino acid identity* with Cry15Aa1
TIC1498 (SEQ ID NO:2)	159	43.1%
TIC1415 (SEQ ID NO:4)	162	42.0%
TIC1497 (SEQ ID NO:6)	164	42.5%
TIC1886 (SEQ ID NO:8)	163	46.3%
TIC1925 (SEQ ID NO:10)	164	42.5%
TIC1414 (SEQ ID NO:12)	159	45.3%
TIC1885 (SEQ ID NO:14)	159	43.2%
TIC1922 (SEQ ID NO:16)	160	41.6%
TIC1422 (SEQ ID NO:18)	156	44.3%
TIC1974 (SEQ ID NO:20)	156	44.3%
TIC2032 (SEQ ID NO:22)	155	40.8%
TIC2120 (SEQ ID NO:24)	158	42.9%
TIC1362 (SEQ ID NO:26)	194	55.3%
TIC2335 (SEQ ID NO:136)	130	38.7%
TIC2334 (SEQ ID NO:138)	129	38.2%

* in a Clustal W alignment

[0106] Cry15Aa1 does not contain any of the signature motifs (SEQ ID NOs:31-122, 140 and 141) shared by the proteins of the present invention. Cry15Aa1 does not exhibit the proteolytic cleavage sites [2], [3], and [4] shared by the proteins of the present invention as shown in Figure 1. Cry15Aa1 exhibits a calculated isoelectric point of about 7.3 pI, in contrast to the proteins of the present invention which each exhibits a calculated isoelectric point of about 5 to 6 pI. Cry15Aa1 exhibits only 3 *positive* charges at neutral pH, whereas the proteins of the present invention exhibit calculated from 3 to 10 *negative* charges at neutral pH.

[0107] The proteins of the present invention can be used to produce antibodies that bind specifically to this genus of proteins and can be used to screen for and to find other members of the genus.

[0108] Nucleotide sequences encoding these proteins can be used as probes and primers for screening to identify other members of the genus using thermal or isothermal amplification and/or hybridization methods, *e. g.*, oligonucleotides as set forth in SEQ ID NOs:127-134, and oligonucleotides hybridizing to sequence encoding the signature motifs of the present invention. Nucleotide sequence homologs, i.e., insecticidal proteins encoded by nucleotide sequences that hybridize to each or any of the sequences disclosed herein under stringent hybridization conditions, are specifically intended to be included within the scope of the present invention. The present invention also provides a method for detecting a first nucleotide sequence that hybridizes to a second nucleotide sequence, wherein the first nucleotide sequence encodes an insecticidal protein or insecticidal fragment thereof and

hybridizes under stringent hybridization conditions to the second nucleotide sequence. In such case the second nucleotide sequence can be any of the sequences disclosed herein under stringent hybridization conditions. Nucleotide coding sequences hybridize to one another under appropriate hybridization conditions and the proteins encoded by these nucleotide sequences cross react with antiserum raised against any one of the other proteins. Stringent hybridization conditions, as defined herein, comprise at least hybridization at 42°C followed by two washes for five minutes each at room temperature with 2X SSC, 0.1% SDS, followed by two washes for thirty minutes each at 65°C in 0.5X SSC, 0.1% SDS. Of course, one skilled in the art will recognize that, due to the redundancy of the genetic code, many other sequences are capable of encoding such related proteins, and those sequences, to the extent that they function to express insecticidal proteins either in *Bacillus* strains or in plant cells, are intended to be encompassed by the present invention, recognizing of course that many such redundant coding sequences will not hybridize under these conditions to the native *Bt* sequences encoding TIC1498, TIC1415, TIC1497, TIC1886, TIC1925, TIC1414, TIC1885, TIC1922, TIC1422, TIC1974, TIC2032, TIC2120, TIC1362, TIC2335, and TIC2334.

[0109] In certain embodiments, a recombinant polypeptide exhibiting insect inhibitory activity against a Lepidopteran and/or Hemipteran insect species is within the scope of the present invention, which polypeptide is encoded by a polynucleotide segment that hybridizes under stringent hybridization conditions to one or more of the nucleotide sequences set forth in any of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:135, or SEQ ID NO:137, or the complement thereof.

[0110] An aspect of this invention provides methods for discovering related proteins, and such methods include the sequencing of *Bt* genomes, assembly of sequence data, the identification and cloning of *Bt* genes encoding such insect inhibitory proteins, and the expression and testing of new *Bt* proteins to assay for insect inhibitory activity. Another aspect of this invention employs molecular methods to engineer and clone commercially useful proteins comprising chimeras of proteins and improved variants from the genus of insect inhibitory proteins, *e. g.*, the chimeras can be assembled from segments in each of the various proteins that are within the spaces between the signature motifs to derive improved embodiments. The proteins of the present invention can be subjected to alignment to each other and to other *Bt* insect inhibitory proteins, and segments of each such protein can be identified that may be useful for substitution between the aligned proteins, resulting in the

construction of chimeric proteins. Such chimeric proteins can be subjected to pest bioassay analysis and characterized for the presence of increased bioactivity or expanded target pest spectrum compared to the parent proteins from which each such segment in the chimera was derived. The insect inhibitory activity of the polypeptides can be further engineered for improved activity to a particular pest or to a broader spectrum of pests by swapping domains or segments with other proteins.

[0111] One skilled artisan understands the concept of amino acid substitution, and recognizes that this requires experimentation that is not routine, as there are amino acid positions that can accept substitution without apparent affect to the structure or function of the protein; however, in surprising circumstances, even a conservative substitution may be determined to significantly alter the structure or function of the protein, and it is often unknown with precision the positions in the amino acid segments that would accept such changes. Accordingly, amino acid substitutions at positions along the length of the protein sequence that affect function can be identified by alanine scanning mutagenesis, and such positions can often be useful for points of amino acid insertions and/or deletions, or N- or C-terminal deletions. Accordingly, the proteins of the present invention include functionally equivalent fragments (N- or C- terminal deletions) of the proteins represented by the amino acid sequences of the present invention. N-terminal protein fragments (SEQ ID NOs:123-126, 16) of TIC1497 and TIC1922 have demonstrated insect inhibitory activity (Table 2 and Examples 6, 10, and 11, respectively). Corresponding N-terminal protein fragments for any member of the genus is contemplated.

[0112] Proteins functionally equivalent (having substantially equivalent insect inhibitory activity) to the proteins of the present invention include proteins with conservative amino acid substitutions in the protein sequences of the present invention. In such amino acid sequences, one or more amino acids in the starting sequence is (are) substituted with another amino acid(s), the charge and polarity of which is similar to that of the native amino acid, *i. e.*, as exemplified herein a conservative amino acid substitution, resulting in a conservative change from the perspective of charge and polarity, but which may result in a change in the bioactivity of the protein, preferably increasing the activity of the protein compared to the starting protein with the original amino acid at such positions, or resulting in a change in the variant protein with reference to the spectrum of biological activity and without any loss of insect inhibitory activity. An example of proteins that can entertain substituted amino acids or terminal deletions to obtain biological equivalents include, but are not limited to, the protein sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID

NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, and SEQ ID NOs:123-126.

[0113] Enrichment of the proteins of the present invention either in plants or by a process that includes culturing recombinant *Bt* cells under conditions to express/produce recombinant polypeptide/proteins of the present invention is contemplated. Such a process can include preparation by desiccation, lyophilization, homogenization, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of recombinant Bacillus thuringiensis cells expressing/producing said recombinant polypeptide. Such a process can result in a *Bt* cell extract, cell suspension, cell homogenate, cell lysate, cell supernatant, cell filtrate, or cell pellet. By obtaining the recombinant polypeptides/proteins so produced, a composition that includes the recombinant polypeptides/proteins can include bacterial cells, bacterial spores, and parasporal inclusion bodies and can be formulated for various uses, including agricultural insect inhibitory spray products or as insect inhibitory formulations in diet bioassays.

[0114] It is intended that an insect inhibitory composition/formulation comprising the aforementioned recombinant polypeptide/protein is within the scope of the present invention. In certain embodiments, such composition may further comprise at least one pesticidal agent that exhibits insect inhibitory activity against the same Lepidopteran and/or Hemipteran insect species but is different from the recombinant polypeptide. Such agent is selected from the group consisting of an insect inhibitory protein, an insect inhibitory dsRNA molecule, and an ancillary protein. Examples of such agents include, but are not limited to, a TIC807 protein, a TIC853 protein, a AXMI-171 protein, and a Cry51Aa1 protein. Other compositions are contemplated for combining with the proteins of the present invention, and with the combinations of proteins provided above. For example, topically applied pesticidal chemistries that are designed for controlling pests that are also controlled by the proteins of the present invention can be used with the proteins of the present invention in seed treatments, spray on/drip on/or wipe on formulations that can be applied directly to the soil (a soil drench), applied to growing plants expressing the proteins of the present invention, or formulated to be applied to seed containing one or more transgenes encoding one or more of the proteins of the present invention. Such formulations for use in seed treatments can be applied with various stickers and tackifiers known in the art. Such formulations may contain pesticides that are synergistic in mode of action with the proteins of the present invention, meaning that the formulation pesticides act through a different mode of action to control the same or similar pests that are controlled by the proteins of the present invention, or that such

pesticides act to control pests within a broader host range, such as lepidopteran or Hemipteran species or other plant pest species such as coleopteran species that are not effectively controlled by the proteins of the present invention.

