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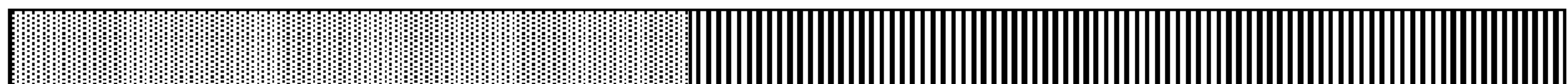
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(54) **Titre : PROCEDES ET COMPOSITIONS PERMETTANT DE RENFORCER L'ACTIVITE DES ENDOTOXINES CRY**
(54) **Title: METHODS AND COMPOSITIONS TO ENHANCE ACTIVITY OF CRY ENDOTOXINS**

Fig. 1

N-terminus

C-terminus



MBP

Bt Cry Protein

E. coli MBP-BT Cry protein fusion

(57) **Abrégé/Abstract:**

Methods and compositions for enhancing the resistance of plants to plant pests are provided. Chimeric pesticidal polypeptides and nucleic acid molecules encoding the chimeric pesticidal polypeptides are provided. The chimeric pesticidal polypeptides comprising a solubility-enhancing polypeptide operably linked to a polypeptide comprising pesticidal activity. The nucleic acid molecules can be used in expression cassettes for making transformed plants with enhanced resistance to plant pests. Further provided are transformed plants, plant tissues, plant cells, other host cells, and seeds as well as pesticidal compositions.

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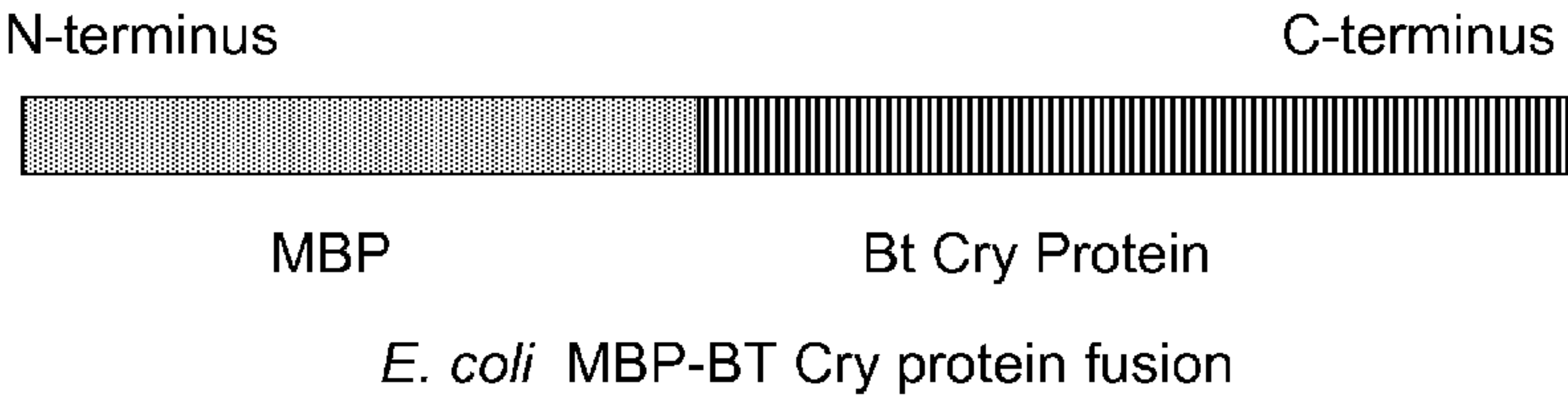
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(54) Title: METHODS AND COMPOSITIONS TO ENHANCE ACTIVITY OF CRY ENDOTOXINS

Fig. 1



(57) Abstract: Methods and compositions for enhancing the resistance of plants to plant pests are provided. Chimeric pesticidal polypeptides and nucleic acid molecules encoding the chimeric pesticidal polypeptides are provided. The chimeric pesticidal polypeptides comprising a solubility-enhancing polypeptide operably linked to a polypeptide comprising pesticidal activity. The nucleic acid molecules can be used in expression cassettes for making transformed plants with enhanced resistance to plant pests. Further provided are transformed plants, plant tissues, plant cells, other host cells, and seeds as well as pesticidal compositions.

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METHODS AND COMPOSITIONS TO ENHANCE ACTIVITY OF CRY ENDOTOXINS

5 CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit US Provisional Application No. 61/713,844, filed October 15, 2012, which is incorporated herein by reference in their entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

10 An official copy of the Sequence Listing submitted electronically as an ASCII formatted Sequence Listing with a file named "3113PCT_SequenceListing.txt," created on September 17, 2013, having a size of 158 kb and filed concurrently with the Specification is part of the Specification and is incorporated herein by reference as if set forth in its entirety.

15

FIELD OF THE INVENTION

The invention relates generally to plant molecular biology and plant pest control, and more particularly to compositions and methods for enhancing the activity of pesticidal polypeptides from *Bacillus* spp. and for protecting a plant from a plant
20 pest, particularly an insect pest.

BACKGROUND OF THE INVENTION

Pests, such as insect pests, are a major factor in the loss of the world's agricultural crops. For example, corn rootworm and boll weevil damage can be economically devastating to agricultural producers. Insect pest-related agricultural
25 crop loss from corn rootworm alone has reached one billion dollars a year.

Traditionally, the primary methods for controlling insect pests, such as corn rootworm, are crop rotation and application of broad-spectrum, synthetic, chemical pesticides. However, consumers and government regulators alike are becoming increasingly concerned with environmental hazards associated with producing and
30 using chemical pesticides. Because of such concerns, regulators have banned or limited the use of some of the more hazardous chemical pesticides. Thus, there is substantial interest in developing alternatives to chemical pesticides that present a lower risk of pollution and environmental hazards and that provide a greater target
35 specificity than is characteristic of chemical pesticides.

Certain species in the genus *Bacillus* have polypeptides that possess pesticidal activity against a broad range of insect pests including those in the orders Lepidoptera, Diptera, Coleoptera, Hemiptera and others. For example, *Bacillus thuringiensis* and *Bacillus popilliae* are among the most successful species
5 discovered to date having pesticidal activity. Such pesticidal activity also has been attributed to strains of *Bacillus larvae*, *Bacillus lentimorbus*, *Bacillus sphaericus* and *Bacillus cereus*. See, *Biotechnology Handbook 2: Bacillus* (Harwood ed., Plenum Press 1989); and Int'l Patent Application Publication No. WO 96/10083.

Pesticidal polypeptides from *Bacillus* spp. include the crystal (Cry)
10 endotoxins, cytolytic (Cyt) endotoxins, vegetative proteins (VIPs) and the like. See, e.g., Bravo *et al.* (2007) *Toxicon* 49:423-435. The Cry endotoxins (also called δ -endotoxins) have been isolated from various strains of *B. thuringiensis*. A common characteristic of the Cry endotoxins is their expression during the stationary phase of growth, as they generally accumulate in a mother cell compartment to form a crystal
15 inclusion that can account for 23-30% of the dry weight of sporulated cells. The Cry endotoxins initially are produced in an inactive protoxin form, which are proteolytically converted into an active endotoxin through the action of proteases in an insect's gut. Once active, the endotoxins bind to the gut epithelium and form cation-selective channels that cause cell lysis and subsequent death. See, Carroll *et al.* (1997) *J.*
20 *Invertebr. Pathol.* 70:41-49; Oppert (1999) *Arch. Insect Biochem. Phys.* 42:1-12; and Rukmini *et al.* (2000) *Biochimie* 82:109-116.

Although Cry endotoxins often are highly effective against insect pests, some insect pests are not affected by them or show low susceptibility. Likewise, some insect pests have developed resistance to the Cry endotoxins, which threatens their
25 effectiveness. Methods to address these problems include enhancing or expanding Cry endotoxin activity by site-directed mutagenesis, by introducing cleavage sites in specific regions of the endotoxin or by deleting small fragments from the amino-terminus of the endotoxin. See, e.g., Abdullah & Dean (2004) *Appl. Environ. Microbiol.* 70:3769-3771; Pardo-López *et al.* (2009) *Peptides* 30:589-595;
30 Rajamohan *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:14338-14343; and Wu *et al.* (2000) *FEBS Lett.* 473 227-232. These methods, however, can be time consuming and not certain to produce a desired result.

For the foregoing reasons, there is a need for compositions and methods to enhance the pesticidal activity of Cry endotoxins.

BRIEF SUMMARY OF THE INVENTION

Compositions and methods are provided for enhancing pesticidal activity of Cry endotoxins and for protecting a plant from plant pest such as insect pests. The compositions comprise chimeric pesticidal polypeptide-encoding nucleic acid molecules, variants and fragments thereof, as well as chimeric pesticidal polypeptides, active variants and fragments thereof. The chimeric pesticidal polypeptides of the invention comprise a solubility-enhancing polypeptide fused to a Cry endotoxin or biologically active fragment thereof. Also provided are expression cassettes or polynucleotide constructs comprising a nucleotide sequence encoding a chimeric pesticidal polypeptide of the invention, as well as bacteria, plants, plant organs, plants tissues, plant parts, plant cells and seeds comprising the expression cassette or polynucleotide nucleotide construct encoding the chimeric pesticidal polypeptide. Further provided are pesticidal compositions comprising at least one chimeric pesticidal polypeptide of the invention.

Methods are provided for enhancing the pesticidal activity of Cry endotoxins. The methods involve making a chimeric pesticidal polypeptide comprising the amino acid sequence of a solubility-enhancing polypeptide operably linked to an amino acid sequence of a Cry endotoxin or biologically active fragment thereof. Such chimeric pesticidal polypeptide can be produced by fusing amino acid sequence of the solubility-enhancing polypeptide to the amino acid sequence of a Cry endotoxin or biologically active fragment thereof. Alternatively, the methods involve making a polynucleotide construct comprising a nucleotide sequence encoding the chimeric pesticidal polypeptide and transforming an organism or non-human host cell of interest with the polynucleotide construct for expression of the chimeric pesticidal polypeptide. The nucleotide sequence encoding the chimeric pesticidal polypeptide can be produced by, for example, operably linking a nucleotide sequence encoding a solubility-enhancing polypeptide to a nucleotide sequence encoding a Cry endotoxin or biologically active fragment thereof. Typically, the polynucleotide construct further comprises a promoter that drives expression in the organism or host cell, wherein the promoter is operably linked to the nucleotide sequence encoding the chimeric pesticidal polypeptide.

Thus, methods are provided for producing a polynucleotide construct comprising a nucleotide sequence encoding a chimeric pesticidal polypeptide, which comprises an amino acid sequence of solubility-enhancing polypeptide fused to an amino acid sequence of a Cry endotoxin or biologically active fragment thereof. The methods involve operably linking a nucleotide sequence encoding solubility-

enhancing polypeptide to a nucleotide sequence encoding a Cry endotoxin or biologically active fragment thereof. The polynucleotide construct may additionally comprise an operably linked promoter for expression of the chimeric pesticidal polypeptide in a non-human host cell of interest, particularly a plant cell. Such a polynucleotide construct finds use, for example, in methods for expressing the chimeric pesticidal polypeptide in a plant transformed therewith.

The present invention further provides methods for making plants with enhanced resistance to at least one pest. The methods involved transforming a plant or at least one plant cell with a polynucleotide construct comprising a nucleotide sequence encoding a chimeric pesticidal polypeptide of the invention. Typically, the nucleotide sequence encoding a chimeric pesticidal polypeptide will be operably linked to a promoter that drives expression in a plant cell. The methods can further involve regenerating the plant or the at least one plant cell into a transformed plant, wherein the regenerated plant expresses the chimeric pesticidal polypeptide. Such a transformed plant comprises enhanced resistance to at least one plant pest, particularly an insect pest, when compared to the resistance of a control plant.

The methods also involve applying a composition such as a pesticidal composition comprising the chimeric pesticidal polypeptide or active variant or fragment thereof, to the environment of an insect pest, particularly on or in the vicinity of a plant by, for example, spraying, dusting, broadcasting or seed coating to protect the plant from the insect pest.

Further provided are transformed plants, plant cells and other host cells, and seeds comprising a nucleotide sequence encoding the chimeric pesticidal polypeptide of the invention.

The following embodiments are encompassed by the present invention:

1. A method of enhancing pesticidal activity of a Cry endotoxin, the method comprising operably linking a first amino acid sequence of a solubility-enhancing polypeptide to a second amino acid sequence of a Cry endotoxin, whereby a chimeric pesticidal polypeptide is produced, the chimeric pesticidal polypeptide comprising the first amino acid sequence operably linked to second amino acid sequence.

2. The method of embodiment 1, wherein the solubility-enhancing polypeptide is selected from the group consisting of a maltose-binding protein (MBP), a thioredoxin, a transcription elongation factor NusA, a glutathione-S-transferase (GST), a mistic, a small ubiquitin-related modifier (SUMO), a protein disulfide isomerase DsbC, and a thiol:disulfide interchange protein DsbD.

3. The method of embodiment 1 or 2, wherein the solubility-enhancing polypeptide is a MBP.

4. The method of embodiment 3, wherein the MBP is selected from the group consisting of MBPs having an accession number set forth in Table 1.

5 5. The method of embodiment 1 or 2, wherein the solubility-enhancing protein is NusA.

6. The method of embodiment 1 or 2, wherein the solubility-enhancing protein is thioredoxin.

10 7. The method of embodiment 1, wherein the solubility-enhancing polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 4, 6, 34, 35, and 36.

8. The method of any one of embodiments 1-7, wherein the Cry endotoxin is selected from the group consisting of Cry endotoxins set forth in Table 2.

15 9. The method of any one of embodiments 1-8, wherein the Cry endotoxin comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 12, and 14.

10. The method of embodiment 1, wherein the chimeric pesticidal polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 2, 16, 18, 21, 23, 25, 27, and 33.

20 11. The method of embodiment 1, wherein the chimeric pesticidal polypeptide is encoded by a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26, and 32.

25 12. The method of any one of embodiments 1-11, wherein the chimeric pesticidal polypeptide comprises a linker amino sequence between the first amino acid sequence and the second amino acid sequence.

13. The method of embodiment 1, wherein the linker amino acid sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 28, 29, 30, and 31.

30 14. The method of any one of embodiments 1-13, further comprising operably linking the linker amino acid sequence between the first amino acid sequence and the amino acid sequence, whereby the chimeric pesticidal polypeptide comprises in linear order the first amino acid sequence, the linker amino acid sequence, and the second amino acid sequence.

35 15. The method of any one of embodiments 1-15, wherein the chimeric pesticidal polypeptide comprises increased pesticidal activity against at least one

pest, when compared to the pesticidal activity of the Cry endotoxin against the at least one pest.

16. The method of embodiment 15, wherein the at least one pest is an insect pest.

5 17. The method of embodiment 16, wherein the insect pest is an insect pest from the order Coleoptera or Lepidoptera.

18. The method of embodiment 16 or 17, wherein the insect pest is from the genus *Diabrotica*.

10 19. The method of embodiment 16, wherein the insect pest is western corn rootworm.

20. The method of embodiment 16, wherein the insect pest is black cutworm.

15 21. A chimeric pesticidal polypeptide comprising a first amino acid sequence of a solubility-enhancing polypeptide operably linked to a second amino acid sequence of a Cry endotoxin.

22. The chimeric pesticidal polypeptide of embodiment 21, wherein the operably linked first and second amino sequences comprise an amino acid sequence selected from the group consisting of:

20 (a) the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33;

(b) an amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32; and

25 (c) an amino acid sequence comprising at least 80% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33, wherein the nucleotide sequence encodes a polypeptide having pesticidal activity.

23. The chimeric pesticidal polypeptide of embodiment 21 or 22, wherein the solubility-enhancing polypeptide is a MBP.

30 24. The chimeric pesticidal polypeptide of embodiment 23, wherein the MBP is selected from the group consisting of MBPs having an accession number set forth in Table 1.

25. The chimeric pesticidal polypeptide of embodiment 21 or 22, wherein the solubility-enhancing protein is a NusA.

35 26. The chimeric pesticidal polypeptide of embodiment 21 or 22, wherein the solubility-enhancing protein is a thioredoxin.

27. The chimeric pesticidal polypeptide of embodiment 21, wherein the first amino acid sequence comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 4, 6, 34, 35, and 36.

5 28. The chimeric pesticidal polypeptide of any one of embodiments 21-27, wherein the Cry endotoxin is selected from the group consisting of Cry endotoxins set forth in Table 2.

29. The chimeric pesticidal polypeptide of embodiment 21, wherein the Cry endotoxin comprises an amino acid sequence selected from the group consisting
10 of SEQ ID NOS: 8, 10, 12, and 14.

30. The chimeric pesticidal polypeptide of any one of embodiments 21-29, wherein the chimeric pesticidal polypeptide further comprises a linker amino sequence operably linked between the first amino acid sequence and the second amino acid sequence.

15 31. The chimeric pesticidal polypeptide of embodiment 30, wherein the linker amino acid sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 28, 29, 30, and 31.

32. The chimeric pesticidal polypeptide of any one of embodiments 21-31, wherein the chimeric pesticidal polypeptide comprises increased pesticidal activity
20 against at least one pest, when compared to the pesticidal activity of the Cry endotoxin against the at least one pest.

33. The chimeric pesticidal polypeptide of embodiment 32, wherein the at least one pest is an insect pest.

34. The chimeric pesticidal polypeptide of embodiment 33, wherein the
25 insect pest is an insect pest from the order Coleoptera or Lepidoptera.

35. The chimeric pesticidal polypeptide of embodiment 33, wherein the insect pest is from the genus *Diabrotica*.

36. The chimeric pesticidal polypeptide of embodiment 33, wherein the insect pest is western corn rootworm.

30 37. The chimeric pesticidal polypeptide of embodiment 33, wherein the insect pest is black cutworm.

38. A nucleic acid molecule comprising a nucleotide sequence encoding a chimeric pesticidal polypeptide, the chimeric pesticidal polypeptide comprising a first amino acid sequence of a solubility-enhancing polypeptide operably linked to a
35 second amino acid sequence of a Cry endotoxin.

39. The nucleic acid molecule of embodiment 38, wherein the nucleotide sequence comprises a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32;

5 (b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33;

(c) a nucleotide sequence comprising at least 80% nucleotide sequence identity to SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32, wherein the nucleotide sequence encodes a polypeptide having pesticidal activity; and

10 (e) a nucleotide sequence encoding an amino acid sequence comprising at least 80% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33, wherein the nucleotide sequence encodes a polypeptide having pesticidal activity.

15 40. The nucleic acid molecule of embodiment 38 or 39, wherein the solubility-enhancing polypeptide is a MBP.

41. The nucleic acid molecule of embodiment 40, wherein the MBP is selected from the group consisting of MBPs having an accession number set forth in Table 1.

20 42. The nucleic acid molecule of embodiment 38 or 39, wherein the solubility-enhancing protein is a NusA.

43. The nucleic acid molecule of embodiment 38 or 39, wherein the solubility-enhancing protein is a thioredoxin.

25 44. The nucleic acid molecule of embodiment 38 or 39, wherein the first amino acid sequence comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 4, 6, 34, 35 and 36.

45. The nucleic acid molecule of any one of embodiments 38-44, wherein the Cry endotoxin is selected from the group consisting of Cry endotoxins set forth in Table 2.

30 46. The nucleic acid molecule of any one of embodiments 38-44, wherein the second amino acid sequence encodes a Cry endotoxin selected from the group consisting of Cry endotoxins set forth in Table 2.

47. The nucleic acid molecule of embodiment 38, wherein the second amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 12 and 14.

35 48. The nucleic acid molecule of any one of embodiments 38-47, wherein the chimeric pesticidal polypeptide further comprises a linker amino sequence

operably linked between the first amino acid sequence and the second amino acid sequence.

49. The nucleic acid molecule of embodiment 48, wherein the linker amino acid sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 28, 29, 30, and 31.

50. The nucleic acid molecule of any one of embodiments 38-49, wherein the chimeric pesticidal polypeptide comprises increased pesticidal activity against at least one pest, when compared to the pesticidal activity of the Cry endotoxin against the at least one pest.

51. The nucleic acid molecule of any one of embodiments 38-50, wherein the at least one pest is an insect pest.

52. The nucleic acid molecule of embodiment 51, wherein the insect pest is an insect pest from the order Coleoptera or Lepidoptera.

53. The nucleic acid molecule of embodiment 51, wherein the insect pest is from the genus *Diabrotica*.

54. The nucleic acid molecule of embodiment 51, wherein the insect pest is western corn rootworm.

55. The nucleic acid molecule of embodiment 51, wherein the insect pest is black cutworm.

56. An expression cassette comprising a promoter that drives expression in a host cell operably linked to a nucleic acid molecule of any one of embodiments 38-55.

57. The expression cassette of embodiment 56, wherein the host cell is a plant cell.

58. The expression cassette of embodiment 57, wherein the promoter is selected from the group consisting of a chemical-inducible promoter, constitutive promoter, pest-inducible promoter, tissue-specific promoter and wound-inducible promoter.

59. The expression cassette of embodiment 58, wherein the tissue-specific promoter is selected from the group consisting of a leaf-preferred promoter, root-preferred promoter, seed-preferred promoter, stalk-preferred promoter and vascular tissue-preferred promoter.

60. A vector comprising the expression cassette of any one of embodiments 56-59.

61. A transformed plant, plant part, plant cell or seed comprising in its genome the expression cassette of any one of embodiments 56-60.

62. The transformed plant, plant part or plant host cell of embodiment 61, wherein the nucleotide sequence is stably incorporated into the genome of the transformed plant, plant part, plant cell or seed.

63. A pesticidal composition comprising an effective amount of a chimeric
5 pesticidal polypeptide of any one of embodiments 21-37 or an active variant or fragment thereof having pesticidal activity.

64. The pesticidal composition of embodiment 63, further comprising bacteria expressing a nucleotide sequence encoding the chimeric pesticidal polypeptide or a biologically active fragment or variant thereof.

10 65. The pesticidal composition of embodiment 63, further comprising at least one agricultural protectant selected from the group consisting of an acaricide, bactericide, fertilizer or micronutrient donor, fungicide, insecticide, nematocide and semiochemical.

15 66. The pesticidal composition of embodiment 65, wherein the semiochemical is selected from the group consisting of an allomone, attractant, feeding pheromone, kairomone, repellent and stimulant.

67. A method of protecting a plant from an insect pest, the method comprising providing an effective amount of a pesticidal composition of any one of embodiments 63-66 to reduce insect pest-related damage to the plant.

20 68. The method of embodiment 67, wherein the pesticidal composition is applied by a procedure selected from the group consisting of spraying, dusting, broadcasting and seed coating.

