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(54) Titre : GENES PESTICIDES ET LEURS PROCEDES D'UTILISATION
(54) Title: PESTICIDAL GENES AND METHODS OF USE

(57) Abrégé/Abstract:
Compositions having pesticidal activity and methods for their use are provided. Compositions include isolated and recombinant polypeptides having pesticidal activity, recombinant and synthetic nucleic acid molecules encoding the polypeptides, DNA constructs and vectors comprising the nucleic acid molecules, host cells comprising the vectors, and antibodies to the polypeptides. Nucleotide sequences encoding the polypeptides can be used in DNA constructs or expression cassettes for transformation and expression in organisms of interest. The compositions and methods provided are useful for producing organisms with enhanced pest resistance or tolerance. Transgenic plants and seeds comprising a nucleotide sequence that encodes a pesticidal protein of the invention are also provided. Such plants are resistant to insects and other pests. Methods are provided for producing the various polypeptides disclosed herein, and for using those polypeptides for controlling or killing a pest. Methods and kits for detecting polypeptides of the invention in a sample are also included.

Abstract

Compositions having pesticidal activity and methods for their use are provided. Compositions include isolated and recombinant polypeptides having pesticidal activity, recombinant and synthetic nucleic acid molecules encoding the polypeptides, DNA constructs and vectors comprising the nucleic acid molecules, host cells comprising the vectors, and antibodies to the polypeptides. Nucleotide sequences encoding the polypeptides can be used in DNA constructs or expression cassettes for transformation and expression in organisms of interest. The compositions and methods provided are useful for producing organisms with enhanced pest resistance or tolerance. Transgenic plants and seeds comprising a nucleotide sequence that encodes a pesticidal protein of the invention are also provided. Such plants are resistant to insects and other pests. Methods are provided for producing the various polypeptides disclosed herein, and for using those polypeptides for controlling or killing a pest. Methods and kits for detecting polypeptides of the invention in a sample are also included.

PESTICIDAL GENES AND METHODS OF USE

FIELD

[0001] The invention is drawn to methods and compositions for controlling pests, particularly plant pests.

CROSS REFERENCE TO RELATED APPLICATION

[0002] This application claims the benefit of U.S. Provisional Application Serial No. 62/149,164, filed April 17, 2015, the contents of this application is herein incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

[0003] The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named AgB011.PCT_seq_listing.txt, created on April 12, 2016, and having a size of 1.11 MB and is filed concurrently with the specification. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety..

BACKGROUND

[0004] Pests, plant diseases, and weeds can be serious threats to crops. Losses due to pests and diseases have been estimated at 37% of the agricultural production worldwide, with 13% due to insects, bacteria and other organisms.

[0005] Toxins are virulence determinants that play an important role in microbial pathogenicity and/or evasion of the host immune response. Toxins from the gram-positive bacterium Bacillus, particularly Bacillus thuringiensis, have been used as insecticidal proteins. Current strategies use the genes expressing these toxins to produce transgenic crops. Transgenic crops expressing insecticidal protein toxins are used to combat crop damage from insects.

[0006] While the use of Bacillus toxins has been successful in controlling insects, resistance to Bt toxins has developed in some target pests in many parts of the world where such toxins have been used intensively. One way of solving this problem is sowing Bt crops with alternating rows of regular non Bt crops (refuge). An alternative method to avoid or slow down development of insect resistance is stacking insecticidal genes with different modes of action against insects in transgenic plants. The current strategy of using transgenic crops expressing insecticidal protein toxins is placing increasing emphasis on the discovery of novel toxins, beyond those already derived from the bacterium Bacillus thuringiensis. These toxins may prove useful as alternatives to those derived from B. thuringiensis for deployment in insect- and pest-resistant transgenic plants. Thus, new toxin proteins are needed.

SUMMARY

[0007] Compositions having pesticidal activity and methods for their use are provided. Compositions include isolated and recombinant polypeptide sequences having pesticidal activity, recombinant and synthetic nucleic acid molecules encoding the pesticidal polypeptides, DNA constructs comprising the nucleic acid molecules, vectors comprising the nucleic acid molecules, host cells comprising the vectors, and antibodies to the pesticidal polypeptides. Nucleotide sequences encoding the polypeptides provided herein can be used in DNA constructs or expression cassettes for transformation and expression in organisms of interest, including microorganisms and plants.

[0008] The compositions and methods provided herein are useful for the production of organisms with enhanced pest resistance or tolerance. These organisms and compositions comprising the organisms are desirable for agricultural purposes. Transgenic plants and seeds comprising a nucleotide sequence that encodes a pesticidal protein of the invention are also provided. Such plants are resistant to insects and other pests.

[0009] Methods are provided for producing the various polypeptides disclosed herein, and for using those polypeptides for controlling or killing a pest. Methods and kits for detecting polypeptides of the invention in a sample are also included.

DETAILED DESCRIPTION

[0010] The present inventions now will be described more fully hereinafter. These inventions may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements.

[0011] Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

I. Polynucleotides and Polypeptides

[0012] Compositions and method for conferring pesticidal activity to an organism are provided. The modified organism exhibits pesticidal resistance or tolerance. Recombinant pesticidal proteins, or polypeptides and fragments and variants thereof that retain pesticidal activity, are provided and include those set forth in SEQ ID NOs: 1-229. The pesticidal proteins are biologically active (e.g., pesticidal) against pests including insects, fungi, nematodes, and the like. Nucleotides encoding the pesticidal polypeptides, including for example, SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162,

163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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 or active fragments or variants thereof, can be used to produce transgenic organisms, such as plants and microorganisms. In specific embodiments, nucleotides encoding the polypeptide include, for example, 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199 or an active variant or fragment thereof. The pesticidal proteins are biologically active (for example, are pesticidal) against pests including insects, fungi, nematodes, and the like. Polynucleotides encoding the pesticidal polypeptides, including for example, SEQ ID NOS: 1-229 or active fragments or variants thereof, can be used to produce transgenic organisms, such as plants and microorganisms. The transformed organisms are characterized by genomes that comprise at least one stably incorporated DNA construct comprising a coding sequence for a pesticidal protein disclosed herein. In some embodiments, the coding sequence is operably linked to a promoter that drives expression of the encoded pesticidal polypeptide. Accordingly, transformed microorganisms, plant cells, plant tissues, plants, seeds, and plant parts are provided. A summary of various polypeptides, active variants and fragments thereof, and polynucleotides encoding the same are set forth below in Table 1. As noted in Table 1, various forms of polypeptides are provided. Full length pesticidal polypeptides, as well as, modified versions of the original full-length sequence (i.e., variants) are provided. Table 1 further denotes "CryBP1" sequences. Such sequences (SEQ ID NO: 190) comprise accessory polypeptides that can be associated with some of the toxin genes. In such instances, the CryBP1 sequences can be used alone or in combination with any of the pesticidal polypeptides provided herein. Table 1 further provides Split-Cry C-terminus polypeptides (SEQ ID NO: 21, 66, 94, 142, 150 and 165). Such sequences comprise the sequence of a downstream protein that has homology to the C-terminal end of the Cry class of toxin genes and are usually found after a Cry gene that is not full-length and is missing the expected C-terminal region.

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Table 1. Summary of SEQ ID NOs, Gene Class, and Variants Thereof

Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
APG00001	1	2, 3			US_7923602_B2-35 (28.6% identity, 43.8% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					AEH76817.1 (28.0% identity, 43.0% similarity)			
					BAC06484.1 (27.9% identity, 44.2% similarity)			
					C12AA_BACTU (27.3% identity, 44.1% similarity)			
APG00003	4	5, 6			Q2HWE8_BACTU (34.5% identity, 52.6% similarity)	Cry	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_74 (33.9% identity, 51.5% similarity)			
					Cry39Aa1 (30.5% identity, 46.3% similarity)			
APG00004	7	8			US_7105332_B2-14 (52.1% identity, 65.6% similarity)	Cry	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US_7329736_B2-2 (49.6% identity, 62.5% similarity)			
					B7NZX8_BACTU (48.0% identity, 60.3% similarity)			
					Cry8Ba1 (29.4% identity, 37.4% similarity)			
APG00006	9	10, 288			APG00201 (79.7% identity, 88.2% similarity)	Mtx	80, 85, 90, 95, 96, 97, 98, 99	90, 95, 96, 97, 98, 99
					J8YPM2_BACCE (79.0% identity, 86.2% similarity)			
					US20130227743A1_100 (77.5% identity, 85.3% similarity)			
					APG00036 (76.1% identity, 83.7% similarity)			
					APG00022 (75.4% identity, 84.3% similarity)			
					US20130227743A1_60 (44.9% identity, 49.5% similarity)			

Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					A8LVM9_SALAI (25.2% identity, 39.7% similarity)			
APG00007	11	12, 13, 14			AEH76820.1 (47.0% identity, 60.1% similarity)	Cry	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					BAB78602.1 (44.5% identity, 57.8% similarity)			
					US20100298211A1_8 (44.5% identity, 57.1% similarity)			
					Cry32Da1 (37.0% identity, 50.4% similarity)			
APG00009	15	16			WP_002166885.1 (26.4% identity, 40.5% similarity)	Mtx	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					C3ICE4_BACTU (25.9% identity, 37.5% similarity)			
					J8Y0J8_BACCE (25.9% identity, 37.3% similarity)			
					WP_033690552.1 (23.8% identity, 34.8% similarity)			
APG00011	17	18			APG00035 (75.9% identity, 84.7% similarity)	Bin	80, 85, 90, 95, 96, 97, 98, 99	90, 95, 96, 97, 98, 99
					K0FZN9_BACTU (75.3% identity, 85.0% similarity)			
					J8HJ33_BACCE (75.1% identity, 85.0% similarity)			
					US20130227743A1_6 (70.0% identity, 78.8% similarity)			
					Cry35Ab1 (23.8% identity, 39.9% similarity)			
APG00012	19	20		21	US_2013_0227743_A1_194 (67.2% identity, 76.2% similarity)	Cry	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	80, 85, 90, 95, 96, 97, 98, 99
					US_8461415_B2-43 (46.5% identity, 53.1% similarity)			
					US_8461415_B2-42 (37.1% identity, 42.4% similarity)			
					Cry44Aa (18.1% identity, 29.8% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
APG00013	22	23			WP_001036192.1 (87.5% identity, 93.4% similarity)	Mtx	90, 95, 96, 97, 98, 99	95, 96, 97, 98, 99
					WP_000163136.1 (85.8% identity, 93.1% similarity)			
					WP_000790613.1 (59.0% identity, 74.9% similarity)			
					APG00234 (57.7% identity, 73.3% similarity)			
					WP_003290257.1 (48.6% identity, 64.7% similarity)			
APG00014	24				WP_033694890.1 (34.1% identity, 45.1% similarity)	Mtx	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20120278954A1_22 (33.1% identity, 49.5% similarity)			
					AF316145_1 (30.8% identity, 48.4% similarity)			
					US_5308760_A-9 (27.9% identity, 42.3% similarity)			
APG00015	25				ACF35049.1 (53.0% identity, 67.6% similarity)	Cyt2	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					WP_000288253.1 (53.0% identity, 67.6% similarity)			
					AF398463_1 (50.4% identity, 64.5% similarity)			
					Cyt2Bc1 (49.5% identity, 67.8% similarity)			
APG00016	26	27			AF038048_1 (26.7% identity, 40.2% similarity)	Mtx	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20120278954A1_30 (25.6% identity, 41.7% similarity)			
					AGP18056.1 (24.8% identity, 40.0% similarity)			
					Cry45Aa (24.2% identity, 38.6% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
APG00017	28				US_8513493_B2-47 (32.1% identity, 53.1% similarity)	Cyt	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					Cyt2Ca1 (29.4% identity, 40.3% similarity)			
APG00018	29	30			APG00205 (97.8% identity, 99.0% similarity)	Mtx	75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					C3IAI7_BACTU (71.7% identity, 81.5% similarity)			
					J8HRD0_BACCE (65.6% identity, 74.8% similarity)			
					C3FB42_BACTU (48.1% identity, 63.4% similarity)			
					WP_018669999.1 (48.1% identity, 63.0% similarity)			
APG00019	31	32			APG00272 (80.2% identity, 81.8% similarity)	Mtx	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					WP_034679607.1 (38.4% identity, 50.8% similarity)			
					US_8829279_B2-2 (29.7% identity, 46.2% similarity)			
					US_8829279_B2-61 (29.3% identity, 45.7% similarity)			
					US20130227743A1_66 (28.9% identity, 45.8% similarity)			
APG00020	33	34			J8H0D9_BACCE (81.1% identity, 83.8% similarity)	Mtx	85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					US20130227743A1_110 (46.4% identity, 63.9% similarity)			
					WP_000844425.1 (27.1% identity, 44.0% similarity)			
					C3ICE4_BACTU (27.0% identity, 44.1% similarity)			
APG00021	35	36			APG00091 (93.6% identity, 96.4% similarity)	Mtx	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					BAD22577.1 (31.5% identity, 46.6% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US_8461421_B2-102 (31.2% identity, 46.4% similarity)			
					US_6063756_A-3 (28.4% identity, 47.7% similarity)			
					Cry15Aa1 (23.5% identity, 37.3% similarity)			
APG00022	37	38			APG00201 (78.8% identity, 85.9% similarity)	Mtx	75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					APG00036 (78.8% identity, 84.4% similarity)			
					APG00006 (75.4% identity, 84.3% similarity)			
					J8YPM2_BACCE (71.8% identity, 81.4% similarity)			
					US20130227743A1_100 (71.4% identity, 81.4% similarity)			
					US20130227743A1_60 (48.9% identity, 51.8% similarity)			
					WP_037788316.1 (21.8% identity, 35.2% similarity)			
APG00024	39	40			WP_016078427.1 (98.3% identity, 98.3% similarity)	Cry6	99	99
					WP_000240776.1 (95.3% identity, 97.2% similarity)			
					WP_000240775.1 (94.4% identity, 96.4% similarity)			
					Cry6Ba1 (28.4% identity, 47.9% similarity)			
APG00025	41	42			K0G027_BACTU (92.9% identity, 96.7% similarity)	Cry70B	95, 96, 97, 98, 99	97, 98, 99
					ADO51070.1 (92.6% identity, 96.4% similarity)			
					T1WCQ4_BACTU (87.6% identity, 93.2% similarity)			
					Cry70Bb1 (85.9% identity, 92.0% similarity)			
					APG00027 (56.3% identity, 71.4% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
APG00026	43	44, 45, 46			R8DHS1_BACCE (72.9% identity, 84.0% similarity)	Cry	75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					APG00109 (69.2% identity, 79.5% similarity)			
					US_7919272_B2-14 (64.1% identity, 76.5% similarity)			
					US20130227743A1_30 (63.6% identity, 72.5% similarity)			
					Cry24Ba1 (27.0% identity, 40.3% similarity)			
APG00028	47	48			EP_1947184-8.01 (30.4% identity, 44.2% similarity)	Cry	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					EP_1947184-6.01 (29.4% identity, 43.2% similarity)			
					F0PZN0_BACT0 (24.4% identity, 33.9% similarity)			
					Cry4Cc1 (24.1% identity, 35.0% similarity)			
APG00029	49				APG00100 (67.8% identity, 80.5% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					AFB18319.1 (26.3% identity, 43.3% similarity)			
					US_5518897_A-1.01 (26.3% identity, 43.1% similarity)			
					US_6071877_A-7 (26.3% identity, 43.1% similarity)			
					Cry11Ba1 (23.8% identity, 37.5% similarity)			
APG00030	50	51			US_8461415_B2-42 (72.5% identity, 85.3% similarity)	Cry	75, 80, 85, 90, 95, 96, 97, 98, 99	90, 95, 96, 97, 98, 99
					APG00096 (72.1% identity, 84.8% similarity)			
					APG00114 (64.1% identity, 81.1% similarity)			
					US_8461415_B2-43 (61.3% identity, 70.6% similarity)			

Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US_2013_0227743_A1_194 (42.3% identity, 48.4% similarity)			
					Cry42Aa1 (22.6% identity, 35.5% similarity)			
APG00031	52	53			APG00127 (99.5% identity, 99.7% similarity)	Cry	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					B7NZX8_BACTU (54.3% identity, 68.6% similarity)			
					B8K1J3_BACTU (35.3% identity, 44.7% similarity)			
					AAQ73470.1 (35.0% identity, 44.4% similarity)			
					Cry8Ja1 (34.4% identity, 43.4% similarity)			
APG00032	54	55, 56			WP_019419510.1 (40.7% identity, 55.0% similarity)	Cry	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					K0G027_BACTU (36.2% identity, 53.0% similarity)			
					ADO51070.1 (36.1% identity, 52.9% similarity)			
					Cry70Bb1 (34.5% identity, 52.1% similarity)			
APG00033	57				APG00104 (84.1% identity, 89.0% similarity)	Vip	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	80, 85, 90, 95, 96, 97, 98, 99
					APG00077 (76.5% identity, 84.1% similarity)			
					KEZ80024.1 (65.7% identity, 77.5% similarity)			
					US_6204435-4 (24.2% identity, 41.4% similarity)			
					Vip3Aa49 (23.9% identity, 40.6% similarity)			
APG00035	58	59			K0FZN9_BACTU (90.9% identity, 96.0% similarity)	Bin	95, 96, 97, 98, 99	97, 98, 99
					J8HJ33_BACCE (87.9% identity, 94.9% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US20130227743A1_6 (80.7% identity, 86.6% similarity)			
					APG00011 (75.9% identity, 84.7% similarity)			
					Cry35Ac2 (21.9% identity, 40.7% similarity)			
APG00036	60	61, 229			APG00201 (79.3% identity, 85.9% similarity)	Mtx	75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					APG00022 (78.8% identity, 84.4% similarity)			
					APG00006 (76.1% identity, 83.7% similarity)			
					J8YPM2_BACCE (74.4% identity, 83.7% similarity)			
					US20130227743A1_100 (73.8% identity, 83.1% similarity)			
					US20130227743A1_60 (47.7% identity, 49.3% similarity)			
					US_8461421_B2-117 (22.6% identity, 36.6% similarity)			
APG00040	62	63			Cry54Ba1 (31.8% identity, 45.1% similarity)	Cry	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
APG00041	64	65		66	APG00145 (77.4% identity, 83.5% similarity)	Cry	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	80, 85, 90, 95, 96, 97, 98, 99
					AEH76822.1 (67.0% identity, 77.8% similarity)			
					J8N7T9_BACCE (65.8% identity, 77.7% similarity)			
					APG00130 (58.4% identity, 66.1% similarity)			
					X2J6C3_BACTU (56.0% identity, 68.9% similarity)			
					APG00140 (55.7% identity, 68.0% similarity)			
					Cry42Aa1 (37.5% identity, 52.6% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
APG00042	67	68			N1LIK5_9BACI (67.1% identity, 80.8% similarity)	Cry	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					N1LSG2_9BACI (31.2% identity, 49.1% similarity)			
					N1LPH2_9BACI (29.9% identity, 46.0% similarity)			
					Cry42Aa1 (25.4% identity, 37.1% similarity)			
APG00043	69	70			US_2013_0227743_A1_178 (27.1% identity, 42.2% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					WP_017762581.1 (25.4% identity, 37.2% similarity)			
					WP_017762616.1 (24.0% identity, 39.6% similarity)			
					WP_017762619.1 (21.9% identity, 35.2% similarity)			
APG00044	71	72			J8HQM8_BACCE (50.4% identity, 64.3% similarity)	Cry	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					BAD35166.1 (44.6% identity, 58.4% similarity)			
					BAD35163.1 (42.2% identity, 55.5% similarity)			
					Cry73Aa (38.4% identity, 51.8% similarity)			
APG00045	73	74, 75, 76			APG00110 (61.0% identity, 71.8% similarity)	Cry	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					R8DLK4_BACCE (49.8% identity, 62.7% similarity)			
					US_8461421_B2-100 (30.0% identity, 47.3% similarity)			
					R8EX84_BACCE (29.2% identity, 42.7% similarity)			
					Cry70Ba1 (22.5% identity, 38.0% similarity)			
APG00047	77				J8F3U1_BACCE (33.1% identity, 43.7% similarity)	Bin	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US_8829279_B2-4 (23.8% identity, 40.1% similarity)			
					US_8829279_B2-39 (23.2% identity, 37.4% similarity)			
					Cry35Ab1 (19.3% identity, 31.6% similarity)			
APG00049	78	79			WP_003290257.1 (91.5% identity, 93.8% similarity)	Mtx	95, 96, 97, 98, 99	95, 96, 97, 98, 99
					WP_008180054.1 (55.7% identity, 69.0% similarity)			
					WP_000790613.1 (55.0% identity, 67.6% similarity)			
					WP_016099228.1 (54.6% identity, 69.6% similarity)			
APG00050	80				CAJ86541.1 (31.3% identity, 44.4% similarity)	Bin	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					CAJ86542.1 (31.0% identity, 44.8% similarity)			
					P12964.1 (28.9% identity, 40.4% similarity)			
					Cry36Aa1 (21.0% identity, 34.9% similarity)			
APG00051	81	82			C3ICE4_BACTU (31.6% identity, 47.8% similarity)	Mtx	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					J8Y0J8_BACCE (31.6% identity, 47.8% similarity)			
					WP_033690552.1 (29.9% identity, 44.7% similarity)			
					J7XTF7_BACCE (29.9% identity, 44.4% similarity)			
APG00053	83				WP_017762616.1 (28.3% identity, 44.2% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_200 (22.7% identity, 38.9% similarity)			
					WP_017762581.1 (22.5% identity, 35.6% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					WP_017762619.1 (22.4% identity, 34.2% similarity)			
APG00054	84	85			APG00068 (68.4% identity, 77.4% similarity)	Cry32	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	75, 80, 85, 90, 95, 96, 97, 98, 99
					ADK66923.1 (59.6% identity, 69.3% similarity)			
					Cry32Ab1 (56.9% identity, 67.1% similarity)			
					APG00185 (50.1% identity, 61.2% similarity)			
					US_8461421_B2-38_1 (60.5% identity, 70.5% similarity)			
					US_8461421_B2-39_1 (60.4% identity, 71.0% similarity)			
APG00055	86	87			US_8796026_B2-6 (80.0% identity, 86.5% similarity)	Mtx	85, 90, 95, 96, 97, 98, 99	90, 95, 96, 97, 98, 99
					WP_000875423.1 (78.6% identity, 86.2% similarity)			
					US_8796026_B2-4 (77.8% identity, 87.6% similarity)			
					US_8796026_B2-8 (77.6% identity, 86.3% similarity)			
					APG00174 (70.3% identity, 80.5% similarity)			
APG00057	88	89			N1LT74_9BACI (47.0% identity, 63.0% similarity)	Cry	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					N1LSG2_9BACI (34.6% identity, 53.7% similarity)			
					N1LPH2_9BACI (32.9% identity, 47.5% similarity)			
					Cry41Aa1 (24.2% identity, 38.0% similarity)			
APG00060	90	91			WP_017154552.1 (57.8% identity, 62.5% similarity)	Bin	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					KEZ80012.1 (50.4% identity, 57.6% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					WP_003308586.1 (41.3% identity, 49.6% similarity)			
					Cry49Aa1 (15.3% identity, 24.5% similarity)			
APG00061	92	93		94	US20130227743A1_198 (24.5% identity, 39.9% similarity)	Cry	25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					T1WCQ4_BACTU (21.5% identity, 31.9% similarity)			
					K0G027_BACTU (21.3% identity, 31.9% similarity)			
					Cry70Bb1 (20.6% identity, 31.1% similarity)			
APG00063	95				CAJ86541.1 (39.9% identity, 53.2% similarity)	Bin	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					CAJ86542.1 (39.4% identity, 52.2% similarity)			
					US_6555655_B1-14 (34.1% identity, 45.5% similarity)			
					Cry35Ab3 (19.3% identity, 34.1% similarity)			
APG00069	96	97, 98			N1LSG2_9BACI (29.1% identity, 40.6% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					N1LPH2_9BACI (27.6% identity, 36.9% similarity)			
					N1LT74_9BACI (27.0% identity, 38.5% similarity)			
					Cry41Ab1 (20.3% identity, 29.3% similarity)			
APG00077	99	100			APG00033 (76.5% identity, 84.1% similarity)	Vip	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	80, 85, 90, 95, 96, 97, 98, 99
					APG00104 (70.9% identity, 79.8% similarity)			
					KEZ80024.1 (67.4% identity, 78.3% similarity)			
					US_8334431_B2-14 (24.7% identity, 42.5% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US_6204435-4 (24.2% identity, 42.2% similarity)			
					Vip3Aa49 (23.9% identity, 41.7% similarity)			
APG00080	101	102, 103, 104			US_8759619_B2-17 (23.1% identity, 32.6% similarity)	Cry	25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US_7923602_B2-6 (21.4% identity, 31.2% similarity)			
					AEH76817.1 (20.5% identity, 29.9% similarity)			
					Cry21Ba1 (19.5% identity, 30.5% similarity)			
APG00081	105	106			APG00066 (83.1% identity, 90.3% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					CAC80985.1 (29.7% identity, 46.3% similarity)			
					Cry56Aa2 (28.5% identity, 44.4% similarity)			
APG00082	107	108, 109, 110			J8MY88_BACCE (48.5% identity, 56.4% similarity)	Cry	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US_8759619_B2-25 (38.5% identity, 48.7% similarity)			
					US_8759619_B2-23 (37.4% identity, 47.5% similarity)			
					Cry32Ea1 (36.6% identity, 46.8% similarity)			
APG00083	111	112			BAC06484.1 (37.4% identity, 53.6% similarity)	Cry	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					CR5BA_BACTU (36.7% identity, 51.0% similarity)			
					AFJ04417.1 (36.6% identity, 51.1% similarity)			
					WP_023521141.1 (36.6% identity, 51.1% similarity)			
APG00086	113	114, 115			N1LPH2_9BACI (67.8% identity, 81.5% similarity)	Cry	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99

Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					APG00122 (62.8% identity, 73.8% similarity)			
					N1LSG2_9BACI (61.0% identity, 72.9% similarity)			
					N1LT74_9BACI (33.3% identity, 52.2% similarity)			
					Cry41Ba1 (26.8% identity, 40.5% similarity)			
APG00088	116	117			A9VV88_BACWK (67.1% identity, 76.8% similarity)	Cry	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_24 (64.0% identity, 73.4% similarity)			
					WP_025988975.1 (51.5% identity, 57.3% similarity)			
					Cry8Ca3 (21.6% identity, 31.8% similarity)			
APG00089	118	119			US_8759619_B2-25 (51.5% identity, 62.4% similarity)	Cry32	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US_8318900_B2-87 (50.3% identity, 59.0% similarity)			
					Cry32Ab1 (49.0% identity, 60.1% similarity)			
APG00091	120	121			APG00021 (93.6% identity, 96.4% similarity)	Mtx	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					BAD22577.1 (30.8% identity, 46.2% similarity)			
					US_8461421_B2-102 (30.8% identity, 45.8% similarity)			
					WP_029440439.1 (27.1% identity, 40.9% similarity)			
					Cry33Aa1 (26.0% identity, 40.3% similarity)			
APG00092	122	123			R8S542_BACCE (46.0% identity, 62.8% similarity)	Mtx	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					R8S773_BACCE (43.1% identity, 60.6% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					R8R7A7_BACCE (40.2% identity, 60.3% similarity)			
					A0A015NB99_BACTU (31.4% identity, 48.4% similarity)			
APG00093	124				US20140096281A1_15 (45.6% identity, 57.7% similarity)	Cry	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20140096281A1_16 (45.5% identity, 57.7% similarity)			
					US20140096281A1_14 (45.4% identity, 57.4% similarity)			
					Cry42Aa1 (24.5% identity, 36.7% similarity)			
APG00096	125	126, 127			US_8461415_B2-42 (89.2% identity, 93.3% similarity)	Cry	90, 95, 96, 97, 98, 99	95, 96, 97, 98, 99
					US_8461415_B2-43 (72.7% identity, 75.8% similarity)			
					APG00030 (72.1% identity, 84.8% similarity)			
					APG00114 (66.1% identity, 80.8% similarity)			
					US_2013_0227743_A1_194 (47.4% identity, 49.9% similarity)			
					Cry42Aa1 (22.9% identity, 37.1% similarity)			
APG00098	128	129			US_8318900_B2-95 (31.9% identity, 46.7% similarity)	Mtx	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_84 (31.0% identity, 44.8% similarity)			
					ADE27985.1 (30.7% identity, 44.3% similarity)			
					BAJ05397.1 (28.0% identity, 44.8% similarity)			
APG00100	130				APG00029 (67.8% identity, 80.5% similarity)	Cry	25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					B8PS57_BACTU (23.8% identity, 39.9% similarity)			

Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					AFB18319.1 (23.7% identity, 39.5% similarity)			
					WP_000390241.1 (23.7% identity, 39.4% similarity)			
					Cry11Ba1 (23.0% identity, 37.0% similarity)			
APG00102	131				EP_2130839-1.01 (34.6% identity, 50.3% similarity)	Cry	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					W4EWR0_9BACI (34.5% identity, 50.2% similarity)			
					WP_033728958.1 (34.2% identity, 49.7% similarity)			
					Cry2Af2 (31.2% identity, 47.8% similarity)			
APG00103	132	133			J8JK75_BACCE (47.6% identity, 59.4% similarity)	Cry	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					J8N2R5_BACCE (47.3% identity, 59.5% similarity)			
					US20140096281A1_12 (28.1% identity, 34.1% similarity)			
					Cry32Ea1 (25.8% identity, 34.8% similarity)			
APG00106	134				EJS10693.1 (73.3% identity, 82.1% similarity)	Vip	75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					WP_033733438.1 (69.6% identity, 76.9% similarity)			
					AGT29561.1 (58.9% identity, 69.8% similarity)			
					Vip1Da1 (26.8% identity, 42.1% similarity)			
APG00109	135	136, 137, 138			R8DHS1_BACCE (92.1% identity, 93.1% similarity)	Cry	95, 96, 97, 98, 99	95, 96, 97, 98, 99
					US20130227743A1_30 (76.4% identity, 78.5% similarity)			
					APG00026 (69.2% identity, 79.5% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					K0FZJ7_BACTU (63.0% identity, 70.6% similarity)			
					Cry24Ba1 (25.8% identity, 38.8% similarity)			
APG00111	139	140, 141		142	ACF15199.1 (52.0% identity, 61.7% similarity)	Cry	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					APG00204 (50.7% identity, 60.1% similarity)			
					US_7351881_B2-25 (50.1% identity, 61.0% similarity)			
					US_8044266_B2-3 (49.5% identity, 59.2% similarity)			
					Cry39Aa1 (33.0% identity, 45.6% similarity)			
APG00122	143	144, 145			N1LSG2_9BACI (76.2% identity, 83.9% similarity)	Cry	80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					APG00086 (62.8% identity, 73.8% similarity)			
					N1LPH2_9BACI (59.5% identity, 72.8% similarity)			
					N1LT74_9BACI (33.6% identity, 51.0% similarity)			
					Cry41Ba1 (26.4% identity, 41.8% similarity)			
APG00123	146	147, 148, 149		150	J8HQM8_BACCE (24.8% identity, 37.9% similarity)	Cry	25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					WP_033694850.1 (23.6% identity, 39.3% similarity)			
					WP_000774801.1 (22.9% identity, 36.6% similarity)			
					Cry41Ab1 (22.6% identity, 36.1% similarity)			
APG00125	151	152			US_7105332_B2-12 (31.4% identity, 47.0% similarity)	Cry	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					ACQ91256.1 (20.3% identity, 29.4% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					W0LR53_BACTU (20.0% identity, 28.8% similarity)			
					Cry4Cb1 (19.6% identity, 28.8% similarity)			
APG00126	153	154			WP_016110460.1 (92.6% identity, 95.1% similarity)	Cy1	95, 96, 97, 98, 99	96, 97, 98, 99
					APG00128 (55.2% identity, 70.8% similarity)			
					WP_016110459.1 (54.4% identity, 70.0% similarity)			
					US_6686452-2 (39.1% identity, 54.7% similarity)			
					Cyt2Ba15 (36.2% identity, 53.6% similarity)			
APG00127	155	156			APG00031 (99.5% identity, 99.7% similarity)	Cry	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					B7NZX8_BACTU (54.7% identity, 68.6% similarity)			
					B8K1J3_BACTU (35.5% identity, 44.7% similarity)			
					AAQ73470.1 (35.3% identity, 44.4% similarity)			
					Cry8Ja1 (34.7% identity, 43.4% similarity)			
APG00128	157				WP_016110459.1 (94.1% identity, 97.5% similarity)	Cyt	95, 96, 97, 98, 99	98, 99
					APG00126 (55.2% identity, 70.8% similarity)			
					WP_016110460.1 (53.0% identity, 69.3% similarity)			
					US_7786351_B2-349 (42.4% identity, 57.6% similarity)			
					Cyt2Ba15 (39.8% identity, 56.8% similarity)			
APG00129	158	159			W8YCZ9_BACTU (95.8% identity, 98.3% similarity)	Mtx	96, 97, 98, 99	99

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US20130227743A1_106 (22.9% identity, 40.0% similarity)			
APG00133	160	161			W0LR53_BACTU (21.4% identity, 30.8% similarity)	Cry	25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					ACQ91256.1 (21.3% identity, 30.7% similarity)			
					ACU57500.1 (21.2% identity, 30.1% similarity)			
					Cry4Cb1 (21.0% identity, 30.4% similarity)			
APG00142	162				US20100298211A1_9 (22.8% identity, 42.6% similarity)	Cry	25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					AFB18319.1 (21.4% identity, 37.7% similarity)			
					US20130227743A1_38 (21.3% identity, 37.6% similarity)			
					Cry18Aa1 (20.7% identity, 31.6% similarity)			
APG00145	163	164		165	APG00041 (77.4% identity, 83.5% similarity)	Cry	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	80, 85, 90, 95, 96, 97, 98, 99
					AEH76822.1 (65.9% identity, 75.9% similarity)			
					J8N7T9_BACCE (64.9% identity, 75.0% similarity)			
					APG00130 (57.1% identity, 63.1% similarity)			
					X2J6C3_BACTU (54.4% identity, 67.8% similarity)			
					APG00140 (54.1% identity, 66.5% similarity)			
					Cry42Aa1 (37.7% identity, 53.2% similarity)			
APG00146	166	167			C3GC23_BACTU (94.4% identity, 95.9% similarity)	Mtx	95, 96, 97, 98, 99	96, 97, 98, 99

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US20130227743A1_102 (62.7% identity, 77.3% similarity)			
					WP_036654376.1 (44.4% identity, 58.8% similarity)			
					W2E623_9BACL (43.3% identity, 57.3% similarity)			
APG00147	168	169			J8HH32_BACCE (33.6% identity, 49.2% similarity)	Bin	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					WP_002090518.1 (29.3% identity, 45.4% similarity)			
					R8CM29_BACCE (29.3% identity, 44.9% similarity)			
					Cry49Ab1 (17.8% identity, 29.7% similarity)			
APG00148	170	171			US20130227743A1_112 (44.0% identity, 61.3% similarity)	Mtx	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_114 (40.1% identity, 58.3% similarity)			
					WP_000239374.1 (26.9% identity, 43.2% similarity)			
					AIK29697.1 (25.1% identity, 40.2% similarity)			
APG00149	172	173			R8S3D4_BACCE (29.4% identity, 42.7% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_34 (29.2% identity, 46.5% similarity)			
					US_7521235_B2-2 (28.8% identity, 42.2% similarity)			
					Cry8Ga2 (18.8% identity, 27.8% similarity)			
APG00151	174	175			US20130227743A1_40 (60.8% identity, 69.2% similarity)	Bin	65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_48 (36.8% identity, 45.8% similarity)			
					AEH76820.1 (34.4% identity, 43.8% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					Cry4Ba4 (26.4% identity, 37.3% similarity)			
APG00161	176	177, 178, 179			APG00085 (78.4% identity, 86.5% similarity)	Cry	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US_8461415_B2-47 (37.2% identity, 50.1% similarity)			
					US_8461415_B2-62 (37.1% identity, 49.6% similarity)			
					US_8461415_B2-49 (35.9% identity, 49.9% similarity)			
					Cry8Ia1 (23.5% identity, 35.2% similarity)			
APG00167	180				CAJ86541.1 (24.9% identity, 36.7% similarity)	Bin	25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					CAJ86542.1 (24.7% identity, 35.9% similarity)			
					Cry36Aa1 (22.6% identity, 36.0% similarity)			
APG00169	181	182			Cry54Aa2 (32.8% identity, 44.2% similarity)	Cry	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
APG00174	183	184, 185			US_8796026_B2-6 (74.8% identity, 83.7% similarity)	Mtx	75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					WP_000875423.1 (74.5% identity, 84.3% similarity)			
					WP_003275939.1 (73.6% identity, 83.1% similarity)			
					US_8796026_B2-4 (72.1% identity, 81.7% similarity)			
					APG00055 (70.3% identity, 80.5% similarity)			
APG00179	186	187			US_8759619_B2-24 (30.6% identity, 46.0% similarity)	Cry	35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					J8N7T9_BACCE (28.0% identity, 41.6% similarity)			
					X2J6C3_BACTU (27.7% identity, 42.0% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					Cry73Aa (27.0% identity, 40.0% similarity)			
APG00185	188	189	190		APG00068 (51.3% identity, 61.1% similarity)	Cry32	80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					Cry32Ea1 (50.5% identity, 61.3% similarity)			
					APG00054 (50.1% identity, 61.2% similarity)			
					US_8759619_B2-9_1 (75.3% identity, 81.6% similarity)			
					US_8461421_B2-26_1 (61.6% identity, 72.6% similarity)			
					US_8318900_B2-32_1 (58.3% identity, 66.9% similarity)			
APG00191	191				KEZ80012.1 (65.9% identity, 78.8% similarity)	Bin	70, 75, 80, 85, 90, 95, 96, 97, 98, 99	80, 85, 90, 95, 96, 97, 98, 99
					WP_017154552.1 (61.5% identity, 75.4% similarity)			
					APG00090 (61.1% identity, 73.6% similarity)			
					WP_003308586.1 (47.8% identity, 62.7% similarity)			
					Cry49Ab1 (21.9% identity, 33.8% similarity)			
APG00199	192				US20100298211A1_9 (43.3% identity, 59.7% similarity)	Cry	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US20130227743A1_16 (42.4% identity, 59.9% similarity)			
					C3I9T3_BACTU (40.9% identity, 57.6% similarity)			
					Cry11Aa1 (25.4% identity, 40.2% similarity)			
APG00201	193	194			APG00006 (79.7% identity, 88.2% similarity)	Mtx	80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					APG00036 (79.3% identity, 85.9% similarity)			

Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					APG00022 (78.8% identity, 85.9% similarity)			
					J8YPM2_BACCE (75.7% identity, 84.6% similarity)			
					US20130227743A1_100 (75.3% identity, 84.5% similarity)			
					US20130227743A1_60 (46.6% identity, 51.1% similarity)			
					A8LVM9_SALAI (24.6% identity, 38.4% similarity)			
APG00202	195	196			APG00208 (88.6% identity, 93.2% similarity)	Mtx	90, 95, 96, 97, 98, 99	95, 96, 97, 98, 99
					US20130227743A1_120 (88.1% identity, 91.9% similarity)			
					J8F337_BACCE (71.6% identity, 81.0% similarity)			
					US20130227743A1_122 (32.9% identity, 48.8% similarity)			
					R8TCG2_BACCE (23.2% identity, 36.7% similarity)			
APG00205	197	198			APG00018 (97.8% identity, 99.0% similarity)	Mtx	75, 80, 85, 90, 95, 96, 97, 98, 99	85, 90, 95, 96, 97, 98, 99
					C3IAI7_BACTU (70.7% identity, 80.9% similarity)			
					J8HRD0_BACCE (64.6% identity, 74.2% similarity)			
					WP_018669999.1 (48.1% identity, 63.0% similarity)			
					H0UDJ3_BRELA (47.6% identity, 62.0% similarity)			
APG00206	199	200			US20130227743A1_198 (25.0% identity, 42.3% similarity)	Cry	30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					US_8461415_B2-42 (20.2% identity, 36.1% similarity)			
					US_8461415_B2-43 (19.6% identity, 31.7% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					Cry41Ab1 (17.9% identity, 31.0% similarity)			
APG00208	201	202			APG00202 (88.6% identity, 93.2% similarity)	Mtx	85, 90, 95, 96, 97, 98, 99	90, 95, 96, 97, 98, 99
					US20130227743A1_120 (84.1% identity, 89.4% similarity)			
					J8F337_BACCE (71.6% identity, 80.8% similarity)			
					US20130227743A1_122 (32.0% identity, 49.5% similarity)			
					R8TCG2_BACCE (24.2% identity, 37.9% similarity)			
APG00222	203	204, 205, 206			US20130227743A1_48 (53.5% identity, 67.1% similarity)	Cry	55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					AEH76820.1 (49.3% identity, 62.3% similarity)			
					US20100298211A1_8 (46.0% identity, 58.1% similarity)			
					Cry32Ea1 (43.8% identity, 56.9% similarity)			
APG00234	207	208, 209			WP_001036192.1 (59.5% identity, 74.8% similarity)	Mtx	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	75, 80, 85, 90, 95, 96, 97, 98, 99
					WP_000163136.1 (59.2% identity, 74.5% similarity)			
					WP_000790613.1 (58.7% identity, 71.9% similarity)			
					APG00013 (57.7% identity, 73.3% similarity)			
					WP_003290257.1 (49.0% identity, 64.8% similarity)			
APG00272	210	211			APG00019 (80.2% identity, 81.8% similarity)	Mtx	45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99	60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99
					WP_034679607.1 (43.8% identity, 59.1% similarity)			
					US_8829279_B2-61 (34.4% identity, 53.4% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
					US_8829279_B2-2 (34.2% identity, 53.4% similarity)			
					C3HSG6_BACTU (33.5% identity, 54.0% similarity)			
APG00299	212	213			US20130227743A1_108 (93.6% identity, 97.3% similarity)	Mtx	95, 96, 97, 98, 99	98, 99
					R8CLR6_BACCE (93.0% identity, 96.6% similarity)			
					J9BNC9_BACCE (92.6% identity, 97.0% similarity)			
					J8E9X3_BACCE (92.3% identity, 96.6% similarity)			
					APG00010 (57.9% identity, 74.5% similarity)			
APG00526	214	215, 216, 217			APG00025 (93.9% identity, 96.5% similarity)	Cry70	95,96,97,98,99	97,98,99
					WP_000162158.1 (93.9% identity, 96.4% similarity)			
					Cry70Ba1 (93.6% identity, 96.2% similarity)			
					APG00728 (92.5% identity, 95.2% similarity)			
					AGU12794.1 (89.7% identity, 94.3% similarity)			
APG00717	218				APG00029 (96.8% identity, 98.6% similarity)	Cry	30,35,40,45,50,55,60,65,70,75,80,85,90,95,96,97,98,99	45,50,55,60,65,70,75,80,85,90,95,96,97,98,99
					APG00100 (69.4% identity, 80.1% similarity)			
					Cry11Aa4 (25.6% identity, 43.1% similarity)			

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Gene Name	Full-length SEQ ID No.	Modified SEQID No.(s)	CryBP1 SEQ ID No.	Split-Cry C-terminus SEQ ID No.	Homologs	Gene Class	Polypeptides of the invention (and polynucleotides encoding the same) include those having the % sequence identity listed below	Polypeptides of the invention (and polynucleotides encoding the same) include those having the similarity set forth below
APG00728	219	220, 221, 222			APG00025 (93.8% identity, 97.0% similarity)	Cry70	95,96,97,98,99	97,98,99
					WP_000162158.1 (92.8% identity, 96.3% similarity)			
					Cry70Ba1 (92.5% identity, 96% similarity)			
					APG00526 (92.5% identity, 95.2% similarity)			
					AGU12794.1 (86.7% identity, 92.6% similarity)			
APG00847	223	224, 225			APG00201 (97.0% identity, 98.7% similarity)	Mtx	80,85,90,95,96,97,98,99	85,90,95,96,97,98,99
					APG00006 (79.0% identity, 88.2% similarity)			
					APG00022 (77.7% identity, 85.1% similarity)			
					WP_000963933.1 (75.1% identity, 84.9% similarity)			
					US_2013_0227743_A1-100 (75.0% identity, 84.9% similarity)			
APG00982	226	227			APG00092 (98.2% identity, 99.1% similarity)	Mtx	50,55,60,65,70,75,80,85,90,95,96,97,98,99	65,70,75,80,85,90,95,96,97,98,99
					WP_016098287.1 (45.0% identity, 62.6% similarity)			
					WP_016098181.1 (42.8% identity, 61.1% similarity)			
					WP_016099611.1 (39.9% identity, 60.1% similarity)			
					WP_033699741.1 (32.4% identity, 50.0% similarity)			

i. Classes of Pesticidal proteins

[0013] The pesticidal proteins provided herein and the nucleotide sequences encoding them are useful in methods for impacting pests. That is, the compositions and methods of the invention find use in agriculture for controlling or killing pests, including pests of many crop plants. The pesticidal proteins provided herein are toxin proteins from bacteria and exhibit activity against certain pests. The pesticidal proteins are from several classes of toxins including Cry, Cyt, BIN, and Mtx toxins. See, for example, Table 1 for the specific protein classifications of the various SEQ ID NOS provided herein. In addition, reference is made throughout this disclosure to Pfam database entries. The Pfam database is a database of protein families, each represented by multiple sequence alignments and a profile hidden Markov model. Finn *et al.* (2014) *Nucl. Acid Res.* Database Issue 42:D222-D230.

[0014] *Bacillus thuringiensis* (Bt) is a gram-positive bacterium that produces insecticidal proteins as crystal inclusions during its sporulation phase of growth. The proteinaceous inclusions of *Bacillus thuringiensis* (Bt) are called crystal proteins or δ-endotoxins (or Cry proteins), which are toxic to members of the class Insecta and other invertebrates. Similarly, Cyt proteins are parasporal inclusion proteins from Bt that exhibits hemolytic (cytolitic) activity or has obvious sequence similarity to a known Cyt protein. These toxins are highly specific to their target organism, and are innocuous to humans, vertebrates, and plants.