[0115] The aforementioned composition/formulation can further comprise an agriculturally-acceptable carrier, such as a bait, a powder, dust, pellet, granule, spray, emulsion, a colloidal suspension, an aqueous solution, a *Bacillus* spore/crystal preparation, a seed treatment, a recombinant plant cell/plant tissue/seed/plant transformed to express one or more of the proteins, or bacterium transformed to express one or more of the proteins. Depending on the level of insect inhibitory or insecticidal inhibition inherent in the recombinant polypeptide and the level of formulation to be applied to a plant or diet assay, the composition/formulation can include various by weight amounts of the recombinant polypeptide, *e.g.* from 0.0001% to 0.001% to 0.01% to 1% to 99% by weight of the recombinant polypeptide.

[0116] The proteins of the invention can be combined in formulations for topical application to plant surfaces, to the soil, in formulations for seed treatments, in formulations with other agents toxic to the target pests of Hemipteran and Lepidopteran species. Such agents include but are not limited to, a TIC807 protein, a TIC853 protein, a AXMI-171 protein, and a Cry51Aa1 protein which each are effective in controlling the same Hemipteran pests that are controlled by the insect inhibitory proteins of the present invention.

[0117] It is also intended that a method of controlling a Lepidopteran and/or Hemipteran species pest is within the scope of the present invention. Such method comprises the steps of contacting the pest with an insect inhibitory amount of the recombinant polypeptide/protein. In certain embodiments, Lepidopteran and Hemipteran species pest is in a crop field.

[0118] An embodiment of the invention includes recombinant polynucleotides that encode the insect inhibitory protein members of the genus. With reference to a "recombinant" polynucleotide, it is intended that a polynucleotide molecule is made by human means or intervention through molecular biology engineering techniques, which can include the amplification or replication of such molecules upon introduction into a host cell, and the subsequent extraction and/or purification of the polynucleotide from the representative host cell. Polynucleotide embodiments of the present invention include ribonucleic acids (RNA) and deoxyribonucleic acids (DNA). Proteins of the present invention can be expressed from DNA constructs in which the open reading frame encoding the protein is operably linked to elements such as a promoter and any other regulatory elements functional for expression in that particular system for which the construct is intended. For example, plant-expressible

promoters can be operably linked to protein encoding sequences for expression of the protein in plants, and *Bt*-expressible promoters can be operably linked to the protein encoding sequences for expression of the protein in *Bt*. Other useful elements that can be operably linked to the protein encoding sequences include, but are not limited to, enhancers, introns, protein immobilization tags (HIS-tag), target sites for post-translational modifying enzymes, dsRNA coding segments, siRNAs, miRNAs, ribosomal binding sites, leader elements, and miRNA target sites.

[0119] Exemplary recombinant polynucleotide molecules provided herewith include, but are not limited to, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:135.

[0120] An aspect of the invention provides a recombinant DNA construct that includes one or more aforementioned polynucleotides, which can additionally be engineered with transcribable or non-transcribable regions or both. Such regions are operably assembled to promote expression of DNA to RNA through either *in vivo* or *in vitro* systems, thereby producing the novel RNA transcript embodiments of the present invention. The present invention features RNA transcripts that include, but are not limited to, the protein-encoding RNA and additional RNA regions that are translatable, non-translatable, or both. Such additional RNA regions include translatable regions engineered to translate to terminal or intra-peptide regions, and non-translatable regions engineered to either promote transcription, translation, or both.

[0121] In certain embodiments, the aforementioned recombinant DNA construct is in an expression cassette for use in an *E. coli* or *Bt* expression system. Expression cassettes are typically designed with a promoter at the 5' end of the cassette, upstream of a desired polynucleotide segment encoding a protein of the present invention. A promoter can consist of multiple different promoter elements operably linked to provide for the initiation of transcription of the sequences encoding a protein of the invention. The DNA sequence consisting of the promoter–protein-encoding DNA can be operably linked at its 3' end to a transcriptional termination signal sequence functional in an *E. coli* and/or *Bt* cell to produce the recombinant DNA construct.

[0122] In certain embodiments, the aforementioned recombinant DNA construct is in an expression cassette for expression in plants. Expression cassettes are designed with a promoter at the 5' end of the cassette, upstream of a desired polynucleotide segment encoding

a protein of the present invention. 5' untranscribed DNA can comprise a promoter which can consist of multiple different promoter and enhancer elements operably linked to provide for the initiation of transcription of downstream sequences including sequences encoding the polypeptides of the invention. One or more transcribed but non-translated DNA sequence(s) can be operably linked 3' to the promoter in the expression cassette, including leader and/or intron sequence(s). An intron sequence is optionally provided 3' to the leader sequence or in some cases within the open reading frame encoding the desired protein. A polynucleotide segment encoding an optional translocation polypeptide (a signal peptide or a chloroplast transit peptide, for example) may be inserted 5' to the coding sequence of the protein of the present invention for localizing the protein of the invention to a particular subcellular position. The nucleotide sequence encoding the protein of the present invention is optionally operably positioned within the aforementioned expression cassette, along with any requisite operably linked polyadenylation (polyA) and/or transcriptional termination sequence functional in plant cells. The aforementioned elements are arranged contiguously and can be used in various combinations depending on the desired expression outcome.

[0123] The present invention features promoters functional in plants including, but not limited to, constitutive, non-constitutive, spatially-specific, temporally-specific, tissue-specific, developmentally-specific, inducible, and viral promoters. Examples of promoters functional in plants include corn sucrose synthetase 1, corn alcohol dehydrogenase 1, corn light harvesting complex, corn heat shock protein, pea small subunit RuBP carboxylase, Ti plasmid mannopine synthase, Ti plasmid nopaline synthase, petunia chalcone isomerase, bean glycine rich protein 1, Potato patatin, lectin, CaMV 35S, the S E9 small subunit RuBP carboxylase, carnation etched ring virus, and dahlia mosaic virus promoter.

[0124] A recombinant DNA construct comprising the protein encoding sequences can also further comprise a region of DNA that encodes for one or more insect inhibitory agents which can be configured to concomitantly express or co-express with DNA sequence encoding the protein of the present invention, a protein different from the aforementioned protein, an insect inhibitory dsRNA molecule, or an ancillary protein. Ancillary proteins include co-factors, enzymes, binding-partners, or other insect inhibitory agents that function synergistically to aid in the effectiveness of an insect inhibitory agent, for example, by aiding its expression, influencing its stability in plants, optimizing free energy for oligomerization, augmenting its toxicity, and increasing its spectrum of activity.

[0125] A recombinant polynucleotide or recombinant DNA construct comprising the protein-coding sequence can be delivered to host cells by vectors. Methods for transferring

recombinant DNA constructs to and from host cells, including *E. coli*, *B. thuringiensis*, and Agrobacterium species, are known in the art. Such vectors are designed to promote the uptake of vector DNA and to further provide expression of DNA to RNA to protein in *in vitro* or *in vivo* systems, either transiently or stably. Examples of the vectors include, but are not limited to, a plasmid, baculovirus, artificial chromosome, virion, cosmid, phagemid, phage, or viral vector. Such vectors can be used to achieve stable or transient expression of the protein encoding sequence in a host cell; and, if the case may be, subsequent expression to polypeptide. An exogenous recombinant polynucleotide or recombinant DNA construct that comprises the protein encoding sequence and that is introduced into a host cell is also referred to herein as a "transgene".

[0126] Plasmids can be designed to replicate in *E. coli* or *B. thuringiensis*, or both. Such plasmids contain genetic elements that allow for the replication and maintenance of such plasmids and for the expression of transgenes, *e.g.* aforementioned recombinant DNA constructs, in either species.

[0127] Plant transformation vectors can be designed to allow for the Agrobacterium-mediated transfer of a T-DNA, *i.e.* transferred DNA comprising aforementioned recombinant DNA constructs. Such plant transformation vectors contain genetic elements that allow for the replication and maintenance of such plasmid vectors in *E. coli* and/or Agrobacterium and are essential for transfer of the T-DNA into a plant genome.

[0128] Transgenic host cells comprising recombinant DNA constructs encoding toxin proteins of the present invention are also contemplated. A transgenic host cell can be further defined as a prokaryotic host cell, *i.e.* a bacterial cell, *e. g.*, *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus laterosperous*, Escherichia, Salmonella, Agrobacterium, Pseudomonas, or Rhizobium cell, or a eukaryotic host cell, *e. g.*, a plant cell, and each of these types of cells is also referred to herein as a microbial cell, a microbe, or a microorganism.

[0129] As used herein a "host cell" means a cell that is transformed or transfected with exogenous recombinant DNA, *e.g.* by electroporation or by Agrobacterium-mediated transformation or by bombardment using microparticles coated with recombinant DNA, or by transduction or by plasmid transfer or by other means. A host cell of this invention can be a transformed bacterium, *e.g. E. coli* host cell or *Bt* host cell or Agrobacterium host cell, or a plant host cell.

[0130] Accordingly, a host cell of can be an originally-transformed plant cell that exists as a microorganism or as a progeny plant cell that is regenerated into differentiated tissue, *e. g.*,

into a transgenic plant with stably-integrated, non-natural recombinant DNA, or seed or pollen derived from a progeny transgenic plant. As used herein a "transgenic plant" includes a plant, plant part, plant cells or seed whose genome has been altered by the stable integration of recombinant DNA. A transgenic plant includes a plant regenerated from an originally-transformed plant cell and progeny transgenic plants from later generations or crosses of a transformed plant. Accordingly, examples of plant parts are leaf, a branch, a bark, a blade, a pollen grain, a stalk, a cell, a stem, a flower, a sepal, a fruit, a root, or a seed.

