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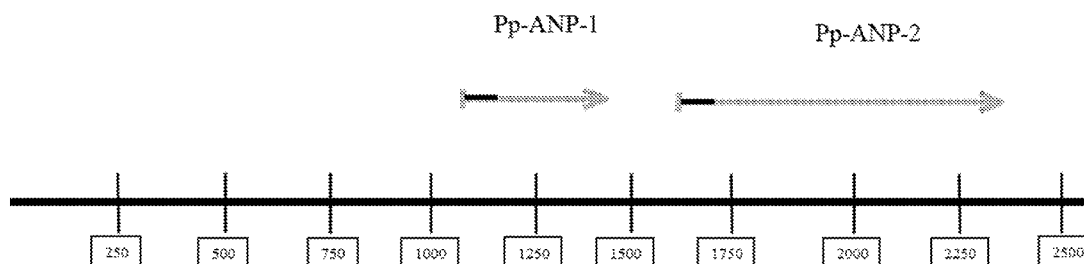
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(54) Title: NEMATICIDAL PROTEIN FROM PSEUDOMONAS

Figure 1



(57) **Abstract:** The present invention relates to nematode-responsive polypeptides, nucleotide sequences encoding the same and their use in creating or enhancing pathogen resistance in plants. Nucleic acid constructs comprising a nematode-control sequence operably linked to a promoter are disclosed as well as vectors, plant cells, plants, and transformed seeds containing such constructs. Methods for the use of such constructs in repressing or inducing expression of a nematode-control sequence in a plant are also provided. In addition, methods are provided for conferring or improving pathogen resistance in plants by repression or induction of nematode-control sequences or by spatially and temporally directing expression to pathogen invasion.



NEMATOCIDAL PROTEIN FROM PSEUDOMONAS

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

5 The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named "2062USPSP2_SequenceList created on January 19, 2017 and having a size of 18.4 kilobytes, 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.

FIELD

10 This invention relates to compositions and methods useful in creating or enhancing nematode resistance in plants. Additionally, the invention relates to plants and other organisms which have been genetically transformed with the compositions of the invention.

BACKGROUND

15 Plants are continually attacked by a diverse range of phytopathogenic organisms. Pathogen infection, such as nematode infection, is a significant problem in the farming of many agriculturally significant crops. Important nematodes in agriculture are the sedentary endoparasites, which include the genera, *Meloidogyne* (root-knot nematodes) and *Heterodera* and *Globodera* (cyst nematodes) (Sasser and Freckman (1987) *A world perspective on Nematology: The role of the society*. IN: J. A. Veech and D. W. Dickson, (Eds.) Vistas on Nematology. Maryland: Society of Nematologists, Inc. pp. 7-14). For example, soybean cyst nematode (*Heterodera glycines*, hereafter referred to as "SCN") is a widespread pest that causes substantial damage to
20 soybeans every year. Such damage is the result of the stunting of the soybean plant caused by the cyst nematode. The stunted plants have smaller root systems, show symptoms of mineral deficiencies in their leaves, and wilt easily. The soybean cyst nematode is believed to be responsible for yield losses in soybeans that are estimated to be in excess of \$1 billion per year in North America. Other pathogenic nematodes of significance to agriculture include the potato cyst
25 nematodes *Globodera rostochiensis* and *Globodera pallida*, which are key pests of the potato, while the beet cyst nematode *Heterodera schachtii* is a major problem for sugar beet growers in Europe and the United States.

30 Currently, plant-parasitic nematodes are generally controlled by chemical nematicides, crop rotation, bio-control and growing resistant cultivars. The primary method of controlling nematodes has
35 been through the application of highly toxic chemical compounds. The widespread use of chemical

compounds poses many problems with regard to the environment because of the non-selectivity of the compounds and the development of insect resistance to the chemicals.

Additional obstacles to pest control are posed by certain pests. For example, it is known that certain nematodes, such as SCN, can inhibit the expression of certain plant genes at the nematode feeding site (see Gheysen and Fenoll (2002) *Annu Rev Phytopathol* 40:191-219). Thus, in implementing a transgenic approach to pest control, an important factor is to increase the expression of desirable genes in response to pathogen attack. Consequently, there is a continued need for the controlled expression of genes deleterious to pests in response to plant damage.

One promising method for nematode control is the production of transgenic plants that are resistant to nematode infection and reproduction. For example, with the use of nematode-inducible promoters, plants can be genetically altered to express nematicidal proteins in response to exposure to nematodes. See, for example, U.S. Patent No. 6,252,138. Alternatively, some methods use a combination of both nematode-inducible and nematode-repressible promoters to obtain nematode resistance. Thus, WO 92/21757 discusses the use of a two promoter system for disrupting nematode feeding sites where one nematode-inducible promoter drives expression of a toxic product that kills the plant cells at the feeding site while the other nematode-repressible promoter drives expression of a gene product that inactivates the toxic product of the first promoter under circumstances in which nematodes are not present, thereby allowing for tighter control of the deleterious effects of the toxic product on plant tissue. Similarly, with the use of proteins having a deleterious effect on nematodes, plants can be genetically altered to express such deleterious proteins in response to nematode attack.

Consequently, there is a continued need for the identification of nematode-control genes for use in promoting nematode resistance.

SUMMARY

Compositions and methods involved in promoting nematode resistance in plants are provided. The compositions include nucleic acid molecules comprising a sequence useful in nematode control. The embodiments further include DNA constructs comprising nucleic acid sequences, operably linked to regulatory promoters, including polynucleotide sequences encoding proteins useful in nematode control, or combinations of these novel sequences of the embodiments with other nucleotide sequences, as well as vectors and transformed plant cells, plants and seeds comprising these constructs. The polypeptides of the embodiments include two proteins shown to exhibit nematicidal activity against nematodes, including *C. elegans*. Amino acid sequences of these proteins are provided as well as purified proteins themselves. Polynucleotides having nucleic acid sequences encoding these polypeptides are also provided. The DNA sequences encoding these proteins can be used to transform plants, bacteria, fungi, yeasts, and other organisms for the control of pests.

The polynucleotides of the invention, or at least 20 contiguous bases therefrom, may be used as probes to isolate and identify similar genes in other microbial species.

In one aspect, this invention relates to DNA sequences isolated from *Pseudomonas*. These sequences alone, or in combination with other sequences, can be used to improve nematode resistance in a plant. In another embodiment of the present invention, DNA constructs and transformation vectors comprising the isolated nucleotide sequences are disclosed. The transformation vectors can be used to transform plants and express the nematode control genes in the transformed cells. In this manner, the nematode resistance of plants can be improved. Transformed cells as well as regenerated transgenic plants and seeds containing and expressing the isolated DNA sequences and protein products are also provided.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows the 2.6 kb genomic DNA fragment from *Pseudomonas fluorescens* strain D3. The two arrows on top show positions and orientations of the two open reading frames encoding the two anti-nematode proteins, Pp-ANP-1 and Pp-ANP-2. The dark bars within the two arrows indicate the predicted signal peptides of the two proteins.

Figure 2 shows *C. elegans* assay with *E. coli* cells containing empty pQE80 vector as control, or *E. coli* cells containing Pp-ANP-1 and Pp-ANP-2. Images showing *C. elegans* after incubating J2 worms in *E. coli* different cells for 2 days.

Figure 3 shows *C. elegans* brood sizes after incubated single L4 worms in *E. coli* OP50 strain, *E. coli* with empty vector pQE80 and *E. coli* containing Pp-ANP-1 and Pp-ANP-2 for 5 days.

Figure 4 shows Pp-ANP inhibiting activity on other free-living nematodes. (A), (B), and (C): *E. coli* Transformed with empty vector; (D), (E), and (F): *E. coli* expressing Pp-ANP-1 and Pp-ANP-2.

Figure 5 shows amino acid sequence alignment of Pp-ANP-1, Pp-ANP-3, and Pp-ANP-5; alignment of Pp-ANP-2, Pp-ANP-4, and Pp-ANP-6.

DETAILED DESCRIPTION

The embodiments of the disclosure provide, *inter alia*, compositions and methods for promoting pathogen resistance in plants, more particularly for improving nematode resistance of plants. The compositions include nucleic acid molecules comprising sequences useful in improving nematode resistance in plants. These compositions may be transferred into plants to confer or improve nematode resistance in the transformed plants. The phrase, "confer or improve nematode or other such pathogen resistance," means that the proteins, DNA, or RNA sequences, either alone or in combination with other proteins or sequences, enhance resistance of a plant to certain nematodes and nematode-caused damage. In

this manner, resistance to certain nematodes and other such pathogens can be enhanced or improved in the transformed plant when at least one of the sequences disclosed herein is provided.

The compositions comprise nucleic acid molecules comprising sequences of genes isolated from *Pseudomonas*, and the polypeptides encoded thereby. Particularly, the nucleotide and amino acid sequences for two *Pseudomonas* peptides are provided in SEQ ID NOs: 2 and 3 with the corresponding protein set forth in SEQ ID NOs: 6 and 7; in SEQ ID NOs: 8 and 9 with the corresponding protein set forth in SEQ ID NOs: 12 and 13; and in SEQ ID NOs: 10 and 11 with the corresponding protein set forth in SEQ ID NOs: 14 and 15. Methods are provided for the expression the sequences disclosed herein in a host plant. Some methods involve stably transforming a plant with a nucleotide sequence capable of modulating the plant's nematode resistance, operably linked with a promoter capable of driving expression of a gene in a plant cell.

Nematodes may include parasitic nematodes such as root-knot, cyst and lesion nematodes, including certain *Heterodera* spp., *Meloidogyne* spp. and *Globodera* spp.; particularly members of the cyst nematodes, including, but not limited to, *Heterodera schachtii* (beet cyst nematode); *Heterodera avenae* (cereal cyst nematode) and *Globodera rostochiensis* and *Globodera pailida* (potato cyst nematodes). Lesion nematodes include *Pratylenchus* spp. Other examples of nematodes and similar pathogens contemplated in the present invention are given elsewhere herein.

The nematicidal sequences disclosed herein, when assembled within a DNA construct such that they are operably linked to a promoter capable of directing expression in the plant, enable increased nematode resistance in the cells of a plant stably transformed with this nucleic acid molecule. The terminology, "operably linked," is intended to mean a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the polynucleotide sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

A nucleotide sequence useful in improving nematode resistance is referred to herein as a "nematode-resistance sequence." A "nematode-resistance sequence" is intended to mean a sequence coding for an RNA and/or a protein or polypeptide that, when expressed, either inhibits, prevents, or repels nematode infection or invasion of a plant cell or the nematode growth and development within plant tissues, thereby limiting the spread and reproduction of the nematode. Such a nematode resistance protein of the embodiments will reduce the disease symptoms resulting from certain nematode challenge by at least about 5% to about 50%, at least about 10% to about 60%, at least about 30% to about 70%, at least about 40% to about 80%, or at least about 50% to about 90% or greater. Such sequences include sequences encoding nematode-resistance proteins and cytotoxic proteins or polypeptides that disrupt cell metabolism, the byproducts of which are essential for

nematode survival and/or reproduction. Expression of such sequences allows a plant to avoid the disease symptoms associated with certain nematode infections, or prevent or minimize certain nematodes from causing disease and associated disease symptoms. These sequences may function as nematicides that are as nematode-killing sequences. Such killing may occur by direct action on
5 nematodes, or by action on the cells of the plant on which the nematodes feed to kill those cells, thereby depriving the infecting nematodes of a site of entry or of feeding. Alternatively, such nematicides may act on other surrounding tissue to cause the release of nematode toxins from that tissue. Examples of nematode-resistance sequences are provided in, for example, U.S. Patent Nos. 5,750,386; 5,994,627; 6,006,470; and 6,228,992, incorporated herein by reference. Other examples of
10 nematode resistance genes include Oryzacystatin-1 and cowpea trypsin inhibitor (Urwin *et al.* (1998) *Planta* 204: 472-479); Rhg (Webb *et al.* (1995) *Theor. Appl. Genet.* 91: 574-581); Hsl (Cai *et al.* (1997) *Science* 275: 832-834); and CRE3 (Lagudah *et al.* (1997) *Genome* 40: 650-665).

The compositions and methods disclosed herein provide for isolated polynucleotides comprising nucleotide sequences encoding the amino acid sequences shown in SEQ ID NOs: 6, 7, and
15 10-15 and their conservatively modified variants. Further provided are polypeptides having an amino acid sequence encoded by a nucleic acid molecule described herein, for example those polypeptides comprising the sequences set forth in SEQ ID NOs: 6, 7, and 10-15, and fragments and variants thereof. The present invention further provides for isolated polynucleotides comprising the sequences shown in SEQ ID NOs: 1-3, and 8-11.

The embodiments encompass isolated or substantially purified nucleic acid or protein compositions. An "isolated" or "purified" nucleic acid molecule or protein, or biologically active portion thereof, 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. In some embodiments, an "isolated" polynucleotides is free of sequences (such
25 as other protein-encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, 0.4 kb, 0.3 kb, 0.2 kb, or 0.1 kb, or 50, 40, 30, 20, or 10 nucleotides that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the
30 nucleic acid is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5%, (by dry weight) of contaminating protein. When the protein of the invention or biologically active portion thereof is recombinantly produced, culture medium may represent less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

Fragments and variants of the disclosed nucleotide sequences are encompassed by the present invention. Fragments and variants of proteins encoded by the disclosed nucleotide sequences are also encompassed. By "fragment" is intended a portion of the nucleotide sequence or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native protein and hence affect development, developmental pathways, stress responses, and/or disease resistance by retaining nematicidal activity. Alternatively, fragments of a nucleotide sequence that are useful as hybridization probes generally do not encode fragment proteins retaining biological activity. Thus, fragments of a nucleotide sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the full-length nucleotide sequence encoding the proteins of the invention.

A fragment of a nucleotide sequence that encodes a biologically active portion of a nematicidal protein of the invention will encode at least 12, 25, 30, 50, 75, 100, 125, 150, 175, 200, 225, or 250 contiguous amino acids, or up to the total number of amino acids present in a full-length protein .

Fragments of a nucleotide sequence that are useful as hybridization probes or PCR primers generally need not encode a biologically active portion of a protein. Thus, a fragment of a nucleotide sequence may encode a biologically active portion of a nematicidal protein, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. A biologically active portion of a nematicidal protein can be prepared by isolating a portion of the nucleotide sequences of the invention, expressing the encoded portion (e.g., by recombinant expression in vitro), and assessing the activity of the resulting protein. Nucleic acid molecules that are fragments of a nucleotide sequence of the embodiments comprise at least 16, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400 or 2500 contiguous nucleotides or up to the number of nucleotides present in a full-length nucleic acid sequence encoding an nematicidal polypeptide disclosed herein, depending upon the intended use. "Contiguous nucleotides" is used herein to refer to nucleotide residues that are immediately adjacent to one another.

A "variant" is intended to mean a substantially similar sequence. For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the polypeptides of the embodiments. Naturally occurring allelic variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically-derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a nematicidal protein of the embodiments. Generally, variants of a particular nucleotide sequence of the invention will have at least

40%, 50%, 60%, 70%, generally at least 75%, 80%, 85%, or about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to that particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters.

A "variant" protein is a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the embodiments are biologically active, that is, they continue to possess the desired nematicidal activity of the native protein. Such variants may result from, for example, genetic polymorphism or from manipulation. Biologically active variants of a native protein of the embodiments will have at least 40%, 50%, 60%, 70%, generally at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs described elsewhere herein using default parameters. A biologically active variant of a protein of the embodiments may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue. As used herein, reference to a particular nucleotide or amino acid sequence shall include all modified variants as described herein.

In some embodiments variants include polypeptides that differ in amino acid sequence due to mutagenesis. Variant proteins encompassed by the disclosure are biologically active, that is they continue to possess the desired biological activity (i.e. nematicidal activity) of the native protein. In some embodiments, the variant will have at least about 10%, at least about 30%, at least about 50%, at least about 70%, at least about 80% or more of the nematicidal activity of the native protein. In some embodiments, the variants may have improved activity over the native protein.

The proteins of the embodiments may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the proteins of the embodiments can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods in Enzymol.* 154:367-382; U.S. Patent No. 4,873,192; Walker and Gastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.* (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be made.

Thus, the genes and nucleotide sequences disclosed herein include both naturally occurring sequences as well as mutant forms. Likewise, the proteins of the embodiments encompass both naturally-occurring proteins as well as variations and modified forms thereof. Such variants will continue to possess the desired nematicidal activity. It is recognized that variants need not retain all of the activities and/or properties of the native protein.

Variant nucleotide sequences 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 coding sequences can be manipulated to create a new sequence encoding a peptide that possesses 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 genes of the embodiments and other known genes to obtain a new gene coding for a protein with an improved property of interest, such as an increased nematicidal effect. 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* 370:389391; Cramer *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; Cramer *et al.* (1998) *Nature* 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

Variant nucleotide sequences can also be evaluated by comparison of the percent sequence identity shared by the polypeptides they encode. For example, isolated nucleic acids which encode a polypeptide with a given percent sequence identity to the polypeptide of SEQ ID NO: 6, 7, and 10-15 are disclosed. The percentage of identity to a reference sequence is at least 50% and, rounded upwards to the nearest integer, can be expressed as an integer selected from the group of integers consisting of from 50 to 99. Thus, for example, the percentage of identity to a reference sequence can be at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

The following terms are used to describe the sequence relationships between two or more polynucleotides or polypeptides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", and, (d) "percentage of sequence identity."