[0015] The structure of the Cry toxins reveals five conserved amino acid blocks, concentrated mainly in the center of the domain or at the junction between the domains. The Cry toxin consists of three domains, each with a specific function. Domain I is a seven α-helix bundle in which a central helix is completely surrounded by six outer helices. This domain is implicated in channel formation in the membrane. Domain II appears as a triangular column of three anti-parallel β–sheets, which are similar to antigen–binding regions of immunoglobulins. Domain III contains anti-parallel β–strands in a β sandwich form. The N-terminal part of the toxin protein is responsible for its toxicity and specificity and contains five conserved regions. The C-terminal part is

usually highly conserved and probably responsible for crystal formation. See, for example, U.S. Patent No. 8,878,007.

[0016] Strains of *B. thuringiensis* show a wide range of specificity against different insect orders (Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Phthiraptera or Mallophaga, and Acari) and other invertebrates (Nemathelminthes, Platyhelminthes, and Sarocomastebrates). The cry proteins have been classified into groups based on toxicity to various insect and invertebrate groups. Generally, Cry I demonstrates toxicity to lepidopterans, Cry II to lepidopterans and dipterans, CryIII to coleopterans, Cry IV to dipterans, and Cry V and Cry VI to nematodes. New Cry proteins can be identified and assigned to a Cry group based on amino acid identity. See, for example, Bravo, A. (1997) *J. of Bacteriol.* 179:2793-2801; Bravo *et al.* (2013) *Microb. Biotechnol.* 6:17-26, herein incorporated by reference.

[0017] Over 750 different *cry* gene sequences have been classified into 73 groups (Cry1–Cry73), with new members of this gene family continuing to be discovered (Crickmore *et al.* (2014) www.btnomenclature.info/). The *cry* gene family consists of several phylogentically non-related protein families that may have different modes of action: the family of three-domain Cry toxins, the family of mosquitocidal Cry toxins, the family of the binary-like toxins, and the Cyt family of toxins (Bravo et al., 2005). Some Bt strains produce additional insecticidal toxins, the VIP toxins. See, also, Cohen *et al.* (2011) *J. Mol. Biol.* 413:4-814; Crickmore *et al.* (2014) *Bacillus thuringiensis* toxin nomenclature, found on the world wide web at lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/; Crickmore *et al.* (1988) *Microbiol. Mol. Biol. Rev.* 62: 807-813; Gill *et al.* (1992) *Ann. Rev. Entomol.* 37: 807-636; Goldbert *et al.* (1997) *Appl. Environ. Microbiol.* 63:2716-2712; Knowles *et al.* (1992) *Proc. R. Soc. Ser. B.* 248: 1-7; Koni *et al.* (1994) *Microbiology* 140: 1869-1880; Lailak *et al.* (2013) *Biochem. Biophys. Res. Commun.* 435: 216-221; Lopez-Diaz *et al.* (2013) *Environ. Microbiol.* 15: 3030-3039; Perez *et al.* (2007) *Cell. Microbiol.* 9: 2931-2937; Promdonkoy *et al.* (2003) *Biochem. J.* 374: 255-259; Rigden (2009) *FEBS Lett.* 583: 1555-1560; Schnepf *et al.* (1998) *Microbiol. Mol. Biol. Rev.* 62: 775-806; Soberon *et al.* (2013) *Peptides* 41: 87-93; Thiery *et al.* (1998) *J. Am. Mosq. Control Assoc.* 14: 472-476;

Thomas *et al.* (1983) FEBS Lett. 154: 362-368; Wirth *et al.* (1997) *Proc. Natl. Acad. Sci.* U.S.A. 94: 10536-10540; Wirth *et al* (2005) *Appl. Environ. Microbiol.* 71: 185-189; and, Zhang *et al.* (2006) *Biosci. Biotechnol. Biochem.* 70: 2199-2204; each of which is herein incorporated by reference in their entirety.

[0018] Cyt designates a parasporal crystal inclusion protein from *Bacillus thuringiensis* with cytolytic activity, or a protein with sequence similarity to a known Cyt protein. (Crickmore *et al.* (1998) *Microbiol. Mol. Biol. Rev.* 62: 807-813). The gene is denoted by *cyt*. These proteins are different in structure and activity from Cry proteins (Gill *et al.* (1992) *Annu. Rev. Entomol.* 37: 615-636). The Cyt toxins were first discovered in *B. thuringiensis* subspecies *israelensis* (Goldberg *et al.* (1977) *Mosq. News.* 37: 355-358). There are 3 Cyt toxin families including 11 holotype toxins in the current nomenclature (Crickmore *et al.* (2014) *Bacillus thuringiensis* toxin nomenclature found on the world wide web at lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/). The majority of the *B. thuringiensis* isolates with *cyt* genes show activity against dipteran insects (particularly mosquitoes and black flies), but there are also *cyt* genes that have been described in *B. thuringiensis* strains targeting lepidopteran or coleopteran insects (Guerchicoff *et al.* (1997) *Appl. Environ. Microbiol.* 63: 2716-2721).

[0019] The structure of Cyt2A, solved by X-ray crystallography, shows a single domain where two outer layers of α-helix wrap around a mixed β-sheet. Further available crystal structures of Cyt toxins support a conserved α-β structural model with two α-helix hairpins flanking a β-sheet core containing seven to eight β-strands. (Cohen *et al.* (2011) *J. Mol. Biol.* 413: 80 4-814) Mutagenic studies identified β-sheet residues as critical for toxicity, while mutations in the helical domains did not affect toxicity (Adang *et al.*; Diversity of *Bacillus thuringiensis* Crystal Toxins and Mechanism of Action. In: T. S. Dhadialla and S. S. Gill, eds, *Advances in Insect Physiology, Vol. 47*, Oxford: Academic Press, 2014, pp. 39-87.) The representative domain of the Cyt toxin is a δ-endotoxin, Bac_thur_toxin (Pfam PF01338).

[0020] There are multiple proposed models for the mode of action of Cyt toxins, and it is still an area of active investigation. Some Cyt proteins (Cyt1A) have been shown to require the presence of accessory proteins for crystallization. Cyt1A and Cyt2A protoxins

are processed by digestive proteases at the same sites in the N- and C-termini to a stable toxin core. Cyt toxins then interact with non-saturated membrane lipids, such as phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. For Cyt toxins, pore-formation and detergent-like membrane disruption have been proposed as non-exclusive mechanisms; and it is generally accepted that both may occur depending on toxin concentration, with lower concentrations favoring oligomeric pores and higher concentrations leading to membrane breaks. (Butko (2003) *Appl. Environ. Microbiol.* 69: 2415-2422) In the pore-formation model, the Cyt toxin binds to the cell membrane, inducing the formation of cation-selective channels in the membrane vesicles leading to colloid-osmotic lysis of the cell. (Knowles *et al.* (1989) FEBS Lett. 244: 259-262; Knowles *et al.* (1992) *Proc. R. Soc. Ser. B.* 248: 1-7 and Promdonkoy *et al.* (2003) *Biochem. J.* 374: 255-259). In the detergent model, there is a nonspecific aggregation of the toxin on the surface of the lipid bilayer leading to membrane disassembly and cell death. (Butko (2003) *supra*; Manceva *et al.* (2005) *Biochem.* 44: 589-597).

[0021] Multiple studies have shown synergistic activity between Cyt toxins and other *B. thuringiensis* toxins, particularly the Cry, Bin, and Mtx toxins. This synergism has even been shown to overcome an insect's resistance to the other toxin. (Wirth 1997, Wirth 2005, Thiery 1998, Zhang 2006) The Cyt synergistic effect for Cry toxins is proposed to involve Cyt1A binding to domain II of Cry toxins in solution or on the membrane plane to promote formation of a Cry toxin pre-pore oligomer. Formation of this oligomer is independent of the Cyt oligomerization, binding or insertion. (Lailak 2013, Perez 2007, Lopez-Diaz 2013)

[0022] A number of pesticidal proteins unrelated to the Cry proteins are produced by some strains of *B. thuringiensis* and *B. cereus* during vegetative growth (Estruch *et al.* (1996) *Proc Natl Acad Sci USA* 93:5389–5394; Warren *et al.* (1994) WO 94/21795). These vegetative insecticidal proteins, or Vips, do not form parasporal crystal proteins and are apparently secreted from the cell. The Vips are presently excluded from the Cry protein nomenclature because they are not crystal-forming proteins. The term VIP is a misnomer in the sense that some *B. thuringiensis* Cry proteins are also produced during vegetative growth as well as during the stationary and sporulation phases, most notably

Cry3Aa. The location of the Vip genes in the *B. thuringiensis* genome has been reported to reside on large plasmids that also encode cry genes (Mesrati *et al.* (2005) *FEMS Microbiol. Lett.* 244(2):353-8). A web-site for the nomenclature of Bt toxins can be found on the world wide web at lifesci.sussex.ac.uk with the path "/home/Neil_Crickmore/Bt/" and at: "btnomenclature.info/". See also, Schnepf *et al.* (1998) *Microbiol. Mol. Biol. Rev.* 62(3):775-806. Such references are herein incorporated by reference.

[0023] To date four categories of Vips have been identified. Some Vip genes form binary two-component protein complexes; an "A" component is usually the "active" portion, and a "B" component is usually the "binding" portion. (Pfam pfam.xfam.org/family/PF03495). The Vip1 and Vip4 proteins generally contain binary toxin B protein domains. Vip2 proteins generally contain binary toxin A protein domains.

[0024] The Vip1 and Vip2 proteins are the two components of a binary toxin that exhibits toxicity to coleopterans. Vip1Aa1 and Vip2Aa1 are very active against corn rootworms, particularly *Diabrotica virgifera* and *Diabrotica longicornis* (Han *et al.* (1999) *Nat. Struct. Biol.* 6:932–936; Warren GW (1997) "Vegetative insecticidal proteins: novel proteins for control of corn pests" In: Carozzi NB, Koziel M (eds) *Advances in insect control, the role of transgenic plants*; Taylor & Francis Ltd, London, pp 109–21). The membrane-binding 95 kDa Vip1 multimer provides a pathway for the 52 kDa Vip2 ADP-ribosylase to enter the cytoplasm of target western corn rootworm cells (Warren (1997) *supra*). The NAD-dependent ADP-ribosyltransferase Vip2 likely modifies monomeric actin at Arg177 to block polymerization, leading to loss of the actin cytoskeleton and eventual cell death due to the rapid subunit ex-change within actin filaments in vivo (Carlier M. F. (1990) *Adv. Biophys.* 26:51–73).

[0025] Like Cry toxins, activated Vip3A toxins are pore-forming proteins capable of making stable ion channels in the membrane (Lee *et al.* (2003) *Appl. Environ. Microbiol.* 69:4648–4657). Vip3 proteins are active against several major lepidopteran pests (Rang *et al.* (2005) *Appl. Environ. Microbiol.* 71(10):6276-6281; Bhalla *et al.* (2005) *FEMS Microbiol. Lett.* 243:467–472; Estruch *et al.* (1998) WO 9844137; Estruch *et al.* (1996) *Proc Natl Acad Sci USA* 93:5389–5394; Selvapandiyan *et al.* (2001) *Appl. Environ*

Microbiol. 67:5855–5858; Yu *et al.* (1997) *Appl. Environ Microbiol.* 63:532–536). Vip3A is active against *Agrotis ipsilon, Spodoptera frugiperda, Spodoptera exigua, Heliothis virescens*, and *Helicoverpa zea* (Warren *et al.* (1996) WO 96/10083; Estruch *et al.* (1996) *Proc Natl Acad Sci USA* 93:5389–5394). Like Cry toxins, Vip3A proteins must be activated by proteases prior to recognition at the surface of the midgut epithelium of specific membrane proteins different from those recognized by Cry toxins.

[0026] The MTX family of toxin proteins is characterized by the presence of a conserved domain, ETX_MTX2 (pfam 03318). Members of this family share sequence homology with the mosquitocidal toxins Mtx2 and Mtx3 from *Bacillus sphaericus*, as well as with the epsilon toxin ETX from *Clostridium perfringens* (Cole *et al.* (2004) *Nat. Struct. Mol. Biol.* 11: 797-8; Thanabalu *et al.* (1996) *Gene* 170:85-9). The MTX-like proteins are structurally distinct from the three-domain Cry toxins, as they have an elongated and predominately β-sheet-based structure. However, similar to the three-domain toxins, the MTX-like proteins are thought to form pores in the membranes of target cells (Adang *et al.* (2014) *supra*). Unlike the three-domain Cry proteins, the MTX-like proteins are much smaller in length, ranging from 267 amino acids (Cry23) to 340 amino acids (Cry15A).

[0027] To date, only 15 proteins belonging to the family of MTX-like toxins have been assigned Cry names, making this a relatively small class compared to the three-domain Cry family (Crickmore *et al.* (2014) *supra*; Adang *et al.* (2014) *supra*). The members of the MTX-like toxin family include Cry15, Cry23, Cry33, Cry38, Cry45, Cry46, Cry51, Cry60A, Cry60B, and Cry64. This family exhibits a range of insecticidal activity, including activity against insect pests of the Lepidopteran and Coleopteran orders. Some members of this family may form binary partnerships with other proteins, which may or may not be required for insecticidal activity.

[0028] Cry15 is a 34 kDA protein that was identified in *Bacillus thuringiensis* serovar thompsoni HD542; it occurs naturally in a crystal together with an unrelated protein of approximately 40 kDa. The gene encoding Cry15 and its partner protein are arranged together in an operon. Cry15 alone has been shown to have activity against lepidopteran insect pests including *Manduca sexta*, *Cydia pomonella*, and *Pieris rapae*, with the

presence of the 40 kDA protein having been shown to increase activity of Cry15 only against *C. pomonella* (Brown K. and Whiteley H. (1992) *J. Bacteriol.* 174:549-557; Naimov *et al.* (2008) *Appl. Environ. Microbiol.* 74:7145–7151). Further studies are needed to elucidate the function of the partner protein of Cry15. Similarly, Cry23 is a 29 kDA protein that has been shown to have activity against the coleopteran pests *Tribolium castaneum* and *Popillia japonica* together with its partner protein Cry37 (Donovan *et al.* (2000) US Patent No. 6,063,756).

[0029] New members of the MTX-like family are continuing to be identified. An ETX_MTX toxin gene was recently identified in the genome of *Bacillus thuringiensis* serovar tolworthi strain Na205-3. This strain was found to be toxic against the lepidpoteran pest *Helicoverpa armigera*, and it also contained homologs of Cry1, Cry11, Vip1, Vip2, and Vip3 (Palma *et al.* (2014) *Genome Announc.* 2(2): e00187-14. Published online Mar 13, 2014 at doi: 10.1128/genomeA.00187-14; PMCID: PMC3953196). Because the MTX-like proteins have a unique domain structure relative to the three-domain Cry proteins, they are believed to possess a unique mode of action, thereby making them a valuable tool in insect control and the fight against insect resistance.

[0030] Bacterial cells produce large numbers of toxins with diverse specificity against host and non-host organisms. Large families of binary toxins have been identified in numerous bacterial families, including toxins that have activity against insect pests. (Poopathi and Abidha (2010) *J. Physiol. Path.* 1(3): 22-38). *Lysinibacillus sphaericus* (*Ls*), formerly *Bacillus sphaericus*, (Ahmed *et al.* (2007) *Int. J. Syst. Evol. Microbiol.* 57:1117-1125) is well-known as an insect biocontrol strain. *Ls* produces several insecticidal proteins, including the highly potent binary complex BinA/BinB. This binary complex forms a parasporal crystal in *Ls* cells and has strong and specific activity against dipteran insects, specifically mosquitos. In some areas, insect resistance to existing *Ls* mosquitocidal strains has been reported. The discovery of new binary toxins with different target specificity or the ability to overcome insect resistance is of significant interest.

[0031] The Ls binary insecticidal protein complex contains two major polypeptides, a 42 kDa polypeptide and a 51 kDa polypepdide, designated BinA and BinB, respectively

(Ahmed *et al.*(2007) *supra*). The two polypeptides act synergistically to confer toxicity to their targets. Mode of action involves binding of the proteins to receptors in the larval midgut. In some cases, the proteins are modified by protease digestion in the larval gut to produce activated forms. The BinB component is thought to be involved in binding, while the BinA component confers toxicity (Nielsen-LeRoux *et al.* (2001) *Appl. Environ. Microbiol.* 67(11):5049–5054). When cloned and expressed separately, the BinA component is toxic to mosquito larvae, while the BinB component is not. However, co-administration of the proteins markedly increases toxicity (Nielsen-LeRoux *et al.* (2001) *supra*).

[0032] A small number of Bin protein homologs have been described from bacterial sources. Priest *et al.* (1997) *Appl. Environ. Microbiol.* 63(4):1195-1198 describe a hybridization effort to identify new Ls strains, although most of the genes they identified encoded proteins identical to the known BinA/BinB proteins. The BinA protein contains a defined conserved domain known as the Toxin 10 superfamily domain. This toxin domain was originally defined by its presence in BinA and BinB. The two proteins both have the domain, although the sequence similarity between BinA and BinB is limited in this region (<40%). The Cry49Aa protein, which also has insecticidal activity, also has this domain (described below).

[0033] The Cry48Aa/Cry49Aa binary toxin of Ls has the ability to kill *Culex quinquefasciatus* mosquito larvae. These proteins are in a protein structural class that has some similarity to the Cry protein complex of Bacillus thuringiensis (Bt), a well-known insecticidal protein family. The Cry34/Cry35 binary toxin of Bt is also known to kill insects, including Western corn rootworm, a significant pest of corn. Cry34, of which several variants have been identified, is a small (14 kDa) polypeptide, while Cry35 (also encoded by several variants) is a 44 kDa polypeptide. These proteins have some sequence homology with the BinA/BinB protein group and are thought to be evolutionarily related (Ellis *et a*l. (2002) *Appl. Environ. Microbiol.* 68(3):1137-1145).

[0034] Phosphoinositide phospholipase C proteins (PI-PLC; also phosphotidylinositol phospholipase C) are members of the broader group of phospholipase C proteins. Many of these proteins play important roles in signal transduction as part of normal cell

physiology. Several important bacterial toxins also contain domains with similarity to these proteins (Titball, R.W. (1993) *Microbiological Reviews*. 57(2):347-366). Importantly, these proteins are implicated in signal amplification during intoxication of insect cells by Bt Cry proteins (Valaitis, A.P. (2008) *Insect Biochemistry and Molecular Biology*. 38: 611-618).

[0035] The PI-PLC toxin class occurs in *Bacillus* isolates, commonly seen in co-occurrence with homologs to other described toxin classes, such as Binary Toxins. This class of sequences has homology to phosphatidylinositol phosphodiesterases (also referred to as phosphatidylinositol-specific phospholipase C – PI-PLC). The crystal structure and its active site were solved for *B. cereus* PI-PLC by Heinz *et al* (Heinz, *et. al.*, (1995) *The EMBO Journal*. 14(16): 3855-3863). The roles of the *B. cereus* PI-PLC active site amino acid residues in catalysis and substrate binding were investigated by Gässler *et al* using site-directed mutagenesis, kinetics, and crystal structure analysis (Gässler, *et. al.*, (1997) *Biochemistry*. 36(42):12802-13).

[0036] These PI-PLC toxin proteins contain a PLC-like phosphodiesterase, TIM beta/alpha-barrel domain (IPR017946) and/or a Phospholipase C, phosphatidylinositol-specific, X domain (IPR000909) (also referred to as the PI-PLC X-box domain). We have also seen proteins with these domains in combination with other typical *Bacillus* protein toxin domains. This list includes most commonly a lectin domain (IPR000772), a sugar-binding domain that can be present in one or more copies and is thought to bind cell membranes, as well as the Insecticidal crystal toxin (IPR008872) (also referred to as Toxin10 or P42), which is the defining domain of the Binary Toxin.

[0037] Previously, toxins of this PI-PLC class were defined in U.S. Patent No. 8,318,900 B2 SEQ ID NOs 30 (DNA) and 79 (amino acid), in U.S. Patent Publication No. 20110263488A1 SEQ ID NOs 8 (DNA) and 9 (amino acid), and in U.S. Patent No. 8,461,421B2 SEQ ID NOs 3 (DNA) and 63 (amino acid).

[0038] Provided herein are pesticidal proteins from these classes of toxins. The pesticidal proteins are classified by their structure, homology to known toxins and/or their pesticidal specificity.

ii. Variants and Fragments of Pesticidal Proteins and Polynucleotides Encoding the Same

[0039] Pesticidal proteins or polypeptides of the invention include those set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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 and fragments and variants thereof. By "pesticidal toxin" or "pesticidal protein" or "pesticidal polypeptide" is intended a toxin or protein or polypeptide that has activity against one or more pests, including, insects, fungi, nematodes, and the like such that the pest is killed or controlled.

[0040] An "isolated" or "purified" polypeptide or protein, or biologically active portion thereof, is substantially or essentially free from components that normally accompany or interact with the polypeptide or protein as found in its naturally occurring environment. Thus, an isolated or purified polypeptide or protein is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of contaminating protein. When the protein of the invention or biologically active portion thereof is recombinantly produced, optimally culture medium represents less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

[0041] The term "fragment" refers to a portion of a polypeptide sequence of the invention. "Fragments" or "biologically active portions" include polypeptides comprising a sufficient number of contiguous amino acid residues to retain the biological activity, i.e., have pesticidal activity. Fragments of the pesticidal proteins include those that are shorter than the full-length sequences, either due to the use of an alternate downstream start site, or due to processing that produces a shorter protein having pesticidal activity. Processing may occur in the organism the protein is expressed in, or in the pest after ingestion of the protein. Examples of fragments of the proteins can be found in Table 1. A biologically active portion of a pesticidal protein can be a polypeptide that is, for example, 10, 25, 50, 100, 150, 200, 250 or more amino acids in length of any one of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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. Such biologically active portions can be prepared by recombinant techniques and evaluated for pesticidal activity. As used here, a fragment comprises at least 8 contiguous amino acids of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156,

157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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.

[0042] Bacterial genes, including those encoding the pesticidal proteins disclosed herein, quite often possess multiple methionine initiation codons in proximity to the start of the open reading frame. Often, translation initiation at one or more of these start codons will lead to generation of a functional protein. These start codons can include ATG codons. However, bacteria such as *Bacillus sp.* also recognize the codon GTG as a start codon, and proteins that initiate translation at GTG codons contain a methionine at the first amino acid. On rare occasions, translation in bacterial systems can initiate at a TTG codon, though in this event the TTG encodes a methionine. Furthermore, it is not often determined *a priori* which of these codons are used naturally in the bacterium. Thus, it is understood that use of one of the alternate methionine codons may also lead to generation of pesticidal proteins. These pesticidal proteins are encompassed in the present invention and may be used in the methods disclosed herein. It will be understood that, when expressed in plants, it will be necessary to alter the alternate start codon to ATG for proper translation.

