

APPLICATION FOR PATENT

FOR

NUCLEOTIDE SEQUENCES ENCODING INSECTICIDAL PROTEINS

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BACKGROUND OF THE INVENTION

The present invention provides novel coding sequences for use in plants. The coding sequences encode a chimeric insecticidal protein toxic to a wide range of lepidopteran species crop pests.

Commercial formulations of naturally occurring *B. thuringiensis* isolates have long been used for the biological control of agricultural insect pests. Bt spores and crystals obtained from fermentation of *Bacillus thuringiensis* species are concentrated and formulated for foliar application according to conventional agricultural practices.

Members of the family of Cry1 crystal proteins are known to exhibit bioactivity against lepidopteran insect larvae and are useful as agents for controlling lepidopteran insect pests. The precursor form of Cry1 δ -endotoxins consist of two approximately equal sized segments. The carboxy-terminal portion of the precursor protein, or pro-toxin segment, stabilizes crystal formation and exhibits no insecticidal activity. The amino-terminal half of the precursor protein comprises the toxin segment of the Cry1 protein and, based on alignment of conserved or substantially conserved sequences within Cry1 family members, can be further sub-divided into three structural domains. These three sub-domains are based on a three dimensional crystallographic structural model of a Cry1A δ -endotoxin in which the three sub-domains were referred to as Domain I, Domain II, and Domain III, respectively as measured from the amino terminus of the protein toxin segment. Domain I comprises about the first third of the active toxin segment and has been shown to be essential for channel formation (Thompson et al., 1995). Domains II and III respectively comprise about the central and carboxy-terminal segments of the active toxin portion. Domains II and III have both been implicated in receptor binding and insect species specificity, depending on the insect and δ -endotoxin being examined (Thompson et al., 1995).

The likelihood of arbitrarily creating a chimeric protein with enhanced properties from the reassortment of the domain structures of the numerous native insecticidal crystal proteins known in the art is remote. This is a result of the complex nature of protein structure, folding, oligomerization, and activation including correct proteolytic processing of the chimeric precursor, if expressed in such form, to release an insecticidal toxin segment. Only by careful selection of specific target regions within each parental protein for inclusion into a chimeric structure can functional insecticidal toxins be constructed that exhibit improved insecticidal activity in comparison to the parental proteins from which the chimeras are derived. Experience has shown that reassembly of the toxin domains, i.e., assembly of a

chimeric toxin consisting of domain I, II, and III of any two or more toxins that are different from each other, results in the construction of a protein that exhibits faulty crystal formation and/or the complete lack of any detectable insecticidal activity directed to a preferred target insect pest species. In some instances, a chimeric toxin will exhibit good crystal formation properties, yet exhibit no detectable insecticidal activity. Only by trial and error are effective insecticidal chimeras formulated, and even then, the skilled artisan is not certain to end up with a chimera that exhibits insecticidal activity that is equivalent to or improved in comparison to any single parental toxin protein from which the constituents of the chimera may have been derived.

The literature reports examples of the construction or assembly of chimeric proteins from two or more Bt insecticidal crystal protein precursors, yet not all exhibited insecticidal or crystal forming properties that were equivalent to or improved in comparison to the precursor proteins from which the chimeras were derived. (Bosch et al. (WO95/06730); Thompson et al. (WO95/30753); Thompson et al. (WO95/30752); Malvar et al. (WO98/22595); Gilroy et al. (US Patent No. 5,128,130); Gilroy (US Patent No. 5,055,294); Lee et al. (1992) Gene 267:3115-3121; Honee et al. (1991) Mol. Microbiol. 5 :2799-2806; Schnepf et al. (1990) J. Biol. Chem. 265:20923-20930; Perlak et al. (1990) Bio/Technol. 8:939-9943; Perlak et al (1993) Plant Mol. Biol. 22:313-321).

Expression of *B. thuringiensis* δ -endotoxins in transgenic corn plants has proven to be an effective means of controlling agriculturally important insect pests (Perlak *et al.* 1990; 1993). Transgenic crops expressing *B. thuringiensis* δ -endotoxins enable growers to significantly reduce the time and costs associated with applications of topically applied chemical insecticides. Use of transgenes encoding *B. thuringiensis* δ -endotoxins is particularly advantageous. Crop plants expressing *B. thuringiensis* δ -endotoxins in areas under heavy insect pressure exhibit improved yields that are better than otherwise similar non-transgenic commercial plant varieties. However, it is anticipated that insects may evolve resistance to *B. thuringiensis* δ -endotoxins expressed in transgenic plants. Such resistance, should it become widespread, would clearly limit the commercial value of germplasm containing genes encoding such *B. thuringiensis* δ -endotoxins. One possible way of increasing the effectiveness of the transgenic insecticides against target pests and contemporaneously reducing the development of insecticide-resistant pests would be to ensure that transgenic crops express high levels of *B. thuringiensis* δ -endotoxins (McGaughey and Whalon 1993; Roush 1994). In addition, having a repository of

insecticidal genes that are effective against groups of insect pests and which manifest their effects through different modes of action can safeguard against any development of resistance. Expression in a plant of two or more insecticidal compositions toxic to the same insect species, each insecticide being expressed at levels high enough to effectively delay the onset of resistance, would be another way to achieve control of the development of resistance. Examples of such insecticides useful in such combinations include but are not limited to Bt toxins, *Xenorhabdus* sp. or *Photorhabdus* sp. insecticidal proteins, deallergenized and de-glycosylated patatin proteins and/or permuteins, plant lectins, and the like. Achieving co-expression of multiple insecticidally active proteins in the same plant, and/or high expression levels of those insecticidal proteins without causing undesirable plant morphological effects has been elusive.

Only a handful of the more than two-hundred and fifty individual insecticidal proteins that have been identified from *Bacillus thuringiensis* species have been tested for expression in plants. Several Cry1's, Cry3's, Cry2Aa and Cry2Ab, binary toxins Cry33/34 and Cry23/37, and a Cry9 have been successfully expressed in plants. Cry1 proteins represent the largest class of proteins that have been expressed in plants, but none have been expressed at high levels. It was necessary to target the Cry2Ab to the chloroplast to avoid undesirable phytotoxic effects. The majority of acres planted in recombinant plants express Cry1A proteins. The likelihood of the onset of resistance to Cry1A proteins by targeted insect pest species is substantially higher than it would be if a resistance management allele was also expressed along with the *cry1* allele, or if the *cry1* allele was expressed at high levels. Therefore it is desirable that alternative toxin genes be developed for expression in plants as supplements and replacements for those being used presently in the first and second generations of transgenic insect resistant plants.