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison

window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two polynucleotides. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

(c) As used herein, "sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the

two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

5 The nucleotide sequences disclosed herein may be used to isolate corresponding sequences from other microbes, particularly other bacteria. In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences based on their sequence homology to the sequences set forth herein. Sequences isolated based on their sequence identity to the nucleotide sequences set forth herein or to fragments thereof are encompassed. Such sequences include sequences that are orthologs of the
10 disclosed sequences. "Orthologs" are genes derived from a common ancestral gene and are found in different species as a result of speciation. Genes found in different species are considered orthologs when their nucleotide sequences and/or their encoded protein sequences share substantial identity as defined elsewhere herein. Functions of orthologs are often highly conserved among species. Thus, isolated sequences that have nematocidal activity, or sequences which encode a nematocidal protein and
15 which hybridize under stringent conditions to the sequences disclosed herein, or to fragments thereof, are encompassed by the embodiments.

In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any plant of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in
20 Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press Plainview, New York), hereinafter "Sambrook." See also Innis *et al.*, eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Known methods of PCR include, but are not
25 limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

In hybridization techniques, all or part of a known nucleotide sequence is used as a probe that selectively hybridizes to other corresponding nucleotide sequences present in a population of cloned genomic DNA fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen organism.
30 The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ³²P, or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the sequences of the invention. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook, *supra*.

For example, an entire sequence disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding nematode-response sequences. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among nematode-response sequences and may be at least about 10 or 15 or 17 nucleotides in length or at least about 20 or 22 or 25 nucleotides in length. Such probes may be used to amplify corresponding sequences from a chosen organism by PCR. This technique may be used to isolate additional coding sequences from a desired organism or as a diagnostic assay to determine the presence of coding sequences in an organism. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook, *supra*

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different under different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length or less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3. Incubation should be at a temperature of least about 30 °C for short probes (e.g., 10 to 50 nucleotides) and at least about 60 °C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulfate) at 37 °C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl, 0.3 M trisodium citrate) at 50 to 55 °C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37 °C, and a wash in 0.5X to 1X SSC at 55 to 60 °C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37 °C, and a final wash in 0.1X SSC at 60 to 65 °C for at least about 20 minutes. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours.

Specificity is typically a function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m (thermal melting point) can be approximated from the equation of Meinkoth and Wahl ((1984) *Anal. Biochem.* 138:267-284): $T_m = 81.5\text{ }^{\circ}\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of

monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1 °C for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the T_m can be decreased 10 °C. Generally, stringent conditions are selected to be about 5 °C lower than the T_m for the specific sequence and its complement at a defined ionic strength and pH.

In general, sequences that encode a nematocidal protein and which hybridize to the nucleotide sequences disclosed herein will be at least about 40% homologous, about 50% or 60% homologous, about 70% homologous, and even about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 98%, 99% or more homologous with the disclosed sequences. That is, the sequence identity of the sequences may be from about 40% to 50% identical, about 60% to 70% or 75%, and even about 80%, 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, or higher, so that the sequences may differ by only 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue or by 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleic acid. In some embodiment, the polynucleotides disclosed here in encode a nematocidal protein sufficiently homologous to the amino acid sequence of SEQ ID NOs: 6, 7, and 10-15. "Sufficiently homologous" is used herein to refer to an amino acid sequence that has at least about 50%, 55%, 60%, 65%, 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% or greater sequence homology compared to a reference sequence using one of the alignment programs described herein using standard parameters.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 10 (available from Genetics Computer Group (GCG), Accelrys, Inc., San Diego, CA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins *et al.* (1988) *Gene* 73:237-244 (1988); Higgins *et al.* (1989) *CABIOS* 5:151-153; Corpet *et al.* (1988) *Nucleic Acids Res.* 16:10881-90; Huang *et al.* (1992) *CABIOS* 8:155-65; and Pearson *et al.* (1994) *Meth. Mol. Biol.* 24:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and

Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide 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 (in BLAST 2.0) 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, or PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. Alignment may also be performed manually by inspection.

Compositions and methods for improving resistance to nematodes are provided. The compositions comprise the genes encoding nematocidal proteins and the proteins themselves as disclosed herein. "Resistance," in the context of pathogen-resistance, disease-resistance, or nematode resistance, is intended to mean that the impact on the plant of the particular pathogen, disease, and/or nematode attack is diminished or entirely avoided. That is, in a plant showing resistance, pathogens are prevented from causing plant diseases and the associated disease symptoms, or alternatively, some or all of the disease symptoms caused by the pathogen are minimized or lessened. This includes, but is not limited to, the ability of a host to prevent certain nematode reproduction. Genes encoding disease resistance traits include, generally, detoxification genes, such as against fumonisin (U.S. Patent No. 5,792,931); avirulence (*avr*) and disease resistance (*R*) genes (Jones *et al.* (1994) *Science* 266:789; Martin *et al.* (1993) *Science* 262:1432; and Mindrinis *et al.* (1994) *Cell* 78:1089); and the like. In some embodiments, the expression of a nematocidal gene product of the embodiments, driven by a heterologous promoter, may be induced in response to disease or stress or attack and confers disease resistance; i.e., production of the nematocidal gene product lessens the symptoms that would ordinarily result in a plant.

The polynucleotides of the embodiments are useful in methods directed to creating or enhancing certain nematode resistance in a plant. Improved nematode resistance may be accomplished by stably transforming a plant of interest with a nucleic acid molecule that comprises a nucleic acid sequence identified herein which encodes a nematocidal protein, operably linked to a promoter sequence capable of directing the expression of nucleotide sequences in plants, or by the use of such transformed plants or other products to produce nematocidal compositions.

A "nematocidal composition" is intended to mean a composition of the invention having antipathogenic activity, thus being capable of suppressing, controlling, and/or killing the invading pathogenic organism. A nematocidal composition of the embodiments will reduce the disease

symptoms resulting from certain pathogen or nematode challenge by at least about 5% to about 50%, at least about 10% to about 60%, at least about 30% to about 70%, at least about 40% to about 80%, or at least about 50% to about 90% or greater. Hence, the methods of the embodiments can be utilized to protect plants from disease, particularly those diseases that are caused by certain plant pathogenic nematodes.

Assays that measure nematicidal activity are commonly known in the art, as are methods to quantitate disease resistance in plants following pathogen infection. These assays may be used to measure the activity of the polypeptides of the embodiments. Such techniques include, measuring over time, the pathogen biomass, and the overall percentage of decayed plant tissues. Also contemplated are antipathogenic assays directed at nematodes. Such assays are known to the skilled artisan, and may include assays directed at specific characteristics of nematode pathogen infections, such as assays directed at nematode feeding site formation. Such assays include those disclosed in U.S. Patent Nos. 6,008,436.

Pathogens include nematodes, such as plant parasitic nematodes such as root-knot, cyst, and lesion nematodes, including certain *Heterodera* and *Globodera* spp; particularly *Globodera rostochiensis* and *globodera pallida* (potato cyst nematodes); *Heterodera schachtii* (beet cyst nematode); and *Heterodera avenae* (cereal cyst nematode).

Other nematodes of interest include, but are not limited to, *Anguina* spp. (i.e., *A. agrostis*, *A. tritici*), *Aphelenchoides* spp. (i.e., *A. besseyi*, *A. composticola*, *A. fragariae*, *A. ritzemabosi*), *Acrobeloides* spp., *Belonolaimus* spp. (i.e., *B. longicaudatus*), *Cacopaurus* spp. (i.e., *C. pestis*), *Criconemella* spp. (i.e., *C. curvata*, *C. macrodora*), *Ditylenchus* spp. (i.e., *D. dipsaci*, *D. destructor*, *D. myceliophagus*, *D. angustus*), *Globodera* spp. (i.e. *G. rostochiensis*, *G. pallida*, *G. tabacum*), *Helicotylenchus* spp. (i.e., *H. multicinctus*, *H. pseudorobustus*, *H. dihystra*, *H. indicus*), *Hemicriconemoides* spp. (i.e., *H. kanayaensis*, *H. gaddi*, *H. chitwoodi*), *Hemicycliophora* spp., (i.e., *H. arenaria*, *H. biosphaera*, *H. poranga*), *Heterodera* spp. (i.e., *H. oryzae*, *H. schachtii*, *H. cajani*, *H. trifolii*, *H. avenae*), *Hirschmanniella* spp. (i.e., *H. belli*, *H. gracilis*, *H. oryzae*), *Hoplolaimus* spp. (i.e. *H. seinhorsti*, *H. columbus*, *H. galeatus*), *Longidorus* spp. (i.e., *L. breviannulatus*), *Meloidogyne* spp. (i.e., *M. hapla*, *M. incognita*, *M. javanica*, *M. arenaria*, *M. naasi*, *M. exigua*, *M. artiellia*), *Nacobbus* spp. (i.e., *N. aberrans*, *N. dorsalis*), *Paratrichodorus* spp. (i.e., *P. anemones*, *P. allius*, *P. porosus*, *P. minor*, *P. christiei*), *Panagrellus redivivus*, *Paratylenchus* spp. (i.e., *P. curvatus*, *P. hamatus*), *Pratylenchus* spp. (i.e., *P. coffeae*, *P. brachyurus*, *P. vulnus*, *P. goodeyi*, *P. penetrans*), *Pristionchus pacificus*, *Radopholus* spp. (i.e. *R. similis*, *R. citrophilus*), *Rhadinaphelenchus cocophilus*, *Rotylenchulus* spp. (i.e., *R. reniformis*, *R. macrodoratus*), *Scutellonema* spp. (i.e., *S. abberans*, *S. bradys*), *Subanguina* spp. (i.e., *S. radiculicola*, *S. picridis*), *Trichodorus* spp. (i.e. *T. primitivus*, *T. obtusus*), *Tylenchorhynchus* spp. (i.e., *T. brassicae*, *T. claytoni*), *Tylenchulus semipenetrans*, *Xiphinema* spp. (i.e. *X. americanum*, *X. elongatum*, *X. ifacolum*).

Methods for increasing pathogen resistance in a plant are provided. In some embodiments, the methods involve stably transforming a plant with a DNA construct comprising an anti-pathogenic nucleotide sequence of the embodiments operably linked to a promoter that drives expression in a plant. While the choice of promoter will depend on the desired timing and location of expression of the nematicidal or other nucleotide sequences, desirable promoters include constitutive and pathogen-inducible promoters. These methods may find use in agriculture, particularly in limiting the impact of certain nematodes on crop plants. Thus, transformed plants, plant cells, plant tissues and seeds thereof are provided by the embodiments.

Additionally, the compositions of the embodiments can be used in formulations for their disease resistance activities. The proteins of the invention can be formulated with an acceptable carrier into a nematicidal composition(s) that is, for example: a suspension, a solution, an emulsion, a dusting powder, a dispersible granule, a wettable powder, an emulsifiable concentrate, an aerosol, an impregnated granule, an adjuvant, a coatable paste, or an encapsulation in, for example, polymer substances.

The compositions and methods of the embodiments function to inhibit or prevent plant disease caused by certain nematodes. The gene products may accomplish their anti-pathogenic effects by suppressing, controlling, and/or killing the invading nematode(s). It is recognized that the embodiments are not dependent upon a particular mechanism of defense. Rather, the compositions and methods of the embodiments work to increase resistance of the plant to nematodes independent of how that resistance is increased or achieved.

The methods of the embodiments can be used with other methods available in the art for enhancing disease resistance in plants. Similarly, in addition to being used singly, the nematode-resistance sequences described herein may be used in combination with sequences encoding other proteins or agents to protect against plant diseases and pathogens. Other plant defense proteins include, but are not limited to, those described in U.S. Patent Nos. 6,586,657; 6,476,292; and U.S. Application Serial No. 09/256,158, filed February 24, 1999, now abandoned.

In certain embodiments the nucleic acid sequences disclosed herein can be stacked with any combination of polynucleotide sequences of interest in order to create plants with a desired phenotype. For example, the polynucleotides disclosed herein may be stacked with any other polynucleotides disclosed herein, such as any combination of or with other genes implicated in phytic acid metabolic pathways such as phytase; Lpa1, Lpa2 (see U.S. Patent Nos. 5,689,054 and 6,111,168); myo-inositol 1-phosphate synthase (MI1PS), inositol polyphosphate kinase (IPPK), and myo-inositol monophosphatase (IMP) (see WO 99/05298) and the like. The combinations generated can also include multiple copies of any one of the polynucleotides of interest. The polynucleotides disclosed herein can also be stacked with any other gene or combination of genes to produce plants with a variety of desired trait combinations including but not limited to traits desirable for animal feed such as high oil

genes (*e.g.*, U.S. Patent No. 6,232,529); balanced amino acids (*e.g.* hordothionins (U.S. Patent Nos. 5,990,389; 5,885,801; 5,885,802; and 5,703,409); barley high lysine (Williamson et al. (1987) *Eur. J. Biochem.* 165:99-106; and WO 98/20122); and high methionine proteins (Pedersen et al. (1986) *J. Biol. Chem.* 261:6279; Kirihara et al. (1988) *Gene* 71:359; and Musumura et al. (1989) *Plant Mol. Biol.* 12: 123)); increased digestibility (*e.g.*, modified storage proteins (U.S. Application Serial No. 10/053,410, filed November 7, 2001); and thioredoxins (U.S. Application Serial No. 10/005,429, filed December 3, 2001)). The polynucleotides disclosed herein can also be stacked with traits desirable for insect, disease or herbicide resistance (*e.g.*, *Bacillus thuringiensis* toxic proteins (U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; Geiser et al (1986) *Gene* 48:109); lectins (Van Damme et al. (1994) *Plant Mol. Biol.* 24:825); fumonisin detoxification genes (U.S. Patent No. 5,792,931); avirulence and disease resistance genes (Jones et al. (1994) *Science* 266:789; Martin et al. (1993) *Science* 262:1432; Mindrinos et al. (1994) *Cell* 78:1089); acetolactate synthase (ALS) mutants that lead to herbicide resistance such as the S4 and/or Hra mutations; inhibitors of glutamine synthase such as phosphinothricin or basta (*e.g.*, bar gene); and glyphosate resistance (EPSPS gene)); and traits desirable for processing or process products such as high oil (*e.g.*, U.S. Patent No. 6,232,529); modified oils (*e.g.*, fatty acid desaturase genes (U.S. Patent No. 5,952,544; WO 94/11516)); modified starches (*e.g.*, ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (SDBE)); and polymers or bioplastics (*e.g.*, U.S. patent No. 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase, and acetoacetyl-CoA reductase (Schubert et al. (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)). The polynucleotides disclosed herein may be combined with polynucleotides providing agronomic traits such as male sterility (*e.g.*, see U.S. Patent No. 5,583,210), stalk strength, flowering time, or transformation technology traits such as cell cycle regulation or gene targeting (*e.g.* WO 99/61619; WO 00/17364; WO 99/25821), the disclosures of which are herein incorporated by reference.

The polynucleotides disclosed herein may be stacked with transgenes that confer resistance to insects including, but not limited to, genes encoding a *Bacillus thuringiensis* protein, a derivative thereof or a synthetic polypeptide modeled thereon. See, for example, Geiser, *et al.*, (1986) *Gene* 48:109, who disclose the cloning and nucleotide sequence of a Bt delta-endotoxin gene. Moreover, DNA molecules encoding delta-endotoxin genes can be purchased from American Type Culture Collection (Rockville, Md.), for example, under ATCC® Accession Numbers 40098, 67136, 31995 and 31998. Other non-limiting examples of *Bacillus thuringiensis* transgenes being genetically engineered are given in the following patents and patent applications: US Patent Numbers 5,188,960; 5,689,052; 5,880,275; 5,986,177; 6,023,013, 6,060,594, 6,063,597, 6,077,824, 6,620,988, 6,642,030, 6,713,259, 6,893,826, 7,105,332; 7,179,965, 7,208,474; 7,227,056, 7,288,643, 7,323,556, 7,329,736, 7,449,552, 7,468,278, 7,510,878, 7,521,235, 7,544,862, 7,605,304, 7,696,412, 7,629,504, 7,705,216, 7,772,465,

7,790,846, 7,858,849 and WO 1991/14778; WO 1999/31248; WO 2001/12731; WO 1999/24581 and WO 1997/40162.

