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(54) Title: USE OF CRY 14 FOR THE CONTROL OF NEMATODE PESTS

(57) Abstract: Compositions and methods for conferring nematicidal activity to bacteria, plants, plant cells, tissues and seeds are provided. In particular, methods for killing or controlling a nematode pest population, particularly a Pratylenchus spp., e.g., Pratylenchus brachyurus population, are provided. The methods include contacting the nematode pest with a pesticidally-effective amount of a polypeptide comprising a nematicidal toxin, particularly a nematicidal toxin active against a Pratylenchus spp. nematode, e.g. Pratylenchus brachyurus. Further included are methods for increasing yield in plants by expressing the toxin of the invention.

USE OF **CRY14** FOR THE CONTROL OF NEMATODE PESTS

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Serial No. 62/438,420, filed December 22, 2016, the contents of which are herein incorporated by reference in their entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named "APA16-6020WOSEQLIST.txt", created on October 3, 2017, and having a size of 40 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 OF THE INVENTION

This invention relates to the field of molecular biology. Provided are methods for the control of nematode pests using Cry14.

BACKGROUND OF THE INVENTION

Nematodes are active, flexible, elongate, organisms that live on rnoist surfaces or in liquid environments, including films of water within soil and moist tissues within other organisms. Many species of nematodes have evolved to be very successful parasites of plants and animals and are responsible for significant economic losses in agriculture and livestock and for morbidity and mortality in humans (Whitehead (1998) Plant Nematode Control. CAB International, New York).

It is estimated that parasitic nematodes cost the horticulture and agriculture industries in excess of $78 billion worldwide a year, based on an estimated average 12% annual loss spread across all major crops. For example, it is estimated that nematodes cause soybean losses of approximately $3.2 billion annually worldwide (Barker et ai. (1994) Plant and Soil Nematodes: Societal Impact and Focus for the Future. The Committee on National Needs and Priorities in Nematology. Cooperative State Research Service, US Department of Agriculture

and Society of Nematologists). In the high-acreage crop markets, nematode damage is greatest in soybeans and cotton. There are however, dozens of additional crops that suffer from significant nematode infestation including potato, pepper, onion, citrus, coffee, sugarcane, greenhouse ornamentals and golf course turf grasses.

Nematodes are known to affect the yield, growth, and health of crops and plants. The physiological changes in the host plant's roots caused by larvae and/or adult nematodes can lead to the formation of galls, which causes a disruption of the vascular system of the plant's roots. Root elongation can stop completely and inadequate supply of water and nutrients provided by the reduced root system can result, causing foliage chlorosis and/or wilt, as well as stunting of growth, any of which can result in low yield or death. In addition, nematodes can cause physiological effects leading to an increase in the susceptibility of plant roots to bacteria and/or fungi attack, including bacteria and/or fungi the plant would otherwise resist. Such attack can lead to extensive secondary decay and rotting.

The root lesion nematode *Pratylenchus brachyurus* has become an increasingly important pathogen of soybean. It has a broad host range and is widely distributed in tropical and subtropical regions, especially in Brazil, Africa, and the Southern United States. *Pratylenchus brachyurus* has become a concern among cotton and soybean growers in the Brazilian Cen-ado region and is considered the main nematode pathogen of soybean in the region. In soybean, this nematode can reduce yields 30 to 50%, with greater damage being observed on sandy soils. There are currently no *P. brachyurus*-resistant soybean varieties identified to date. Although several soybean genotypes have been studied for *Pratylenchus brachyurus* resistance, and some cultivars identified with increased tolerance, breeding resistant cultivars against *P.brachyurus* is difficult due to the fact that this nematode is polyphagous and lacks a close interaction with its hosts (Machado (2014) Current Agricultural Science and Technology 20:26-35; Antonio et al. (2012) Soil productivity losses in area infested by the nematoid of the root lesions in Vera, MT. In: Brazilian Congress of Soy, 6, 2012, Cuiaba. Abstracts. Londrina: Embrapa Soja, 4pp; Rios et al. (2016) Ciencia Rural 46:580-584; Lima et al., 2017, Chapter 6 in the book: Soybean - The Basis of Yield, Biomass and Productivity; Edited by Minobu Kasai, ISBN 978-953-51-31 18-2, Print ISBN 978-953-51-3117-5, InTech; Inornoio et al. (2011) Sucessao de culturas sob pivo central para controle de fitonematoides: variaçao populacional, patogenicidade e estimativa de perdas. Tropical Plant Pathology 36: 178-1 85).

Methods for controlling infestations by nematodes have been provided in several forms. Biological and cultural control methods, including plant quarantines, have been attempted in numerous instances. Genetic resistance to certain nematodes is available in some commercial cultivars (e.g., soybeans), but these are restricted in number and the availability of cultivars with both desirable agronomic features and resistance is limited. Furthermore, the production of nematode resistant commercial varieties by conventional plant breeding based on genetic recombination through sexual crosses is a slow process and is often further hampered by a lack of appropriate germplasm.

Chemical means of controlling plant parasitic nematodes continue to be essential for many crops which lack adequate natural resistance. However, chemical agents are often not selective, and some exert their effects on non-target organisms, effectively disrupting populations of beneficial microorganisms, for a period of time following application of the agent. Chemical agents may persist in the environment and only be slowly metabolized.

Thus, there exists a need for additional means for controlling nematode populations in agriculturally-important plants.

SUMMARY OF INVENTION

Compositions and methods for conferring nematicidal activity' to plants, plant cells, tissues and seeds are provided. In particular, methods for killing or controlling a nematode pest population, particularly a lesion nematode such as Pratylenchus sp, e.g., *Pratylenchus hrachyurus,* population, are provided. The invention further provides control of root knot nematode *{Meloidogyne* spp. soybean pest nematodes, including but not limited to *Meloidogyne incognita, Meloidogyne arenaria, Meloidogyne hapla,* or *Meloidogyne javanica,* or any combination thereof), reniform nematode *(Rotylenchulus reniformis)* and Lance nematode *(Hopiolaimus* spp. such as *H. columbus, H. galeatus,* and *H. magmstylus).* The methods comprise contacting the nematode pest with a pesticidally-effective amount of a polypeptide comprising a nematicidal toxin, particularly a nematicidal toxin active against a Pratylenchus spp. nematode, e.g. *Pratylenchus hrachyurus,* a root knot nematode, a reniform nematode, or a Lance nematode. In various embodiments, the nematicidal toxin comprises the amino acid sequence of SEQ ID NO: 1 or 2, or pesticidally-effective variants or fragments thereof. In some embodiments, the method for protecting a plant or cell thereof from a nematode pest population, particularly a Pratylenchus spp. nematode, e.g. *Pratylenchus hrachyurus,* a root knot nematode, a reniform nematode, or a Lance nematode, comprises

expressing in a plant or cell thereof a nucleic acid encoding SEQ ID NO: 1 or 2, or a variant or fragment thereof, wherein the nucleic acid is operably linked to a promoter capable of directing expression of the nucleic acid in a plant cell.

Further comprised are methods for increasing yield in a plant comprising growing in a field a plant or a seed thereof having stably incorporated into its genome a DNA construct comprising a nucleic acid operably linked to a promoter capable of directing expression of the nucleic acid in a plant cell, wherein the nucleic acid encodes SEQ ID NO: 1 or 2, or a pesticidally-effective variant or fragment thereof.

The compositions and methods of the invention are useful for the production of organisms with enhanced nematode, e.g. Pratyienchus spp., root knot nematode, reniform nematode, or Lance nematode resistance or tolerance. These organisms and compositions comprising the organisms are desirable for agricultural purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. *Pratyienchus* resistance greenhouse assay in the USA

Elite soybean plants with EE-GM4 control *Pratyienchus brachyurus* in US greenhouse assays. Soybean plants expressing SEQ ID NO:2 ("EE-GM4") were compared to other elite soybean lines: one SCN susceptible Maturity Group (MG) 3 line ("THORNE"), one MG3 SCN susceptible line, one MG 6.2 SCN susceptible line and one MG9 SCN susceptible line ("Susc WT" shows the average for these 3 lines), one MG3 SCN resistant line (with the rhgl resistance allele from PI88788, "SCN Res (PI88788)"), and one MG 6.2 SCN resistant line with the rhgl and Rhg4 SCN resistance from Peking ("SCN Res (Peking)"). Plotted are the average numbers of *Pratyienchus* in roots 30 days after infestation (5 plants per entiy), also showing the variation observed across variaties (as typically seen in greenhouse assays). Results show ~85% control of Pratyienchus across EE-GM4 lines. Soybean lines with native SCN resistance (from Peking or PI88788) do not control *Pratyienchus brachyurus*.

Figure 2. *Pratyienchus* resistance greenhouse assay in Brazil

Soybean plants with EE-GM4 ("EE-GM4") significantly reduce *Pratyienchus brachyurus* in soybean roots. *Pratyienchus brachyurus* were isolated from local fields in Brazil. EE-GM4 plants (in two different US elite lines (both maturity group 6.2, one SCN-susceptible and one with Peking SCN-resistance ("EE-GM4")) and five Brazilian soybean lines, with limited *Pratyienchus* control ("Brazil lines"), one Brazilian line, labeled as low Rf (reproductive

factor) for *Pratylenchus* ("BRS 7380 (low Rf)"), one US elite line (maturity group 6.2) that is SCN-susceptible ("SCN Susc") and one US elite line of MG 6.2 with Peking SCN-resistance ("SCN Res (Peking)") were evaluated for *Pratylenchus* control in a greenhouse assay in Brazil. Plotted are the averages of those entries, also showing the variation observed across varieties (as typically seen in greenhouse assays). One Brazilian soybean line (BRS 7380), showed ~ 89% reduction of *Pratylenchus*. EE-GM4 lines gave ~79% control of *Pratylenchus*. Soybean lines that carry Peking native resistance to SCN do not control *Pratylenchus brachyiirus.*

DETAILED DESCRIPTION

The present invention is drawn to methods for regulating nematode resistance in organisms, particularly plants or plant cells. By "resistance" is intended that the nematode is killed upon ingestion or other contact with the polypeptides of the invention is impaired in the movement, feeding, reproduction, or other functions of the nematode. Controlling plant-parasitic nematode populations in a plant or seed thereof will improve nodulation, germination, root development, emergence, and health, including resistance to or protection from disease, including bacterial or fungal disease, which is an important benefit of methods disclosed and described herein. Thus, methods as described herein are useful for controlling nematode populations, particularly Pratylenchus spp. nematode populations, e.g., Pratylenchus bracliyurus, root knot nematode, reniform nematode, or Lance nematode, which provide improved general plant health, nutrition and/or improved agronomical benefit of a plant and/or seed. Any benefit related to nematode population control, such as, for example, reduction in total number/area of nematodes, reduction in nematode eggs/area, or reduction in damage to the plant, can be an agronomical benefit of the present invention. Secondary-benefits of controlling the nematode populations include, without limitation, improved root development (e.g., improved root or root hair growth), improved yield, faster emergence, improved plant stress management including increased stress tolerance and/or improved recovery from stress, increased mechanical strength, improved drought resistance, reduced fungal disease infection, and improved plant health. Combinations of any of these benefits can also be obtained.

The methods of the present invention involve transformation of organisms or use of organisms comprising a heterologous nucleotide sequence encoding a nematicidal protein of the invention. The methods described herein are useful for controlling or killing nematode

pest populations and for producing compositions with nematicidai activity against nematode pests.

By "pesticidal toxin" or "pestieidal protein," or '"nematicidai activity" or '"nematicidai toxin" is intended a toxin that has activity against one or more nematode pests, including, but not limited to, Pratylenchus spp., including *Pralylenchus alleni, Pratylenchus brachyurus, Pratyienchus coffeae, Pratylenchus crenatus, Pratylenchus dulscus, Pratylenchus fallax, Pratylenchus flakkensis, Pratylenchus goodeyi, Pratyienchus hexincisus, Pratylenchus loosi, Pratylenchus minutus, Pratylenchus rnulchandi, Pratylenchus musicola, Pratylenchus neglectus, Pratyienchus penetrans, Pratyienchus pratensis, Pratylenchus reniformia, Pratylenchus scribneri, Pratylenchus thornei, Pratylenchus vulnus,* and *Pratylenchus zeae.* Nematicidai proteins include amino acid sequences deduced from the full-length nucleotide sequences disclosed herein, and amino acid sequences that are shorter than the full-length sequences, either due to the use of an alternate downstream start site, or due to processing that produces a shorter protein having nematicidai activity. Processing may occur in the organism the protein is expressed in, or in the pest after ingestion of the protein.

In specific embodiments, the nematicidai protein comprises a Cry 14 protein. In various embodiments, the Cry 14 protein is Cryl4Aal (GENBANK accession number AAA21516) or Ctyl4Abl (GENBANK accession number KC 156652). In some embodiments, the Cry 14Aal protein encompasses the amino acid sequence set forth in SEQ ID NO: 1, as well as variants and fragments thereof. In other embodiments, the Cryl4Abl protein encompasses the amino acid sequence set forth in SEQ ID NO:2, as well as variants and fragments thereof. Exemplary nucleotide sequences encoding SEQ ID NO: 1 are set forth in SEQ ID NO:3 and 5. Exemplary nucleotide sequences encoding SEQ ID NO:2 are set forth in SEQ ID NO: 4 and 6.

Thus, provided herein are methods for killing or controlling a nematode pest population, e.g. a Pratylenchus spp. population, e.g., Pratylenchus brachyurus, root knot nematode, reniform nematode, or Lance nematode, comprising contacting the nematode pest, or exposing the nematode pest to, a composition comprising the nematicidai toxin of the invention. In specific embodiments, the nematicidai protein comprises the Cry 14 protein set forth in SEQ ID NO: 1 or 2, as well as variants and fragments thereof.