[0043] In various embodiments the pesticidal proteins provided herein include amino acid sequences deduced from the full-length nucleotide sequences and amino acid sequences that are shorter than the full-length sequences due to the use of an alternate downstream start site. Thus, the nucleotide sequence of the invention and/or vectors, host cells, and plants comprising the nucleotide sequence of the invention (and methods of making and using the nucleotide sequence of the invention) may comprise a nucleotide sequence encoding an alternate start site.

[0044] It is recognized that modifications may be made to the pesticidal polypeptides provided herein creating variant proteins. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques. Alternatively, native, as yet-unknown or as yet unidentified polynucleotides and/or polypeptides structurally

and/or functionally-related to the sequences disclosed herein may also be identified that fall within the scope of the present invention. Conservative amino acid substitutions may be made in nonconserved regions that do not alter the function of the pesticidal proteins. Alternatively, modifications may be made that improve the activity of the toxin.

Modification of Cry toxins by domain III swapping has resulted in some cases in hybrid toxins with improved toxicities against certain insect species. Thus, domain III swapping could be an effective strategy to improve toxicity of Cry toxins or to create novel hybrid toxins with toxicity against pests that show no susceptibility to the parental Cry toxins. Site-directed mutagenesis of domain II loop sequences may result in new toxins with increased insecticidal activity. Domain II loop regions are key binding regions of initial Cry toxins that are suitable targets for the mutagenesis and selection of Cry toxins with improved insecticidal properties. Domain I of the Cry toxin may be modified to introduce protease cleavage sites to improve activity against certain pests. Strategies for shuffling the three different domains among large numbers of *cry* genes and high throughput bioassay screening methods may provide novel Cry toxins with improved or novel toxicities.

[0045] As indicated, fragments and variants of the polypeptides disclosed herein will retain pesticidal activity. Pesticidal activity comprises the ability of the composition to achieve an observable effect diminishing the occurrence or an activity of the target pest, including for example, bringing about death of at least one pest, or a noticeable reduction in pest growth, feeding, or normal physiological development. Such decreases in numbers, pest growth, feeding or normal development can comprise any statistically significant decrease, including, for example a decrease of about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90%, 95% or greater. It is recognized that the pesticidal activity may be different or improved relative to the activity of the native protein, or it may be unchanged, so long as pesticidal activity is retained. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) *J. Econ. Entomol.* 83:2480-2485; Andrews *et al.* (1988) *Biochem. J.* 252:199-206; Marrone *et al.* (1985) *J. of Economic Entomology* 78:290-293; and U.S. Pat. No. 5,743,477, all of which are herein incorporated by reference in their entirety.

[0046] Polypeptide variants of this disclosure include polypeptides having an amino acid sequence that is at least about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% identical to the amino acid sequence of any of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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 and retain pesticidal activity. Note, Table 1 provides non-limiting examples of variant polypeptides (and polynucleotide encoding the same) for each of SEQ ID NOS: 1-229. A biologically active variant of a pesticidal polypeptide of the invention may differ by as few as about 1-15 amino acid residues, as few as about 1-10, such as about 6-10, as few as 5, as few as 4, as few as 3, as few as 2, or as few as 1 amino acid residue. In specific embodiments, the polypeptides can comprise an N'-terminal or a C'-terminal truncation, which can comprise at least a deletion of 10, 15, 20, 25, 30, 35, 40, 45, 50 amino acids or more from either the N' or C' terminal end of the polypeptide. Table 2 provides protein domains found in SEQ ID NOs: 1-229 based on PFAM data. Both the domain description and the positions within a given SEQ ID NO are provided in Table 2. In specific embodiments, the active variant comprising any one of SEQ ID NOs: 1-229 can comprise at least 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 1-229 and further comprises at least one of the conserved domain set forth in Table 2. For example, in one embodiment, the active

variant will comprise at least 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1, and further comprises the native amino acids at positions 82-294.

Table 2. Summary of PFAM domains in each of SEQ ID NOs: 1-229

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
PG00001	Seq ID 1		PF03945	Endotoxin N	82	294
			PF03944	Endotoxin C	471	608
APG00001 modified	Seq ID 2	Alternate start	PF03945	Endotoxin N	75	287
			PF03944	Endotoxin C	464	601
APG00001 modified	Seq ID 3	Alternate start and 3` Truncation	PF03945	Endotoxin N	75	287
			PF03944	Endotoxin C	464	600
APG00003	Seq ID 4		PF03945	Endotoxin N	73	299
			PF00555	Endotoxin M	304	504
			PF03944	Endotoxin C	514	649
APG00003 modified	Seq ID 5	Alternate start	PF03945	Endotoxin N	68	294
			PF00555	Endotoxin M	299	499
			PF03944	Endotoxin C	509	644
APG00003 modified	Seq ID 6	3` Truncation	PF03945	Endotoxin N	73	299
			PF00555	Endotoxin M	304	504
			PF03944	Endotoxin C	514	648
APG00004	Seq ID 7		PF03945	Endotoxin N	70	292
			PF00555	Endotoxin M	297	506
			PF03944	Endotoxin C	516	658
APG00004 modified	Seq ID 8	3` Truncation	PF03945	Endotoxin N	70	292
			PF00555	Endotoxin M	297	506
			PF03944	Endotoxin C	516	657
APG00006	Seq ID 9		PF03318	ETX MTX2	35	259
APG00006 modified	Seq ID 10	Signal peptide removed	PF03318	ETX MTX2	16	239
APG00007	Seq ID 11		PF03945	Endotoxin N	75	338
			PF00555	Endotoxin M	346	524
			PF03944	Endotoxin C	541	680
APG00007 modified	Seq ID 12	Alternate start	PF03945	Endotoxin N	62	325
			PF00555	Endotoxin M	333	511
			PF03944	Endotoxin C	528	667

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
APG00007 modified	Seq ID 13	Alternate start and 3' Truncation	PF03945	Endotoxin N	62	325
			PF00555	Endotoxin M	333	512
			PF03944	Endotoxin C	528	666
APG00007 modified	Seq ID 14	3' Truncation	PF03945	Endotoxin N	75	338
			PF00555	Endotoxin M	346	525
			PF03944	Endotoxin C	541	679
APG00009	Seq ID 15		PF03318	ETX MTX2	159	372
APG00009 modified	Seq ID 16	Signal peptide removed	PF03318	ETX MTX2	127	325
APG00011	Seq ID 17		PF14200	RicinB lectin 2	2	102
			PF05431	Toxin 10	156	353
APG00011 modified	Seq ID 18	Alternate start	PF14200	RicinB lectin 2	1	98
			PF05431	Toxin 10	152	349
APG00012	Seq ID 19		PF03945	Endotoxin N	56	302
APG00012 modified	Seq ID 20	Alternate start	PF03945	Endotoxin N	53	299
APG00013	Seq ID 22		PF03318	ETX MTX2	24	293
APG00013 modified	Seq ID 23	Signal peptide removed	PF03318	ETX MTX2	10	261
APG00015	Seq ID 25		PF01338	Bac thur toxin	19	240
APG00016	Seq ID 26		PF03318	ETX MTX2	52	290
APG00016 modified	Seq ID 27	Alternate start	PF03318	ETX MTX2	39	276
APG00017	Seq ID 28		PF01338	Bac thur toxin	10	205
APG00018	Seq ID 29		PF03318	ETX MTX2	71	309
APG00018 modified	Seq ID 30	Signal peptide removed	PF03318	ETX MTX2	36	274
APG00019	Seq ID 31		PF03318	ETX MTX2	36	254
APG00019 modified	Seq ID 32	Signal peptide removed	PF03318	ETX MTX2	10	228
APG00020	Seq ID 33		PF03318	ETX MTX2	107	251
APG00020 modified	Seq ID 34	Signal peptide removed	PF03318	ETX MTX2	75	260
APG00021	Seq ID 35		PF03318	ETX MTX2	43	256
APG00021 modified	Seq ID 36	Alternate start	PF03318	ETX MTX2	38	251
APG00022	Seq ID 37		PF03318	ETX MTX2	32	254
APG00022 modified	Seq ID 38	Alternate start	PF03318	ETX MTX2	29	251
APG00024	Seq ID 39		PF05791	Bacillus HBL	66	207
APG00024 modified	Seq ID 40	Alternate start	PF05791	Bacillus HBL	60	201
APG00025	Seq ID 41		PF03945	Endotoxin N	100	340

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
			PF03944	Endotoxin C	532	669
APG00025 modified	Seq ID 42	3' Truncation	PF03945	Endotoxin N	100	340
			PF03944	Endotoxin C	532	668
APG00026	Seq ID 43		PF03945	Endotoxin N	61	296
			PF00555	Endotoxin M	307	514
			PF03944	Endotoxin C	524	657
			PF14200	RicinB lectin 2	702	804
APG00026 modified	Seq ID 44	Alternate start and 3' Truncation	PF03945	Endotoxin N	58	293
			PF00555	Endotoxin M	304	511
			PF03944	Endotoxin C	521	654
APG00026 modified	Seq ID 45	Alternate start	PF03945	Endotoxin N	58	293
			PF00555	Endotoxin M	304	511
			PF03944	Endotoxin C	521	654
			PF14200	RicinB lectin 2	699	801
APG00026 modified	Seq ID 46	3' Truncation	PF03945	Endotoxin N	61	296
			PF00555	Endotoxin M	307	514
			PF03944	Endotoxin C	524	657
APG00028	Seq ID 47		PF03945	Endotoxin N	61	291
			PF00555	Endotoxin M	296	498
			PF03944	Endotoxin C	508	641
APG00028 modified	Seq ID 48	3' Truncation	PF03945	Endotoxin N	61	291
			PF00555	Endotoxin M	296	498
			PF03944	Endotoxin C	508	640
APG00029	Seq ID 49		PF03945	Endotoxin N	32	248
APG00030	Seq ID 50		PF03945	Endotoxin N	58	304
			PF00030	Crystall	735	815
			PF00652	Ricin B lectin	825	958
APG00030 modified	Seq ID 51	Alternate start	PF03945	Endotoxin N	48	294
			PF00030	Crystall	725	805
			PF00652	Ricin B lectin	815	948
APG00031	Seq ID 52		PF03945	Endotoxin N	65	287
			PF00555	Endotoxin M	292	500
			PF03944	Endotoxin C	510	653
APG00031 modified	Seq ID 53	3' Truncation	PF03945	Endotoxin N	65	287
			PF00555	Endotoxin M	292	500
			PF03944	Endotoxin C	510	652
APG00032	Seq ID 54		PF03945	Endotoxin N	100	355

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
			PF03944	Endotoxin C	550	676
APG00032 modified	Seq ID 55	Signal peptide removed and 3` Truncation	PF03945	Endotoxin N	70	325
			PF03944	Endotoxin C	520	645
APG00032 modified	Seq ID 56	Signal peptide removed	PF03945	Endotoxin N	70	325
			PF03944	Endotoxin C	520	646
APG00033	Seq ID 57		PF12495	Vip3A N	16	188
			PF02018	CBM 4 9	543	656
APG00035	Seq ID 58		PF14200	RicinB lectin 2	53	150
			PF05431	Toxin 10	156	353
APG00035 modified	Seq ID 59	Alternate start	PF14200	RicinB lectin 2	44	146
			PF05431	Toxin 10	152	349
APG00036	Seq ID 60		PF03318	ETX MTX2	30	252
APG00036 modified	Seq ID 61	Alternate start	PF03318	ETX MTX2	30	252
APG00040	Seq ID 62		PF03945	Endotoxin N	63	302
			PF00555	Endotoxin M	307	522
			PF03944	Endotoxin C	532	667
APG00040 modified	Seq ID 63	3` Truncation	PF03945	Endotoxin N	63	302
			PF00555	Endotoxin M	307	522
			PF03944	Endotoxin C	532	666
APG00041	Seq ID 64		PF03945	Endotoxin N	63	315
			PF00555	Endotoxin M	322	507
			PF03944	Endotoxin C	517	650
			PF00652	Ricin B lectin	662	789
APG00041 modified	Seq ID 65	3` Truncation	PF03945	Endotoxin N	63	315
			PF00555	Endotoxin M	322	507
			PF03944	Endotoxin C	517	649
APG00042	Seq ID 67		PF03945	Endotoxin N	43	301
			PF03944	Endotoxin C	509	642
APG00042 modified	Seq ID 68	3` Truncation	PF03945	Endotoxin N	43	301
			PF03944	Endotoxin C	509	641
APG00043	Seq ID 69		PF03945	Endotoxin N	128	333
APG00043 modified	Seq ID 70	Signal peptide removed	PF03945	Endotoxin N	91	296
APG00044	Seq ID 71		PF03945	Endotoxin N	45	267
			PF00555	Endotoxin M	272	455
			PF03944	Endotoxin C	465	606
			PF14200	RicinB lectin 2	649	748

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
APG00044 modified	Seq ID 72	3` Truncation	PF03945	Endotoxin N	45	267
			PF00555	Endotoxin M	272	455
			PF03944	Endotoxin C	465	605
APG00045	Seq ID 73		PF03945	Endotoxin N	120	359
			PF03944	Endotoxin C	550	685
			PF01473	CW binding 1	737	754
			PF01473	CW binding 1	767	783
			PF01473	CW binding 1	795	812
			PF01473	CW binding 1	825	841
APG00045 modified	Seq ID 74	Signal peptide removed and 3` Truncation	PF03945	Endotoxin N	96	335
			PF03944	Endotoxin C	526	660
APG00045 modified	Seq ID 75	Signal peptide removed	PF03945	Endotoxin N	96	335
			PF03944	Endotoxin C	526	661
			PF01473	CW binding 1	713	730
			PF01473	CW binding 1	743	759
			PF01473	CW binding 1	771	788
			PF01473	CW binding 1	801	817
APG00045 modified	Seq ID 76	3` Truncation	PF03945	Endotoxin N	120	359
			PF03944	Endotoxin C	550	684
APG00047	Seq ID 77		PF05431	Toxin 10	76	267
APG00049	Seq ID 78		PF03318	ETX MTX2	28	288
APG00049 modified	Seq ID 79	Signal peptide removed	PF03318	ETX MTX2	9	260
APG00050	Seq ID 80		PF05431	Toxin 10	213	407
APG00051	Seq ID 81		PF03318	ETX MTX2	122	297
APG00051 modified	Seq ID 82	Signal peptide removed	PF03318	ETX MTX2	78	254
APG00053	Seq ID 83		PF03945	Endotoxin N	76	281
			PF01473	CW binding 1	297	311
			PF01473	CW binding 1	380	395
			PF01473	CW binding 1	434	448
APG00054	Seq ID 84		PF03945	Endotoxin N	63	302
			PF00555	Endotoxin M	307	522
			PF03944	Endotoxin C	532	666
APG00054 modified	Seq ID 85	3` Truncation	PF03945	Endotoxin N	63	302
			PF00555	Endotoxin M	307	522
			PF03944	Endotoxin C	532	665
APG00055	Seq ID 86		PF03318	ETX MTX2	96	334

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
APG00055 modified	Seq ID 87	Signal peptide removed	PF03318	ETX MTX2	67	305
APG00057	Seq ID 88		PF03945	Endotoxin N	66	319
			PF00555	Endotoxin M	324	520
			PF03944	Endotoxin C	530	666
APG00057 modified	Seq ID 89	3' Truncation	PF03945	Endotoxin N	66	319
			PF00555	Endotoxin M	324	520
			PF03944	Endotoxin C	530	665
APG00060	Seq ID 90		PF00652	Ricin B lectin	9	83
			PF05431	Toxin 10	78	278
APG00060 modified	Seq ID 91	Alternate start	PF00652	Ricin B lectin	8	82
			PF05431	Toxin 10	77	277
APG00061	Seq ID 92		PF03945	Endotoxin N	65	315
			PF03944	Endotoxin C	528	667
APG00061 modified	Seq ID 93	3` Truncation	PF03945	Endotoxin N	65	315
			PF03944	Endotoxin C	528	666
APG00061 Split-Cry C-term	Seq ID 94		PF14200	RicinB lectin 2	355	460
APG00063	Seq ID 95		PF05431	Toxin 10	220	412
APG00069	Seq ID 96		PF03945	Endotoxin N	1	133
			PF00555	Endotoxin M	138	335
			PF03944	Endotoxin C	345	495
APG00069 modified	Seq ID 97	Alternate start	PF03945	Endotoxin N	1	133
			PF00555	Endotoxin M	138	335
			PF03944	Endotoxin C	345	495
APG00069 modified	Seq ID 98	3' Truncation	PF03945	Endotoxin N	1	133
			PF00555	Endotoxin M	138	335
			PF03944	Endotoxin C	345	494
APG00077	Seq ID 99		PF12495	Vip3A N	16	188
			PF02018	CBM 4 9	549	663
APG00077 modified	Seq ID 100	Alternate start	PF12495	Vip3A N	14	186
			PF02018	CBM 4 9	547	661
APG00080	Seq ID 101		PF03945	Endotoxin N	2	173
			PF03944	Endotoxin C	346	481
APG00080 modified	Seq ID 102	Alternate start	PF03945	Endotoxin N	2	173
			PF03944	Endotoxin C	346	481
APG00080	Seq ID 103	Alternate start	PF03945	Endotoxin N	2	173

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
modified		and 3` Truncation	PF03944	Endotoxin C	346	479
APG00080 modified	Seq ID 104	3` Truncation	PF03945	Endotoxin N	2	173
			PF03944	Endotoxin C	346	479
APG00081	Seq ID 105		PF03945	Endotoxin N	75	309
			PF00555	Endotoxin M	317	516
			PF03944	Endotoxin C	536	699
APG00081 modified	Seq ID 106	3` Truncation	PF03945	Endotoxin N	75	309
			PF00555	Endotoxin M	317	516
			PF03944	Endotoxin C	536	698
APG00082	Seq ID 107		PF03945	Endotoxin N	120	337
			PF03945	Endotoxin N	330	416
			PF00555	Endotoxin M	421	639
			PF03944	Endotoxin C	649	789
APG00082 modified	Seq ID 108	Alternate start	PF03945	Endotoxin N	110	327
			PF03945	Endotoxin N	320	406
			PF00555	Endotoxin M	411	629
			PF03944	Endotoxin C	639	779
APG00082 modified	Seq ID 109	Alternate start and 3` Truncation	PF03945	Endotoxin N	110	327
			PF03945	Endotoxin N	320	406
			PF00555	Endotoxin M	411	629
			PF03944	Endotoxin C	639	778
APG00082 modified	Seq ID 110	3' Truncation	PF03945	Endotoxin N	120	337
			PF03945	Endotoxin N	330	416
			PF00555	Endotoxin M	421	639
			PF03944	Endotoxin C	649	788
APG00083	Seq ID 111		PF03945	Endotoxin N	65	297
			PF03944	Endotoxin C	495	632
APG00083 modified	Seq ID 112	3` Truncation	PF03945	Endotoxin N	65	297
			PF03944	Endotoxin C	495	631
APG00086	Seq ID 113		PF03945	Endotoxin N	71	281
			PF03945	Endotoxin N	283	330
			PF00555	Endotoxin M	337	545
			PF03944	Endotoxin C	555	687
APG00086 modified	Seq ID 114	Alternate start	PF03945	Endotoxin N	71	281
			PF03945	Endotoxin N	283	330
			PF00555	Endotoxin M	337	545
			PF03944	Endotoxin C	555	687

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
APG00086 modified	Seq ID 115	3` Truncation	PF03945	Endotoxin N	71	281
			PF03945	Endotoxin N	283	330
			PF00555	Endotoxin M	337	545
			PF03944	Endotoxin C	555	686
APG00088	Seq ID 116		PF03945	Endotoxin N	38	264
			PF00555	Endotoxin M	269	481
			PF03944	Endotoxin C	491	621
APG00088 modified	Seq ID 117	3` Truncation	PF03945	Endotoxin N	38	264
			PF00555	Endotoxin M	269	481
			PF03944	Endotoxin C	491	620
APG00089	Seq ID 118		PF03945	Endotoxin N	68	320
			PF00555	Endotoxin M	325	430
			PF03944	Endotoxin C	501	638
APG00089 modified	Seq ID 119	3` Truncation	PF03945	Endotoxin N	68	320
			PF00555	Endotoxin M	325	431
			PF03944	Endotoxin C	501	637
APG00091	Seq ID 120		PF03318	ETX MTX2	36	256
APG00091 modified	Seq ID 121	Alternate start	PF03318	ETX MTX2	32	251
APG00092	Seq ID 122		PF03318	ETX MTX2	56	319
APG00092 modified	Seq ID 123	Signal peptide removed	PF03318	ETX MTX2	26	290
APG00093	Seq ID 124		PF03945	Endotoxin N	27	267
			PF00555	Endotoxin M	272	463
			PF03944	Endotoxin C	473	541
APG00096	Seq ID 125		PF03945	Endotoxin N	56	301
			PF00030	Crystall	652	731
			PF00030	Crystall	732	814
			PF14200	RicinB lectin 2	854	961
APG00096 modified	Seq ID 126	Alternate start	PF03945	Endotoxin N	53	298
			PF00030	Crystall	649	728
			PF00030	Crystall	729	811
			PF14200	RicinB lectin 2	851	958
APG00096 modified	Seq ID 127	Alternate start and 3` Truncation	PF03945	Endotoxin N	53	298
			PF00030	Crystall	649	728
			PF00030	Crystall	729	811
APG00098	Seq ID 128		PF03318	ETX MTX2	5	309
APG00098 modified	Seq ID 129	Alternate start	PF03318	ETX MTX2	5	273

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
APG00100	Seq ID 130		PF03945	Endotoxin N	30	246
APG00102	Seq ID 131		PF03945	Endotoxin N	57	157
			PF03945	Endotoxin N	144	308
			PF09131	Endotoxin mid	310	509
APG00103	Seq ID 132		PF03945	Endotoxin N	64	302
			PF00555	Endotoxin M	307	529
			PF03944	Endotoxin C	539	682
APG00103 modified	Seq ID 133	3' Truncation	PF03945	Endotoxin N	64	302
			PF00555	Endotoxin M	307	529
			PF03944	Endotoxin C	539	681
APG00106	Seq ID 134		PF07691	PA14	15	142
			PF03495	Binary toxB	185	600
			PF09259	Fve	831	920
APG00109	Seq ID 135		PF03945	Endotoxin N	104	339
			PF00555	Endotoxin M	350	558
			PF03944	Endotoxin C	568	705
			PF14200	RicinB lectin 2	748	849
APG00109 modified	Seq ID 136	Alternate start and 3` Truncation	PF03945	Endotoxin N	52	287
			PF00555	Endotoxin M	298	506
			PF03944	Endotoxin C	516	648
APG00109 modified	Seq ID 137	Alternate start	PF03945	Endotoxin N	52	287
			PF00555	Endotoxin M	298	506
			PF03944	Endotoxin C	516	653
			PF14200	RicinB lectin 2	696	797
APG00109 modified	Seq ID 138	3` Truncation	PF03945	Endotoxin N	104	339
			PF00555	Endotoxin M	350	558
			PF03944	Endotoxin C	568	700
APG00111	Seq ID 139		PF03945	Endotoxin N	147	384
			PF00555	Endotoxin M	389	585
			PF03944	Endotoxin C	595	735
APG00111 modified	Seq ID 140	Alternate start	PF03945	Endotoxin N	56	293
			PF00555	Endotoxin M	298	494
			PF03944	Endotoxin C	504	644
APG00111 modified	Seq ID 141	3' Truncation	PF03945	Endotoxin N	147	384
			PF00555	Endotoxin M	389	585
			PF03944	Endotoxin C	595	734
APG00122	Seq ID 143		PF03945	Endotoxin N	67	320