SUMMARY OF THE INVENTION

The invention provides isolated nucleotide sequences for expression in plants encoding an insecticidal protein exhibiting lepidopteran insect inhibitory properties. SEQ ID NO:1 is an example of such nucleotide sequences consisting of a *cry1A.105* gene and encodes an insect inhibitory Cry1A.105 protein. SEQ ID NO:1 is similar to SEQ ID NO:3, both encoding a Cry1A.105 protein. SEQ ID NO:1 is preferred for use in a dicotyledonous cells, while SEQ ID NO:3 is preferred for use in monocotyledonous cells. SEQ ID NO:4 is encoded from SEQ ID NO:3 and is identical in amino acid sequence to SEQ ID NO:2. The isolated nucleotide sequence is intended to include sequences that exhibit at least from about 88% to about 90% or greater nucleotide sequence identity to the sequence as set forth at SEQ ID NO:1, or that hybridize to SEQ ID NO:1 under stringent hybridization conditions. The isolated nucleotide sequence is also intended to include sequences that exhibit at least about 90% nucleotide sequence identity to the sequence as set forth at SEQ ID NO:3, or that hybridize to SEQ ID NO:3 under stringent hybridization conditions.

The invention also provides an isolated and purified insecticidal protein exhibiting inhibitory activity directed to lepidopteran insect species. The insecticidal protein is designated herein at least as the toxin portion of Cry1A.105 and exhibits an amino acid sequence as set forth in SEQ ID NO:2. The full length precursor protein consisting of about 1177 amino acids as set forth in SEQ ID NO:2 is also referred to as an insecticidal Cry1A.105 protein, however any fragment of the precursor protein that exhibits insecticidal bioactivity is intended to be referred to as an insecticidal Cry1A.105 protein, and includes at least a Cry1A.105 insecticidal protein corresponding to an amino acid sequence segment from about amino acid 1 through about amino acid 612 as set forth in SEQ ID NO:2, and may also include a segment from about amino acid 2 through about amino acid 610. Any composition consisting of an insecticidally effective amount of the insecticidal protein is intended to be within the scope of the invention.

The invention also provides an expression cassette for use in expressing an insecticidal protein as set forth in SEQ ID NO:2 in a host cell. The expression cassette preferably contains a promoter functional in the intended host cell which is linked to and regulates the expression of a nucleotide sequence encoding an insecticidal segment of a Cry1A.105 protein. Exemplary expression cassettes are provided herein as set forth at SEQ ID NO:5 and SEQ ID NO:7, intended for use in a dicot plant cell or a monocot plant cell, respectively. The promoter and the coding sequence are operably linked and function

together in the host cell. The expression cassette can be intended for use in any host cell, but is preferably for use in a bacterial cell, a fungal cell, a mammalian cell, or a plant cell. Bacterial cells are preferably selected from the group consisting of a *Bacillus* species cell, a *Enterobacteriaceae* species cell, a *Pseudomonas* species cell, a *Clostridium* species cell, and a *Rhizobium* species cell, and a *Agrobacterium* species cell. If the host cell is a plant cell, it is preferable that it is a cell chosen from a crop species of plant cell, preferably either a dicotyledonous plant or a monocotyledonous plant cell. Examples of dicotyledonous plant cells are alfalfa, apple, apricot, asparagus, bean, berry, blackberry, blueberry, canola, carrot, cauliflower, celery, cherry, chickpea, citrus tree, cotton, cowpea, cranberry, cucumber, cucurbit, egg plant, fruit tree, grape, lemon, lettuce, linseed, melon, mustard, nut bearing tree, okra, orange, pea, peach, peanut, pear, plum, potato, soybeans, squash, strawberry, sugar beet, sunflower, sweet potato, tobacco, tomato, turnip, and vegetable. Monocotyledonous plant cells examples are corn, wheat, oat, rice, sorghum, milo, buckwheat, rye, grass (fescue, timothy, brome, orchard, St. Augustine, Bermuda, bentgrass), and barley. Expression cassettes intended for use in a plant cell typically contain in operable linkage sequences that regulate the levels and efficiencies of expression of an intended substance, such as a Cry1A.105 insecticidal protein. Such sequences may be an expression enhancer sequence, an untranslated leader sequence, an intron sequence, a chloroplast targeting peptide encoding sequence, and a transcription termination and polyadenylation sequence.

The expression cassette is preferably incorporated into a vector for use in stabilizing the maintenance of the Cry1A.105 coding sequence within the host cell. A vector can be any number of structures known in the art, but is typically a plasmid or replicon into which the expression cassette is constructed or inserted prior to incorporation into the host cell. A vector is intended to include but not be limited to a plasmid, a cosmid, a bacmid, a phagemid, a YAC, a BAC, a suicide vector, an insertion sequence, a transposon, or even a linear nucleotide sequence to which the expression cassette is linked or in which the expression cassette is embedded.

Transgenic plants resistant to lepidopteran insect infestation are an embodiment of the present invention. Such plants contain a nucleotide sequence that encodes a Cry1A.105 insecticidal protein as set forth in SEQ ID NO:2 at least from about amino acid 2 to about amino acid 612. The transgenic plant is effective in controlling lepidopteran insect infestations brought about by insects such as leaf rollers, cutworms, armyworms, borers, bagworms, and any forage feeder. Preferred pests are fall armyworms, European corn borers, corn earworms (cotton bollworms), southwestern corn borers, and black cutworms.

The present invention is intended to include the progeny and seed or fruits or product yielded from the transgenic plant of the present invention, so long as the nucleotide sequence of the present invention encoding a Cry1A.105 insecticidal segment is maintained within the heritable and/or plastid genome of the cells of the plant, its progeny, seed, and the like.

5 The present invention also provides one or more methods for controlling lepidopteran insect infestation of a plant by providing in the diet of an insect pest a composition that contains an insecticidally effective amount of an insecticidal Cry1A.105 protein. One such composition would be plant cells that have been or are descended from a plant cell transformed with a nucleic acid sequence that encodes an insecticidal segment of a
10 Cry1A.105 amino acid sequence as set forth in SEQ ID NO:2. A transgenic plant generated from a plant cell transformed to contain an expression cassette, exemplified as set forth at SEQ ID NO:5 and SEQ ID NO:7, which contains a sequence encoding a Cry1A.105 insecticidal amino acid sequence, would be one means for providing an insecticidal composition in the diet of the insect. Another means would be to produce an insecticidally
15 effective amount of a Cry1A.105 protein in a bacterial or fungal cell and provide the bacterial cell or fungal cell or a purified amount of the Cry1A.105 protein in the diet of one or more target insect pests susceptible to the Cry1A.105 protein.

 A method of identifying a nucleotide sequence encoding a Cry1A.105 amino acid sequence in a biological sample is provided. The method consists of contacting a sample
20 being tested for the presence of the Cry1A.105 coding sequence with a polynucleotide probe that binds with specificity to the Cry1A.105 coding sequence. In particular, the probe sequence binds, or hybridizes to, a Cry1A.105 coding sequence under stringent hybridization conditions. Detecting binding in a reaction mix is diagnostic for the presence of the Cry1A.105 coding sequence.