Isolated Nucleic Acid Molecules, and Variants and Fragments Thereof

One aspect of the invention pertains to isolated, recombinant or chimeric nucleic acid molecules comprising nucleotide sequences encoding nematicidai proteins and polypeptides

or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify nucleic acid molecules encoding proteins with regions of sequence homology. Also encompassed herein are nucleotide sequences capable of hybridizing to the nucleotide sequences of the invention under stringent conditions as defined elsewhere herein. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., recombinant DNA, cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. The term "recombinant" encompasses polynucleotides or polypeptides that have been manipulated with respect to the native polynucleotide or polypeptide, such that the polynucleotide or polypeptide differs (e.g., in chemical composition or structure) from what is occurring in nature. An "isolated nucleic acid (sequence/molecule)" or "isolated DNA (sequence/molecule)", as used herein, refers to a nucleic acid or DNA (sequence/molecule) which is no longer in the natural environment it was isolated from, e.g., the nucleic acid sequence in another bacterial host or in a plant genome, or a nucleic acid or DNA (sequence/molecule) fused to DNA or nucleic acid (sequence/molecule) from another origin, such as when contained in a chimeric gene under the control of a (heterologous) plant-expressible promoter. Any nucleic acid or DNA of this invention, including any primer, can also be non-naturally-occurring, such as a nucleic acid or DNA with a sequence identical to a sequence occurring in nature, but having a label (missing from the naturally-occurring counterpart), or with a sequence having at least one nucleotide addition or replacement or at least one internal nucleotide deletion compared to a naturally-existing nucleotide, or with a sequence having a sequence identity below 100 % (not identical) to a naturally-existing nucleic acid or DNA or a fragment thereof, or a nucleic acid or DNA with a sequence consisting of nucleotide sequences from different origins that do not occur together in nature (a chimeric or hybrid DNA), or a man-made synthetic nucleic acid or DNA with a sequence different from the natural nucleic acid or DNA or a fragment thereof.

An isolated, recombinant or chimeric nucleic acid (or DNA) is used herein to refer to a nucleic acid (or DNA) that is no longer in its natural environment, for example in an *in vitro* or in a recombinant bacterial or plant host cell. In some embodiments, an isolated, recombinant or chimeric nucleic acid is free of sequences (preferably 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 purposes of the invention, "isolated" when used to refer to nucleic acid molecules

excludes isolated chromosomes. For example, in various embodiments, the isolated delta-endotoxin encoding nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the ceil from which the nucleic acid is derived. In various embodiments, a delta-endotoxin 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 non-delta-endotoxin protein (also referred to herein as a "contaminating protein"). In some embodiments, the recombinant nucleic acid of the invention comprises one or more nucleotide substitutions relative to SEQ ID NO:3-6, or a variant or fragment thereof.

Nucleotide sequences encoding the proteins of the present invention include the sequence set forth in SEQ ID NO:3-6, and variants, fragments, and complements thereof. By "complement" is intended a nucleotide sequence that is sufficiently complementary to a given nucleotide sequence such that it can hybridize to the given nucleotide sequence to thereby form a stable duplex. The corresponding amino acid sequences for the nematicidal proteins encoded by these nucleotide sequences are set forth in SEQ ID NO: 1 and 2.

Nucleic acid molecules that are fragments of these nucleotide sequences encoding nematicidal proteins are also encompassed by the present invention. By "fragment" is intended a portion of the nucleotide sequence encoding a nematicidal protein. 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. Nucleic acid molecules that are fragments of a nucleotide sequence encoding a nematicidal protein comprise at least about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1350, 1400 contíguous nucleotides, or up to the number of nucleotides present in a full-length nucleotide sequence encoding a nematicidal protein disclosed herein, depending upon the intended use. By "contiguous" nucleotides is intended nucleotide residues that are immediately adjacent to one another. Fragments of the nucleotide sequences of the present invention will encode protein fragments that retain the biological activity of the nematicidal protein and, hence, retain pesticidal activity against a nematode pest. Thus, biologically-active fragments of the polypeptides disclosed herein are also encompassed. By "retains activity" is intended that the fragment will have at least about 30%, at least about 50%, at least about 70%, 80%, 90%, 95% or higher of the pesticidal activity of the nematicidal protein. Methods for measuring nematicidal activity are well known in the art and are also described herein.

A fragment of a nucleotide sequence encoding a nematicidal protein that encodes a biologically active portion of a protein of the invention will encode at least about 15, 25, 30, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, or 1150 contiguous amino acids, or up to the total number of amino acids present in a full-length nematicidal protein of the invention. In some embodiments, the fragment is a proteolytic cleavage fragment. For example, the proteolytic cleavage fragment may have an N-terminal or a C-terminal truncation of at least about 30 amino acids, at least about 40 amino acids, at least about 50, at least about 100 amino acids, about 120, about 130, about 140, about 150, or about 160 amino acids relative to SEQ ID NO: 1 or 2. In some embodiments, the fragments encompassed herein result from the removal of the C-terminal crystallization domain, e.g., by proteolysis, or by insertion of a stop codon in the coding sequence. In further embodiments, the fragments encompassed herein comprise an N-terminal truncation and the N-terminal truncations may comprise a methionine residue at the truncated N-tenninus.

In various embodiments, the nucleic acid of the invention comprises a degenerate nucleic acid of SEQ ID NQ:3-6, wherein said degenerate nucleotide sequence encodes the same ammo acid sequence as SEQ ID NO: 1 or 2.

Preferred nematicidal proteins of the present invention are encoded by a nucleotide sequence sufficiently identical to the nucleotide sequence of SEQ ID NO:3-6, or the nematicidal proteins are sufficiently identical to the amino acid sequence set forth in SEQ ID NO: 1 or 2. By "sufficiently identical" is intended an amino acid or nucleotide sequence that has at least about 60% or 65% sequence identity, about 70% or 75% sequence identity, about 80% or 85% sequence identity, about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity compared to a reference sequence using one of the alignment programs described herein using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent identity = number of identical positions/total number of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are the same length. In another embodiment, the percent identity is calculated across the entirety of the

reference sequence (i.e., the sequence disclosed herein as any of SEQ ID NO: 1-6). The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent identity, typically exact matches are counted. A gap, i.e. a position in an alignment where a residue is present in one sequence but not in the other, is regarded as a position with non-identical residues.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A nonlimiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the BLASTN and BLASTX programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to pesticidai-Iike nucleic acid molecules of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to nematicidal protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra.* When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., BLASTX and BLASTN) can be used. Alignment may also be performed manually by inspection.

Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the ClustaiW algorithm (Higgins *et al.* (1994) *Nucleic Adds Res.* 22:4673-4680). ClustaiW compares sequences and aligns the entirety of the amino acid or DNA sequence, and thus can provide data about the sequence conservation of the entire amino acid sequence. The ClustaiW algorithm is used in several commercially available DNA/amino acid analysis software packages, such as the ALIGNX module of the Vector NTI Program Suite (Invitrogen Corporation, Carlsbad, CA). After alignment of amino acid sequences with ClustaiW, the percent amino acid identity can be assessed. A non-limiting example of a software program useful for analysis of ClustaiW alignments is GENEDOC™. GENEDOC™ (Karl Nicholas) allows assessment of amino acid (or DNA) similarity and identity between multiple proteins. Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program

(version 2.0), which is part of the GCG Wisconsin Genetics Software Package, Version 10 (available from Aecelrys, Inc., 9685 Scranton Rd., San Diego, CA, USA). When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Unless otherwise stated, GAP Version 10, which uses the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol* 48(3):443-453, will be used to determine sequence identity or similarity 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 or % similarity for an amino acid sequence using GAP weight of 8 and length weight of *2*, and the BLOSUM62 scoring program. Equivalent programs may also be used. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

The invention also encompasses variant nucleic acid molecules. "Variants" of the nematicidal protein encoding nucleotide sequences include those sequences that encode the nematicidal proteins disclosed herein but that differ conservatively because of the degeneracy of the genetic code as well as those that are sufficiently identical as discussed above. Naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the nematicidal proteins disclosed in the present invention as discussed below. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, pesticidal activity against a nematode pest. By "retains activity" is intended that the variant will have at least about 30%, at least about 50%, at least about 70%, or at least about 80% of the pesticidal activity of the native protein. Methods for measuring pesticidal activity against a nematode pest are well known in the art and described elsewhere herein.

The skilled artisan will further appreciate that changes can be introduced by mutation of the nucleotide sequences of the invention thereby leading to changes in the amino acid sequence of the encoded nematicidal proteins, without altering the biological activity of the proteins. Thus, variant isolated nucleic acid molecules can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide

sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention.

For example, conservative amino acid substitutions may be made at one or more, predicted, nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a nematicidal protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

Delta-endotoxins generally have five conserved sequence domains, and three conserved structural domains (see, for example, de Maagd *etal.* (2001) *Trends Genetics* 17: 193-199). The first conserved structural domain consists of seven alpha helices and is involved in membrane insertion and pore formation. Domain II consists of three beta-sheets arranged in a Greek key configuration, and domain III consists of two antiparallel beta-sheets in "jelly-roll" formation (de Maagd *el al.,* 2001, supra). Domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity.

Amino acid substitutions may be made in nonconserved regions that retain function. In general, such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif, where such residues are essential for protein activity. Examples of residues that are conserved and that may be essential for protein activity include, for example, residues that are identical between all proteins contained in an alignment of similar or related toxins to the sequences of the invention (e.g., residues that are identical in an alignment of homologous proteins). Examples of residues that are conserved but that may allow conservative amino acid substitutions and still retain activity include, for example, residues that have only conservative substitutions between all

proteins contained in an alignment of similar or related toxins to the sequences of the invention (e.g., residues that have only conservative substitutions between all proteins contained in the alignment homologous proteins). However, one of skill in the art would understand that functional variants may have minor conserved or nonconserved alterations in the conserved residues.

Alternatively, variant nucleotide sequences can be made by introducing mutations randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for ability to confer pesticidal activity against a nematode pest to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinant!}, and the activity of the protein can be determined using standard assay techniques.

Using methods such as PCR, hybridization, and the like corresponding pesticidal sequences can be identified, such sequences having substantial identity to the sequences of the invention (e.g., at least about 70%, at least about 75%, 80%, 85%, 90%, 95% or more sequence identity across the entirety of the reference sequence) and having or conferring pesticidal activity against a nematode pest. See, for example, Sambrook and Russell (2001) *Molecular Cloning: A Laboratory Manual.* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) and Innis, *et al.* (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, NY).

In a hybridization method, all or part of the pesticidal nucleotide sequence can be used to screen cDNA or genomic libraries. Methods for construction of such cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook and Russell, 2001, *supra.* The so-called hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P, or any other detectable marker, such as other radioisotopes, a fluorescent compound, an enzyme, or an enzyme co-factor. Probes for hybridization can be made by labeling synthetic oligonucleotides based on the known nematicidal protein-encoding nucleotide sequence disclosed herein. Degenerate primers designed on the basis of conserved nucleotides or amino acid residues in the nucleotide sequence or encoded amino acid sequence can additionally be used. The probe typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, at least about 25, at least about 50, 75, 100, 125, 150, 175, or 200 consecutive nucleotides of nucleotide sequence encoding a nematicidal protein of the invention or a fragment or variant thereof. Methods for

the preparation of probes for hybridization are generally known in the art and are disclosed in Sanibrook and Russell, 2001, *supra* herein incorporated by reference.

For example, an entire nematicidal sequence disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding nernaticidal protein-like sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique and are preferably at least about 10 nucleotides in length, or at least about 20 nucleotides in length. Such probes may be used to amplify corresponding pesticidal sequences from a chosen organism or sample 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, Sarnbrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Thus, the present invention encompasses probes for hybridization, as well as nucleotide sequences capable of hybridization to all or a portion of a nucleotide sequence of the invention (e.g., at least about 300 nucleotides, at least about 400, at least about 400, 450, 500, 1000, 1200, 1500, 2000, 2500, 3000, 3500, or up to the full length of a nucleotide sequence disclosed herein). 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 in 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, preferably 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 and the temperature is at 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. Exemplar}' low stringency conditions include hybridization with a buffer

solution of 30 to 35% fomiamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M tnsodmm 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. Exemplar}' high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. 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 the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the Tm can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138: 267-284: T_m = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% forrn) - 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 thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (Tm); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *laboratory Techniques in Biochemistry and Molecular Biology— Hybridization with Nucleic Acid Probes,* Part **I,** Chapter 2 (Elsevier, New York); and Ausubel *el ciL* , eds. (1995) *Current Protocols in Molecular Biology,* Chapter 2 (Greene Publishing and

Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Isolated Proteins and Variants and Fragments Thereof

Nematicidal proteins are also encompassed within the present invention. By "nematicidal protein" is intended a protein having the amino acid sequence set forth in SEQ ID NO: 1 or 2. Fragments, biologically active portions, and variants thereof are also provided, and may be used to practice the methods of the present invention. An "isolated protein" or a "recombinant protein" is used to refer to a protein that is no longer in its natural environment, for example *in vitro* or in a recombinant bacterial or plant host cell. In some embodiments, the recombinant protein is a variant of SEQ ID NO: 1 or 2, wherein the variant comprises at least one amino acid substitution, deletion, or insertion relative to SEQ ID NO: 1 or 2.

"Fragments" or "biologically active portions" include polypeptide fragments comprising amino acid sequences sufficiently identical to the amino acid sequence set forth in SEQ ID NO: 1 or 2, and that exhibit pesticidal activity against a nematode pest. A biologically active portion of a nematicidal protein can be a polypeptide that is, for example, 10, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, or more amino acids in length. Such biologically active portions can be prepared by recombinant tectmiques and evaluated for pesticidal activity against a nematode pest. Methods for measuring pesticidal activity against a nematode pest are well known in the art (see, for example, US Patent Application Publication No. US 20160066584) and described elsewhere herein. As used here, a fragment comprises at least 8 contíguous amino acids of SEQ ID NO: 1 or 2. The invention encompasses other fragments, however, such as any fragment in the protein greater than about 10, 20, 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350 or more amino acids in length.