25 69. The method of embodiment 67 or 68, wherein the chimeric pesticidal polypeptide has pesticidal activity against an insect pest in the order Coleoptera, an insect pest in the order Lepidoptera or both.

70. The method of any one of embodiments 67-69, wherein the insect pest is selected from the group consisting of species in the genus *Diabrotica*.

30 71. A plant comprising a polynucleotide construct stably incorporated in its genome, the polynucleotide construct comprising a nucleotide sequence operably linked to a promoter that drives expression in the plant, wherein the nucleotide sequence encodes a chimeric pesticidal polypeptide comprising a first amino acid sequence of a solubility-enhancing polypeptide operably linked to a second amino acid sequence of a Cry endotoxin.

72. The plant of embodiment 71, wherein the nucleotide sequence is selected from the group consisting of:

(a) the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32;

5 (b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33;

(c) a nucleotide sequence comprising at least 80% nucleotide sequence identity to SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32, wherein the nucleotide sequence encodes a polypeptide having pesticidal activity; and

10 (e) a nucleotide sequence encoding an amino acid sequence comprising at least 80% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33, wherein the nucleotide sequence encodes a polypeptide having pesticidal activity.

15 73. The plant of embodiment 71 or 72, wherein the solubility-enhancing polypeptide is MBP.

74. The plant of embodiment 73, wherein the MBP is selected from the group consisting of MBPs having an accession number set forth in Table 1.

75. The plant of embodiment 71 or 72, wherein the solubility-enhancing protein is NusA.

20 76. The plant of embodiment 71 or 72, wherein the solubility-enhancing protein is thioredoxin.

77. The plant of embodiment 71, wherein the first amino acid sequence comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 4, 6, 34, 35 and 36.

25 78. The plant of any one of embodiments 71-77, wherein the Cry endotoxin is selected from the group consisting of Cry endotoxins set forth in Table 2.

79. The plant of any one of embodiments 71-78, wherein the second amino acid sequence encodes a Cry endotoxin selected from the group consisting of Cry endotoxins set forth in Table 2.

30 80. The plant of embodiment 71 or 72, wherein the second amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 12 and 14.

35 81. The plant of any one of embodiments 71-80, wherein the chimeric pesticidal polypeptide further comprises a linker amino sequence operably linked between the first amino acid sequence and the second amino acid sequence.

82. The nucleic acid molecule of embodiment 81, wherein the linker amino acid sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 28, 29, 30 and 31.

5 83. The plant of any one of embodiments 71-82, wherein the chimeric pesticidal polypeptide comprises increased pesticidal activity against at least one pest, when compared to the pesticidal activity of the Cry endotoxin against the at least one pest.

84. The plant of embodiment 83, wherein the at least one pest is an insect pest.

10 85. The plant of embodiment 84, wherein the insect pest is an insect pest from the order Coleoptera or Lepidoptera.

86. The plant of embodiment 84, wherein the insect pest is from the genus *Diabrotica*.

15 87. The plant of embodiment 84, wherein the insect pest is western corn rootworm.

88. The plant of embodiment 84, wherein the insect pest is black cutworm.

89. The plant of any one of embodiments 71-88, wherein the plant is a monocot.

20 90. The plant of embodiment 89, wherein the monocot is barley, maize, rice, rye, sorghum, sugarcane or wheat.

91. The plant of any one of embodiments 71-88, wherein the plant is a dicot.

92. The plant of embodiment 91, wherein the dicot is alfalfa, *Brassica*, cotton, soybean or sunflower.

25 93. The plant of any one of embodiment 71-92, wherein the plant is a seed.

94. A method of protecting a plant, plant part or plant host cell from an insect pest, the method comprising the steps of:

30 (a) introducing into the plant, plant part or plant host cell an expression cassette of any one of embodiments 56-59; and

(b) regenerating the plant, plant part or plant host cell into a morphologically normal fertile plant, wherein the plant or part thereof comprises a chimeric pesticidal polypeptide.

95. The method of embodiment 94, wherein the chimeric pesticidal polypeptide has pesticidal activity against an insect pest in the order Coleoptera, an insect pest in the order Lepidoptera or both.

96. The method of embodiment 94, wherein the insect pest is selected
5 from the group consisting of species in the genus *Diabrotica*.

97. A method of enhancing the resistance of a plant to at least one pest, the method comprising introducing into a plant or at least one plant cell a polynucleotide construct comprising a nucleotide sequence operably linked to a promoter that drives expression in the plant, wherein the nucleotide sequence
10 encodes a chimeric pesticidal polypeptide comprising a first amino acid sequence of a solubility-enhancing polypeptide operably linked to a second amino acid sequence of a Cry endotoxin.

98. The method of embodiment 97, wherein the nucleotide sequence comprises a nucleotide sequence selected from the group consisting of:

15 (a) the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32;

(b) a nucleotide sequence encoding an amino acid sequence comprising the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33;

20 (c) a nucleotide sequence comprising at least 80% nucleotide sequence identity to SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32, wherein the nucleotide sequence encodes a polypeptide having pesticidal activity; and

(e) a nucleotide sequence encoding an amino acid sequence comprising at least 80% amino acid sequence identity to the amino acid sequence
25 set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33, wherein the nucleotide sequence encodes a polypeptide having pesticidal activity.

99. The method of embodiment 97 or 98, wherein solubility-enhancing polypeptide is MBP.

100. The method of embodiment 99, wherein the MBP is selected from the
30 group consisting of MBPs having an accession number set forth in Table 1.

101. The method of embodiment 97 or 98, wherein the solubility-enhancing protein is NusA.

102. The method of embodiment 97 or 98, wherein the solubility-enhancing protein is thioredoxin.

103. The method of embodiment 97, wherein the first amino acid sequence comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 4, 6, 34, 35 and 36.

104. The method of any one of embodiments 97-103, wherein the Cry
5 endotoxin is selected from the group consisting of Cry endotoxins set forth in Table 2.

105. The method of any one of embodiments 97-104, wherein the second amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 12 and 14.

106. The method of any one of embodiments 97-105, wherein the chimeric
10 pesticidal polypeptide further comprises a linker amino sequence operably linked between the first amino acid sequence and the second amino acid sequence.

107. The method of embodiment 106, wherein the linker amino acid sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 28, 29, 30 and 31.

108. The method of any one of embodiments 97-107, wherein the chimeric
15 pesticidal polypeptide comprises increased pesticidal activity against at least one pest, when compared to the pesticidal activity of the Cry endotoxin against the at least one pest.

109. The method of any one of embodiments 97-108, further comprising
20 regenerating a plant comprising the polynucleotide construct.

110. The method of any one of embodiments 97-109, wherein the plant has enhanced resistance to at least one pest, when compared to a control plant lacking the polynucleotide construct.

111. The method of embodiment 110, wherein the at least one pest is an
25 insect pest.

112. The method of embodiment 111, wherein the insect pest is an insect pest from the order Coleoptera or Lepidoptera.

113. The method of embodiment 111, wherein the insect pest is from the genus *Diabrotica*.

114. The method of embodiment 111, wherein the insect pest is western
30 corn rootworm.

115. The method of embodiment 111, wherein the insect pest is black cutworm.

116. The method of any one of embodiments 97-115, wherein the plant is a
35 monocot.

117. The method of embodiment 116, wherein the monocot is barley, maize, rice, rye, sorghum, sugarcane or wheat.

118. The method of any one of embodiments 97-115, wherein the plant is a dicot.

5 119. The method of embodiment 118, wherein the dicot is alfalfa, Brassica, cotton, soybean or sunflower.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The present invention will be better understood and features, aspects and advantages other than those set forth above will become apparent when consideration is given to the following detailed description thereof. Such detailed description makes reference to the following drawings, wherein:

FIG. 1 depicts the structure of *E. coli* maltose-binding protein (MBP)-Bt Cry protein fusion of the present invention.  = maltose binding protein  = Bt Cry protein.

DETAILED DESCRIPTION OF THE INVENTION

20 The present inventions now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the inventions are shown. Indeed, these inventions may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout.

25 Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although 30 specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

The present invention is based on the discovery that pesticidal activity of Cry endotoxins can be enhanced by operably linking a solubility-enhancing polypeptide, such as, for example, a maltose-binding protein (MBP), to a Cry endotoxin or 35 insecticidally active fragment thereof. Moreover, insect pest resistance can be overcome by operably linking an MBP to a Cry endotoxin by the methods disclosed

herein. The present invention therefore provides compositions and methods for enhancing Cry endotoxin activity, for overcoming plant pest resistance, and for protecting plants from pests, especially insect pests.

While the invention does not depend on a particular biological mechanism, the fusion of a solubility-enhancing polypeptide to a Cry endotoxin or insecticidally active fragment thereof may act to raise the solubility of the Cry protein, particularly in the insect gut environment, more particularly in the gut environment of a Coleopteran insect. That is the solubility of the fusion protein has a higher solubility at a slightly acidic pH than the Cry protein has at the same pH. In a preferred embodiment of the invention, with activated Cry toxin that is used in making the fusion protein of the invention has an isoelectric point (pI) from about pH 7 to about pH 8. It is recognized that in the digestive system in Lepidopteran insects, pH is relatively high. In such alkaline conditions, an activated Bt Cry toxin typically is soluble but in the slightly acidic pH of the Coleopteran gut environment in which pH is slightly acidic, solubility of the activated Bt Cry toxin can be much lower.

Compositions and methods are provided for enhancing the pesticidal activity of Cry endotoxins by operably linking a solubility-enhancing protein to a Cry endotoxin and for protecting plants from insect pests. The compositions and methods disclosed herein include recombinant nucleic acid molecules that encode chimeric pesticidal polypeptides, expression cassettes and other nucleotide constructs including the nucleic acid molecules described herein organisms transformed with the nucleic acid molecules described herein, isolated chimeric pesticidal polypeptides, and pesticidal compositions having the chimeric pesticidal polypeptides, as well as methods of using the same. The compositions and methods therefore find use in enhancing pesticidal activity of pesticidal proteins and in protecting plants from insect pests.

As used herein, a "solubility-enhancing polypeptide" is any polypeptide that when operably linked to a Cry endotoxin or insecticidally active fragment thereof enhances the pesticidal activity of the Cry endotoxin or insecticidally active fragment against at least one insect pest, preferably a Coleopteran insect pest, when compared to the pesticidal activity of the same Cry endotoxin or insecticidally active fragment thereof in the absence of an operably linked solubility-enhancing polypeptide. In certain embodiments, the solubility enhancing polypeptides of the present invention are capable of increasing the solubility of an operable linked Cry endotoxin or insecticidally active fragment thereof at a slightly acidic pH that is found, for example, in the gut environment of a Coleopteran insect. Preferably, a chimeric

pesticidal polypeptide of the present invention, which comprises a solubility-enhancing polypeptide of the present invention operably linked to a Cry endotoxin or insecticidally active fragment thereof, comprises an increased solubility in an environment that has a slightly acidic pH such as, for example, the gut environment
5 of a Coleopteran insect, than the same Cry endotoxin or insecticidally active fragment thereof lacking the operably linked solubility-enhancing polypeptide.

As used herein, "pest" or "plant pest" means an organism that interferes with or is harmful to plant development and/or growth. Such plant pests include, but are not limited to, nematodes, insect, viruses, viroids, mites, fungal pathogens, bacteria
10 and any other plant pests disclosed herein. Accordingly, the polynucleotides and polypeptides of the invention can be used to enhance resistance of plants to plant pests. One of skill in the art, however, understands that not all polypeptides are equally effective against all plant pests. The chimeric pesticidal polypeptides described herein display activity against plant pests such as insect pests, which may
15 include economically important agronomic, forest, greenhouse, nursery ornamentals, food and fiber, public and animal health, domestic and commercial structure, household and stored product pests. Therefore, of interest herein are chimeric pesticidal polypeptides for use in protecting plants from insect pests.

As used herein, "pesticidal polypeptide" means a peptide, polypeptide or
20 protein that has biological activity against insect pests (*i.e.*, is pesticidal).

As used herein, "pesticidal" or "pesticidal activity" means capable of killing insect pests. Likewise, "pesticidal" or "pesticidal activity" means capable of inhibiting insect growth. As such, the chimeric pesticidal polypeptides described herein are capable of inhibiting growth or reproduction of or of killing, at least one plant pest,
25 particularly at least one insect pest.

By "insecticidal activity" is intended the ability of a polypeptide of the invention or a composition comprising the polypeptide (or other agent, chemical or composition) to inhibit the growth of or damage to a plant caused by, at least one insect pest.

As used herein, "enhance" and the like means increasing an activity/effectiveness of a pesticidal polypeptide such as a Cry endotoxin against a particular pest by operably linking the Cry endotoxin to a solubility-enhancing polypeptide, where the activity/effectiveness of the resulting chimeric pesticidal polypeptide is increased by about 1% to about 5%, about 5% to about 10%, about
30 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about

80%, about 80% to about 90% or about 90% to about 100% when compared to a wild-type Cry endotoxin. Alternatively, the activity/effectiveness of the chimeric pesticidal polypeptide is increased by about 1%, about 2%, about 3%, about 4%, about 5%, 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 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more when compared to the wild-type Cry endotoxin. In addition, "enhance" can mean that the host pest range of the pesticidal polypeptide is expanded to include additional pests.

As used herein, "about" means within a statistically meaningful range of a value such as a stated concentration, length, molecular weight, percentage, pH, time frame, temperature or volume. Such a value or range can be within an order of magnitude, typically within 20%, more typically within 10%, and even more typically within 5% of a given value or range. The allowable variation encompassed by "about" will depend upon the particular system under study, and can be readily appreciated by one of skill in the art.

As used herein, "chimeric polypeptide" or "chimeric pesticidal polypeptide" means a polypeptide having a first amino acid sequence derived from a first source operably linked, covalently or non-covalently, to a second amino acid sequence derived from a second source, where the first and second source are not the same. A first source and a second source that are not the same can include two different organisms or two different proteins from the same organism or a biological source and a synthetic source or even two different synthetic sources. A biological source can include any non-synthetically produced nucleotide or amino acid sequence (e.g., a genomic or cDNA sequence, a plasmid or viral vector, a native virion or a mutant or analog). A synthetic source can include a nucleotide or amino acid sequence produced chemically and not by a biological source (e.g., solid phase synthesis of amino acid sequences). The chimeric pesticidal polypeptide can be produced by expressing a recombinant nucleic acid molecule encoding a polypeptide having at least two parts or can be produced synthetically.

A chimeric pesticidal polypeptide of the present can further comprise, for example, a linker molecule (also referred to here as a "linker") between the first and second amino acid sequences. Examples of linkers that can be used in the methods of the present invention are the NEB pMAL, SA, NusA, and TrxA linkers having the amino acid sequences set forth in SEQ ID NOS: 28, 29, 30, and 31, respectively.

Solubility-enhancing polypeptides of the present include, but are not limited to, maltose-binding protein (MBP), thioredoxin (e.g., TrxA), transcription elongation factor NusA, glutathione-S-transferase (GST), mistic, small ubiquitin-related modifier (SUMO), protein disulfide isomerase DsbC, and thiol:disulfide interchange protein DsbD, and variants and fragments thereof that when operably linked to a Cry endotoxin or insecticidally active fragment thereof enhance the pesticidal activity of an operably linked Cry endotoxin or insecticidally active fragment against at least one insect pest, preferably a Coleopteran insect pest, when compared to the pesticidal activity of the same Cry endotoxin or insecticidally active fragment thereof in the absence of an operably linked solubility-enhancing polypeptide.

As used herein, "maltose-binding protein" or "MBP" means a polypeptide that is a member of the maltodextrin transport system that binds maltodextrins (e.g., maltose, maltotriose and trehalose) with micromolar affinity and that is essential for an energy-dependent translocation of maltodextrins through a cytoplasmic membrane of some prokaryotes. See, Boos & Shuman (1998) *Microbiol. Mol. Biol. Rev.* 62:204-229. For example, in *Escherichia coli*, the *malE* gene encodes a 396 amino acid residue pre-MBP, which subsequently is processed into MBP upon cleavage of a 26 amino acid N-terminal signal peptide. See, e.g., Duplay *et al.* (1984) *J. Biol. Chem.* 259:10606-10613.

In one embodiment of the invention, the chimeric pesticidal polypeptide comprises an amino acid sequence of an MBP operably linked to an amino acid sequence of a Cry endotoxin including, for example, the chimeric pesticidal polypeptides comprising the amino acid sequences set forth in SEQ ID NOS: 2, 21, 23, 25, 27, and 33 and encoded by the nucleotide sequences set forth in SEQ ID NOS: 1, 20, 22, 24, 26, and 32, respectively.

In another embodiment, the chimeric pesticidal polypeptide comprises an amino acid sequence of NusA operably linked to an amino acid sequence of a Cry endotoxin including, for example, the chimeric pesticidal polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 16 and encoded by the nucleotide sequence set forth in SEQ ID NO: 15.

In yet another embodiment, the chimeric pesticidal polypeptide comprises an amino acid sequence of thioredoxin operably linked to an amino acid sequence of a Cry endotoxin including, for example, the chimeric pesticidal polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 18 and encoded by the nucleotide sequence set forth in SEQ ID NO: 17.

The present invention comprises the use of an solubility-enhancing polypeptide that when fused to a Cry endotoxin polypeptide increases the pesticidal activity of against at least one pest, preferably an insect pest, more preferably an insect pest from the order Coleoptera or Lepidoptera, even more preferably an insect pest from the genus *Diabrotica*, most preferably the western corn rootworm (*Diabrotica virgifera virgifera*). In certain embodiments, the solubility-enhancing polypeptide selected from the group consisting of MBP, NusA, and TrxA.

The present invention does not depend on the use of a particular solubility-enhancing polypeptide. Any solubility-enhancing polypeptide that, when fused to a Cry endotoxin polypeptide, is capable increasing the pesticidal activity of the Cry endotoxin polypeptide against at least one pest can be used in the methods and compositions disclosed herein. Such solubility enhancing polypeptides include, for example, pre-proteins and mature proteins, and variants and fragments thereof. In certain embodiments, the solubility-enhancing polypeptide is MBP. MBPs of the present invention include, but are not limited, full-length MBP (also referred to as "pre-MBP", a mature form of an MBP, a fragment of a full-length or mature MBP or a variant. Unless expressed stated herein or otherwise apparent from the context, the terms "maltose-binding protein" and "MBP" encompass such full-length and mature forms of MBPs as well as fragments and variants thereof.

The amino acid and/or nucleotide sequences for a number of MBPs have been disclosed and can be used in the compositions and methods of the present invention including, but not limited to, the following those MBPs having the following accession numbers in Table 1.

Table 1

GenBank Accession Number*				
NP_290668	ZP_03064501	CBG37227	NP_709885	ACI74011
YP_002415175	YP_001726921	EFK20320	ZP_02999303	YP_312946
ZP_06651607	ACC91724	NP_756856	ACF57854	ACF57853
ABO28850	AAB86559	AAC83813	BAI57431	AAQ93661
ACI46135	ACI46133	AAK55118	AAB87675	ZP_02904048
YP_002385139	ZP_06356417	YP_003367151	YP_002639794	NP_458527
YP_001338045	ACY91424	ZP_03381315	YP_219095	AAX68014
AAL23053	NP_463094	ZP_04558600	YP_002228802	ZP_02700756
YP_002149143	CAA38189	CBK84491	ZP_06390929	YP_001455393
YP_003610806	YP_001174979	YP_001572421	YP_002241024	P18815
YP_003212142	YP_001436222	YP_001480694	ZP_06712852	ZP_04616043
ZP_04638283	ZP_06191619	ZP_04626936	ZP_04633703	ZP_04613648

GenBank Accession Number*				
NP_667372	ZP_04641958	YP_001161578	YP_001008013	ZP_04625082
YP_003294268	YP_002931694	ZP_06638363	BAE44434	YP_003743846
YP_003039205	NP_927811	ZP_05729913	YP_003714385	YP_003469920
YP_003729949	YP_003520206	YP_002891253	ZP_03366279	ZP_05920010
YP_003007381	ZP_06636620	YP_001343631	YP_003255618	ZP_04976824
YP_089260	ZP_06157313	YP_002474876	YP_001447347	ZP_01984309
ZP_04754507	ZP_06177188	NP_800911	ZP_06180148	ZP_02477508
ZP_01258612	ZP_05878943	ZP_05629984	YP_001053928	YP_132094
ZP_05882865	ZP_02194153	ZP_01221443	ZP_05927269	NP_763457
ZP_05943387	ZP_01865449	ZP_05717553	ZP_06032225	ZP_06079586
ZP_05239461	YP_002812521	NP_233329	ZP_01970977	ZP_04918366
ZP_05719582	ZP_05886194	ZP_05117778	ZP_06941932	ZP_04415908
ZP_01950896	ZP_01676462	ZP_01897017	YP_001343632	YP_132089
ZP_06938154	YP_003007380	ZP_03352864	ZP_01221449	ZP_00988394
ZP_01811558	YP_856203	YP_001142458	NP_936112	ZP_01062901
NP_763137	YP_206757	ZP_00988462	ZP_04403894	ZP_05120494
ZP_01947956	ZP_01062959	ZP_01865441	YP_431775	ZP_01895948
YP_001173956	ZP_03375554	YP_001188617	ZP_00135549	ZP_03699982
YP_003269532	YP_003548264	ZP_01893753	ZP_01112736	YP_431868
ZP_01157584	ZP_01115171	ZP_03369842	ZP_01955972	ZP_01955970
ZP_06938864	YP_002352611	YP_002250432	YP_002534909	NP_229009
YP_001245135	YP_002534307	YP_001739017	YP_001244560	YP_003346096
YP_002250571	CAA72193	NP_229635	YP_002352749	YP_001470540
NP_623418	ZP_05493657	YP_001662811	YP_002941091	YP_003672376
YP_146557	ZP_04392773	YP_003677351	ZP_05336993	YP_003477456
AAY89718	YP_001568299	YP_001410291	ZP_03385039	YP_001568464
YP_001124738	YP_604964	YP_003514612	ZP_03146818	ZP_04152680
ZP_04158384	YP_001305848	YP_001142178	ZP_04307633	YP_002509363
ZP_06220504	ZP_04121933	ZP_04204735	ZP_03232052	YP_003148220
ZP_04086064	ZP_04258265	ZP_04296475	ZP_02397670	ZP_00236932
ZP_04187645	ZP_03105903	ZP_03238270	YP_896372	YP_038073
YP_085351	NP_846464	ZP_04128078	ZP_04285672	YP_003186266
ZP_02948002	YP_002447583	CAB65651	NP_980358	ZP_04302212
ZP_03493519	ZP_02878398	ZP_04176050	ZP_05183404	ZP_04290906
ZP_04199019	ZP_04263630	YP_001646638	ZP_04229423	ZP_04170363
YP_002334500	BAB40635	ZP_04218748	ZP_02866107	YP_697032
YP_003258733	ZP_02953587	YP_177014	YP_051264	ZP_02638879
YP_003018517	ZP_04607307	ZP_03828721	YP_003116555	ZP_03832162
YP_002316535	NP_294284	NP_563259	YP_002785582	YP_002886868