25 A method of identifying an insecticidal fragment of a Cry1A.105 protein in a sample is also provided. The method consists of contacting a sample being tested for the presence of a Cry1A.105 insecticidal fragment with an antibody that binds specifically to the insecticidal fragment. Detecting the binding in a reaction mix is diagnostic for the presence of the Cry1A.105 protein in the sample.

30 Chimeric or hybrid insecticidal proteins are also provided. Such hybrids are composed of two or more different insecticidal proteins, each of which exhibits insecticidal activity directed to at least one member of the same insect species. The hybrid insecticidal protein is made up of parts of each of the different insecticidal proteins. Segments of insecticidal proteins used in constructing the hybrid consist of from at least about 50 to at

least about 200 contiguous amino acids selected from the contiguous amino acids making up any one of the different insecticidal proteins. A Cry1A.105 insecticidal protein as set forth in SEQ ID NO:2 from about amino acid position 2 through about amino acid position 612 is intended to be included within the group of the different insecticidal proteins from which a
5 segment may be selected for constructing a hybrid insecticidal protein.

Various advantages and features of the present invention being apparent, the nature of the invention may be more clearly understood by reference to the following detailed description, the examples, and to the appended claims.

Brief Description of the Sequences

SEQ ID NO:1 is a synthetic sequence for expression of a Cry1A.105 insecticidal protein, preferably in a dicot cell.

5 SEQ ID NO:2 is a Cry1A.105 protein encoded from the nucleotide sequence as set forth at SEQ ID NO:1.

SEQ ID NO:3 is a synthetic sequence for expression of a Cry1A.105 insecticidal protein, preferably in a monocot cell.

SEQ ID NO:4 is a Cry1A.105 protein encoded from the nucleotide sequence as set forth at SEQ ID NO:3.

10 SEQ ID NO:5 represents a nucleotide sequence consisting of an expression cassette that functions in a plant cell, and preferably in a dicot plant cell, for expressing a Cry1A.105 insecticidal protein.

SEQ ID NO:6 represents a Cry1A.105 insecticidal protein encoded by a segment within the expression cassette as set forth in SEQ ID NO:5.

15 SEQ ID NO:7 represents a nucleotide sequence consisting of an expression cassette that functions in a plant cell, and preferably in a monocot plant cell, for expressing a Cry1A.105 insecticidal protein.

SEQ ID NO:8 represents a Cry1A.105 insecticidal protein encoded by a segment within the expression cassette as set forth in SEQ ID NO:7.

Detailed Description of the Invention

In accordance with the present invention, the inventors have constructed nucleotide sequences that encode a novel insecticidal protein identified herein as a Cry1A.105 protein. It has been identified that the Cry1A.105 amino acid sequence, set forth in SEQ ID NO:2, exhibits properties that provide advantages over naturally occurring Bt insecticidal proteins that are toxic to lepidopteran insect species. In particular, the Cry1A.105 protein can be expressed at high levels in both monocot and dicot plants without most transgenic events exhibiting phytotoxic effects as a result of the increased levels of expression compared to effects observed when naturally occurring Cry1 proteins are expressed in plants. In addition, the Cry1A.105 protein form stable crystals when expressed in *Bacillus thuringiensis*, likely because of the stabilizing effect of the Cry1Ac protoxin segment linked to the toxin moiety of the chimeric Cry1A.105 protein. In addition, the Cry1A.105 insecticidal protein exhibits a range of insecticidal bioactivity directed to lepidopteran species that is not observed with other naturally occurring Cry1 proteins that have been identified to date. Therefore, expression of the Cry1A.105 protein in transgenic plants results in increased numbers of morphologically normal transgenic events expressing higher levels of an analogue of a Cry1 toxin that exhibits a broad range of control of lepidopteran insect pest species for any event that is selected for commercial development. Such events should result in the advantage of delaying the onset of resistance to the Cry1A toxin analogue, and when combined with a second toxin that is toxic to one or more of the insect pest species to which the Cry1A analogue is also toxic and that exerts its mode of action in a way that is different from that of the Cry1A analogue, any likelihood of the development of resistance to either toxin is anticipated to be extremely remote.

The inventors have constructed at least two different nucleotide sequences for use in plants, each nucleotide sequence encoding the same Cry1A.105 insecticidal protein. The first (or amino terminal) about two thirds of the insecticidal portion of the Cry1A.105 protein consists of amino acid sequences derived from a Cry1Ab amino acid sequence. This sequence is linked to the carboxy-terminus of the toxin portion and a part of the protoxin domain of an amino acid sequence derived from an insecticidal Cry1 protein obtained from an Ecogen *Bt aizawai* strain EG6346 (Chambers et al., 1991, J. Bacteriol. 173:3966-3976). The Cry1A.105 toxin segment is linked then to a segment that is substantially a Cry1Ac protoxin peptide sequence. The inventors demonstrated that this construction provides a unique amino acid sequence that exhibits surprisingly improved insecticidal properties when compared to the properties exhibited by the protein from which the chimera is derived.

Furthermore, the Cry1A.105 precursor protein exhibits excellent crystal forming properties and is efficiently solubilized and processed to the active toxin form in the gut of specific targeted lepidopteran insect pests.

The nucleotide sequences embodied herein have been constructed using methods set forth in US Patents No. 5,500,365, and 5,689,052, in particular by avoiding certain inimical sequences in the coding sequence that have been observed to be problematic for expression of heterologous gene sequences in plant cells. The segment encoding the toxin portion of the Cry1A.105 protein consists of nucleotides as set forth in SEQ ID NO:1 and SEQ ID NO:3 from about position 1 through about position 1830, more or less. The sequence as set forth at SEQ ID NO:1 was constructed for use in dicotyledonous plant species, and in particular, in cotton plants. The sequence as set forth at SEQ ID NO:3 was constructed for expression in monocotyledonous plants, and in particular, in maize or corn plant species.

Nucleotide sequences of the present invention exhibit an overall identity of about 94.3% to each other and are identical from about nucleotide position 1330 through about nucleotide position 3534. The segment of each of these nucleotide sequences encoding the toxin portion of the Cry1A.105 protein exhibits, from about nucleotide position 1 through about nucleotide position 1830, about 88.9% identity to each other. The segment of these nucleotide sequences encoding the first two domain structures of the Cry1A.105 protein is substantially more diverse and exhibits only about 84.7% identity to each other.

The inventors have constructed transgenic plant events using these sequences.