By "variants" is intended proteins or polypeptides having an amino acid sequence that is at least about 60%, 65%, about 70%, 75%, about 80%, 85%, about 90%, 91%, 92%, 93%, 94%, 95%, 96%>, 97%, 98% or 99% identical to the amino acid sequence of any of SEQ ID NO: 1 or 2. Variants also include polypeptides encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of SEQ ID NO:3-6, or a complement thereof, under stringent conditions. Variants include polypeptides that differ in amino acid sequence due to

mutagenesis. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, retaining pesticidal activity against a nematode pest. In some embodiments, the variants have improved activity relative to the native protein. Methods for measuring pesticidal activity against a nematode pest are well known in the art (see, for example, US Patent Application Publication No. US 20160066584) and described elsewhere herein.

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

In various embodiments of the present invention, nematicidal proteins include amino acid sequences deduced from the full-length nucleotide sequences disclosed herein, and amino acid sequences that are shorter than the full-length sequences due to the use of an alternate downstream start site.

Antibodies to the polypeptides of the present invention, or to variants or fragments thereof, are also encompassed. Methods for producing antibodies are well known in the art (see, for example, Harlow and Lane (1988) *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; U.S. Patent No. 4,196,265).

Thus, one aspect of the invention concerns antibodies, single-chain antigen binding molecules, or other proteins that specifically bind to one or more of the protein or peptide molecules of the invention and their homoiogs, fusions or fragments. In a particularly preferred embodiment, the antibody specifically binds to a protein having the amino acid sequence set forth in SEQ ID NO: 1 or 2 or a fragment thereof. In another embodiment, the antibody specifically binds to a fusion protein comprising an amino acid sequence selected from the amino acid sequence set forth in SEQ ID NO: 1 or 2 or a fragment thereof. In

various embodiments, the antibody that specifically binds to the protein of the invention or a fusion protein comprising the protein of the invention is a non-naturally occurring antibody.

Antibodies of the invention may be used to quantitatively or qualitatively detect the protein or peptide molecules of the invention, or to detect post translational modifications of the proteins. As used herein, an antibody or peptide is said to "specifically bind" to a protein or peptide molecule of the invention if such binding is not competitively inhibited by the presence of non-related molecules.

The antibodies of the invention may be contained within a kit useful for detection of the protein or peptide molecules of the invention. The invention further comprises a method of detecting the protein or peptide molecule of the invention (particularly a protein encoded by the amino acid sequence set forth in SEQ ID NO: 1 or 2, including variants or fragments thereof that are capable of specifically binding to the antibody of the invention) comprising contacting a sample with the antibody of the invention and determining whether the sample contains the protein or peptide molecule of the invention. Methods for utilizing antibodies for the detection of a protein or peptide of interest are known in the art.

Altered or Improved Variants

It is recognized that DNA sequences of a nematicidal protein may be altered by various methods, and that these alterations may result in DNA sequences encoding proteins with amino acid sequences different than that encoded by a nematicidal protein of the present invention. This protein may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions of one or more amino acids of SEQ ID NO: 1 or 2, including up to about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 155, or more amino acid substitutions, deletions or insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a nematicidal protein can be prepared by mutations in the DNA. This may also be accomplished by one of several forms of mutagenesis and/or in directed evolution. In some aspects, the changes encoded in the amino acid sequence will not substantially affect the function of the protein. Such variants will possess the desired pesticidal activity against a nematode pest. However, it is understood that the ability of a nematicidal protein to confer pesticidal activity against a nematode pest may be improved by the use of such techniques

upon the compositions of this invention. For example, one may express a nernaticidal protein in host cells that exhibit high rates of base misincorporation during DNA replication, such as XL-1 Red (Stratagene, La Jolla, CA). After propagation in such strains, one can isolate the DNA (for example by preparing plasmid DNA, or by amplifying by PCR and cloning the resulting PCR fragment into a vector), culture the nernaticidal protein mutations in a non-mutagenic strain, and identify mutated genes with pesticidai activity against a nematode pest, for example by performing an assay to test for pesticidai activity against a nematode pest. Such assays can include contacting plants with one or more pests and determining the plant's ability to survive and/or cause the death of the pests. See, for example, US Patent Application Publication No. US 20160066584).

Alternatively, alterations may be made to the protein sequence of many proteins at the amino or carboxy terminus without substantially affecting activity. This can include insertions, deletions, or alterations introduced by modem molecular methods, such as PCR, including PCR amplifications that alter or extend the protein coding sequence by virtue of inclusion of ammo acid encoding sequences in the oligonucleotides utilized in the PCR amplification. Alternatively, the protein sequences added can include entire protein-coding sequences, such as those used commonly in the art to generate protein fusions. Such fusion proteins are often used to (1) increase expression of a protein of interest (2) introduce a binding domain, enzymatic activity, or epitope to facilitate either protein purification, protein detection, or other experimental uses known in the art (3) target secretion or translation of a protein to a subcellular organelle, such as the periplasmic space of Gram-negative bacteria, or the endoplasmic reticulum of eukaryotic cells, the latter of which often results in glycosylation of the protein.

Variant nucleotide and amino acid sequences of the present invention also encompass sequences derived from mutagenic and recombinogenic procedures such as DNA shuffling. With such a procedure, one or more different nernaticidal protein coding regions can be used to create a new nernaticidal protein possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo.* For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between a pesticidai gene of the invention and other known pesticidai genes to obtain a new gene coding for a protein with an improved property of interest, such as an increased insecticidal activity. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad.*

Sci. USA 91:10747-10751; Stemmer (1994) *Nature* 370:389-391; Cramen *et a/*. (1997) *Nature Biotech.* 15:436-438; Moore *etal.* (1997) / *Mol. Biol.* 272:336-347; Zhang *etal. (1997) Proc. Natl. Acad. Sci. USA* 94:4504-4509; Cramen *etal.* (1998) *Nature* 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

Domain swapping or shuffling is another mechanism for generating altered nematicidal proteins. Domains may be swapped between nematicidai proteins, resulting in hybrid or chimeric toxins with improved pesticidal activity against a nematode pest or target spectrum. Methods for generating recombinant proteins and testing them for pesticidal activity against a nematode pest are well known in the art (see, for example, Naimov *et al.* (2001) *Appl. Environ. Microbiol.* 67:5328-5330; de Maagd *et al.* (1996) *Appl. Environ. Microbiol.* 62:1537-1543; Ge *etal.* (1991) *J. Biol. Chem.* 266:17954-17958; Schnepf *etal.* (1990) *J. Biol. Chem.* 265:20923-20930; Rang *ei al.* 91999) *Appl. Environ. Microbiol.* 65:2918-2925).

In yet another embodiment, variant nucleotide and/or amino acid sequences can be obtained using one or more of error-prone PCR, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis, permutational mutagenesis, synthetic ligation reassembly (SLR), recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation, and the like.

Vectors

A pesticidal sequence of the invention may be provided in an expression cassette for expression in a host cell of interest, e.g. a plant cell or a microbe. By '"plant expression cassette" is intended a DNA construct that is capable of resulting in the expression of a protein from an open reading frame in a plant cell. Typically these contain a promoter and a coding sequence. Often, such constructs will also contain a 3' untranslated region. Such constructs may contain a "signal sequence" or "leader sequence" to facilitate co-translational

or post-translational transport of the peptide to certain intracellular structures such as the chloroplast (or other plastid), endoplasmic reticulum, or Goigi apparatus.

By "signal sequence" is intended a sequence that is known or suspected to result in cotransiational or post-translational peptide transport across the cell membrane. In eukaryotes, this typically involves secretion into the Golgi apparatus, with some resulting glycosylation. Insecticidai toxins of bacteria are often synthesized as protoxins, which are protolytically activated in the gut of the target pest (Chang (1987) *Methods Enzymol.* 153:507-516). In some embodiments of the present invention, the signal sequence is located in the native sequence, or may be derived from a sequence of the invention. By "leader sequence" is intended any sequence that when translated, results in an amino acid sequence sufficient to trigger co-translational transport of the peptide chain to a subcellular organelle. Thus, this includes leader sequences targeting transport and/or glycosylation by passage into the endoplasmic reticulum, passage to vacuoles, plastids including chloroplasts, mitochondria, and the like. Thus, further provided herein is a polypeptide comprising an amino acid sequence of the present invention that is operablv linked to a heterologous leader or signal sequence.

By "plant transformation vector" is intended a DNA molecule that is necessary for efficient transformation of a plant cell. Such a molecule may consist of one or more plant expression cassettes, and may be organized into more than one "vector" DNA molecule. For example, binary vectors are plant transformation vectors that utilize two non-contiguous DNA vectors to encode all requisite cis- and trans-acting functions for transformation of plant cells (Hellens and Mullmeaux (2000) *Trends in Plant Science* 5:446-451). "Vector" refers to a nucleic acid construct designed for transfer between different host cells. "Expression vector" refers to a vector that has the ability to incorporate, integrate and express heterologous DNA sequences or fragments in a foreign cell. The cassette will include 5' and/or 3' regulatory sequences operably linked to a sequence of the invention. By "operably linked" is intended 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 nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. In some embodiments, the nucleotide sequence is operably linked to a heterologous promoter capable of directing expression of said nucleotide sequence in a host cell, such as a microbial host cell or a plant host cell. The cassette may additionally contain at least one additional gene to be cotransformed into the

organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

In various embodiments, the nucleotide sequence of the invention is operably linked to a heterologous promoter, e.g., a plant promoter. "Promoter" refers to a nucleic acid sequence that functions to direct transcription of a downstream coding sequence. The promoter together with other transcriptional and translational regulator}' nucleic acid sequences (also termed "control sequences") are necessary for the expression of a DNA sequence of interest.

Such an expression cassette is provided with a plurality of restriction sites for insertion of the pesticidal sequence to be under the transcriptional regulation of the regulatory regions.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a DNA sequence of the invention, and a translational and transcriptional termination region (i.e., termination region) functional in plants. The promoter may be native or analogous, or foreign or heterologous, to the plant host and/or to the DNA sequence of the invention. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. Where the promoter is "native" or "homologous" to the plant host, it is intended that the promoter is found in the native plant into which the promoter is introduced. Where the promoter is "foreign" or "heterologous" to the DNA sequence of the invention, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked DNA sequence of the invention. The promoter may be inducible or constitutive. It may be naturally-occurring, may be composed of portions of various naturally-occurring promoters, or may be partially or totally synthetic. Guidance for the design of promoters is provided by studies of promoter structure, such as that of Harley and Reynolds (1987) *Nucleic Acids Res.* 15:2343-2361. Also, the location of the promoter relative to the transcription start may be optimized. See, e.g., Roberts *et al.* (1979) *Proc. Natl. Acad. Sci. USA,* 76:760-764. Many suitable promoters for use in plants are well known in the art.

For instance, suitable constitutive promoters for use in plants include: the promoters from plant viruses, such as the peanut chiorotic streak caulimovirus (PC1SV) promoter (U.S. Pat. No. 5,850,019); the 35S promoter from cauliflower mosaic vims (CaMV) (Odell *et al.* (1985) *Nature* 313:810-812); the 35S promoter described in Kay *et al.* (1987) *Science* 236: 1299-1 302; promoters of Chlorella virus methyltransferase genes (U.S. Pat. No. 5,563,328) and the full-length transcript promoter from figwort mosaic virus (FMV) (U.S. Pat. No.

5,378,6 19): the promoters from such genes as rice actin (McElroy *et al.* (1990) *Plant Cell* 2:163-171 and U.S. Patent 5,641,876): ubiquitm (Christensen *et al.* (1989) *Plant Moi. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689) and Grefen *et al.*(20 10) *Plant J*, 64:355-365; pEMU (Last *et al.* (1991) *Theor. Appl. Genet.* 81:581-588): MAS (Velten *et al.* (1984) *EMBO J*. 3:2723-2730 and U.S. Patent 5,5 10,474); maize H3 histone (Lepetit *et al* (1992) *Mol. Gen. Genet.* 231:276-285 and Atanassova *et al.* (1992) *Plant J*. 2(3):291-300); Brassica napus ALS3 (PCT application W097/41228); a plant ribulose-biscarboxylase/oxygenase (RuBisCO) small subunitgene; the circovirus (AU 689 311) or the Cassava vein mosaic virus (CsVMV, US 7,053,205); promoters from soybean (Pbdc6 or Pbdc7, described in WO/2014/150449 or ubiquitin 3 promoter described in US Patent No. 7393948 and US Patent No. 8395021); and promoters of various *Agrohacterhim* genes (see U.S. Pat. Nos. 4,771,002; 5,102,796; 5,182,200; and 5,428,147).

Suitable inducible promoters for use in plants include: the promoter from the ACE1 system which responds to copper (Mett *et al.* (1993) *PNAS* 90:4567-4571); the promoter of the maize In2 gene which responds to benzenesulfonamide herbicide safeners (Hershey *et al.* (1991) *Mol. Gen. Genetics* 227:229-237 and Gal/ *et al.* (1994) *Mol. Gen. Genetics* 243:32-38); and the promoter of the Tet repressor from TnlO (Gatz *et al.* (1991) *Mol Gen. Genet.* 227:229-237). Another inducible promoter for use in plants is one that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter of this type is the inducible promoter from a steroid hormone gene, the transcriptional activity of which is induced by a glucocorticosteroid hormone (Schena *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88: 10421) or the recent application of a chimeric transcription activator, XVE, for use in an estrogen receptor-based inducible plant expression system activated by estradiol (Zuo *et al.* (2000) *Plant J.*, 24:265-273). Oilier inducible promoters for use in plants are described in EP 332104, PCT WO 93/21334 and PCT WO 97/06269 which are herein incorporated by reference in their entirety. Promoters composed of portions of other promoters and partially or totally synthetic promoters can also be used. See, e.g., Ni *et al.* (1995) *Plant J*. 7:661-676 and PCT WO 95/14098 describing such promoters for use in plants.