GenBank Accession Number*				
ZP_06681977	ZP_05667047	ZP_05664221	ZP_03981553	ZP_06699376
YP_001488817	YP_001375943	ZP_00603967	ZP_05132228	YP_699603
YP_002574412	YP_001814130	YP_003445304	ZP_04878976	ZP_03055079
ZP_01861955	YP_002918149	YP_002240053	YP_001334109	YP_001813248
ZP_06549508	ZP_06329195	ZP_04864724	YP_001179261	YP_144918
YP_039671	NP_645005	NP_370738	YP_003465347	ZP_05877552
ZP_04016117	YP_001439475	BAI87082	ADI96692	CBI48099
YP_850342	YP_005257	ZP_00233442	YP_003613139	ZP_06315382
YP_946709	NP_391341	AAD42742	EFK15520	ZP_05300115
ZP_05298949	YP_001695468	ZP_02185856	YP_830242	YP_288893
NP_471563	YP_003208908	YP_002958966	YP_001836789	ZP_01827852
YP_415662	NP_359509	BAB18102	YP_002739191	YP_003154662
ZP_03497002	YP_003696753	YP_003684577	ZP_07053052	ZP_05231671
ZP_02711433	ZP_01821820	ZP_01821214	NP_346527	ZP_01408156
ZP_02186034	BAC10980	YP_003336687	NP_670540	YP_071605
ZP_06640983	YP_003723548	ZP_04853514	YP_002743432	YP_001399898
YP_077886	YP_002038696	NP_391296	ZP_06611384	ZP_06198009
YP_003339007	YP_003729851	ZP_03958786	ZP_01170933	YP_081353
YP_001719722	ZP_06059512	YP_001449426	ZP_06872846	ZP_04851795
YP_003241054	YP_002473273	YP_001396549	YP_920853	ZP_06190079
BAI87023	YP_001664668	YP_003704868	ZP_06899649	NP_693481
YP_003314160	YP_003334475	ZP_05647136	YP_003203952	YP_056254
ZP_06014674	ZP_05687198	YP_003506902	ZP_04449975	YP_002958266
YP_002948859	YP_003677653	ZP_06428097	YP_003781558	ZP_03925962
YP_001199499	NP_579667	YP_003003618	YP_003678645	ZP_03225712
ZP_01460386	YP_003326738	YP_002744243	YP_002746719	YP_002123617
AAA26922	YP_003477807	ZP_02329884	ZP_04431587	ZP_01461944
NP_242885	YP_003161478	ZP_06533915	YP_001137791	YP_003009537
NP_125870	YP_002308182	ZP_06885100	ZP_04876799	YP_001559408
YP_003699585	ZP_01130620	YP_184184	ZP_05621839	NP_243792
YP_002883083	YP_003637265	YP_003425427	YP_003061750	YP_002997014
ZP_07076764	ZP_05749331	YP_602654	NP_269430	NP_737480
ZP_06808450	YP_003009727	YP_002565304	YP_598732	YP_280516
YP_002881503	NP_784007	YP_002246441	ZP_02045060	YP_002509787
ZP_06608272	YP_002136599	ZP_06363693	EFK02214	ZP_05431814
YP_136875	ZP_06662656	YP_001880661	YP_177522	ZP_06048003
YP_002387348	ZP_01983428	YP_003229678	ZP_04402673	YP_467337
ZP_06365288	CAL69747	YP_002881799	ZP_04412511	ZP_01951248

* The amino acid and corresponding nucleotide sequences of the accession numbers in Table 1 are herein incorporated by reference.

In certain embodiments of the invention, the MBP comprises the amino acid sequence set forth in SEQ ID NO: 4 or 6 or fragment or variant thereof. Any nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4 or 6 or fragment or variant thereof, can be used in the methods and compositions of the present invention. In some embodiments of the invention, the nucleotide sequences of the invention will be optimized for expression in a host organism or cell of interest, particularly a plant, more particularly a crop plant, most particularly a maize plant.

As used herein, "Cry endotoxin" means a δ -endotoxin encoded by *cry* (crystal protein) genes that are located mainly on large plasmids in members of the genus *Bacillus*, although chromosomally encoded Cry endotoxins have been reported. See, Ben-Dov *et al.* (1996) *Appl. Environ. Microbiol.* 62:3140-3145; Berry *et al.* (2002) *Appl. Environ. Microbiol.* 68:5082-5095; Gonz  les *et al.* (1981) *Plasmid* 5:351-365; Lereclus *et al.* (1982) *Mol. Gen. Genet.* 186:391-398; and Trisrisook *et al.* (1990) *Appl. Environ. Microbiol.* 56:1710-1716. Cry endotoxins do not have a broad spectrum of activity, so they typically do not kill beneficial insects. Furthermore, Cry endotoxins are non-toxic to mammals, including humans, domesticated animals and wildlife.

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

As used herein, "insect pest" means an organism in the phylum Arthropoda that interferes with or is harmful to plant development and/or growth, and more specifically means an organism in the class Insecta. The class Insecta can be divided into two groups historically treated as subclasses: (1) wingless insects, known as Apterygota; and (2) winged insects, known as Pterygota. The insect pests can be adults, larvae or even ova. A preferred developmental stage for testing for pesticidal activity is larvae or other immature form of the insect pest. Methods of rearing insect larvae and performing bioassays are well known in the art. See, *e.g.*, Czapla & Lang (1990) *J. Econ. Entomol.* 83:2480-2485; Griffith & Smith (1977) *J. Aust. Ent. Soc.* 16:366; and Keiper & Foote (1996) *Hydrobiologia* 339:137-139; as

well as US Patent No. 5,351,643. For example, insect pests can be reared in total darkness at about 20°C to about 30°C and from about 30% to about 70% relative humidity.

5 Compositions comprising chimeric pesticidal polypeptide-encoding nucleic acid molecules and chimeric pesticidal polypeptides also are provided. The compositions include chimeric pesticidal polypeptides that comprise a solubility-enhancing polypeptide fused to a Cry endotoxin or biologically active fragment thereof. Nucleotide sequences that encode the chimeric pesticidal polypeptides can be derived from the chimeric pesticidal polypeptide sequence and produced using
10 any method known in the art. The compositions also include variants and fragments of the chimeric pesticidal-encoding nucleic acid molecules and chimeric pesticidal polypeptides. The isolated, chimeric pesticidal-encoding nucleic acid molecules can be used to create transgenic organisms, such as plants, that are resistant to an insect pest susceptible to the pesticidal polypeptide.

15 The presently disclosed methods and compositions provides for chimeric pesticidal polypeptides with improved efficacy and nucleic acid molecules encoding the chimeric pesticidal polypeptides. The chimeric pesticidal polypeptides comprise solubility-enhancing polypeptide fused (i.e., operably linked) to a Cry endotoxin or biologically active fragment thereof and have enhanced pesticidal activity against at
20 least one plant pest, when compared to the pesticidal activity of the Cry endotoxin or biologically active fragment thereof that has not been fused to a solubility-enhancing polypeptide.

As used herein, "pesticide," "pesticidal polypeptide," or "pesticidal protein" mean a polypeptide that is capable of killing the pest or inhibiting its growth, feeding
25 or reproduction. One of skill in the art understands that not all substances or mixtures thereof are equally effective against all pests. "Chimeric pesticidal polypeptides" or "chimeric pesticidal proteins" are "pesticidal polypeptides" or "pesticidal proteins", to which a solubility-enhancing polypeptide has been fused as disclosed herein. Of particular interest herein are pesticidal polypeptides and
30 chimeric pesticidal polypeptides that act as insecticides and thus have biological activity against insect pests.

As used herein, "pest" means an organism that interferes with or is harmful to plant development and/or growth. Examples of pests include, but are not limited to, algae, arachnids (e.g., acarids including mites and ticks), bacteria (e.g., plant
35 pathogens including *Xanthomonas* spp. and *Pseudomonas* spp.), crustaceans (e.g., pillbugs and sowbugs); fungi (e.g., members in the phylum Ascomycetes or

Basidiomycetes, and fungal-like organisms including Oomycetes such as *Pythium* spp. and *Phytophthora* spp.), insects, mollusks (e.g., snails and slugs), nematodes (e.g., soil-transmitted nematodes including *Clonorchis* spp., *Fasciola* spp., *Heterodera* spp., *Globodera* spp., *Opisthorchis* spp. and *Paragonimus* spp.),
 5 protozoans (e.g., *Phytomonas* spp.), viruses (e.g., *Comovirus* spp., *Cucumovirus* spp., *Cytorhabdovirus* spp., *Luteovirus* spp., *Nepovirus* spp., *Potyvirus* spp., *Tobamovirus* spp., *Tombusvirus* spp. and *Tospovirus* spp.), viroids, parasitic plants, and weeds.

Of particular interest herein are insect pests. As used herein, "insect pest"
 10 means an organism in the phylum Arthropoda that interferes with or is harmful to plant development and/or growth, and more specifically means an organism in the class Insecta. The class Insecta can be divided into two groups historically treated as subclasses: (1) wingless insects, known as Apterygota; and (2) winged insects, known as Pterygota. Examples of insect pests include, but are not limited to, insects
 15 in the orders Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mallophaga orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera and Thysanura, particularly Coleoptera and Lepidoptera. While technically not insects, arthropods such as arachnids, especially in the order Acari, are included in "insect pest." Insect pests include economically important
 20 agronomic, forest, greenhouse, nursery ornamentals, food and fiber, public and animal health, domestic and commercial structure, household, and stored product pests.

Insects of the order Lepidoptera include, but are not limited to, armyworms, cutworms, loopers, and heliothines in the family Noctuidae *Agrotis ipsilon* Hufnagel
 25 (black cutworm); *A. orthogonia* Morrison (western cutworm); *A. segetum* Denis & Schiffermüller (turnip moth); *A. subterranea* Fabricius (granulate cutworm); *Alabama argillacea* Hübner (cotton leaf worm); *Anticarsia gemmatilis* Hübner (velvetbean caterpillar); *Athetis mindara* Barnes and McDunnough (rough skinned cutworm); *Earias insulana* Boisduval (spiny bollworm); *E. vittella* Fabricius (spotted bollworm);
 30 *Egira (Xylomyges) curialis* Grote (citrus cutworm); *Euxoa messoria* Harris (darksided cutworm); *Helicoverpa armigera* Hübner (American bollworm); *H. zea* Boddie (corn earworm or cotton bollworm); *Heliothis virescens* Fabricius (tobacco budworm); *Hypena scabra* Fabricius (green cloverworm); *Hyponeuma taltula* Schaus; (*Mamestra configurata* Walker (bertha armyworm); *M. brassicae* Linnaeus (cabbage
 35 moth); *Melanchra picta* Harris (zebra caterpillar); *Mocis latipes* Guenée (small mocis moth); *Pseudaletia unipuncta* Haworth (armyworm); *Pseudoplusia includens* Walker

(soybean looper); *Richia albicosta* Smith (Western bean cutworm); *Spodoptera frugiperda* JE Smith (fall armyworm); *S. exigua* Hübner (beet armyworm); *S. litura* Fabricius (tobacco cutworm, cluster caterpillar); *Trichoplusia ni* Hübner (cabbage looper); borers, casebearers, webworms, coneworms, and skeletonizers from the families Pyralidae and Crambidae such as *Achroia grisella* Fabricius (lesser wax moth); *Amyelois transitella* Walker (naval orangeworm); *Anagasta kuehniella* Zeller (Mediterranean flour moth); *Cadra cautella* Walker (almond moth); *Chilo partellus* Swinhoe (spotted stalk borer); *C. suppressalis* Walker (striped stem/rice borer); *C. terrenellus* Pagenstecher (sugarcane stem borer); *Corcyra cephalonica* Stainton (rice moth); *Crambus caliginosellus* Clemens (corn root webworm); *C. teterrellus* Zincken (bluegrass webworm); *Cnaphalocrocis medinalis* Guenée (rice leaf roller); *Desmia funeralis* Hübner (grape leaf folder); *Diaphania hyalinata* Linnaeus (melon worm); *D. nitidalis* Stoll (pickleworm); *Diatraea flavipennella* Box; *D. grandiosella* Dyar (southwestern corn borer), *D. saccharalis* Fabricius (sugarcane borer); *Elasmopalpus lignosellus* Zeller (lesser cornstalk borer); *Eoreuma loftini* Dyar (Mexican rice borer); *Ephestia elutella* Hübner (tobacco (cacao) moth); *Galleria mellonella* Linnaeus (greater wax moth); *Hedylepta accepta* Butler (sugarcane leafroller); *Herpetogramma licarsisalis* Walker (sod webworm); *Homoeosoma electellum* Hulst (sunflower moth); *Loxostege sticticalis* Linnaeus (beet webworm); *Maruca testulalis* Geyer (bean pod borer); *Orthaga thyrsalis* Walker (tea tree web moth); *Ostrinia nubilalis* Hübner (European corn borer); *Plodia interpunctella* Hübner (Indian meal moth); *Scirpophaga incertulas* Walker (yellow stem borer); *Udea rubigalis* Guenée (celery leaf tier); and leafrollers, budworms, seed worms, and fruit worms in the family Tortricidae *Acleris gloverana* Walsingham (Western blackheaded budworm); *A. variana* Fernald (Eastern blackheaded budworm); *Adoxophyes orana* Fischer von Rösslerstamm (summer fruit tortrix moth); *Archips* spp. including *A. argyrospila* Walker (fruit tree leaf roller) and *A. rosana* Linnaeus (European leaf roller); *Argyrotaenia* spp.; *Bonagota salubricola* Meyrick (Brazilian apple leafroller); *Choristoneura* spp.; *Cochylis hospes* Walsingham (banded sunflower moth); *Cydia latiferreana* Walsingham (filbertworm); *C. pomonella* Linnaeus (codling moth); *Endopiza viteana* Clemens (grape berry moth); *Eupoecilia ambiguella* Hübner (vine moth); *Grapholita molesta* Busck (oriental fruit moth); *Lobesia botrana* Denis & Schiffermüller (European grape vine moth); *Platynota flavedana* Clemens (variegated leafroller); *P. stultana* Walsingham (omnivorous leafroller); *Spilonota ocellana* Denis & Schiffermüller (eyespotted bud moth); and *Suleima helianthana* Riley (sunflower bud moth).

Selected other agronomic pests in the order Lepidoptera include, but are not limited to, *Alsophila pometaria* Harris (fall cankerworm); *Anarsia lineatella* Zeller (peach twig borer); *Anisota senatoria* J.E. Smith (orange striped oakworm); *Antheraea pernyi* Guérin-Méneville (Chinese Oak Silkmoth); *Bombyx mori* Linnaeus (Silkworm); *Bucculatrix thurberiella* Busck (cotton leaf perforator); *Colias eurytheme* Boisduval (alfalfa caterpillar); *Datana integerrima* Grote & Robinson (walnut caterpillar); *Dendrolimus sibiricus* Tschetwerikov (Siberian silk moth), *Ennomos subsignaria* Hübner (elm spanworm); *Erannis tiliaria* Harris (linden looper); *Erechthias flavistriata* Walsingham (sugarcane bud moth); *Euproctis chrysorrhoea* Linnaeus (browntail moth); *Harrisina americana* Guérin-Méneville (grapeleaf skeletonizer); *Heliothis subflexa* Guenée; *Hemileuca oliviae* Cockrell (range caterpillar); *Hyphantria cunea* Drury (fall webworm); *Keiferia lycopersicella* Walsingham (tomato pinworm); *Lambdina fiscellaria fiscellaria* Hulst (Eastern hemlock looper); *L. fiscellaria lugubrosa* Hulst (Western hemlock looper); *Leucoma salicis* Linnaeus (satin moth); *Lymantria dispar* Linnaeus (gypsy moth); *Malacosoma* spp.; *Manduca quinquemaculata* Haworth (five spotted hawk moth, tomato hornworm); *M. sexta* Haworth (tomato hornworm, tobacco hornworm); *Operophtera brumata* Linnaeus (winter moth); *Orgyia* spp.; *Paleacrita vernata* Peck (spring cankerworm); *Papilio cresphontes* Cramer (giant swallowtail orange dog); *Phryganidia californica* Packard (California oakworm); *Phyllocnistis citrella* Stainton (citrus leafminer); *Phyllonorycter blancardella* Fabricius (spotted tentiform leafminer); *Pieris brassicae* Linnaeus (large white butterfly); *P. rapae* Linnaeus (small white butterfly); *P. napi* Linnaeus (green veined white butterfly); *Platyptilia carduidactyla* Riley (artichoke plume moth); *Plutella xylostella* Linnaeus (diamondback moth); *Pectinophora gossypiella* Saunders (pink bollworm); *Pontia protodice* Boisduval & Leconte (Southern cabbageworm); *Sabulodes aegrotata* Guenée (omnivorous looper); *Schizura concinna* J.E. Smith (red humped caterpillar); *Sitotroga cerealella* Olivier (Angoumois grain moth); *Telchin licus* Drury (giant sugarcane borer); *Thaumetopoea pityocampa* Schiffermüller (pine processionary caterpillar); *Tineola bisselliella* Hummel (webbing clothesmoth); *Tuta absoluta* Meyrick (tomato leafminer) and *Yponomeuta padella* Linnaeus (ermine moth).

Of interest are larvae and adults of the order Coleoptera including weevils from the families Anthribidae, Bruchidae, and Curculionidae including, but not limited to: *Anthonomus grandis* Boheman (boll weevil); *Cylindrocopturus adspersus* LeConte (sunflower stem weevil); *Diaprepes abbreviatus* Linnaeus (Diaprepes root weevil); *Hypera punctata* Fabricius (clover leaf weevil); *Lissorhoptrus oryzophilus*

Kuschel (rice water weevil); *Metamasius hemipterus hemipterus* Linnaeus (West Indian cane weevil); *M. hemipterus sericeus* Olivier (silky cane weevil); *Sitophilus granarius* Linnaeus (granary weevil); *S. oryzae* Linnaeus (rice weevil); *Smicronyx fulvus* LeConte (red sunflower seed weevil); *S. sordidus* LeConte (gray sunflower seed weevil); *Sphenophorus maidis* Chittenden (maize billbug); *S. livis* Vaurie (sugarcane weevil); *Rhabdoscelus obscurus* Boisduval (New Guinea sugarcane weevil); flea beetles, cucumber beetles, rootworms, leaf beetles, potato beetles, and leafminers in the family Chrysomelidae including, but not limited to: *Chaetocnema ectypa* Horn (desert corn flea beetle); *C. pulicaria* Melsheimer (corn flea beetle); *Colaspis brunnea* Fabricius (grape colaspis); *Diabrotica barberi* Smith & Lawrence (northern corn rootworm); *D. undecimpunctata howardi* Barber (southern corn rootworm); *D. virgifera virgifera* LeConte (western corn rootworm); *Leptinotarsa decemlineata* Say (Colorado potato beetle); *Oulema melanopus* Linnaeus (cereal leaf beetle); *Phyllotreta cruciferae* Goeze (corn flea beetle); *Zygogramma exclamationis* Fabricius (sunflower beetle); beetles from the family Coccinellidae including, but not limited to: *Epilachna varivestis* Mulsant (Mexican bean beetle); chafers and other beetles from the family Scarabaeidae including, but not limited to: *Antitrogon parvulus* Britton (Childers cane grub); *Cyclocephala borealis* Arrow (northern masked chafer, white grub); *C. immaculata* Olivier (southern masked chafer, white grub); *Dermolepida albohirtum* Waterhouse (Greyback cane beetle); *Euetheola humilis rugiceps* LeConte (sugarcane beetle); *Lepidiota frenchi* Blackburn (French's cane grub); *Tomarus gibbosus* De Geer (carrot beetle); *T. subtropicus* Blatchley (sugarcane grub); *Phyllophaga crinita* Burmeister (white grub); *P. latifrons* LeConte (June beetle); *Popillia japonica* Newman (Japanese beetle); *Rhizotrogus majalis* Razoumowsky (European chafer); carpet beetles from the family Dermestidae; wireworms from the family Elateridae, *Eleodes* spp., *Melanotus* spp. including *M. communis* Gyllenhal (wireworm); *Conoderus* spp.; *Limonius* spp.; *Agriotes* spp.; *Ctenicera* spp.; *Aeolus* spp.; bark beetles from the family Scolytidae; beetles from the family Tenebrionidae; beetles from the family Cerambycidae such as, but not limited to, *Migdolus fryanus* Westwood (longhorn beetle); and beetles from the Buprestidae family including, but not limited to, *Aphanisticus cochinchinae seminulum* Obenberger (leaf-mining buprestid beetle).

Adults and immatures of the order Diptera are of interest, including leafminers *Agromyza parvicornis* Loew (corn blotch leafminer); midges including, but not limited to: *Contarinia sorghicola* Coquillett (sorghum midge); *Mayetiola destructor* Say (Hessian fly); *Neolasioptera murtfeldtiana* Felt, (sunflower seed midge); *Sitodiplosis*

mosellana Géhin (wheat midge); fruit flies (Tephritidae), *Oscinella frit* Linnaeus (frit flies); maggots including, but not limited to: *Delia* spp. including *Delia platura* Meigen (seedcorn maggot); *D. coarctata* Fallen (wheat bulb fly); *Fannia canicularis* Linnaeus, *F. femoralis* Stein (lesser house flies); *Meromyza americana* Fitch (wheat stem maggot); *Musca domestica* Linnaeus (house flies); *Stomoxys calcitrans* Linnaeus (stable flies)); face flies, horn flies, blow flies, *Chrysomya* spp.; *Phormia* spp.; and other muscoid fly pests, horse flies *Tabanus* spp.; bot flies *Gastrophilus* spp.; *Oestrus* spp.; cattle grubs *Hypoderma* spp.; deer flies *Chrysops* spp.; *Melophagus ovinus* Linnaeus (keds); and other *Brachycera*, mosquitoes *Aedes* spp.; *Anopheles* spp.; *Culex* spp.; black flies *Prosimulium* spp.; *Simulium* spp.; biting midges, sand flies, sciarids, and other *Nematocera*.

Included as insects of interest are those of the order Hemiptera such as, but not limited to, the following families: Adelgidae, Aleyrodidae, Aphididae, Asterolecaniidae, Cercopidae, Cicadellidae, Cicadidae, Cixiidae, Coccidae, Coreidae, Dactylopiidae, Delphacidae, Diaspididae, Eriococcidae, Flatidae, Fulgoridae, Issidae, Lygaeidae, Margarodidae, Membracidae, Miridae ortheziidae, Pentatomidae, Phoenicococcidae, Phylloxeridae, Pseudococcidae, Psyllidae, Pyrrhocoridae and Tingidae.