Genes encoding nematocidal proteins may also be stacked including but are not limited to: insecticidal proteins from *Pseudomonas* sp. such as PSEEN3174 (Monalysin, (2011) *PLoS Pathogens*, 7:1-13), from *Pseudomonas protegens* strain CHA0 and Pf-5 (previously *fluorescens*) (Pechy-Tarr, (2008) *Environmental Microbiology* 10:2368-2386: GenBank Accession No. EU400157); from *Pseudomonas Taiwanensis* (Liu, *et al.*, (2010) *J. Agric. Food Chem.* 58:12343-12349) and from *Pseudomonas pseudoalcaligenes* (Zhang, *et al.*, (2009) *Annals of Microbiology* 59:45-50 and Li, *et al.*, (2007) *Plant Cell Tiss. Organ Cult.* 89:159-168); insecticidal proteins from *Photorhabdus* sp. and *Xenorhabdus* sp. (Hinchliffe, *et al.*, (2010) *The Open Toxinology Journal* 3:101-118 and Morgan, *et al.*, (2001) *Applied and Envir. Micro.* 67:2062-2069), US Patent Number 6,048,838, and US Patent Number 6,379,946; a PIP-1 polypeptide of US Patent Publication Number US2014-0007297-A1; an AfIP-1A and/or AfIP-1B polypeptides of US Patent Publication Number US2014-0033361; a PHI-4 polypeptides of US Serial Number 13/839702; PIP-47 polypeptides of PCT Serial Number PCT/US14/51063; a PHI-4 polypeptide of US patent Publication US20140274885 or PCT Patent Publication WO2014/150914; a PIP-72 polypeptide of PCT Serial Number PCT/US14/55128; the insecticidal proteins of PCT Serial Number PCT/US14/49923; and δ -endotoxins including, but not limited to, the Cry1, Cry2, Cry3, Cry4, Cry5, Cry6, Cry7, Cry8, Cry9, Cry10, Cry11, Cry12, Cry13, Cry14, Cry15, Cry16, Cry17, Cry18, Cry19, Cry20, Cry21, Cry22, Cry23, Cry24, Cry25, Cry26, Cry27, Cry28, Cry29, Cry30, Cry31, Cry32, Cry33, Cry34, Cry35, Cry36, Cry37, Cry38, Cry39, Cry40, Cry41, Cry42, Cry43, Cry44, Cry45, Cry 46, Cry47, Cry49, Cry 51, Cry52, Cry 53, Cry 54, Cry55, Cry56, Cry57, Cry58, Cry59, Cry60, Cry61, Cry62, Cry63, Cry64, Cry65, Cry66, Cry67, Cry68, Cry69, Cry70, Cry71 and Cry72 classes of δ -endotoxin genes and the *B. thuringiensis* cytolytic Cyt1 and Cyt2 genes. Members of these classes of *B. thuringiensis* insecticidal proteins include, but are not limited to Cry1Aa1 (Accession # AAA22353); Cry1Aa2 (Accession # Accession # AAA22552); Cry1Aa3 (Accession # BAA00257); Cry1Aa4 (Accession # CAA31886); Cry1Aa5 (Accession # BAA04468); Cry1Aa6 (Accession # AAA86265); Cry1Aa7 (Accession # AAD46139); Cry1Aa8 (Accession # I26149); Cry1Aa9 (Accession # BAA77213); Cry1Aa10 (Accession # AAD55382); Cry1Aa11 (Accession # CAA70856); Cry1Aa12 (Accession # AAP80146); Cry1Aa13 (Accession # AAM44305); Cry1Aa14 (Accession # AAP40639); Cry1Aa15 (Accession # AAY66993); Cry1Aa16 (Accession # HQ439776); Cry1Aa17 (Accession # HQ439788); Cry1Aa18 (Accession # HQ439790); Cry1Aa19 (Accession # HQ685121); Cry1Aa20 (Accession # JF340156); Cry1Aa21 (Accession # JN651496); Cry1Aa22 (Accession # KC158223); Cry1Ab1 (Accession # AAA22330); Cry1Ab2 (Accession # AAA22613); Cry1Ab3 (Accession # AAA22561); Cry1Ab4 (Accession # BAA00071); Cry1Ab5 (Accession # CAA28405); Cry1Ab6 (Accession # AAA22420);

Cry1Ab7 (Accession # CAA31620); Cry1Ab8 (Accession # AAA22551); Cry1Ab9 (Accession # CAA38701); Cry1Ab10 (Accession # A29125); Cry1Ab11 (Accession # I12419); Cry1Ab12 (Accession # AAC64003); Cry1Ab13 (Accession # AAN76494); Cry1Ab14 (Accession # AAG16877); Cry1Ab15 (Accession # AAO13302); Cry1Ab16 (Accession # AAK55546); Cry1Ab17 (Accession # AAT46415); Cry1Ab18 (Accession # AAQ88259); Cry1Ab19 (Accession # AAW31761); Cry1Ab20 (Accession # ABB72460); Cry1Ab21 (Accession # ABS18384); Cry1Ab22 (Accession # ABW87320); Cry1Ab23 (Accession # HQ439777); Cry1Ab24 (Accession # HQ439778); Cry1Ab25 (Accession # HQ685122); Cry1Ab26 (Accession # HQ847729); Cry1Ab27 (Accession # JN135249); Cry1Ab28 (Accession # JN135250); Cry1Ab29 (Accession # JN135251); Cry1Ab30 (Accession # JN135252); Cry1Ab31 (Accession # JN135253); Cry1Ab32 (Accession # JN135254); Cry1Ab33 (Accession # AAS93798); Cry1Ab34 (Accession # KC156668); Cry1Ab-like (Accession # AAK14336); Cry1Ab-like (Accession # AAK14337); Cry1Ab-like (Accession # AAK14338); Cry1Ab-like (Accession # ABG88858); Cry1Ac1 (Accession # AAA22331); Cry1Ac2 (Accession # AAA22338); Cry1Ac3 (Accession # CAA38098); Cry1Ac4 (Accession # AAA73077); Cry1Ac5 (Accession # AAA22339); Cry1Ac6 (Accession # AAA86266); Cry1Ac7 (Accession # AAB46989); Cry1Ac8 (Accession # AAC44841); Cry1Ac9 (Accession # AAB49768); Cry1Ac10 (Accession # CAA05505); Cry1Ac11 (Accession # CAA10270); Cry1Ac12 (Accession # I12418); Cry1Ac13 (Accession # AAD38701); Cry1Ac14 (Accession # AAQ06607); Cry1Ac15 (Accession # AAN07788); Cry1Ac16 (Accession # AAU87037); Cry1Ac17 (Accession # AAX18704); Cry1Ac18 (Accession # AAY88347); Cry1Ac19 (Accession # ABD37053); Cry1Ac20 (Accession # ABB89046); Cry1Ac21 (Accession # AAY66992); Cry1Ac22 (Accession # ABZ01836); Cry1Ac23 (Accession # CAQ30431); Cry1Ac24 (Accession # ABL01535); Cry1Ac25 (Accession # FJ513324); Cry1Ac26 (Accession # FJ617446); Cry1Ac27 (Accession # FJ617447); Cry1Ac28 (Accession # ACM90319); Cry1Ac29 (Accession # DQ438941); Cry1Ac30 (Accession # GQ227507); Cry1Ac31 (Accession # GU446674); Cry1Ac32 (Accession # HM061081); Cry1Ac33 (Accession # GQ866913); Cry1Ac34 (Accession # HQ230364); Cry1Ac35 (Accession # JF340157); Cry1Ac36 (Accession # JN387137); Cry1Ac37 (Accession # JQ317685); Cry1Ad1 (Accession # AAA22340); Cry1Ad2 (Accession # CAA01880); Cry1Ae1 (Accession # AAA22410); Cry1Af1 (Accession # AAB82749); Cry1Ag1 (Accession # AAD46137); Cry1Ah1 (Accession # AAQ14326); Cry1Ah2 (Accession # ABB76664); Cry1Ah3 (Accession # HQ439779); Cry1Ai1 (Accession # AAO39719); Cry1Ai2 (Accession # HQ439780); Cry1A-like (Accession # AAK14339); Cry1Ba1 (Accession # CAA29898); Cry1Ba2 (Accession # CAA65003); Cry1Ba3 (Accession # AAK63251); Cry1Ba4 (Accession # AAK51084); Cry1Ba5 (Accession # ABO20894); Cry1Ba6 (Accession # ABL60921); Cry1Ba7 (Accession # HQ439781); Cry1Bb1 (Accession # AAA22344); Cry1Bb2 (Accession # HQ439782); Cry1Bc1 (Accession # CAA86568); Cry1Bd1 (Accession # AAD10292); Cry1Bd2 (Accession # AAM93496);

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Cry2Ac7 (Accession # CAL18690); Cry2Ac8 (Accession # CAM09325); Cry2Ac9 (Accession # CAM09326); Cry2Ac10 (Accession # ABN15104); Cry2Ac11 (Accession # CAM83895); Cry2Ac12 (Accession # CAM83896); Cry2Ad1 (Accession # AAF09583); Cry2Ad2 (Accession # ABC86927); Cry2Ad3 (Accession # CAK29504); Cry2Ad4 (Accession # CAM32331); Cry2Ad5 (Accession # CAO78739); Cry2Ae1 (Accession # AAQ52362); Cry2Af1 (Accession # ABO30519); Cry2Af2 (Accession # GQ866915); Cry2Ag1 (Accession # ACH91610); Cry2Ah1 (Accession # EU939453); Cry2Ah2 (Accession # ACL80665); Cry2Ah3 (Accession # GU073380); Cry2Ah4 (Accession # KC156702); Cry2Ai1 (Accession # FJ788388); Cry2Aj (Accession #); Cry2Ak1 (Accession # KC156660); Cry2Ba1 (Accession # KC156658); Cry3Aa1 (Accession # AAA22336); Cry3Aa2 (Accession # AAA22541); Cry3Aa3 (Accession # CAA68482); Cry3Aa4 (Accession # AAA22542); Cry3Aa5 (Accession # AAA50255); Cry3Aa6 (Accession # AAC43266); Cry3Aa7 (Accession # CAB41411); Cry3Aa8 (Accession # AAS79487); Cry3Aa9 (Accession # AAW05659); Cry3Aa10 (Accession # AAU29411); Cry3Aa11 (Accession # AAW82872); Cry3Aa12 (Accession # ABY49136); Cry3Ba1 (Accession # CAA34983); Cry3Ba2 (Accession # CAA00645); Cry3Ba3 (Accession # JQ397327); Cry3Bb1 (Accession # AAA22334); Cry3Bb2 (Accession # AAA74198); Cry3Bb3 (Accession # I15475); Cry3Ca1 (Accession # CAA42469); Cry4Aa1 (Accession # CAA68485); Cry4Aa2 (Accession # BAA00179); Cry4Aa3 (Accession # CAD30148); Cry4Aa4 (Accession # AFB18317); Cry4A-like (Accession # AAY96321); Cry4Ba1 (Accession # CAA30312); Cry4Ba2 (Accession # CAA30114); Cry4Ba3 (Accession # AAA22337); Cry4Ba4 (Accession # BAA00178); Cry4Ba5 (Accession # CAD30095); Cry4Ba-like (Accession # ABC47686); Cry4Ca1 (Accession # EU646202); Cry4Cb1 (Accession # FJ403208); Cry4Cb2 (Accession # FJ597622); Cry4Cc1 (Accession # FJ403207); Cry5Aa1 (Accession # AAA67694); Cry5Ab1 (Accession # AAA67693); Cry5Ac1 (Accession # I34543); Cry5Ad1 (Accession # ABQ82087); Cry5Ba1 (Accession # AAA68598); Cry5Ba2 (Accession # ABW88931); Cry5Ba3 (Accession # AFJ04417); Cry5Ca1 (Accession # HM461869); Cry5Ca2 (Accession # ZP_04123426); Cry5Da1 (Accession # HM461870); Cry5Da2 (Accession # ZP_04123980); Cry5Ea1 (Accession # HM485580); Cry5Ea2 (Accession # ZP_04124038); Cry6Aa1 (Accession # AAA22357); Cry6Aa2 (Accession # AAM46849); Cry6Aa3 (Accession # ABH03377); Cry6Ba1 (Accession # AAA22358); Cry7Aa1 (Accession # AAA22351); Cry7Ab1 (Accession # AAA21120); Cry7Ab2 (Accession # AAA21121); Cry7Ab3 (Accession # ABX24522); Cry7Ab4 (Accession # EU380678); Cry7Ab5 (Accession # ABX79555); Cry7Ab6 (Accession # ACI44005); Cry7Ab7 (Accession # ADB89216); Cry7Ab8 (Accession # GU145299); Cry7Ab9 (Accession # ADD92572); Cry7Ba1 (Accession # ABB70817); Cry7Bb1 (Accession # KC156653); Cry7Ca1 (Accession # ABR67863); Cry7Cb1 (Accession # KC156698); Cry7Da1 (Accession # ACQ99547); Cry7Da2 (Accession # HM572236); Cry7Da3 (Accession # KC156679); Cry7Ea1 (Accession # HM035086); Cry7Ea2 (Accession # HM132124); Cry7Ea3 (Accession #

EEM19403); Cry7Fa1 (Accession # HM035088); Cry7Fa2 (Accession # EEM19090); Cry7Fb1 (Accession # HM572235); Cry7Fb2 (Accession # KC156682); Cry7Ga1 (Accession # HM572237); Cry7Ga2 (Accession # KC156669); Cry7Gb1 (Accession # KC156650); Cry7Gc1 (Accession # KC156654); Cry7Gd1 (Accession # KC156697); Cry7Ha1 (Accession # KC156651); Cry7Ia1 (Accession # KC156665); Cry7Ja1 (Accession # KC156671); Cry7Ka1 (Accession # KC156680); Cry7Kb1 (Accession # BAM99306); Cry7La1 (Accession # BAM99307); Cry8Aa1 (Accession # AAA21117); Cry8Ab1 (Accession # EU044830); Cry8Ac1 (Accession # KC156662); Cry8Ad1 (Accession # KC156684); Cry8Ba1 (Accession # AAA21118); Cry8Bb1 (Accession # CAD57542); Cry8Bc1 (Accession # CAD57543); Cry8Ca1 (Accession # AAA21119); Cry8Ca2 (Accession # AAR98783); Cry8Ca3 (Accession # EU625349); Cry8Ca4 (Accession # ADB54826); Cry8Da1 (Accession # BAC07226); Cry8Da2 (Accession # BD133574); Cry8Da3 (Accession # BD133575); Cry8Db1 (Accession # BAF93483); Cry8Ea1 (Accession # AAQ73470); Cry8Ea2 (Accession # EU047597); Cry8Ea3 (Accession # KC855216); Cry8Fa1 (Accession # AAT48690); Cry8Fa2 (Accession # HQ174208); Cry8Fa3 (Accession # AFH78109); Cry8Ga1 (Accession # AAT46073); Cry8Ga2 (Accession # ABC42043); Cry8Ga3 (Accession # FJ198072); Cry8Ha1 (Accession # AAW81032); Cry8Ia1 (Accession # EU381044); Cry8Ia2 (Accession # GU073381); Cry8Ia3 (Accession # HM044664); Cry8Ia4 (Accession # KC156674); Cry8Ib1 (Accession # GU325772); Cry8Ib2 (Accession # KC156677); Cry8Ja1 (Accession # EU625348); Cry8Ka1 (Accession # FJ422558); Cry8Ka2 (Accession # ACN87262); Cry8Kb1 (Accession # HM123758); Cry8Kb2 (Accession # KC156675); Cry8La1 (Accession # GU325771); Cry8Ma1 (Accession # HM044665); Cry8Ma2 (Accession # EEM86551); Cry8Ma3 (Accession # HM210574); Cry8Na1 (Accession # HM640939); Cry8Pa1 (Accession # HQ388415); Cry8Qa1 (Accession # HQ441166); Cry8Qa2 (Accession # KC152468); Cry8Ra1 (Accession # AFP87548); Cry8Sa1 (Accession # JQ740599); Cry8Ta1 (Accession # KC156673); Cry8-like (Accession # FJ770571); Cry8-like (Accession # ABS53003); Cry9Aa1 (Accession # CAA41122); Cry9Aa2 (Accession # CAA41425); Cry9Aa3 (Accession # GQ249293); Cry9Aa4 (Accession # GQ249294); Cry9Aa5 (Accession # JX174110); Cry9Aa like (Accession # AAQ52376); Cry9Ba1 (Accession # CAA52927); Cry9Ba2 (Accession # GU299522); Cry9Bb1 (Accession # AAV28716); Cry9Ca1 (Accession # CAA85764); Cry9Ca2 (Accession # AAQ52375); Cry9Da1 (Accession # BAA19948); Cry9Da2 (Accession # AAB97923); Cry9Da3 (Accession # GQ249293); Cry9Da4 (Accession # GQ249297); Cry9Db1 (Accession # AAX78439); Cry9Dc1 (Accession # KC156683); Cry9Ea1 (Accession # BAA34908); Cry9Ea2 (Accession # AAO12908); Cry9Ea3 (Accession # ABM21765); Cry9Ea4 (Accession # ACE88267); Cry9Ea5 (Accession # ACF04743); Cry9Ea6 (Accession # ACG63872); Cry9Ea7 (Accession # FJ380927); Cry9Ea8 (Accession # GQ249292); Cry9Ea9 (Accession # JN651495); Cry9Eb1 (Accession # CAC50780); Cry9Eb2 (Accession # GQ249298); Cry9Eb3 (Accession # KC156646);