In one embodiment of this invention, a promoter sequence specific for particular regions or tissues of plants can be used to express the nernaticidal proteins of the invention, such as promoters specific for seeds (Datla, R. et al., 1997, Biotechnology Ann. Rev. 3, 269-296), especially the napin promoter (EP 255 378 A l), the phaseolin promoter, the glutemn promoter, the helianthinin promoter (WO92/17580), the albumin promoter (WO98/45460),

the oleosin promoter (W098/45461), the SAT1 promoter or the SAT3 promoter (PCT/US98/06978).

Use may also be made of an inducible promoter advantageously chosen from the phenylalanine ammonia lyase (PAL), HMG-CoA reductase (HMG), chitinase, glucanase, proteinase inhibitor (PI), PR1 family gene, nopaline synthase (nos) and vspB promoters (US 5 670 349, Table 3), the HMG2 promoter (US 5 670 349), the apple beta-galactosidase (ABG1) promoter and the apple aminocyclopropane carboxylate synthase (ACC synthase) promoter (W098/45445). Multiple promoters can be used in the constmcis of the invention, including in succession.

The promoter may include, or be modified to include, one or more enhancer elements. In some embodiments, the promoter may include a plurality of enhancer elements. Promoters containing enhancer elements provide for higher levels of transcription as compared to promoters that do not include them. Suitable enhancer elements for use in plants include the PC1SV enhancer element (U.S. Pat. No. 5,850,019), the CaMV 35S enhancer element (U.S. Pat. Nos. 5,106,739 and 5,164,316) and the FMV enhancer element (Mam *etal.* (1997) *Transgenic Res.* 6:143-156): the translation activator of the tobacco mosaic vims (TMV) described in Application WO87/07644, or of the tobacco etch virus (TEV) described by Carrington & Freed 1990, J. Virol. 64: 1590-1597, for example, or introns such as the adhl intron of maize or intron 1 of rice actin. See also PCT W096/23898, WO20 12/02 1794, WO2012/021797, WO201 1/084370, and WO201 1/028914.

Often, such constructs can contain 5' and 3' untranslated regions. Such constructs may contain a "signal sequence" or "leader sequence" to facilitate co-translational or post-translational transport of the peptide of interest to certain intracellular structures such as the chloroplast (or other plastid), endoplasmic reticulum, or Golgi apparatus, or to be secreted. For example, the constnict can be engineered to contain a signal peptide to facilitate transfer of the peptide to the endoplasmic reticulum. By "signal sequence" is intended a sequence that is known or suspected to result in cotranslational or post-translational peptide transport across the cell membrane. In eukaryotes, this typically involves secretion into the Golgi apparatus, with some resulting glycosylation. By "leader sequence" is intended any sequence that, when translated, results in an amino acid sequence sufficient to trigger co-translational transport of the peptide chain to a sub-cellular organelle. Tlms, this includes leader sequences targeting transport and/or glycosylation by passage into the endoplasmic reticulum, passage to vacuoles, plastids including chloroplasts, mitochondria, and the like. It

may also be preferable to engineer the plant expression cassette to contain an intron, such that mRNA processing of the intron is required for expression.

By "3' untranslated region" is intended a polynucleotide located downstream of a coding sequence. Polyadenylation signal sequences and other sequences encoding regulatory signals capable of affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor are 3' untranslated regions. By "5' untranslated region" is intended a polynucleotide located upstream of a coding sequence.

Other upstream or downstream untranslated elements include enhancers. Enhancers are polynucleotides that act to increase the expression of a promoter region. Enhancers are well known in the art and include, but are not limited to, the SV40 enhancer region and the 35S enhancer element.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous to the promoter, the DNA sequence of interest, the plant host, or any combination thereof). 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.* (199!) *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 host cell (synthetic DNA sequence). That is, the genes can be synthesized using host cell-preferred codons for improved expression, or may be synthesized using codons at a host-preferred codon usage frequency. Expression of the open reading frame of the synthetic DNA sequence in a cell results in production of the polypeptide of the invention. Synthetic DNA sequences can be useful to simply remove unwanted restriction endonuclease sites, to facilitate DNA cloning strategies, to alter or remove any potential codon bias, to alter or improve GC content, to remove or alter alternate reading frames, and/or to alter or remove intron/exon splice recognition sites, polyadenylation sites, Shine-Delgamo sequences, unwanted promoter elements and the like that may be present in a native DNA sequence. Generally, the GC content of the gene will be increased. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92: 1-1 1 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for example,

U.S. Patent Nos. 5,380,831, and 5,436,391, U.S. Patent Publication No. 20090137409, and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

It is also possible that synthetic DNA sequences may be utilized to introduce other improvements to a DNA sequence, such as introduction of an intron sequence, creation of a DNA sequence that in expressed as a protein fusion to organelle targeting sequences, such as chloroplast transit peptides, apoplast/vacuolar targeting peptides, or peptide sequences that result in retention of the resulting peptide in the endoplasmic reticulum. Thus, in one embodiment, the nematicidal protein is targeted to the chloroplast for expression. In this manner, where the nematicidal protein is not directly inserted into the chloroplast, the expression cassette will additionally contain a nucleic acid encoding a transit peptide to direct the nematicidal protein to the chloroplasts. Such transit peptides are known in the art. See, for example, Von Heijne *et al*. (1991) *Plant Mol. Biol. Rep.* 9:104-126; Clark *etal.* (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 *etal.* (1986) *Science* 233:478-481.

The pesticidal gene to be targeted to the chloroplast may be optimized for expression in the chloroplast to account for differences in codon usage between the plant nucleus and this organelle. In this manner, the nucleic acids of interest may be synthesized using chloroplast-preferred codons. See, for example, U.S. Patent No. 5,380,831, herein incorporated by reference.

Plant Transformation

Methods of the invention involve introducing a nucleotide construct into a plant. By 'Introducing" is intended to present 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 invention do not require that a particular method for introducing a nucleotide construct to a plant is used, 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.

By "plant" is intended whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, propagules, embryos and progeny of the same. Plant cells can be differentiated or undifferentiated (e.g. callus, suspension culture cells, protoplasts, leaf cells, root cells, phloem cells, pollen).

"Transgenic plants" or "transformed plants" or "stably transformed" plants or cells or tissues refers to plants that have incorporated or integrated exogenous nucleic acid sequences or DNA fragments into the plant cell. These nucleic acid sequences include those that are exogenous, or not present in the untransformed plant cell, as well as those that may be endogenous, or present in the untransformed plant cell. "Heterologous" generally refers to the nucleic acid sequences that are not endogenous to the cell or part of the native genome in which they are present, and have been added to the cell by infection, transfection, microinjection, electroporation, microprojection, or the like.

The transgenic plants of the invention express one or more of the novel toxin sequences disclosed herein. In some embodiments, the protein or nucleotide sequence of the invention is advantageously combined in plants with other genes which encode proteins or RNAs that confer useful agronomic properties to such plants. Among the genes which encode proteins or RNAs that confer useful agronomic properties on the transformed plants, mention can be made of the DNA sequences encoding proteins which confer tolerance to one or more herbicides, and others which confer tolerance to certain insects, those which confer tolerance to certain diseases, DNAs that encodes RNAs that provide nematode or insect control, and the like. Such genes are in particular described in published PCT Patent Applications WO91/02071 and WO95/06128 and in U.S. Patents 7,923,602 and US Patent Application Publication No. 20100166723, each of which is herein incorporated by reference in its entirety. In various embodiments, the transgenic plant further comprises one or more additional genes for insect resistance (e.g., Cryl, such as members of the CrylA, CrylB, Cry1C, CrylD, CryIE, and Cry1F families; Cry2, such as members of the Cry2A family; Cry9, such as members of the Cry9A, Cry9B, Cry9C, Cry9D, Cry9E, and Cry9F families; etc.). It will be understood by one of skill in the art that the transgenic plant may comprise any gene imparting an agronomic trait of interest.

Among the DNA sequences encoding proteins which confer tolerance to certain herbicides on the transformed plant cells and plants, mention can be made of a bar or PAT gene or the *Streptomyces coelicolor* gene described in WO2009/152359 which confers tolerance to glufosinate herbicides, a gene encoding a suitable EPSPS which confers tolerance to herbicides having EPSPS as a target, such as glyphosate and its salts (US 4,535,060, US 4,769,061, US 5,094,945, US 4,940,835, US 5,188,642, US 4,971,908, US 5,145,783, US 5,310,667, US 5,312,910, US 5,627,061, US 5,633,435), a gene encoding gljphosate-n-acetyltransferase (for example, US 8,222,489, US 8,088,972, US 8,044,261, US 8,021,857, US 8,008,547, US 7,999,152, US 7,998,703, US 7,863,503, US 7,714,188, US

7,709,702, US 7,666,644, US 7,666,643, US 7,531,339, US 7,527,955, and US 7,405,074), a gene encoding glyphosate oxydoreductase (for example, US 5,463,175), or a gene encoding an HPPD inhibitor-tolerant protein (for example, the HPPD inhibitor tolerance genes described in WO 2004/055191, WO 199638567, US 6791014, WO201 1/068567, WO201 1/076345, WO201 1/085221, WO201 1/094205, WO20 11/068567, WO201 1/094199, WO201 1/094205, WO201 1/145015, WO20 12/056401, and WO2014/043435).

Among the DNA sequences encoding a suitable EPSPS which confer tolerance to the herbicides which have EPSPS as a target, mention will more particularly be made of the gene which encodes a plant EPSPS, in particular maize EPSPS, particularly a maize EPSPS which comprises two mutations, particularly a mutation at amino acid position 102 and a mutation at amino acid position 106 (WO2004/074443), and which is described in Patent Apphcation US 6566587, hereinafter named double mutant maize EPSPS or 2mEPSPS, or the gene which encodes an EPSPS isolated from Agrobacterium and which is described by sequence ID No. 2 and sequence ID No. 3 of US Patent 5,633,435, also named CP4.

Among the DNA sequences encoding a suitable EPSPS which confer tolerance to the herbicides which have EPSPS as a target, mention will more particularly be made of the gene which encodes an EPSPS GRG23 from Arthrobacter globiformis, but also the mutants GRG23 ACE !, GRG23 ACE2, or GRG23 ACE3, particularly the mutants or variants of GRG23 as described in WO2008/1 00353, such as GRG23(ace3)Rl 73K of SEQ ID No. 29 in WO2008/100353.

In the case of the DNA sequences encoding EPSPS, and more particularly encoding the above genes, the sequence encoding these enzymes is advantageously preceded by a sequence encoding a transit peptide, in particular the "optimized transit peptide" described in US Patent 5,510,471 or 5,633,448.

Exemplar}, herbicide tolerance traits that can be combined with the nucleic acid sequence of the invention further include at least one ALS (acetolactate synthase) inhibitor (WO2007/024782); a mutated Arabidopsis ALS/AHAS gene (U.S. Patent 6,855,533); genes encoding 2,4-D-monooxygenases conferring tolerance to 2,4-D (2,4-dichlorophenoxyacetic acid) by metabolization (U.S. Patent 6,153,401); and, genes encoding Dicamba monooxygenases conferring tolerance to dicamba (3,6-dichloro-2-methoxybenzoic acid) by metabolization (US 2008/0119361 and US 2008/0120739).

In various embodiments, the nucleic acid of the invention is stacked with one or more herbicide tolerant genes, including one or more HPPD inhibitor herbicide tolerant genes, and/or one or more genes tolerant to glyphosate and/or glufosinate.

Among the DNA sequences encoding proteins concerning properties of tolerance to insects, mention will more particularly be made of the Bt proteins widely described in the literature and well known to those skilled in the art. Mention will also be made of proteins extracted from bacteria such as *Photorhabdus* (W097/17432 & WO98/08932).

Among such DNA sequences encoding proteins of interest which confer novel properties of tolerance to insects, mention will more particularly be made of the Bt Cry or VIP proteins widely described in the literature and well known to those skilled in the art. These include the Cry IF protein or hybrids derived from a Cry IF protein (e.g., the hybrid CrylA-CrylF proteins described in US 6,326,169; US 6,281,016: US 6,218,188, or toxic fragments thereof), the Cry 1A-type proteins or toxic fragments thereof, preferably the Cryl Ac protein or hybrids derived from the Cry 1Ac protein (e.g., the hybrid Cry lAb-Cry 1Ac protein described in US 5,880,275) or the CrylAb or Bt2 protein or insecticidai fragments thereof as described in EP451878, the Cry2Ae, Cry2Af or Cry2Ag proteins as described in WO2002/057664 or toxic fragments thereof, the Cry 1A.105 protein descnbed in WO 2007/1 40256 (SEQ ID No. 7) or a toxic fragment thereof, the VIP3 Aa19 protein of NCBI accession ABG20428, the VIP3Aa20 protein of NCBI accession ABG20429 (SEQ ID No. 2 in WO 2007/142840), the VIP3A proteins produced in the COT202 or COT203 cotton events (WO2005/054479 and WO2005/054480, respectively), the Ciy proteins as described in WO200 1/47952, the VIP3Aa protein or a toxic fragment thereof as described in Estruch et al. (1996), Proc Natl Acad Sci U S A. 28;93(1 1):5389-94 and US 6,291,156, the insecticidai proteins from *Xenorhabdus* (as described in WO98/50427), *Serratia* (particularly from *S. entomophila)* or *Photorhabdus* species strains, such as Tc-proteins from Photorhabdus as described in WO98/08932 (e.g., Waterfield et al, 2001, Appl Environ Microbiol. 67(1 1):5017-24; Ffrench-Constant and Bowen, 2000, Cell Mol Life Sci.; 57(5):828-33). Also any variants or mutants of any one of these proteins differing in some (1-10, preferably 1-5) amino acids from any of the above sequences, particularly the sequence of their toxic fragment, or which are fused to a transit peptide, such as a plastid transit peptide, or another protein or peptide, is included herein.