Agronomically important members from the order Hemiptera include, but are not limited to: *Acrosternum hilare* Say (green stink bug); *Acyrtosiphon pisum* Harris (pea aphid); *Adelges* spp. (adelgids); *Adelphocoris rapidus* Say (rapid plant bug); *Anasa tristis* De Geer (squash bug); *Aphis craccivora* Koch (cowpea aphid); *A. fabae* Scopoli (black bean aphid); *A. gossypii* Glover (cotton aphid, melon aphid); *A. maidiradicis* Forbes (corn root aphid); *A. pomi* De Geer (apple aphid); *A. spiraecola* Patch (spirea aphid); *Aulacaspis tegalensis* Zehntner (sugarcane scale); *Aulacorthum solani* Kaltenbach (foxglove aphid); *Bemisia tabaci* Gennadius (tobacco whitefly, sweetpotato whitefly); *B. argentifolii* Bellows & Perring (silverleaf whitefly); *Blissus leucopterus leucopterus* Say (chinch bug); *Blotomatidae* spp.; *Brevicoryne brassicae* Linnaeus (cabbage aphid); *Cacopsylla pyricola* Foerster (pear psylla); *Calocoris norvegicus* Gmelin (potato capsid bug); *Chaetosiphon fragaefolii* Cockerell (strawberry aphid); *Cimicidae* spp.; *Coreidae* spp.; *Corythuca gossypii* Fabricius (cotton lace bug); *Cyrtopeltis modesta* Distant (tomato bug); *C. notatus* Distant (suckfly); *Deois flavopicta* Stål (spittlebug); *Dialeurodes citri* Ashmead (citrus whitefly); *Diaphnocoris chlorionis* Say (honeylocust plant bug); *Diuraphis noxia* Kurdjumov/Mordvilko (Russian wheat aphid); *Duplachionaspis divergens* Green (armored scale); *Dysaphis plantaginea* Paaserini (rosy apple aphid); *Dysdercus*

- suturellus* Herrich-Schäffer (cotton stainer); *Dysmicoccus boninsis* Kuwana (gray sugarcane mealybug); *Empoasca fabae* Harris (potato leafhopper); *Eriosoma lanigerum* Hausmann (woolly apple aphid); *Erythroneoura* spp. (grape leafhoppers); *Eumetopina flavipes* Muir (Island sugarcane planthopper); *Eurygaster* spp.;
- 5 *Euschistus servus* Say (brown stink bug); *E. variolarius* Palisot de Beauvois (one-spotted stink bug); *Graptostethus* spp. (complex of seed bugs); and *Hyalopterus pruni* Geoffroy (mealy plum aphid); *Icerya purchasi* Maskell (cottony cushion scale); *Labopidicola allii* Knight (onion plant bug); *Laodelphax striatellus* Fallen (smaller brown planthopper); *Leptoglossus corculus* Say (leaf-footed pine seed bug);
- 10 *Leptodictya tabida* Herrich-Schaeffer (sugarcane lace bug); *Lipaphis erysimi* Kaltenbach (turnip aphid); *Lygocoris pabulinus* Linnaeus (common green capsid); *Lygus lineolaris* Palisot de Beauvois (tarnished plant bug); *L. hesperus* Knight (Western tarnished plant bug); *L. pratensis* Linnaeus (common meadow bug); *L. rugulipennis* Poppius (European tarnished plant bug); *Macrosiphum euphorbiae*
- 15 Thomas (potato aphid); *Macrostelus quadrilineatus* Forbes (aster leafhopper); *Magicicada septendecim* Linnaeus (periodical cicada); *Mahanarva fimbriolata* Stål (sugarcane spittlebug); *M. posticata* Stål (little cicada of sugarcane); *Melanaphis sacchari* Zehntner (sugarcane aphid); *Melanaspis glomerata* Green (black scale); *Metopolophium dirhodum* Walker (rose grain aphid); *Myzus persicae* Sulzer (peach-
- 20 potato aphid, green peach aphid); *Nasonovia ribisnigri* Mosley (lettuce aphid); *Nephotettix cincticeps* Uhler (green leafhopper); *N. nigropictus* Stål (rice leafhopper); *Nezara viridula* Linnaeus (southern green stink bug); *Nilaparvata lugens* Stål (brown planthopper); *Nysius ericae* Schilling (false chinch bug); *Nysius raphanus* Howard (false chinch bug); *Oebalus pugnax* Fabricius (rice stink bug); *Oncopeltus fasciatus*
- 25 Dallas (large milkweed bug); *Orthops campestris* Linnaeus; *Pemphigus* spp. (root aphids and gall aphids); *Peregrinus maidis* Ashmead (corn planthopper); *Perkinsiella saccharicida* Kirkaldy (sugarcane delphacid); *Phylloxera devastatrix* Pergande (pecan phylloxera); *Planococcus citri* Risso (citrus mealybug); *Plesiocoris rugicollis* Fallen (apple capsid); *Poecillocapsus lineatus* Fabricius (four-lined plant bug);
- 30 *Pseudatomoscelis seriatus* Reuter (cotton fleahopper); *Pseudococcus* spp. (other mealybug complex); *Pulvinaria elongata* Newstead (cottony grass scale); *Pyrilla perpusilla* Walker (sugarcane leafhopper); *Pyrrhocoridae* spp.; *Quadraspidiotus perniciosus* Comstock (San Jose scale); *Reduviidae* spp.; *Rhopalosiphum maidis* Fitch (corn leaf aphid); *R. padi* Linnaeus (bird cherry-oat aphid); *Saccharicoccus*
- 35 *sacchari* Cockerell (pink sugarcane mealybug); *Scaptacoris castanea* Perty (brown root stink bug); *Schizaphis graminum* Rondani (greenbug); *Sipha flava* Forbes

(yellow sugarcane aphid); *Sitobion avenae* Fabricius (English grain aphid); *Sogatella furcifera* Horvath (white-backed planthopper); *Sogatodes oryzae* Muir (rice delphacid); *Spanagonicus albofasciatus* Reuter (whitemarked fleahopper); *Therioaphis maculata* Buckton (spotted alfalfa aphid); *Tinidae* spp.; *Toxoptera*
 5 *aurantii* Boyer de Fonscolombe (black citrus aphid); and *T. citricida* Kirkaldy (brown citrus aphid); *Trialeurodes abutiloneus* (bandedwinged whitefly) and *T. vaporariorum* Westwood (greenhouse whitefly); *Trioza diospyri* Ashmead (persimmon psylla); and *Typhlocyba pomaria* McAtee (white apple leafhopper).

Also included are adults and larvae of the order Acari (mites) such as *Aceria*
 10 *tosichella* Keifer (wheat curl mite); *Panonychus ulmi* Koch (European red mite); *Petrobia latens* Müller (brown wheat mite); *Steneotarsonemus bancrofti* Michael (sugarcane stalk mite); spider mites and red mites in the family Tetranychidae, *Oligonychus grypus* Baker & Pritchard, *O. indicus* Hirst (sugarcane leaf mite), *O. pratensis* Banks (Banks grass mite), *O. stickneyi* McGregor (sugarcane spider mite);
 15 *Tetranychus urticae* Koch (two spotted spider mite); *T. mcdanieli* McGregor (McDaniel mite); *T. cinnabarinus* Boisduval (carmine spider mite); *T. turkestani* Ugarov & Nikolski (strawberry spider mite), flat mites in the family Tenuipalpidae, *Brevipalpus lewisi* McGregor (citrus flat mite); rust and bud mites in the family Eriophyidae and other foliar feeding mites and mites important in human and animal
 20 health, i.e. dust mites in the family Epidermoptidae, follicle mites in the family Demodicidae, grain mites in the family Glycyphagidae, ticks in the order Ixodidae. *Ixodes scapularis* Say (deer tick); *I. holocyclus* Neumann (Australian paralysis tick); *Dermacentor variabilis* Say (American dog tick); *Amblyomma americanum* Linnaeus (lone star tick); and scab and itch mites in the families Psoroptidae, Pyemotidae, and
 25 Sarcoptidae.

Insect pests of the order Thysanura are of interest, such as *Lepisma saccharina* Linnaeus (silverfish); *Thermobia domestica* Packard (firebrat).

Additional arthropod pests covered include: spiders in the order Araneae such as *Loxosceles reclusa* Gertsch & Mulaik (brown recluse spider); and the *Latrodectus*
 30 *mactans* Fabricius (black widow spider); and centipedes in the order Scutigleromorpha such as *Scutigera coleoptrata* Linnaeus (house centipede). In addition, insect pests of the order Isoptera are of interest, including those of the termitidae family, such as, but not limited to, *Cornitermes cumulans* Kollar, *Cylindrotermes nordenskioeldi* Holmgren and *Pseudacanthotermes militaris* Hagen
 35 (sugarcane termite); as well as those in the Rhinotermitidae family including, but not limited to *Heterotermes tenuis* Hagen. Insects of the order Thysanoptera are also of

interest, including but not limited to thrips, such as *Stenchaetothrips minutus* van Deventer (sugarcane thrips).

The insect pests can be adults, larvae or even ova. A preferred developmental stage for testing for pesticidal activity is larvae or other immature form of the insect pest. Methods of rearing insect larvae and performing bioassays are well known in the art. See, e.g., Czapla & Lang (1990) *J. Econ. Entomol.* 83:2480-2485; Griffith & Smith (1977) *J. Aust. Ent. Soc.* 16:366; Keiper & Foote (1996) *Hydrobiologia* 339:137-139; and US Patent No. 5,351,643. For example, insect pests can be reared in total darkness at about 20°C to about 30°C and from about 30% to about 70% relative humidity.

The novel chimeric pesticidal polypeptides can exhibit improved pesticidal activity when compared to a pesticidal polypeptide lacking a solubility-enhancing polypeptide. As used herein, the term "improved pesticidal activity" refers to a polypeptide that has enhanced pesticidal activity following the presently disclosed methods relative to the activity of the corresponding pesticidal polypeptide lacking a solubility-enhancing polypeptide as made by the methods disclosed herein and/or to a polypeptide that is effective against a broader range of pests, and/or a polypeptide having specificity for a pest that is not susceptible to the toxicity of the polypeptide prior to modification of its sequence using the presently disclosed methods. A finding of improved or enhanced pesticidal activity requires a demonstration of an increase of pesticidal activity of at least 10%, against the pest target or at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 100%, 150%, 200% or 300% or greater increase of pesticidal activity relative to the pesticidal activity of the polypeptide prior to the addition of a solubility-enhancing polypeptide, as determined against the same pest.

For example, an improved pesticidal activity is provided where a wider or narrower range of pests is impacted by the polypeptide relative to the range of pests that is affected by the polypeptide prior to sequence modification. A wider range of impact may be desirable where versatility is desired, while a narrower range of impact may be desirable where, for example, beneficial insects might otherwise be impacted by use or presence of the pesticidal polypeptide. While the invention is not bound by any particular mechanism of action, an improved pesticidal activity may also be provided by changes in one or more characteristics of a polypeptide; for example, the stability or longevity of a polypeptide in an insect gut may be increased relative to the stability or longevity of a corresponding wild-type protein.

A “subject plant or plant cell” is one in which genetic alteration, such as transformation, has been affected as to a gene of interest or is a plant or plant cell which is descended from a plant or cell so altered and which comprises the alteration. A “control” or “control plant” or “control plant cell” provides a reference point for measuring changes in phenotype of the subject plant or plant cell.

A control plant or plant cell may comprise, for example: (a) a wild-type plant or cell, i.e., of the same genotype as the starting material for the genetic alteration which resulted in the subject plant or cell; (b) a plant or plant cell of the same genotype as the starting material but which has been transformed with a null construct (i.e. with a construct which has no known effect on the trait of interest, such as a construct comprising a marker gene); (c) a plant or plant cell which is a non-transformed segregant among progeny of a subject plant or plant cell; (d) a plant or plant cell genetically identical to the subject plant or plant cell but which is not exposed to conditions or stimuli that would induce expression of the gene of interest; or (e) the subject plant or plant cell itself, under conditions in which the gene of interest is not expressed.

As noted above, the methods involve generating novel chimeric pesticidal polypeptide sequences and the nucleotide sequences that encode such polypeptides. The novel chimeric pesticidal polypeptide sequences are produced by making a fusion protein comprising an amino acid sequence of a solubility-enhancing polypeptide operably linked to an amino acid sequence of a pesticidal protein, particularly a cry protein.

Any pesticidal protein can be used in the presently disclosed methods. In some embodiments, the pesticidal protein is a δ -endotoxin of *Bacillus* spp. The specific activity of δ -endotoxins is considered highly beneficial. Unlike most insecticides, the δ -endotoxins do not have a broad spectrum of activity, so they typically do not kill beneficial insects. Furthermore, the δ -endotoxins are non-toxic to mammals, including humans, domesticated animals, and wildlife. In particular embodiments, the δ -endotoxin is a Cry protein.

It is well known that naturally occurring δ -endotoxins are synthesized by *B. thuringiensis* sporulating cells as a proteinaceous crystalline inclusion protoxin. Upon being ingested by susceptible insect larvae, the microcrystals dissolve in the midgut, and the protoxin is transformed into a biologically active moiety by proteases characteristic of digestive enzymes located in the insect gut. The activated δ -endotoxin binds with high affinity to protein receptors on brush-border membrane vesicles. The epithelial cells lining the midgut are the primary target of the endotoxin

and are rapidly destroyed as a consequence of membrane perforation resulting from the formation of gated, cation-selective channels by the toxin. Both Cry and Cyt toxins are pore-forming toxins. The α -helix regions of the Cry toxins form the trans-membrane pore, whereas Cyt toxins insert into the membrane by forming a β -barrel comprised of β -sheets from each monomer.

Bt Cry proteins have five conserved sequence domains, and three conserved structural domains (see, e.g., de Maagd *et al.* (2001) *Trends Genetics* 17:193-199). The most amino-terminal conserved structural domain (Domain I) consists of seven alpha helices, with a central hydrophobic helix- α 5 encircled by six other amphipathic helices, and is involved in membrane insertion and pore formation. The second conserved structural domain (Domain II) consists of three antiparallel beta-sheets implicated in cell binding, and the most carboxy-terminal conserved structural domain (Domain III) consists of a beta-sandwich. Exposed regions in domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity. The location and properties of these domains are known to those of skill in the art. See, for example, Grochulski *et al.* (1995) *J Mol Biol* 254:447-464; Morse, Yamamoto, and Stroud (2001) *Structure* 9:409-417; Li *et al.* (1991) *Nature* 353:815-821; Galitsky *et al.* (2001) *Acta Cryst* D57:1101-1109; Boonserm *et al.* (2006) *J Bacteriol* 188:3391-3401; Boonserm *et al.* (2005) *J Mol Biol* 348:363-382; and Guo *et al.* (2009) *J Struct Biol* 168:259-266.

Bt Cyt proteins have a single α - β domain comprising two outer layers of α -helix hairpins wrapped around a β -sheet (Li, Koni, and Ellar (1996) *J Mol Biol* 257:129-152; and Cohen *et al.* (2008) *J Mol Biol* 380:820-827). The β -sheet is involved in membrane insertion.

A list of some known δ -endotoxins (Cry and Cyt endotoxins) and their GenBank Accession Nos. are listed in Table 2, which can be used as a source for nucleic and amino acid sequences for use in the methods disclosed herein.

Table 2

Endotoxin	Accession #		Endotoxin	Accession #
Cry1Aa1	AAA22353		Cry1Aa2	AAA22552
Cry1Aa3	BAA00257		Cry1Aa4	CAA31886
Cry1Aa5	BAA04468		Cry1Aa6	AAA86265
Cry1Aa7	AAD46139		Cry1Aa8	I26149
Cry1Aa9	BAA77213		Cry1Aa10	AAD55382
Cry1Aa11	CAA70856		Cry1Aa12	AAP80146

Endotoxin	Accession #		Endotoxin	Accession #
Cry1Aa13	AAM44305		Cry1Aa14	AAP40639
Cry1Aa15	AAY66993		Cry1Aa16	HQ439776
Cry1Aa17	HQ439788		Cry1Aa18	HQ439790
Cry1Aa19	HQ685121		Cry1Aa20	JF340156
Cry1Aa21	JN651496		Cry1Aa22	KC158223
Cry1Ab1	AAA22330		Cry1Ab2	AAA22613
Cry1Ab3	AAA22561		Cry1Ab4	BAA00071
Cry1Ab5	CAA28405		Cry1Ab6	AAA22420
Cry1Ab7	CAA31620		Cry1Ab8	AAA22551
Cry1Ab9	CAA38701		Cry1Ab10	A29125
Cry1Ab11	I12419		Cry1Ab12	AAC64003
Cry1Ab13	AAN76494		Cry1Ab14	AAG16877
Cry1Ab15	AAO13302		Cry1Ab16	AAK55546
Cry1Ab17	AAT46415		Cry1Ab18	AAQ88259
Cry1Ab19	AAW31761		Cry1Ab20	ABB72460
Cry1Ab21	ABS18384		Cry1Ab22	ABW87320
Cry1Ab23	HQ439777		Cry1Ab24	HQ439778
Cry1Ab25	HQ685122		Cry1Ab26	HQ847729
Cry1Ab27	JN135249		Cry1Ab28	JN135250
Cry1Ab29	JN135251		Cry1Ab30	JN135252
Cry1Ab31	JN135253		Cry1Ab32	JN135254
Cry1Ab33	AAS93798		Cry1Ab34	KC156668
Cry1Ab-like	AAK14336		Cry1Ab-like	AAK14337
Cry1Ab-like	AAK14338		Cry1Ab-like	ABG88858
Cry1Ac1	AAA22331		Cry1Ac2	AAA22338
Cry1Ac3	CAA38098		Cry1Ac4	Cry1Ac4
Cry1Ac5	AAA22339		Cry1Ac6	AAA86266
Cry1Ac7	AAB46989		Cry1Ac8	AAC44841
Cry1Ac9	AAB49768		Cry1Ac10	CAA05505
Cry1Ac11	CAA10270		Cry1Ac12	I12418
Cry1Ac13	AAD38701		Cry1Ac14	AAQ06607
Cry1Ac15	AAN07788		Cry1Ac16	AAU87037
Cry1Ac17	AAX18704		Cry1Ac18	AAY88347
Cry1Ac19	ABD37053		Cry1Ac20	ABB89046
Cry1Ac21	AAY66992		Cry1Ac22	ABZ01836
Cry1Ac23	CAQ30431		Cry1Ac24	ABL01535
Cry1Ac25	FJ513324		Cry1Ac26	FJ617446
Cry1Ac27	FJ617447		Cry1Ac28	ACM90319
Cry1Ac29	DQ438941		Cry1Ac30	GQ227507
Cry1Ac31	GU446674		Cry1Ac32	HM061081
Cry1Ac33	GQ866913		Cry1Ac34	HQ230364

Endotoxin	Accession #		Endotoxin	Accession #
Cry1Ac35	JF340157		Cry1Ac36	JN387137
Cry1Ac37	JQ317685		Cry1Ad1	AAA22340
Cry1Ad2	CAA01880		Cry1Ae1	AAA22410
Cry1Af1	AAB82749		Cry1Ag1	AAD46137
Cry1Ah1	AAQ14326		Cry1Ah2	ABB76664
Cry1Ah3	HQ439779		Cry1Ai1	AAO39719
Cry1Ai2	HQ439780		Cry1A-like	AAK14339
Cry1Ba1	CAA29898		Cry1Ba2	CAA65003
Cry1Ba3	AAK63251		Cry1Ba4	AAK51084
Cry1Ba5	ABO20894		Cry1Ba6	ABL60921
Cry1Ba7	HQ439781		Cry1Bb1	AAA22344
Cry1Bb2	HQ439782		Cry1Bc1	CAA86568
Cry1Bd1	AAD10292		Cry1Bd2	AAM93496
Cry1Be1	AAC32850		Cry1Be2	AAQ52387
Cry1Be3	ACV96720		Cry1Be4	HM070026
Cry1Bf1	CAC50778		Cry1Bf2	AAQ52380
Cry1Bg1	AAO39720		Cry1Bh1	HQ589331
Cry1Bi1	KC156700		Cry1Ca1	CAA30396
Cry1Ca2	CAA31951		Cry1Ca3	AAA22343
Cry1Ca4	CAA01886		Cry1Ca5	CAA65457
Cry1Ca6	AAF37224		Cry1Ca7	AAG50438
Cry1Ca8	AAM00264		Cry1Ca9	AAL79362
Cry1Ca10	AAN16462		Cry1Ca11	AAX53094
Cry1Ca12	HM070027		Cry1Ca13	HQ412621
Cry1Ca14	JN651493		Cry1Cb1	M97880
Cry1Cb2	AAG35409		Cry1Cb3	ACD50894
Cry1Cb-like	AAX63901		Cry1Da1	CAA38099
Cry1Da2	I76415		Cry1Da3	HQ439784
Cry1Db1	CAA80234		Cry1Db2	AAK48937
Cry1Dc1	ABK35074		Cry1Ea1	CAA37933
Cry1Ea2	CAA39609		Cry1Ea3	AAA22345
Cry1Ea4	AAD04732		Cry1Ea5	A15535
Cry1Ea6	AAL50330		Cry1Ea7	AAW72936
Cry1Ea8	ABX11258		Cry1Ea9	HQ439785
Cry1Ea10	ADR00398		Cry1Ea11	JQ652456
Cry1Eb1	AAA22346		Cry1Fa1	AAA22348
Cry1Fa2	AAA22347		Cry1Fa3	HM070028
Cry1Fa4	HM439638		Cry1Fb1	CAA80235
Cry1Fb2	BAA25298		Cry1Fb3	AAF21767
Cry1Fb4	AAC10641		Cry1Fb5	AAO13295
Cry1Fb6	ACD50892		Cry1Fb7	ACD50893