SEQ ID NO:1 was introduced into a plasmid vector containing an expression cassette consisting of a enhanced Figwort Mosaic Virus promoter (eFMV) sequence operably linked to a *Petunia hybrida* Hsp70 untranslated leader sequence (Ph.Hsp70, a.k.a., DnaK), an *Arabidopsis thaliana* ribulose bis phosphate carboxylase small subunit chloroplast targeting peptide coding sequence, and a *Pisum sativum* E9 ribulose bis phosphate carboxylase small subunit gene transcription termination and polyadenylation sequence. The Cry1A.105 coding sequence as set forth at SEQ ID NO:1 was inserted into this expression cassette in frame with and immediately adjacent to the 3' end coding sequence of the targeting peptide coding sequence, and upstream of the E9 termination sequence. The nucleotide sequence of the resulting expression cassette is set forth at SEQ ID NO:5. A segment of the vector containing the Cry1A.105 expression cassette linked to a second expression cassette containing a plant expressible GUS marker was excised and used to generate transgenic cotton events using biolistic methods. Transgenic events were tested in bioassay for insecticidal activity against several different lepidopteran pest species and were determined to

exhibit significantly better insect controlling properties than previously existing insect resistant cotton plants containing only Cry1Ac or a combination of Cry1Ac and Cry2Ab proteins. In addition, some of the Cry1A.105 transgenic cotton events exhibited levels of Cry1A.105 protein accumulation exceeding 10 to 20 parts per million throughout the growing season, even in cotton bolls, and without exhibiting any phytotoxic effects on the plant or reproductive tissues. This is in contrast to other Cry1 proteins that have been tested previously, which generally were only capable of levels of accumulation to less than about 10 parts per million, whether or not targeted to the chloroplast. Phytotoxic effects were observed when other Cry1 type proteins were tested in cotton, especially when levels of Cry1 accumulation approached or exceeded about 10 ppm.

SEQ ID NO:3 was introduced into a plasmid vector containing an expression cassette consisting of an enhanced Cauliflower Mosaic Virus promoter (eCaMV) sequence operably linked to a *Triticum aestivum* major chlorophyll a/b binding protein gene untranslated leader sequence and an *Oryza sativa* actin intron sequence, and a *Triticum aestivum* hsp17 gene transcription termination and polyadenylation sequence. The Cry1A.105 coding sequence as set forth at SEQ ID NO:3 was inserted into this expression cassette immediately adjacent to and 3' of the intron sequence and upstream of the termination sequence. The nucleotide sequence of the resulting expression cassette is set forth at SEQ ID NO:7. The vector also contains a glyphosate herbicide selectable marker that was used to select events transformed with the Cry1A.105 expression cassette. Maize events selected after transformation with the Cry1A.105 expression cassette were tested in bioassays against several lepidopteran pest species and determined to exhibit a wide range of insecticidal activity that was not prevalent with events transformed with other Bt insecticidal proteins such as Cry1Ab. The fall armyworm and black cutworm activities exhibited by events expressing insecticidal levels of Cry1A.105 coupled with the Cry1A.105 insecticidal activity directed to corn earworm and corn borer equivalent to or greater than that of events expressing Cry1Ab, provides a broader spectrum of insecticidal activity for Cry1A.105 events.

The nucleotide sequences of the present invention are exemplary. Other nucleotide sequences are capable of expressing a Cry1A.105 insecticidal protein fragment in a plant cell, and still other nucleotide sequences are capable of being designed that express well in other types of host cells. Without limiting the scope of the disclosure, it is intended that a nucleotide sequence for use in expression of a Cry1A.105 insecticidal fragment exhibit at least about 85%, or at least about 90%, or at least about 95%, or at least about 99% or greater nucleotide sequence identity to the nucleotide sequences exemplified herein. Other

nucleotide sequences intended for expression of a Cry1A.105 insecticidal fragment in a host cell other than a plant cell can be of any percentage identity or similarity to the exemplified nucleotide sequences. Nucleotide sequences can vary because of the redundancy of the genetic code, and so it is possible to synthesize any number of nucleic acid sequences that encode any part of the amino acid sequence set forth in SEQ ID NO:2, and all of these sequences are intended to be within the scope of the present invention. Any isolated and purified nucleic acid sequence encoding at least an insecticidal fragment of a Cry1.105 protein is intended to be within the scope of the disclosure, as well as any composition in which the nucleic acid can be detected by antibody, by nucleic acid probe, or by one or more pairs of primers designed to produce an amplicon consisting of such sequence.

The nucleic acid sequence exemplified herein and expressed in maize consists only of a Cry1A.105 precursor protein coding sequence, while the sequence expressed in cotton consists of a chloroplast targeted Cry1A.105 precursor protein coding sequence. The expression of Cry1 proteins in plants has proven to be problematic. It is not known whether or if any particular Cry1 protein will be expressed well in any particular plant, and so trial and error experimentation is required. Some Cry1 proteins expressed in corn will result in phytotoxic effects, and so targeting the protein to the chloroplast sometimes alleviates such effects. Similar circumstances are observed with cotton plant expression of Cry1 proteins. The examples herein are not intended to teach that Cry1A.105 expression is only possible in maize if localized to the cytoplasmic space, and similarly, are not intended to teach that Cry1A.105 expression is only possible in cotton if localized to the plastid. The examples are intended to teach that either method of protein localization functions with this protein to achieve morphologically normal plants that exhibit high levels of Cry1A.105 protein expression and accumulation, and that exhibit commercial levels of resistance to a broad range of Lepidopteran insect plant pests in the genus' selected from the groups consisting of Anticarsia, Pseudoplusia, Rachiplusia, Helicoverpa, Heliothis, Spodoptera, Epinotia, and Armigera. It is believed that any plastid targeting peptide coding sequence would function effectively for directing the precursor Cry1A.105 protein to the plastid/chloroplast.

Untranslated leader sequences, introns and 3' transcription termination and polyadenylation sequences are known in the art, and the skilled artisan would understand that in certain circumstances, expression can be enhanced or stabilized by incorporating these sequences into the expression cassettes. A number of such sequences are known in the art and are intended to be included within the scope of the present disclosure. Similarly, promoters that function to achieve the regulated expression of a linked sequence are known in

the art and are also intended to be included within the scope of the present disclosure. Promoters can be selected for use to drive expression of a linked sequence in any number of combinations of parameters, including but not limited to temporal control of expression, spatial or tissue specific control of expression, and to control the amount of a particular gene product desired to be accumulated within a particular plant cell or tissue.

The isolated and purified protein comprising an insecticidal fragment of the Cry1A.105 amino acid sequence is also intended to be within the scope of the present invention. Variants are also intended to be within the scope of the invention so long as the amino acid substitution or substitutions effecting the variation are generally conservative with respect to the substituted amino acid(s), and the substitution(s) does not result in a reduction of insecticidal bioactivity or range of species specificity. It is intended that an insecticidal fragment of a Cry1A.105 protein is a segment of the amino acid sequence as set forth in SEQ ID NO:2 from about amino acid position 1 through about amino acid position 650, or from about amino acid position 2 through about amino acid position 612, or from about amino acid position 5 through about amino acid position 610, or from about amino acid position 10 through about amino acid position 600. Alternatively, it is intended that an insecticidal fragment of a Cry1A.105 protein consist of from about 550 to about 650 contiguous amino acids selected from the group consisting of amino acid residues 1 through about 650 as set forth at SEQ ID NO:2. The full length precursor protein, consisting of amino acid residue 1 through about residue 3534, exhibits excellent crystal formation properties and is tolerated well by both monocot and dicot plant species. The precursor protein also exhibits excellent stability when in crystalline form, and also exhibits excellent solubility at alkaline pH, in particular alkaline pH within a range of from about 8.0 to about 12.0, or from about 8.5 to about 11.5, or from about pH 9.0 to about pH 11.0.