In various embodiments, the nucleic acid of the invention can be combined in plants with one or more genes conferring a desirable trait, such as herbicide tolerance, insect tolerance, drought tolerance, nematode control, water use efficiency, nitrogen use efficiency, improved nutritional value, disease resistance, improved photosynthesis, improved fiber quality, stress tolerance, improved reproduction, and the like.

Particularly useful transgenic events which may be combined with the genes of the current invention in plants of the same species (e.g., by crossing or by re-transforming a plant containing another transgenic event with a chimeric gene of the invention), include Event BPS-CV127-9 (soybean, herbicide tolerance, deposited as NCIMB No. 41603, described in WO20 10/080829); Event DAS21606-3 / 1606 (soybean, herbicide tolerance, deposited as PTA-1 1028, descnbed in WO20 12/033794), Event DAS-44406-6 / pDAB8264.44.06.1 (soybean, herbicide tolerance, deposited as PTA-1 1336, described in WO201 2/075426), Event DAS-14536-7 /pDAB829 1.45.36.2 (soybean, herbicide tolerance, deposited as PTA-1 1335, described in WO20 12/075429), Event DAS68416 (soybean, herbicide tolerance, deposited as ATCC PTA-10442, descnbed in WO201 1/066384 or WO201 1/066360); Event DP-305423-1 (soybean, quality trait, not deposited, descnbed in US-A 2008-312082 or WO2008/054747); Event DP-356043-5 (soybean, herbicide tolerance, deposited as ATCC PTA-8287, described in US-A 2010-0184079 or WO2008/002872); Event FG72 (soybean, herbicide tolerance, deposited as PTA-1 1041, described in WO201 1/0634 13), Event LL27 (soybean, herbicide tolerance, deposited as NCIMB41 658, descnbed in WO2006/108674 or US-A 2008-320616); Event LL55 (soybean, herbicide tolerance, deposited as NCIMB 41660, described in WO 2006/108675 or US-A 2008-196127); Event MON87701 (soybean, insect control, deposited as ATCC PTA-8194, descnbed in US-A 2009-1 30071 or WO2009/Q64652); Event MON87705 (soybean, quality trait - herbicide tolerance, deposited as ATCC PTA-9241, described in US-A 2010-0080887 or WO2010/037016); Event MON87708 (soybean, herbicide tolerance, deposited as ATCC PTA-9670, described in WO201 1/034704); Event MON87712 (soybean, yield, deposited as PTA-1 0296, descnbed in WO20 12/05 l 199), Event MON87754 (soybean, quality trait, deposited as ATCC PTA-9385, described in WO2010/024976); Event MON87769 (soybean, quality trait, deposited as ATCC PTA-8911, described in US-A 201 1-0067141 or WO2009/102873); Event MON89788 (soybean, herbicide tolerance, deposited as ATCC PTA-6708, described in US-A 2006-282915 or WO2006/130436); Event SYHT0H2 / SYN-000H2-5 (soybean, herbicide tolerance, deposited as PTA-1 1226, described in WO20 12/082548), event EE-GM3 / FG72 (soybean, herbicide tolerance, ATCC Accession N° PTA-1 1041) optionally stacked with event EE-GM1/LL27 or event EE-GM2/LL55 (WO201 1/0634 13A2); Event DAS-68416-4 (soybean, herbicide tolerance, ATCC Accession N° PTA-10442, WO2011/066360A1); Event DAS-68416-4 (soybean, herbicide tolerance, ATCC Accession N° PTA-10442, WO201 1/066384A1); Event DAS-21 606-3 (soybean, herbicide tolerance, ATCC Accession No. PTA-1 1028, WO2012/033794A2); Event MON-87712-4 (soybean, quality trait, ATCC

Accession N°. PTA-10296, WO2012/051199A2); Event DAS-44406-6 (soybean, stacked herbicide tolerance, ATCC Accession N°. PTA-11336, WO2012/075426A1); Event DAS-14536-7 (soybean, stacked herbicide tolerance, ATCC Accession N°. PTA-1 1335, WO2012/075429A1); Event SYN-000H2-5 (soybean, herbicide tolerance, ATCC Accession N°. PTA-1 1226, WO2012/082548A2); Event 8264.44.06.1 (soybean, stacked herbicide tolerance, Accession N° PTA-11336, WO2012075426A2); Event 8291.45.36.2 (soybean, stacked herbicide tolerance, Accession N°. PTA-1 1335, WO2012075429A2); Event SYHT0H2 (soybean, ATCC Accession N°. PTA-1 1226, WO2012/082548A2); Event pDAB8264.42.32. 1 (soybean, stacked herbicide tolerance, ATCC Accession N° PTA-1 1993, WO2013/010094A1).

Further, provided herein is a method for producing a soybean plant or seed comprising a nucleotide sequence encoding SEQ ID NO: 1 or 2 combined with another SCN resistance locus/gene, such as by combining a soybean plant or seed comprising a nucleotide sequence encoding SEQ ID NO: I or 2 with another SCN resistance locus/gene occurring in the same soybean plant/seed, and planting seed comprising a nucleotide sequence encoding SEQ ID NO: 1 or 2 and said other SCN resistance locus/gene. In one embodiment, the plants, ceils or seeds of the invention contain one or more other SCN resistance loci/genes that occur in soybean, to get a combination of different SCN resistance sources in the soybean plants, cells or seeds of the invention. Several soybean SCN resístance loci or genes are known and one or more of those can be combined with a plant comprising SEQ ID NO: 1 or 2 in the same plant, cell or seed, such as any one of the SCN resistance genes/loci from the resístance sources PI 88788, PI 548402 (Peking), PI 437654 (Hartwig or CystX®), or any combination thereof, or one or more of the native SCN resistance loci/genes *rhgl, rhgl-b, rhg2, rhg3, Rhg4, Rhg5*, qSCNll, cqSCN-003, cqSCN-005, cqSCN-006, cqSCN-007, or any of the SCN resistance loci identified on any one of soybean chromosomes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or any combination thereof (Kim et al. 2016, Theor. Appl. Genet. 129(12):2295-2311; Kim and Diers 2013, Crop Science 53:775-785; Kazi et al. 2010, Tboor. Appl. Gen. 120(3):633-644; Glover et al. 2004, Crop Science 44(3):936-941; www.soybase.org; Concibido etal. 2004, Crop Science 44:1121-1 131; Webb et al. 1995, Theor. Appl. Genet. 91:574-581). Also, in one embodiment the plants or seeds of the invention are combined with one or more SCN resistance loci in soybean obtained from any one of SCN resistance sources PI 548316, PI 567305, PI 437654, PI 90763, PI 404198B, PI 88788, PI 468916 , PI 5675 16C, PI 209332, PI 438489B, PI 89772, Peking, PI 548402, PI

404198A, PI 561389B, PI 629013, PI 507471, PI 633736, PI 507354, PI 404166, PI 437655, PI 467312, PI 567328, PI 22897, or PI 494182.

Transfomiation of plant cells can be accomplished by one of several techniques known in the art. The pesticidal gene of the invention may be modified to obtain or enhance expression in plant cells. Typically a construct that expresses such a protein would contain a promoter to drive transcription of the gene, as well as a 3' untranslated region to allow transcription termination and polyadenylation. The organization of such constructs is well known in the art. In some instances, it may be useful to engineer the gene such that the resulting peptide is secreted, or otherwise targeted within the plant ceil. For example, the gene can be engineered to contain a signal peptide to facilitate transfer of the peptide to the endoplasmic reticulum. It may also be preferable to engineer the plant expression cassette to contain an intron, such that mRNA processing of the intron is required for expression.

Typically this "plant expression cassette" will be inserted into a "plant transformation vector". This plant transfomiation vector may be comprised of one or more DNA vectors needed for achieving plant transfomiation. For example, it is a common practice in the art to utilize plant transformation vectors that are comprised of more than one contiguous DNA segment. These vectors are often referred to in the art as "binary vectors." Binary vectors as well as vectors with helper plasmids are most often used for *Agrobacterium-medi&ted* transformation, where the size and complexity of DNA segments needed to achieve efficient transformation is quite large, and it is advantageous to separate functions onto separate DNA molecules. Binary vectors typically contain a plasmid vector that contains the cis-acting sequences required for T-DNA transfer (such as left border and right border), a selectable marker that is engineered to be capable of expression in a plant cell, and a "gene of interest" (a gene engineered to be capable of expression in a plant cell for which generation of transgenic plants is desired). Also present on this plasmid vector are sequences required for bacterial replication. The cis-acting sequences are arranged in a fashion to allow efficient transfer into plant cells and expression therein. For example, the selectable marker gene and the pesticidal gene are located between the left and right borders. Often a second plasmid vector contains the trans-acting factors that mediate T-DNA transfer from *Agrobacterium* to plant cells. This plasmid often contains the virulence functions (Vir genes) that allow infection of plant cells by *Agrobacterium*, and transfer of DNA by cleavage at border sequences and vir-mediated DNA transfer, as is understood in the art (Hellens and Mullmeaux (2000) *Trends in Plant Science* 5:446-451). Several types of *Agrobacterium* strains (e.g. LBA4404, GV3101, EHA101, EHA105, etc.) can be used for plant

transformation. The second plasrnid vector is not necessary for transforming the plants by other methods such as microprojection, microinjection, electroporation, polyethylene glycol, etc.

In general, plant transformation methods involve transferring heterologous DNA into target plant cells (e.g. immature or mature embryos, suspension cultures, undifferentiated callus, protoplasts, etc.), followed by applying a maximum threshold level of appropriate selection (depending on the selectable marker gene) to recover the transformed plant cells from a group of untransformed cell mass. Explants are typically transferred to a fresh supply of the same medium and cultured routinely. Subsequently, the transformed cells are differentiated into shoots after placing on regeneration medium supplemented with a maximum threshold level of selecting agent. The shoots are then transferred to a selective rooting medium for recovering rooted shoot or plantiet. The transgenic plantiet then grows into a mature plant and produces fertile seeds (e.g. Hiei *et al.* (1994) *The Plant Journal* 6:271-282; Ishida *et al.* (1996) *Nature Biotechnology* 14:745-750). Explants are typically transferred to a fresh supply of the same medium and cultured routinely. A general description of the techniques and methods for generating transgenic plants are found in Ayres and Park (1994) *Critical Reviews in Plant Science* 13:219-239 and Bommineni and Jauhar (1997) *Maydica* 42: 107-120. Since the transformed material contains many cells; both transformed and non-transformed cells are present in any piece of subjected target callus or tissue or group of cells. The ability to kill non-transformed cells and allow transformed cells to proliferate results in transformed plant cultures. Often, the ability to remove non-transformed cells is a limitation to rapid recover}' of transformed plant cells and successful generation of transgenic plants.

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. Generation of transgenic plants may be performed by one of several methods, including, but not limited to, microinjection, electroporation, direct gene transfer, introduction of heterologous DNA by *Agrobacterium* into plant cells (*Agrobacter um-mediated* transformation), bombardment of plant cells with heterologous foreign DNA adhered to particles, ballistic particle acceleration, aerosol beam transformation (U.S. Published Application No. 20010026941; U.S. Patent No. 4,945,050; International Publication No. WO 91/00915; U.S. Published Application No. 2002015066), Led transformation, and various other non-particle direct-mediated methods to transfer DNA.

Methods for transformation of chloroplasts are known in the art. See, for example, Svab *etal.* (1990) *Proc. Natl. Acad. Sci. USA* 87:8526-8530; Svab and Maliga (1993) *Proc. Natl. Acad. Sci. USA* 90:913-917; Svab and Maliga (1993) *EMBO J*. 12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the piastid genome through homologous recombination. Additionally, plastid transformation can be accomplished by transactivation of a silent plastid-bome transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride *et al.* (1994) *Proc. Nail. Acad. Sci. USA* 91:7301-7305.

Following integration of heterologous foreign DNA into plant cells, one then applies a maximum threshold level of appropriate selection in the medium to kill the urrtransformed cells and separate and proliferate the putatively transformed cells that survive from this selection treatment by transferring regularly to a fresh medium. By continuous passage and challenge with appropriate selection, one identifies and proliferates the cells that are transformed with the plasmid vector. Molecular and biochemical methods can then be used to confirm the presence of the integrated heterologous gene of interest into the genome of the transgenic plant.

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

Evaluation of Plant Transformation

Following introduction of heterologous foreign DNA into plant cells, the transformation or integration of heterologous gene in the plant genome is confirmed by various methods such as analysis of nucleic acids, proteins and metabolites associated with the integrated gene.

PCR analysis is a rapid method to screen transformed cells, tissue or shoots for the presence of incorporated gene at the earlier stage before transplanting into the soil (Sambrook and Russell (2001) *Molecular Cloning: A laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). PCR is carried out using oligonucleotide primers specific to the gene of interest or *Agrohacteriurn* vector background, etc.