Cry9Ec1 (Accession # AAC63366); Cry9Ed1 (Accession # AAX78440); Cry9Ee1 (Accession # GQ249296); Cry9Ee2 (Accession # KC156664); Cry9Fa1 (Accession # KC156692); Cry9Ga1 (Accession # KC156699); Cry9-like (Accession # AAC63366); Cry10Aa1 (Accession # AAA22614); Cry10Aa2 (Accession # E00614); Cry10Aa3 (Accession # CAD30098); Cry10Aa4 (Accession # AFB18318); Cry10A-like (Accession # DQ167578); Cry11Aa1 (Accession # AAA22352); Cry11Aa2 (Accession # AAA22611); Cry11Aa3 (Accession # CAD30081); Cry11Aa4 (Accession # AFB18319); Cry11Aa-like (Accession # DQ166531); Cry11Ba1 (Accession # CAA60504); Cry11Bb1 (Accession # AAC97162); Cry11Bb2 (Accession # HM068615); Cry12Aa1 (Accession # AAA22355); Cry13Aa1 (Accession # AAA22356); Cry14Aa1 (Accession # AAA21516); Cry14Ab1 (Accession # KC156652); Cry15Aa1 (Accession # AAA22333); Cry16Aa1 (Accession # CAA63860); Cry17Aa1 (Accession # CAA67841); Cry18Aa1 (Accession # CAA67506); Cry18Ba1 (Accession # AAF89667); Cry18Ca1 (Accession # AAF89668); Cry19Aa1 (Accession # CAA68875); Cry19Ba1 (Accession # BAA32397); Cry19Ca1 (Accession # AFM37572); Cry20Aa1 (Accession # AAB93476); Cry20Ba1 (Accession # ACS93601); Cry20Ba2 (Accession # KC156694); Cry20-like (Accession # GQ144333); Cry21Aa1 (Accession # I32932); Cry21Aa2 (Accession # I66477); Cry21Ba1 (Accession # BAC06484); Cry21Ca1 (Accession # JF521577); Cry21Ca2 (Accession # KC156687); Cry21Da1 (Accession # JF521578); Cry22Aa1 (Accession # I34547); Cry22Aa2 (Accession # CAD43579); Cry22Aa3 (Accession # ACD93211); Cry22Ab1 (Accession # AAK50456); Cry22Ab2 (Accession # CAD43577); Cry22Ba1 (Accession # CAD43578); Cry22Bb1 (Accession # KC156672); Cry23Aa1 (Accession # AAF76375); Cry24Aa1 (Accession # AAC61891); Cry24Ba1 (Accession # BAD32657); Cry24Ca1 (Accession # CAJ43600); Cry25Aa1 (Accession # AAC61892); Cry26Aa1 (Accession # AAD25075); Cry27Aa1 (Accession # BAA82796); Cry28Aa1 (Accession # AAD24189); Cry28Aa2 (Accession # AAG00235); Cry29Aa1 (Accession # CAC80985); Cry30Aa1 (Accession # CAC80986); Cry30Ba1 (Accession # BAD00052); Cry30Ca1 (Accession # BAD67157); Cry30Ca2 (Accession # ACU24781); Cry30Da1 (Accession # EF095955); Cry30Db1 (Accession # BAE80088); Cry30Ea1 (Accession # ACC95445); Cry30Ea2 (Accession # FJ499389); Cry30Fa1 (Accession # ACI22625); Cry30Ga1 (Accession # ACG60020); Cry30Ga2 (Accession # HQ638217); Cry31Aa1 (Accession # BAB11757); Cry31Aa2 (Accession # AAL87458); Cry31Aa3 (Accession # BAE79808); Cry31Aa4 (Accession # BAF32571); Cry31Aa5 (Accession # BAF32572); Cry31Aa6 (Accession # BAI44026); Cry31Ab1 (Accession # BAE79809); Cry31Ab2 (Accession # BAF32570); Cry31Ac1 (Accession # BAF34368); Cry31Ac2 (Accession # AB731600); Cry31Ad1 (Accession # BAI44022); Cry32Aa1 (Accession # AAG36711); Cry32Aa2 (Accession # GU063849); Cry32Ab1 (Accession # GU063850); Cry32Ba1 (Accession # BAB78601); Cry32Ca1 (Accession # BAB78602); Cry32Cb1 (Accession # KC156708); Cry32Da1 (Accession # BAB78603); Cry32Ea1 (Accession # GU324274); Cry32Ea2 (Accession # KC156686); Cry32Eb1 (Accession # KC156663); Cry32Fa1 (Accession # KC156656);

Cry32Ga1 (Accession # KC156657); Cry32Ha1 (Accession # KC156661); Cry32Hb1 (Accession # KC156666); Cry32Ia1 (Accession # KC156667); Cry32Ja1 (Accession # KC156685); Cry32Ka1 (Accession # KC156688); Cry32La1 (Accession # KC156689); Cry32Ma1 (Accession # KC156690); Cry32Mb1 (Accession # KC156704); Cry32Na1 (Accession # KC156691); Cry32Oa1 (Accession # KC156703); Cry32Pa1 (Accession # KC156705); Cry32Qa1 (Accession # KC156706); Cry32Ra1 (Accession # KC156707); Cry32Sa1 (Accession # KC156709); Cry32Ta1 (Accession # KC156710); Cry32Ua1 (Accession # KC156655); Cry33Aa1 (Accession # AAL26871); Cry34Aa1 (Accession # AAG50341); Cry34Aa2 (Accession # AAK64560); Cry34Aa3 (Accession # AAT29032); Cry34Aa4 (Accession # AAT29030); Cry34Ab1 (Accession # AAG41671); Cry34Ac1 (Accession # AAG50118); Cry34Ac2 (Accession # AAK64562); Cry34Ac3 (Accession # AAT29029); Cry34Ba1 (Accession # AAK64565); Cry34Ba2 (Accession # AAT29033); Cry34Ba3 (Accession # AAT29031); Cry35Aa1 (Accession # AAG50342); Cry35Aa2 (Accession # AAK64561); Cry35Aa3 (Accession # AAT29028); Cry35Aa4 (Accession # AAT29025); Cry35Ab1 (Accession # AAG41672); Cry35Ab2 (Accession # AAK64563); Cry35Ab3 (Accession # AY536891); Cry35Ac1 (Accession # AAG50117); Cry35Ba1 (Accession # AAK64566); Cry35Ba2 (Accession # AAT29027); Cry35Ba3 (Accession # AAT29026); Cry36Aa1 (Accession # AAK64558); Cry37Aa1 (Accession # AAF76376); Cry38Aa1 (Accession # AAK64559); Cry39Aa1 (Accession # BAB72016); Cry40Aa1 (Accession # BAB72018); Cry40Ba1 (Accession # BAC77648); Cry40Ca1 (Accession # EU381045); Cry40Da1 (Accession # ACF15199); Cry41Aa1 (Accession # BAD35157); Cry41Ab1 (Accession # BAD35163); Cry41Ba1 (Accession # HM461871); Cry41Ba2 (Accession # ZP_04099652); Cry42Aa1 (Accession # BAD35166); Cry43Aa1 (Accession # BAD15301); Cry43Aa2 (Accession # BAD95474); Cry43Ba1 (Accession # BAD15303); Cry43Ca1 (Accession # KC156676); Cry43Cb1 (Accession # KC156695); Cry43Cc1 (Accession # KC156696); Cry43-like (Accession # BAD15305); Cry44Aa (Accession # BAD08532); Cry45Aa (Accession # BAD22577); Cry46Aa (Accession # BAC79010); Cry46Aa2 (Accession # BAG68906); Cry46Ab (Accession # BAD35170); Cry47Aa (Accession # AAY24695); Cry48Aa (Accession # CAJ18351); Cry48Aa2 (Accession # CAJ86545); Cry48Aa3 (Accession # CAJ86546); Cry48Ab (Accession # CAJ86548); Cry48Ab2 (Accession # CAJ86549); Cry49Aa (Accession # CAH56541); Cry49Aa2 (Accession # CAJ86541); Cry49Aa3 (Accession # CAJ86543); Cry49Aa4 (Accession # CAJ86544); Cry49Ab1 (Accession # CAJ86542); Cry50Aa1 (Accession # BAE86999); Cry50Ba1 (Accession # GU446675); Cry50Ba2 (Accession # GU446676); Cry51Aa1 (Accession # ABI14444); Cry51Aa2 (Accession # GU570697); Cry52Aa1 (Accession # EF613489); Cry52Ba1 (Accession # FJ361760); Cry53Aa1 (Accession # EF633476); Cry53Ab1 (Accession # FJ361759); Cry54Aa1 (Accession # ACA52194); Cry54Aa2 (Accession # GQ140349); Cry54Ba1 (Accession # GU446677); Cry55Aa1 (Accession # ABW88932); Cry54Ab1 (Accession # JQ916908); Cry55Aa2 (Accession # AAE33526); Cry56Aa1 (Accession # ACU57499); Cry56Aa2 (Accession #

GQ483512); Cry56Aa3 (Accession # JX025567); Cry57Aa1 (Accession # ANC87261); Cry58Aa1 (Accession # ANC87260); Cry59Ba1 (Accession # JN790647); Cry59Aa1 (Accession # ACR43758); Cry60Aa1 (Accession # ACU24782); Cry60Aa2 (Accession # EAO57254); Cry60Aa3 (Accession # EEM99278); Cry60Ba1 (Accession # GU810818); Cry60Ba2 (Accession # EAO57253); Cry60Ba3 (Accession # EEM99279); Cry61Aa1 (Accession # HM035087); Cry61Aa2 (Accession # HM132125); Cry61Aa3 (Accession # EEM19308); Cry62Aa1 (Accession # HM054509); Cry63Aa1 (Accession # BAI44028); Cry64Aa1 (Accession # BAJ05397); Cry65Aa1 (Accession # HM461868); Cry65Aa2 (Accession # ZP_04123838); Cry66Aa1 (Accession # HM485581); Cry66Aa2 (Accession # ZP_04099945); Cry67Aa1 (Accession # HM485582); Cry67Aa2 (Accession # ZP_04148882); Cry68Aa1 (Accession # HQ113114); Cry69Aa1 (Accession # HQ401006); Cry69Aa2 (Accession # JQ821388); Cry69Ab1 (Accession # JN209957); Cry70Aa1 (Accession # JN646781); Cry70Ba1 (Accession # ADO51070); Cry70Bb1 (Accession # EEL67276); Cry71Aa1 (Accession # JX025568); Cry72Aa1 (Accession # JX025569).

Examples of δ -endotoxins also include but are not limited to Cry1A proteins of US Patent Numbers 5,880,275 and 7,858,849; a DIG-3 or DIG-11 toxin (N-terminal deletion of α -helix 1 and/or α -helix 2 variants of Cry proteins such as Cry1A) of US Patent Numbers 8,304,604 and 8,304,605; Cry1B of US Patent Application Serial Number 10/525,318; Cry1C of US Patent Number 6,033,874; Cry1F of US Patent Numbers 5,188,960, 6,218,188; Cry1A/F chimeras of US Patent Numbers 7,070,982; 6,962,705 and 6,713,063); a Cry2 protein such as Cry2Ab protein of US Patent Number 7,064,249); a Cry3A protein including but not limited to an engineered hybrid insecticidal protein (eHIP) created by fusing unique combinations of variable regions and conserved blocks of at least two different Cry proteins (US Patent Application Publication Number 2010/0017914); a Cry4 protein; a Cry5 protein; a Cry6 protein; Cry8 proteins of US Patent Numbers 7,329,736, 7,449,552, 7,803,943, 7,476,781, 7,105,332, 7,378,499 and 7,462,760; a Cry9 protein such as such as members of the Cry9A, Cry9B, Cry9C, Cry9D, Cry9E, and Cry9F families; a Cry15 protein of Naimov, *et al.*, (2008) *Applied and Environmental Microbiology* 74:7145–7151; a Cry22, a Cry34Ab1 protein of US Patent Numbers 6,127,180, 6,624,145 and 6,340,593; a CryET33 and CryET34 protein of US Patent Numbers 6,248,535, 6,326,351, 6,399,330, 6,949,626, 7,385,107 and 7,504,229; a CryET33 and CryET34 homologs of US Patent Publication Number 2006/0191034, 2012/0278954, and PCT Publication Number WO 2012/139004; a Cry35Ab1 protein of US Patent Numbers 6,083,499, 6,548,291 and 6,340,593; a Cry46 protein, a Cry 51 protein, a Cry binary toxin; a TIC901 or related toxin; TIC807 of US 2008/0295207; ET29, ET37, TIC809, TIC810, TIC812, TIC127, TIC128 of PCT US 2006/033867; TIC807 of US2040194351, TIC853 toxins of US Patent 8,513,494, AXMI-027, AXMI-036, and AXMI-038 of US Patent Number 8,236,757; AXMI-031, AXMI-039, AXMI-040, AXMI-049 of US7,923,602; AXMI-018, AXMI-020, and AXMI-021 of WO 2006/083891; AXMI-010 of WO

2005/038032; AXMI-003 of WO 2005/021585; AXMI-008 of US 2004/0250311; AXMI-006 of US 2004/0216186; AXMI-007 of US 2004/0210965; AXMI-009 of US 2004/0210964; AXMI-014 of US 2004/0197917; AXMI-004 of US 2004/0197916; AXMI-028 and AXMI-029 of WO 2006/119457; AXMI-007, AXMI-008, AXMI-0080rf2, AXMI-009, AXMI-014 and AXMI-004 of WO

5 2004/074462; AXMI-150 of US Patent Number 8,084,416; AXMI-205 of US20110023184; AXMI-011, AXMI-012, AXMI-013, AXMI-015, AXMI-019, AXMI-044, AXMI-037, AXMI-043, AXMI-033, AXMI-034, AXMI-022, AXMI-023, AXMI-041, AXMI-063, and AXMI-064 of US 2011/0263488; AXMI-R1 and related proteins of US 2010/0197592; AXMI221Z, AXMI222z, AXMI223z, AXMI224z and AXMI225z of WO 2011/103248; AXMI218, AXMI219, AXMI220,

10 AXMI226, AXMI227, AXMI228, AXMI229, AXMI230, and AXMI231 of WO11/103247; AXMI-115, AXMI-113, AXMI-005, AXMI-163 and AXMI-184 of US Patent Number 8,334,431; AXMI-001, AXMI-002, AXMI-030, AXMI-035, and AXMI-045 of US 2010/0298211; AXMI-066 and AXMI-076 of US20090144852; AXMI128, AXMI130, AXMI131, AXMI133, AXMI140, AXMI141, AXMI142, AXMI143, AXMI144, AXMI146, AXMI148, AXMI149, AXMI152, AXMI153, AXMI154,

15 AXMI155, AXMI156, AXMI157, AXMI158, AXMI162, AXMI165, AXMI166, AXMI167, AXMI168, AXMI169, AXMI170, AXMI171, AXMI172, AXMI173, AXMI174, AXMI175, AXMI176, AXMI177, AXMI178, AXMI179, AXMI180, AXMI181, AXMI182, AXMI185, AXMI186, AXMI187, AXMI188, AXMI189 of US Patent Number 8,318,900; AXMI079, AXMI080, AXMI081, AXMI082, AXMI091, AXMI092, AXMI096, AXMI097, AXMI098, AXMI099,

20 AXMI100, AXMI101, AXMI102, AXMI103, AXMI104, AXMI107, AXMI108, AXMI109, AXMI110, AXMI111, AXMI112, AXMI114, AXMI116, AXMI117, AXMI118, AXMI119, AXMI120, AXMI121, AXMI122, AXMI123, AXMI124, AXMI1257, AXMI1268, AXMI127, AXMI129, AXMI164, AXMI151, AXMI161, AXMI183, AXMI132, AXMI138, AXMI137 of US 2010/0005543; AXMI221 of US20140196175; AXMI345 of US 20140373195; and Cry proteins such

25 as Cry1A and Cry3A having modified proteolytic sites of US Patent Number 8,319,019; and a Cry1Ac, Cry2Aa and Cry1Ca toxin protein from *Bacillus thuringiensis* strain VBTS 2528 of US Patent Application Publication Number 2011/0064710. Other Cry proteins are well known to one skilled in the art (see, Crickmore, *et al.*, "Bacillus thuringiensis toxin nomenclature" (2011), at lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/intro.html which can be accessed on the world-wide web

30 using the "www" prefix). The insecticidal activity of Cry proteins is well known to one skilled in the art (for review, see, van Franckenhuysen, (2009) *J. Invert. Path.* 101:1-16). The use of Cry proteins as transgenic plant traits is well known to one skilled in the art and Cry-transgenic plants including but not limited to Cry1Ac, Cry1Ac+Cry2Ab, Cry1Ab, Cry1A.105, Cry1F, Cry1Fa2, Cry1F+Cry1Ac, Cry2Ab, Cry3A, mCry3A, Cry3Bb1, Cry34Ab1, Cry35Ab1, Vip3A, mCry3A, Cry9c and CBI-Bt have

35 received regulatory approval (see, Sanahuja, (2011) *Plant Biotech Journal* 9:283-300 and the CERA

(2010) GM Crop Database Center for Environmental Risk Assessment (CERA), ILSI Research Foundation, Washington D.C. at cera-gmc.org/index.php?action=gm_crop_database which can be accessed on the world-wide web using the “www” prefix). More than one pesticidal proteins well known to one skilled in the art can also be expressed in plants such as Vip3Ab & Cry1Fa (US2012/0317682), Cry1BE & Cry1F (US2012/0311746), Cry1CA & Cry1AB (US2012/0311745), Cry1F & CryCa (US2012/0317681), Cry1DA & Cry1BE (US2012/0331590), Cry1DA & Cry1Fa (US2012/0331589), Cry1AB & Cry1BE (US2012/0324606), and Cry1Fa & Cry2Aa, Cry1I or Cry1E (US2012/0324605). Pesticidal proteins also include insecticidal lipases including lipid acyl hydrolases of US Patent Number 7,491,869, and cholesterol oxidases such as from *Streptomyces* (Purcell et al. (1993) *Biochem Biophys Res Commun* 15:1406-1413). Pesticidal proteins also include VIP (vegetative insecticidal proteins) toxins of US Patent Numbers 5,877,012, 6,107,279, 6,137,033, 7,244,820, 7,615,686, and 8,237,020, and the like. Other VIP proteins are well known to one skilled in the art (see, lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/vip.html which can be accessed on the world-wide web using the “www” prefix). Pesticidal proteins also include toxin complex (TC) proteins, obtainable from organisms such as *Xenorhabdus*, *Photorhabdus* and *Paenibacillus* (see, US Patent Numbers 7,491,698 and 8,084,418). Some TC proteins have “stand alone” insecticidal activity and other TC proteins enhance the activity of the stand-alone toxins produced by the same given organism. The toxicity of a “stand-alone” TC protein (from *Photorhabdus*, *Xenorhabdus* or *Paenibacillus*, for example) can be enhanced by one or more TC protein “potentiators” derived from a source organism of a different genus. There are three main types of TC proteins. As referred to herein, Class A proteins (“Protein A”) are stand-alone toxins. Class B proteins (“Protein B”) and Class C proteins (“Protein C”) enhance the toxicity of Class A proteins. Examples of Class A proteins are TcbA, TcdA, XptA1 and XptA2. Examples of Class B proteins are TcaC, TcdB, XptB1Xb and XptC1Wi. Examples of Class C proteins are TccC, XptC1Xb and XptB1Wi. Pesticidal proteins also include spider, snake and scorpion venom proteins. Examples of spider venom peptides include but are not limited to lycotoxin-1 peptides and mutants thereof (US Patent Number 8,334,366).