The protein of the present invention can be purified and used alone in an insecticidally effective amount in any number of compositions intended for use as a lepidopteran pest control agent, or can be combined in an insecticidally effective amount with any number of other pesticidal agents that are different from the Cry1A.105 protein. Such other pesticidal agents are intended to include but not to be limited to other Bt Cry or other insecticidal compositions whether or not toxic to a lepidopteran species including chemical insecticides, fungicidal or fungistatic agents, antibiotics, antibacterial agents, bacteriostatic agents, and nematocidal or nematostatic agents. Such pesticidal combinations including a Cry1A.105 along with any number of other pesticidal agents can be produced by a transgenic cell, or formulated using purified or substantially purified pesticidal agents into a pesticide

composition in a form consisting of a dust, a granular material, an oil suspension, a water suspension, a mixture of oil and water emulsion, or a wettable powder, and then provided in a an agriculturally acceptable carrier for foliar applications. The compositions can be formulated into a seed treatment as well, either together with a Cry1A.105 in the composition
5 intended for inclusion in the seed treatment, or as a composition applied to a seed that is derived from a transgenic plant transformed to express insecticidally effective amounts of a Cry1A.105, so that the seed treatment composition containing pesticidal agents is provided to a target lepidopteran pest along with cells of a plant grown from the seed that are producing pesticidally effective amounts of a Cry1A.105 protein. A combination of insecticidal
10 proteins the each are toxic to the same insect species and yet manifest their toxicity effects through different modes of action would be a particularly useful combination of pesticidal agents for controlling lepidopteran species or delaying the onset of resistance to any single pesticidal agent otherwise effective against a particular lepidopteran species. An exemplary combination of such proteins would be a Cry1A.105 protein of the present invention, i.e., a
15 first insecticidal protein, coupled with at least a second insecticidal protein different from the first. Such different insecticidal proteins include but are not limited to other lepidopteran Bt. crystalline proteins (other Cry1's, Cry2's, Cry5's, Cry9's), VIP proteins, lepidopteran insecticidal proteins referred to as TIC proteins, and insecticidal proteins produced by *Xenorhabdus* and *Photorhabdus* species of bacteria. Providing in the diet of an insect pest a
20 combination of one or more insecticidal proteins along with an agent designed for achieving dsRNA mediated gene suppression of one or more genes essential for insect survival is a particularly useful combination of pesticidal agents for controlling lepidopteran species or delaying the onset of resistance to any single pesticidal agent otherwise effective against a particular lepidopteran species.

25 Plants transformed with the nucleotide sequences of the present invention are provided as another embodiment of the present invention. Methods for stably introducing DNA into plant cells is known in the art, and includes but is not limited to vacuum infiltration, *Agrobacterium* or *Rhizobium* mediated transformation, electroporation, and various ballistic methods. DNA introduced into plants is generally targeted for insertion into
30 the nuclear chromosome, although insertion into the chloroplast or plastid DNA can be achieved. DNA introduced into plants is generally linked to or associated with a sequence that provides a means for identifying or selecting the cell or cells that have been stably transformed with the DNA of interest, including but not limited to scoreable markers such as fluorescence or light emitting genes and genes encoding pigments or enzymes that, in the

presence of the appropriate substrate, impart a colorimetric feature to the transformed cell or cells, or by including selectable markers that allow a positive selection of transformed cells and tissue, providing a growth advantage to the transformed cells and essentially causing the non-transformed cells or tissue to become static or to die. Such selectable markers include but are not limited to genes encoding basta, bar, methotrexate resistance, neomycin phosphotransferase, glyphosate insensitive EPSPS enzymes, glyphosate oxidoreductase (GOX) enzymes, *E. coli* *phnO* or its equivalent, and the like.

Vectors and other types of sequences designed for maintaining, manipulating, and/or shepherding the exemplified nucleotide sequences while being manipulated in the laboratory or for introduction into a host cell are also included within the scope of the invention, and are intended to include but not be limited to phages, plasmids, bacmids, yacmids, cosmids, and the like.

Transformed plants are also within the scope of the present invention. Plants transformed to contain a nucleotide sequence encoding at least an insecticidal fragment of a Cry1A.105 protein are specifically enabled by the present disclosure. Both monocot and dicot plants are envisioned to be within the scope of the present invention. Monocots are intended to include but not be limited to corn, wheat, oat, rice, sorghum, milo, buckwheat, rye, grass (fescue, timothy, brome, orchard, St. Augustine, Bermuda, bentgrass), and barley, and dicot plants are intended to include at least alfalfa, apple, apricot, asparagus, bean, berry, blackberry, blueberry, canola, carrot, cauliflower, celery, cherry, chickpea, citrus tree, cotton, cowpea, cranberry, cucumber, cucurbit, egg plant, fruit tree, grape, lemon, lettuce, linseed, melon, mustard, nut bearing tree, okra, orange, pea, peach, peanut, pear, plum, potato, soybeans, squash, strawberry, sugar beet, sunflower, sweet potato, tobacco, tomato, turnip, and vegetable. Produce from these plants as well as seeds and tissues produced from these plants are specifically included within the present invention, so long as the seed, tissue, or produce contains a transgene encoding an insecticidal fragment of a Cry1A.105 protein.

Methods for detecting, in a biological sample, a Cry1A.105 protein or a nucleotide sequence encoding an insecticidal fragment of a Cry1A.105 protein are provided by the present invention. Cry1A.105 can be used to immunize animals to produce antibodies specific for Cry1A.105 epitopes. Cry1A.105 specific antibodies can be used to detect the presence of Cry1A.105 in a biological sample. Methods for detecting the binding of an antibody to an antigen are known in the art. Detecting the binding of an antibody to a Cry1A.105 epitope in a biological sample is diagnostic for the presence of the protein in the sample.

Nucleotide sequences encoding a Cry1A.105 insecticidal fragment can be detected as well. Synthetic nucleotide probes can be used to bind to a target sequence, i.e., a nucleotide sequence encoding a Cry1A.105 insecticidal fragment. Methods for detecting the binding of a probe to a target sequence are known in the art. Detecting the binding of a probe to the target Cry1A.105 coding sequence is diagnostic for the presence of the coding sequence in the sample.