[0131] Transgenic plants expressing the protein(s) of the invention exhibit pest tolerance. Such plants and its cells include alfalfa, banana, barley, bean, berry, brassica, broccoli, cabbage, cactus, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, clover, coconut, coffee, corn, cotton, cucumber, cucurbit, Douglas fir, eggplant, eucalyptus, flax, fruit, garlic, grape, hops, kapok, leek, legume, lettuce, Loblolly pine, melons, millets, nut, oat, olive, onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeonpea, pine, poplar, potato, pumpkin, Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, succulent, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, tree, triticale, turf grass, vegetable, watermelon, and wheat plants and cells.

[0132] Nucleotides sequences can be constructed that are useful for expression of these proteins in plant cells, and such plant cells can be regenerated into transgenic plants that can produce seeds containing such nucleotide sequences which can be commercialized, bred together with other transgenic plants expressing different *Bt* insect inhibitory proteins or other agents toxic to crop pests.

[0133] Plant cells can be transformed by multiple mechanisms that are within the skill of the art including but not limited to bacterial transformation systems such as Agrobacterium or Rhizobacterium, electroporation, ballistic mediated systems, and the like. Microprojectile bombardment methods are illustrated in US Patents 5,015,580 (soybean); 5,550,318 (corn); 5,538,880 (corn); 5,914,451 (soybean); 6,160,208 (corn); 6,399,861 (corn); 6,153,812 (wheat) and 6,365,807 (rice) and Agrobacterium-mediated transformation is described in US Patents 5,159,135 (cotton); 5,824,877 (soybean); 5,463,174 (canola); 5,591,616 (corn); 5,846,797 (cotton); 6,384,301 (soybean), 7,026,528 (wheat) and 6,329,571 (rice), US Patent Application Publication 2004/0087030 A1 (cotton), and US Patent Application Publication 2001/0042257 A1 (sugar beet) and in Arencibia et al. (1998) Transgenic Res. 7:213-222 (sugarcane) and other more recent methods described in US Patent Application Publications 2009/0138985A1 (soybean), 2008/0280361A1 (soybean), 2009/0142837A1 (corn),

2008/0282432 (cotton), 2008/0256667 (cotton), 2003/0110531 (wheat), US Patents 5,750,871 (canola), 7,026,528 (wheat), and 6,365,807 (rice). Transformation of plant material can be practiced in tissue culture on a nutrient media, *e. g.*, a mixture of nutrients that will allow cells to grow in vitro. Recipient cell targets include, but are not limited to, meristem cells, hypocotyls, calli, immature embryos and gametic cells such as microspores, pollen, sperm and egg cells. Callus may be initiated from tissue sources including, but not limited to, immature embryos, hypocotyls, seedling apical meristems, microspores and the like. Cells containing a transgenic nucleus are grown into transgenic plants.

[0134] In addition to direct transformation of a plant material with a recombinant DNA, a transgenic plant cell nucleus can be prepared by crossing a first plant having cells with a transgenic nucleus with recombinant DNA with a second plant lacking the transgenic nucleus. For example, recombinant DNA can be introduced into a nucleus from a first plant line that is amenable to transformation to transgenic nucleus in cells that are grown into a transgenic plant which can be crossed with a second plant line to introgress the recombinant DNA into the second plant line. A transgenic plant with recombinant DNA providing an enhanced trait, *e. g.*, enhanced yield, can be crossed with transgenic plant line having other recombinant DNA that confers another trait, for example herbicide resistance or pest resistance, to produce progeny plants having recombinant DNA that confers both traits. Typically, in such breeding for combining traits the transgenic plant donating the additional trait is a male line and the transgenic plant carrying the base traits is the female line. The progeny of this cross will segregate such that some of the plants will carry the DNA for both parental traits and some will carry DNA for one parental trait; such plants can be identified by markers associated with parental recombinant DNA, *e. g.*, marker identification by analysis for recombinant DNA or, in the case where a selectable marker is linked to the recombinant DNA, by application of the selecting agent such as a herbicide for use with a herbicide tolerance marker, or by selection for the insect inhibitory trait. Progeny plants carrying DNA for both parental traits can be crossed back into the female parent line multiple times, for example usually 6 to 8 generations, to produce a progeny plant with substantially the same genotype as one original transgenic parental line but for the recombinant DNA of the other transgenic parental line.

[0135] In the practice of plant transformation, exogenous DNA is typically introduced into only a small percentage of target plant cells in any one transformation experiment. Cells of this invention can be directly tested to confirm stable integration of the exogenous DNA by a variety of well-known DNA detection methods or by a variety of well-known bioactivity

assays that test for insect inhibitory activity (further described in the examples section). Marker genes can be used to provide an efficient system for identification of those cells that are stably transformed by receiving and integrating a recombinant DNA molecule into their genomes. Preferred marker genes provide selective markers which confer resistance to a selective agent, such as an antibiotic or an herbicide. Any of the herbicides to which plants of this invention can be made resistant can be used as agents for selective markers. Potentially transformed cells are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene is integrated and expressed at sufficient levels to permit cell survival. Cells may be tested further to confirm stable integration of the exogenous DNA. Commonly used selective marker genes include those conferring resistance to antibiotics such as kanamycin and paromomycin (nptII), hygromycin B (aph IV), spectinomycin (aadA) and gentamycin (aac3 and aacC4) or resistance to herbicides such as glufosinate (bar or pat), dicamba (DMO) and glyphosate (aroA or EPSPS). Examples of such selectable markers are illustrated in US Patents 5,550,318, 5,633,435, 5,780,708, and 6,118,047. Markers which provide an ability to visually screen transformants can also be employed, for example, a gene expressing a colored or fluorescent protein such as a luciferase or green fluorescent protein (GFP).

[0136] Plant cells that survive exposure to the selective agent, or plant cells that have been scored positive in a screening assay, may be cultured in regeneration media and allowed to mature into plants. Developing plants regenerated from transformed plant cells can be transferred to plant growth mix, and hardened off, for example, in an environmentally controlled chamber at about 85% relative humidity, 600 ppm CO_2, and 25 to 250 microeinsteins m 2 s-1 of light, prior to transfer to a greenhouse or growth chamber for maturation. These growth conditions vary among plant species and are known to those skilled in the art. Plants are regenerated from about 6 weeks to 10 months after a transformant is identified, depending on the initial tissue, and plant species. Plants may be pollinated using conventional plant breeding methods known to those of skill in the art and seed produced, for example self-pollination is commonly used with transgenic corn. The regenerated transformed plant or its progeny seed or plants can be tested for expression of the recombinant DNA and selected for the presence of insect inhibitory activity.

[0137] Transgenic plants encoding and expressing one or more of the proteins of the present invention are grown to (i) generate transgenic plants having an enhanced trait as compared to a control plant and (ii) produce transgenic seed and haploid pollen of this invention. Such plants with enhanced traits are identified by selection of transformed plants or progeny seed

for the enhanced trait. For efficiency a selection method is designed to evaluate multiple transgenic plants (events) comprising the recombinant DNA, for example multiple plants from 2 to 20 or more transgenic events. Transgenic plants grown from transgenic seed provided herein demonstrate improved agronomic traits that contribute to increased insect inhibitory tolerance or increased harvest yield or other traits that provide increased plant value, including, for example, improved seed or boll quality. Of particular interest are cotton, alfalfa, corn, soy, or sugarcane plants having enhanced insect inhibitory resistance against one or more insects of the orders Lepidoptera and/or Hemiptera. Of particular interest are cotton plants having enhanced insect inhibitory resistance against an insect of the order Hemiptera.

[0138] The invention provides methods to produce a plant and harvest a crop from seed comprising a recombinant polynucleotide molecule encoding the insect inhibitory polypeptides of the present invention. Of particular interest are cotton, alfalfa, corn, soy, or sugarcane plants having enhanced insect inhibitory resistance against an insect(s) of the order Lepidoptera and/or Hemiptera. The method includes the steps of crossing an insect resistant plant expressing the recombinant polypeptides of the present invention with another plant, obtaining at least one progeny plant derived from this cross, and selecting progeny that expresses the recombinant polypeptides of the present invention wherein said progeny is resistant against an insect. This includes the steps of planting the seed, producing a crop from plants grown from the seed, and harvesting the crop, wherein at least 50% of the crop comprises seed comprising the recombinant polynucleotide molecule.

[0139] In an aspect of the invention, a transgenic plant cell, a transgenic plant, and transgenic plant parts comprising a recombinant polynucleotide (i.e. transgene) that expresses any one or more of the protein encoding sequences are provided herein. It is intended that "bacterial cell" or "bacterium" can include, but are not limited to, an *Agrobacterium*, a *Bacillus*, an *Escherichia*, a *Salmonella*, a *Pseudomonas*, or a *Rhizobium* cell. It is intended that "plant cell" or "plant" include an alfalfa, banana, barley, bean, broccoli, cabbage, brassica, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, coconut, coffee, corn, clover, cotton, a cucurbit, cucumber, Douglas fir, eggplant, eucalyptus, flax, garlic, grape, hops, leek, lettuce, Loblolly pine, millets, melons, nut, oat, olive, onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeonpea, pine, potato, poplar, pumpkin, Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, triticale, turf grass, watermelon, and wheat plant

cell or plant. In certain embodiments, transgenic plants and transgenic plant parts regenerated from a transgenic plant cell are provided. In certain embodiments, the transgenic plants can be obtained from a transgenic seed. In certain embodiments, transgenic plant parts can be obtained by cutting, snapping, grinding or otherwise disassociating the part from the plant. In certain embodiments, the plant part can be a seed, a boll, a leaf, a flower, a stem, a root, or any portion thereof. In certain embodiments, a transgenic plant part provided herein is a non-regenerable portion of a transgenic plant part. As used in this context, a "non-regenerable" portion of a transgenic plant part is a portion that cannot be induced to form a whole plant or that cannot be induced to form a whole plant that is capable of sexual and/or asexual reproduction. In certain embodiments, a non-regenerable portion of a plant part is a portion of a transgenic seed, boll, leaf, flower, stem, or root.