In some embodiments the stacked trait may be in the form of silencing of one or more polynucleotides of interest resulting in suppression of one or more target pest polypeptides. In some embodiments the silencing is achieved through the use of a suppression DNA construct.

In some embodiments one or more polynucleotides encoding the polypeptides of the insecticidal polypeptides of the disclosure or fragments or variants thereof may be stacked with one or more polynucleotides encoding one or more polypeptides having insecticidal activity or agronomic traits as set forth supra and optionally may further include one or more polynucleotides providing for gene silencing of one or more target polynucleotides as discussed infra.

“Suppression DNA construct” is a recombinant DNA construct which when transformed or stably integrated into the genome of the plant expresses a silencing element, and results in “silencing” of a target gene in the plant. The target gene may be endogenous or transgenic to the plant. “Silencing,” as used herein with respect to the target gene, refers generally to the suppression of levels of mRNA or protein/enzyme expressed by the target gene, and/or the level of the enzyme activity or protein functionality. The term “suppression” includes lower, reduce, decline, decrease, inhibit, eliminate and prevent. “Silencing” or “gene silencing” does not specify mechanism and is inclusive, and not limited to, anti-sense, cosuppression, viral-suppression, hairpin suppression, stem-loop suppression, RNAi-based approaches and small RNA-based approaches.

A suppression DNA construct may comprise a region derived from a target gene of interest and may comprise all or part of the nucleic acid sequence of the sense strand (or antisense strand) of the target gene of interest. Depending upon the approach to be utilized, the region may be 100% identical or less than 100% identical (e.g., at least 50% or any integer between 51% and 100% identical) to all or part of the sense strand (or antisense strand) of the gene of interest.

Silencing elements are well-known in the art, are readily constructed once the target gene of interest is selected, and include, without limitation, cosuppression constructs, antisense constructs, viral-suppression constructs, hairpin suppression constructs, stem-loop suppression constructs, double-stranded RNA-producing constructs, and more generally, RNAi (RNA interference) constructs and small RNA constructs such as siRNA (short interfering RNA) constructs and miRNA (microRNA) constructs.

In some embodiments, a silencing element may comprise hairpin structures that incorporate all or part, of an mRNA encoding sequence in a complementary orientation that results in a potential “stem-loop” structure for the expressed RNA (PCT Publication WO 1999/53050). In this case the stem is formed by polynucleotides corresponding to the gene of interest inserted in either sense or antisense orientation with respect to the promoter and the loop is formed by some polynucleotides of the gene of interest, which do not have a complement in the construct. This increases the frequency of cosuppression or silencing in the recovered transgenic plants. For review of hairpin suppression, see, Wesley, *et al.*, (2003) *Methods in Molecular Biology, Plant Functional Genomics: Methods and Protocols* 236:273-286.

Nucleic acid molecules including silencing elements for targeting the vacuolar ATPase H subunit, useful for controlling a coleopteran pest population and infestation as described in US Patent Application Publication 2012/0198586. PCT Publication WO 2012/055982 describes ribonucleic acid (RNA or double stranded RNA) that inhibits or down regulates the expression of a target gene that encodes: an insect ribosomal protein such as the ribosomal protein L19, the ribosomal protein L40 or the ribosomal protein S27A; an insect proteasome subunit such as the Rpn6 protein, the Pros 25, the

Rpn2 protein, the proteasome beta 1 subunit protein or the Pros beta 2 protein; an insect β -coatamer of the COPI vesicle, the γ -coatamer of the COPI vesicle, the β' -coatamer protein or the ζ -coatamer of the COPI vesicle; an insect Tetraspanine 2 A protein which is a putative transmembrane domain protein; an insect protein belonging to the actin family such as Actin 5C; an insect ubiquitin-5E protein; an insect Sec23 protein which is a GTPase activator involved in intracellular protein transport; an insect crinkled protein which is an unconventional myosin which is involved in motor activity; an insect crooked neck protein which is involved in the regulation of nuclear alternative mRNA splicing; an insect vacuolar H⁺-ATPase G-subunit protein and an insect Tbp-1 such as Tat-binding protein. PCT publication WO 2007/035650 describes ribonucleic acid (RNA or double stranded RNA) that inhibits or down regulates the expression of a target gene that encodes Snf7. US Patent Application publication 2011/0054007 describes polynucleotide silencing elements targeting RPS10. US Patent Application publication 2014/0275208 and US2015/0257389 describe polynucleotide silencing elements targeting RyanR and PAT3. PCT publications WO 2016/060911, WO 2016/060912, WO 2016/060913, and WO 2016/060914 describe polynucleotide silencing elements targeting COPI coatamer subunit nucleic acid molecules that confer resistance to Coleopteran and Hemipteran pests. US Patent Application Publications 2012/029750, US 20120297501, and 2012/0322660 describe interfering ribonucleic acids (RNA or double stranded RNA) that functions upon uptake by an insect pest species to down-regulate expression of a target gene in said insect pest, wherein the RNA comprises at least one silencing element wherein the silencing element is a region of double-stranded RNA comprising annealed complementary strands, one strand of which comprises or consists of a sequence of nucleotides which is at least partially complementary to a target nucleotide sequence within the target gene. US Patent Application Publication 2012/0164205 describe potential targets for interfering double stranded ribonucleic acids for inhibiting invertebrate pests including: a Chd3 Homologous Sequence, a Beta-Tubulin Homologous Sequence, a 40 kDa V-ATPase Homologous Sequence, a EF1 α Homologous Sequence, a 26S Proteasome Subunit p28 Homologous Sequence, a Juvenile Hormone Epoxide Hydrolase Homologous Sequence, a Swelling Dependent Chloride Channel Protein Homologous Sequence, a Glucose-6-Phosphate 1-Dehydrogenase Protein Homologous Sequence, an Act42A Protein Homologous Sequence, a ADP-Ribosylation Factor 1 Homologous Sequence, a Transcription Factor IIB Protein Homologous Sequence, a Chitinase Homologous Sequences, a Ubiquitin Conjugating Enzyme Homologous Sequence, a Glyceraldehyde-3-Phosphate Dehydrogenase Homologous Sequence, an Ubiquitin B Homologous Sequence, a Juvenile Hormone Esterase Homolog, and an Alpha Tubulin Homologous Sequence.

These stacked combinations can be created by any method including but not limited to cross breeding plants by any conventional or TopCross methodology, or genetic transformation. If the traits are stacked by genetically transforming the plants, the polynucleotide sequences of interest can be

combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of the polynucleotide of interest. This may be combining with any combination of other suppression cassettes or overexpression cassettes to generate the desired combination of traits in the plant.

In some embodiments, the nucleic acid molecules comprising nematocidal sequences of the invention are provided in expression cassettes or DNA constructs for expression in the plant of interest. Such cassettes will include 5' and 3' regulatory sequences operably linked to a sequence of the embodiments. The cassette may additionally contain at least one additional gene to be co-transformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional initiation region (i.e., a promoter), translational initiation region, a polynucleotide of the embodiments, a translational termination region and, optionally, a transcriptional termination region functional in the host organism. The regulatory regions (i.e., promoters, transcriptional regulatory regions, and translational termination regions) and/or the polynucleotide of the embodiments may be native/analogous to the host cell or to each other. Alternatively, the regulatory regions and/or the polynucleotide of the embodiments may be heterologous to the host cell or to each other. Where the promoter is "foreign" or "heterologous" to the sequence of the embodiments, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked sequence of the embodiments. For example, a promoter operably linked to a heterologous polynucleotide is from a species different from the species from which the polynucleotide was derived, or, if from the same/analogous species, one or both are substantially modified from their original form and/or genomic locus, or the promoter is not the native promoter for the operably linked polynucleotide.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, or may be derived from another source. 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 Acid Res.* 15:9627-9639. Where appropriate, the gene(s) may be optimized for increased expression in the

transformed plant. That is, the genes can be synthesized using plant-preferred codons for improved expression. Methods are available in the art for synthesizing plant-preferred genes. Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to enhance expression in a given host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, Omega prime (the 5'-leader sequence of tobacco mosaic virus RNA, *Nucleic Acids Res* 1987 Apr 24;15(8):3257-73), EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison *et al.* (1986) *Virology* 154:9-20); MDMV leader (Maize Dwarf Mosaic Virus); and human immunoglobulin heavy-chain binding protein (BiP), (Macejak *et al.* (1991) *Nature* 353:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling *et al.* (1987) *Nature* 325:622-625); tobacco mosaic virus leader (TMV) (Gallie *et al.* (1989) in *Molecular Biology of RNA*, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology* 81:382-385). See also, Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968. Other methods known to enhance translation can also be utilized, for example, introns, and the like.

In those instances where it is desirable to have the expressed product of the heterologous nucleotide sequence of interest directed to a particular organelle, such as the chloroplast or mitochondrion, or secreted at the cell's surface or extracellularly, the expression cassette may further comprise a coding sequence for a transit peptide. Such transit peptides are well known in the art and include, but are not limited to, the transit peptide for the acyl carrier protein, the small subunit of RUBISCO, plant EPSP synthase, and the like.

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, re-substitutions, e.g., transitions and transversions, may be involved.

An expression cassette may 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). See generally, Yarranton (1992) *Curr. Opin. Biotech.* 3:506-511; Christopherson *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6314-6318; Yao *et al.* (1992) *Cell* 71:63-72; Reznikoff (1992) *Mol. Microbiol.* 6:2419-2422; Barkley *et al.* (1980) in *The Operon*, pp. 177-220; Hu *et al.* (1987) *Cell* 48:555-566; Brown *et al.* (1987) *Cell* 49:603-612; Figge *et al.* (1988) *Cell* 52:713-722; Deuschle *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:5400-5404; Fuerst *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2549-2553; Deuschle *et al.* (1990) *Science* 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:1917-1921; Labow *et al.* (1990) *Mol. Cell. Biol.* 10:3343-3356; Zambretti *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3952-3956; Baim *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:5072-5076; Wyborski *et al.* (1991) *Nucleic Acids Res.* 19:4647-4653; Hillenand-Wissman (1989) *Topics Mol. Struc. Biol.* 10:143-162; Degenkolb *et al.* (1991) *Antimicrob. Agents Chemother.* 35:1591-1595; Kleinschmidt *et al.* (1988) *Biochemistry* 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Oliva *et al.* (1992) *Antimicrob. Agents Chemother.* 36:913-919; Hlavka *et al.* (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); Gill *et al.* (1988) *Nature* 334:721-724.

Nematode-resistance may be directly selected by inoculating nematodes into the transformed protoplasts, cells, or tissues. Promoters may be selected based on the desired outcome. The nucleic acids can be combined with constitutive, tissue-preferred, or other promoters for expression in plants. Constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Patent No. 6,072,050; the core 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); ALS promoter (U.S. Patent No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 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.

Generally, it will be beneficial to express the gene from an inducible promoter, particularly from a pathogen-inducible promoter, such as UCP3, (see U.S. Patent No. 7,041,873) or a nematode-repressible promoter, such as SCP1 (see U.S. Patent No. 7,041,873). Such

promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. 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. See U.S. Patent No. 6,429,362,.

A "nematode-regulated" promoter is a promoter whose transcription initiation activity is either induced or repressed in response to a nematode or other pathogen stimulus. Thus, a nematode-inducible promoter increases expression of an operably linked nucleotide sequence in the presence of a nematode stimulus. In contrast, a nematode-repressible promoter decreases the transcription of an operably linked nucleotide sequence in the presence of a nematode stimulus. Nematode-regulated promoters provide a means for improved regulation of genetically engineered nematode resistance in plants. It is known that expression of a toxin gene product in nematode feeding sites can potentially harm uninfected plant cells in tissues adjacent to those sites. Thus, it can be beneficial to additionally alter the transgenic plant to express a product that counteracts excessive production of the toxin. See, for example, the methods disclosed in WO 92/21757.

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau *et al.* (1987) *Plant Mol. Biol.* 9:335-342; Matton *et al.* (1989) *Molecular Plant-Microbe Interactions* 2:325-331; Somssich *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somssich *et al.* (1988) *Mol. Gen. Genet.* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977. See also, 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; U.S. Patent No. 5,750,386 (nematode-inducible).

Additionally, as nematodes cause damage to the roots by feeding, a wound-inducible promoter may be used in the constructions of the invention. Such wound-inducible promoters include potato proteinase inhibitor (*pin II*) gene (Ryan (1990) *Ann. Rev. Phytopath.* 28:425-449; Duan *et al.* (1996) *Nature Biotechnology* 14:494-498); *wun1* and *wun2*, US 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); WIPI (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).

Root-preferred promoters are known and may be selected from the many available from the literature or isolated *de novo* from various compatible species. See, for example, those disclosed in U.S. Application Serial No. 10/104,706 (Isoflavone synthase promoter); 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 in the GRP 1.8

gene of French bean); Sanger *et al.* (1990) *Plant Mol. Biol.* 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); and Miao *et al.* (1991) *Plant Cell* 3(1):1122 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also Bogusz *et al.* (1990) *Plant Cell* 2(7):633-641, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomentosa* are described. The promoters of these genes were linked to a B-glucuronidase reporter gene and introduced into both the nonlegume *Nicotiana tabacum* and the legume *Lotus corniculatus*, and in both instances root-specific promoter activity was preserved. Leach and Aoyagi ((1991) *Plant Science* 79(1):69-76)) describe their analysis of the promoters of the highly expressed *rolC* and *rolD* root-inducing genes of *Agrobacterium rhizogenes*. They concluded that enhancer and tissue-preferred DNA determinants are dissociated in those promoters. Teeri *et al.* (1989) used gene fusion to *lacZ* to show that the *Agrobacterium* T-DNA gene encoding octopine synthase is especially active in the epidermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in leaf tissue, an especially desirable combination of characteristics for use with an insecticidal or larvicidal gene (see *EMBO J.* 8(2):343-350). The TR1' gene, fused to *nptII* (neomycin phosphotransferase II), showed similar characteristics. Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster *et al.* (1995) *Plant Mol. Biol.* 29(4):759-772); and *rolB* promoter (Capana *et al.* (1994) *Plant Mol. Biol.* 25(4):681-691. 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. Root-preferred sorghum (*Sorghum bicolor*) RCc3 promoters are disclosed in US Patent Application US20120210463.

Where low level expression is desired, weak promoters will be used. Generally, a "weak promoter" is a promoter that drives expression of a coding sequence at a low level. A "low level" is intended to mean expression at levels of about 1/1000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts per cell. Alternatively, it is recognized that weak promoters also include promoters that are expressed in only a few cells and not in others to give a total low level of expression. Where a promoter is expressed at unacceptably high levels, portions of the promoter sequence can be deleted or modified to decrease expression levels. Such weak constitutive promoters include, for example, the core promoter of the Rsyn7 promoter (WO 99/43838 and U.S. Patent No. 6,072,050), the core 35S CaMV promoter, and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142, and 6,177,611, herein incorporated by reference.

The above list of promoters is not meant to be limiting. Any appropriate promoter can be used in the embodiments.

The methods of the embodiments involve introducing a nucleotide construct into a plant. As used herein, "introducing" is intended to mean presenting to the plant the nucleotide construct in such a manner that the construct gains access to the interior of a cell of the plant. The methods of the embodiments do not depend on a particular method for introducing a nucleotide construct to a plant, only that the nucleotide construct gains access to the interior of at least one cell of the plant. Methods for introducing nucleotide constructs into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods. "Stable transformation" is intended to mean that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by progeny thereof. "Transient transformation" is intended to mean that a nucleotide construct introduced into a plant does not integrate into the genome of the plant.