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
			PF00555	Endotoxin M	327	537
			PF03944	Endotoxin C	547	680
APG00122 modified	Seq ID 144	Alternate start	PF03945	Endotoxin N	67	320
			PF00555	Endotoxin M	327	537
			PF03944	Endotoxin C	547	680
APG00122 modified	Seq ID 145	3' Truncation	PF03945	Endotoxin N	67	320
			PF00555	Endotoxin M	327	537
			PF03944	Endotoxin C	547	679
APG00123	Seq ID 146		PF03945	Endotoxin N	66	303
			PF03945	Endotoxin N	342	383
			PF00555	Endotoxin M	388	492
			PF03944	Endotoxin C	621	761
APG00123 modified	Seq ID 147	Alternate start and 3' Truncation	PF03945	Endotoxin N	33	270
			PF03945	Endotoxin N	309	350
			PF00555	Endotoxin M	355	460
			PF03944	Endotoxin C	588	727
APG00123 modified	Seq ID 148	Alternate start	PF03945	Endotoxin N	33	270
			PF03945	Endotoxin N	309	350
			PF00555	Endotoxin M	355	459
			PF03944	Endotoxin C	588	728
APG00123 modified	Seq ID 149	3' Truncation	PF03945	Endotoxin N	66	303
			PF03945	Endotoxin N	342	383
			PF00555	Endotoxin M	388	493
			PF03944	Endotoxin C	621	760
APG00125	Seq ID 151		PF03945	Endotoxin N	58	291
			PF00555	Endotoxin M	296	492
			PF03944	Endotoxin C	502	633
APG00125 modified	Seq ID 152	3' Truncation	PF03945	Endotoxin N	58	291
			PF00555	Endotoxin M	296	492
			PF03944	Endotoxin C	502	632
APG00126	Seq ID 153		PF01338	Bac thur toxin	20	240
APG00126 modified	Seq ID 154	Alternate start	PF01338	Bac thur toxin	14	234
APG00127	Seq ID 155		PF03945	Endotoxin N	65	287
			PF00555	Endotoxin M	292	500
			PF03944	Endotoxin C	510	653
APG00127 modified	Seq ID 156	3' Truncation	PF03945	Endotoxin N	65	287
			PF00555	Endotoxin M	292	500

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
			PF03944	Endotoxin C	510	652
APG00128	Seq ID 157		PF01338	Bac thur toxin	13	234
APG00129	Seq ID 158		PF03318	ETX MTX2	119	351
APG00129 modified	Seq ID 159	Alternate start	PF03318	ETX MTX2	119	351
APG00133	Seq ID 160		PF03945	Endotoxin N	65	299
			PF00555	Endotoxin M	304	510
			PF03944	Endotoxin C	520	657
APG00133 modified	Seq ID 161	3` Truncation	PF03945	Endotoxin N	65	299
			PF00555	Endotoxin M	304	510
			PF03944	Endotoxin C	520	656
APG00142	Seq ID 162		PF03945	Endotoxin N	124	349
APG00145	Seq ID 163		PF03945	Endotoxin N	63	315
			PF00555	Endotoxin M	322	507
			PF03944	Endotoxin C	517	656
			PF05588	Botulinum HA-17	670	784
APG00145 modified	Seq ID 164	3' Truncation	PF03945	Endotoxin N	63	315
			PF00555	Endotoxin M	322	507
			PF03944	Endotoxin C	517	655
APG00146	Seq ID 166		PF03318	ETX MTX2	70	298
APG00146 modified	Seq ID 167	Signal peptide removed	PF03318	ETX MTX2	39	266
APG00147	Seq ID 168		PF14200	RicinB lectin 2	96	206
			PF05431	Toxin 10	215	409
APG00147 modified	Seq ID 169	Signal peptide removed	PF14200	RicinB lectin 2	65	175
			PF05431	Toxin 10	184	378
APG00148 APG00149	Seq ID 170 Seq ID 172		PF03945	Endotoxin N	71	300
			PF00555	Endotoxin M	306	482
			PF03944	Endotoxin C	492	658
APG00149 modified	Seq ID 173	3' Truncation	PF03945	Endotoxin N	71	300
			PF00555	Endotoxin M	306	482
			PF03944	Endotoxin C	492	657
APG00151	Seq ID 174		PF14200	RicinB lectin 2	45	146
			PF05431	Toxin 10	152	350
APG00151 modified	Seq ID 175	Alternate start	PF14200	RicinB lectin 2	45	146
			PF05431	Toxin 10	152	350
APG00161	Seq ID 176		PF03945	Endotoxin N	75	297

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
			PF00555	Endotoxin M	302	510
			PF03944	Endotoxin C	520	658
			PF14200	RicinB lectin 2	697	807
APG00161 modified	Seq ID 177	Alternate start	PF03945	Endotoxin N	70	292
			PF00555	Endotoxin M	297	505
			PF03944	Endotoxin C	515	653
			PF14200	RicinB lectin 2	692	802
APG00161 modified	Seq ID 178	Alternate start and 3` Truncation	PF03945	Endotoxin N	70	292
			PF00555	Endotoxin M	297	505
			PF03944	Endotoxin C	515	652
APG00161 modified	Seq ID 179	3` Truncation	PF03945	Endotoxin N	75	297
			PF00555	Endotoxin M	302	510
			PF03944	Endotoxin C	520	657
APG00167	Seq ID 180		PF14200	RicinB lectin 2	38	142
			PF05431	Toxin 10	316	510
APG00169	Seq ID 181		PF03945	Endotoxin N	59	290
			PF00555	Endotoxin M	295	501
			PF03944	Endotoxin C	511	653
APG00169 modified	Seq ID 182	3` Truncation	PF03945	Endotoxin N	59	290
			PF00555	Endotoxin M	295	501
			PF03944	Endotoxin C	511	652
APG00174	Seq ID 183		PF03318	ETX MTX2	112	348
APG00174 modified	Seq ID 184	Alternate start	PF03318	ETX MTX2	96	332
APG00174 modified	Seq ID 185	Signal peptide removed	PF03318	ETX MTX2	67	303
APG00179	Seq ID 186		PF03945	Endotoxin N	49	281
			PF00555	Endotoxin M	286	490
			PF03944	Endotoxin C	500	647
APG00179 modified	Seq ID 187	3` Truncation	PF03945	Endotoxin N	49	281
			PF00555	Endotoxin M	286	490
			PF03944	Endotoxin C	500	646
APG00185	Seq ID 188		PF03945	Endotoxin N	64	299
			PF00555	Endotoxin M	304	527
			PF03944	Endotoxin C	537	675
APG00185 modified	Seq ID 189	3` Truncation	PF03945	Endotoxin N	64	299
			PF00555	Endotoxin M	304	527
			PF03944	Endotoxin C	537	674

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
APG00185 CryBP1	Seq ID 190		PF07029	CryBP1	61	222
APG00191	Seq ID 191		PF00652	Ricin B lectin	70	173
			PF05431	Toxin 10	167	362
			PF03495	Binary toxB	234	352
APG00199	Seq ID 192		PF03945	Endotoxin N	101	322
APG00201	Seq ID 193		PF03318	ETX MTX2	36	258
APG00201 modified	Seq ID 194	Alternate start	PF03318	ETX MTX2	29	251
APG00202	Seq ID 195		PF03318	ETX MTX2	146	375
APG00202 modified	Seq ID 196	Signal peptide removed	PF03318	ETX MTX2	100	329
APG00205	Seq ID 197		PF03318	ETX MTX2	71	309
APG00205 modified	Seq ID 198	Signal peptide removed	PF03318	ETX MTX2	36	274
APG00206	Seq ID 199		PF03945	Endotoxin N	62	307
			PF03944	Endotoxin C	515	670
APG00206 modified	Seq ID 200	3' Truncation	PF03945	Endotoxin N	62	307
			PF03944	Endotoxin C	515	669
APG00208	Seq ID 201		PF03318	ETX MTX2	147	375
APG00208 modified	Seq ID 202	Alternate start	PF03318	ETX MTX2	131	359
APG00222	Seq ID 203		PF03945	Endotoxin N	43	284
			PF00555	Endotoxin M	289	509
			PF03944	Endotoxin C	519	658
APG00222 modified	Seq ID 204	Alternate start	PF03945	Endotoxin N	42	283
			PF00555	Endotoxin M	288	508
			PF03944	Endotoxin C	518	657
APG00222 modified	Seq ID 205	Alternate start and 3' Truncation	PF03945	Endotoxin N	42	283
			PF00555	Endotoxin M	288	508
			PF03944	Endotoxin C	518	656
APG00222 modified	Seq ID 206	3' Truncation	PF03945	Endotoxin N	43	284
			PF00555	Endotoxin M	289	509
			PF03944	Endotoxin C	519	657
APG00234	Seq ID 207		PF03318	ETX MTX2	45	315
APG00234 modified	Seq ID 208	Alternate start	PF03318	ETX MTX2	32	298
APG00234 modified	Seq ID 209	Signal peptide removed	PF03318	ETX MTX2	8	265
APG00272	Seq ID 210		PF03318	ETX MTX2	36	297
APG00272 modified	Seq ID 211	Signal peptide removed	PF03318	ETX MTX2	10	271

APG ID	Seq ID	Modification Type	PFAM Domain	Domain Description	Domain Position	
					Start	Stop
APG00299	Seq ID 212		PF03318	ETX MTX2	20	277
APG00299 modified	Seq ID 213	Signal peptide removed	PF03318	ETX MTX2	9	253
APG00526	Seq ID 214		PF03945	Endotoxin N	100	340
			PF03944	Endotoxin C	532	669
APG00526 modified	Seq ID 215	Signal peptide removed	PF03945	Endotoxin N	70	310
			PF03944	Endotoxin C	502	639
APG00526 modified	Seq ID 216	3' Truncation	PF03945	Endotoxin N	100	340
			PF03944	Endotoxin C	532	668
APG00526 modified	Seq ID 217	Signal peptide removed and 3' Truncation	PF03945	Endotoxin N	70	310
			PF03944	Endotoxin C	502	638
APG00717	Seq ID 218		PF03945	Endotoxin N	32	248
APG00728	Seq ID 219		PF03945	Endotoxin N	100	340
			PF03944	Endotoxin C	532	669
APG00728 modified	Seq ID 220	3' Truncation	PF03945	Endotoxin N	100	340
			PF03944	Endotoxin C	532	668
APG00728 modified	Seq ID 221	Signal peptide removed	PF03945	Endotoxin N	70	310
			PF03944	Endotoxin C	502	639
APG00728 modified	Seq ID 222	Signal peptide removed and 3' Truncation	PF03945	Endotoxin N	70	310
			PF03944	Endotoxin C	502	638
APG00847	Seq ID 223		PF03318	ETX MTX2	33	258
APG00847 modified	Seq ID 224	Alternate start	PF03318	ETX MTX2	26	251
APG00847 modified	Seq ID 225	Signal peptide removed	PF03318	ETX MTX2	13	238
APG00982	Seq ID 226		PF03318	ETX MTX2	56	319
APG00982 modified	Seq ID 227	Signal peptide removed	PF03318	ETX MTX2	26	290
APG00006 modified	Seq ID 228	Alternate start	PF03318	ETX MTX2	28	252
APG00036 modified	Seq ID 229	Alternate start	PF03318	ETX MTX2	27	249

[0047] Recombinant or synthetic nucleic acids encoding the pesticidal polypeptides disclosed herein are also provided. Of particular interest are nucleic acid sequences that have been designed for expression in a plant of interest. That is, the nucleic acid sequence can be optimized for increased expression in a host plant. A pesticidal protein of the invention can be back-translated to produce a nucleic acid comprising codons optimized for expression in a particular host, for example, a crop plant. In another

embodiment, the polynucleotides encoding the polypeptides provided herein may be optimized for increased expression in the transformed plant. That is, the polynucleotides can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92:1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference. Expression of such a coding sequence by the transformed plant (e.g., dicot or monocot) will result in the production of a pesticidal polypeptide and confer increased resistance in the plant to a pest. Recombinant and synthetic nucleic acid molecules encoding the pesticidal proteins of the invention do not include the naturally occurring bacterial sequence encoding the protein.

[0048] A "recombinant polynucleotide" or "recombinant nucleic acid" comprises a combination of two or more chemically linked nucleic acid segments which are not found directly joined in nature. By "directly joined" is intended the two nucleic acid segments are immediately adjacent and joined to one another by a chemical linkage. In specific embodiments, the recombinant polynucleotide comprises a polynucleotide of interest or a variant or fragment thereof such that an additional chemically linked nucleic acid segment is located either 5', 3' or internal to the polynucleotide of interest. Alternatively, the chemically-linked nucleic acid segment of the recombinant polynucleotide can be formed by deletion of a sequence. The additional chemically linked nucleic acid segment or the sequence deleted to join the linked nucleic acid segments can be of any length, including for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or greater nucleotides. Various methods for making such recombinant polynucleotides include chemical synthesis or by the manipulation of isolated segments of polynucleotides by genetic engineering techniques. In specific embodiments, the recombinant polynucleotide can comprise a recombinant DNA sequence or a recombinant RNA sequence. A "fragment of a recombinant polynucleotide or nucleic acid" comprises at least one of a combination of two or more chemically linked amino acid segments which are not found directly joined in nature.

[0049] Fragments of a polynucleotide (RNA or DNA) may encode protein fragments that retain activity. In specific embodiments, a fragment of a recombinant polynucleotide or a recombinant polynucleotide construct comprises at least one junction of the two or more chemically linked or operably linked nucleic acid segments which are not found directly joined in nature A fragment of a polynucleotide that encodes a biologically active portion of a polypeptide that retains pesticidal activity will encode at least 25, 30, 40, 50, 60, 70, 75, 80, 90, 100, 110, 120, 125, 130, 140, 150, 160, 170, 175, 180, contiguous amino acids, or up to the total number of amino acids present in a full-length polypeptide as set forth in SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229. In specific embodiments, such polypeptide fragments are active fragments, and in still other embodiments, the polypeptide fragment comprises a recombinant polypeptide fragment. As used herein, a fragment of a recombinant polypeptide comprises at least one of a combination of two or more chemically linked amino acid segments which are not found directly joined in nature.

[0050] The term "variants" as used herein is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a deletion and/or addition of one or more nucleotides at one or more internal sites within the native polynucleotide and/or a substitution of one or more nucleotides at one or more sites in the native polynucleotide. As used herein, a "native" polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively.

[0051] Variants of a particular polynucleotide of the invention (i.e., the reference polynucleotide) can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant polynucleotide and the polypeptide encoded by the reference polynucleotide. Thus, for example, an isolated polynucleotide that encodes a polypeptide with a given percent sequence identity to the polypeptide of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229 are disclosed. Percent sequence identity between any two polypeptides can be calculated using sequence alignment programs and parameters described elsewhere herein. Where any given pair of polynucleotides of the invention is evaluated by comparison of the percent sequence identity shared by the two polypeptides they encode, the percent sequence identity between the two encoded polypeptides is at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159,

160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229. In other embodiments, the variant of the polynucleotide provided herein differs from the native sequence by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides.

[0052] Variant polynucleotide and proteins also encompass sequences and proteins derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different pesticidal protein disclosed herein (SEQ ID NO: 1-209) is manipulated to create a new pesticidal protein possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between the pesticidal sequences provided herein and other known pesticidal genes to obtain a new gene coding for a protein with an improved property of interest, such as an increased K_m in the case of an enzyme. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751; Stemmer (1994) *Nature* 370:389-391; Crameri *et al.* (1997) *Nature Biotech.* 15:436-438; Moore *et al.* (1997) *J. Mol. Biol.* 272:336-347; Zhang *et al. (1997) Proc. Natl. Acad. Sci. USA* 94:4504-4509; Crameri *et al.* (1998) *Nature* 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458. A "shuffled" nucleic acid is a nucleic acid produced by a shuffling procedure such as any shuffling procedure set forth herein. Shuffled nucleic acids are produced by recombining (physically or virtually) two or more nucleic acids (or character strings), for example in an artificial, and optionally recursive, fashion. Generally, one or more screening steps are used in shuffling processes to identify nucleic acids of interest; this screening step can be performed before or after any recombination step. In some (but not all) shuffling embodiments, it is desirable to perform multiple rounds of recombination prior to selection to increase the diversity of the pool to be screened. The overall process of recombination and selection are optionally repeated recursively. Depending on context, shuffling can refer to an overall process of

recombination and selection, or, alternately, can simply refer to the recombinational portions of the overall process.

[0053] In one embodiment, a method of obtaining a polynucleotide that encodes an improved polypeptide comprising pesticidal activity is provided, wherein the improved polypeptide has at least one improved property over any one of SEQ ID NOS: 1-229. Such methods can comprise (a) recombining a plurality of parental polynucleotides to produce a library of recombinant polynucleotides encoding recombinant pesticidal polypeptides; (b) screening the library to identify a recombinant polynucleotide that encodes an improved recombinant pesticidal polypeptide that has an enhanced property improved over the parental polynucleotide; (c) recovering the recombinant polynucleotide that encodes the improved recombinant pesticidal polypeptide identified in (b); and, (d) repeating steps (a), (b) and (c) using the recombinant polynucleotide recovered in step (c) as one of the plurality of parental polynucleotides in repeated step (a).

iii. Sequence Comparisons

[0054] As used herein, the term "identity" or "percent identity" when used with respect to a particular pair of aligned amino acid sequences, refers to the percent amino acid sequence identity that is obtained by counting the number of identical matches in the alignment and dividing such number of identical matches by the length of the aligned sequences. As used herein, the term "similarity" or "percent similarity" when used with respect to a particular pair of aligned amino acid sequences, refers to the sum of the scores that are obtained from a scoring matrix for each amino acid pair in the alignment divided by the length of the aligned sequences.

[0055] Unless otherwise stated, identity and similarity will be calculated by the Needleman-Wunsch global alignment and scoring algorithms (Needleman and Wunsch (1970) *J. Mol. Biol.* 48(3):443-453) as implemented by the "needle" program, distributed as part of the EMBOSS software package (Rice,P. Longden,I. and Bleasby,A., EMBOSS: The European Molecular Biology Open Software Suite, 2000, *Trends in Genetics* 16, (6) pp. 276-277, versions 6.3.1 available from EMBnet at embnet.org/resource/emboss and emboss.sourceforge.net, among other sources) using default gap penalties and scoring

matrices (EBLOSUM62 for protein and EDNAFULL for DNA). Equivalent programs may also be used. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by needle from EMBOSS version 6.3.1.

[0056] Additional mathematical algorithms are known in the art and can be utilized for the comparison of two sequences. See, for example, the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the BLAST programs of Altschul et al. (1990) J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the BLASTN program (nucleotide query searched against nucleotide sequences) to obtain nucleotide sequences homologous to pesticidal-like nucleic acid molecules of the invention, or with the BLASTX program (translated nucleotide query searched against protein sequences) to obtain protein sequences homologous to pesticidal nucleic acid molecules of the invention. BLAST protein searches can be performed with the BLASTP program (protein query searched against protein sequences) to obtain amino acid sequences homologous to pesticidal protein molecules of the invention, or with the TBLASTN program (protein query searched against translated nucleotide sequences) to obtain nucleotide sequences homologous to pesticidal protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra.* When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., BLASTX and BLASTN) can be used. Alignment may also be performed manually by inspection.

[0057] Two sequences are "optimally aligned" when they are aligned for similarity scoring using a defined amino acid substitution matrix (e.g., BLOSUM62), gap existence penalty and gap extension penalty so as to arrive at the highest score possible for that pair of sequences. Amino acid substitution matrices and their use in quantifying the similarity

between two sequences are well-known in the art and described, e.g., in Dayhoff et al. (1978) "A model of evolutionary change in proteins." In "Atlas of Protein Sequence and Structure," Vol. 5, Suppl. 3 (ed. M. O. Dayhoff), pp. 345-352. Natl. Biomed. Res. Found., Washington, D.C. and Henikoff et al. (1992) Proc. Natl. Acad. Sci. USA 89:10915-10919. The BLOSUM62 matrix is often used as a default scoring substitution matrix in sequence alignment protocols. The gap existence penalty is imposed for the introduction of a single amino acid gap in one of the aligned sequences, and the gap extension penalty is imposed for each additional empty amino acid position inserted into an already opened gap. The alignment is defined by the amino acids positions of each sequence at which the alignment begins and ends, and optionally by the insertion of a gap or multiple gaps in one or both sequences, so as to arrive at the highest possible score. While optimal alignment and scoring can be accomplished manually, the process is facilitated by the use of a computer-implemented alignment algorithm, e.g., gapped BLAST 2.0, described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-3402, and made available to the public at the National Center for Biotechnology Information Website (www.ncbi.nlm.nih.gov). Optimal alignments, including multiple alignments, can be prepared using, e.g., PSI-BLAST, available through www.ncbi.nlm.nih.gov and described by Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-3402.

[0058] With respect to an amino acid sequence that is optimally aligned with a reference sequence, an amino acid residue "corresponds to" the position in the reference sequence with which the residue is paired in the alignment. The "position" is denoted by a number that sequentially identifies each amino acid in the reference sequence based on its position relative to the N-terminus. For example, in SEQ ID NO: 1 position 1 is L, position 2 is S, position 3 is F, etc. When a test sequence is optimally aligned with SEQ ID NO: 1, a residue in the test sequence that aligns with the F at position 3 is said to "correspond to position 3" of SEQ ID NO: 1. Owing to deletions, insertion, truncations, fusions, etc., that must be taken into account when determining an optimal alignment, in general the amino acid residue number in a test sequence as determined by simply counting from the N-terminal will not necessarily be the same as the number of its corresponding position in the reference sequence. For example, in a case where there is a deletion in an aligned test sequence, there will be no amino acid that corresponds to a

position in the reference sequence at the site of deletion. Where there is an insertion in an aligned reference sequence, that insertion will not correspond to any amino acid position in the reference sequence. In the case of truncations or fusions there can be stretches of amino acids in either the reference or aligned sequence that do not correspond to any amino acid in the corresponding sequence.

iv. Antibodies

[0059] Antibodies to the polypeptides of the present invention, or to variants or fragments thereof, are also encompassed. Methods for producing antibodies are well known in the art (see, for example, Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; and U.S. Pat. No. 4,196,265). These antibodies can be used in kits for the detection and isolation of toxin polypeptides. Thus, this disclosure provides kits comprising antibodies that specifically bind to the polypeptides described herein, including, for example, polypeptides having the sequence of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229.