[0140] Also provided herein are methods of making transgenic plants that comprise insect inhibitory amounts of the protein(s) of the present invention. Such plants can be made by introducing a recombinant polynucleotide that encodes any of the proteins provided herein into a plant cell, and selecting a plant derived from said plant cell that expresses an insect inhibitory amount of the proteins. Plants can be derived from the plant cells by regeneration, seed, pollen, or meristem transformation techniques.

[0141] Also provided herein is the use of a transgenic plant that expresses an insect inhibitory amount of one or more of the proteins of the present invention to control a Lepidopteran and/or a Hemipteran species pest. Any of the aforementioned transgenic plants can be used in methods for protecting a plant from insect infestation provided herein.

[0142] Also provided herein is the use of any of the aforementioned transgenic host cells to produce the proteins of the present invention.

[0143] Additional aspects of the invention include methods and/or kits for detecting DNA, RNA, or protein of the present invention, methods for identifying members of the genus of proteins described herein, methods for identifying novel proteins related to genus family members, methods for testing for control of insect growth and/or infestation, and methods for providing such control to plants and other recipient hosts. These proteins can be used to produce antibodies that bind specifically to this class/genus of protein and these antibodies can be used to screen and find other members of the genus. An antibody by itself, or in a mixture of antibodies, that binds specifically to a target of the recombinant polypeptides of the present invention is contemplated; and, the method of using this antibody by itself, or in a mixture of antibodies, to detect or quantify proteins sharing epitopes of the proteins of the present invention is also contemplated. Such a method to detect or quantify can include the

steps of contacting a sample with the antibody and using detection means well known in the art to detect the binding of antibody to polypeptide target in the sample. Where one or more epitopes are contemplated and their combination used in such a method, the binding of an antibody or mixture of antibodies recognizing different epitopes can identify a polypeptide exhibiting homology to the recombinant polypeptides of the present invention.

[0144] Kits for detecting the presence of a polypeptide target in a sample suspected of containing the polypeptide target are provided. Such kits would include a reagent(s) used for epitope detection and a control reagent(s) to show that the detection was operating within statistical variances. Reagent storage, instructions for detection means and use of reagents, and additional parts and tools that can be included in such kits are contemplated.

[0145] The polynucleotide segments encoding the proteins of the present invention, *i.e.* the proteins of the described genus, particularly the segments derived from wild type *Bt* strains, can be used as probes and primers for screening for and identifying other members within the genus using thermal amplification and/or hybridization methods. Nucleotide probes or primers can vary in length, sequence, concentration, backbone, and formulation depending on the sample detection method used. The present invention features primers and probes that can be used to detect and isolate homologous genes that encode for insect inhibitory protein members of the genus. A DNA detection kit is contemplated providing a skilled artisan to more easily perform the detection and/or isolation of homologous genes of the present invention. The invention provides for use of such kits and methods and for novel genes and the insect inhibitory polypeptides encoded by such genes that are detected and isolated by the aforementioned detection means.

[0146] The invention further provides for methods of testing the polypeptides of the present invention for insect inhibitory activity, herein termed "bioassay". Described herein are qualitative insect bioassays that measure growth inhibition, mortality, or a combination of both. The insect orders tested in the following examples include Coleoptera, Diptera, Lepidoptera, and Hemiptera. The diet recipe and preparation, the preparation of test and control samples, the insect preparation, and the procedures for conducting assays are typically dependent upon the type and size of the insect and/or pest being subjected to any particular evaluation. Such methods are illustrated and described in detail in the following examples.

[0147] In certain embodiments, plant product can comprise commodity or other products of commerce derived from a transgenic plant or transgenic plant part, where the commodity or other products can be tracked through commerce by detecting nucleotide segments or expressed RNA or proteins that encode or comprise distinguishing portions of the proteins of

the present invention. Such commodity or other products of commerce include, but are not limited to, plant parts, biomass, oil, meal, sugar, animal feed, flour, flakes, bran, lint, processed seed, and seed.

[0148] Also provided herewith are processed plant products that comprise a detectable amount of a recombinant nucleotide encoding any one of the proteins of the present invention, an insect inhibitory fragment thereof, or any distinguishing portion thereof. In certain embodiments, the processed product is selected from the group consisting of plant biomass, oil, meal, animal feed, flour, flakes, bran, lint, hulls, and processed seed. In certain embodiments, the processed product is non-regenerable. In certain embodiments, a distinguishing portion thereof can comprise any polynucleotide encoding at least 20, 30, 50 or 100 amino acids of the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 136 or 138.

[0149] Also provided herein are methods of controlling insects. Such methods can comprise growing a plant comprising an insect inhibitory amount of the protein of the present invention. In certain embodiments, such methods can further comprise any one or more of: (i) applying any composition comprising or encoding the proteins of the present invention to the plant or a seed that gives rise to the plant; and/or (ii) transforming the plant or a plant cell that gives rise to the plant with a polynucleotide encoding the proteins of the present invention. In certain embodiments, the plant is a transiently or stably transformed transgenic plant comprising a transgene that expresses an insect inhibitory amount of the protein of the present invention. In certain embodiments, the plant is a non-transgenic plant to which a composition comprising the protein of the present invention has been applied.

[0150] Other features and advantages of the invention will be apparent from the following detailed description, examples, and claims.

EXAMPLES

[0151] In view of the foregoing, those of skill in the art should appreciate that changes can be made in the specific aspects which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention. Thus, specific details disclosed herein are not to be interpreted as limiting.

Example 1: Discovery of insect inhibitory proteins

[0152] Various *Bt* strains exhibiting distinctive attributes, *e.g.* inferred toxicity, proteomic diversity, and morphological variations when compared to each other, were identified, and

DNA was obtained from each such strain and prepared for DNA sequencing. DNA sequence information was generated for each such strain, raw sequence reads were processed, contigs were assembled from processed reads, open reading frames were identified, and deduced amino acid sequences were analyzed.

Example 2: Cloning and expressing insect inhibitory proteins

[0153] This example illustrates the cloning of polynucleotide segments encoding insect inhibitory proteins, and insertion into and expression in recombinant host cells.

[0154] Nucleotide segments were obtained by amplification from corresponding genomic samples from which each open reading frame was identified in Example 1. Amplified nucleotide segments were inserted into a recombinant plasmid and transformed into an acrystalliferous *Bt* host cell or into an *E. coli* expression strain, and the resulting recombinant strain(s) were observed to express a recombinant protein.

[0155] Recombinant proteins exemplified herein were observed to exhibit insect inhibitory properties to a variety of pest species as described in Examples 3 - 13 below. Nucleotide sequences as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:135, and SEQ ID NO:137 were confirmed to encode proteins having amino acid sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:26, SEQ ID NO:136, and SEQ ID NO:138 (respectively, TIC1498, TIC1415, TIC1497, TIC1886, TIC1414, TIC1922, TIC1422, TIC1974, TIC1362, TIC2335, and TIC2334).

[0156] Recombinant plasmids and strains were also constructed to contain polynucleotide segments having the sequences as set forth in SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:21, and SEQ ID NO:23, and were confirmed to encode proteins having amino acid sequences as set forth in SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:22, and SEQ ID NO:24 (respectively, TIC1925, TIC1885, TIC2032, and TIC2120).

Example 3: Lepidopteran activity of TIC1886

[0157] This example illustrates the *Helicoverpa zea* (*Hz*) activity exhibited by a sample from a recombinant strain expressing recombinant protein TIC1886 having deduced amino acid sequence of SEQ ID NO:8.

[0158] A diet of 16.5% (w/v) of "Multiple Species" diet (Southland Products, 201 Stuart Island Road, Lake Village, Arkansas 71653) was prepared in a 14% (w/v) agar base (Serva

#11393). The agar base was melted and blended with diet and purified water to volume (1.4% (w/v) agar). Diet suspension was dispensed into individual bioassay compartments.

[0159] A test sample of recombinant protein TIC1886 was prepared as described in example 2, and overlaid over 24 compartmentalized diet surfaces approximating about 2890 ug/mL per compartment. A buffer control sample was overlaid onto 96 compartmentalized diet surfaces. Together, 120 compartmentalized diet surfaces comprise the test set of this example prepared for *Helicoverpa zea* bioassay.

[0160] A single neonate larva was transferred to the diet surface of each individual compartment of the test set of this example (120 total neonates) and each compartment sealed with a ventilated cover. The test-set was placed in a controlled environment at 27°C and 60% RH with no light for 5-7 days and scored for mortality and stunting. Stunting was visually estimated in comparison to untreated insects and was scored as significantly stunted (>67 % stunting), moderately stunted (33-67% stunted), or stunted (<33%).

[0161] No stunting or mortality was observed with buffer control samples. Mortality was observed against 24 *Hz* larvae at 2890 ug/mL TIC1886. It was concluded that the protein TIC1886 demonstrated *Helicoverpa zea* (corn earworm) activity (see Figure 2 and Table 2).

[0162] The lepidopteran bioassay procedure described in this example was also applied to a combination of larvae from *Ostrinia nubilalis*, *Diatraea saccharalis*, *Diatraea grandiosella*, and *Anticarsia gemmatalis* species using the proteins of the present invention (from Example 2, TIC1498, TIC1415, TIC1497, TIC1414, TIC1422, and TIC1362), and the results are described in examples 4-7.