Endotoxin	Accession #		Endotoxin	Accession #
Cry1Ga1	CAA80233		Cry1Ga2	CAA70506
Cry1Gb1	AAD10291		Cry1Gb2	AAO13756
Cry1Gc1	AAQ52381		Cry1Ha1	CAA80236
Cry1Hb1	AAA79694		Cry1Hb2	HQ439786
Cry1H-like	AAF01213		Cry1Ia1	CAA44633
Cry1Ia2	AAA22354		Cry1Ia3	AAC36999
Cry1Ia4	AAB00958		Cry1Ia5	CAA70124
Cry1Ia6	AAC26910		Cry1Ia7	AAM73516
Cry1Ia8	AAK66742		Cry1Ia9	AAQ08616
Cry1Ia10	AAP86782		Cry1Ia11	CAC85964
Cry1Ia12	AAV53390		Cry1Ia13	ABF83202
Cry1Ia14	ACG63871		Cry1Ia15	FJ617445
Cry1Ia16	FJ617448		Cry1Ia17	GU989199
Cry1Ia18	ADK23801		Cry1Ia19	HQ439787
Cry1Ia20	JQ228426		Cry1Ia21	JQ228424
Cry1Ia22	JQ228427		Cry1Ia23	JQ228428
Cry1Ia24	JQ228429		Cry1Ia25	JQ228430
Cry1Ia26	JQ228431		Cry1Ia27	JQ228432
Cry1Ia28	JQ228433		Cry1Ia29	JQ228434
Cry1Ia30	JQ317686		Cry1Ia31	JX944038
Cry1Ia32	JX944039		Cry1Ia33	JX944040
Cry1Ib1	AAA82114		Cry1Ib2	ABW88019
Cry1Ib3	ACD75515		Cry1Ib4	HM051227
Cry1Ib5	HM070028		Cry1Ib6	ADK38579
Cry1Ib7	JN571740		Cry1Ib8	JN675714
Cry1Ib9	JN675715		Cry1Ib10	JN675716
Cry1Ib11	JQ228423		Cry1Ic1	AAC62933
Cry1Ic2	AAE71691		Cry1Id1	AAD44366
Cry1Id2	JQ228422		Cry1Ie1	AAG43526
Cry1Ie2	HM439636		Cry1Ie3	KC156647
Cry1Ie4	KC156681		Cry1If1	AAQ52382
Cry1Ig1	KC156701		Cry1I-like	AAC31094
Cry1I-like	ABG88859		Cry1Ja1	AAA22341
Cry1Ja2	HM070030		Cry1Ja3	JQ228425
Cry1Jb1	AAA98959		Cry1Jc1	AAC31092
Cry1Jc2	AAQ52372		Cry1Jd1	CAC50779
Cry1Ka1	AAB00376		Cry1Ka2	HQ439783
Cry1La1	AAS60191		Cry1La2	HM070031
Cry1Ma1	FJ884067		Cry1Ma2	KC156659
Cry1Na1	KC156648		Cry1Nb1	KC156678
Cry1-like	AAC31091		Cry2Aa1	AAA22335

Endotoxin	Accession #		Endotoxin	Accession #
Cry2Aa2	AAA83516		Cry2Aa3	D86064
Cry2Aa4	AAC04867		Cry2Aa5	CAA10671
Cry2Aa6	CAA10672		Cry2Aa7	CAA10670
Cry2Aa8	AAO13734		Cry2Aa9	AAO13750
Cry2Aa10	AAQ04263		Cry2Aa11	AAQ52384
Cry2Aa12	ABI83671		Cry2Aa13	ABL01536
Cry2Aa14	ACF04939		Cry2Aa15	JN426947
Cry2Ab1	AAA22342		Cry2Ab2	CAA39075
Cry2Ab3	AAG36762		Cry2Ab4	AAO13296
Cry2Ab5	AAQ04609		Cry2Ab6	AAP59457
Cry2Ab7	AAZ66347		Cry2Ab8	ABC95996
Cry2Ab9	ABC74968		Cry2Ab10	EF157306
Cry2Ab11	CAM84575		Cry2Ab12	ABM21764
Cry2Ab13	ACG76120		Cry2Ab14	ACG76121
Cry2Ab15	HM037126		Cry2Ab16	GQ866914
Cry2Ab17	HQ439789		Cry2Ab18	JN135255
Cry2Ab19	JN135256		Cry2Ab20	JN135257
Cry2Ab21	JN135258		Cry2Ab22	JN135259
Cry2Ab23	JN135260		Cry2Ab24	JN135261
Cry2Ab25	JN415485		Cry2Ab26	JN426946
Cry2Ab27	JN415764		Cry2Ab28	JN651494
Cry2Ac1	CAA40536		Cry2Ac2	AAG35410
Cry2Ac3	AAQ52385		Cry2Ac4	ABC95997
Cry2Ac5	ABC74969		Cry2Ac6	ABC74793
Cry2Ac7	CAL18690		Cry2Ac8	CAM09325
Cry2Ac9	CAM09326		Cry2Ac10	ABN15104
Cry2Ac11	CAM83895		Cry2Ac12	CAM83896
Cry2Ad1	AAF09583		Cry2Ad2	ABC86927
Cry2Ad3	CAK29504		Cry2Ad4	CAM32331
Cry2Ad5	CAO78739		Cry2Ae1	AAQ52362
Cry2Af1	ABO30519		Cry2Af2	GQ866915
Cry2Ag1	ACH91610		Cry2Ah1	EU939453
Cry2Ah2	ACL80665		Cry2Ah3	GU073380
Cry2Ah4	KC156702		Cry2Ai1	FJ788388
Cry2Aj			Cry2Ak1	KC156660
Cry2Ba1	KC156658		Cry3Aa1	AAA22336
Cry3Aa2	AAA22541		Cry3Aa3	CAA68482
Cry3Aa4	AAA22542		Cry3Aa5	AAA50255
Cry3Aa6	AAC43266		Cry3Aa7	CAB41411
Cry3Aa8	AAS79487		Cry3Aa9	AAW05659
Cry3Aa10	AAU29411		Cry3Aa11	AAW82872

Endotoxin	Accession #		Endotoxin	Accession #
Cry3Aa12	ABY49136		Cry3Ba1	CAA34983
Cry3Ba2	CAA00645		Cry3Ba3	JQ397327
Cry3Bb1	AAA22334		Cry3Bb2	AAA74198
Cry3Bb3	I15475		Cry3Ca1	CAA42469
Cry4Aa1	CAA68485		Cry4Aa2	BAA00179
Cry4Aa3	CAD30148		Cry4Aa4	AFB18317
Cry4A-like	AAAY96321		Cry4Ba1	CAA30312
Cry4Ba2	CAA30114		Cry4Ba3	AAA22337
Cry4Ba4	BAA00178		Cry4Ba5	CAD30095
Cry4Ba-like	ABC47686		Cry4Ca1	EU646202
Cry4Cb1	FJ403208		Cry4Cb2	FJ597622
Cry4Cc1	FJ403207		Cry5Aa1	AAA67694
Cry5Ab1	AAA67693		Cry5Ac1	I34543
Cry5Ad1	ABQ82087		Cry5Ba1	AAA68598
Cry5Ba2	ABW88931		Cry5Ba3	AFJ04417
Cry5Ca1	HM461869		Cry5Ca2	ZP_04123426
Cry5Da1	HM461870		Cry5Da2	ZP_04123980
Cry5Ea1	HM485580		Cry5Ea2	ZP_04124038
Cry6Aa1	AAA22357		Cry6Aa2	AAM46849
Cry6Aa3	ABH03377		Cry6Ba1	AAA22358
Cry7Aa1	AAA22351		Cry7Ab1	AAA21120
Cry7Ab2	AAA21121		Cry7Ab3	ABX24522
Cry7Ab4	EU380678		Cry7Ab5	ABX79555
Cry7Ab6	ACI44005		Cry7Ab7	ADB89216
Cry7Ab8	GU145299		Cry7Ab9	ADD92572
Cry7Ba1	ABB70817		Cry7Bb1	KC156653
Cry7Ca1	ABR67863		Cry7Cb1	KC156698
Cry7Da1	ACQ99547		Cry7Da2	HM572236
Cry7Da3	KC156679		Cry7Ea1	HM035086
Cry7Ea2	HM132124		Cry7Ea3	EEM19403
Cry7Fa1	HM035088		Cry7Fa2	EEM19090
Cry7Fb1	HM572235		Cry7Fb2	KC156682
Cry7Ga1	HM572237		Cry7Ga2	KC156669
Cry7Gb1	KC156650		Cry7Gc1	KC156654
Cry7Gd1	KC156697		Cry7Ha1	KC156651
Cry7Ia1	KC156665		Cry7Ja1	KC156671
Cry7Ka1	KC156680		Cry7Kb1	BAM99306
Cry7La1	BAM99307		Cry8Aa1	AAA21117
Cry8Ab1	EU044830		Cry8Ac1	KC156662
Cry8Ad1	KC156684		Cry8Ba1	AAA21118
Cry8Bb1	CAD57542		Cry8Bc1	CAD57543

Endotoxin	Accession #		Endotoxin	Accession #
Cry8Ca1	AAA21119		Cry8Ca2	AAR98783
Cry8Ca3	EU625349		Cry8Ca4	ADB54826
Cry8Da1	BAC07226		Cry8Da2	BD133574
Cry8Da3	BD133575		Cry8Db1	BAF93483
Cry8Ea1	AAQ73470		Cry8Ea2	EU047597
Cry8Ea3	KC855216		Cry8Fa1	AAT48690
Cry8Fa2	HQ174208		Cry8Fa3	AFH78109
Cry8Ga1	AAT46073		Cry8Ga2	ABC42043
Cry8Ga3	FJ198072		Cry8Ha1	AAW81032
Cry8Ia1	EU381044		Cry8Ia2	GU073381
Cry8Ia3	HM044664		Cry8Ia4	KC156674
Cry8Ib1	GU325772		Cry8Ib2	KC156677
Cry8Ja1	EU625348		Cry8Ka1	FJ422558
Cry8Ka2	ACN87262		Cry8Kb1	HM123758
Cry8Kb2	KC156675		Cry8La1	GU325771
Cry8Ma1	HM044665		Cry8Ma2	EEM86551
Cry8Ma3	HM210574		Cry8Na1	HM640939
Cry8Pa1	HQ388415		Cry8Qa1	HQ441166
Cry8Qa2	KC152468		Cry8Ra1	AFP87548
Cry8Sa1	JQ740599		Cry8Ta1	KC156673
Cry8-like	FJ770571		Cry8-like	ABS53003
Cry9Aa1	CAA41122		Cry9Aa2	CAA41425
Cry9Aa3	GQ249293		Cry9Aa4	GQ249294
Cry9Aa5	JX174110		Cry9Aa like	AAQ52376
Cry9Ba1	CAA52927		Cry9Ba2	GU299522
Cry9Bb1	AAV28716		Cry9Ca1	CAA85764
Cry9Ca2	AAQ52375		Cry9Da1	BAA19948
Cry9Da2	AAB97923		Cry9Da3	GQ249293
Cry9Da4	GQ249297		Cry9Db1	AAX78439
Cry9Dc1	KC156683		Cry9Ea1	BAA34908
Cry9Ea2	AAO12908		Cry9Ea3	ABM21765
Cry9Ea4	ACE88267		Cry9Ea5	ACF04743
Cry9Ea6	ACG63872		Cry9Ea7	FJ380927
Cry9Ea8	GQ249292		Cry9Ea9	JN651495
Cry9Eb1	CAC50780		Cry9Eb2	GQ249298
Cry9Eb3	KC156646		Cry9Ec1	AAC63366
Cry9Ed1	AAX78440		Cry9Ee1	GQ249296
Cry9Ee2	KC156664		Cry9Fa1	KC156692
Cry9Ga1	KC156699		Cry9-like	AAC63366
Cry10Aa1	AAA22614		Cry10Aa2	E00614
Cry10Aa3	CAD30098		Cry10Aa4	AFB18318

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

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

Endotoxin	Accession #		Endotoxin	Accession #
Cry52Ba1	FJ361760		Cry53Aa1	EF633476
Cry53Ab1	FJ361759		Cry54Aa1	ACA52194
Cry54Aa2	GQ140349		Cry54Ba1	GU446677
Cry55Aa1	ABW88932		Cry54Ab1	JQ916908
Cry55Aa2	AAE33526		Cry56Aa1	ACU57499
Cry56Aa2	GQ483512		Cry56Aa3	JX025567
Cry57Aa1	ANC87261		Cry58Aa1	ANC87260
Cry59Ba1	JN790647		Cry59Aa1	ACR43758
Cry60Aa1	ACU24782		Cry60Aa2	EAO57254
Cry60Aa3	EEM99278		Cry60Ba1	GU810818
Cry60Ba2	EAO57253		Cry60Ba3	EEM99279
Cry61Aa1	HM035087		Cry61Aa2	HM132125
Cry61Aa3	EEM19308		Cry62Aa1	HM054509
Cry63Aa1	BAI44028		Cry64Aa1	BAJ05397
Cry65Aa1	HM461868		Cry65Aa2	ZP_04123838
Cry66Aa1	HM485581		Cry66Aa2	ZP_04099945
Cry67Aa1	HM485582		Cry67Aa2	ZP_04148882
Cry68Aa1	HQ113114		Cry69Aa1	HQ401006
Cry69Aa2	JQ821388		Cry69Ab1	JN209957
Cry70Aa1	JN646781		Cry70Ba1	ADO51070
Cry70Bb1	EEL67276		Cry71Aa1	JX025568
Cry72Aa1	JX025569		Cyt1Aa	X03182
Cyt1Ab	X98793		Cyt1B	U37196
Cyt2A	Z14147		Cyt2B	U52043

* The amino acid and corresponding nucleotide sequences of the accession numbers in Table 2 are herein incorporated by reference.

Examples of δ -endotoxins also include but are not limited to Cry1A proteins of
5 US Patent Numbers 5,880,275 and 7,858,849; a DIG-3 or DIG-11 toxin (N-terminal
deletion of α -helix 1 and/or α -helix 2 variants of cry proteins such as Cry1A, Cry3A)
of US Patent Numbers 8,304,604, 8,304,605 and 8,476,226; Cry1B of US Patent
Application Serial Number 10/525,318; Cry1C of US Patent Number 6,033,874;
Cry1F of US Patent Numbers 5,188,960 and 6,218,188; Cry1A/F chimeras of US
10 Patent Numbers 7,070,982; 6,962,705 and 6,713,063); a Cry2 protein such as
Cry2Ab protein of US Patent Number 7,064,249); a Cry3A protein including but not
limited to an engineered hybrid insecticidal protein (eHIP) created by fusing unique
combinations of variable regions and conserved blocks of at least two different Cry
proteins (US Patent Application Publication Number 2010/0017914); a Cry4 protein;
15 a Cry5 protein; a Cry6 protein; Cry8 proteins of US Patent Numbers 7,329,736,

7,449,552, 7,803,943, 7,476,781, 7,105,332, 7,378,499 and 7,462,760; a Cry9 protein such as such as members of the Cry9A, Cry9B, Cry9C, Cry9D, Cry9E and Cry9F families; a Cry15 protein of Naimov, *et al.*, (2008) *Applied and Environmental Microbiology*, 74:7145-7151; a Cry22, a Cry34Ab1 protein of US Patent Numbers 6,127,180, 6,624,145 and 6,340,593; a CryET33 and cryET34 protein of US Patent Numbers 6,248,535, 6,326,351, 6,399,330, 6,949,626, 7,385,107 and 7,504,229; a CryET33 and CryET34 homologs of US Patent Publication Number 2006/0191034, 2012/0278954, and PCT Publication Number WO 2012/139004; a Cry35Ab1 protein of US Patent Numbers 6,083,499, 6,548,291 and 6,340,593; a Cry46 protein, a Cry 51 protein, a Cry binary toxin; a TIC901 or related toxin; TIC807 of US Patent Application Publication Number 2008/0295207; ET29, ET37, TIC809, TIC810, TIC812, TIC127, TIC128 of PCT US 2006/033867; TIC853 toxins of US Patent 8,513,494, AXMI-027, AXMI-036, and AXMI-038 of US Patent Number 8,236,757; AXMI-031, AXMI-039, AXMI-040, AXMI-049 of US Patent Number 7,923,602; AXMI-018, AXMI-020 and AXMI-021 of WO 2006/083891; AXMI-010 of WO 2005/038032; AXMI-003 of WO 2005/021585; AXMI-008 of US Patent Application Publication Number 2004/0250311; AXMI-006 of US Patent Application Publication Number 2004/0216186; AXMI-007 of US Patent Application Publication Number 2004/0210965; AXMI-009 of US Patent Application Number 2004/0210964; AXMI-014 of US Patent Application Publication Number 2004/0197917; AXMI-004 of US Patent Application Publication Number 2004/0197916; AXMI-028 and AXMI-029 of WO 2006/119457; AXMI-007, AXMI-008, AXMI-0080rf2, AXMI-009, AXMI-014 and AXMI-004 of WO 2004/074462; AXMI-150 of US Patent Number 8,084,416; AXMI-205 of US Patent Application Publication Number 2011/0023184; AXMI-011, AXMI-012, AXMI-013, AXMI-015, AXMI-019, AXMI-044, AXMI-037, AXMI-043, AXMI-033, AXMI-034, AXMI-022, AXMI-023, AXMI-041, AXMI-063 and AXMI-064 of US Patent Application Publication Number 2011/0263488; AXMI-R1 and related proteins of US Patent Application Publication Number 2010/0197592; AXMI221Z, AXMI222z, AXMI223z, AXMI224z and AXMI225z of WO 2011/103248; AXMI218, AXMI219, AXMI220, AXMI226, AXMI227, AXMI228, AXMI229, AXMI230 and AXMI231 of WO 2011/103247; AXMI-115, AXMI-113, AXMI-005, AXMI-163 and AXMI-184 of US Patent Number 8,334,431; AXMI-001, AXMI-002, AXMI-030, AXMI-035 and AXMI-045 of US Patent Application Publication Number 2010/0298211; AXMI-066 and AXMI-076 of US Patent Application Publication Number 2009/0144852; AXMI128, AXMI130, AXMI131, AXMI133, AXMI140, AXMI141, AXMI142, AXMI143, AXMI144, AXMI146, AXMI148, AXMI149, AXMI152, AXMI153, AXMI154, AXMI155, AXMI156,

AXMI157, AXMI158, AXMI162, AXMI165, AXMI166, AXMI167, AXMI168, AXMI169, AXMI170, AXMI171, AXMI172, AXMI173, AXMI174, AXMI175, AXMI176, AXMI177, AXMI178, AXMI179, AXMI180, AXMI181, AXMI182, AXMI185, AXMI186, AXMI187, AXMI188, AXMI189 of US Patent Number 8,318,900; AXMI079, AXMI080, AXMI081, AXMI082, AXMI091, AXMI092, AXMI096, AXMI097, AXMI098, AXMI099, AXMI100, AXMI101, AXMI102, AXMI103, AXMI104, AXMI107, AXMI108, AXMI109, AXMI110, AXMI111, AXMI112, AXMI114, AXMI116, AXMI117, AXMI118, AXMI119, AXMI120, AXMI121, AXMI122, AXMI123, AXMI124, AXMI1257, AXMI1268, AXMI127, AXMI129, AXMI164, AXMI151, AXMI161, AXMI183, AXMI132, AXMI138, AXMI137 of US Patent Application Publication Number 2010/0005543, cry proteins such as Cry1A and Cry3A having modified proteolytic sites of US Patent Number 8,319,019; a Cry1Ac, Cry2Aa and Cry1Ca toxin protein from *Bacillus thuringiensis* strain VBTS 2528 of US Patent Application Publication Number 2011/0064710. Other Cry proteins are well known to one skilled in the art (see, Crickmore, *et al.*, "Bacillus thuringiensis toxin nomenclature" (2011), at lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/ which can be accessed on the world-wide web using the "www" prefix). The insecticidal activity of Cry proteins is well known to one skilled in the art (for review, see, van Franckenhuysen, (2009) *J. Invert. Path.* 101:1-16). The use of Cry proteins as transgenic plant traits is well known to one skilled in the art and Cry-transgenic plants including but not limited to plants expressing Cry1Ac, Cry1Ac+Cry2Ab, Cry1Ab, Cry1A.105, Cry1F, Cry1Fa2, Cry1F+Cry1Ac, Cry2Ab, Cry3A, mCry3A, Cry3Bb1, Cry34Ab1, Cry35Ab1, Vip3A, mCry3A, Cry9c and CBI-Bt have received regulatory approval (see, Sanahuja, (2011) *Plant Biotech Journal* 9:283-300 and the CERA (2010) GM Crop Database Center for Environmental Risk Assessment (CERA), ILSI Research Foundation, Washington D.C. at cera-gmc.org/index.php?action=gm_crop_database, which can be accessed on the world-wide web using the "www" prefix).

The primary structure of a pesticidal polypeptide can be used in its native form or modified in order to introduce amino acid residues (either through addition or substitution) that contribute to or are suspected of contributing to the pesticidal activity of at least one pesticidal polypeptide. Functional data and results from structure-function analyses can be consulted to identify sequences and/or amino acid residues that contribute to pesticidal activity that should be retained or introduced into the candidate polypeptide sequence. Those residues that contribute to or are suspected of contributing to pesticidal activity include those residues that enhance

efficacy or those that dictate pesticidal specificity, including those residues that narrow or broaden the range of pests of pesticidal proteins.

For example, the aromaticity of the tyrosine and phenylalanine residues at position 249 and 264, respectively, in helix 7 of the *Bt* Cry4Ba toxin has been found to be important for toxicity (Tiewsiiri and Angsuthanasombat (2007) *J Biochem Mol Biol* 40:163-171). Thus, in those embodiments wherein the candidate polypeptide is homologous with the *Bt* Cry4Ba toxin, the residues at the positions corresponding to positions 249 and 264 of Cry4Ba should be maintained if they are aromatic or modified if they are non-aromatic.

Further, additional mutations can be introduced into pesticidal polypeptide sequence to improve the pesticidal activity. For example, as described in U.S. Patent Nos. 7,462,760 and 7,105,332 (each of which are herein incorporated by reference in its entirety), mutations can be introduced into the candidate polypeptide sequence to destroy proteolytic sites to protect the polypeptide from degradative digestion, for example, by plant proteases. As a further example, the toxicity of *Bt* Cry proteins can be improved by introducing at least one more protease-sensitive site (e.g., trypsin cleavage site) into the region located between alpha helices 3 and 4 of domain 1 of the endotoxin protein (see U.S. Patent Application Publication No. US2004/0091505, which is herein incorporated by reference in its entirety). As another non-limiting example, a protease-sensitive site that is readily cleaved by insect chymotrypsin, e.g., a chymotrypsin found in the bertha armyworm or the corn earworm (Hegedus *et al.* (2003) *Arch. Insect Biochem. Physiol.* 53: 30-47; and Lenz *et al.* (1991) *Arch. Insect Biochem. Physiol.* 16: 201-212), may be added to the candidate polypeptide sequence to provide improved toxicity to the polypeptide.

In certain embodiments of the invention, the Cry protein comprises the amino acid sequence set forth in SEQ ID NO: 8, 10, 12 or 14 or fragment or variant thereof. Any nucleotide sequence encoding the amino acid sequence set forth in 8, 10, 12 or 14 or fragment or variant thereof, can be used in the methods and compositions of the present invention including, but not limited to the nucleotides sequences encoding SEQ ID NOS: 8, 10, 12, and 14 which are set forth in SEQ ID NOS: 7, 9, 11, and 13, respectively. In some embodiments of the invention, the nucleotide sequences of the invention will be optimized for expression in a host organism or cell of interest, particularly a plant, more particularly a crop plant, most particularly a maize plant.