II. Pests

[0060] The compositions and methods provided herein are useful against a variety of pests. "Pests" includes but is not limited to, insects, fungi, bacteria, nematodes, acarids, protozoan pathogens, animal-parasitic liver flukes, and the like. Pests of particular interest are insect pests, particularly insect pests that cause significant damage to

agricultural plants. Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthroptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, or nematodes. In non-limiting embodiments, the insect pest comprises Western corn rootworm, *Diabrotica virgifera virgifera*; Fall armyworm, *Spodoptera frugiperda;* Colorado potato beetle, *Leptinotarsa decemlineata*; Corn earworm, *Helicoverpa zea* (in North America same species attacks cotton and called cotton bollworm); European corn borer, *Ostrinia nubilalis*; Black cutworm, *Agrotis ipsilon*; Diamondback moth, *Plutella xylostella*; Velvetbean caterpillar, *Anticarsia gemmatalis*; Southwestern corn borer, *Diatraea grandiosella;* Cotton bollworm, *Helicoverpa armigera* (found other than USA in rest of the world); Southern green stinkbug, *Nezara viridula*; Green stinkbug, *Chinavia halaris*; Brown marmorated stinkbug, *Halyomorpha halys; and* Brown stinbug, *Euschistus servus Euschistus heros* (Neotropical brown stink bug OR soy stink bug) ; *Piezodorus guildinii* (red-banded stink bug); *Dichelops melacanthus* (no common name) and/or *Dichelops furcatus* (no common name); an aphid, such as a soybean aphid . In other embodiments, the pest comprises a nematode including, but not limited to, *Meloidogyne hapla* (Northern root-knot nematode); *Meloidogyne enterolobii*, *Meloidogyne arenaria* (peanut root-knot nematode); and *Meloidogyne javanica.*

[0061] The term "insect pests" as used herein refers to insects and other similar pests such as, for example, those of the order Acari including, but not limited to, mites and ticks. Insect pests of the present invention include, but are not limited to, insects of the order Lepidoptera, e.g. *Achoroia grisella, Acleris gloverana, Acleris variana, Adoxophyes orana, Agrotis ipsilon, Alabama argillacea, Alsophila pometaria, Amyelois transitella, Anagasta kuehniella, Anarsia lineatella, Anisota senatoria, Antheraea pernyi, Anticarsia gemmatalis*, Archips sp., Argyrotaenia sp., *Athetis mindara, Bombyx mori, Bucculatrix thurberiella, Cadra cautella*, Choristoneura sp., *Cochylls hospes, Colias eurytheme, Corcyra cephalonica, Cydia latiferreanus, Cydia pomonella, Datana integerrima, Dendrolimus sibericus*, Desmiafeneralis, *Diaphania hyalinata, Diaphania nitidalis, Diatraea grandiosella, Diatraea saccharalis, Ennomos subsignaria, Eoreuma loftini, Esphestia elutella, Erannis tilaria, Estigmene acrea, Eulia salubricola, Eupocoellia ambiguella, Eupoecilia ambiguella, Euproctis chrysorrhoea, Euxoa*

messoria, *Galleria mellonella*, *Grapholita molesta*, *Harrisina americana*, *Helicoverpa subflexa*, *Helicoverpa zea*, *Heliothis virescens*, *Hemileuca oliviae*, *Homoeosoma electellum*, *Hyphantia cunea*, *Keiferia lycopersicella*, *Lambdina fiscellaria fiscellaria*, *Lambdina fiscellaria lugubrosa*, *Leucoma salicis*, *Lobesia botrana*, *Loxostege sticticalis*, *Lymantria dispar*, *Macalla thyrisalis*, Malacosoma sp., *Mamestra brassicae*, *Mamestra configurata*, *Manduca quinquemaculata*, *Manduca sexta*, *Maruca testulalis*, *Melanchra picta*, *Operophtera brumata*, Orgyia sp., *Ostrinia nubilalis*, *Paleacrita vernata*, *Papilio cresphontes*, *Pectinophora gossypiella*, *Phryganidia californica*, *Phyllonorycter blancardella*, *Pieris napi*, *Pieris rapae*, *Plathypena scabra*, *Platynota flouendana*, *Platynota stultana*, *Platyptilia carduidactyla*, *Plodia interpunctella*, *Plutella xylostella*, *Pontia protodice*, *Pseudaletia unipuncta*, *Pseudoplasia includens*, *Sabulodes aegrotata*, *Schizura concinna*, *Sitotroga cerealella*, *Spilonta ocellana*, Spodoptera sp., *Thaurnstopoea pityocampa*, *Tinsola bisselliella*, *Trichoplusia hi*, *Udea rubigalis*, *Xylomyges curiails*, and *Yponomeuta padella*.

[0062] Insect pests also include insects selected from the orders Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthroptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, Coleoptera.

[0063] Insect pests of the invention for the major crops include, but are not limited to: Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zeae*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*, surgarcane borer; western corn rootworm, e.g., *Diabrotica virgifera virgifera*; northern corn rootworm, e.g., *Diabrotica longicornis barberi*; southern corn rootworm, e.g., *Diabrotica undecimpunctata howardi*; Melanotus spp., wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white grub); *Popillia japonica*, Japanese beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Anuraphis maidiradicis*, corn root aphid; *Euschistus heros* (Neotropical brown stink bug OR soy stink bug) ; *Piezodorus guildinii* (red-banded stink bug); *Dichelops melacanthus* (no common name); *Dichelops furcatus*

(no common name) ; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot; *Agromyza parvicornis*, corn blotch leafminer; *Anaphothrips obscrurus*, grass thrips; *Solenopsis milesta*, thief ant; *Tetranychus urticae*, two spotted spider mite; Sorghum: *Chilo partellus*, sorghum borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, leser cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*, white grub; *Eleodes, Conoderus*, and Aeolus spp., wireworms; *Oulema melanopus*, cereal leaf beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*; corn leaf aphid; *Sipha flava*, yellow sugarcane aphid; chinch bug, e.g., *Blissus leucopterus leucopterus*; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, two-spotted spider mite; Wheat: *Pseudaletia unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Agrotis orthogonia*, pale western cutworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf beetle; *Hypera punctata*, clover leaf weevil; southern corn rootworm, e.g., *Diabrotica undecimpunctata howardi*; Russian wheat aphid; *Schizaphis graminum*, greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Mayetiola destructor*, Hessian fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella fusca*, tobacco thrips; *Cephus cinctus*, wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower: *Cylindrocupturus adspersus*, sunflower stem weevil; *Smicronyx fulus*, red sunflower seed weevil; *Smicronyx sordidus*, gray sunflower seed weevil; *Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower moth; *Zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis virescens*, tobacco budworm; *Helicoverpa zea*, cotton bollworm; *Spodoptera exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; boll weevil, e.g., *Anthonomus grandis*; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis seriatus*, cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus lineolaris*, tarnished plant bug;

Melanoplus femurrubrum, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Thrips tabaci*, onion thrips; *Franklinkiella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, two-spotted spider mite; Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea*, grape colaspis; *Lissorhoptrus oryzophilus*, rice water weevil; *Sitophilus oryzae*, rice weevil; *Nephotettix nigropictus*, rice leafhoper; chinch bug, e.g., *Blissus leucopterus leucopterus*; *Acrosternum hilare*, green stink bug; Soybean: *Pseudoplusia includens*, soybean looper; *Anticarsia gemmatalis*, velvetbean caterpillar; *Plathypena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, tobacco budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; *Thrips tabaci*, onion thrips; *Tetranychus turkestani*, strawberry spider mite; *Tetranychus urticae*, two-spotted spider mite; Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; chinch bug, e.g., *Blissus leucopterus leucopterus*; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Jylemya platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Vrevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, crucifer flea beetle; *Phyllotreta striolata*, striped flea beetle; *Phyllotreta nemorum*, striped turnip flea beetle; *Meligethes aeneus*, rapeseed beetle; and the pollen beetles *Meligethes rufimanus*, *Meligethes nigrescens*, *Meligethes canadianus*, and *Meligethes viridescens*; Potato: *Leptinotarsa decemlineata*, Colorado potato beetle.

[0064] The methods and compositions provided herein may be effective against Hemiptera such as *Lygus hesperus*, *Lygus lineolaris*, *Lygus pratensis*, *Lygus rugulipennis Popp*, *Lygus pabulinus*, *Calocoris norvegicus*, *Orthops compestris*, *Plesiocoris rugicollis*, *Cyrtopeltis modestus*, *Cyrtopeltis notatus*, *Spanagonicus albofasciatus*, *Diaphnocoris chlorinonis*, *Labopidicola allii*, *Pseudatomoscelis seriatus*, *Adelphocoris rapidus*, *Poecilocapsus lineatus*, *Blissus leucopterus*, *Nysius ericae*, *Nysius raphanus*, *Euschistus*

servus, *Nezara viridula*, Eurygaster, Coreidae, Pyrrhocoridae, Tinidae, Blostomatidae, Reduviidae, and Cimicidae. Pests of interest also include *Araecerus fasciculatus*, coffee bean weevil; *Acanthoscelides obtectus*, bean weevil; *Bruchus rufmanus*, broadbean weevil; *Bruchus pisorum*, pea weevil; *Zabrotes subfasciatus*, Mexican bean weevil; *Diabrotica balteata*, banded cucumber beetle; *Cerotoma trifurcata*, bean leaf beetle; *Diabrotica virgifera*, Mexican corn rootworm; *Epitrix cucumeris*, potato flea beetle; *Chaetocnema confinis*, sweet potato flea beetle; *Hypera postica*, alfalfa weevil; *Anthonomus quadrigibbus*, apple curculio; *Sternechus paludatus*, bean stalk weevil; *Hypera brunnipennis*, Egyptian alfalfa weevil; *Sitophilus granaries*, granary weevil; *Craponius inaequalis*, grape curculio; *Sitophilus zeamais*, maize weevil; *Conotrachelus nenuphar*, plum curculio; *Euscepes postfaciatus*, West Indian sweet potato weevil; *Maladera castanea*, Asiatic garden beetle; *Rhizotrogus majalis*, European chafer; *Macrodactylus subspinosus*, rose chafer; *Tribolium confusum*, confused flour beetle; *Tenebrio obscurus*, dark mealworm; *Tribolium castaneum*, red flour beetle; *Tenebrio molitor*, yellow mealworm.

[0065] Nematodes include parasitic nematodes such as root-knot, cyst, and lesion nematodes, including Heterodera spp., Meloidogyne spp., and Globodera spp.; particularly members of the cyst nematodes, including, but not limited to, *Heterodera glycines* (soybean cyst nematode); *Heterodera schachtii* (beet cyst nematode); *Heterodera avenae* (cereal cyst nematode); and *Globodera rostochiensis* and *Globodera pailida* (potato cyst nematodes). Lesion nematodes include Pratylenchus spp.

[0066] Insect pests may be tested for pesticidal activity of compositions of the invention in early developmental stages, e.g., as larvae or other immature forms. The insects may be reared in total darkness at from about 20°C to about 30°C and from about 30% to about 70% relative humidity. Bioassays may be performed as described in Czapla and Lang (1990) *J. Econ. Entomol.* 83 (6): 2480-2485. See, also the experimental section herein.

III. Expression Cassettes

[0067] Polynucleotides encoding the pesticidal proteins provided herein can be provided in expression cassettes for expression in an organism of interest. The cassette

will include 5' and 3' regulatory sequences operably linked to a polynucleotide encoding a pesticidal polypeptide provided herein that allows for expression of the polynucleotide. The cassette may additionally contain at least one additional gene or genetic element to be cotransformed into the organism. Where additional genes or elements are included, the components are operably linked. Alternatively, the additional gene(s) or element(s) can be provided on multiple expression cassettes. Such an expression cassette is provided with a plurality of restriction sites and/or recombination sites for insertion of the polynucleotides to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain a selectable marker gene.

[0068] The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a pesticidal polynucleotide of the invention, and a transcriptional and translational termination region (i.e., termination region) functional in the organism of interest, i.e., a plant or bacteria. The promoters of the invention are capable of directing or driving expression of a coding sequence in a host cell. The regulatory regions (i.e., promoters, transcriptional regulatory regions, and translational termination regions) may be endogenous or heterologous to the host cell or to each other. As used herein, "heterologous" in reference to a sequence is a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence.

[0069] Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon *et al.* (1991) *Genes Dev.* 5:141-149; Mogen *et al.* (1990) *Plant Cell* 2:1261-1272; Munroe *et al.* (1990) *Gene* 91:151-158; Ballas *et al.* (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi *et al.* (1987) *Nucleic Acids Res.* 15:9627-9639.

[0070] Additional regulatory signals include, but are not limited to, transcriptional initiation start sites, operators, activators, enhancers, other regulatory elements, ribosomal

binding sites, an initiation codon, termination signals, and the like. See, for example, U.S. Pat. Nos. 5,039,523 and 4,853,331; EPO 0480762A2; Sambrook et al. (1992) Molecular Cloning: A Laboratory Manual, ed. Maniatis et al. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), hereinafter "Sambrook 11"; Davis et al., eds. (1980) Advanced Bacterial Genetics (Cold Spring Harbor Laboratory Press), Cold Spring Harbor, N.Y., and the references cited therein.

[0071] In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

[0072] A number of promoters can be used in the practice of the invention. The promoters can be selected based on the desired outcome. The nucleic acids can be combined with constitutive, inducible, tissue-preferred, or other promoters for expression in the organism of interest. See, for example, promoters set forth in WO 99/43838 and in US Patent Nos: 8,575,425; 7,790,846; 8,147,856; 8,586832; 7,772,369; 7,534,939; 6,072,050; 5,659,026; 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611; herein incorporated by reference.

[0073] For expression in plants, constitutive promoters also include CaMV 35S promoter (Odell *et al.* (1985) *Nature* 313:810-812); rice actin (McElroy *et al.* (1990) *Plant Cell* 2:163-171); ubiquitin (Christensen *et al.* (1989) *Plant Mol. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last *et al.* (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten *et al.* (1984) *EMBO J.* 3:2723-2730). Inducible promoters include those that drive expression of pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen. See, for example, Redolfi *et al.* (1983) *Neth. J. Plant Pathol.* 89:245-254; Uknes *et al.* (1992) *Plant Cell* 4:645-656; and Van Loon (1985) *Plant Mol. Virol.* 4:111-116; and WO 99/43819, herein incorporated by reference. Promoters that are expressed locally at or near the site of

pathogen infection may also be used (Marineau *et al.* (1987) *Plant Mol. Biol.* 9:335-342; Matton *et al.* (1989) *Molecular Plant-Microbe Interactions* 2:325-331; Somsisch *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somsisch *et al.* (1988) *Mol. Gen. Genet.* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977; Chen *et al.* (1996) *Plant J.* 10:955-966; Zhang *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:2507-2511; Warner *et al.* (1993) *Plant J.* 3:191-201; Siebertz *et al.* (1989) *Plant Cell* 1:961-968; Cordero *et al.* (1992) *Physiol. Mol. Plant Path.* 41:189-200; U.S. Patent No. 5,750,386 (nematode-inducible); and the references cited therein).

[0074] Wound-inducible promoters may be used in the constructions of the invention. Such wound-inducible promoters include pin II promoter (Ryan (1990) *Ann. Rev. Phytopath.* 28:425-449; Duan *et al.* (1996) *Nature Biotechnology* 14:494-498); wun1 and wun2 (U.S. Patent No. 5,428,148); win1 and win2 (Stanford *et al.* (1989) *Mol. Gen. Genet.* 215:200-208); systemin (McGurl *et al.* (1992) *Science* 225:1570-1573); WIP1 (Rohmeier *et al.* (1993) *Plant Mol. Biol.* 22:783-792; Eckelkamp *et al.* (1993) *FEBS Letters* 323:73-76); MPI gene (Corderok *et al.* (1994) *Plant J.* 6(2):141-150); and the like, herein incorporated by reference.

[0075] Tissue-preferred promoters for use in the invention include those set forth in Yamamoto *et al.* (1997) *Plant J.* 12(2):255-265; Kawamata *et al.* (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen *et al.* (1997) *Mol. Gen Genet.* 254(3):337-343; Russell *et al.* (1997) *Transgenic Res.* 6(2):157-168; Rinehart *et al.* (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp *et al.* (1996) *Plant Physiol.* 112(2):525-535; Canevascini *et al.* (1996) *Plant Physiol.* 112(2):513-524; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35(5):773-778; Lam (1994) *Results Probl. Cell Differ.* 20:181-196; Orozco *et al.* (1993) *Plant Mol Biol.* 23(6):1129-1138; Matsuoka *et al.* (1993) *Proc Natl. Acad. Sci. USA* 90(20):9586-9590; and Guevara-Garcia *et al.* (1993) *Plant J.* 4(3):495-505.

[0076] Leaf-preferred promoters include those set forth in Yamamoto *et al.* (1997) *Plant J.* 12(2):255-265; Kwon *et al.* (1994) *Plant Physiol.* 105:357-67; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35(5):773-778; Gotor *et al.* (1993) *Plant J.* 3:509-18; Orozco *et al.* (1993) *Plant Mol. Biol.* 23(6):1129-1138; and Matsuoka *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90(20):9586-9590.

[0077] Root-preferred promoters are known and include those in Hire *et al.* (1992) *Plant Mol. Biol.* 20(2):207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner (1991) *Plant Cell* 3(10):1051-1061 (root-specific control element); Sanger *et al.* (1990) *Plant Mol. Biol.* 14(3):433-443 (mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); and Miao *et al.* (1991) *Plant Cell* 3(1):11-22 (cytosolic glutamine synthetase (GS)); Bogusz *et al.* (1990) *Plant Cell* 2(7):633-641; Leach and Aoyagi (1991) *Plant Science* (Limerick) 79(1):69-76 (rolC and rolD); Teeri *et al.* (1989) *EMBO J.* 8(2):343-350; Kuster *et al.* (1995) *Plant Mol. Biol.* 29(4):759-772 (the VfENOD-GRP3 gene promoter); and, Capana *et al.* (1994) *Plant Mol. Biol.* 25(4):681-691 (rolB promoter). See also U.S. Patent Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

[0078] "Seed-preferred" promoters include both "seed-specific" promoters (those promoters active during seed development such as promoters of seed storage proteins) as well as "seed-germinating" promoters (those promoters active during seed germination). See Thompson *et al.* (1989) *BioEssays* 10:108. Seed-preferred promoters include, but are not limited to, Cim1 (cytokinin-induced message); cZ19B1 (maize 19 kDa zein); milps (myo-inositol-1-phosphate synthase) (see WO 00/11177 and U.S. Patent No. 6,225,529). Gamma-zein is an endosperm-specific promoter. Globulin 1 (Glb-1) is a representative embryo-specific promoter. For dicots, seed-specific promoters include, but are not limited to, bean β-phaseolin, napin, β-conglycinin, soybean lectin, cruciferin, and the like. For monocots, seed-specific promoters include, but are not limited to, maize 15 kDa zein, 22 kDa zein, 27 kDa zein, gamma-zein, waxy, shrunken 1, shrunken 2, Globulin 1, etc. See also WO 00/12733, where seed-preferred promoters from *end1* and *end2* genes are disclosed.

[0079] For expression in a bacterial host, promoters that function in bacteria are well-known in the art. Such promoters include any of the known crystal protein gene promoters, including the promoters of any of the pesticidal proteins of the invention, and promoters specific for *B. thuringiensis* sigma factors. Alternatively, mutagenized or recombinant crystal protein-encoding gene promoters may be recombinantly engineered and used to promote expression of the novel gene segments disclosed herein.

[0080] The expression cassette can also comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). Additional selectable markers are known and any can be used in the practice of the invention. See, for example, PCT/US2015/066648, filed on December 18, 2015, herein incorporated by reference in its entirety, which discloses glufosinate resistance sequences that can be employed as selectable markers.

IV. Methods, Host Cells and Plant Cells

[0081] As indicated, DNA constructs comprising nucleotide sequences encoding the pesticidal proteins or active variants or fragment thereof can be used to transform plants of interest or other organisms of interest. Methods for transformation involve introducing a nucleotide construct into a plant. By "introducing" is intended to introduce the nucleotide construct to the plant or other host cell in such a manner that the construct gains access to the interior of a cell of the plant or host cell. The methods of the invention do not require a particular method for introducing a nucleotide construct to a plant or host cell, only that the nucleotide construct gains access to the interior of at least one cell of the plant or the host organism. Methods for introducing nucleotide constructs into plants and other host cells are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

[0082] The methods result in a transformed organisms, such as a plant, including whole plants, as well as plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, propagules, embryos and progeny of the same. Plant cells can be differentiated or undifferentiated (e.g. callus, suspension culture cells, protoplasts, leaf cells, root cells, phloem cells, pollen).

[0083] "Transgenic plants" or "transformed plants" or "stably transformed" plants or cells or tissues refers to plants that have incorporated or integrated a polynucleotide encoding at least one pesticidal polypeptide of the invention. It is recognized that other

exogenous or endogenous nucleic acid sequences or DNA fragments may also be incorporated into the plant cell. *Agrobacterium*-and biolistic-mediated transformation remain the two predominantly employed approaches. However, transformation may be performed by infection, transfection, microinjection, electroporation, microprojection, biolistics or particle bombardment, electroporation, silica/carbon fibers, ultrasound mediated, PEG mediated, calcium phosphate co-precipitation, polycation DMSO technique, DEAE dextran procedure, Agro and viral mediated(Caulimoriviruses, Geminiviruses, RNA plant viruses), liposome mediated and the like.

[0084] Transformation protocols as well as protocols for introducing polypeptides or polynucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Methods for transformation are known in the art and include those set forth in US Patent Nos: 8,575,425; 7,692,068; 8,802,934; 7,541,517; each of which is herein incorporated by reference. See, also, Rakoczy-Trojanowska, M. (2002) *Cell Mol Biol Lett.* 7:849-858; Jones *et al.* (2005) *Plant Methods* 1:5; Rivera *et al.* (2012) *Physics of Life Reviews* 9:308-345; Bartlett *et al.* (2008) *Plant Methods* 4:1-12; Bates, G.W. (1999) *Methods in Molecular Biology* 111:359-366; Binns and Thomashow (1988) *Annual Reviews in Microbiology* 42:575-606; Christou, P. (1992) *The Plant Journal* 2:275-281; Christou, P. (1995) *Euphytica* 85:13-27; Tzfira *et al.* (2004) *TRENDS in Genetics* 20:375-383; Yao *et al.* (2006) *Journal of Experimental Botany* 57:3737-3746; Zupan and Zambryski (1995) *Plant Physiology* 107:1041-1047; Jones *et al.* (2005) *Plant Methods* 1:5;

[0085] Transformation may result in stable or transient incorporation of the nucleic acid into the cell. "Stable transformation" is intended to mean that the nucleotide construct introduced into a host cell integrates into the genome of the host cell and is capable of being inherited by the progeny thereof. "Transient transformation" is intended to mean that a polynucleotide is introduced into the host cell and does not integrate into the genome of the host cell.