Example 4: Lepidopteran activity of TIC1498, TIC1497, and TIC1422

[0163] Using the methods and bioassay techniques described in Example 3, recombinant proteins TIC1498 (SEQ ID NO:2), TIC1497 (SEQ ID NOs:6), and TIC1422 (SEQ ID NO:18) were tested against neonates of *Ostrinia nubilalis* (*On*), *Diatraea saccharalis* (*Ds*), *Diatraea grandiosella* (*Dg*), and *Anticarsia gemmatalis* (*Ag*) insect species.

[0164] TIC1498 exhibited mortality against *Ostrinia nubilalis*, and survivors were significantly stunted, over 24 larvae at 2500 ug/mL. TIC1497 exhibited mortality against *Ostrinia nubilalis*, and survivors were significantly stunted, over 24 larvae at 3700 ug/mL. TIC1422 exhibited mortality against *Ostrinia nubilalis*, and survivors were significantly stunted, over 24 larvae at 1300 ug/mL. It was concluded that TIC1498, TIC1497, and TIC1422 demonstrated activity (see Figure 2 and Table 2) against *Ostrinia nubilalis* (European corn borer).

[0165] TIC1498 exhibited mortality against *Diatraea saccharalis*, and survivors were significantly stunted, over 24 larvae at 3000 ug/mL. TIC1497 exhibited mortality against *Diatraea saccharalis*, and survivors were significantly stunted, over 24 larvae at 2000 ug/mL. TIC1422exhibited 100% mortality to *Diatraea saccharalis*, over 24 larvae at 300 ug/mL. It was concluded that recombinant proteins TIC1498, TIC1497, and TIC1422 demonstrated activity (see Figure 2 and Table 2) against *Diatraea saccharalis* (surgarcane borer).

[0166] TIC1498 exhibited mortality against *Diatraea grandiosella*, and survivors were moderately stunted, over 24 larvae at 3000 ug/mL. TIC1497 exhibited mortality rate against *Diatraea grandiosella*, and survivors were significantly stunted, over 24 larvae at 2000 ug/mL. TIC1422 exhibited mortality against *Diatraea grandiosella*, over 24 larvae at 300 ug/mL. It was concluded that TIC1498, TIC1497, and TIC1422 demonstrated activity (see Figure 2 and Table 2) against *Diatraea grandiosella* (southwestern corn borer).

[0167] TIC1498 exhibited mortality against *Anticarsia gemmatalis*, and survivors were significantly stunted, over 48 larvae at 2500-3000 ug/mL. TIC1497 exhibited mortality against *Anticarsia gemmatalis*, and survivors were moderately to significantly stunted, over 48 larvae at 2000-3700 ug/mL. TIC1422 exhibited mortality against against *Anticarsia gemmatalis*, and survivors were significantly stunted, over 48 larvae at 300-1300 ug/mL. It was concluded that TIC1498, TIC1497, and TIC1422 demonstrated activity (see Figure 2 and Table 2) against *Anticarsia gemmatalis* (velvetbean caterpillar).

Example 5: Lepidopteran activity of TIC1415

[0168] TIC1415 (SEQ ID NO:4) was tested against *Ostrinia nubilalis* (*On*) and *Anticarsia gemmatalis* (*Ag*) insect species neonates. TIC1415 exhibited mortality against *Ostrinia nubilalis*, and survivors were moderately stunted, over 24 larvae at 1500 ug/mL. It was concluded that TIC1415 demonstrated activity (see Figure 2 and Table 2) against *Ostrinia nubilalis* (European corn borer).

[0169] TIC1415 exhibited mortality against *Anticarsia gemmatalis*, and survivors were moderately stunted, over 24 larvae at 1500 ug/mL. It was concluded that TIC1415 demonstrated activity (see Figure 2 and Table 2) against *Anticarsia gemmatalis* (velvetbean caterpillar).

Example 6: Lepidopteran activity of TIC1414

[0170] TIC1414 (SEQ ID NO:12) was tested against *Anticarsia gemmatalis* (*Ag*) insect species neonates. TIC1414 exhibited mortality against *Anticarsia gemmatalis*, and survivors

were stunted, over 24 larvae at 870 ug/mL. It was concluded that TIC1414 demonstrated activity (see Figure 2 and Table 2) against *Anticarsia gemmatalis* (velvetbean caterpillar).

Example 7: Lepidopteran activity of TIC1362

[0171] TIC1362 (SEQ ID NO:26) was tested against *Diatraea saccharalis* (*Ds*) and *Diatraea grandiosella* (*Dg*) insect species neonates. TIC1362 exhibited mortality against *Diatraea saccharalis*, and survivors were significantly stunted, over 24 larvae at 400 ug/mL. TIC1362 demonstrated *Diatraea saccharalis* (velvetbean caterpillar) activity (see Figure 2 and Table 2).

[0172] TIC1362 exhibited 100% mortality against *Diatraea grandiosella* over 24 larvae at 400 ug/mL. TIC1362 demonstrated *Diatraea grandiosella* (southwestern corn borer) activity (see Figure 2 and Table 2).

Example 8: Hemipteran activity of TIC1498

[0173] This example illustrates insect inhibitory activity of TIC1498 (SEQ ID NO:2) when provided in the diet of hemipteran insects, including but not limited to members of the Heteroptera miridae, including the genus Lygus, *e. g.*, *Lygus hesperus* and *Lygus lineolaris*. This example more specifically illustrates the *Lygus hesperus* (*Lh*) and *Lygus lineolaris* (*Ll*) activity exhibited by a sample from a recombinant strain expressing the recombinant protein TIC1498.

[0174] A diet of 7.81% (w/v) of "Lygus Diet" diet (Bio-Serv #F9644B, One 8th Street, Suite One, Frenchtown, NJ 08825) and liquid contents of two whole fresh eggs was prepared. The diet was cooled and stored under moisture controlled conditions and at 4°C until ready for use. This diet preparation was used within 2 days of preparation.

[0175] Test samples containing TIC1498 protein were prepared encapsulated (~40 uL) between stretched Parafilm and Mylar sheets that were heat-sealed (sachets).

[0176] Lygus hesperus and Lygus lineolaris eggs were incubated at 24°C until they reached between 0 to about 12 hours pre-hatch stage. Pre-hatch eggs were soaked and rinsed in sterile water, then placed in confined proximity to the prepared sachets in a controlled environment at 24°C and 60% RH with no light for 4-7 days and scored for percent mortality and stunting of any survivors. Stunting was visually estimated in comparison to untreated insects and was scored as significantly stunted (>67 % stunting), moderately stunted (33-67% stunted), or stunted (<33%).

[0177] At 10 ug/mL TIC1498, mortality was observed against *Lygus lineolaris*, and survivors stunted, over 24 neonate nymphs. At 50 ug/mL TIC1498, mortality was observed against *Lygus lineolaris*, and survivors stunted, over 24 neonate nymphs. TIC1498 exhibited mortality against *Lygus lineolaris* at 100 ug/mL, and survivors were moderately stunted, over 24 neonate nymphs.

[0178] At 10 ug/mL TIC1498, mortality was observed against *Lygus hesperus*, and survivors stunted, over 24 neonate nymphs. At 50 ug/mL TIC1498, mortality was observed against *Lygus hesperus*, and survivors stunted, over 24 neonate nymphs. TIC1498 exhibited mortality against *Lygus hesperus* at 100 ug/mL, and survivors were moderately stunted, over 24 neonate nymphs. At 2300 ug/mL TIC1498, 100% mortality was observed against *Lygus hesperus*, over 24 neonate nymphs.

[0179] TIC1498 demonstrated both *Lygus lineolaris* (tarnished plant bug) and *Lygus hesperus* (Western tarnish plant bug) activity (see Figure 2 and Table 2). The hemipteran bioassay procedure described in this example was also performed using TIC1415, TIC1497, TIC1886, TIC1414, TIC1922, TIC1974, and TIC1362.

Example 9: Hemipteran activity of TIC1922 and TIC1974

[0180] TIC1922 (SEQ ID NO:16) and TIC1974 (SEQ ID NO:20) were tested against *Lygus lineolaris* (*Ll*). TIC1922 was tested in 3 groups of 24 sachets, and exhibitedmortality against *Lygus lineolaris*, and survivors exhibited stunting at 3000 ug/mL. TIC1974 did not exhibit mortality against *Lygus lineolaris*, but survivors were stunted, in an evaluation of 24 neonate nymphs at 3000 ug/mL. TIC1922 and TIC1974 demonstrated *Lygus lineolaris* (tarnished plant bug) activity (see Figure 2 and Table 2).

Example 10: Hemipteran activity of TIC1497

[0181] TIC1497 (SEQ ID NO:6) was tested against *Lygus hesperus* (*Lh*). TIC1497 exhibited mortality against *Lygus hesperus*, and survivors were moderately stunted, in an experiment evaluating 48 neonate nymphs at 2000 ug/mL.

[0182] Preparations of TIC1497 fragments were made by treating TIC1497 with thermolysin, chymotrypsin, trypsin, or Glu-C, resulting in TIC1497 fragments exhibiting masses of 32411 Da (SEQ ID NO:64), 32557 Da (SEQ ID NO:62), 34225 Da (SEQ ID NO:61), and 34485 Da (SEQ ID NO:63). The protein eluate from the thermolysin treated preparation was isolated (TIC1497.32411) on an ion exchange column and used in bioassays against Hemipteran species.

[0183] TIC1497.32411 exhibited mortality against *Lygus lineolaris*, and survivors were moderately stunted, in an experiment using 24 neonate nymphs at 100 ug/mL. TIC1497.32411 exhibited 100% mortality at a dose of 1000 ug/mL.