Methods of measuring pesticidal activity by insect bioassays are well known in the art. See, e.g., Brooke *et al.* (2001) *Bull. Entomol. Res.* 91:265-272; Chen *et al.*

(2007) *Proc. Natl. Acad. Sci. USA* 104:13901-13906; Crespo *et al.* (2008) *Appl. Environ. Microb.* 74:130-135; Khambay *et al.* (2003) *Pest Manag. Sci.* 59:174-182; Liu & Dean (2006) *Protein Eng. Des. Sel.* 19:107-111; Marrone *et al.* (1985) *J. Econ. Entomol.* 78:290-293; Robertson *et al.*, *Pesticide Bioassays with Arthropods* (2nd ed., CRC Press 2007); Scott & McKibben (1976) *J. Econ. Entomol.* 71:343-344; Strickman (1985) *Bull. Environ. Contam. Toxicol.* 35:133-142; and Verma *et al.* (1982) *Water Res.* 16 525-529; as well as US Patent No. 6,268,181. Examples of insect bioassays include, but are not limited to, pest mortality, pest weight loss, pest repellency, pest attraction, and other behavioral and physical changes of the pest after feeding and exposure to a pesticide or pesticidal polypeptide for an appropriate length of time. General methods include addition of the pesticide, pesticidal polypeptide or an organism having the pesticidal polypeptide to the diet source in an enclosed container. See, e.g., U.S. Patent Nos. 6,339,144 and 6,570,005.

Further, a nucleic acid encoding the novel chimeric pesticidal polypeptide can be derived from the amino acid sequence and can be generated using any method known in the art. Therefore, novel isolated pesticidal polypeptides and isolated nucleic acid molecules encoding the same are provided. An "isolated" or "purified" polynucleotide or protein or biologically active fragment thereof, is substantially or essentially free from components that normally accompany or interact with the polynucleotide or protein as found in its naturally occurring environment. Thus, an isolated or purified polynucleotide or protein is substantially free of other cellular material or culture medium when produced by recombinant techniques or substantially free of chemical precursors or other chemicals when chemically synthesized. Optimally, an "isolated" polynucleotide is free of sequences (optimally protein encoding sequences) that naturally flank the polynucleotide (i.e., sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide is derived. For example, in various embodiments, the isolated polynucleotide can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequence that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, 5% or 1% (by dry weight) of contaminating protein. When the protein of the invention or biologically active fragment thereof is recombinantly produced, optimally culture medium represents less than about 30%, 20%, 10%, 5% or 1% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

Further provided are novel chimeric pesticidal polypeptides comprising fragments and variants of a solubility-enhancing polypeptide and/or pesticidal protein as well as nucleic acid molecules encoding such novel chimeric pesticidal polypeptides. Fragments and variants of the novel chimeric pesticidal polypeptides and chimeric pesticidal polypeptide-encoding nucleic acid molecules are also provided. By "fragment" is intended a portion of the polynucleotide or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a polynucleotide may encode protein fragments that retain solubility-enhancing activity and/or pesticidal activity. Alternatively, fragments of a polynucleotide that are useful as hybridization probes generally do not encode fragment proteins retaining a solubility-enhancing and/or pesticidal activity. Thus, fragments of a nucleotide sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the full-length polynucleotide encoding the novel chimeric pesticidal polypeptides.

A fragment of a pesticide-encoding polynucleotide that encodes a biologically active fragment of a solubility-enhancing polypeptide or pesticidal protein will encode at least 15, 25, 30, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000 or 1,100 contiguous amino acids or up to the total number of amino acids present in a chimeric pesticidal polypeptide, a solubility-enhancing polypeptide or a pesticidal protein of the invention. Fragments of a chimeric pesticidal polypeptide, solubility-enhancing polypeptide or a pesticidal protein that are useful as hybridization probes or PCR primers generally need not encode a biologically active fragment of a chimeric pesticidal polypeptide, a solubility-enhancing polypeptide or a pesticidal protein.

Thus, a fragment of a chimeric pesticidal polypeptide, a solubility-enhancing polypeptide or a pesticidal protein may encode a biologically active fragment of a pesticidal protein or it may be a fragment that can be used as a hybridization probe or PCR primer using methods well known in the art and disclosed elsewhere herein. A biologically active fragment of a pesticidal protein can be prepared by isolating a portion of one of the pesticide-encoding polynucleotides, expressing the encoded portion of the pesticidal protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the pesticidal protein. Polynucleotides that are fragments of a chimeric pesticidal polypeptide, a solubility-enhancing polypeptide or a pesticidal protein sequence comprise at least 16, 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2500 or 3000

contiguous nucleotides or up to the number of nucleotides present in a full-length pesticide-encoding polynucleotide discovered using the methods disclosed herein.

"Variants" is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a polynucleotide having deletions (i.e., truncations) at the 5' and/or 3' end; deletion and/or addition of one or more nucleotides at one or more internal sites in the native polynucleotide; and/or substitution of one or more nucleotides at one or more sites in the native polynucleotide. As used herein, a "native" polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively. For polynucleotides, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the chimeric pesticidal polypeptide, solubility-enhancing polypeptides or pesticidal proteins of the invention. Naturally occurring allelic variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant polynucleotides also include synthetically derived polynucleotides, such as those generated, for example, by using site-directed mutagenesis but which still encode a chimeric pesticidal polypeptide, a solubility-enhancing polypeptide or a pesticidal protein of the invention. Generally, variants of a particular polynucleotide of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that particular polynucleotide as determined by sequence alignment programs and parameters as described elsewhere herein.

Variants of a particular polynucleotide of the invention (i.e., the reference polynucleotide) can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant polynucleotide and the polypeptide encoded by the reference polynucleotide. Thus, for example, an isolated polynucleotide that encodes a polypeptide with a given percent sequence identity to the polypeptide of SEQ ID NO: 2 is disclosed. Percent sequence identity between any two polypeptides can be calculated using sequence alignment programs and parameters described elsewhere herein. Where any given pair of polynucleotides of the invention is evaluated by comparison of the percent sequence identity shared by the two polypeptides they encode, the percent sequence identity between the two encoded polypeptides is at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity.

"Variant" protein is intended to mean a protein derived from the native protein by deletion (so-called truncation) of one or more amino acids at the N-terminal and/or C-terminal end of the native protein; deletion and/or addition of one or more amino acids at one or more internal sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, pesticidal activity as described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a chimeric pesticidal polypeptide of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs and parameters described elsewhere herein. A biologically active variant of a protein of the invention may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2 or even 1 amino acid residue.

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

The deletions, insertions, and substitutions of the novel chimeric pesticidal polypeptide sequences, pesticidal polypeptide sequences and solubility-enhancing polypeptide sequences encompassed herein are not expected to produce radical

changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. That is, the pesticidal activity can be evaluated using an insect feeding
5 bioassays as described elsewhere herein. Preferably, the biological activity of a solubility-enhancing polypeptide or fragment or variant thereof is evaluated by operably linking the solubility-enhancing polypeptide, fragment or variant to a pesticidal polypeptide and assaying the pesticidal activity of the resulting chimeric pesticidal polypeptide using the methods disclosed herein or otherwise known in the
10 art. The pesticidal activity of the chimeric pesticidal polypeptide can be compared to the pesticidal activity of the pesticidal peptide (i.e., without the solubility-enhancing polypeptide, fragment or variant) to determine if the solubility-enhancing polypeptide, fragment or variant increases the insecticidal activity the chimeric pesticidal polypeptide. Preferably, in any insect feeding assays in which the chimeric pesticidal
15 polypeptide its corresponding pesticidal polypeptide, the amount of the respective polypeptides that are fed to the insects will be adjusted to take into the account the large molecular weight of the chimeric pesticidal polypeptide such that pesticidal activity comparisons will effectively be made on a per molecule or per mole basis. Furthermore, it is recognized that insecticidal activity of chimeric pesticidal
20 polypeptides with different solubility-enhancing polypeptides or fragments or variants of a solubility-enhancing polypeptide can also evaluated and compared to one another in a like manner and preferably with adjustments for any differences in molecular weights between the chimeric pesticidal polypeptides that are being compared.

25 Variant polynucleotides and proteins also encompass sequences and proteins derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different pesticide-coding sequences can be manipulated to create a new pesticidal polypeptide possessing the desired properties and these new pesticidal proteins can be used in the methods disclosed
30 herein to make chimeric pesticidal polypeptides. 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*. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad.*
35 *Sci. USA* 91:10747-10751; Stemmer (1994) *Nature* 370:389-391; Crameri *et al.* (1997) *Nature Biotech.* 15:436-438; Moore *et al.* (1997) *J. Mol. Biol.* 272:336-347;

Zhang *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:4504-4509; Crameri *et al.* (1998) *Nature* 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

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

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

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

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent sequence identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local alignment algorithm of Smith *et al.* (1981) *Adv. Appl. Math.* 2:482; the global alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-local alignment method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; 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.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the GCG Wisconsin Genetics Software Package, Version 10 (available from Accelrys Inc., 9685 Scranton Road,

San Diego, California, USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins *et al.* (1988) *Gene* 73:237-244 (1988); Higgins *et al.* (1989) *CABIOS* 5:151-153; Corpet *et al.* (1988) *Nucleic Acids Res.* 16:10881-90; Huang *et al.* (1992) *CABIOS* 8:155-65; and Pearson *et al.* (1994) *Meth. Mol. Biol.* 24:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (*e.g.*, BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.nlm.nih.gov. Alignment may also be performed manually by inspection.

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

GAP uses the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453, to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of

matched bases and the fewest gaps. It allows for the provision of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the GCG Wisconsin Genetics Software Package for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 200. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or greater.

GAP presents one member of the family of best alignments. There may be many members of this family, but no other member has a better quality. GAP displays four figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the GCG Wisconsin Genetics Software Package is BLOSUM62 (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

(c) As used herein, "sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well

known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

In hybridization techniques, all or part of a known polynucleotide is used as a probe that selectively hybridizes to other corresponding polynucleotides present in a population of cloned genomic DNA fragments or cDNA fragments (*i.e.*, genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments or other oligonucleotides, and may be labeled with a detectable group such as ³²P or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the babyboom polynucleotide. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

For example, the entire pesticide-encoding polynucleotide or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding pesticide-encoding polynucleotides and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among pesticide-encoding polynucleotide sequences and are optimally at least about 10 nucleotides in length, and most optimally at least

about 20 nucleotides in length. Such probes may be used to amplify corresponding pesticide-encoding polynucleotides from a chosen organism by PCR. This technique may be used to isolate additional coding sequences from a desired organism or as a diagnostic assay to determine the presence of coding sequences in an organism.

5 Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

10 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
15 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, optimally less than 500 nucleotides in length.

20 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
25 addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1%
30 SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a 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. The duration of the wash
35 time will be at least a length of time sufficient to reach equilibrium.

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 T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5^{\circ}\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61$
 5 $(\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, $\%GC$ is the percentage of guanosine and cytosine nucleotides in the DNA, $\% \text{ form}$ 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
 10 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 $\geq 90\%$ 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
 15 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 (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15 or
 20 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 optimal to increase the SSC
 25 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 *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York).
 30 See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

The use of the term "polynucleotide" is not intended to limit the present invention to polynucleotides comprising DNA. Those of ordinary skill in the art will recognize that polynucleotides can comprise ribonucleotides and combinations of
 35 ribonucleotides and deoxyribonucleotides. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules and synthetic analogues.

The polynucleotides of the invention also encompass all forms of sequences including, but not limited to, single-stranded forms, double-stranded forms, hairpins, stem-and-loop structures, and the like.

Nucleic acids encoding the novel chimeric pesticidal polypeptides can be
5 used in DNA constructs for expression in various hosts, including plants. Thus, expression cassettes comprising the novel chimeric pesticidal polypeptide-encoding nucleic acids for expression in the organism of interest are provided. The cassette will include 5' and 3' regulatory sequences operably linked to a novel chimeric
10 pesticidal polypeptide-encoding polynucleotide. "Operably linked" is intended to mean a functional linkage between two or more elements. For example, an operable linkage between a polynucleotide of interest and a regulatory sequence (i.e., a promoter) is a functional link that allows for expression of the polynucleotide of interest. In the case of fusion proteins or fusion polypeptides, an operable linkage between, for example, a first amino acid sequence of interest and a second amino
15 acid of interest results in a single amino acid sequence, which comprises both the first and second amino acid sequences, and wherein the C-terminal amino acid of the first amino acid sequence is covalently attached to the N-terminal amino acid of the second amino acid sequence by a peptide bond. Operably linked elements may be contiguous or non-contiguous. The cassette may additionally contain at least one
20 additional polynucleotide of interest to be cotransformed into the organism. Alternatively, the additional polynucleotide(s) of interest can be provided on multiple expression cassettes. Such an expression cassette is provided with a plurality of restriction sites and/or recombination sites for insertion of the pesticide-encoding polynucleotide to be under the transcriptional regulation of the regulatory regions.
25 The expression cassette may additionally contain selectable marker genes.

The chimeric pesticidal polypeptides of the present invention comprise an amino acid sequence of a solubility-enhancing polypeptide operably linked to an amino acid sequence of a Cry endotoxin. It is recognized that in such an operable linkage, the amino acid sequence of the solubility-enhancing polypeptide can be
30 contiguous to the amino acid sequence of the Cry endotoxin or can be separated by an intervening linker or other amino acid sequence. Likewise, the chimeric pesticidal polypeptide-encoding nucleic acid molecules of the present invention comprise a nucleotide sequence encoding a solubility-enhancing polypeptide operably linked to a nucleotide sequence encoding a Cry endotoxin. It is further recognized that in such
35 an operable linkage, the nucleotide sequence encoding the solubility-enhancing polypeptide can be contiguous to the nucleotide sequence encoding the Cry

endotoxin or can be separated by an intervening linker-encoding nucleotide sequence or other nucleotide sequence.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a novel pesticide-
5 encoding polynucleotide, and a transcriptional and translational termination region (i.e., termination region) functional in the organism to which the expression cassette is introduced. The regulatory regions (i.e., promoters, transcriptional regulatory regions, and translational termination regions) and/or the pesticide-encoding polynucleotide may be native/analogous to the host cell or to each other.
10 Alternatively, the regulatory regions and/or the pesticide-encoding polynucleotide may be heterologous to the host cell or to each other. As used herein, "heterologous" in reference to a sequence is a sequence that originates from a foreign species or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For
15 example, a promoter operably linked to a heterologous polynucleotide is from a species different from the species from which the polynucleotide was derived or, if from the same/analogous species, one or both are substantially modified from their original form and/or genomic locus or the promoter is not the native promoter for the operably linked polynucleotide.

20 The termination region may be native with the transcriptional initiation region, may be native with the operably linked pesticidal polynucleotide 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 pesticidal polynucleotide of interest, the plant host or any combination thereof. Convenient termination regions are available from the
25 Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon *et al.* (1991) *Genes Dev.* 5:141-149; Mogen *et al.* (1990) *Plant Cell* 2:1261-1272; Munroe *et al.* (1990) *Gene* 91:151-158; Ballas *et al.* (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi *et al.* (1987)
30 *Nucleic Acids Res.* 15:9627-9639.

Where appropriate, the polynucleotides may be optimized for increased expression in the transformed plant. That is, the polynucleotides can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92:1-11 for a discussion of host-preferred codon
35 usage. Methods are available in the art for synthesizing plant-preferred genes. See,

for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious
5 polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin
10 secondary mRNA structures.

The expression cassettes may additionally contain 5' leader sequences. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein *et al.* (1989) *Proc. Natl.*
15 *Acad. Sci. USA* 86:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Gallie *et al.* (1995) *Gene* 165(2):233-238), MDMV leader (Maize Dwarf Mosaic Virus) (*Virology* 154:9-20), and human immunoglobulin heavy-chain binding protein (BiP) (Macejak *et al.* (1991) *Nature* 353:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling *et al.* (1987) *Nature*
20 325:622-625); tobacco mosaic virus leader (TMV) (Gallie *et al.* (1989) in *Molecular Biology of RNA*, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology* 81:382-385). See also, Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968.

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

The chimeric pesticidal polypeptide-encoding nucleic acid molecules, expression cassettes comprising the same or the chimeric pesticidal polypeptides can be introduced into an organism or host cell, particularly a non-human host cell.
35 Host cells may be prokaryotic cells such as *E. coli* or eukaryotic cells such as yeast,

insect, amphibian or mammalian cells. In some examples, host cells are monocotyledonous or dicotyledonous plant cells.

The nucleic acid encoding the novel chimeric pesticidal polypeptides can be introduced into microorganisms that multiply on plants (epiphytes) to deliver chimeric
5 pesticidal polypeptides to potential target pests. Alternatively, the chimeric pesticidal polypeptides can be directly introduced into such microorganisms. Epiphytes, for example, can be gram-positive or gram-negative bacteria.

Root-colonizing bacteria, for example, can be isolated from the plant of interest by methods known in the art. Specifically, a *Bacillus cereus* strain that
10 colonizes roots can be isolated from roots of a plant (see, for example, Handelsman *et al.* (1991) *Appl. Environ. Microbiol.* 56:713-718). Nucleic acids encoding the pesticidal proteins of the invention or the chimeric pesticidal polypeptides can be introduced into a root-colonizing *Bacillus cereus* by standard methods known in the art.

15 Nucleic acids encoding chimeric pesticidal polypeptides can be introduced, for example, into the root-colonizing *Bacillus* by means of electrotransformation. Specifically, nucleic acids encoding the pesticidal proteins can be cloned into a shuttle vector, for example, pHT3101 (Lerecius *et al.* (1989) *FEMS Microbiol. Letts.* 60: 211-218. The shuttle vector pHT3101 containing the coding sequence for the
20 particular chimeric pesticidal polypeptide-encoding nucleic acid can, for example, be transformed into the root-colonizing *Bacillus* by means of electroporation (Lerecius *et al.* (1989) *FEMS Microbiol. Letts.* 60: 211-218).

Expression systems can be designed so that chimeric pesticidal polypeptides are secreted outside the cytoplasm of gram-negative bacteria, such as *E. coli*, for
25 example. Advantages of having chimeric pesticidal polypeptides secreted are: (1) avoidance of potential cytotoxic effects of the chimeric pesticidal polypeptides expressed; and (2) improvement in the efficiency of purification of the chimeric pesticidal polypeptides, including, but not limited to, increased efficiency in the recovery and purification of the protein per volume cell broth and decreased time
30 and/or costs of recovery and purification per unit protein.

Chimeric pesticidal polypeptides can be made to be secreted in *E. coli*, for example, by fusing an appropriate *E. coli* signal peptide to the amino-terminal end of the pesticidal protein. Signal peptides recognized by *E. coli* can be found in proteins already known to be secreted in *E. coli*, for example the OmpA protein (Ghrayeb *et al.* (1984) *EMBO J*, 3:2437-2442). OmpA is a major protein of the *E. coli* outer
35 membrane, and thus its signal peptide is thought to be efficient in the translocation

process. Also, the OmpA signal peptide does not need to be modified before processing as may be the case for other signal peptides, for example lipoprotein signal peptide (Duffaud *et al.* (1987) *Meth. Enzymol.* 153: 492).

Chimeric pesticidal polypeptides of the invention can be fermented in a bacterial host and the resulting bacteria processed and used as a microbial spray in the same manner that *Bacillus thuringiensis* strains have been used as insecticidal sprays. In the case of a chimeric pesticidal polypeptide(s) that is secreted from *Bacillus*, the secretion signal is removed or mutated using procedures known in the art. Such mutations and/or deletions prevent secretion of the chimeric pesticidal polypeptide(s) into the growth medium during the fermentation process. The chimeric pesticidal polypeptides are retained within the cell, and the cells are then processed to yield the encapsulated chimeric pesticidal polypeptides. Any suitable microorganism can be used for this purpose. *Pseudomonas* has been used to express *Bacillus thuringiensis* endotoxins as encapsulated proteins and the resulting cells processed and sprayed as an insecticide (Gaertner *et al.* (1993), in: *Advanced Engineered Pesticides*, ed. Kim).

Alternatively, the chimeric pesticidal polypeptides are produced by introducing a heterologous nucleic acid encoding the chimeric pesticidal polypeptide into a cellular host. Expression of the heterologous nucleic acid results, directly or indirectly, in the intracellular production and maintenance of the pesticide. These cells are then treated under conditions that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s). The resulting product retains the toxicity of the toxin. These naturally encapsulated chimeric pesticidal polypeptides 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.

The novel chimeric pesticidal polypeptide-encoding polynucleotides and chimeric pesticidal polypeptides can be introduced into plant cells or plants to produce a transgenic plant that is resistant to pests that are susceptible to the chimeric pesticidal polypeptide.

The novel chimeric pesticidal polypeptide-encoding polynucleotides can be combined with constitutive, tissue-preferred or other promoters for expression in plants. The promoters can be selected based on the desired outcome.

Such constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and

U.S. Patent No. 6,072,050; the core CaMV 35S promoter (Odell *et al.* (1985) *Nature* 313:810-812); rice actin (McElroy *et al.* (1990) *Plant Cell* 2:163-171); ubiquitin (Christensen *et al.* (1989) *Plant Mol. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last *et al.* (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten *et al.* (1984) *EMBO J.* 3:2723-2730); ALS promoter (U.S. Patent No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611.

Generally, it will be beneficial to express the gene from an inducible promoter, particularly from a pathogen-inducible promoter. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi *et al.* (1983) *Neth. J. Plant Pathol.* 89:245-254; Uknes *et al.* (1992) *Plant Cell* 4:645-656; and Van Loon (1985) *Plant Mol. Virol.* 4:111-116. See also WO 99/43819, herein incorporated by reference.

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau *et al.* (1987) *Plant Mol. Biol.* 9:335-342; Matton *et al.* (1989) *Molecular Plant-Microbe Interactions* 2:325-331; Somsisch *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somsisch *et al.* (1988) *Mol. Gen. Genet.* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977. See also, Chen *et al.* (1996) *Plant J.* 10:955-966; Zhang *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:2507-2511; Warner *et al.* (1993) *Plant J.* 3:191-201; Siebertz *et al.* (1989) *Plant Cell* 1:961-968; U.S. Patent No. 5,750,386 (nematode-inducible); and the references cited therein. Of particular interest is the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero *et al.* (1992) *Physiol. Mol. Plant Path.* 41:189-200).