[0086] Methods for transformation of chloroplasts are known in the art. See, for example, Svab et al. (1990) Proc. Nail. Acad. Sci. USA 87:8526-8530; Svab and Maliga (1993) Proc. Natl. Acad. Sci. USA 90:913-917; Svab and Maliga (1993) EMBO J.

12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination. Additionally, plastid transformation can be accomplished by transactivation of a silent plastid-borne transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91:7301-7305.

[0087] The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick et al. (1986) Plant Cell Reports 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved. In this manner, the present invention provides transformed seed (also referred to as "transgenic seed") having a nucleotide construct of the invention, for example, an expression cassette of the invention, stably incorporated into their genome.

[0088] In specific embodiments, the sequences provided herein can be targeted to specific sites within the genome of the host cell or plant cell. Such methods include, but are not limited to, meganucleases designed against the plant genomic sequence of interest (D'Halluin *et al.* 2013 *Plant Biotechnol J*); CRISPR-Cas9, TALENs, and other technologies for precise editing of genomes (Feng, *et al.* Cell Research 23:1229-1232, 2013, Podevin, *et al. Trends Biotechnology*, online publication, 2013, Wei *et al.*, *J Gen Genomics*, 2013, Zhang *et al* (2013) WO 2013/026740); Cre-lox site-specific recombination (Dale *et al.* (1995) *Plant J* 7:649-659; Lyznik, *et al.* (2007) *Transgenic Plant J* 1:1-9; FLP-FRT recombination (Li *et al.* (2009) *Plant Physiol* 151:1087-1095); Bxb1-mediated integration (Yau *et al. Plant J* (2011) 701:147-166); zinc-finger mediated integration (Wright *et al.* (2005) *Plant J* 44:693-705); Cai *et al.* (2009) *Plant Mol Biol* 69:699-709); and homologous recombination (Lieberman-Lazarovich and Levy (2011) *Methods Mol Biol* 701: 51-65); Puchta (2002) *Plant Mol Biol* 48:173-182).

[0089] The sequence provided herein may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (maize), sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, and oilseed rape, Brassica sp., alfalfa, rye, millet, safflower, peanuts, sweet potato, cassaya, coffee, coconut, pineapple, citrus trees, cocoa, tea, banana, avocado, fig, guava, mango, olive, papaya, cashew, macadamia, almond, oats, vegetables, ornamentals, and conifers.

[0090] Vegetables include, but are not limited to, tomatoes, lettuce, green beans, lima beans, peas, and members of the genus Curcumis such as cucumber, cantaloupe, and musk melon. Ornamentals include, but are not limited to, azalea, hydrangea, hibiscus, roses, tulips, daffodils, petunias, carnation, poinsettia, and chrysanthemum. Preferably, plants of the present invention are crop plants (for example, maize, sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, oilseed rape, etc.).

[0091] As used herein, the term plant includes plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers, and the like. Grain is intended to mean the mature seed produced by commercial growers for purposes other than growing or reproducing the species. Progeny, variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced polynucleotides. Further provided is a processed plant product or byproduct that retains the sequences disclosed herein, including for example, soymeal.

[0092] In another embodiment, the genes encoding the pesticidal proteins can be used to transform insect pathogenic organisms. Such organisms include baculoviruses, fungi, protozoa, bacteria, and nematodes. Microorganism hosts that are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplana) of one or more crops of interest may be selected. These microorganisms are selected so as to be capable

of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the pesticidal protein, and desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

[0093] Such microorganisms include archaea, bacteria, algae, and fungi. Of particular interest are microorganisms such as bacteria, e.g., Bacillus, Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylius, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc, and Alcaligenes. Fungi include yeast, e.g., Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula, and Aureobasidium. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, Agrobacteria, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioti*, *Alcaligenes entrophus*, *Clavibacter xyli* and *Azotobacter vinlandir* and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces rosues*, *S. odorus*, *Kluyveromyces veronae*, *Aureobasidium pollulans*, *Bacillus thuringiensis*, *Escherichia coli*, *Bacillus subtilis*, and the like.

[0094] Illustrative prokaryotes, both Gram-negative and gram-positive, include Enterobacteriaceae, such as Escherichia, Erwinia, Shigella, Salmonella, and Proteus; Bacillaceae; Rhizobiceae, such as Rhizobium; Spirillaceae, such as photobacterium, Zymomonas, Serratia, Aeromonas, Vibrio, Desulfovibrio, Spirillum; Lactobacillaceae; Pseudomonadaceae, such as Pseudomonas and Acetobacter; Azotobacteraceae and Nitrobacteraceae. Fungi include Phycomycetes and Ascomycetes, e.g., yeast, such as Saccharomyces and Schizosaccharomyces; and Basidiomycetes yeast, such as Rhodotorula, Aureobasidium, Sporobolomyces, and the like.

[0095] Genes encoding pesticidal proteins can be introduced by means of electrotransformation, PEG induced transformation, heat shock, transduction, conjugation, and the like. Specifically, genes encoding the pesticidal proteins can be

cloned into a shuttle vector, for example, pHT3101 (Lerecius *et al.* (1989) *FEMS Microbiol. Letts.* 60: 211-218. The shuttle vector pHT3101 containing the coding sequence for the particular pesticidal protein gene can, for example, be transformed into the root-colonizing Bacillus by means of electroporation (Lerecius *et al.* (1989) *FEMS Microbiol. Letts.* 60: 211-218).

[0096] Expression systems can be designed so that pesticidal proteins are secreted outside the cytoplasm of gram-negative bacteria by fusing an appropriate signal peptide to the amino-terminal end of the pesticidal protein. Signal peptides recognized by *E. coli* include the OmpA protein (Ghrayeb *et al.* (1984) *EMBO J*, 3: 2437-2442).

[0097] Pesticidal proteins and active variants thereof can be fermented in a bacterial host and the resulting bacteria processed and used as a microbial spray in the same manner that *Bacillus thuringiensis* strains have been used as insecticidal sprays. In the case of a pesticidal protein(s) that is secreted from Bacillus, the secretion signal is removed or mutated using procedures known in the art. Such mutations and/or deletions prevent secretion of the pesticidal protein(s) into the growth medium during the fermentation process. The pesticidal proteins are retained within the cell, and the cells are then processed to yield the encapsulated pesticidal proteins.

[0098] Alternatively, the pesticidal proteins are produced by introducing heterologous genes into a cellular host. Expression of the heterologous gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. These cells are then treated under conditions that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s). The resulting product retains the toxicity of the toxin. These naturally encapsulated pesticidal proteins may then be formulated in accordance with conventional techniques for application to the environment hosting a target pest, e.g., soil, water, and foliage of plants. See, for example U.S. Patent No. 6,468,523 and U.S. Publication No. 20050138685, and the references cited therein. In the present invention, a transformed microorganism (which includes whole organisms, cells, spore(s), pesticidal protein(s), pesticidal component(s), pest-impacting component(s), mutant(s), living or dead cells and cell components, including mixtures of living and dead cells and cell components, and including broken cells and

cell components) or an isolated pesticidal protein can be formulated with an acceptable carrier into a pesticidal or agricultural composition(s) that is, for example, a suspension, a solution, an emulsion, a dusting powder, a dispersible granule, a wettable powder, and an emulsifiable concentrate, an aerosol, an impregnated granule, an adjuvant, a coatable paste, and also encapsulations in, for example, polymer substances.

[0099] Agricultural compositions may comprise a polypeptide, a recombinogenic polypeptide or a variant or fragment thereof, as disclosed herein. The agricultural composition disclosed herein may be applied to the environment of a plant or an area of cultivation, or applied to the plant, plant part, plant cell, or seed.

[0100] Such compositions disclosed above may be obtained by the addition of a surface-active agent, an inert carrier, a preservative, a humectant, a feeding stimulant, an attractant, an encapsulating agent, a binder, an emulsifier, a dye, a UV protectant, a buffer, a flow agent or fertilizers, micronutrient donors, or other preparations that influence plant growth. One or more agrochemicals including, but not limited to, herbicides, insecticides, fungicides, bactericides, nematicides, molluscicides, acaracides, plant growth regulators, harvest aids, and fertilizers, can be combined with carriers, surfactants or adjuvants customarily employed in the art of formulation or other components to facilitate product handling and application for particular target pests. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g., natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders, or fertilizers. The active ingredients of the present invention are normally applied in the form of compositions and can be applied to the crop area, plant, or seed to be treated. For example, the compositions of the present invention may be applied to grain in preparation for or during storage in a grain bin or silo, etc. The compositions of the present invention may be applied simultaneously or in succession with other compounds. Methods of applying an active ingredient of the present invention or an agrochemical composition of the present invention that contains at least one of the pesticidal proteins produced by the bacterial strains of the present invention include, but are not limited to, foliar application,

seed coating, and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

[0101] Suitable surface-active agents include, but are not limited to, anionic compounds such as a carboxylate of, for example, a metal; a carboxylate of a long chain fatty acid; an N-acylsarcosinate; mono or di-esters of phosphoric acid with fatty alcohol ethoxylates or salts of such esters; fatty alcohol sulfates such as sodium dodecyl sulfate, sodium octadecyl sulfate or sodium cetyl sulfate; ethoxylated fatty alcohol sulfates; ethoxylated alkylphenol sulfates; lignin sulfonates; petroleum sulfonates; alkyl aryl sulfonates such as alkyl-benzene sulfonates or lower alkylnaphtalene sulfonates, e.g., butyl-naphthalene sulfonate; salts of sulfonated naphthalene-formaldehyde condensates; salts of sulfonated phenol-formaldehyde condensates; more complex sulfonates such as the amide sulfonates, e.g., the sulfonated condensation product of oleic acid and N-methyl taurine; or the dialkyl sulfosuccinates, e.g., the sodium sulfonate of dioctyl succinate. Non-ionic agents include condensation products of fatty acid esters, fatty alcohols, fatty acid amides or fatty-alkyl- or alkenyl-substituted phenols with ethylene oxide, fatty esters of polyhydric alcohol ethers, e.g., sorbitan fatty acid esters, condensation products of such esters with ethylene oxide, e.g., polyoxyethylene sorbitar fatty acid esters, block copolymers of ethylene oxide and propylene oxide, acetylenic glycols such as 2,4,7,9-tetraethyl-5-decyn-4,7-diol, or ethoxylated acetylenic glycols. Examples of a cationic surface-active agent include, for instance, an aliphatic mono-, di-, or polyamine such as an acetate, naphthenate or oleate; or oxygen-containing amine such as an amine oxide of polyoxyethylene alkylamine; an amide-linked amine prepared by the condensation of a carboxylic acid with a di- or polyamine; or a quaternary ammonium salt.

[0102] Examples of inert materials include but are not limited to inorganic minerals such as kaolin, phyllosilicates, carbonates, sulfates, phosphates, or botanical materials such as cork, powdered corncobs, peanut hulls, rice hulls, and walnut shells.

[0103] The compositions of the present invention can be in a suitable form for direct application or as a concentrate of primary composition that requires dilution with a suitable quantity of water or other diluant before application. The pesticidal concentration

will vary depending upon the nature of the particular formulation, specifically, whether it is a concentrate or to be used directly. The composition contains 1 to 98% of a solid or liquid inert carrier, and 0 to 50% or 0.1 to 50% of a surfactant. These compositions will be administered at the labeled rate for the commercial product, for example, about 0.01 lb-5.0 lb. per acre when in dry form and at about 0.01 pts.-10 pts. per acre when in liquid form.

[0104] In a further embodiment, the compositions, as well as the transformed microorganisms and pesticidal proteins, provided herein can be treated prior to formulation to prolong the pesticidal activity when applied to the environment of a target pest as long as the pretreatment is not deleterious to the pesticidal activity. Such treatment can be by chemical and/or physical means as long as the treatment does not deleteriously affect the properties of the composition(s). Examples of chemical reagents include but are not limited to halogenating agents; aldehydes such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride; alcohols, such as isopropanol and ethanol; and histological fixatives, such as Bouin's fixative and Helly's fixative (see, for example, Humason (1967) *Animal Tissue Techniques* (W.H. Freeman and Co.).

[0105] In one aspect, pests may be killed or reduced in numbers in a given area by application of the pesticidal proteins of the invention to the area. Alternatively, the pesticidal proteins may be prophylactically applied to an environmental area to prevent infestation by a susceptible pest. Preferably the pest ingests, or is contacted with, a pesticidally-effective amount of the polypeptide. By "pesticidally-effective amount" is intended an amount of the pesticide that is able to bring about death to at least one pest, or to noticeably reduce pest growth, feeding, or normal physiological development. This amount will vary depending on such factors as, for example, the specific target pests to be controlled, the specific environment, location, plant, crop, or agricultural site to be treated, the environmental conditions, and the method, rate, concentration, stability, and quantity of application of the pesticidally-effective polypeptide composition. The formulations or compositions may also vary with respect to climatic conditions, environmental considerations, and/or frequency of application and/or severity of pest infestation.

[0106] The active ingredients are normally applied in the form of compositions and can be applied to the crop area, plant, or seed to be treated. Methods are therefore provided for providing to a plant, plant cell, seed, plant part or an area of cultivation, an effective amount of the agricultural composition comprising the polypeptide, recombinogenic polypeptide or an active variant or fragment thereof. By "effective amount" is intended an amount of a protein or composition sufficient to kill or control the pest or result in a noticeable reduction in pest growth, feeding, or normal physiological development. Such decreases in pest numbers, pest growth, pest feeding or pest normal development can comprise any statistically significant decrease, including, for example a decrease of about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90%, 95% or greater. For example, the compositions may be applied to grain in preparation for or during storage in a grain bin or silo, etc. The compositions may be applied simultaneously or in succession with other compounds. Methods of applying an active ingredient or an agrochemical composition comprising at least one of the polypeptides, recombinogenic polypeptides or variants or fragments thereof as disclosed herein, include but are not limited to, foliar application, seed coating, and soil application.

[0107] Methods for increasing plant yield are provided. The methods comprise providing a plant or plant cell expressing a polynucleotide encoding the pesticidal polypeptide sequence disclosed herein and growing the plant or a seed thereof in a field infested with (or susceptible to infestation by) a pest against which said polypeptide has pesticidal activity. In some embodiments, the polypeptide has pesticidal activity against a lepidopteran, coleopteran, dipteran, hemipteran, or nematode pest, and said field is infested with a lepidopteran, hemipteran, coleopteran, dipteran, or nematode pest. As defined herein, the "yield" of the plant refers to the quality and/or quantity of biomass produced by the plant. By "biomass" is intended any measured plant product. An increase in biomass production is any improvement in the yield of the measured plant product. Increasing plant yield has several commercial applications. For example, increasing plant leaf biomass may increase the yield of leafy vegetables for human or animal consumption. Additionally, increasing leaf biomass can be used to increase production of plant-derived pharmaceutical or industrial products. An increase in yield can comprise any statistically significant increase including, but not limited to, at least a 1% increase,

at least a 3% increase, at least a 5% increase, at least a 10% increase, at least a 20% increase, at least a 30%, at least a 50%, at least a 70%, at least a 100% or a greater increase in yield compared to a plant not expressing the pesticidal sequence. In specific methods, plant yield is increased as a result of improved pest resistance of a plant expressing a pesticidal protein disclosed herein. Expression of the pesticidal protein results in a reduced ability of a pest to infest or feed.

[0108] The plants can also be treated with one or more chemical compositions, including one or more herbicide, insecticides, or fungicides.

[0109] Non-limiting embodiments include:

[0110] 1. An isolated polypeptide having insecticidal activity, comprising:

(a) a polypeptide comprising an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229; or

(b) a polypeptide comprising an amino acid sequence having at least the percent sequence identity set forth in Table 1 to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106,

107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229.

[0111] 2. The polypeptide of embodiment 1, wherein said polypeptide comprises the amino acid sequence set forth in SEQ ID Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229.

[0112] 3. The polypeptide of embodiment 1 or 2, further comprising heterologous amino acid sequences.

[0113] 4. A composition comprising the polypeptide of any one of embodiments 1 to 3.

[0114] 5. A recombinant nucleic acid molecule that encodes the polypeptide of any one of embodiments 1 to 3, wherein said recombinant nucleic acid molecule is not the naturally occurring sequence encoding said polypeptide.

[0115] 6. The recombinant nucleic acid of embodiment 5, wherein said nucleic acid molecule is a synthetic sequence that has been designed for expression in a plant.

[0116] 7. The recombinant nucleic acid molecule of embodiment 5 or 6, wherein said nucleic acid molecule is operably linked to a promoter capable of directing expression in a plant cell.

[0117] 8. The recombinant nucleic acid molecule of embodiment 5 or 6, wherein said nucleic acid molecule is operably linked to a promoter capable of directing expression in a bacteria.

[0118] 9. A host cell that contains the recombinant nucleic acid molecule of any one of embodiments 5 to 8.

[0119] 10. The host cell of embodiment 9, wherein said host cell is a bacterial host cell.

[0120] 11. A DNA construct comprising a promoter that drives expression in a plant cell operably linked to a recombinant nucleic acid molecule comprising:

(a) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229; or,

(b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least the percent sequence identity set forth in Table 1 to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229.

[0121] 12. The DNA construct of embodiment 11, wherein said nucleotide sequence is a synthetic DNA sequence that has been designed for expression in a plant.

[0122] 13. A vector comprising the DNA construct of embodiment 11 or 12.

[0123] 14. A host cell that contains the DNA construct of embodiment 11 or 12 or the vector of embodiment 13.

[0124] 15. The host cell of embodiment 13 or 14, wherein the host cell is a plant cell.

[0125] 16. A transgenic plant comprising the host cell of embodiment 15.

[0126] 17. A composition comprising the host cell of any one of embodiments 9, 10, 14, or 15.

[0127] 18. The composition of embodiment 17, wherein said composition is selected from the group consisting of a powder, dust, pellet, granule, spray, emulsion, colloid, and solution.

[0128] 19. The composition of embodiment 17 or 18, wherein said composition comprises from about 1% to about 99% by weight of said polypeptide.

[0129] 20. A method for controlling a pest population comprising contacting said population with a pesticidal-effective amount of the composition of any one of embodiments 17 to 19.

[0130] 21. A method for killing a pest population comprising contacting said population with a pesticidal-effective amount of the composition of any one of embodiments 17 to 19.

[0131] 22. A method for producing a polypeptide with pesticidal activity, comprising culturing the host cell of any one of embodiments 9, 10, 14, or 15 under conditions in which the nucleic acid molecule encoding the polypeptide is expressed.

[0132] 23. A plant having stably incorporated into its genome a DNA construct comprising a nucleotide sequence that encodes a protein having pesticidal activity, wherein said nucleotide sequence comprises:

(a) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229; or,

(b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least the percent sequence identity set forth in Table 1 to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117,

118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229.

[0133] 24. A transgenic seed of the plant of embodiment 23.

[0134] 25. A method for protecting a plant from an insect pest, comprising expressing in a plant or cell thereof a nucleotide sequence that encodes a pesticidal polypeptide, wherein said nucleotide sequence comprising:

(a) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229; or,

(b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least the percent sequence identity set forth in Table 1 to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229.

[0135] 26. The method of embodiment 25, wherein said plant produces a pesticidal polypeptide having pesticidal activity against a lepidopteran or coleopteran pest.

[0136] 27. A method for increasing yield in a plant comprising growing in a field a plant or seed thereof having stably incorporated into its genome a DNA construct comprising a promoter that drives expression in a plant operably linked to a nucleotide sequence that encodes a pesticidal polypeptide, wherein said nucleotide sequence comprises:

(a) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229; or,

(b) a nucleotide sequence that encodes a polypeptide comprising an amino acid

sequence having at least the percent sequence identity set forth in Table 1 to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133,134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 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, or 229.

[0137] 28. A method of obtaining a polynucleotide that encodes an improved polypeptide comprising pesticidal activity is provided, wherein the improved polypeptide has at least one improved property over any one of SEQ ID NOS: 1-229 comprising:

(a) recombining a plurality of parental polynucleotides comprising SEQ ID NO: 1-229 or an active variant or fragment thereof to produce a library of recombinant polynucleotides encoding recombinant pesticidal polypeptides;

(b) screening the library to identify a recombinant polynucleotide that encodes an improved recombinant pesticidal polypeptide that has an enhanced property improved over the parental polynucleotide;

(c) recovering the recombinant polynucleotide that encodes the improved recombinant pesticidal polypeptide identified in (b); and,

(d) repeating steps (a), (b) and (c) using the recombinant polynucleotide recovered in step (c) as one of the plurality of parental polynucleotides in repeated step (a).

[0138] The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Experiment 1: Discovery of novel genes by sequencing and DNA analysis

[0139] Microbial cultures were grown in liquid culture in standard laboratory media. Cultures were grown to saturation (16 to 24 hours) before DNA preparation. DNA was extracted from bacterial cells by detergent lysis, followed by binding to a silica matrix and washing with an ethanol buffer. Purified DNA was eluted from the silica matrix with a mildly alkaline aqueous buffer.

[0140] DNA for sequencing was tested for purity and concentration by spectrophotometry. Sequencing libraries were prepared using the Nextera XT library preparation kit according to the manufacturer's protocol. Sequence data was generated on a HiSeq 2000 according to the Illumina HiSeq 2000 System User Guide protocol.

[0141] Sequencing reads were assembled into draft genomes using the CLC Bio Assembly Cell software package. Following assembly, gene calls were made by several methods and resulting gene sequences were interrogated to identify novel homologs of pesticidal genes. Novel genes were identified by BLAST, by domain composition, and by pairwise alignment versus a target set of pesticidal genes. A summary of such sequences is set forth in Table 1.

[0142] Genes identified in the homology search were amplified from bacterial DNA by PCR and cloned into bacterial expression vectors containing fused in-frame purification tags. Cloned genes were expressed in E. coli and purified by column chromatography. Purified proteins were assessed in insect diet bioassay studies to identify active proteins.

[0143] Insect diet bioassays were performed using a wheat germ and agar artificial diet to which purified protein were applied as a surface treatment. Insect larvae were applied to treated diet and monitored for mortality.

[0144] Insect diet bioassays were performed using a sucrose liquid diet contained in a membrane sachet to which purified protein was added. Insect nymphs were allowed to feed on the diet sachet and were monitored for mortality. Insects tested in bioassays

included the Brown Stink Bug (BSB), *Euschistus servus*, and the Southern Green Stink Bug (SGSB), *Nezara viridula*. Data is listed in the below in Table 3.

Table 3.

Gene	Expression Level	Test 1	Test 2	Test 3
APG00059	Very Low (<10 ppm)	+ BSB	+ SGSB	+ SGSB
APG00046	High (>500ppm)	+ BSB	+ BSB	
APG00002	Low (50 ppm)	+ SGSB	+ SGSB	+ SGSB

BSB = Brown Stink Bug, SGSB = Southern Green Stink Bug

Example 2. Heterologous Expression in E. coli

[0145] Each open reading frame set forth in Tables 4 and 5 was cloned into an *E. coli* expression vector containing a maltose binding protein (pMBP). The expression vector was transformed into BL21*RIPL. An LB culture supplemented with carbenicillin was inoculated with a single colony and grown overnight at 37°C using 0.5% of the overnight culture, a fresh culture was inoculated and grown to logarithmic phase at 37°C. The culture was induced using 250 mM IPTG for 18 hours at 16°C. The cells were pelleted and resuspended in 10mM Tris pH7.4 and 150 mM NaCl supplemented with protease inhibitors. The protein expression was evaluated by SDS-PAGE.