[0184] TIC1497.32411 exhibited mortality against *Lygus lineolaris*, and survivors were moderately stunted, in an experiment using 24 neonate nymphs at a dose of 2300 ug/mL.

[0185] TIC1497 demonstrated *Lygus hesperus* (Western tarnish plant bug) activity (see Figure 2 and Table 2). The fragment TIC1497.32411 demonstrated both *Lygus lineolaris* (tarnished plant bug) and *Lygus hesperus* (Western tarnish plant bug) activity (see Figure 2 and Table 2).

Example 11: Hemipteran activity of TIC1886, TIC1415, TIC1414, and TIC1362

[0186] TIC1886 (SEQ ID NO:8), TIC1415 (SEQ ID NO:4), TIC1414 (SEQ ID NO:12), and TIC1362 (SEQ ID NO:24) were tested against *Lygus lineolaris* and *Lygus hesperus*. TIC1886 exhibited mortality against both *Lygus lineolaris* and *Lygus hesperus*, at a dose equivalent to 124 ug/mL. TIC1415 exhibited mortality against *Lygus lineolaris* and *Lygus hesperus* at a dose equivalent to 150 ug/mL and survivors were stunted. TIC1414 exhibited mortality against *Lygus lineolaris* at a dose equivalent to 95 ug/mL. TIC1362 exhibited mortality against *Lygus lineolaris* at a dose equivalent to 370 ug/mL.

[0187] TIC1886, TIC1415, and TIC1362 demonstrated both *Lygus lineolaris* (tarnished plant bug) and *Lygus hesperus* (Western tarnish plant bug) activity. TIC1414 demonstrated *Lygus lineolaris* (tarnished plant bug) activity (see Figure 2 and Table 2)

Example 12: Insect inhibitory activities of other protein members

[0188] Other protein members from the genus of the present invention, such as but not limited to TIC1925 (SEQ ID NO:10), TIC1885 (SEQ ID NO:14), TIC2032 (SEQ ID NO:22), and TIC2120 (SEQ ID NO:24), are prepared for bioassay against pests of plants, including a pest from the phylum Nematoda, a pest from Lepidoptera, and a pest from Hemiptera.

Example 13: Protein expression in plants

[0189] This example illustrates expression of proteins of the present invention in plants. Polynucleotide segments for use in expression of the proteins of the present invention in plants can be produced according to the methods set forth in US Patent No. 7,741,118. For example, toxin proteins having the amino acid sequence as set forth in SEQ ID NO:4, SEQ ID NO:12, SEQ ID NO:18, and SEQ ID NO:26 can be produced in plants from

polynucleotide segments having the sequence as set forth respectively in SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30. Polynucleotide segments designed for use in plants and encoding the proteins of the present invention, including the sequences as set forth in SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30 are operably linked to the requisite expression elements for expression in plants, and transformed into the genome of plant cells, preferably cotton, alfalfa, corn, and soybean cells.

[0190] It is intended that polynucleotide segments (or polynucleotide molecules) encoding each of the following enumerated proteins, insect inhibitory fragments thereof, and proteins exhibiting the degree of identity specified herein above to one or more of these enumerated proteins, be used alone or in combinations with each other, or in combinations with other toxin proteins or toxic agents such as dsRNA mediated gene suppression molecules designed to work in synergistic or synonymous ways with the proteins of the present invention, to achieve plants and plant cells protected from pest infestation, particularly insect pest infestation. The specific enumerated proteins within the scope of the invention include TIC1498 (SEQ ID NO:2), TIC1415 (SEQ ID NO:4), TIC1497 (SEQ ID NO:6), TIC1886 (SEQ ID NO:8), TIC1925 (SEQ ID NO:10), TIC1414 (SEQ ID NO:12), TIC1885 (SEQ ID NO:14), TIC1922 (SEQ ID NO:16), TIC1422 (SEQ ID NO:18), TIC1974 (SEQ ID NO:20), TIC2032 (SEQ ID NO:22), TIC2120 (SEQ ID NO:24), TIC1362 (SEQ ID NO:26), TIC2335 (SEQ ID NO:136), TIC2334 (SEQ ID NO:138) and insect inhibitory fragments thereof, such as but not limited to, TIC1497.34225 (SEQ ID NO:61), TIC1497.32557 (SEQ ID NO:62), TIC1497.34485 (SEQ ID NO:63), and TIC1497.32411 (SEQ ID NO:64).

[0191] For instance, proteins of the TIC1415 genus of the present invention can be combined with other pesticidal agents, including pesticidal agents targeting pests which overlap with pests targeted by TIC1415 proteins. Additionally, other pesticidal agents may include agents that target pests that do not overlap with pests targeted by TIC1415 proteins. In either case, it is intended that TIC1415 proteins be used alone or combined with other pesticidal agents. In the examples described below, TIC1415 was co-expressed with a TIC807 toxin protein in cotton plants and *in planta* bioassays were conducted. In addition to TIC807 toxin proteins, other pesticidal agents that can be used in combination with TIC1415 proteins include (1) hemipteran-centric agents, e.g. dsRNA directed towards hemipteran orthologs of *Nilaparvata lugens* V-ATPase-E, *21E01*(Li, Jie et al., 2011, Pest Manag Sci); dsRNA directed towards hemipteran orthologs of five different genes – actin ortholog, ADP/ATP translocase, α-tubulin, ribosomal protein L9 (RPL9) and V-ATPase A subunit (Upadhyay, S. K., et al., 2011, J. Biosci. 36(1), p.153-161); AXMI-171 (US20100298207A1); *Bt* endotoxins such as

Cry3A, Cry4Aa, Cry11Aa, and Cyt1Aa, which were found to exhibit low to moderate toxicity on the pea aphid, Acyrthosiphon pisum, in terms of both mortality and growth rate (Porcar, M. et al., Applied and Environmental Microbiology, July 2009, p.4897–4900, Vol. 75, No. 14); (2) other Coleopteran pesticidal agents, e.g. DIG11 and DIG5; Cry7; eCry3.1Ab; mCry3A; Cry8; Cry34/Cry35; and Cry3 toxins generally; and (3) other Lepidopteran pesticidal agents, e.g. DIG2; Cry1 toxins; Cry1A.105; Cry2 toxins, particularly Cry2A toxins; Cry1F toxins; VIP3 toxins; and Cry9 toxins. Transgenic crop events expressing other pesticidal agents can also be used in combination with crop events expressing TIC1415 proteins, examples of which include MON88017, MON89034, MON863, MON15985, MON531, MON757, COT102, TC1507, DAS59122-7, 3006-210-23, 281-24-236, T304-40, GHB119, COT67B, MIR162; corn event 5307, and the like. Such combinations with events expressing one or more proteins of the TIC1415 genus proteins provide more durable pest protection, provide a resistance management strategy for target pest control, and reduce farmer inputs, saving considerable expense in time and monetary value.

[0192] Recombinant plants are generated from transformed plant cells of this example, and the recombinant plants or their progeny are evaluated for resistance to pest infestation, such as tolerance to Hemiptera and/or Lepidoptera. Transgenic plants and seed are selected that provide pest resistance, such as to Hemiptera and Lepidoptera, and such plants and seed are advanced for further development.

Example 14: *In-planta* bioassay of TIC1415

[0193] In this study, toxin protein TIC1415 having the amino acid sequence as set forth in SEQ ID NO:4 was produced in plants from polynucleotide segments having the sequence as set forth in SEQ ID NO:27. DNA having the sequence of SEQ ID NO:27 encoding TIC1415 was cloned into an Agrobacterium-mediated plant transformation vector along with requisite promoter and regulatory elements for transformation and expression in cotton cells. Transgenic cotton plants (recombinant cotton plants) were produced and tested for efficacy. Regenerated (R0) transgenic plants were transferred to soil and tissue samples selected from transformation events that were low in copy number and expressing TIC1415 protein. Lyophilized tissue samples of R0 plants from three events were weighed and combined 1:50 and 1:100 (weight:buffer) of 25 mM Sodium-carb/bicarb buffered at pH 10.5 to extract soluble protein from the tissue. Samples were confirmed by Western blot for presence of TIC1415 protein. Sample extracts were fed to *Lygus lineolaris* using the bioactivity assay described in Example 8. Extract from DP393 cotton tissue absent of TIC1415 protein was

also prepared as negative control. Sample extracts from all three events exhibited mortality against *Lygus lineolaris* and survivors were stunted. Mortality and stunting scores were significant compared to bioactivity scores of insects fed with sample extracts from the DP393 negative control.

[0194] R0 plants were grown and self-pollinated to obtain seed homozygous for the introduced transgenic DNA. Homozygous plants from three different single copy events were selected and five seed per event planted and evaluated in a whole plant caging assay. Plants were grown to flowering stage and each whole cotton plant was enclosed in a mesh cage made from perforated plastic. Two pairs of male and female *Lygus hesperus* adults were placed into each cage and allowed to reproduce. Resulting insect progeny were allowed to infest the caged cotton plants for 3 weeks. At the end of the 21 day period, Lygus insects at various stages of development were counted and average means calculated on a per plant basis. Plants from all three events had significantly less insects compared to the DP393 negative control. See Table 4.

Table 4. In-planta bioassay of TIC1415.

Event	Mean 3rd Instar or <	Mean 4th Instar Nymphs	Mean 5th Instar Nymphs	Mean Live 2nd Gen. Adults	Mean Total 2nd Gen. Lygus	Students t Grouping (p<0.05)
84	0.20	0.00	0.60	0.00	0.80	B
52	0.00	0.40	1.60	1.20	3.20	B
39	0.40	0.40	2.00	1.60	4.40	B
DP393 (negative)	3.60	5.20	9.70	9.50	28.0	A

Mean 2nd Generation *Lygus* Recovered from Five Cotton Plants per Event in a Caged Whole Plant Assay. Events with the same letter do not have statistically different 2nd generation Lygus numbers (p<0.05, Students t).