Synthetic nucleotide primers can be used in thermal amplification reactions to produce an amplicon from a biological sample suspected of containing a nucleotide sequence encoding an insecticidal fragment of a Cry1A.105 protein. The presence of an amplicon produced in such a thermal amplification reaction is diagnostic for the presence of the nucleotide sequence in the sample. Particularly useful sequences as probes which are diagnostic for detecting the presence of the Cry1A.105 coding sequences of the present invention in a biological sample are sequences that correspond to or are perfectly complementary to (1) nucleotide position 1401-1420 as set forth at SEQ ID NO:1 or SEQ ID NO:3, or (2) nucleotide position 1821 – 1840 as set forth at SEQ ID NO:1 or SEQ ID NO:3. These sequences correspond to (1) the 20 nucleotides spanning the sequence encoding the junction between Domain II and Domain III of the segments of different insecticidal proteins used for constructing the insecticidal portion of the proteins of the present invention, and (2) the 20 nucleotides spanning the sequence encoding the junction between Domain III and the protoxin coding segment of the different protein coding segments used for constructing the coding sequence of the pre-pro-toxin Cry1Ab.105 protein. Nucleotide sequences that are, or are complementary to, either of these segments of DNA (1401-1420 or 1821-1840) can be used as probes for detecting the presence of these coding sequences in biological samples. The detecting of such binding is diagnostic for the presence of such coding sequences in a biological sample. Other sequences as will be recognized by the skilled artisan that flank either side of these segments of DNA can be used as primers for amplifying various sized amplicon segments from such biological samples, and such amplicons are diagnostic for the presence of such coding sequences in the sample. For example, a first primer sequence corresponding to the nucleotide sequence set forth at SEQ ID NO:1 from position 1201-1220 could be used as a forward primer in a thermal amplification reaction with a second primer sequence corresponding to the reverse complement of the nucleotide sequence as set forth at SEQ ID NO:1 from position 1581-1600. Such primers when used together in a thermal amplification reaction with a biological sample containing SEQ ID NO:1 would result in the synthesis of an amplicon corresponding to SEQ ID NO:1 from nucleotide position 1201

through 1600, i.e., a 400 nucleotide amplicon, which would contain the 20 nucleotide segment from nucleotide position 1401 – 1420 as set forth in SEQ ID NO:1, and would therefore be diagnostic for the presence of the Cry1A.105 coding sequence in such sample.

5 A kit for detecting the presence of a Cry1A.105 or detecting the presence of a nucleotide sequence encoding a Cry1A.105 in a sample is provided. The kit is provided along with all reagents and control samples necessary for carrying out a method for detecting the intended agent, as well as instructions for use.

10 The following examples describe preferred embodiments of the invention. Other embodiments within the scope of the claims will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

EXAMPLES

Example 1.

5 This example illustrates synthetic nucleotide sequences encoding an insecticidal Cry1A.105 protein.

A nucleotide sequence as set forth at SEQ ID NO:1 encoding a Cry1A.105 insecticidal protein was constructed for use in dicotyledonous plants. The amino acid sequence translation is set forth at SEQ ID NO:2. The toxin encoding segment consists of nucleotides from about position 1 through about position 1830, more or less.

10 A nucleotide sequence as set forth at SEQ ID NO:3 encoding a Cry1A.105 amino acid sequence was constructed for expression in monocotyledonous plants. The amino acid sequence translation is set forth at SEQ ID NO:4. The toxin encoding segment consists of nucleotide from about position 1 through about position 1830, more or less.

15 The nucleotide sequences as set forth at SEQ ID NO:1 and SEQ ID NO:3 are substantial equivalents of each other. SEQ ID NO:1 and SEQ ID NO:3 exhibit an overall identity of about 94.3%. The two coding sequences are identical from about nucleotide position 1330 through the nucleotide position 3534. The toxin encoding portion of each sequence consists of from about nucleotide position 1 through nucleotide position 1830, and these segments exhibit about 88.9% identity to each other. The substantial differences
20 between the two sequences lie within from about nucleotide position 1 through about nucleotide position 1329, or about the first two thirds of the segment encoding the toxin portion of the Cry1A.105 protein. The two sequences exhibit about 84.7% identity throughout this segment.

25 An *E. coli* strain (TOP10, Invitrogen, Inc.) transformed with a plasmid designated as pMON70522 containing a beta-lactamase selectable marker and a sequence as set forth at SEQ ID NO:3 encoding a Cry1A.105 was deposited on August 31, 2005, with the Agriculture Research Culture Collection (NRRL) International Depository Authority at 1815 North University Street, in Peoria, Illinois 61604 U.S.A., according to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent
30 Procedures and was designated as NRRL B-30873.

Example 2.

This example illustrates transgenic cotton plants expressing a Cry1A.105 protein.

Delta and Pineland DP50 cotton seeds were surface sterilized and germinated overnight. Meristem explants were isolated and the primary leaves were removed by micro dissection. Dissected explants were placed in a targeting medium such that the meristems were oriented perpendicular to the direction of the particle delivery. The transformation vector, pMON47740, comprises an expression cassette having a nucleotide sequence set forth in SEQ ID NO:9. A *KpnI* fragment containing a GUS marker gene under the control of an e35S promoter and a chloroplast targeted Cry1A.105 coding sequence under the control of an eFMV promoter was excised from this plasmid and isolated by HPLC and used for gun transformation of the cotton meristem explants. Purified DNA containing both the Cry1A.105 expression cassette and the GUS marker was precipitated onto microscopic gold beads and coated in a thin layer onto a Mylar sheet. The DNA was accelerated into the meristem tissue by electric discharge particle delivery under a partial vacuum. Following bombardment, explants were de-targeted onto hormone-free media without a selective agent. Leaf tissues from regenerated plantlets were sampled and assayed for expression of the GUS marker. Transgenic plants exhibiting a high level of GUS expression were sent to the greenhouse for further screens. These plants were again tested for expression of GUS and negative portions of the plants were pruned off. This cycle of sampling and pruning of GUS-negative tissues was repeated until all sectors of from each plant were positive for the GUS marker. The plants were then maintained under standard greenhouse conditions until seed harvest.

Tissues obtained from F1 GUS positive transgenic cotton plants were tested in bioassays for insecticidal activity against cotton bollworm (CBW) and fall armyworm (FAW). Previously generated isogenic cotton plants expressing insecticidal levels of Cry1Ac or a combination of Cry1Ac and Cry2Ab were used as positive controls and a non-transgenic isoline was used as the negative control.

CBW square assays were used as one means for determining insecticidal activity of the transgenic cotton plants. (Adamczyk et al., (2001) J. Econ. Entomol. 94:284-290; Kranthi et al (2005) Current Science 89:291-298). Squares of leaf tissue (match head size or larger) were collected and placed individually in assay wells. Each square was infested with a single third-instar CBW larva. The number of surviving insects was recorded five days after infestation.

CBW boll assays were also used to determine the insecticidal activity of boll tissue collected from the transgenic plants. 8 hard green bolls (post bloom) from each event were

collected and placed in individual cups and infested with third instar CBW larvae. The number of surviving insects was recorded five days after infestation.