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

Chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of the chemical induces gene expression or a chemical-repressible promoter, where application of the chemical represses gene expression. Chemical-inducible promoters are known in the art and include, but are not limited to, the maize In2-2 promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco PR-1a promoter, which is activated by salicylic acid. Other chemical-regulated promoters of interest include steroid-responsive promoters (see, for example, the glucocorticoid-inducible promoter in Schena *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:10421-10425 and McNellis *et al.* (1998) *Plant J.* 14(2):247-257) and tetracycline-inducible and tetracycline-repressible promoters (see, for example, Gatz *et al.* (1991) *Mol. Gen. Genet.* 227:229-237, and U.S. Patent Nos. 5,814,618 and 5,789,156), herein incorporated by reference.

Tissue-preferred promoters can be utilized to target enhanced chimeric pesticidal polypeptide expression within a particular plant tissue. Tissue-preferred promoters include Yamamoto *et al.* (1997) *Plant J.* 12(2):255-265; Kawamata *et al.* (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen *et al.* (1997) *Mol. Gen. Genet.* 254(3):337-343; Russell *et al.* (1997) *Transgenic Res.* 6(2):157-168; Rinehart *et al.* (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp *et al.* (1996) *Plant Physiol.* 112(2):525-535; Canevascini *et al.* (1996) *Plant Physiol.* 112(2):513-524; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35(5):773-778; Lam (1994) *Results Probl. Cell Differ.* 20:181-196; Orozco *et al.* (1993) *Plant Mol Biol.* 23(6):1129-1138; Matsuoka *et al.* (1993) *Proc Natl. Acad. Sci. USA* 90(20):9586-9590; and Guevara-Garcia *et al.* (1993) *Plant J.* 4(3):495-505. Such promoters can be modified, if necessary, for weak expression.

Leaf-preferred promoters are known in the art. See, for example, Yamamoto *et al.* (1997) *Plant J.* 12(2):255-265; Kwon *et al.* (1994) *Plant Physiol.* 105:357-67; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35(5):773-778; Gotor *et al.* (1993) *Plant J.* 3:509-18; Orozco *et al.* (1993) *Plant Mol. Biol.* 23(6):1129-1138; and Matsuoka *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90(20):9586-9590.

Root-preferred promoters are known and can be selected from the many available from the literature or isolated de novo from various compatible species. See, for example, Hire *et al.* (1992) *Plant Mol. Biol.* 20(2):207-218 (soybean root-

specific glutamine synthetase gene); Keller and Baumgartner (1991) *Plant Cell* 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger *et al.* (1990) *Plant Mol. Biol.* 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); and Miao *et al.* 5 (1991) *Plant Cell* 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also Bogusz *et al.* (1990) *Plant Cell* 2(7):633-641, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomentosa* are 10 described. The promoters of these genes were linked to a β -glucuronidase reporter gene and introduced into both the nonlegume *Nicotiana tabacum* and the legume *Lotus corniculatus*, and in both instances root-specific promoter activity was preserved. Leach and Aoyagi (1991) describe their analysis of the promoters of the highly expressed rolC and rolD root-inducing genes of *Agrobacterium rhizogenes* 15 (see *Plant Science* (Limerick) 79(1):69-76). They concluded that enhancer and tissue-preferred DNA determinants are dissociated in those promoters. Teeri *et al.* (1989) used gene fusion to lacZ to show that the *Agrobacterium* T-DNA gene encoding octopine synthase is especially active in the epidermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in 20 leaf tissue, an especially desirable combination of characteristics for use with an insecticidal or larvicidal gene (see *EMBO J.* 8(2):343-350). The TR1' gene, fused to *nptII* (neomycin phosphotransferase II) showed similar characteristics. Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster *et al.* (1995) *Plant Mol. Biol.* 29(4):759-772); and rolB promoter (Capana *et al.* (1994) *Plant* 25 *Mol. Biol.* 25(4):681-691. See also U.S. Patent Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

"Seed-preferred" promoters include both "seed-specific" promoters (those promoters active during seed development such as promoters of seed storage proteins) as well as "seed-germinating" promoters (those promoters active during 30 seed germination). See Thompson *et al.* (1989) *BioEssays* 10:108, herein incorporated by reference. Such seed-preferred promoters include, but are not limited to, Cim1 (cytokinin-induced message); cZ19B1 (maize 19 kDa zein); milps (myo-inositol-1-phosphate synthase) (see WO 00/11177 and U.S. Patent No. 6,225,529; herein incorporated by reference). Gamma-zein is an endosperm-specific 35 promoter. Globulin 1 (Glb-1) is a representative embryo-specific promoter. For dicots, seed-specific promoters include, but are not limited to, bean β -phaseolin,

napin, β -conglycinin, soybean lectin, cruciferin, and the like. For monocots, seed-specific promoters include, but are not limited to, maize 15 kDa zein, 22 kDa zein, 27 kDa zein, gamma-zein, waxy, shrunken 1, shrunken 2, Globulin 1, etc. See also WO 00/12733, where seed-preferred promoters from *end1* and *end2* genes are disclosed;
 5 herein incorporated by reference.

Where low level expression is desired, weak promoters will be used. Generally, by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By low level is intended at levels of about 1/1000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts.
 10 Alternatively, it is recognized that weak promoters also encompasses promoters that are expressed in only a few cells and not in others to give a total low level of expression. Where a promoter is expressed at unacceptably high levels, portions of the promoter sequence can be deleted or modified to decrease expression levels.

Such weak constitutive promoters include, for example, the core promoter of
 15 the Rsyn7 promoter (WO 99/43838 and U.S. Patent No. 6,072,050), the core 35S CaMV promoter, and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142. See also, U.S. Patent No. 6,177,611, herein incorporated by reference.

20 The expression cassette can also comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to
 25 herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). Additional selectable markers include phenotypic markers such as β -galactosidase and fluorescent proteins such as green fluorescent protein (GFP) (Su et al. (2004) *Biotechnol Bioeng* 85:610-9 and Fetter et al. (2004) *Plant Cell* 16:215-28), cyan florescent protein (CYP) (Bolte et al. (2004) *J. Cell*
 30 *Science* 117:943-54 and Kato et al. (2002) *Plant Physiol* 129:913-42), and yellow florescent protein (PhiYFP™ from Evrogen, see, Bolte et al. (2004) *J. Cell Science* 117:943-54). For additional selectable markers, see generally, Yarranton (1992) *Curr. Opin. Biotech.* 3:506-511; Christopherson et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6314-6318; Yao et al. (1992) *Cell* 71:63-72; Reznikoff (1992) *Mol. Microbiol.* 6:2419-
 35 2422; Barkley et al. (1980) in *The Operon*, pp. 177-220; Hu et al. (1987) *Cell* 48:555-566; Brown et al. (1987) *Cell* 49:603-612; Figge et al. (1988) *Cell* 52:713-722; Deuschle

et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5400-5404; Fuerst et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2549-2553; Deuschle et al. (1990) *Science* 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:1917-1921; Labow et al. (1990) *Mol. Cell. Biol.* 10:3343-3356; Zambretti et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:3952-3956; Baim et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:5072-5076; Wyborski et al. (1991) *Nucleic Acids Res.* 19:4647-4653; Hillenand-Wissman (1989) *Topics Mol. Struc. Biol.* 10:143-162; Degenkolb et al. (1991) *Antimicrob. Agents Chemother.* 35:1591-1595; Kleinschmidt et al. (1988) *Biochemistry* 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Oliva et al. (1992) *Antimicrob. Agents Chemother.* 36:913-919; Hlavka et al. (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); Gill et al. (1988) *Nature* 334:721-724. Such disclosures are herein incorporated by reference.

The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

In one embodiment, the polynucleotide of interest is targeted to the chloroplast for expression. In this manner, where the polynucleotide of interest is not directly inserted into the chloroplast, the expression cassette will additionally contain a nucleic acid encoding a transit peptide to direct the gene product of interest 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 et al. (1989) *J. Biol. Chem.* 264:17544-17550; Della-Cioppa et al. (1987) *Plant Physiol.* 84:965-968; Romer et al. (1993) *Biochem. Biophys. Res. Commun.* 196:1414-1421; and Shah et al. (1986) *Science* 233:478-481.

Chloroplast targeting sequences are known in the art and include the chloroplast small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) (de Castro Silva Filho et al. (1996) *Plant Mol. Biol.* 30:769-780; Schnell et al. (1991) *J. Biol. Chem.* 266(5):3335-3342); 5-(enolpyruvyl)shikimate-3-phosphate synthase (EPSPS) (Archer et al. (1990) *J. Bioenerg. Biomemb.* 22(6):789-810); tryptophan synthase (Zhao et al. (1995) *J. Biol. Chem.* 270(11):6081-6087); plastocyanin (Lawrence et al. (1997) *J. Biol. Chem.* 272(33):20357-20363); chorismate synthase (Schmidt et al. (1993) *J. Biol. Chem.* 268(36):27447-27457); and the light harvesting chlorophyll a/b binding protein (LHBP) (Lamppa et al. (1988) *J. Biol. Chem.* 263:14996-14999). See also Von Heijne et al. (1991) *Plant Mol. Biol. Rep.* 9:104-126; Clark et al. (1989) *J. Biol. Chem.* 264:17544-17550; Della-Cioppa et al. (1987)

Plant Physiol. 84:965-968; Romer *et al.* (1993) *Biochem. Biophys. Res. Commun.* 196:1414-1421; and Shah *et al.* (1986) *Science* 233:478-481.

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

The polynucleotides of interest 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 polynucleotide of
15 interest may be synthesized using chloroplast-preferred codons. See, for example, U.S. Patent No. 5,380,831, herein incorporated by reference.

The methods of the invention involve introducing a polypeptide or polynucleotide into a plant or other organism. "Introducing" is intended to mean presenting to the organism the polynucleotide or polypeptide in such a manner that
20 the sequence gains access to the interior of a cell of the organism. The methods of the invention do not depend on a particular method for introducing a sequence into an organism, only that the polynucleotide or polypeptides gains access to the interior of at least one cell of the organism. Methods for introducing polynucleotides or polypeptides into plants are known in the art including, but not limited to, stable
25 transformation methods, transient transformation methods, and virus-mediated methods.

"Stable transformation" is intended to mean that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof. "Transient transformation" is intended to mean that
30 a polynucleotide is introduced into the plant and does not integrate into the genome of the plant or a polypeptide is introduced into a plant.

Transformation protocols as well as protocols for introducing polypeptides or polynucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of
35 introducing polypeptides and polynucleotides into plant cells include microinjection (Crossway *et al.* (1986) *Biotechniques* 4:320-334), electroporation (Riggs *et al.*

(1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606, *Agrobacterium*-mediated transformation (U.S. Patent No. 5,563,055 and U.S. Patent No. 5,981,840), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, U.S. Patent Nos. 4,945,050; U.S. Patent No. 5,879,918; U.S. Patent No. 5,886,244; and, 5,932,782; Tomes *et al.* (1995) in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe *et al.* (1988) *Biotechnology* 6:923-926); and Lec1 transformation (WO 00/28058). Also see Weissinger *et al.* (1988) *Ann. Rev. Genet.* 22:421-477; Sanford *et al.* (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou *et al.* (1988) *Plant Physiol.* 87:671-674 (soybean); McCabe *et al.* (1988) *Bio/Technology* 6:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh *et al.* (1998) *Theor. Appl. Genet.* 96:319-324 (soybean); Datta *et al.* (1990) *Biotechnology* 8:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology* 6:559-563 (maize); U.S. Patent Nos. 5,240,855; 5,322,783; and, 5,324,646; Klein *et al.* (1988) *Plant Physiol.* 91:440-444 (maize); Fromm *et al.* (1990) *Biotechnology* 8:833-839 (maize); Hooykaas-Van Slogteren *et al.* (1984) *Nature (London)* 311:763-764; U.S. Patent No. 5,736,369 (cereals); Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet *et al.* (1985) in *The Experimental Manipulation of Ovule Tissues*, ed. Chapman *et al.* (Longman, New York), pp. 197-209 (pollen); Kaeppler *et al.* (1990) *Plant Cell Reports* 9:415-418 and Kaeppler *et al.* (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin *et al.* (1992) *Plant Cell* 4:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford (1995) *Annals of Botany* 75:407-413 (rice); Osjoda *et al.* (1996) *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

In specific embodiments, the chimeric pesticidal polypeptide-encoding sequences of the invention can be provided to a plant using a variety of transient transformation methods. Such transient transformation methods include, but are not limited to, the introduction of the pesticidal protein or variants and fragments thereof directly into the plant or the introduction of the a pesticide-encoding transcript into the plant. Such methods include, for example, microinjection or particle bombardment. See, for example, Crossway *et al.* (1986) *Mol Gen. Genet.* 202:179-185; Nomura *et al.* (1986) *Plant Sci.* 44:53-58; Hepler *et al.* (1994) *Proc. Natl. Acad. Sci.* 91: 2176-2180 and Hush *et al.* (1994) *The Journal of Cell Science* 107:775-784, all of which are herein incorporated by reference. Alternatively, the pesticide-encoding

polynucleotide can be transiently transformed into the plant using techniques known in the art. Such techniques include a viral vector system and the precipitation of the polynucleotide in a manner that precludes subsequent release of the DNA. Thus, the transcription from the particle-bound DNA can occur, but the frequency with which it is released to become integrated into the genome is greatly reduced. Such methods include the use of particles coated with polyethylimine.

In other embodiments, the novel chimeric pesticidal polypeptide-encoding polynucleotide may be introduced into plants by contacting plants with a virus or viral nucleic acids. Generally, such methods involve incorporating a nucleotide construct within a viral DNA or RNA molecule. Methods for introducing polynucleotides into plants and expressing a protein encoded therein, involving viral DNA or RNA molecules, are known in the art. See, for example, U.S. Patent Nos. 5,889,191, 5,889,190, 5,866,785, 5,589,367, 5,316,931, and Porta *et al.* (1996) *Molecular Biotechnology* 5:209-221; herein incorporated by reference.

The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Reports* 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting progeny 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 polynucleotide of the invention, for example, an expression cassette of the invention, stably incorporated into their genome.

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

The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plant species of interest

include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (e.g., *B. napus*, *B. rapa*, *B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet
5 (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea*
10 spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar
15 beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables
ornamentals, and conifers.

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

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

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

The present invention also provides a pesticidal composition comprising an effective amount of at least one chimeric pesticidal polypeptide of the invention to reduce pest damage to the plant and methods of protecting a plant from a pest
10 involving providing to the plant or to the vicinity of the plant an effective amount of a pesticidal composition. By "effective amount" and "effective concentration" is intended an amount and concentration, respectively, that is sufficient to kill or inhibit the growth of a plant pest. Methods for determining an "effective amount" and "effective concentration" are known to those of ordinary skill in art and include, for
15 example, assays involving placing varying amounts of concentrations of the active ingredient (e.g., a chimeric pesticidal polypeptide) in contact with and/or in the vicinity of a pest and monitoring the survival and/or growth of the pest over time.

As described above, the effective amount of a chimeric pesticidal polypeptide can vary depending on the formulation and method in which the formulation is
20 applied to the plant or plant environment. As such, bacteria can be transformed with a nucleotide sequence encoding a chimeric pesticidal polypeptide and can be used in the pesticidal compositions as described herein. Thus, the pesticidal composition can be an organism that is transformed to express a chimeric pesticidal polypeptide. Alternatively, a chimeric pesticidal polypeptide can be purified from the bacteria as
25 described above. In some embodiments, the pesticidal composition or pesticidal formulation can include other agricultural protectants, as described above. As used herein, the term "pesticidal composition" and "pesticidal formulation" are equivalent terms that have the same meaning unless indicated otherwise or apparent from the context as used.

30 As described above, the effective amount of a chimeric pesticidal polypeptide can vary depending on the formulation and method in which the formulation is applied to the plant or plant environment. As such, bacteria can be transformed with a nucleotide sequence encoding a chimeric pesticidal polypeptide and can be used in the pesticidal compositions as described herein. Thus, the pesticidal composition
35 can be an organism that is transformed to express a chimeric pesticidal polypeptide. Alternatively, the chimeric pesticidal polypeptide can be purified from the bacteria as

described above. In some embodiments, the pesticidal compositions or pesticidal formulation can include other agricultural protectants, as described above.

As described above, the pesticidal composition can be, for example, a dust, emulsion, solid (e.g., particle or pellets) or liquid.

5 The pesticidal composition can be provided to the plant by applying the pesticidal composition directly to the environment of the plant or to the vicinity of the plant such as, for example, in the soil or other growth medium surrounding the plant to protect the plant from pest attacks. For example, the pesticidal composition can be applied directly to the plant by atomizing, broadcasting, coating or pouring,
10 dusting spraying, scattering, soil drenching, sprinkling or seed coating at the time when the pest such as, for example, an insect pest has begun to appear on the plant or before the appearance of insect pests as a protective measure.

 Alternatively, the pesticidal composition can be introduced into irrigation water and then applied to the plant during watering. It is preferred to obtain good control of
15 pest in the early stages of plant growth as this is the time when the plant can be most severely damaged. To maintain protection as plants grow and to obtain the greatest protection of large plants, repeated applications of the pesticidal composition can be beneficial.

 The number of applications and the rate of application depend on the intensity
20 of infestation by the corresponding pest. When the pesticidal composition not only includes the chimeric pesticidal polypeptide and/or a chimeric pesticidal polypeptide-expressing bacteria, but also includes another agricultural protectant, the formulation can be applied to the crop area or plant to be treated simultaneously or in succession (i.e., sequentially).

25 The pesticidal composition will reduce pest-related damage by at least about 5% to about 50%, at least about 10% to about 60%, at least about 30% to about 70%, at least about 40% to about 80% or at least about 50% to about 90% or greater. Hence, the methods can be utilized to protect plants from pests. Protection may vary from a slight decrease in plant damage caused by the pest (e.g., partial
30 inhibition) to total decrease such that the plant is unaffected by the presence of the pest.

 The present invention therefore provides methods of protecting plants, plant parts and plant host cells by providing a pesticidal composition comprising a chimeric pesticidal polypeptide.

35 In certain embodiments the polynucleotides of the present invention can be stacked with any combination of polynucleotide sequences of interest in order to

create plants with a desired trait. A trait, as used herein, refers to the phenotype derived from a particular sequence or groups of sequences. For example, the polynucleotides of the present invention may be stacked with any other polynucleotides encoding polypeptides having pesticidal and/or insecticidal activity, such as other *Bacillus thuringiensis* toxic proteins (described in U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; and Geiser *et al.* (1986) *Gene* 48:109), lectins (Van Damme *et al.* (1994) *Plant Mol. Biol.* 24:825, pentin (described in U.S. Patent No. 5,981,722), and the like. The combinations generated can also include multiple copies of any one of the polynucleotides of interest. The polynucleotides of the present invention can also be stacked with any other gene or combination of genes to produce plants with a variety of desired trait combinations including, but not limited to, traits desirable for animal feed such as high oil genes (e.g., U.S. Patent No. 6,232,529); balanced amino acids (e.g., hordothionins (U.S. Patent Nos. 5,990,389; 5,885,801; 5,885,802; and 5,703,409); barley high lysine (Williamson *et al.* (1987) *Eur. J. Biochem.* 165:99-106; and WO 98/20122) and high methionine proteins (Pedersen *et al.* (1986) *J. Biol. Chem.* 261:6279; Kirihaara *et al.* (1988) *Gene* 71:359; and Musumura *et al.* (1989) *Plant Mol. Biol.* 12:123)); increased digestibility (e.g., modified storage proteins (U.S. Application Serial No. 10/053,410, filed November 7, 2001); and thioredoxins (U.S. Application Serial No. 10/005,429, filed December 3, 2001)); the disclosures of which are herein incorporated by reference.

The polynucleotides of the present invention can also be stacked with traits desirable for disease or herbicide resistance (e.g., fumonisin detoxification genes, U.S. Patent No. 5,792,931); avirulence and disease resistance genes (Jones *et al.* (1994) *Science* 266:789; Martin *et al.* (1993) *Science* 262:1432; Mindrinis *et al.* (1994) *Cell* 78:1089); acetolactate synthase (ALS) mutants that lead to herbicide resistance such as the S4 and/or Hra mutations; inhibitors of glutamine synthase such as phosphinothricin or basta (e.g., bar gene); and glyphosate resistance (EPSPS gene); and traits desirable for processing or process products such as high oil (e.g., U.S. Patent No. 6,232,529); modified oils (e.g., fatty acid desaturase genes (U.S. Patent No. 5,952,544; WO 94/11516)); modified starches (e.g., ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE), and starch debranching enzymes (SDBE)); and polymers or bioplastics (e.g., U.S. Patent No. 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase, and acetoacetyl-CoA reductase (Schubert *et al.* (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)); the disclosures of which are

herein incorporated by reference. One could also combine the polynucleotides of the present invention with polynucleotides providing agronomic traits such as male sterility (e.g., see U.S. Patent No. 5,583,210), stalk strength, flowering time or transformation technology traits such as cell cycle regulation or gene targeting (e.g.,
5 WO 99/61619, WO 00/17364, and WO 99/25821); the disclosures of which are herein incorporated by reference.

These stacked combinations can be created by any method including, but not limited to, cross-breeding plants by any conventional or TopCross methodology or genetic transformation. If the sequences are stacked by genetically transforming the
10 plants, the polynucleotide sequences of interest can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes.
15 For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of the
20 polynucleotide of interest. This may be combined with any combination of other suppression cassettes or overexpression cassettes to generate the desired combination of traits in the plant. It is further recognized that polynucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840,
25 WO99/25855, and WO99/25853, all of which are herein incorporated by reference.

Various changes in phenotype are of interest including modifying the fatty acid composition in a plant, altering the amino acid content of a plant, altering a plant's pathogen defense mechanism, and the like. These results can be achieved by providing expression of heterologous products or increased expression of
30 endogenous products in plants. Alternatively, the results can be achieved by providing for a reduction of expression of one or more endogenous products, particularly enzymes or cofactors in the plant. These changes result in a change in phenotype of the transformed plant.

Genes of interest are reflective of the commercial markets and interests of
35 those involved in the development of the crop. Crops and markets of interest change, and as developing nations open up world markets, new crops and

technologies will emerge also. In addition, as our understanding of agronomic traits and characteristics such as yield and heterosis increase, the choice of genes for transformation will change accordingly. General categories of genes of interest include, for example, those genes involved in information, such as zinc fingers, those
5 involved in communication, such as kinases, and those involved in housekeeping, such as heat shock proteins. More specific categories of transgenes, for example, include genes encoding important traits for agronomics, insect resistance, disease resistance, herbicide resistance, sterility, grain characteristics, and commercial products. Genes of interest include, generally, those involved in oil, starch,
10 carbohydrate or nutrient metabolism as well as those affecting kernel size, sucrose loading, and the like.