The nucleotide constructs of the embodiments may be introduced into plants by contacting plants with a virus or viral nucleic acids. Generally, such methods involve incorporating a nucleotide construct of the invention within a viral DNA or RNA molecule. It is recognized that the nematocidal proteins of the invention may be initially synthesized as part of a viral polyprotein, which later may be processed by proteolysis in vivo or in vitro to produce the desired recombinant protein. Methods for introducing nucleotide constructs into plants and expressing a protein encoded therein, involving viral DNA or RNA molecules, are known in the art. See, for example, U.S. Patent Nos. 5,889,191, 5,889,190, 5,866,785, 5,589,367 and 5,316,931.

A variety of other transformation protocols are contemplated in the embodiments. Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i. e., monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway et al. (1986) *Biotechniques* 4:320-334), electroporation (Riggs et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606, *Agrobacterium*-mediated transformation (U.S. Patent Nos. 5,563,055 and 5,981,840), direct gene transfer (Paszkowski et al. (1984) *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, U.S. Patent Nos. 4,945,050, 5,879,918, 5,886,244, 5,932,782; Tomes et al. (1995) *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, eds. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe et al. (1988) *Biotechnology* 6:923-926); and Lecl transformation (WO 00/28058, published May 18, 2000). Also see Weissinger et al. (1988) *Ann. Rev. Genet.* 22:421-477; Sanford et al. (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou et al. (1988) *Plant Physiol.* 87:671-674 (soybean); McCabe et al. (1988)

Bio/Technology 6:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh et al. (1998) *Theor. Appl. Genet.* 96:319-324 (soybean); Datta et al. (1990) *Biotechnology* 8:736-740 (rice); Klein et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein et al. (1988) *Biotechnology* 6:559-563 (maize); U.S. Patent Nos. 5,240,855, 5,322,783 and 5,324,646; Tomes et al. (1995, *supra*) (maize); Klein et al. (1988) *Plant Physiol.* 91:440-444 (maize); Fromm et al. (1990) *Biotechnology* 8:833-839 (maize); Hooykaas-Van Slogteren et al. (1984) *Nature (London)* 311:763-764; Bowen et al., U.S. Patent No. 5,736,369 (cereals); Bytebier et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet et al. (1985) in *The Experimental Manipulation of Ovule Tissues*, ed. Chapman et al. (Longman, New York), pp. 197-209 (pollen); Kaeppler et al. (1990) *Plant Cell Reports* 9:415-418 and Kaeppler et al. (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin et al. (1992) *Plant Cell* 4:1495-1505 (electroporation); Li et al. (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford (1995) *Annals of Botany* 75:407-413 (rice); Osjoda et al. (1996) *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*).

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 constitutive expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure constitutive expression of the desired phenotypic characteristic has been achieved.

The sequences of the embodiments may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plant species of interest include, but are not limited to, corn (*Zea mays*), *Brassica* spp. (e.g., *B. napus*, *B. rapa*, *B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew

(*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables, ornamentals, and conifers.

Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (e.g., *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and chrysanthemum.

Conifers that may be employed in practicing the present invention include, for example, pines (*Pinus* spp.) such as loblolly pine (*Pinus taeda*), slash pine (*P. elliotii*), ponderosa pine (*P. ponderosa*), lodgepole pine (*P. contorta*), and Monterey pine (*P. radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow cedar (*Chamaecyparis nootkatensis*). Plants of the present invention may be crop plants (for example, alfalfa, sunflower, *Brassica*, cotton, safflower, peanut, sorghum, wheat, millet, tobacco, *etc.*), corn or soybean plants.

Plants interest include grain plants that provide seeds of interest, oil-seed plants, and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, *etc.* Oilseed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, *etc.* Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mung bean, lima bean, fava bean, lentils, chickpea, *etc.*

Derivatives of the coding sequences can be made by site-directed mutagenesis to increase the level of pre-selected amino acids in the encoded polypeptide. Other proteins include methionine-rich plant proteins such as from sunflower seed (Lilley *et al.* (1989) *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, ed. Applewhite (American Oil Chemists Society, Champaign, Illinois), pp. 497-502); corn (Pedersen *et al.* (1986) *J. Biol. Chem.* 261:6279; Kiriara *et al.* (1988) *Gene* 71:359 and rice (Musumura *et al.* (1989) *Plant Mol. Biol.* 12:123). Other agronomically important genes encode latex, Flourey 2, growth factors, seed storage factors, and transcription factors.

In an aspect, the disclosed polynucleotides encoding nematocidal polypeptide compositions can be introduced into the genome of a plant using genome editing technologies, or previously introduced polynucleotides encoding nematocidal polypeptides in the genome of a plant may be edited using genome editing technologies. For example, the disclosed polynucleotides can be introduced into a desired location in the genome of a plant through the use of double-stranded break technologies such

as TALENs, meganucleases, zinc finger nucleases, CRISPR-Cas, and the like. For example, the disclosed polynucleotides can be introduced into a desired location in a genome using a CRISPR-Cas system, for the purpose of site-specific insertion. The desired location in a plant genome can be any desired target site for insertion, such as a genomic region amenable for breeding or may be a target site located in a genomic window with an existing trait of interest. Existing traits of interest could be either an endogenous trait or a previously introduced trait.

In another aspect, where the disclosed polynucleotides encoding nematocidal polypeptides has previously been introduced into a genome, genome editing technologies may be used to alter or modify the introduced polynucleotide sequence. Site specific modifications that can be introduced into the disclosed polynucleotides encoding nematocidal polypeptides compositions include those produced using any method for introducing site specific modification, including, but not limited to, through the use of gene repair oligonucleotides (e.g. US Publication 2013/0019349), or through the use of double-stranded break technologies such as TALENs, meganucleases, zinc finger nucleases, CRISPR-Cas, and the like. Such technologies can be used to modify the previously introduced polynucleotide through the insertion, deletion or substitution of nucleotides within the introduced polynucleotide. Alternatively, double-stranded break technologies can be used to add additional nucleotide sequences to the introduced polynucleotide. Additional sequences that may be added include, additional expression elements, such as enhancer and promoter sequences. In another embodiment, genome editing technologies may be used to position additional insecticidally-active proteins in close proximity to the disclosed polynucleotides encoding nematocidal polypeptide compositions disclosed herein within the genome of a plant, in order to generate molecular stacks of insecticidally-active proteins.

An "altered target site," "altered target sequence," "modified target site," and "modified target sequence" are used interchangeably herein and refer to a target sequence as disclosed herein that comprises at least one alteration when compared to non-altered target sequence. Such "alterations" include, for example: (i) replacement of at least one nucleotide, (ii) a deletion of at least one nucleotide, (iii) an insertion of at least one nucleotide, or (iv) any combination of (i) - (iii).

Chloroplast targeting sequences are known in the art and include the chloroplast small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) (de Castro Silva Filho *et al.* (1996) *Plant Mol. Biol.* 30:769-780; Schnell *et al.* (1991) *J. Biol. Chem.* 266(5):3335-3342); 5-(enolpyruvyl)shikimate-3-phosphate synthase (EPSPS) (Archer *et al.* (1990) *J. Bioenerg. Biomemb.* 22(6):789-810); tryptophan synthase (Zhao *et al.* (1995) *J. Biol. Chem.* 270(11):6081-6087); plastocyanin (Lawrence *et al.* (1997) *J. Biol. Chem.* 272(33):20357-20363); chorismate synthase (Schmidt *et al.* (1993) *J. Biol. Chem.* 268(36):27447-27457); and the light harvesting chlorophyll a/b binding protein (LHBP) (Larripa *et al.* (1988) *J Biol. Chem.* 263:14996-14999). See also Von Heijne *et al.* (1991) *Plant Mol. Biol. Rep.*

9:104-126; Clark *et al.* (1989) *J. Biol. Chem.* 264:17544-17550; Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968; Romer *et al.* (1993) *Biochem. Biophys. Res. Commun.* 196:1414-1421; and Shah *et al.* (1986) *Science* 233:478-481.

The use of the term "nucleotide constructs" herein is not intended to limit the present embodiments to nucleotide constructs comprising DNA. Those of ordinary skill in the art will recognize that nucleotide constructs, particularly polynucleotides and oligonucleotides, comprised of ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides may also be employed in the methods disclosed herein. Thus, the nucleotide constructs of the present invention encompass all nucleotide constructs that can be employed in the methods of the present invention for transforming plants including, but not limited to, those comprised of deoxyribonucleotides, ribonucleotides, and combinations thereof. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules and synthetic analogues. The nucleotide constructs also encompass all forms of nucleotide constructs including, but not limited to, single-stranded forms, double-stranded forms, hairpins, stem-and-loop structures, and the like.

The nucleotide constructs of the embodiments also encompass nucleotide constructs that may be employed in methods for altering or mutating a genomic nucleotide sequence in an organism, including, but not limited to, chimeric vectors, chimeric mutational vectors, chimeric repair vectors, mixed-duplex oligonucleotides, self-complementary chimeric oligonucleotides, and recombinogenic oligonucleobases. Such nucleotide constructs and methods of use, such as, for example, chimeraplasty, are known in the art. Chimeraplasty involves the use of such nucleotide constructs to introduce site-specific changes into the sequence of genomic DNA within an organism. See, U.S. Patent Nos. 5,565,350; 5,731,181; 5,756,325; 5,760,012; 5,795,972; and 5,871,984. See also, WO 98/49350, WO 99/07865, WO 99/25821, and Beetham *et al.* (1999) *Proc. Natl. Acad. Sci. USA* 96:8774-8778.

In some embodiments methods are provided for killing an insect pest, comprising contacting the insect pest with an nematocidally-effective amount of an nematocidal polypeptide of the disclosure.

In some embodiments methods are provided for controlling certain nematode pest population(s), comprising contacting the insect pest population(s) with a nematocidally-effective amount of a recombinant nematocidal polypeptide of the embodiments. In some embodiments methods are provided for controlling certain nematode pest population(s), comprising contacting the nematode pest population(s) with a nematocidally-effective amount of a nematocidal polypeptide of the embodiments. As used herein, "controlling a pest population" or "controls a pest" refers to any effect on a pest that results in limiting the damage that the pest causes. Controlling a pest includes, but is not limited to, killing the pest, inhibiting development of the pest, altering fertility or growth of the pest in such a manner that the pest provides less damage to the plant, decreasing the number of offspring produced, producing less fit pests, producing pests more susceptible to predator attack or deterring the pests from eating the plant.

In some embodiments methods are provided for controlling an pest population resistant to a pesticidal protein, comprising contacting the nematode pest population with an nematocidally-effective amount of a recombinant nematocidal polypeptide of the disclosure. In some embodiments methods are provided for controlling an nematode pest population resistant to a pesticidal protein, comprising
5 contacting the nematode pest population with an nematocidally-effective amount of a polypeptide of the embodiments.

In some embodiments methods are provided for protecting a plant from a pest, comprising expressing in the plant or cell thereof a recombinant polynucleotide encoding an polypeptide of the disclosure. In some embodiments methods are provided for protecting a plant from an pest,
10 comprising expressing in the plant or cell thereof a recombinant polynucleotide encoding pesticidal polypeptide of the embodiments.

To protect and to enhance yield production and trait technologies, seed treatment options can provide additional crop plan flexibility and cost effective control against insects, weeds and diseases. Seed material can be treated, typically surface treated, with a composition comprising combinations of
15 chemical or biological herbicides, herbicide safeners, insecticides, fungicides, germination inhibitors and enhancers, nutrients, plant growth regulators and activators, bactericides, nematocides, avicides and/or molluscicides. These compounds are typically formulated together with further carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. The coatings may be applied by impregnating propagation material with a liquid formulation or by coating with a combined
20 wet or dry formulation. Examples of the various types of compounds that may be used as seed treatments are provided in The Pesticide Manual: A World Compendium, C.D.S. Tomlin Ed., Published by the British Crop Production Council, which is hereby incorporated by reference.

In some embodiments the active ingredients can be applied in the form of compositions and can be applied to the crop area or plant to be treated, simultaneously or in succession, with other compounds.
25 These compounds can be fertilizers, weed killers, Cryoprotectants, surfactants, detergents, pesticidal soaps, dormant oils, polymers, and/or time-release or biodegradable carrier formulations that permit long-term dosing of a target area following a single application of the formulation. They can also be selective herbicides, chemical insecticides, virucides, microbicides, amoebicides, pesticides, fungicides, bacteriocides, nematocides, molluscicides or mixtures of several of these preparations, if desired,
30 together with further agriculturally acceptable carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. 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. Likewise the formulations may be prepared into edible “baits” or fashioned into pest “traps” to permit feeding or
35 ingestion by a target pest of the pesticidal formulation.

Methods of applying an active ingredient or an agrochemical composition that contains at least one of the insecticidal polypeptides of the disclosure produced by the bacterial strains include leaf 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.

The composition may be formulated as a powder, dust, pellet, granule, spray, emulsion, colloid, solution or such like, and may be prepared by such conventional means as desiccation, lyophilization, homogenation, extraction, filtration, centrifugation, sedimentation or concentration of a culture of cells comprising the polypeptide. In all such compositions that contain at least one such pesticidal polypeptide, the polypeptide may be present in a concentration of from about 1% to about 99% by weight.

Lepidopteran, Dipteran, Heteropteran, nematode, Hemiptera or Coleopteran pests may be killed or reduced in numbers in a given area by the methods of the disclosure or 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. "Pesticidally-effective amount" as used herein refers to 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 may also vary with respect to climatic conditions, environmental considerations, and/or frequency of application and/or severity of pest infestation.

Some seed treatments that may be used on crop seed include, but are not limited to, one or more of abscisic acid, acibenzolar-S-methyl, avermectin, amitrol, azaconazole, azospirillum, azadirachtin, azoxystrobin, *Bacillus* spp. (including one or more of *cereus*, *firmus*, *megaterium*, *pumilis*, *sphaericus*, *subtilis* and/or *thuringiensis* species), *bradyrhizobium* spp. (including one or more of *betae*, *canariense*, *elkanii*, *iriomotense*, *japonicum*, *liaonigense*, *pachyrhizi* and/or *yuanmingense*), captan, carboxin, chitosan, clothianidin, copper, cyazypyr, difenoconazole, etidiazole, fipronil, fludioxonil, fluoxastrobin, fluquinconazole, flurazole, fluxofenim, harpin protein, imazalil, imidacloprid, ipconazole, isoflavenoids, lipo-chitoooligosaccharide, mancozeb, manganese, maneb, mefenoxam, metalaxyl, metconazole, myclobutanil, PCNB, penflufen, penicillium, penthiopyrad, permethrin, picoxystrobin, prothioconazole, pyraclostrobin, rynaxypyr, S-metolachlor, saponin, sedaxane, TCMTB, tebuconazole, thiabendazole, thiamethoxam, thiocarb, thiram, tolclofos-methyl, triadimenol, trichoderma, trifloxystrobin, triticonazole and/or zinc. PCNB seed coat refers to EPA Registration Number 00293500419, containing quintozen and terrazole. TCMTB refers to 2-(thiocyanomethylthio) benzothiazole.

Seed varieties and seeds with specific transgenic traits may be tested to determine which seed treatment options and application rates may complement such varieties and transgenic traits in order to enhance yield. For example, a variety with good yield potential but head smut susceptibility may benefit from the use of a seed treatment that provides protection against head smut, a variety with good yield
5 potential but cyst nematode susceptibility may benefit from the use of a seed treatment that provides protection against cyst nematode, and so on. Likewise, a variety encompassing a transgenic trait conferring insect resistance may benefit from the second mode of action conferred by the seed treatment, a variety encompassing a transgenic trait conferring herbicide resistance may benefit from a seed treatment with a safener that enhances the plants resistance to that herbicide, etc. Further, the good root
10 establishment and early emergence that results from the proper use of a seed treatment may result in more efficient nitrogen use, a better ability to withstand drought and an overall increase in yield potential of a variety or varieties containing a certain trait when combined with a seed treatment.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent
15 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.

Although the foregoing embodiments have been described in some detail by way of illustration and example for purposes of clarity of understanding, certain changes and modifications may be practiced within the scope of the appended claims.

20 The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1: *C. elegans* feeding assay

C. elegans L1 larvae were synchronized by bleaching adult worms and hatching eggs overnight (Sulston & Hodgkin, 1988). Multiple bacterial strains were isolated from environmental samples. The strains were grown in 5 mL King's medium overnight at 30 °C with shaking at 225 rpm. 5 to 30 µL of the liquid culture was added into assay wells in 96-well plates. Each assay well contained 120 µL of liquid with ~50 L1 staged *C. elegans*, 30 µg/mL tetracycline, 30 µg/mL chloramphenicol, overnight culture and S-medium. *E. coli* strain OP50 was used as a control. Forty eight hours later, the assay plates were scored under a microscope by checking the worm's growth and development. One strain, named as D3, totally inhibited worm development and all worms were arrested at the L1 stage, indicating that D3 shows nematicidal activity.