Leaf assays were conducted to determine the insecticidal activity of transgenic leaf tissue against FAW. New leaves were taken from terminals of cotton plants. 2 leaf punches, each about 3/4" in diameter, were collected and placed in each of 16 individual assay wells. Each well was infested with a single second or third instar FAW larva. The number of surviving insects was recorded five days after infestation.

Bioassay results are shown in Table 1. The results show that transgenic cotton events expressing Cry1A.105 exhibited greater insecticidal activity than transgenic events expressing either Cry1Ac or a combination of Cry1Ac and Cry2Ab against both FAW and CBW.

Table 1. Bioassay results of FAW and CBW using the transgenic cotton plant tissue.

Plant	FAW (% survival) (leaf tissue)	CBW (% survival) (Square tissue)	CBW (% survival) (Boll tissue)
Cry1Ac/Cry2Ab	74.5	32.0	35.8
Cry1Ac	92.7	35.5	35.8
Isoline	99.6	96.8	54
17238	10.9	9.4	25
17567	0	12.5	12.5
17774	1.6	1.2	0
17875	3.1	4.2	0
18026	1.6	18.8	12.5
18122	7.8	22.9	0

Tobacco budworm and corn earworms were also tested in similar bioassays. In each case, the Cry1A.105 plants exhibited insecticidal activity against these pests as well.

Example 3.

This example illustrates transgenic corn plants expressing a Cry1A.105 protein.

Transgenic corn plants were regenerated from cells transformed with the vector pMON40232. pMON40232 contains an expression cassette having a nucleotide sequence as set forth in SEQ ID NO:7 that contains, in operable linkage, an enhanced CAMV 35S promoter, a wheat CAB leader sequence, a rice actin 1 intron, a Cry1A.105 coding sequence

and a wheat hsp17 gene 3' transcription termination and polyadenylation sequence. A nucleotide sequence encoding an *Arabidopsis thaliana* EPSPS chloroplast targeting sequence (At.EPSES-CTP2) is positioned upstream of and in frame with the Cry1A.105 coding sequence. pMON40232 contains a recombinant gene encoding an EPSPS that is insensitive to the herbicide glyphosate, for use in selection of transgenic events. Transgenic events arising from tissue transformed with pMON40232 were designated as LAJ 105. Transgenic events were screened for the absence of any vector backbone, for the presence of a single simple inserted sequence, and for the intactness of the expression cassette containing the nucleotide sequence encoding the Cry1A.105 protein.

Bioassays were conducted with events that met the limitations of the event screen. LAJ105 transgenic corn plants were compared in the bioassay to an isogenic LH198 negative control and a positive control MON810 variety expressing the insecticidal portion of a Cry1Ab protein. Five leaf disks, each about one centimeter in diameter, were obtained from each of 10 individual Cry1A.105 transgenic events and from the controls. Leaf disks were placed on agar filled wells to keep the plant material turgid. Discs were then subjected to feeding by FAW, black cutworm (BCW), European corn borer (ECB), corn ear worm (CEW), and Southwestern corn borer (SWCB) neonate larvae. A single neonate FAW larvae, a single CEW larvae, two neonate BCW, two neonate SWCB larvae, or four neonate ECB larvae were applied to each well. Feeding damage was evaluated after four days, using a leaf damage rating (LDR) scale from 0 - 11, 0 indicating no visible feeding damage, 11 indicating at least 50% of the disc was eaten, and each point on the scale between 0 and 11 indicating a 5% increase in observed feeding damage to the leaf disc under observation.

Bioassay results indicated that events expressing Cry1A.105 protein exhibited greater insecticidal activity toward FAW, ECB and CEW than the LDR's exhibited by the Cry1Ab control against the same pest larvae. LDR's for these three pests on the Cry1A.105 events was less than 1 while the Cry1Ab control exhibited LDR's ranging from about 8 to about 10. The LDR was consistently between 1 and 2 both for the Cry1A.105 events and for the Cry1Ab control when tested for activity against SWCB, indicating that the Cry1A.105 protein was no more toxic to SWCB than was Cry1Ab. The results of this bioassay supported previous results that indicated that Cry1Ab is ineffective in controlling BCW. The Cry1A.105 events were no more effective against BCW than was the Cry1Ab control. Thus, at the levels of expression of the Cry1A.105 protein *in planta*, these plants would be effective in controlling other lepidopteran genus plant pests including but not

limited to those in the genus *Anticarsia*, *Pseudoplusia*, *Rachiplusia*, *Heliothis*, *Helicoverpa*, *Spodoptera*, *Epinotia*, and *Armigera*.

CLAIMS

What is claimed is:

1. An isolated nucleotide sequence encoding an insecticidal protein comprising an amino acid sequence as set forth in SEQ ID NO:2 from amino acid position 10 through amino acid position 600.
2. The isolated nucleotide sequence of Claim 1 selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3.
3. The isolated nucleotide sequence of Claim 2 for use in expressing said insecticidal protein in a crop plant.
4. The isolated nucleotide sequence of Claim 3 wherein said crop plant is selected from the group consisting of a monocotyledonous crop plant and a dicotyledonous crop plant.
5. The isolated nucleotide sequence of Claim 4 wherein said monocotyledonous plant is selected from the group of plants consisting of corn, wheat, oat, rice, sorghum, milo, buckwheat, rye, grass (fescue, timothy, brome, orchard, St. Augustine, Bermuda, bentgrass), and barley.
6. The isolated nucleotide sequence of Claim 4 wherein said dicotyledonous plant is selected from the group of plants consisting of alfalfa, apple, apricot, asparagus, bean, berry, blackberry, blueberry, canola, carrot, cauliflower, celery, cherry, chickpea, citrus tree, cotton, cowpea, cranberry, cucumber, cucurbit, egg plant, fruit tree, grape, lemon, lettuce, linseed, melon, mustard, nut bearing tree, okra, orange, pea, peach, peanut, pear, plum, potato, soybeans, squash, strawberry, sugar beet, sunflower, sweet potato, tobacco, tomato, turnip, and vegetable.
7. An isolated and purified insecticidal protein comprising an amino acid sequence as set forth in SEQ ID NO: 2 from amino acid position 10 through amino acid position 600.