Plant transformation may be confirmed by Southern blot analysis of genomic DNA (Sambrook and Russell, 2001, *supra).* In general, total DNA is extracted from the transformant, digested with appropriate restriction enzymes, fractionated in an agarose gel and transferred to a nitrocellulose or nylon membrane. The membrane or "blot" is then probed with, for example, radiolabeled ^{32}P target DNA fragment to confirm the integration of introduced gene into the plant genome according to standard techniques (Sambrook and Russell, 2001, *supra).*

In Northern blot analysis, RNA is isolated from specific tissues of transformant, fractionated in a formaldehyde agarose gel, and blotted onto a nylon filter according to standard procedures that are routinely used in the art (Sambrook and Russell, 2001, *supra).* Expression of RNA encoded by the pesticidai gene is then tested by hybridizing the filter to a radioactive probe derived from a pesticidai gene, by methods known in the art (Sambrook and Russell, 2001, *supra).*

Western blot, biochemical assays and the like may be carried out on the transgenic plants to confirm the presence of protein encoded by the pesticidai gene by standard procedures (Sambrook and Russell, 2001, *supra)* using antibodies that bind to one or more epitopes present on the nematicidal protein.

Pesticidai Activity in Plants

In another aspect of the invention, one may generate transgenic plants expressing a nematicidal protein that has pesticidai activity against a nematode pest. Methods described above by way of example may be utilized to generate transgenic plants, but the manner in which the transgenic plant cells are generated is not critical to this invention. Methods known or described in the art such as *Agrohacterium*-mediated transformation, biolistic transformation, and non-particle-mediated methods may be used at the discretion of the experimenter. Plants expressing a nematicidal protein may be isolated by common methods described in the art, for example by transform ation of callus, selection of transformed callus, and regeneration of fertile plants from such transgenic callus. In such process, one may use

any gene as a selectable marker so long as its expression in plant cells confers ability to identify or select for transformed cells.

A number of markers have been developed for use with plant cells, such as resistance to chloramphenicol, the aminoglycoside G418, hygromycin, or the like. Oilier genes that encode a product involved in chloroplast metabolism may also be used as selectable markers. For example, genes that provide resistance to plant herbicides such as glyphosate, bromoxynil, or imidazolinone may find particular use. Such genes have been reported (Stalker *et al*. (1985) *J. Biol. Chem.* 263:6310-6314 (bromoxynil resistance nitrilase gene); and Sathasivan *etal.* (1990) *Nucl. Acids Res.* 18:2188 (AHAS imidazolinone resistance gene). Additionally, the genes disclosed herein are useful as markers to assess transformation of bacterial or plant cells. Methods for detecting the presence of a transgene in a plant, plant organ (e.g., leaves, stems, roots, etc.), seed, plant cell, propagule, embryo or progeny of the same are well known in the art. In one embodiment, the presence of the transgene is detected by testing for pesticidal activity against a nematode pest.

Fertile plants expressing a nematicidal protein may be tested for pesticidal activity against a nematode pest, and the plants showing optimal activity selected for further breeding. Methods are available in the art to assay for pest activity. Generally, the protein is mixed and used in feeding assays. See, for example Marrone *et al.* (1985) *J. of Economic Entomology* 78:290-293.

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

Use in Pesticidal Control

General methods for employing strains comprising a nucleotide sequence of the present invention, or a variant thereof, in pest control or in engineering other organisms as pesticidal agents are known in the art. See, for example U.S. Patent No. 5,039,523 and EP 0480762A2.

Microorganisms can be genetically altered to contain a nucleotide sequence encoding SEQ ID NO: 1 or 2, or nematicidally-active variants or fragments thereof, and protein may be

used for protecting agricultural crops and products from pests. In one aspect of the invention, whole, i.e., imiysed, cells of a toxin (pesticide)-producing organism are treated with reagents that prolong the activity of the toxin produced in the ceil when the cell is applied to the environment of target pest(s).

Alternatively, the pesticide is produced by introducing a pesticidal gene into a cellular host. Expression of the pesticidal gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. In one aspect of this invention, these cells are then treated under conditions that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of the target pest(s). The resulting product retains the toxicity of the toxin. These naturally encapsulated pesticides may then be formulated in accordance with conventional techniques for application to the environment hosting a target pest, e.g., soil, water, and foliage of plants. See, for example EPA 0192319, and the references cited therein. Alternatively, one may formulate the cells expressing a gene of this invention such as to allow application of the resulting material as a pesticide.

The active ingredients of the present invention are normally 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. These compounds can be fertilizers, weed killers, cryoprotectants, surfactants, detergents, pesticidal soaps, dormant oils, polymers, and/or time-release or biodegradable earner fonnulations that permít 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, 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 ingestion by a target pest of the pesticidal formulation.

Methods of applying an active ingredient of the present invention or an agrochemical composition of the present invention that contains at least one of the nematicidal proteins disclosed herein as SEQ ID NO: 1 or 2, or nematicidally-effective variants or fragments thereof, 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, iyophilization, 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.

Nematode pests may be killed or reduced in numbers in a given area by the methods of the invention, or may be prophylactieafly applied to an environmental area to prevent infestation by a susceptible pest. Preferably the pest ingests, or is contacted with, a pesticidally-effective amount of the polypeptide. By "pesticidally-effective amount" or "nematicidaily-effective amount" is intended an amount of the pesticide or nematicide that is able to bring about death to at least one pest, or to noticeably reduce pest growth, feeding, or normal physiological development of the pest or the host plant in which the pest infests. 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 nematicidaily-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.

The pesticide compositions described may be made by formulating either the bacterial cell, the crystal and/or the spore suspension, or the isolated protein component with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, desiccated, or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. The formulated compositions may be in the form of a dust or granular material, or a suspension in oil (vegetable or mineral), or water or oil/water emulsions, or as a wettable powder, or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers can be solid or liquid and are well known in the art. The term "agriculturally-acceptable earner" covers all adjuvants, inert components, dispersants, surfactants, tackifiers, binders, etc. that are ordinarily used in pesticide formulation technology; these are well known to those skilled in pesticide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, e.g.,

by homogeneously mixing, blending and/or grinding the pesticidal composition with suitable adjuvants using conventional formulation techniques. Suitable formulations and application methods are described in U.S. Patent No. 6,468,523, herein incorporated by reference.

Methods for Increasing Plant Yield

Methods for increasing plant yield are provided. The methods comprise providing a plant or plant cell expressing a polynucleotide encoding the nematicidal polypeptide sequence disclosed herein and growing the plant or a seed thereof in a field infested with (or susceptible to infestation by) a nematode pest against which said polypeptide has nematicidal activity. In some embodiments, the Cry14 polypeptide described herein has nematicidal activity against a Pratylenchus spp., and said field is infested with said Pratylenchus spp. In various embodiments, the Pratylenchus spp. is *Pratylenchus brachyiiriis*. In additional embodiments, the nematode is a root knot nematode, a lesion nematode, or a Lance nematode. As defined herein, the "yield" of the plant refers to the quality and/or quantity of biomass produced by the plant. By "biomass" is intended any measured plant product. An increase in biomass production is any improvement in the yield of the measured plant product. Increasing plant yield has several commercial applications. For example, increasing plant leaf biomass may increase the yield of leafy vegetables for human or animal consumption. Additionally, increasing leaf biomass can be used to increase production of plant-derived pharmaceutical or industrial products. An increase in yield can comprise any statistically significant increase including, but not limited to, at least a 1% increase, at least a 3% increase, at least a 5% increase, at least a 10% increase, at least a 20% increase, at least a 30%, at least a 50%, at least a 70%, at least a 100% or a greater increase in yield compared to a plant not expressing the pesticidal protein described herein. In specific methods, plant yield is increased as a result of improved nematode resistance of a plant expressing the nematicidal protein disclosed herein. Expression of the nematicidal protein results in a reduced ability of a pest to infest or feed. In various embodiments, expression of the nematicidal protein results in improved root development (e.g., improved root or root hair growth), improved yield, faster emergence, improved plant stress management including increased stress tolerance and/or improved recovery from stress, increased mechanical strength, improved drought resistance, reduced fungal disease infection, and improved plant health compared to a plant not expressing the nematicidal protein of the invention.

The plants can also be treated with one or more chemical compositions, including one or more herbicide, insecticides, or fungicides. Exemplary chemical compositions include:

Fruits/Vegetables Herbicides: Atrazine, BromaciL Diuron, Glyphosaie, Linuron, Meiribuzin, Simazine, Trifluralin, Fluazifop, Glufosinate, Halosulfuron Gowan, Paraquat, Propyzamide, Sethoxydim, Butafenacil, Halosulfuron, Indazifiam; Fruits/Vegetables Insecticides: Aldicarb , Bacillus thuriengiensis, Carbaryl, Carbofuran, Chloipyrifos, Cypermethrin, Deltamethrin, Abamectin, Cyfluthrin/beta-cyfluthrin, Esfenvalerate, Lambda-cyhalothrin, Acequinocyl, Bifenazate, Methoxyfenozide, Novaiuron, Chromafenozide, Thiacioprid, Dinotefuran, Fluaciypyrim, Spirodiclofen, Gamma-cyhalothrin, Spiromesifen, Spinosad, Rynaxypvr, Cyazypyr, Triflumuron,Spirotetramat, Imidacloprid, Flubendiamide, Thiodicarb, Metaflumizone, Sulfoxaflor, Cyflumetofen, Cyanopyrafen, Clothianidin, Thiamethoxam, Spinotoram, Thiodicarb, Flonicamid, Methiocarb, Emamectin-benzoate, Indoxacarb, Fenamiphos, Pyriproxifen, Fenbutatin-oxid; Fruits/Vegetables Fungicides: Ametoctradin, Azoxystrobin, Benthiavalicarb, Boscalid, Captan, Carbendazim, Chlorothalonil, Copper, Cyazofamid, Cyflufenamid, Cymoxanil, Cyproconazole, Cyprodinil, Difenoconazole, Dimethomorph, Dithianon, Fenamidone, Fenhexamid, Fluazinam, Fludioxonil, Fluopicolide, Fluopyram, Fluoxastrobin, Fluxapyroxad, Folpet, Fosety], Iprodione, Tprovalicarb, Isopyrazam, Kresoxim-methyl, Mancozeb, Mandipropamid, Metaiaxyl/mefenoxam, Metiram, Metrafenone, MyciobutaniL Penconazole, Penthiopyrad, Picoxystrobin, Propamocarb, Propiconazole, Propineb, Proquinazid, Prothioconazole, Pyraclostrobin, Pyrimethanil, Quinoxyfen, Spiroxamine, Sulphur, Tebuconazole, Thiophanate-methyl, Trifloxystrobin; Cereals Herbicides:

2.4-D, Amidosulfuron, Bromoxynil, Carfentrazone-E, Chlorotoluron, Chlorsulfuron, Clodinafop-P, Clopyralid, Dicamba, DicJofop-M, Diflufenican, Fenoxaprop, Florasulam, Fiucarbazone-NA, Flufenacet, Flupyrosulfuron-M, Fluroxypyr, Flurtamone, Glyphosaie, Iodosulfufon, loxynil, Isoproturon, MCPA, Mesosulfuron, Metsulfuron, Pendimethalin, Pinoxaden, Propoxycarbazone, Prosuifocarb, PyroxsuSam, Sulfosulfuron, Thifensulfuron, Tralkoxydim, Triasulfuron, Tribenuron, Trifluralin, Tritosulfuron; Cereals Fungicides: Azoxystrobin, Bixafen, Boscalid, Carbendazim, Chlorothalonil, Cyflufenamid, Cyproconazole, Cyprodinil, Dimoxystrobin, Epoxiconazole, Fenpropidin, Fenpropimorph, Fluopyram, Fluoxastrobin, Fluquinconazole, Fluxapyroxad, isopyrazam, Kresoxim-methyl, Metconazole, Metrafenone, Penthiopyrad, Picoxystrobin, Prochloraz, Propiconazole, Proquinazid, Prothioconazole, Pyraclostrobin, Quinoxyfen, Spiroxamine, Tebuconazole, Thiophanate-methyl , Trifloxystrobin: Cereals Insecticides: Dimethoate, Lambda-cyhalthrin, Deltamethrin, alpha-Cypennethrin, β-cyfluthrin, Bifenthrin, Imidacloprid, Clothianidin, Thiamethoxam, Thiacioprid, Acetamiprid, Dinetofuran, Clorphyriphos, Pirimicarb,