Example 15: Protein bioassay of TIC1415 and a TIC807 Hemipteran toxic protein

[0195] Protein samples were prepared containing various mixtures of TIC1415 and a TIC807 hemipteran toxic protein and tested in bioassay. TIC1415 protein alone and the TIC807 protein alone were also prepared as positive controls. Buffer was used as negative control. Sample mixtures were fed to *Lygus lineolaris* using bioactivity assay. All three preparations containing toxin protein exhibited mortality against *Lygus lineolaris* and survivors were stunted. Mortality and stunting scores were significant compared to bioactivity scores of

insects fed with buffer (see Table 5). The data suggests that there are no antagonistic effects. Additional bioassay tests are performed on mixtures to demonstrate synergistic and/or additive effects.

Table 5. Bioassay data for protein mix: TIC1415 combined with a TIC807 toxin protein

SAMPLE	TIC1415 (ug/mL)	TIC807 (ug/mL)	Mean† Population mortality	T Grouping on mort	Mean† stunting‡ score	T Grouping on stunting
TIC1415 + TIC807	4.35	1	21.79	AB*	0.60	AB*
TIC1415 + TIC807	2.175	1	20.36	B*	0.60	AB*
TIC1415 + TIC807	1.0875	1	12.50	BC	0.60	AB*
TIC1415 + TIC807	4.35	0.5	32.50	A*	0.80	A*
TIC1415 + TIC807	1.75	0.265	7.86	CD	0.40	ABC
TIC1415 + TIC807	0.875	0.265	0.00	D	0.00	C
TIC1415 + TIC807	0.4375	0.265	5.36	CD	0.00	C
TIC1415 + TIC807	4.35	0.25	13.21	BC	0.40	ABC
TIC1415 + TIC807	1.75	0.1325	0.00	D	0.00	C
TIC1415 + TIC807	1.75	0.06625	0.00	D	0.00	C
TIC1415	4.35	0	12.50	BC	0.40	ABC
TIC1415	1.75	0	7.86	CD	0.00	C
TIC807	0	1	0.00	D	0.20	BC
TIC807	0	0.265	2.50	CD	0.00	C
Buffer (negative) control	0	0	0.00	D	0.00	C

†Average (mean) of 5 populations of 8 nymphs per population.
‡ Stunting scores correspond to visual mass ratings where 0 = no difference to negative control, 1 = about 25% less mass, 2 = about 50% less mass, and 3 = about 75% less mass. The average of the stunting scores for each population of eight nymphs is reported.
* At 95% confidence interval.

Example 16: In-planta bioassay of TIC1415 and TIC807

[0196] Transgenic cotton events were designed to co-express respective proteins TIC1415 (SEQ ID NO:4) and a TIC807 protein. Such plants were evaluated in a caged whole plant assay infested with *Lygus lineolaris*. Five plants each from ten events were caged and infested with 2 pairs of male and female *L. lineolaris* per plant. The assay was incubated in a growth chamber under normal environmental conditions for cotton plant development for 21

days. DP393 negative control plants were grown in similar manner. At the end of the 3 week period, *Lygus* of various stages of development were counted. The mean number per plant of *Lygus hesperus* insects at each stage in development were calculated and the results are shown in Table 6. Therein, different plant-expressible promoters were used to drive expression of the transcript encoding TIC1415 in the respective constructs 12 and 13.

Table 6. In-planta data for protein mix: TIC1415 combined with a TIC807 protein toxin

Construct	Event	N	Mean 3rd Instar or <	Mean 4th Instar Nymphs	Mean 5th Instar Nymphs	Mean Live 2nd Gen. Adults	Mean Total 2nd Gen. Lygus	SEM	Tukey Grouping
12	021	5	0.00	0.00	0.00	0.00	0.00	0.00	B
	625	5	0.20	0.20	0.20	0.00	0.60	0.24	B
	830	5	2.20	0.20	0.00	0.00	2.40	1.12	AB
	890	5	4.40	0.00	0.20	0.00	4.60	2.62	AB
	521	5	4.60	0.60	0.00	0.00	5.20	4.27	AB
	980	5	3.40	1.20	1.20	0.00	5.80	4.86	AB
13	426	5	0.00	0.00	0.00	0.00	0.00	0.00	B
	611	5	0.60	0.00	0.00	0.00	0.60	0.60	B
	999	5	0.40	0.00	0.40	0.00	0.80	0.37	B
	356	5	6.20	0.00	0.40	0.00	6.60	4.73	AB
Inbred	DP393 (Negative)	10	7.00	2.50	0.80	0.00	10.30	3.75	A

Mean 2nd Generation *Lygus* Recovered from Five Cotton Plants per Event in a Caged Whole Plant Assay. Events with the same letter do not have statistically different 2nd generation Lygus numbers ($p<0.05$, Students t).

WHAT IS CLAIMED IS:

1. A recombinant polypeptide exhibiting insect inhibitory activity against Hemipteran and/or Lepidopteran pest species, wherein said polypeptide optionally:

(a) exhibits at least from about 47% to about 100% amino acid sequence identity, or any percentage point between 47% and 100%, to one or more of the proteins having the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24;

(b) exhibits at least from about 56% to about 100% amino acid sequence identity, or any percentage point between 56% and 100%, to one or more of the proteins having the amino acid sequence as set forth in any of SEQ ID NO:26, SEQ ID NO:136, or SEQ ID NO:138;

(c) contains in operable position within the polypeptide, at least one of each of six different motif peptide segments in consecutive order M0, M1, M2, M3, M4 and M5, each motif peptide segment exhibiting at least about 80% identity to a consensus sequence specified for the respective motif peptide segment, in which the consensus sequence for motif peptide segment M0 is set forth in SEQ ID NO:31, the consensus sequence for motif peptide segment M1 is set forth in SEQ ID NO:48, the consensus sequence for motif peptide segment M2 is set forth in SEQ ID NO:53, the consensus sequence for motif peptide segment M3 is set forth in SEQ ID NO:62, the consensus sequence for motif peptide segment M4 is set forth in SEQ ID NO:65, and the consensus sequence for motif peptide segment M5 is set forth in SEQ ID NO:139;

(d) contains in operable linkage within the polypeptide, at least one of each of three different motif peptide segments M1t, M2t, and M4t, in consecutive order, wherein each motif peptide segment exhibits at least about 80% identity to a consensus sequence specified for the respective motif peptide segment, and wherein the consensus sequence for motif peptide segment M1t is set forth at SEQ ID NO:70, the consensus sequence for motif peptide segment M2t is

set forth at SEQ ID NO:87, and the consensus sequence for motif peptide segment M4t is set forth at SEQ ID NO:120;

(e) contains an amino acid sequence exhibiting from about 195 to about 386 amino acid identities to the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:136, or SEQ ID NO:138;

(f) contains an amino acid sequence exhibiting at least from about 56% to about 100% identity, or any percentage point between 56% and 100%, to the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, or SEQ ID NO:24;

(g) contain an amino acid sequence exhibiting at least from about 56% to about 100% identity, or any percentage point in between 56% to 100% to the amino acid sequence set forth in any of SEQ ID NO:26, SEQ ID NO:136, and SEQ ID NO:138; or

(h) is encoded by a polynucleotide segment that hybridizes under stringent hybridization conditions to one or more of the nucleotide sequences set forth in any of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:135, or SEQ ID NO:137, or the complement thereof.

2. A recombinant polypeptide comprising a peptide segment exhibiting at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:31, SEQ ID NO:48, SEQ ID NO:53, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:139, SEQ ID NO:70, SEQ ID NO:87, SEQ ID NO:120, and any combination thereof, wherein said polypeptide exhibits insect inhibitory activity against Lepidopteran and/or Hemipteran species.

3. The recombinant polypeptide of claim 1 or 2, wherein said polypeptide comprises a

peptide segment exhibiting at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:31-122, 140 and 141, and any combination thereof.

4. The recombinant polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence exhibiting a number of amino acid identities to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:136, and SEQ ID NO:138, wherein said number is any number from 195 to 386.

5. The recombinant polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence exhibiting at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.2, 99.5, 99.8, or 100% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:136, and SEQ ID NO:138.

6. The recombinant polypeptide of claim 1 or 2, wherein said polypeptide comprises the amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:136, or SEQ ID NO:138, or an insect inhibitory fragment thereof.

7. The recombinant polypeptide of claim 6, wherein said insect inhibitory fragment comprises the amino acid sequence as set forth in SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, and SEQ ID NO:126.

8. The recombinant polypeptide of claim 1 or 2, wherein said Lepidopteran species are selected from the group consisting of *H. zea, O. nubilalis, D. saccharalis, D. grandiosella, A. gemmatalis, S. frugiperda, S. exigua, A. ipsilon, T. ni, P. includens,*

H. virescens, P. xylostella, P. gossypiella, H. armigera, E. lignosellus, and *P. citrella,* and wherein said Hemipteran species are selected from the group consisting of *L. hesperus, L. lineolaris, A. hilare, E. servus, N. viridula, M. persicae, A. glycines, and A. gossypii.*

9. An insect inhibitory composition comprising the recombinant polypeptide of claim 1 or 2.

10. The composition of claim 9, further comprising at least one pesticidal agent, wherein said pesticidal agent exhibits insect inhibitory activity against the same Lepidopteran and/or Hemipteran species but is different from said recombinant polypeptide, wherein said pesticidal agent is selected from the group consisting of an insect inhibitory protein, an insect inhibitory dsRNA molecule, and an ancillary protein.