8. A composition comprising an insecticidally effective amount of the isolated and purified protein of Claim 7.
9. An expression cassette for use in expressing an insecticidal protein as set forth in SEQ ID NO:2 in a host cell, wherein said expression cassette comprises in operable linkage a promoter sequence functional in said host cell and a nucleotide sequence encoding said protein.
10. The expression cassette of Claim 9 wherein said host cell is selected from the group consisting of a bacterial cell, a fungal cell, a mammalian cell, and a plant cell.
11. The expression cassette of Claim 10 wherein
 - (a) said bacterial cell is selected from the group consisting of a *Bacillus* species cell, a *Enterobacteriaceae* species cell, a *Pseudomonas* species cell, a *Clostridium* species cell, and a *Rhizobium* species cell, and a *Agrobacterium* species cell; and
 - (b) said plant cell is selected from the group of plants consisting of a dicotyledonous plant and a monocotyledonous plant, said dicotyledonous plant being further selected from the group consisting of alfalfa, apple, apricot, asparagus, bean, berry, blackberry, blueberry, canola, carrot, cauliflower, celery, cherry, chickpea, citrus tree, cotton, cowpea, cranberry, cucumber, cucurbit, egg plant, fruit tree, grape, lemon, lettuce, linseed, melon, mustard, nut bearing tree, okra, orange, pea, peach, peanut, pear, plum, potato, soybeans, squash, strawberry, sugar beet, sunflower, sweet potato, tobacco, tomato, turnip, and vegetable, and said monocotyledonous plant being further selected from the group consisting of corn, wheat, oat, rice, sorghum, milo, buckwheat, rye, grass (fescue, timothy, brome, orchard, St. Augustine, Bermuda, bentgrass), and barley.
12. The expression cassette of Claim 9 wherein said host cell is a plant cell and said expression cassette further comprises in operable linkage a sequence selected from the group consisting of an expression enhancer sequence, an untranslated leader sequence, an intron sequence, a chloroplast targeting peptide encoding sequence, and a transcription termination and polyadenylation sequence.
13. A vector comprising the expression cassette of any of Claims 9 through Claim 12.

14. A transgenic plant or plant cell resistant to lepidopteran insect infestation comprising a nucleotide sequence encoding an amino acid sequence as set forth in SEQ ID NO:2 from amino acid position 10 through amino acid position 600, wherein said amino acid sequence exhibits insecticidal activity.
15. The transgenic plant or plant cell of claim 14 wherein said transgenic plant is selected from the group consisting of a dicotyledonous plant and a monocotyledonous plant, said dicotyledonous plant being further selected from the group consisting of alfalfa, apple, apricot, asparagus, bean, berry, blackberry, blueberry, canola, carrot, cauliflower, celery, cherry, chickpea, citrus tree, cotton, cowpea, cranberry, cucumber, cucurbit, egg plant, fruit tree, grape, lemon, lettuce, linseed, melon, mustard, nut bearing tree, okra, orange, pea, peach, peanut, pear, plum, potato, soybeans, squash, strawberry, sugar beet, sunflower, sweet potato, tobacco, tomato, turnip, and vegetable, and said monocotyledonous plant being further selected from the group consisting of corn, wheat, oat, rice, sorghum, milo, buckwheat, rye, grass (fescue, timothy, brome, orchard, St. Augustine, Bermuda, bentgrass), and barley.
16. The transgenic plant or plant cell of claim 15 resistant to lepidopteran insect infestation, wherein said lepidopteran insect is selected from the group consisting of a leaf roller, a cutworm, an armyworm, a borer, a bagworm, and a forage feeder.
17. The transgenic plant or plant cell of claim 16, wherein said lepidopteran insect is further selected from the group consisting of a fall armyworm, a European corn borer, a corn earworm (same as cotton bollworm), a southwestern corn borer, and a black cutworm.
18. Progeny or seed of the transgenic plant or plant cell of claim 15, wherein said progeny or seed comprise said nucleotide sequence.
19. A method for controlling lepidopteran insect infestation of a plant by providing in the diet of the insect one or more plant cells transformed with a nucleic acid sequence comprising in operable linkage a plant functional promoter and a nucleotide sequence encoding an amino acid sequence as set forth in SEQ ID NO:2 from amino acid position 10 through amino acid position 600 exhibiting lepidopteran insecticidal activity.

20. The expression cassette of claim 12 comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:7.
21. A method of identifying in a biological sample a nucleotide sequence encoding a Cry1A.105 amino acid sequence, said method comprising contacting said sample with a polynucleotide that hybridizes to the nucleotide sequence under stringent hybridization conditions and detecting the binding of said polynucleotide to said nucleotide sequence, wherein said binding is diagnostic for said nucleotide sequence in said sample.
22. A method of identifying in a sample a Cry1A.105 protein, said method comprising contacting said sample with an antibody that binds specifically to said protein, and detecting the binding, wherein said binding is diagnostic for the presence of said protein in said sample.
23. An isolated nucleotide sequence encoding an insecticidal protein as set forth at SEQ ID NO:2 from about amino acid 1 through about amino acid 612.
24. An isolated nucleotide sequence encoding an insecticidal protein as set forth at SEQ ID NO:2 from about amino acid 1 through about amino acid 610.
25. The isolated nucleotide sequence of Claim 23 exhibiting at least about 90% identity to the nucleotide sequence as set forth at SEQ ID NO:1.
26. The isolated nucleotide sequence of Claim 23 exhibiting at least about 90% identity to the nucleotide sequence as set forth at SEQ ID NO:3.
27. A hybrid insecticidal protein comprising an amino acid segment comprising from about 500 to about 600 contiguous amino acids selected from the amino acid segment as set forth at SEQ ID NO:2 from about amino acid position 10 through about amino acid position 600.

28. A composition comprising an insecticidally effective amount of a Cry1A.105 protein, wherein said composition is selected from the group consisting of a plant cell, a bacterial cell, a fungal cell, a colloid, an emulsion, a seed coating, a bait, and a powder.
29. The composition of claim 28 wherein said Cry1A.105 protein is present in an amount from about 0.5 to about 200 parts per million (PPM).
30. The composition of claim 29 wherein said Cry1A.105 protein is present in an amount from about 0.5 to about 20 PPM.
31. The composition of claim 29 wherein said composition is a plant cell or a group of plant cells.
32. The composition of claim 31 wherein the nucleotide sequence encoding the insecticidally effective amount of said Cry1A.105 protein is present in an amount detectable using a probe that is or is complementary to a sequence selected from the group consisting of SEQ ID NO:1 from nucleotide position 1401-1420 and SEQ ID NO:1 from nucleotide position 1821-1840.
33. The composition of claim 31 wherein said insecticidally effective amount of said protein is sufficient to control a lepidopteran genus plant pest when provided in the diet of said pest, said pest being selected from the group consisting of *Anticarsia*, *Pseudoplusia*, *Rachiplusia*, *Heliothis*, *Helicoverpa*, *Spodoptera*, *Epinotia*, and *Armigera*.
34. A method for protecting a crop in a field from lepidopteran insect infestation comprising growing a transgenic crop plant comprising an insecticidally effective amount of a Cry1A.105 protein insecticidal agent in the diet of the lepidopteran insect to inhibit the insect from surviving on said transgenic crop plant.
35. The method of claim 34, wherein said transgenic crop plant further comprises an additional insecticidal agent toxic to the same insect species as the Cry1A.105 and is selected from the group consisting of a Bacillus toxin, a Xenorhabdus toxin, a Photorhabdus toxin, and a dsRNA specific for suppression of one or more essential genes in said insect species.

36. The method of claim 34 or 35 wherein the yield of said crop is improved compared to the yield of an isogenic crop lacking said insecticidal agent or agents.