Methiocarb, Sulfoxaflor; Maize Herbicides: Atrazine, Alachlor, Bromoxynil, Acetochlor, Dicamba, Clopyralid, (S-)Dimethenamid, Glufosinate, Glyphosate, Isoxaflutole, (S~)Metolachlor, Mesotrione, Nicosulfuron, Primisulfuron, Rimsulfuron, Sulcotrione, Foramsulfuron, Topramezone, Tembotrione, Saflufenacil, Tliiencarbazone, Flufenacet, Pyroxasulfon; Maize Insecticides: Carbofuran, Chlorpyrifos, Bifentlirin, Fipronil, Imidacloprid, Lambda-Cyhaiothrin, Tefluthrin, Terbufos, Thiamethoxam, Ciothianidin, Spiromesifen, Flubendiamide, Triflumuron, Rynaxypyr, Deltaniethrin, Thiodicarb, ß-Cyfluthrin, Cypermethrin, Bifentlirin, Lufenuron, Tebupirirnphos, Ethiprole, Cyazypyr, Thiacloprid, Acetaniiprid, Dinetofuran, Avermectm; Maize Fungicides: Azoxystrobin, Bixafen, Boscalid, Cyproconazoie, Dimoxystrobin, Epoxiconazole, Fenitropan, Fiuopyram, Fluoxastrobin, Fluxapyroxad, Isopyrazam, Metconazole, Penthiopyrad, Picoxystrobin, Propiconazole, Prothioconazole, Pyraclostrobin, Tebuconazole, Trifloxystrobin; Rice Herbicides: Butachlor, Propanil, Azimsulfuron, Bensulfuron, Cyhalofop, Daimuron, Fentrazamide, Imazosulfuron, Mefenacet, Oxaziclomefone, Pyrazosulturon, Pynbuticarb, Quinclorac, Thiobencarb, Indanofan, Flufenacet, Fentrazamide, Halosulfuron, Oxaziclomefone, Benzobicycion, Pyriftalid, Penoxsuiam, Bispynbac, Oxadiargyl, Ethoxysulfuron, Pretilachlor, Mesotrione, Tefuryltrione, Oxadiazone, Fenoxaprop, Pyrimisulfan; Rice Insecticides: Diazinon, Fenobucarb, Benfuracarb, Buprofezin, Dinotefuran, Fipronil, Imidacloprid, Isoprocarb, Thiacloprid, Chromafenozide, Ciothianidin, Ethiprole, Flubendiamide, Rynaxypyr, Deltaniethrin, Acetaniiprid, Thiamethoxam, Cyazypyr, Spinosad, Spinotoram, Emamectin-Benzoate, Cypermethrin, Chlorpyriphos, Etofenprox, Carbofuran, Benfuracarb, Sulfoxaflor; Rice Fungicides: Azoxystrobin, Carbendazim, Carpropamid, Diclocymet, Difenoconazole, Edifenphos, Ferimzone, Gentamycin, Hexaconazole, Hymexazol, Iprobenfos (IBP), Isoprothiolane, Isotianii, Kasugamycin, Mancozeb, Metominostrobin, Orj'sastrobin, Pencycuron, Probenazole, Propiconazole, Propineb, Pyroquilon, Tebuconazole, Thiophanate-methyl, Tiadinil, Tricyclazole, Trifloxystrobin, Validamycin; Cotton Herbicides: Diuron, Fluometuron, MSMA, Oxyfluorfen, Prometiyn, Trifluralin, Carfentrazone, Clethodim, Fluazifop-butyl, Glyphosate, Norflurazon, Pendimethalin, Pyrithiobac-sodium, Trifloxysulfuron, Tepraloxydim, Glufosinate, Fiumioxazin, Thidiazuron; Cotton Insecticides: Acephate. Aidicarb, Chloipyrifos, Cypermethrin, Deltamethrin, Abamectin, Acetamiprid, Emamectin Benzoate, Imidacloprid, Indoxacarb, Lambda-Cyhaiothrin, Spinosad, Thiodicarb, Gamma-Cyhalothrin, Spiromesifen, Pyridalyl, Flonicamid Flubendiamide, Triftumuron,Ryiiaxypyr,Beta-Cyfluthrin,Spirotetramat, Ciothianidin,

Thiamethoxam, Thiacloprid, Dinetofiiran, Flubendiamide, Cyazypyr, Spinosad, Spinotorarn, gamma Cyhalothrin, 4-[[(6-Chlorpyridiη-3-yl)met1^1](2,2-ά ii ıvoΓe hyı)aηıiηo]fiuran-2(5H)-on, Thiodicarb, Avermectin, Fionicamid, Pyridalyl, Spiromesifen, Sulfoxaflor; Cotton Fungicides: Azoxystrobin. Bixafen, Boscalid, Carbendazim, Chlorothalonil, Copper, Cyproconazole, Difenoconazole, Dimoxystrobin, Epoxiconazole, Fenamidone, Fiuazinam, Fluopyram, Fluoxastrobin, Fiuxapyroxad, Iprodione, Isopyrazam, Isotianil, Mancozeb, Maneb, Metominostrobin, Penthiopyrad, Picoxystrobin, Propineb, Prothioconazole, Pyraclostrobin, Quintozene, Tebuconazole, Tetraconazole, Thiophanate-methyl, Trifloxystrobin; Soybean Herbicides: Alachlor, Bentazone, Tnfluralm, Chlorimuron-Ethyi, Cloransulam-Methyl, Fenoxaprop, Fomesafen, Fluazifop, Glyphosate, Imazamox, Imazaquin, Imazethapyr, (S-)Metolachlor, Metribuzin, Pendimethalin, Tepraloxydim, Glufosinate; Soybean insecticides: Lambda-cyhalothrin. Methomyl, Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinetofiiran, Flubendiamide, Rynaxypyr, Cyazypyr, Spinosad, Spinotorarn, Emamectin-Benzoate, Fipronil, Ethiprole, Deltamethrin, β-Cyfluthrin, gamma and lambda Cyhalothrin, 4-[[(6-Chlθϕ γriάiη-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Spirotetramat, Spmodiciofen, Triflumuron, Fionicamid, Thiodicarb, beta-Cyfluthrin; Soybean Fungicides: Azoxystrobin. Bixafen, Boscalid, Carbendazim, Chlorothalonil, Copper, Cyproconazole, Difenoconazole, Dimoxystrobin, Epoxkonazole, Fiuazinam, Fluopyram, Fluoxastrobin, Flutriatol, Fiuxapyroxad, Isopyrazam, Iprodione, Isotianil, Mancozeb, Maneb, Metconazole, Metominostrobin, Myclobutanil, Penthiopyrad, Picoxystrobin, Propiconazole, Propineb, Prothioconazole, Pyraclostrobin, Tebuconazole, Tetraconazole, Thiophanate-methyl, Trifloxystrobin; Sugarbeet Herbicides: Chloridazon, Desmedipham, Ethofiimesate, Phenmedipham, Triallate, Clopyralid, Fluazifop, Lenacil, Metamitron, Quinmerac, Cycioxydim, Triflusailfuron, Tepraloxydim, Quizalofop; Sugarbeet Insecticides: Imidacloprid. Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinetofiiran, Deltamethrin, B-Cyfluthrin, gamma/lambda Cyhalothrin, 4-[[(6-Chioϕ yridin-3-yl)me thyl](2,2-diF uorethyl)anıino]furan-2(5H)-on, Tefluthrin, Rynaxypyr, Cyaxypyr, Fipronil, Carbofuran; Canola Herbicides: Clopyralid. Diclofop, Fluazifop, Glufosinate, Glyphosate, Metazaclilor, Trifluralin Ethametsuifuron, Quinmerac, Quizalofop, Clethodim, Tepraloxydim; Canola Fungicides: Azoxystrobin, Bixafen, Boscalid, Carbendazim, Cyproconazole, Difenoconazole, Dimoxystrobin, Epoxiconazole, Fiuazinam, Fluopyram, Fluoxastrobin, Flusilazole, Fiuxapyroxad, Iprodione, Isopyrazam, Mepiquat-chloride, Metconazole, Metominostrobin, Paclobutrazole, Penthiopyrad., Picoxystrobin, Prochloraz, Prothioconazole, Pyraclostrobin, Tebuconazole, Thiophanate-methyl,

Trifioxystrobin, Vinclozolin; Canola insecticides: Carbofuran, Thiacioprid, Deltamethrin, Imidacloprid, Clothianidin, Thiamethoxam, Acetamiprid, Dinetofuran, β-Cyfluthrin, gamma and lambda Cyhalothrin, tau-Fluvaleriate, Ethiproie, Spinosad, Spinotoram, Flubendiamide, Rynaxypyr, Cyazypyr, 4-ff(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)aminolfuran-2(5H)-on.

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

EXPERIMENTAL EXAMPLES

Example 1. Cryl4Aal expression in soybean

Soybean events expressing Cryl4Aal (SEQ ID NO:3) were developed through Agrobactenum -mediated transformation of Thorne soybean plants using a construct containing a gene encoding a 4-hydroxyphenylpyruvate dioxygenase protein (HPPD) inhibitor tolerant herbicide gene (described in WO20 14043435) and Cry 14Aal . Wild-type Thorne soybean served as the non-nematode resistant control. Cryl4Aal, when expressed in soybean plants, reduces the number of *Pratylenchus brachyurus* that reproduce in the roots compared with wild-type plants. Three independent event lines consistently gave the same result showing nematode reduction upon retest multiple times. In all three events, the number of nematodes was reduced by 60-85%.

Example 2. Cryl4Abl expression in soybean

EE-GM4 soybean events expressing Cryl4Abl (SEQ ID NO:4) were developed through Agrobacterium-mediated transformation of Thorne soybean plants using a construct containing a gene encoding a 4-hydroxyphenyipyruvate dioxygenase protein (HPPD) inhibitor tolerant herbicide gene (described in WO2014043435) and Cryl4Abl . Wild-type Thorne soybean served as the non-nematode resistant control. Cryl4Abl, when expressed in soybean plants, reduces the number of *Pratylenchus brachyurus* that reproduce in the roots compared with wild-type plants. Non-transformed Thome and EE-GM4 seeds were geminated and planted in the greenhouse to check for control of the lesion nematode, *Pratylenchus brachyurus.* *Pratylenchus brachyurus* nematodes (# 1500/plant, different developmental stages) were applied to the plants when 2 weeks old. Thirty days after application, *Pratylenchus* nematodes were extracted from the roots and counted. The average number of nematodes found in the roots of plants containing EE-GM4 were

compared with the average number of *Praiylenchus* nematodes found in the wild-type Thome plant roots. On average about 80-90% fewer *Praiylenchus* nematodes were found in roots of plants containing EE-GM4 when compared with the Thome control roots, indicating significant control of lesion nematodes by soybean event EE-GM4.

Figure 1 shows results from a *Praiylenchus brachyurus* greenhouse assay in the US, comparing elite lines with EE-GM4 in 5 elite soybean lines (one SCN susceptible (MG 1), one SCN resistant (PI88788, MG 3), one SCN susceptible (MG 6.2), one SCN resistant (Peking, MG 6.2), and one SCN susceptible (MG 9) to SCN-susceptible and SCN-resistant US soybean lines. The soybean plants were grown in small cone pots and kept in greenhouses with temperature varying between 25-32°C. *Praiylenchus brachyurus* nematodes, obtained from South Carolina and increased in the greenhouse were used to inoculate plants in the V2-V3 development stage. Approximately 1500 eggs + adults were inoculated per plant and each entry had 5 plants. Thirty days after infestation, nematodes and eggs were extracted from the roots and counted. Each entry was run in two independent experiments. While SCN-susceptible and SCN-resistant US soybean lines did not show control of *Praiylenchus,* plants with EE-GM4 showed about 85% control of *Praiylenchus*.

Figure 2 shows results from a *Praiylenchus brachyurus* greenhouse assay in Brazil, comparing soybean plants with EE-GM4 to Brazil soybean lines with no resistance and 1 low Rf line, and SCN-susceptible and -resistant plants. The soybean lines were grown in small cone pots and kept in greenhouses with temperature varying between 25-32°C. *Praiylenchus brachyurus* nematodes, obtained from Brazil fields and increased in the greenhouse were used to inoculate plants in the V2-V3 development stage. Approximately 1000 eggs + adults were inoculated per plant and each entry had 5 plants. Thirty days after infestation, nematodes and eggs were extracted from the roots and counted. Results shown are from a single experiment. One Brazilian soybean line (BRS 7380), labeled as having a low-reproductive factor for *Praiylenchus,* showed about 89% reduction of *Praiylenchus*. Plants with EE-GM4 gave ~97% control of *Praiylenchus*. Soybean lines that carry native resistance to SCN *(rhgl + Rhg4)* do not control *Praiylenchus brachyurus.*

Also, plants containing EE-GM4 can be used to control root-knot nematodes (RKN) such as *Meloidogyne incognita.* Even though the population of *Meloidogyne incognita* does not infest Thome wild-type soybean very well, Thome plants with EE-GM4 show a further

reduction in the number of RKN eggs/root mass on average, as compared to untransformed Thome plants.

Example 3. Vectoring of Genes for Plant Expression

The coding regions of the invention are connected with appropriate promoter and terminator sequences for expression in plants. Such sequences are well known in the art. Techniques for producing and confirming promoter - gene - terminator constructs also are well known in the art.

In one aspect of the invention, synthetic DNA sequences are designed and generated. These synthetic sequences have altered nucleotide sequence relative to the parent sequence, but encode proteins that are essentially identical to the parent sequence. In some embodiments, the synthetic DNA sequence comprises SEQ ID NO: 3 or 4.

In another aspect of the invention, modified versions of the synthetic genes are designed such that the resulting peptide is targeted to a plant organelle, such as the endoplasmic reticulum or the apoplast. Peptide sequences known to result in targeting of fusion proteins to plant organelles are known in the art. For example, the N-terminal region of the acid phosphatase gene from the White Lupin *Lupinus albus* (GENBANK® ID GI: 14276838, Miller *et al.* (2001) *Plant Physiology* 127: 594-606) is known in the art to result in endoplasmic reticulum targeting of heterologous proteins. If the resulting fusion protein also contains an endoplasmic reticulum retention sequence comprising the peptide N-teiminus-lysine-aspartic acid-glutamic acid-leucine (i.e., the "KDEL" motif, SEQ ID NO: **7)** at the C-terminus, the fusion protein will be targeted to the endoplasmic reticulum. If the fusion protein lacks an endoplasmic reticulum targeting sequence at the C-terminus, the protein will be targeted to the endoplasmic reticulum, but will ultimately be sequestered in the apoplast.

Thus, this gene encodes a fusion protein that contains the N-terminal thirty-one ammo acids of the acid phosphatase gene from the White Lupin *Lupinus albus* (GENBANK® ID GI: 14276838 , Miller *etal,* 2001, *supra)* fused to the N-terminus of the amino acid sequence of the invention, as well as the KDEL (SEQ ID NO: **7)** sequence at the C-terminus. Thus, the resulting protein is predicted to be targeted the plant endoplasmic reticulum upon expression in a plant cell.

The plant expression cassettes described above are combined with an appropriate plant selectable marker to aid in the selection of transformed cells and tissues, and ligated

into plant transformation vectors. These may include binary vectors from *Agrobacterium*-mediated transformation or simple plasmid vectors for aerosol or biolistic transformation.