Example 3. Pesticidal Activity against Coleopteran and Lepidoptera

[0146] *Protein Expression:* Each sequence set forth in Table 4 was expressed in *E. coli* as described in Example 2. 400 mL of LB was inoculated and grown to an OD600 of 0.6. The culture was induced with 0.25mM IPTG overnight at 16°C. The cells were spun down and the cell pellet was resuspend in 5 mL of buffer. The resuspension was sonicated for 2 min on ice.

[0147] *Bioassay:* Fall army worm (FAW), corn ear worm (CEW), European corn borer (ECB) southwestern corn borer (SWCB) and diamond backed moth (DBM or Px) eggs were purchased from a commercial insectary (Benzon Research Inc., Carlisle, PA). The FAW, CEW, ECB and BCW eggs were incubated to the point that eclosion would occur within 12 hrs of the assay setup. SWCB and DBM were introduced to the assay as neonate larvae. Assays were carried out in 24-well trays containing multispecies

lepidopteran diet (Southland Products Inc., Lake Village, AR). Samples of the sonicated lysate were applied to the surface of the diet (diet overlay) and allowed to evaporate and soak into the diet. For CEW, FAW, BCW, ECB and SWCB, a 125 µl of sonicated lysate was added to the diet surface and dried. For DBM, 50 µl of a 1:2 dilution of sonicated lysate was added to the diet surface. The bioassay plates were sealed with a plate sealing film vented with pin holes. The plates were incubated at 26°C at 65% relative humidity (RH) on a 16:8 day:night cycle in a Percival for 5 days. The assays were assessed for level of mortality, growth inhibition and feeding inhibition.

[0148] For the western corn rootworm bioassay, the protein construct/lysate was evaluated in an insect bioassay by dispensing 60 µl volume on the top surface of diet in well/s of 24-well plate (Cellstar, 24-well, Greiner Bio One) and allowed to dry. Each well contained 500 µl diet (Marrone et al., 1985). Fifteen to twenty neonate larvae were introduced in each well using a fine tip paint brush and the plate was covered with membrane (Viewseal, Greiner Bio One). The bioassay was stored at ambient temperature and scored for mortality, and/or growth/feeding inhibition at day 4.

[0149] For Colorado Potato Beetle (CPB) a cork bore size No. 8 leaf disk was excised from potato leaf and was dipped in the protein construct/lysate until thoroughly wet and placed on top of filter disk (Millipore, glass fiber filter, 13 mm). 60 µl dH_2O was added to each filter disk and placed in each well of 24-well plate (Cellstar, 24-well, Greiner Bio One). The leaf disk was allowed to dry and five to seven first instar larvae were introduced in each well using a fine tip paint brush. The plate was covered with membrane (Viewseal, Greiner Bio One) and small hole was punctured in each well of the membrane. The construct was evaluated with four replicates, and scored for mortality and leaf damage on day 3.

[0150] Table 4 provides a summary of pesticidal activity against coleopteran and lepidoptera of the various sequences. Table code: "-" indicates no activity seen; "+" indicates pesticidal activity seen; "NT" indicates not tested; "S" indicates stunt; "SS" indicates slight stunt; "LF" indicates low feeding, "M" indicates mortality.

Table 4. Summary of Pesticidal Activity against Coleopteran and Lepidoptera.

APG	Seq ID	FAW	CEW	BCW	ECB	SWCB	CPB	Px	WCR Mortality (%)
APG00003	5	SS	SS	-	-	SS	NT	-	0-50% mortality
APG00006	10	M, S	-	-	-	-	NT	NT	0-50% mortality
APG00014	24	SS	-	-	-	-	NT	+	80-100% mortality
APG00016	27	S	-	-	-	SS	NT	+	80-100% mortality
APG00024	40	SS	-	-	-	-	NT	NT	80-100% mortality
APG00025	41	-	-	-	-	-	-	-	0-60% mortality
APG00026	45	M, S	SS	-	-	-	-	+	NT
APG00028	47	-	-	-	-	-	-	-	0-60% mortality
APG00029	49	-	-	-	-	SS	+	-	60-100% mortality
APG00030	51	-	-	-	-	SS	NT	-	NT
APG00031	52	-	-	-	-	NT	NT	NT	NT
APG00032	56	SS	-	-	-	-	NT	NT	0-50% mortality
APG00035	59	-	-	-	-	-	-	-	80-100% mortality
APG00040	62	S	-	SS	-	SS	NT	-	50-80% mortality
APG00041	64	-	-	-	-	-	+	-	0-60% mortality
APG00042	67	S	-	-	-	-	NT	NT	NT
APG00047	77	S	-	-	-	-	NT	-	50-80% mortality
APG00049	79	-	SS	-	NT	NT	NT	-	50-80% mortality
APG00049	79	NT	NT	NT	NT	NT	NT	NT	50% mortality
APG00050	80	-	-	-	-	-	+	-	80-100% mortality
APG00055	87	S	-	SS	SS	-	NT	+	80-100% mortality
APG00061	92	SS	-	-	-	-	NT	-	NT
APG00077	100	-	-	-	-	-	-	-	0-60% mortality
APG00080	102	-	-	-	-	-	-	-	0-60% mortality
APG00082	108	-	-	-	-	-	NT	-	80-100% mortality
APG00083	111	-	-	-	-	SS	NT	-	NT
APG00093	124	-	-	-	-	-	-	-	0-60% mortality
APG00098	129	S	-	-	-	S	NT	-	50-80% mortality
APG00102	131	SS	-	-	-	-	NT	NT	NT
APG00103	132	-	-	-	-	-	NT	-	80-100% mortality
APG00106	134	SS	-	-	-	-	+	NT	50-80% mortality
APG00109	136	-	-	-	-	NT	-	NT	0-50% mortality
APG00111	140	-	-	-	-	-	-	-	80-100% mortality
APG00123	148	-	-	-	-	-	+		80-100% mortality
APG00125	151	M, S	-	-	-	-	NT		NT
APG00127	156	-	-	-	-	-	NT		80-100% mortality
APG00128	157	NT	NT	NT	NT	NT	NT	NT	50-100% % mortality
APG00129	159	-	SS	-	-	-	NT		NT
APG00142	162	SS	-	SS	-	SS	NT		80-100% mortality

APG00145	164	-	-	-	-	-	NT		0-50% mortality
APG00146	167	-	-	-	-	-	NT		80-100% mortality
APG00149	172	-	-	-	-	-	NT		80-100% mortality
APG00167	180	HM, S	-	-	-	-	NT		60-100% mortality
APG00169	181	-	-	-	-	-	-		80-100% mortality
APG00174	185	-	-	-	-	-	NT		80-100% mortality
APG00206	199	-	-	-	-	-	NT		80-100% mortality
APG00222	204	-	-	-	-	-	NT		50-80% mortality
APG00234	208	-	-	-	-	-	NT		0-50% mortality
APG00299	213	-	-	-	-	-	-		0-60% mortality

Example 4. Pesticidal Activity against Hemipteran

[0151] *Protein Expression:* Each of the sequences set forth in Table 5 was expressed in *E. coli* as described in Example 2. 400 mL of LB was inoculated and grown to an OD600 of 0.6. The culture was induced with 0.25mM IPTG overnight at 16°C. The cells were spun down and the cell pellet was re-suspend in 5 mL of buffer. The resuspension was sonicated for 2 min on ice.

[0152] Second instar SGSB were obtained from a commercial insectary (Benzon Research Inc., Carlisle, PA). A 50% v/v ratio of sonicated lysate sample to 20% sucrose was employed in the bioassay. Stretched parafilm was used as a feeding membrane to expose the SGSB to the diet/sample mixture. The plates were incubated at 25°C:21°C, 16:8 day:night cycle at 65%RH for 5 days.

[0153] Mortality was scored for each sample. The results are set forth in Table 5. A dashed line indicates no mortality was detected. The proteins listed in Table 5 showed 25% mortality or 75% mortality (as indicated) against southern green stinkbug (1 stinkbug out of 4 died). The negative controls (empty vector expressed binding domain and buffer only) both showed no mortality (0 stinkbugs out of 4).

Table 5.Summary of Pesticidal Activity against Hemipteran

APG	Seq ID	Tested against SGSB
APG00001	3	25%
APG00004	7	25%
APG00015	25	25%

APG00017	28	50%
APG00025	41	25%
APG00029	49	25%
APG00040	62	25%
APG00049	79	25%
APG00096	126	25%
APG00103	132	25%
APG00111	140	25%
APG00149	172	25%
APG00161	177	25%
APG00167	180	25%
APG00174	185	25%
APG00191	191	75%
APG00206	199	25%

Example 5. Transformation of Soybean

[0154] DNA constructs comprising each of SEQ ID NOs: 1-229 or active variants or fragments thereof operably linked to a promoter active in a plant are cloned into transformation vectors and introduced into Agrobacterium as described in PCT application No. PCT/US2015/066702, filed December 18, 2015, herein incorporated by reference in its entirety.

[0155] Four days prior to inoculation, several loops of Agrobacterium are streaked to a fresh plate of YEP* medium supplemented with the appropriate antibiotics** (spectinomycin, chloramphenicol and kanamycin). Bacteria are grown for two days in the dark at 28°C. After two days, several loops of bacteria are transferred to 3 ml of YEP liquid medium with antibiotics in a 125 ml Erlenmeyer flask. Flasks are placed on a rotary shaker at 250 RPM at 28°C overnight. One day before inoculation, 2-3 ml of the overnight culture were transferred to 125 ml of YEP with antibiotics in a 500 ml Erlenmeyer flask. Flasks are placed on a rotary shaker at 250 RPM at 28°C overnight.

[0156] Prior to inoculation, the OD of the bacterial culture is checked at OD 620. An OD of 0.8-1.0 indicates that the culture is in log phase. The culture is centrifuged at 4000 RPM for 10 minutes in Oakridge tubes. The supernatant is discarded and the pellet is re-

suspended in a volume of Soybean Infection Medium (SI) to achieve the desired OD. The cultures are held with periodic mixing until needed for inoculation.

[0157] Two or three days prior to inoculation, soybean seeds are surface sterilized using chlorine gas. In a fume hood, a petri dish with seeds is placed in a bell jar with the lid off. 1.75 ml of 12 N HCl is slowly added to 100 ml of bleach in a 250 ml Erlenmeyer flask inside the bell jar. The lid is immediately placed on top of the bell jar. Seeds are allowed to sterilize for 14-16 hours (overnight). The top is removed from the bell jar and the lid of the petri dish is replaced. The petri dish with the surface sterilized is then opened in a laminar flow for around 30 minutes to disperse any remaining chlorine gas.

[0158] Seeds are imbibed with either sterile DI water or soybean infection medium (SI) for 1-2 days. Twenty to 30 seeds are covered with liquid in a 100x25 mm petri dish and incubated in the dark at 24°C. After imbibition, non-germinating seeds are discarded.

[0159] Cotyledonary explants are processed on a sterile paper plate with sterile filter paper dampened using SI medium employing the methods of U.S. Patent No. 7,473,822, herein incorporated by reference.

[0160] Typically, 16-20 cotyledons are inoculated per treatment. The SI medium used for holding the explants is discarded and replaced with 25 ml of Agrobacterium culture (OD 620=0.8-20). After all explants are submerged, the inoculation is carried out for 30 minutes with periodic swirling of the dish. After 30 minutes, the Agrobacterium culture is removed.

[0161] Co-cultivation plates are prepared by overlaying one piece of sterile paper onto Soybean Co-cultivation Medium (SCC). Without blotting, the inoculated cotyledons are cultured adaxial side down on the filter paper. Around 20 explants can be cultured on each plate. The plates are sealed with Parafilm and cultured at 24°C and around 120 μmoles $m^{-2}s^{-1}$ (in a Percival incubator) for 4-5 days.

[0162] After co-cultivation, the cotyledons are washed 3 times in 25 ml of Soybean Wash Medium with 200 mg/l of cefotaxime and timentin. The cotyledons are blotted on sterile filter paper and then transferred to Soybean Shoot Induction Medium (SSI). The nodal end of the explant is depressed slightly into the medium with distal end kept above

the surface at about 45deg. No more than 10 explants are cultured on each plate. The plates are wrapped with Micropore tape and cultured in the Percival at 24°C and around 120 µmoles $m^{-2}s^{-1}$.

[0163] The explants are transferred to fresh SSI medium after 14 days. Emerging shoots from the shoot apex and cotyledonary node are discarded. Shoot induction is continued for another 14 days under the same conditions.

[0164] After 4 weeks of shoot induction, the cotyledon is separated from the nodal end and a parallel cut is made underneath the area of shoot induction (shoot pad). The area of the parallel cut is placed on Soybean Shoot Elongation Medium (SSE) and the explants cultured in the Percival at 24°C and around 120 µmoles $m^{-2}s^{-1}$. This step is repeated every two weeks for up to 8 weeks as long as shoots continue to elongate.

[0165] When shoots reach a length of 2-3 cm, they are transferred to Soybean Rooting Medium (SR) in a Plantcon vessel and incubated under the same conditions for 2 weeks or until roots reach a length of around 3-4 cm. After this, plants are transferred to soil.

[0166] Note, all media mentioned for soybean transformation are found in Paz et al. (2010) Agrobacterium-mediated transformation of soybean and recovery of transgenic soybean plants; Plant Transformation Facility of Iowa State University, which is herein incorporated by reference in its entirety. (See, agron-www.agron.iastate.edu/ptf/protocol/Soybean.pdf.)

Example 6. Transformation of Maize

[0167] Maize ears are best collected 8-12 days after pollination. Embryos are isolated from the ears, and those embryos 0.8-1.5 mm in size are preferred for use in transformation. Embryos are plated scutellum side-up on a suitable incubation media, such as DN62A5S media (3.98 g/L N6 Salts; 1 mL/L (of 1000X Stock) N6 Vitamins; 800 mg/L L-Asparagine; 100 mg/L Myo-inositol; 1.4 g/L L-Proline; 100 mg/L Casamino acids; 50 g/L sucrose; 1 mL/L (of 1 mg/mL Stock) 2,4-D). However, media and salts other than DN62A5S are suitable and are known in the art. Embryos are incubated overnight at 25°C in the dark. However, it is not necessary per se to incubate the embryos overnight.

[0168] The resulting explants are transferred to mesh squares (30-40 per plate), transferred onto osmotic media for about 30-45 minutes, and then transferred to a beaming plate (see, for example, PCT Publication No. WO/0138514 and U.S. Patent No. 5,240,842). DNA constructs designed to express the GRG proteins of the present invention in plant cells are accelerated into plant tissue using an aerosol beam accelerator, using conditions essentially as described in PCT Publication No. WO/0138514. After beaming, embryos are incubated for about 30 min on osmotic media, and placed onto incubation media overnight at 25°C in the dark. To avoid unduly damaging beamed explants, they are incubated for at least 24 hours prior to transfer to recovery media. Embryos are then spread onto recovery period media, for about 5 days, 25°C in the dark, and then transferred to a selection media. Explants are incubated in selection media for up to eight weeks, depending on the nature and characteristics of the particular selection utilized. After the selection period, the resulting callus is transferred to embryo maturation media, until the formation of mature somatic embryos is observed. The resulting mature somatic embryos are then placed under low light, and the process of regeneration is initiated by methods known in the art. The resulting shoots are allowed to root on rooting media, and the resulting plants are transferred to nursery pots and propagated as transgenic plants.

Example 7. Pesticidal activity against Nematodes

Heterodera glycine's (Soybean Cyst Nematode) *in vitro* assay

[0169] Soybean Cyst Nematodes are dispensed into a 96 well assay plate with a total volume of 100uls and 100 J2 per well. The protein of interest as set forth in any one of SEQ ID NOs: 1-229 is dispensed into the wells and held at room temperature for assessment. Finally, the 96 well plate containing the SCN J2 is analyzed for motility. Data is reported as % inhibition as compared to the controls. Hits are defined as greater or equal to 70% inhibition.

Heterodera glycine's (Soybean Cyst Nematode) on-plant assay

[0170] Soybean plants expressing one or more of SEQ ID NOs: 1-229 are generated as described elsewhere herein. A 3-week-old soybean cutting is inoculated with 5000 SCN eggs per plant. This infection is held for 70 days and then harvested for counting of SCN

cyst that has developed on the plant. Data is reported as % inhibition as compared to the controls. Hits are defined as greater or equal to 90% inhibition.

Meloidogyne incognita (Root-Knot Nematode) *in vitro* assay

[0171] Root-Knot Nematodes are dispensed into a 96 well assay plate with a total volume of 100uls and 100 J2 per well. The protein of interest comprising any one of SEQ ID NOs: 1-229 is dispensed into the wells and held at room temperature for assessment. Finally the 96 well plate containing the RKN J2 is analyzed for motility. Data is reported as % inhibition as compared to the controls. Hits are defined as greater or equal to 70% inhibition.

Meloidogyne incognita (Root-Knot Nematode) on-plant assay

[0172] Soybean plants expressing one or more of SEQ ID NOs: 1-229 are generated as described elsewhere herein. A 3-week-old soybean is inoculated with 5000 RKN eggs per plant. This infection is held for 70days and then harvested for counting of RKN eggs that have developed in the plant. Data is reported as % inhibition as compared to the controls. Hits are defined as greater or equal to 90% inhibition.

Example 8. Additional Assays for Pesticidal Activity

[0173] The various polypeptides set forth in SEQ ID NOs: 1-229 can be tested to act as a pesticide upon a pest in a number of ways. One such method is to perform a feeding assay. In such a feeding assay, one exposes the pest to a sample containing either compounds to be tested or control samples. Often this is performed by placing the material to be tested, or a suitable dilution of such material, onto a material that the pest will ingest, such as an artificial diet. The material to be tested may be composed of a liquid, solid, or slurry. The material to be tested may be placed upon the surface and then allowed to dry. Alternatively, the material to be tested may be mixed with a molten artificial diet, and then dispensed into the assay chamber. The assay chamber may be, for example, a cup, a dish, or a well of a microtiter plate.

[0174] Assays for sucking pests (for example aphids) may involve separating the test material from the insect by a partition, ideally a portion that can be pierced by the sucking mouth parts of the sucking insect, to allow ingestion of the test material. Often

the test material is mixed with a feeding stimulant, such as sucrose, to promote ingestion of the test compound.

[0175] Other types of assays can include microinjection of the test material into the mouth, or gut of the pest, as well as development of transgenic plants, followed by test of the ability of the pest to feed upon the transgenic plant. Plant testing may involve isolation of the plant parts normally consumed, for example, small cages attached to a leaf, or isolation of entire plants in cages containing insects.

[0176] Other methods and approaches to assay pests are known in the art, and can be found, for example in Robertson and Preisler, eds. (1992) Pesticide bioassays with arthropods, CRC, Boca Raton, Fla. Alternatively, assays are commonly described in the journals Arthropod Management Tests and Journal of Economic Entomology or by discussion with members of the Entomological Society of America (ESA). Any one of SEQ ID NOS: 1-229 can be expressed and employed in an assay as set forth in Examples 3 and 4, herein.

[0177] All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0178] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

That which is claimed is:

1. A recombinant polypeptide having pesticidal activity, comprising

(a) a polypeptide comprising an amino acid sequence having at least 90% percent sequence identity to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199; or,

(b) a polypeptide comprising the amino acid sequence set forth in SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199.

2. The polypeptide of claim 1, further comprising a heterologous amino acid sequence.

3. A composition comprising the polypeptide of claim 1 or claim 2.

4. A recombinant nucleic acid molecule encoding an amino acid sequence comprising

(a) at least 90% percent sequence identity to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID Nos: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199; or

(b) the amino acid sequence set forth in SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199;

wherein said recombinant nucleic acid molecule is not a naturally occurring sequence encoding said polypeptide.

5. The recombinant nucleic acid of claim 4, wherein said nucleic acid molecule is a synthetic sequence designed for expression in a plant.

6. The recombinant nucleic acid molecule of claim 4 or claim 5, wherein said nucleic acid molecule is operably linked to a promoter capable of directing expression in a plant cell.

7. The recombinant nucleic acid molecule of any one of claims 4 to 6, wherein said nucleic acid molecule is operably linked to a promoter capable of directing expression in a bacteria.

8. A host cell comprising the recombinant nucleic acid molecule of any one of claims 4 to 7.

9. The host cell of claim 8, wherein said host cell is a bacterial host cell.

10. A DNA construct comprising a promoter that drives expression in a plant cell operably linked to a recombinant nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 90% percent sequence identity to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199.

11. The DNA construct of claim 10, wherein said nucleotide sequence is a synthetic DNA sequence designed for expression in a plant.

12. A vector comprising the DNA construct of claim 10 or claim 11.

13. A host cell comprising the DNA construct of claim 10 or claim 11 or the vector of claim 12.

14. A composition comprising the host cell of claim 13.

15. The composition of claim 14, wherein said composition is selected from the group consisting of a powder, dust, pellet, granule, spray, emulsion, colloid, and solution.

16. The composition of claim 15, wherein said composition comprises from about 1% to about 99% by weight of said polypeptide.

17. A method for controlling a pest population comprising contacting said pest population with a pesticidal-effective amount of the composition of any one of claim 3 or claims 14-16.

18. A method for producing a polypeptide with pesticidal activity comprising culturing the host cell of any one of claims 8, 9, or 13 under conditions in which the nucleic acid molecule encoding the polypeptide is expressed.

19. A plant having stably incorporated into its genome a DNA construct comprising a nucleotide sequence that encodes a protein having pesticidal activity, wherein said nucleotide sequence comprise

(a) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199; or

(b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 90% percent sequence identity to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199.

20. A transgenic seed of the plant of claim 19.

21. A method for protecting a plant from an insect pest, comprising expressing in a plant or cell thereof a nucleotide sequence that encodes a pesticidal polypeptide, wherein said nucleotide sequence comprising

(a) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199; or

(b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 90% percent sequence identity to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199.

22. The method of claim 21, wherein said plant produces a pesticidal polypeptide having pesticidal activity against at least one of a lepidopteran pest, a coleopteran pest, or a hemipteran pest.

23. A method for increasing yield in a plant comprising growing in a field a plant or seed thereof having stably incorporated into its genome a DNA construct comprising a promoter that drives expression in a plant operably linked to a nucleotide sequence that encodes a pesticidal polypeptide, wherein said nucleotide sequence comprises

(a) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 5, 10, 24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199; or

(b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 90% percent sequence identity to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NOs: 5, 10,

24, 27, 40, 41, 45, 47, 49, 51, 52, 56, 59, 62, 64, 67, 77, 79, 80, 87, 92, 100, 102, 108, 111, 124, 129, 131, 132, 134, 136, 140, 148, 151, 156, 157, 159, 162, 164, 167, 172, 180, 181, 185, 199, 204, 208, 3, 7, 25, 28, 41, 49, 62, 79, 126, 132, 140, 172, 177, 180, 185, 191, or 199.

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