Specific PCR primers (SEQ ID NOs: 4 and 5) for rDNA were used to isolate ~1400 bp rDNA sequence from the D3 strain and the 1400bp sequence showed 100% identity to the 16S ribosomal RNA gene from *Pseudomonas protegens* Pf-5 strain.

EXAMPLE 2: Protein purification techniques and assignment of nematicidal activity to specific proteins and obtention of their sequences

D3 cells were grown for 20 hours at 30 °C in four 1-liter flasks containing 250 mL King's medium. Cultures were spun at 10,000 x g for 10 minutes, and cell pellets were stored at -80 °C. Cells were resuspended in a total of 30 mL of 50 mM Hepes, pH 7.2, 10 mM potassium phosphate, 100 mM KCl, 5% ethylene glycol, 1 mM DTT, protease inhibitor cocktail (Sigma P8465, 2 mg/mL). Lysis was attained by extrusion from a French pressure cell at 18,000 PSI. The lysate was then sonicated to reduce the viscosity. The lysate was spun at 100K x g for 30 minutes, in a Beckman MLA 80 rotor, and the supernatant was filtered through a 0.2 micron filter flask. The supernatant was desalted by gel filtration on Sephadex G25 (10 PD-10 columns, 2.5 mL of supernatant/column) equilibrated with 50 mM Hepes, pH 7.2, 20 mM KCl, 5% ethylene glycol (G25 buffer). The gel-filtered crude supernatant (GFC) was passed through an anion exchange resin (HiPrep Q 16/10, GE Health Sciences) equilibrated with G25 buffer. The nematicidal activity was present in the flow-through. Turbidity was observed in the flow-through, and was removed by centrifugation at 100K x g. The flow-through was concentrated 2- to 3-fold by ultra-filtration (AmiconUltra15, YM30 membrane), then gel filtered into Sulfopropyl start buffer (25 mM Hepes, pH 6.9 and 5% EG). This material was then applied to HiPrep Sulfopropyl cation exchange resin (GE Health sciences) equilibrated with start buffer. The material was eluted with a linear gradient to 200 mM KCl in Sulfopropyl start buffer. Active fractions were pooled, concentrated (YM30) to 1 mL, buffer exchanged into 25 mM Hepes, pH 6.9, then chromatographed on

a second, higher-resolution cation exchanger, CM825 (Tosoh). The anti-nematode activity eluted within a shallow gradient of 160 to 200 mM KCl in 25 mM Hepes, pH 6.9.

Active fractions were pooled and concentrated. Recovery of activity at this stage was about 30%. SDS-PAGE analysis of the active fractions from this step shows bands at 60K, 28K and minor bands at 20K and 14K. The activity elutes from Superdex 75 at MW = 60K. The proteins were resolved by reverse phase HPLC using a Jupiter C5 column (Phenomenex) with a shallow gradient of acetonitrile in 0.2% TFA. The proteins were transferred from SDS-PAGE gel to a PVDF membrane and the protein bands were sent for N-terminal sequencing. Two separate peptides based on the relative abundance of amino acids in each cycle of the Edman degradation were composed. Multiple peptide sequences were obtained with Edman degradation.

EXAMPLE 3: Amplification of the specific DNA fragments and ORF results

Degenerate PCR primers were designed based on the peptide sequences. The genomic DNA of the strain D3 was used as a template, and several DNA fragments were amplified with PCR. The fragments were then isolated, cloned, and sequenced. One of the fragments, when translated, shared amino acid sequences with some of the peptides isolated in Example 2. Further genome walking using these fragments resulted in the assembly of a fragment approximately 2.6 kb in length (shown in SEQ ID NO: 1).

The 2.6 kb assembled sequence, when translated, yielded two open reading frames (ORFs) (See Figure 1; SEQ ID NOs: 2 and 3) in the same orientation. The first ORF side contains the N-terminus as well as all the internal amino acid sequence of the first peptide identified in Example 2 and has a 24 amino acid predicted signal sequence at the N-terminus. This ORF encodes a protein (Pp-ANP-1, SEQ ID NO: 6) with a molecular weight of ~12 kDa for full-length protein and ~10 kDa for the mature protein. The second ORF contains the N-terminal as well as all the internal amino acid sequence of the first peptide and has a 24 predicted amino acid signal sequence at the N-terminus. This ORF encodes a protein (Pp-ANP-2, SEQ ID NO: 7) with a molecular weight of ~28 kDa for full length protein and ~26 kDa for the mature protein. The two ORFs are separated by a 170 bp non-translatable sequence. The molecular weight of neither ORF matches the originally predicted 60 kDa weight for the active peptide. The discrepancy may be that during purification of the protein, these two subunits stayed aggregated until the final HPLC step.

A BLAST did not significantly match any sequences in certain databases, while Pp-ANP-2 showed ~30% identity (~40% similarity) to the subunit A of *E. coli* enterotoxin and cholera toxin (Table 1).

Table 1. Protein Identity of Pp-ANP-2 and subunit A of enterotoxins.

	Pp-ANP2	LTh-A	LTp-A	LT-IIa A	LT-IIb A	CT-A
Pp-ANP2		30.5 (39.5)*	30.8 (39.8)*	30.1 (42.0)*	28.9 (40.7)*	29.9 (40.3)*
LTh-A			98.8 (99.2)*	49.8 (63.2)*	50.2 (61.1)*	79.8 (85.7)*
LTp-A				49.8 (63.2)*	51.7 (63.8)*	80.2 (86.4)*
LT-IIa A					71.1 (78.7)*	48.7 (62.8)*
LT-IIb A						48.7 (61.2)*
CT-A						

*Similarity represented in parentheses.

EXAMPLE 4: Subcloning of the two proteins and construct preparation

5 The two ORFs identified in Example 3 were subcloned into the expression vector pQE80 (Qiagen). In order to test if both the proteins are required for activity, two constructs (with and without the N-terminal extension) for each protein and a construct with both proteins including the intervening non-coding sequence were made. Five constructs were prepared as follows:

1. construct containing the full length sequence of Pp-ANP-1;
- 10 2. construct containing the sequence of Pp-ANP-1 without the signal peptide sequence (Pp-ANP-1');;
3. construct containing the full length sequence of Pp-ANP-2;
4. construct containing the sequence of Pp-ANP-2 without the signal peptide sequence (Pp-ANP-2');
- 15 5. construct containing the full length sequence of both Pp-ANP-1, Pp-ANP-2 and the 170 bp spacer sequence between the two ORFs.

These five constructs were transformed into the *E. coli* strain XL-1 Blue (Stratagene) and the empty pQE80 vector was used as a negative control.

20 *E. coli* cells transformed with these constructs were grown in LB medium with carbenicillin overnight at 37 °C 225 at rpm, then the cultures were diluted five fold with fresh LB plus carbenicillin and continuously grown at 37 °C at 225 rpm. When the OD₆₀₀ reached 0.6, IPTG was added to the culture (final IPTG concentration was 1 mM) to induce protein expression. Uninduced cultures were

also prepared as controls. Four hours later, the cultures were collected for running SDS-PAGE and setting up the *C. elegans* assay as described in Example 1. Nematicidal activity was detected only from the construct #5 which had both proteins (Figure 2), under these test conditions. For the brood size test, individual L4 worms were picked from NGM agar plates and transferred into 96-well microtiter plates, with a single worm per well. Each well contained 120 μ L of the same medium as for the anti-nematode assay. After incubation at room temperature for 48h, progeny in each well were counted. The *E. coli* cells containing both Pp-ANP-1 and Pp-ANP-2 significantly reduced the *C. elegans* brood sizes (Figure 3).

In addition, comparable activity could also be detected when cells with full-length Pp-ANP-1 and full-length Pp-ANP-2 were mixed *in vitro* and fed to *C. elegans*. These experiments confirmed that both proteins are required for activity in *C. elegans* under test conditions; N-terminal signal peptides are needed for both proteins to show activity on *C. elegans* under test conditions; and Pp-ANP-1 and Pp-ANP-2 can be expressed separately and the active protein can be reconstituted.

Example 5: *In planta* expression of Pp-ANP proteins and *C. elegans* feeding assays

In order to check if Pp-ANP-1 and/or Pp-ANP-2 can be expressed in plants *N. benthamiana* transient assays were performed with the binary vector constructs. In addition to infiltrating *Agrobacterium* with individual constructs, co-infiltration of two constructs was also performed.

Three days after the infiltration, total protein was extracted from the infiltrated leaves with Tris extraction buffer (100mM Tris pH8.0, 100mM NaCl, 1mM EDTA, 10mM DTT and 1X protease inhibitors). The protein samples were checked with SDS-PAGE. Pp-ANP-2 protein clearly showed on the gel, indicating the high level expression of this protein.

The same infiltrated extracts were fed to *C. elegans* to check for nematicidal activity (as described in Example 1, except protein samples were added and *E. coli* OP50 was used as food for *C. elegans*). Activity was detected only from the Pp-ANP-1/ Pp-ANP-2 co-infiltrated extracts, indicating once again that the two proteins assemble rapidly into an active multi-subunit configuration which is required for activity under test conditions.

Example 6: Anti-nematode activity on Pp-ANP proteins on other free-living nematodes

The effects of Pp-ANP-1 and Pp-ANP-2 were tested on other free living nematodes, *Pristionchus pacificus*, *Panagrellus redivivus*, and *Acroboloides* sp. on agar plates with *E. coli* expressing Pp-ANP-1/ Pp-ANP-2 toxic proteins. Pp-ANP-1/ Pp-ANP-2 clearly showed strong activity on all three nematode species by inhibiting the worm growth and development (Figure 4).

D3 culture, Pp-ANP-1/ Pp-ANP-2 transformed *E. coli* culture and their clear lysate were further tested on certain other targets, including insects (Coleoptera, Lepidoptera, and Hemiptera), plant parasitic nematodes (*Heterodera glycines*), and fungi (*Colletotrichum graminicola* and *Fusarium verticillioides*). No clear detrimental effects were observed under test conditions on these organisms, suggesting that Pp-ANP-1 and Pp-ANP-2 are not general toxins but instead show specificity to free living nematodes.

Example 7: Homolog sequences

Another *Pseudomonas protegens* strain, 14B2, also showed strong inhibition activity on *C. elegans*. PCR and further genome sequence of strain 14B2 revealed that it contains a homolog of Pp-ANP-1 and Pp-ANP-2. The DNA sequences of the two ORFs from 14Be are named as Pp-ANP-3 and Pp-ANP-4 (SEQ ID NO: 8 and SEQ ID NO: 9). Their amino acid sequences are shown in SEQ ID NO: 12 and SEQ ID NO: 13. Pp-ANP-3 showed 84.2% identity to Pp-ANP-1, while Pp-ANP-4 showed 93.8% identity to Pp-ANP-2. Pp-ANP-3 and Pp-ANP-4 were subcloned into *E. coli* as described in Example 3. The *E. coli* cells expressing Pp-ANP-3 and Pp-ANP-4 showed strong inhibition activity on *C. elegans*, indicating Pp-ANP-3 and Pp-ANP-4 are also anti-nematode proteins.

Blast searching of a proprietary bacterial genome database revealed that several other *Pseudomonas protegens* strains contain homologous proteins. The two ORFs from strain JH67972-2 were named as Pp-ANP-5 and Pp-ANP-6 (SEQ ID NOs: 10 and 11, with amino acid sequences represented in SEQ ID NOs: 14, and 15). Pp-ANP-5 showed 85.1% identity to Pp-ANP-1, while Pp-ANP-6 showed 95.0% identity to Pp-ANP-2. The amino acid sequence alignments are showed in Figure 5.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a polynucleotide operably linked to a heterologous promoter, wherein the polynucleotide is selected from the group consisting of:

- a) a sequence set forth in SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11;
- 5 b) a nucleotide sequence comprising an effective number of contiguous nucleotides of the sequence of SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11 so that said nucleotide sequence encodes a polypeptide having nematicidal activity;
- c) a nucleotide sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11, wherein said nucleotide sequence encodes a polypeptide having nematicidal activity;
- 10 d) a nucleic acid molecule comprising a sequence encoding the amino acid sequences set forth in SEQ ID NOs: 6, 7, and 10-15; and
- e) a nucleic acid molecule comprising a sequence encoding an amino acid having at least 90% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 6, 7, and 10-15;
- 15

wherein said polynucleotide encodes a polypeptide having nematicidal activity.

2. A DNA construct comprising the polynucleotide of claim 1.

3. The DNA construct of claim 2, wherein the DNA construct further comprises a second polynucleotide wherein the polynucleotide is selected from the group consisting of:

- a) the sequence set forth in SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11;
- 20 b) a nucleotide sequence comprising an effective number of contiguous nucleotides of the sequence of SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11 so that said nucleotide sequence encodes a polypeptide having nematicidal activity;
- c) a nucleotide sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11, wherein said nucleotide sequence encodes a polypeptide having nematicidal activity;
- 25 d) a nucleic acid molecule comprising a sequence encoding the amino acid sequences set forth in SEQ ID NOs: 6, 7, 12, 13, 14, and 15; and
- e) a nucleic acid molecule comprising a sequence encoding an amino acid having at least 90% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 6, 7, 12, 13, 14, and 15;
- 30

wherein said polynucleotide encodes a polypeptide having nematicidal activity.

4. A plant cell having stably incorporated in its genome the nucleic acid molecule of claim 1.

5. A plant cell having stably incorporated into its genome at least one DNA construct of claim 2.

6. The plant cell of claim 5, wherein said plant cell is from a dicot plant.

7. The plant cell of claim 6, wherein said dicot plant is soybean.

8. The plant cell of claim 5, wherein said plant cell is a root cell.

9. A nematicidal polypeptide comprising:

a) a polypeptide sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15; or

b) a polypeptide comprising at least 85% sequence identity compared to the amino acid sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15;

wherein said polypeptide encodes a polypeptide having nematicidal activity.

10. The polypeptide of claim 9 further comprising a second polypeptide, wherein the second polypeptide is selected from the group consisting of:

a) a polypeptide sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15; or

b) a polypeptide comprising at least 85% sequence identity compared to the amino acid sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15;

wherein said polypeptide encodes a polypeptide having nematicidal activity.

11. A nematicidal composition comprising an agricultural acceptable carrier and a polypeptide selected from the group consisting of:

a) a polypeptide sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15; or

b) a polypeptide comprising at least 85% sequence identity compared to the amino acid sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15;

wherein said polypeptide encodes a polypeptide having nematicidal activity.

12. The composition of claim 11 further comprising a second polypeptide, wherein the second polypeptide is selected from the group consisting of:

a) a polypeptide sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15; or

b) a polypeptide comprising at least 85% sequence identity compared to the amino acid sequence set forth in SEQ ID NO: 6, 7, 12, 13, 14, and 15;

wherein said polypeptide encodes a polypeptide having nematicidal activity.

13. A method for conferring or improving nematode resistance in a plant, said method comprising:

a) transforming a plant cell with a nucleic acid molecule comprising a heterologous sequence operably linked to a regulatory sequence that induces transcription of said heterologous sequence in a plant cell, wherein said heterologous sequence comprises a nucleotide sequence selected from the group consisting of:

i) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11;

ii) a nucleotide sequence comprising an effective number of contiguous nucleotides of the sequence of SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11 wherein said nucleotide sequence encodes a polypeptide having nematicidal activity;

- iii) a nucleotide sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1, 2, 3, 8, 9, 10, and 11, wherein said nucleotide sequence encodes a polypeptide having nematocidal activity;
 - iv) a nucleic acid molecule comprising a sequence encoding the amino acid sequences set forth in SEQ ID NOs: 6, 7, 12, 13, 14, and 15; and
 - v) a nucleic acid molecule comprising a sequence encoding an amino acid having at least 90% sequence identity to the amino acid sequences set forth in SEQ ID NOs: 6, 7, 12, 13, 14, and 15; and
- b) regenerating stably transformed plants.

- 10 14. A method of inhibiting growth or killing an agricultural nematode pest population, comprising contacting the nematode pest population with an effective amount of the polypeptide of claim 9.