In the present invention, an expression cassette including a synthetic gene encoding Cry 14A (SEQ ID NO: 1 or 2) is operably linked to the promoter region of the sucrose synthase 1 gene of *Oryza saliva* (Wang et al. (1992) *Plant Molecular Biology*, 19, 881-885) or the promoter region of the Cauliflower Mosaic Virus 35S transcript (Odell et al. (1985) *Nature* 313, 810-812) and the leader sequence of the chlorophyll a/b binding protein gene of *Petunia hybrid* (Harpster et al. (1988) *Molecular and General Genetics* 212, 182-190). The expression cassettes further comprised the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (Depicker et al. (1982) *Journal of Molecular and Applied Genetics* 1, 561-573) operably linked to the 3' end of the Cry 14 sequence.

Example 4: Soybean transformation

Soybean transformation is achieved using methods well known in the art, such as the one described using the Agrobacterium tumefaciens mediated transformation soybean half-seed explants using essentially the method described by Paz et al. (2006), Plant ceil Rep. 25:206. Transformants are identified using tembotrione as selection marker. The appearance of green shoots was observed, and documented as an indicator of tolerance to the herbicide isoxaflutole or tembotrione. The tolerant transgenic shoots will show normal greening comparable to wild-type soybean shoots not treated with isoxaflutole or tembotrione, whereas wild-type soybean shoots treated with the same amount of isoxaflutole or tembotrione will be entirely bleached. This indicates that the presence of the HPPD protein enables the tolerance to HPPD inhibitor herbicides, like isoxaflutole or tembotrione.

Tolerant green shoots are transferred to rooting media or grafted. Rooted piantlets are transferred to the greenhouse after an acclimation period. Plants containing the transgene are then sprayed with HPPD inhibitor herbicides, as for example with tembotrione at a rate of lOOg AI/ha or with mesotrione at a rate of 300g AI/ha supplemented with ammonium sulfate methyl ester rapeseed oil. Ten days after the application the symptoms due to the application of the herbicide are evaluated and compared to the symptoms observed on wild type plants under the same conditions.

Example 5: Cotton TO plant establishment and selection.

Cotton transformation is achieved using methods well known in the art, especially preferred method in the one described in the PCT patent publication WO 00/71733.

Regenerated plants are transferred to the greenhouse. Following an acclimation period, sufficiently grown plants are sprayed with HPPD inhibitor herbicides as for example tembotrione equivalent to 100 or 200 gAI/ha supplemented with ammonium sulfate and methyl ester rapeseed oil. Seven days after the spray application, the symptoms due to the treatment with the herbicide are evaluated and compared to the symptoms obsen'ed on wild type cotton plants subjected to the same treatment under the same conditions.

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

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

THAT WHICH IS CLAIMED:

1. A **method** for controlling a Pratylenchus spp. nematode pest population comprising contacting said population with a **nematicidally-effective** amount of a polypeptide comprising an amino acid sequence having **at** least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 or 2, wherein said polypeptide has nematicidal activity against the Pratylenchus spp. nematode pest population.

2. The method of claim 1, wherein said Pratylenchus spp. is *Pratylenchus brachyurus.*

3. The method of claim 1 or 2 wherein said plant is a soybean plant.

4. A method for protecting a plant from **a** Pratylenchus spp. nematode pest, comprising expressing **in** a plant or ceil thereof a nucleotide sequence operably linked to a promoter capable **of** directing expression of the nucleotide sequence **in** a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO:3 or 4; and

b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence **of** SEQ **ID** NO: 1 or *2,* wherein said polypeptide has nematicidal activity against said Pratylenchus spp. nematode pest.

5. A method for increasing yield **in** a plant comprising **growing** in a field a plant or a seed thereof having stably **incorporated** into its genome a DNA construct comprising a nucleotide sequence operably linked to a promoter capable of directing expression **of** the nucleotide sequence in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO:3 or 4; and

b) a nucleotide sequence **that** encodes a polypeptide comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence **of** SEQ **ID** NO: 1 or 2, wherein said polypeptide has nematicidal activity against a Pratylenchus spp. nematode pest;

wherein said field is infested with the Pratylenchus spp. nematode pest.

6. The method of claim 5, wherein said Pratylenchus spp. is *Pratylenchus brachyiinis*.

7. The method of claims 4-6, wherein said plant further comprises one or more nucleotide sequences encoding one or more insect toxins.

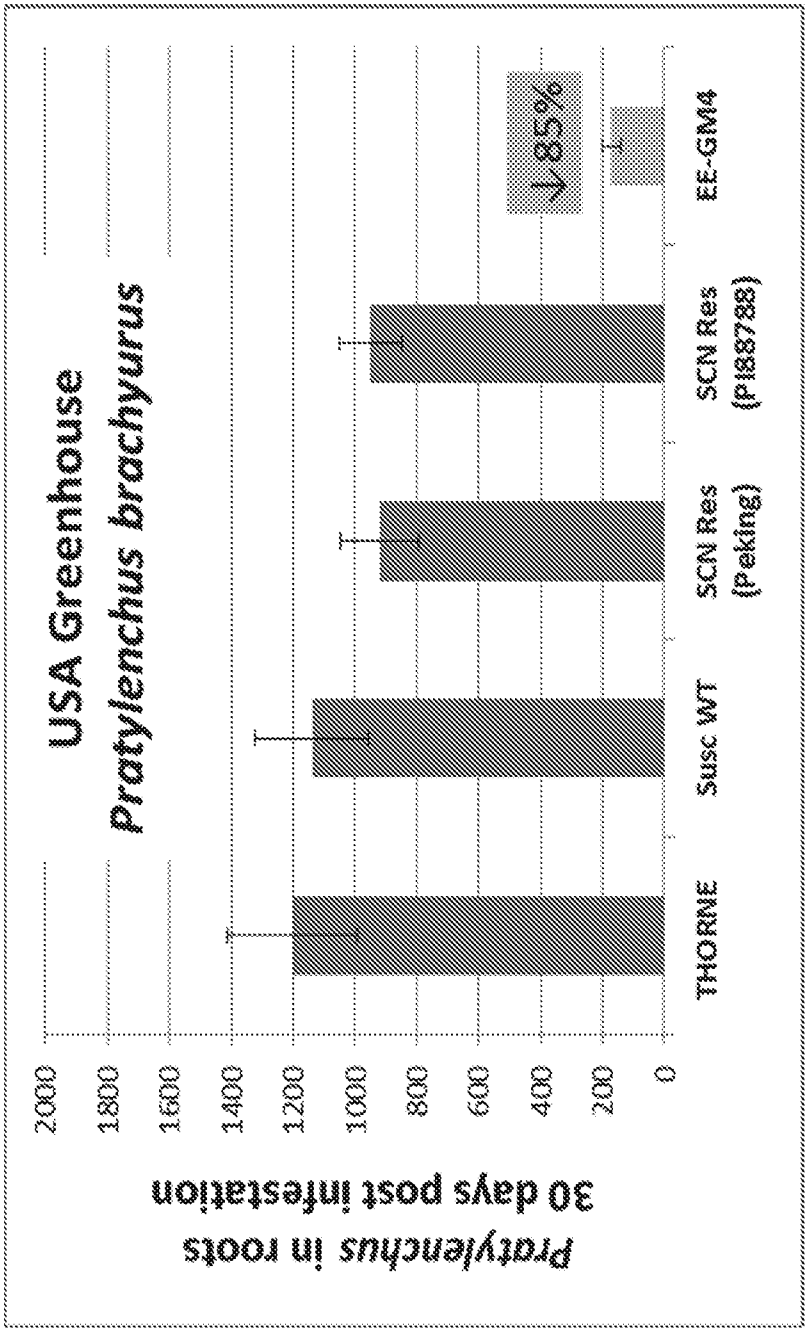
USA Greenhouse
Pratylenchus brachyurus
Pratylenchus in roots
30 days post infestation
2000
1800
1600
1400
1200
1000
800
600
400
200
0
↓85%
THORNE
Susc WT
SCN Res (Peking)
SCN Res (PI88788)
EE-GM4

Fig. 1

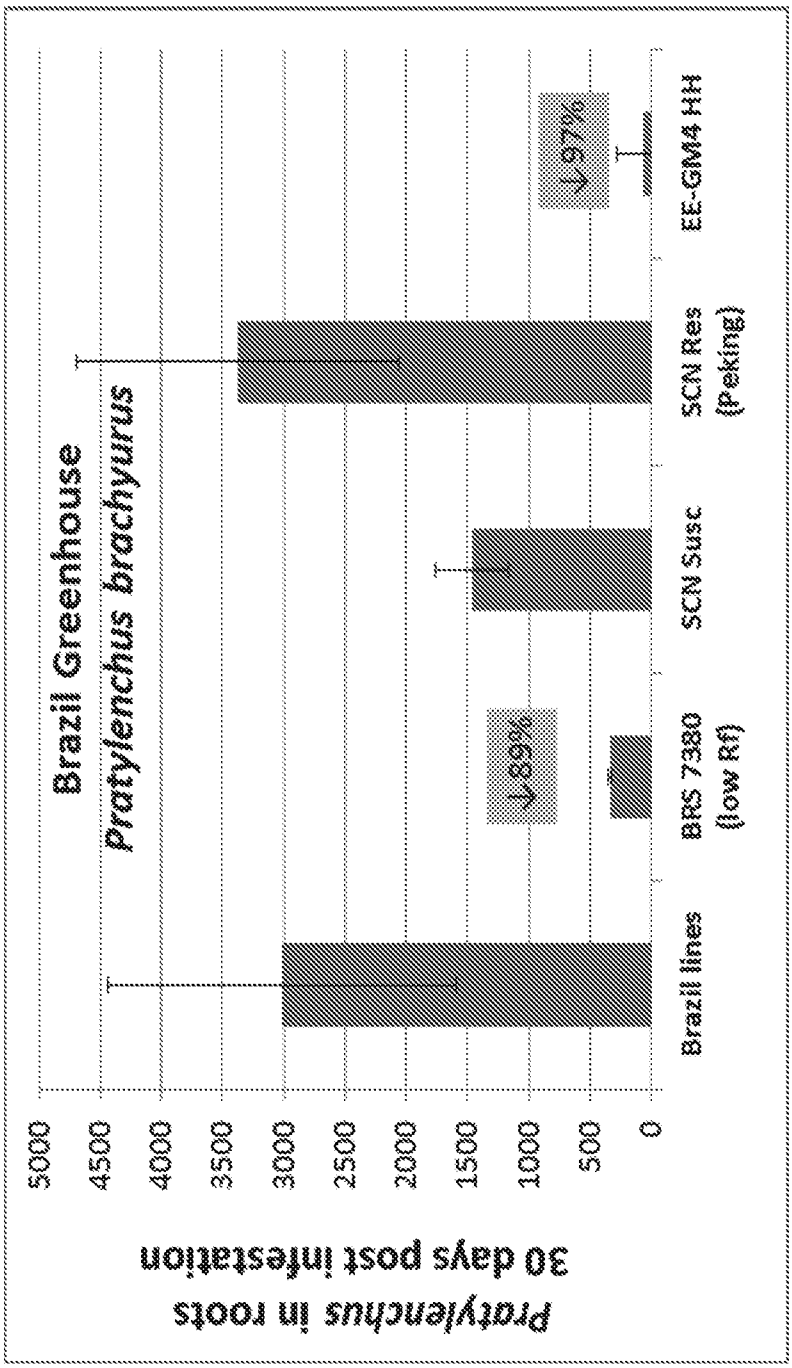
Brazil Greenhouse
Pratylenchus brachyurus
Pratylenchus in roots
30 days post infestation
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
↓89%
↓97%
Brazil lines
BRS 7380 (low Rf)
SCN Susc
SCN Res (Peking)
EE-GM4 HH

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/068070

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C07K14/325 A01N63/02
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 A01N

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 , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/147029 A2 (ATHENIX CORP [US] ; CAROZZI NADINE [US] ; DUCK NICHOLAS [US] ; KAHN THEOD) 21 December 2007 (2007-12-21) page 28, line 18 - line 25 page 33, line 21 - line 27; example 7; sequences 2, 13	1-7
X	WO 2010/027808 A2 (DOW AGROSCIENCES LLC [US] ; NARVA KENNETH E [US] ; HEY TIMOTHY D [US] ; W) 11 March 2010 (2010-03-11) sequence 3	4-7
X	WO 94/16079 A2 (MYCOGEN CORP [US]) 21 July 1994 (1994-07-21) claims 1-22 ; examples 4,6; sequence 6	4-7

-/- .

X Further documents are listed in the continuation of Box C.

X See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 May 2018	17/05/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Maddox, Andrew

9

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/068Q70

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see addi tional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims oould be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only tthhoossee cllaaiimmss ffoorr wwhhiicchh ffee<es were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ TThhee aaddddiittiioonnaall sseeaarrcclh fees were accompanied by the applicant's protest but the applicable protest ffeeee wwaass nnoott ppaaiidd wwiitthhin the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees .

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: l-7(partially)

   Methods for controlling Pratylenchus (yield) based on SEQ ID NO: 1 and 3

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2. claims: l-7(partially)

   Methods for controlling Pratylenchus (yield) based on SEQ ID N0:2 and 4

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 462 721 A2 (MYCOGEN CORP [US]) 27 December 1991 (1991-12-27) page 2, line 49 - line 55 page 5, line 8 - line 11 page 13, line 31 - line 35; sequences 6,8 page 14, line 28 - line 31; example 5; sequences 6,8	1-7
A	WO 93/19604 A1 (RES CORP TECHNOLOGIES INC [US]) 14 October 1993 (1993-10-14) page 5, line 9 - line 26 page 9, line 4 - line 24 claims 7,31	1-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/068070

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