11. The composition of claim 10, wherein said pesticidal agent exhibits insect inhibitory activity against the same Hemipteran species, and wherein said pesticidal agent is selected from the group consisting of a TIC807 protein, a TIC853 protein, a Cry51Aa1 protein, and a AXMI-171 protein.

12. A method of controlling a Lepidopteran and/or Hemipteran species pest, said method comprising contacting said pest with an insect inhibitory amount of the recombinant polypeptide of claim 1 or 2.

13. The method of claim 12, wherein said Lepidopteran and/or Hemipteran species pest is in a crop field.

14. A recombinant polynucleotide comprising a nucleotide sequence encoding the recombinant polypeptide of claim 1 or 2.

15. The recombinant polynucleotide of claim 14, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:135.

16. The recombinant polynucleotide of claim 14, further comprising a nucleotide sequence encoding one or more insect inhibitory agents that are each different from said recombinant polypeptide.

17. The recombinant polynucleotide of claim 16, wherein said nucleotide sequence encodes a TIC807 protein.

19. A transgenic plant cell, plant or plant part comprising the recombinant polynucleotide of claim 14.

20. The transgenic plant cell, plant or plant part of claim 19, wherein said plant is selected from the group consisting of alfalfa, banana, barley, bean, broccoli, cabbage, brassica, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, coconut, coffee, corn, clover, cotton, a cucurbit, cucumber, Douglas fir, eggplant, eucalyptus, flax, garlic, grape, hops, leek, lettuce, Loblolly pine, millets, melons, nut, oat, olive, onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeonpea, pine, potato, poplar, pumpkin, Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, triticale, turf grass, watermelon, and wheat, and wherein said plant part is a seed, a boll, a leaf, a flower, a stem, a root, or any portion thereof.

FIGURE 1

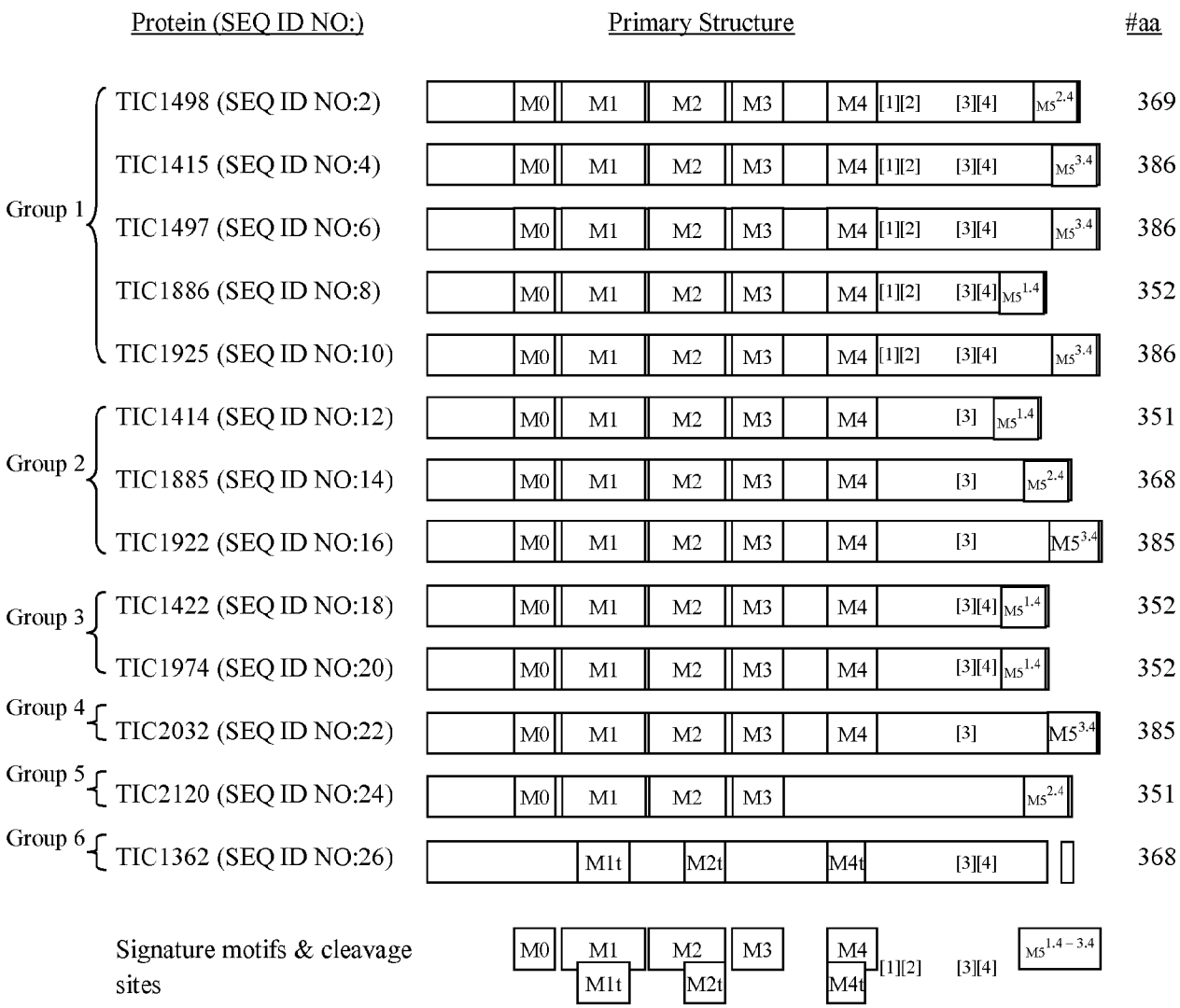
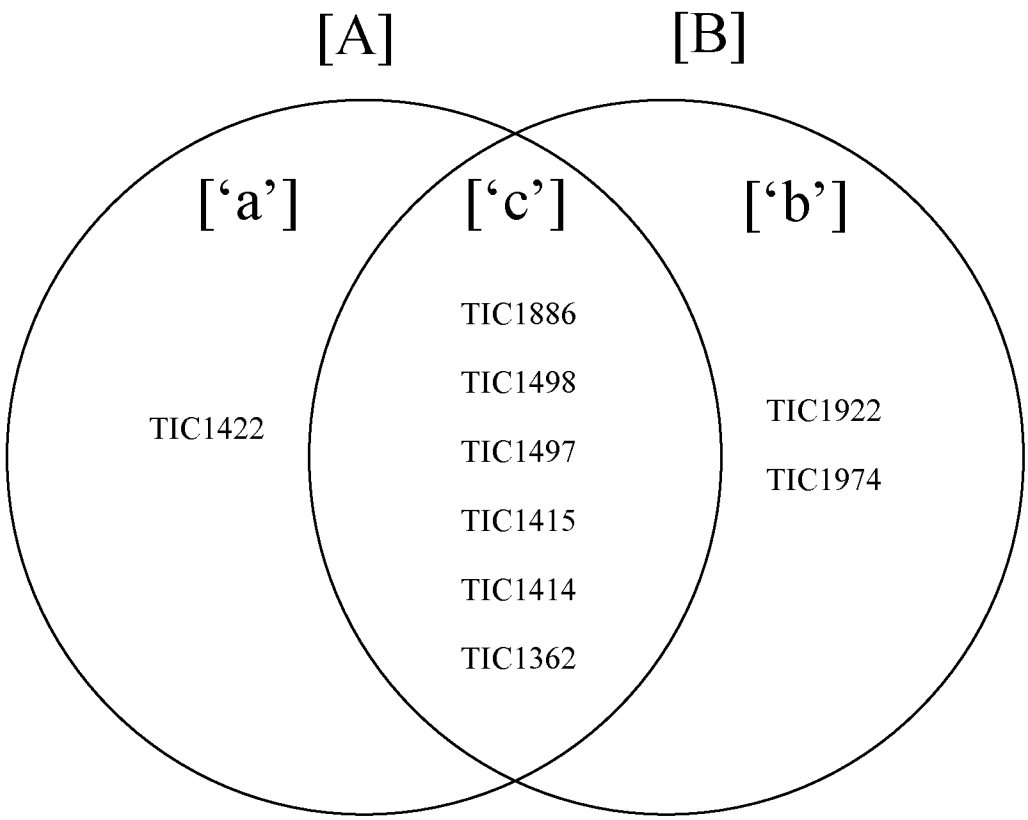

Protein (SEQ ID NO:)
Primary Structure
#aa
Group 1
TIC1498 (SEQ ID NO:2)
TIC1415 (SEQ ID NO:4)
TIC1497 (SEQ ID NO:6)
TIC1886 (SEQ ID NO:8)
TIC1925 (SEQ ID NO:10)
Group 2
TIC1414 (SEQ ID NO:12)
TIC1885 (SEQ ID NO:14)
TIC1922 (SEQ ID NO:16)
Group 3
TIC1422 (SEQ ID NO:18)
TIC1974 (SEQ ID NO:20)
Group 4
TIC2032 (SEQ ID NO:22)
Group 5
TIC2120 (SEQ ID NO:24)
Group 6
TIC1362 (SEQ ID NO:26)
M0
M1
M2
M3
M4
[1][2]
[3][4]
[3]
M1t
M2t
M4t
369
386
386
352
386
351
368
385
352
352
385
351
368
Signature motifs & cleavage sites

FIGURE 2


[A]
[B]
['a']
['c']
['b']
TIC1886
TIC1498
TIC1422
TIC1497
TIC1922
TIC1974
TIC1415
TIC1414
TIC1362