Figure 1

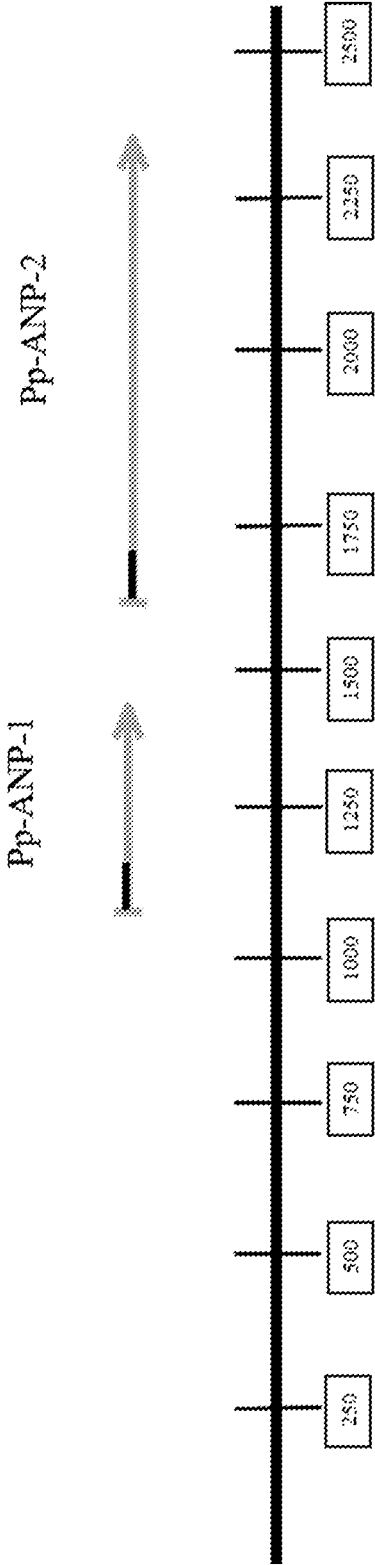
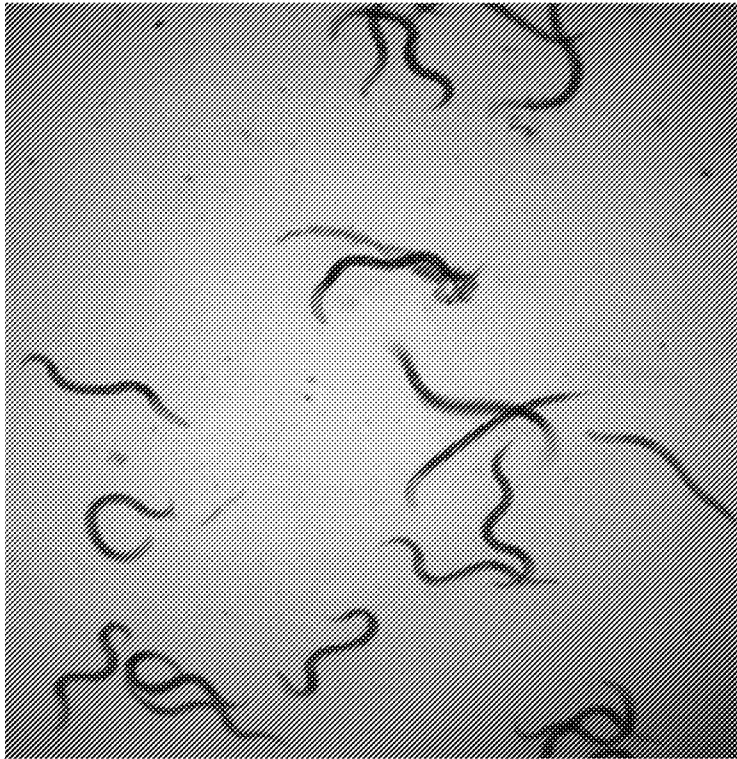
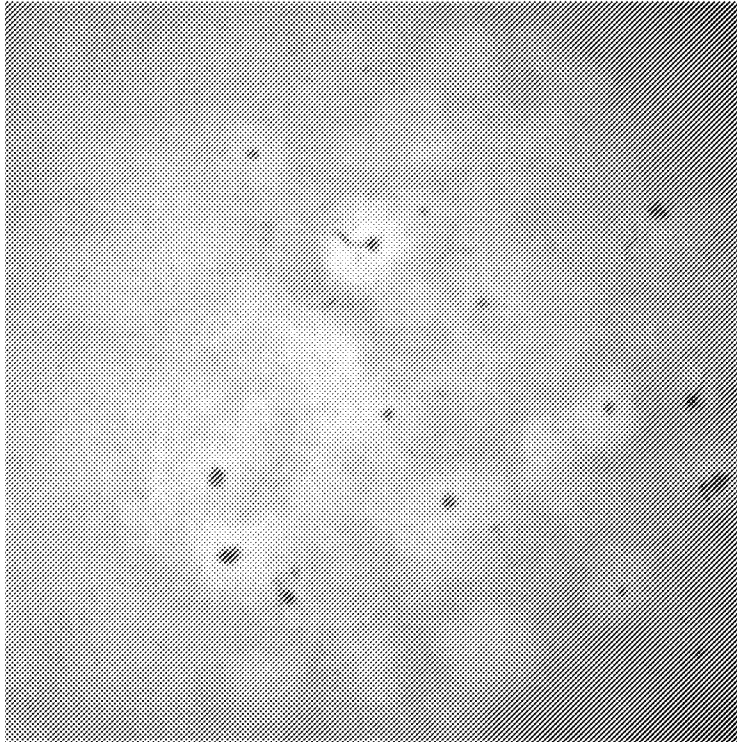


Figure 2

pQE control



pQE with Pp-ANP1 and Pp-ANP-2/2

Figure 3

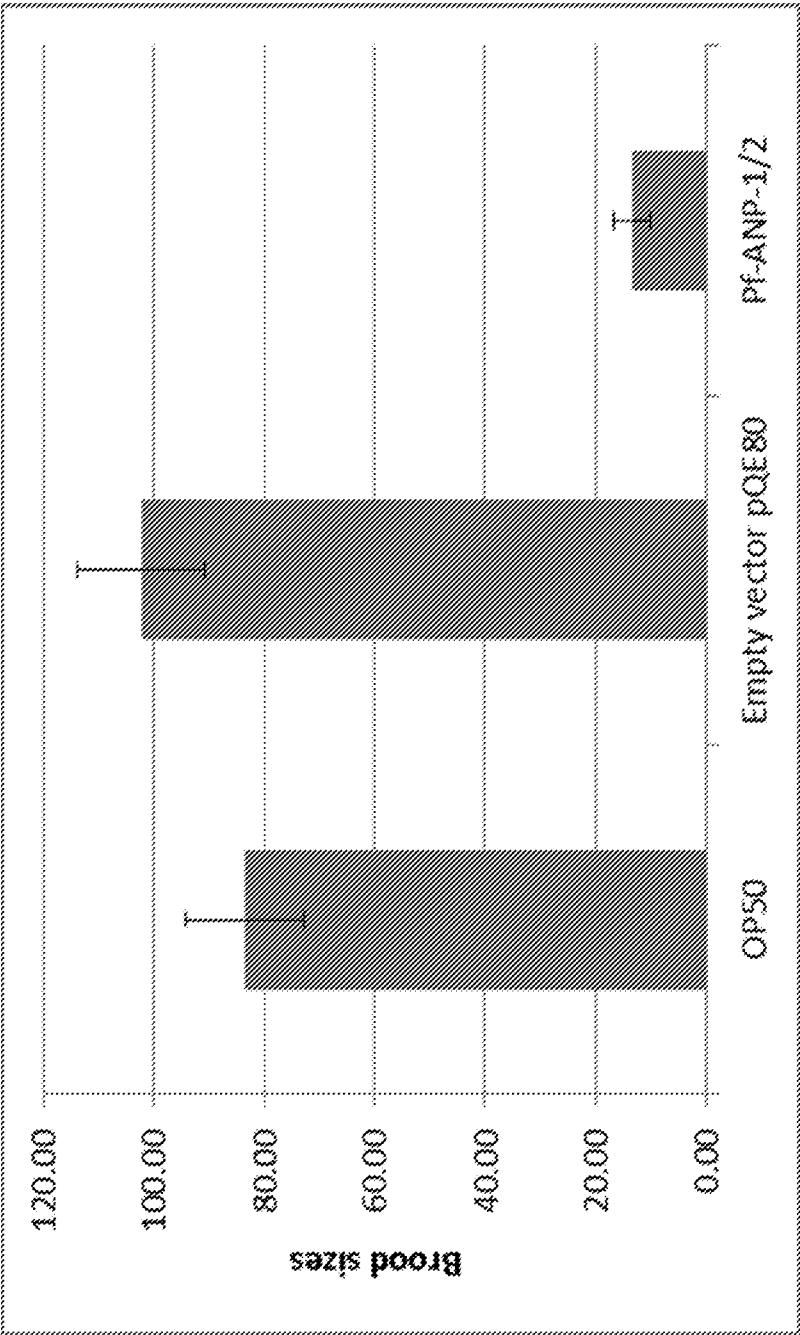


Figure 4

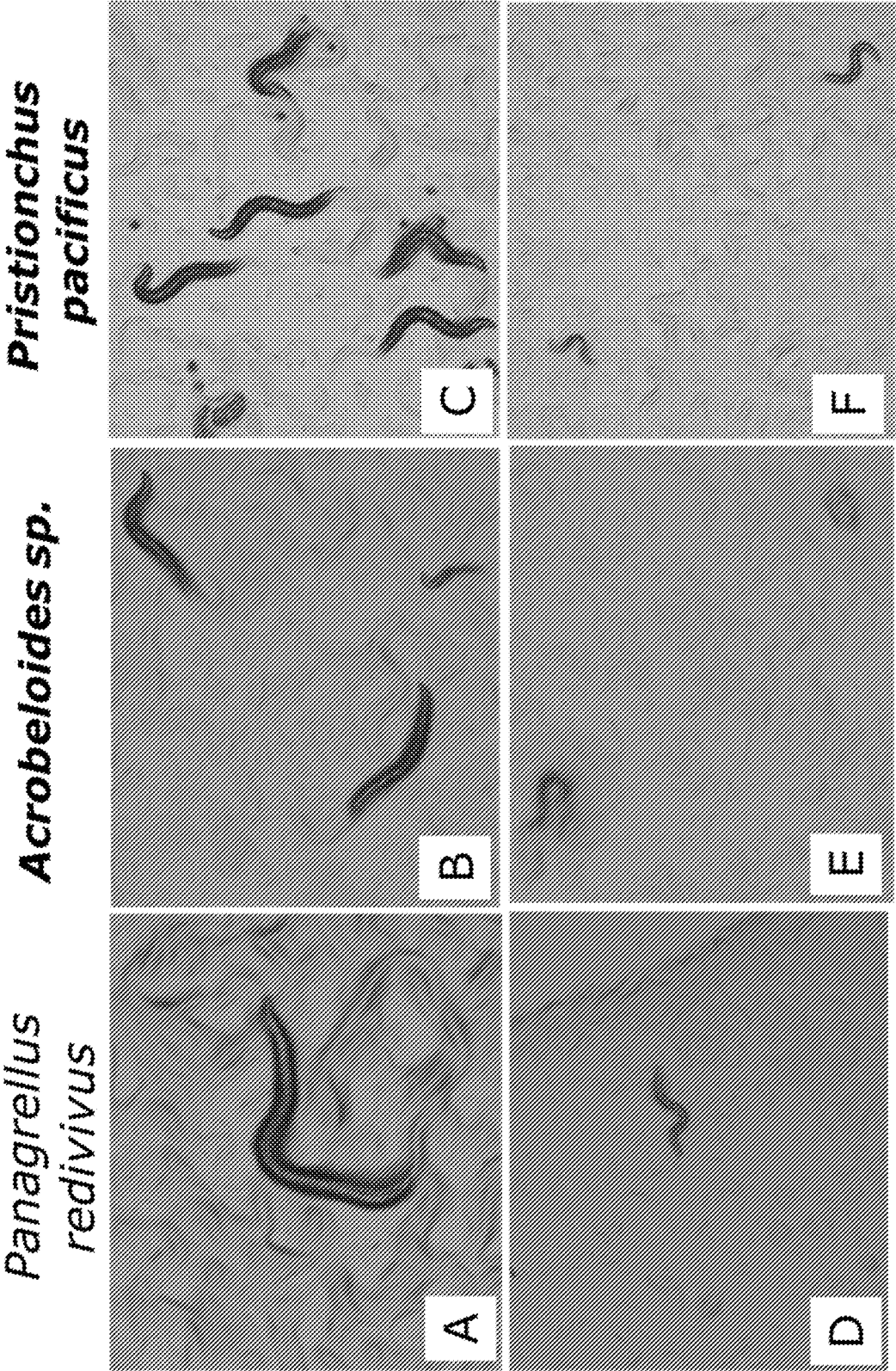


Figure 5

Pp-ANP-1	(1)	1	MR IL VFF I VV	GLVNGGVAAAP V	NCP	GSYSGVHLRYVGYRES	GDYITFA	60
Pp-ANP-3	(1)		MR VFF I	GLVNGGVAAAP	NCP	GSYSGVHLRYVGYRES	GDYITFA	KEDNG
Pp-ANP-5	(1)		MR VFF I	GLVNGGVAAAP	NCP	GSYSGVHLRYVGYRES	GDYITFA	KEDNG
Pp-ANP-1	(61)	61	ALKSS EYWLSNYK D	WAKA	YSGALTALMAGSSLW	INC	SGKA	DQLYIYAY
Pp-ANP-3	(61)		ALKSS EYWLSNYK D	WAKA	YSGALTALMAGSSLW	INC	SGKA	DQLYIYAY
Pp-ANP-5	(61)		ALKSS EYWLSNYK D	WAKA	YSGALTALMAGSSLW	INC	SGKA	DQLYIYAY
Pp-ANP-2	(1)	1	MNMV RFFVFPLI	VSE CS	ADNLPNVYRAVGL	LDTP	PAA	TKKAGGFFPKGMDGSRPNC
Pp-ANP-4	(1)		--M RFFVFPLI	VSE S	ADNLPNVYRAVGL	LDTP	PAA	TKKAGGFFPKGMDGSRPNC
Pp-ANP-6	(1)		---M RFFVFPLI	VSE S	ADNLPNVYRAVGL	LDTP	PAA	TKKAGGFFPKGMDGSRPNC
Pp-ANP-2	(61)	61	PPPNINLWNHTNGAETGLARYDSGYVATTTLR		ALDWLGTHLGNNGYIYHIRPT	PNFID		120
Pp-ANP-4	(59)		PPPNINLWNHTNGAETGLARYDSGYVATTTLR		ALDWLGTHLGNNGYIYHIRPT	PNFID		
Pp-ANP-6	(59)		PPPNINLWNHTNGAETGLARYDSGYVATTTLR		ALDWLGTHLGNNGYIYHIRPT	PNFID		
Pp-ANP-2	(121)	121	VNNTL RYSPHPAEHEAASLGMIHWDQ		VGWERVEYGRVHPFENN	RDYNAQRYGGIVQGI		180
Pp-ANP-4	(119)		VNNTL RYSPHPAEHEAASLGMIHWDQ		VGWERVEYGRVHPFENN	RDYNAQRYGGIVQGI		
Pp-ANP-6	(119)		VNNTL RYSPHPAEHEAASLGMIHWDQ		VGWERVEYGRVHPFENN	RDYNAQRYGGIVQGI		
Pp-ANP-2	(181)	181	PNVQPLAGFPAGHVAWQ	EPWVAFANC	STSSSAYALS	SSSCSPKESSES	YGEEFFYK	240
Pp-ANP-4	(179)		PNVQPLAGFPAGHVAWQ	EPWVAFANC	STSSSAYALS	SSSCSPKESSES	YGEEFFYK	
Pp-ANP-6	(179)		PNVQPLAGFPAGHVAWQ	EPWVAFANC	STSSSAYALS	SSSCSPKESSES	YGEEFFYK	
Pp-ANP-2	(241)	241	QNIGRWLPAAISPILLDN					258
Pp-ANP-4	(239)		QNIGRWLPAAISPILLDN					
Pp-ANP-6	(239)		QNIGRWLPAAISPILLDN					

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/012737

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
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2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

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A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/82 C07K14/21
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2004/248304 A1 (HU XU [US] ET AL) 9 December 2004 (2004-12-09) -----	1-14
A	US 2013/031667 A1 (GRUVER STEVEN D [US] ET AL) 31 January 2013 (2013-01-31) -----	1-14
A	US 2013/029844 A1 (ALTIER DANIEL J [US] ET AL) 31 January 2013 (2013-01-31) -----	1-14
A	JOYCE E LOPER ET AL: "Genomic analysis of antifungal metabolite production by <i>Pseudomonas fluorescens</i> Pf-5", EUROPEAN JOURNAL OF PLANT PATHOLOGY, vol. 119, no. 3, 5 July 2007 (2007-07-05), pages 265-278, XP019550794, KLUWER ACADEMIC PUBLISHERS, DO ISSN: 1573-8469, DOI: 10.1007/S10658-007-9179-8 ----- -/-	1-14



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

27 March 2018

Date of mailing of the international search report

16/04/2018

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Kania, Thomas

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/012737

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PETER BURLINSON ET AL: "Pseudomonas fluorescens NZ17 repels grazing by C. elegans, a natural predator", THE I S M E JOURNAL: MULTIDISCIPLINARY JOURNAL OF MICROBIAL ECOLOGY, vol. 7, no. 6, 21 February 2013 (2013-02-21), pages 1126-1138, XP055462738, United Kingdom ISSN: 1751-7362, DOI: 10.1038/ismej.2013.9 -----	1-14
A	DATABASE Protein [Online] 7 August 2015 (2015-08-07), "Heat-labile enterotoxin alpha chain domain-containing protein", XP002779580, retrieved from NCBI Database accession no. WP 050499198 55% identity to present SEQ ID NO: 6, 13 and 15; abstract -----	9,10
X,P	JUN-ZHI WEI ET AL: "An Enterotoxin-Like Binary Protein from Pseudomonas protegens with Potent Nematicidal Activity", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 83, no. 19, 1 October 2017 (2017-10-01), pages e00942-17, XP055462746, US ISSN: 0099-2240, DOI: 10.1128/AEM.00942-17 the whole document -----	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2018/012737

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004248304	A1	09-12-2004	NONE
US 2013031667	A1	31-01-2013	NONE
US 2013029844	A1	31-01-2013	NONE