Agronomically important traits such as oil, starch, and protein content can be genetically altered in addition to using traditional breeding methods. Modifications include increasing content of oleic acid, saturated and unsaturated oils, increasing
15 levels of lysine and sulfur, providing essential amino acids, and also modification of starch. Hordothionin protein modifications are described in U.S. Patent Nos. 5,703,049, 5,885,801, 5,885,802, and 5,990,389, herein incorporated by reference. Another example is lysine and/or sulfur rich seed protein encoded by the soybean 2S albumin described in U.S. Patent No. 5,850,016, and the chymotrypsin inhibitor from
20 barley, described in Williamson *et al.* (1987) *Eur. J. Biochem.* 165:99-106, the disclosures of which are herein incorporated by reference.

Derivatives of the coding sequences can be made by site-directed mutagenesis to increase the level of preselected amino acids in the encoded polypeptide. For example, the gene encoding the barley high lysine polypeptide
25 (BHL) is derived from barley chymotrypsin inhibitor, U.S. Application Serial No. 08/740,682, filed November 1, 1996, and WO 98/20133, the disclosures of which are herein incorporated by reference. Other proteins include methionine-rich plant proteins such as from sunflower seed (Lilley *et al.* (1989) *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*,
30 ed. Applewhite (American Oil Chemists Society, Champaign, Illinois), pp. 497-502; herein incorporated by reference); corn (Pedersen *et al.* (1986) *J. Biol. Chem.* 261:6279; Kirihaara *et al.* (1988) *Gene* 71:359; both of which are herein incorporated by reference); and rice (Musumura *et al.* (1989) *Plant Mol. Biol.* 12:123, herein incorporated by reference). Other agronomically important genes encode latex,
35 Flourey 2, growth factors, seed storage factors, and transcription factors.

Insect resistance genes may encode resistance to pests that have great yield drag such as rootworm, cutworm, European Corn Borer, and the like. Such genes include, for example, *Bacillus thuringiensis* toxic protein genes (U.S. Patent Nos. 5,366,892; 5,747,450; 5,736,514; 5,723,756; 5,593,881; and Geiser *et al.* (1986) 5 *Gene* 48:109); and the like.

Genes encoding disease resistance traits include detoxification genes, such as against fumonisin (U.S. Patent No. 5,792,931); avirulence (avr) and disease resistance (R) genes (Jones *et al.* (1994) *Science* 266:789; Martin *et al.* (1993) *Science* 262:1432; and Mindrinos *et al.* (1994) *Cell* 78:1089); and the like.

10 Herbicide resistance traits may include genes coding for resistance to herbicides that act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance, in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides that act to inhibit action of 15 glutamine synthase, such as phosphinothricin or basta (e.g., the bar gene); glyphosate (e.g., the EPSPS gene and the GAT gene; see, for example, U.S. Publication No. 20040082770 and WO 03/092360); or other such genes known in the art. The *bar* gene encodes resistance to the herbicide basta, the *nptII* gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS-gene mutants 20 encode resistance to the herbicide chlorsulfuron.

Sterility genes can also be encoded in an expression cassette and provide an alternative to physical detasseling. Examples of genes used in such ways include male tissue-preferred genes and genes with male sterility phenotypes such as QM, described in U.S. Patent No. 5,583,210. Other genes include kinases and those 25 encoding compounds toxic to either male or female gametophytic development.

The quality of grain is reflected in traits such as levels and types of oils, saturated and unsaturated, quality and quantity of essential amino acids, and levels of cellulose. In corn, modified hordothionin proteins are described in U.S. Patent Nos. 5,703,049, 5,885,801, 5,885,802, and 5,990,389.

30 Commercial traits can also be encoded on a gene or genes that could increase for example, starch for ethanol production or provide expression of proteins. Another important commercial use of transformed plants is the production of polymers and bioplastics such as described in U.S. Patent No. 5,602,321. Genes such as β -Ketothiolase, PHBase (polyhydroxybutyrate synthase), and acetoacetyl-CoA reductase (see Schubert *et al.* (1988) *J. Bacteriol.* 170:5837-5847) facilitate 35 expression of polyhydroxyalkanoates (PHAs).

Exogenous products include plant enzymes and products as well as those from other sources including prokaryotes and other eukaryotes. Such products include enzymes, cofactors, hormones, and the like. The level of proteins, particularly modified proteins having improved amino acid distribution to improve the nutrient value of the plant, can be increased. This is achieved by the expression of such proteins having enhanced amino acid content.

Throughout this specification and the claims, the words “comprise,” “comprises,” and “comprising” are used in a non-exclusive sense, except where the context requires otherwise.

As used herein, the term “about,” when referring to a value is meant to encompass variations of, in some embodiments $\pm 50\%$, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, when an amount, concentration or other value or parameter is given as either a range, preferred range or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the presently disclosed subject matter be limited to the specific values recited when defining a range.

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

EXAMPLE 1

Enhancement of the Insecticidal Activity of Cry Proteins Against Western Corn Rootworms and Black Cutworms When Fused to MBP

A computer-designed and artificially synthesized Cry3Aa-type protein called IP3-1 (SEQ ID NO: 8), a truncated RX002 protein (SEQ ID NO: 12) cloned from a Bt strain, a shuffled Cry8Bb-type protein called 2A-12 (SEQ ID NO: 10), and a truncated native Cry1Bd protein called 4c6 (SEQ ID NO: 14) were expressed in *E. coli* BL21 as fusion proteins comprising an operably linked MBP. The amino acid sequences of the MBP-IP3-1, MBP-RX002, MBP-2A-12, and MBP-4c6 fusion proteins are set forth in SEQ ID NOS: 21, 23, 25, and 27, respectively. Nucleotide sequences encoding

the MBP-IP3-1, MBP-RX002, MBP-2A-12, and MBP-4c6 fusion proteins are set forth in SEQ ID NOS: 20, 22, 24, and 26, respectively.

To express MBP with these Bt Cry proteins, corresponding genes were cloned in an NEB pMAL vector and the proteins were purified using amylose resin following the manufacturer's recommended method (New England Biolabs, Inc., Ipswich, MA, USA; Catalog No. E8021L). The target protein eluted from amylose resin was concentrated in Amicon Centricon concentrator (10 kDa cutoff). The final fusion proteins were dissolved in 25 mM HEPES-NaOH buffer pH8 at a concentration around 2 mg/ml. The protein concentration was determined by SDS-PAGE using bovine serum albumin as the reference. The pMAL vector has a protease site specific to Factor Xa that cleaves MBP and the linker off the Cry protein. All Cry proteins included in this application were resistant to Factor Xa. The protease digestion was carried out in the HEPES buffer at 1:25 protease and substrate ration at 20°C for 16 hr. The digestion leading to the complete MBP removal was confirmed by SDS-PAGE.

MBP fusion and MBP free Cry proteins were diluted in 25 mM HEPES-NaOH buffer pH 8 and 10 µL of diluted samples were mixed with 40 µL of molten artificial insect diet made with low temperature melting agarose. The diet mixture was then placed in each well of a 96-well micro-titer plate and allowed to feed with neonate insect larvae. After 4 days at 27°C, the responses of insects towards the Cry proteins were scored using a 0-3 numerical scoring system based on the size and mortality. If no response or normal growth was seen, Score 0 was given. When the growth was somewhat retarded without any mortality, it was Score 1. Score 2 meant partial death (multiple insects were used in each well) and strong growth inhibition. Score 3 indicated the complete mortality. Each treatment was repeated 6 times for possible highest score of 18 (3X6). In this scoring system, Score 9 with 6 repeats of one treatment means the 50% response (9 out of 18) of the treatment and called ILC50 (growth Inhibition and Lethal Concentration for 50% response). The results of these assays are shown in Tables 3 and 4.

The results shown in Tables 3 and 4 with four different Cry proteins demonstrate that MBP-Cry fusion proteins have significantly increased insecticidal activity against both Coleopteran (Western Corn Rootworm) and Lepidopteran (Black Cutworm) insect species, when compared to the insecticidal activity of their respective free Cry proteins. These results further demonstrate the general applicability of the methods of the present invention to enhancing the insecticidal activity of Cry proteins. Table 3 shows ILC50 (based on the size or weight of the Cry

protein portion) values for insecticidal activity against Western Corn Rootworm of MBP-Cry Fusion Proteins and Free Cry Proteins.

Table 3

IP3-1		RX002		2A-12	
<u>MBP-fusion</u>	<u>free Cry</u>	<u>MBP-fusion</u>	<u>free Cry</u>	<u>MBP-fusion</u>	<u>free Cry</u>
7 ppm	103 ppm	89 ppm	No activity*	32 ppm	152 ppm

* Only an 11% response was observed at 2480 ppm.

Table 4 shows the ILC50 values for insecticidal activity against Black Cutworm of a MBP-4c6 Cry Fusion Protein and Free 4c6 Cry Protein.

Table 4

<u>MBP-4c6 fusion</u>	<u>free 4c6</u>
75 ppm	148 ppm

EXAMPLE 2

MBP-Cry Fusion Protein with Increased Activity Against WCRW

A new vector, pMAL-SA (SEQ ID NO: 19), was constructed for making MBP-Cry fusion proteins. This vector is based on NEB pMAL vector. It has the cry gene cloning site delineated with SphI and BamHI recognition sequences at the end of MBP and a specially designed linker called “SA” linker between MBP and the Cry cloning site.

In order to clone a Bt cry gene, the cry coding region is amplified by PCR using appropriate forward and reverse primers. In the forward primer, there is an SphI site over the ATG translation initiation site. In the reverse primer, there is a stop codon at the end of the cry gene coding region and a BamHI site.

A computer-designed cry3Aa sequence, IP3-1, was synthesized and used as the PCR template. The IP3-1 nucleotide sequence is set forth in SEQ ID NO: 7. This IP3-1 gene was cloned in pMAL-SA as described above to produce a plasmid that contains MBP, the SA linker and IP3-1 coding region (SEQ ID NO: 1). The second amino acid residue of IP3-1 was mutated back to Asn from His to produce the MBP-SA-IP3-1 nucleotide sequence set forth in SEQ ID NO: 2.

The MBP-SA-IP3-1 fusion protein was expressed in E. coli BL21 and purified by amylose resin affinity chromatography according to the method described in

Example 1. The column eluate was concentrated in Amicon Centricon and its buffer was exchanged to 25 mM HEPES-NaOH buffer pH 8. The fusion protein was digested with trypsin at 1:25 trypsin-substrate ratio at 37°C for 1 hr. Trypsin cleaves the protein at the end of the linker to liberate the Cry protein from MBP and the SA linker. IP3-1 was resistant to trypsin under this digestion condition. The digestion was confirmed with SDS-PAGE. Both the fusion and MBP-free IP3-1 proteins were assayed against WCRW. Table 5 shows ILC50 values for insecticidal activity against Western Corn Rootworm of a MBP-SA-IP3-1 and MBP-free IP3-1. The assay results in Table 5 demonstrate that there is a significant enhancement of WCRW activity when IP3-1 is operable linked to MBP-SA (i.e., MBP-SA-IP3-1), when compared to the insecticidal activity of MBP-free IP3-1 against WCRW.

Table 5

<u>Protein</u>	<u>ICL50</u>
MBP-SA-IP3-1	6 ppm
MBP-free IP3-1	269 ppm

EXAMPLE 3

Enhancement of Cry3Aa Protein Activity when Fused to NusA and Trx

A computer-designed and artificially synthesized *cry3Aa*-type gene called IP3-1 was cloned in pET-43.1 EK/LIC for NusA fusion and pET-32 for TrxA fusion by following the manufacturer's directions (EMD Biosciences, Madison, WI, USA). These vectors were used to express NusA- IP3-1 and TrxA- IP3-1 fusion proteins. The amino acid sequences of the NusA-IP3-1 and TrxA-IP3-1 fusion proteins are set forth in SEQ ID NOS: 16 and 18, respectively. Nucleotide sequences encoding the NusA-IP3-1 and TrxA-IP3-1 fusion proteins are set forth in SEQ ID NOS: 15 and 17, respectively. The amino acid sequence of IP3-1 is set forth in SEQ ID NO: 8. A nucleotide sequence encoding IP3-1 is set forth in SEQ ID NO: 7.

The fusion proteins were purified by affinity chromatography using Ni-NTA agarose (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's directions. The Cry3Aa protein was then digested away from the tags including NusA, TrxA, 6XHis etc. with enterokinase and the insecticidal activity of the free Cry3A protein was compared with its NusA and TrxA fusion proteins. The insect assay was conducted using WCRW as described above. Table 6 shows the ILC50 values for

insecticidal activity against Western Corn Rootworm of a MBP-SA-IP3-1 and MBP-free IP3-1 protein. The IP3-1 protein sample was produced from the NusA-Cry3Aa fusion by enterokinase digestion. As demonstrated in the results in Table 6, both the NusA-IP3-1-and TrxA-IP3-1-fusion proteins, which comprise a solubility-enhancing polypeptide and a Cry3A protein (IP3-1), have an increased insecticidal activity against WCRW as evidenced by the significantly lower ICL50 values, when compared to insecticidal activity of free IP3-1.

Table 6:

<u>Protein</u>	<u>ICL50</u>
NusA-IP3-1-fusion	26 ppm
TrxA-IP3-1-fusion	31 ppm
Tag free IP3-1	554 ppm

10

EXAMPLE 4**Generation of a MBP-SA-RX002 Transformation Construct**

The maltose-binding protein (MBP-SA) (SEQ ID NO: 4) was fused to the N-terminus of RX002 (SEQ ID NO:11) in a synthetic gene designed for this invention (SEQ ID NO:32 encoding SEQ ID NO:33). The gene was cloned as a BamHI – StuI fragment into a Gateway entry vector containing a plant expression cassette with the BSV(AY) TR PROMOTER – ADH1 INTRON1 sequence and the potato PIN II terminator sequence. The resulting plant expression cassette contains the following components operatively linked together in this order; BSV (AY) TR PRO-ADH1-INTRON1, the MBP-SA-RX002 gene, and the PIN II terminator. The expression cassette is flanked by Gateway *attL3* and *attL4* recombination sites and this entry vector was used to transfer the expression cassette into an *attR3* and *attR4* containing binary destination transformation vector. The final transformation vector contains the MBP-SA-RX002 expression cassette upstream of a cassette containing the maize Ubiquitin1 promoter-5'UTR-Ubiquitin intron1 controlling expression of a PAT selectable marker gene with the 35S terminator sequence.

25

EXAMPLE 5***Agrobacterium*-mediated Transformation of Maize and Regeneration of Transgenic Plants**

30

For *Agrobacterium*-mediated transformation of maize with a promoter sequence of the invention, the method of Zhao was employed (U.S. Patent No.

5,981,840, and PCT patent publication WO98/32326; the contents of which are hereby incorporated by reference). Briefly, immature embryos were isolated from maize and the embryos contacted with a suspension of *Agrobacterium* under conditions whereby the bacteria were capable of transferring the promoter sequence of the invention to at least one cell of at least one of the immature embryos (step 1: the infection step). In this step the immature embryos were immersed in an *Agrobacterium* suspension for the initiation of inoculation. The embryos were co-cultured for a time with the *Agrobacterium* (step 2: the co-cultivation step). The immature embryos were cultured on solid medium following the infection step. Following the co-cultivation period and an optional "resting" step was performed. In this resting step, the embryos were incubated in the presence of at least one antibiotic known to inhibit the growth of *Agrobacterium* without the addition of a selective agent for plant transformants (step 3: resting step). The immature embryos were cultured on solid medium with antibiotic, but without a selecting agent, for elimination of *Agrobacterium* and for a resting phase for the infected cells. Next, inoculated embryos were cultured on medium containing a selective agent and growing transformed callus was recovered (step 4: the selection step). The immature embryos were cultured on solid medium with a selective agent resulting in the selective growth of transformed cells. The callus was then regenerated into plants (step 5: the regeneration step), and calli grown on selective medium were cultured on solid medium to regenerate the plants.

EXAMPLE 6

Expression of MBP-RX002 fusion in Transgenic Maize Tissue

Transgenic events derived from the testing vector were evaluated for expression of MBP-RX002 by Western analysis. Leaf and root material for transgenic maize expressing MBP-RX002 were lyophilized then powdered with 5/32 inch BBs (i.e., birdshot) using a Geno/Grinder 2000 homogenizer at 1700 beats per minute for 30 seconds. 80 μ L of grinding buffer (1X PBS + 0.1% Tween-20 + 1% 2-mercaptoethanol containing Roche cOMplete protease inhibitor (Roche Applied Science, Indianapolis, IN, USA; Catalog No. 04693124001; at one tablet per 7 mL) was added to each sample. Pulverization was repeated for an additional 30 seconds, then the samples were sonicated for 5 minutes at room temperature in a VWR 75D sonicator. After centrifugation at 21,000 g/4°C for 15 minutes, supernatants were collected. Protein concentrations were determined from the supernatants using Thermo Scientific Coomassie Plus Kit (23236) and a

SpectraMAX 190 spectrophotometer. Samples were normalized for total protein in 21 μ L using grinding buffer as diluent. Seven microliters of 4X LDS dye containing 1% 2-mercaptoethanol was added to each sample prior to heating at 80°C for 10 minutes. Twenty-five microliters of sample was loaded per lane on a NuPAGE
5 Novex 4-12% Bis-Tris midi gel (Invitrogen WB1402BOX) and electrophoresed at 200 V for 1 hour. Protein was transferred to a nitrocellulose membrane using an Invitrogen iBlot with a transfer stack (IB301001). The membrane was blocked for 30 minutes in 1X PBS + 0.1% Tween-20 + 5% powdered milk w/v (blocking buffer), then incubated overnight at 4°C with rabbit polyclonal antibody against RX002, diluted in
10 blocking buffer at 1:4000. Membrane was washed 4 x 5 minutes in PBST (1X PBS + 0.1% Tween-20), then incubated for 2 hours with goat anti-rabbit HRP conjugated secondary antibody (Pierce 31460; 10 μ g/mL working stock) at a 1:5000 dilution in blocking buffer. The membrane was washed 4 x 5 minutes in PBST and developed using Thermo Super Signal West Dura Extended Duration Substrate (34076).
15 Visualization of hybridization signal was accomplished using a Fujifilm LAS-4000 imaging system.

The results of this analysis are shown in Table 7. Accumulation of MBP-RX002 was detected in 8 of the 10 events sampled for Western analysis in either root or leaf and root tissue. An 118 kD protein band corresponding to the expected size of
20 the MBP-RX002 fusion was observed in both leaf and root or root tissue demonstrating that the fusion can be expressed *in planta*. In addition to the full length protein, a 75 kD immunoreactive protein band corresponding to the expected size of RX002 was also observed in some events indicating that some proportion of the full-length fusion protein was processed *in planta* to release RX002.

25

Table 7

<u>Event No.</u>	<u>Expression in leaf</u>	<u>Expression in root</u>
Control	-	-
123434603	-	-
123434607	+++	++++
123434610	-	-
123434613	++++	++++
123434614	-	+
123434615	-	++
123434616	++++	++++
123434617	++++	++++
123434618	++++	++++
123434619	-	+++

The article "a" and "an" are used herein to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one or more element.

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

Applicant Ref.: 3113WOPCT

THAT WHICH IS CLAIMED:

1. A method of enhancing pesticidal activity of a Cry endotoxin, the method comprising operably linking a first amino acid sequence of a solubility-enhancing polypeptide to a second amino acid sequence of a Cry endotoxin, whereby a chimeric pesticidal polypeptide is produced, the chimeric pesticidal polypeptide comprising the first amino acid sequence operably linked to second amino acid sequence.

2. The method of claim 1; wherein the solubility-enhancing polypeptide is selected from the group consisting of maltose-binding protein (MBP), thioredoxin, and transcription elongation factor NusA.

3. The method of claim 2, wherein the solubility-enhancing polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 4, 6, 34, 35 and 36.

4. The method of claim 1, 2 or 3, wherein the Cry endotoxin comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 12 and 14.

5. The method of claim 1, 2, 3 or 4, wherein the chimeric pesticidal polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 2, 16, 18, 21, 23, 25, 27 and 33.

6. A chimeric pesticidal polypeptide comprising a first amino acid sequence of a solubility-enhancing polypeptide operably linked to a second amino acid sequence of a Cry endotoxin, wherein the operably linked first and second amino sequences comprise an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33; and

(b) an amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32.

Applicant Ref.: 3113WOPCT

7. A nucleic acid molecule encoding the chimeric pesticidal polypeptide of claim 6.
8. An expression cassette comprising a promoter that drives expression in a host cell operably linked to the nucleic acid molecule of claim 7.
9. A vector comprising the expression cassette of claim 8.
10. A transformed plant, plant part, plant cell or seed comprising in its genome the expression cassette of claim 8.
11. A pesticidal composition comprising an effective amount of a chimeric pesticidal polypeptide of claim 6 or an active variant or fragment thereof having pesticidal activity.
12. A method of protecting a plant from an insect pest, the method comprising providing an effective amount of the pesticidal composition of claim 10 to reduce insect pest-related damage to the plant.
13. A plant comprising a polynucleotide construct stably incorporated in its genome, the polynucleotide construct comprising a nucleotide sequence operably linked to a promoter that drives expression in the plant, wherein the nucleotide sequence encodes a chimeric pesticidal polypeptide comprising a first amino acid sequence of a solubility-enhancing polypeptide operably linked to a second amino acid sequence of a Cry endotoxin, wherein the solubility-enhancing polypeptide is selected from the group consisting of maltose-binding protein (MBP), thioredoxin, and transcription elongation factor NusA.
14. The plant of claim 13, wherein the nucleotide sequence is selected from the group consisting of:
 - (a) the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32; and

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(b) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33.

15. A method of protecting a plant, plant part or plant host cell from an insect pest, the method comprising the steps of:

(a) introducing into the plant, plant part or plant host cell an expression cassette of claim 7; and

(b) regenerating the plant, plant part or plant host cell into a morphologically normal fertile plant, wherein the plant or part thereof comprises a chimeric pesticidal polypeptide.

16. A method of enhancing the resistance of a plant to at least one pest, the method comprising introducing into a plant or at least one plant cell a polynucleotide construct comprising a nucleotide sequence operably linked to a promoter that drives expression in the plant, wherein the nucleotide sequence encodes a chimeric pesticidal polypeptide comprising a first amino acid sequence of a solubility-enhancing polypeptide operably linked to a second amino acid sequence of a Cry endotoxin.

17. The method of claim 16, wherein the solubility-enhancing polypeptide is selected from the group consisting of maltose-binding protein (MBP), thioredoxin, and transcription elongation factor NusA.

18. The method of claim 17, wherein the nucleotide sequence comprises a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence set forth in SEQ ID NO: 1, 15, 17, 20, 22, 24, 26 or 32; and

(b) a nucleotide sequence encoding an amino acid sequence comprising the amino acid sequence set forth in SEQ ID NO: 2, 16, 18, 21, 23, 25, 27 or 33.

Fig. 1

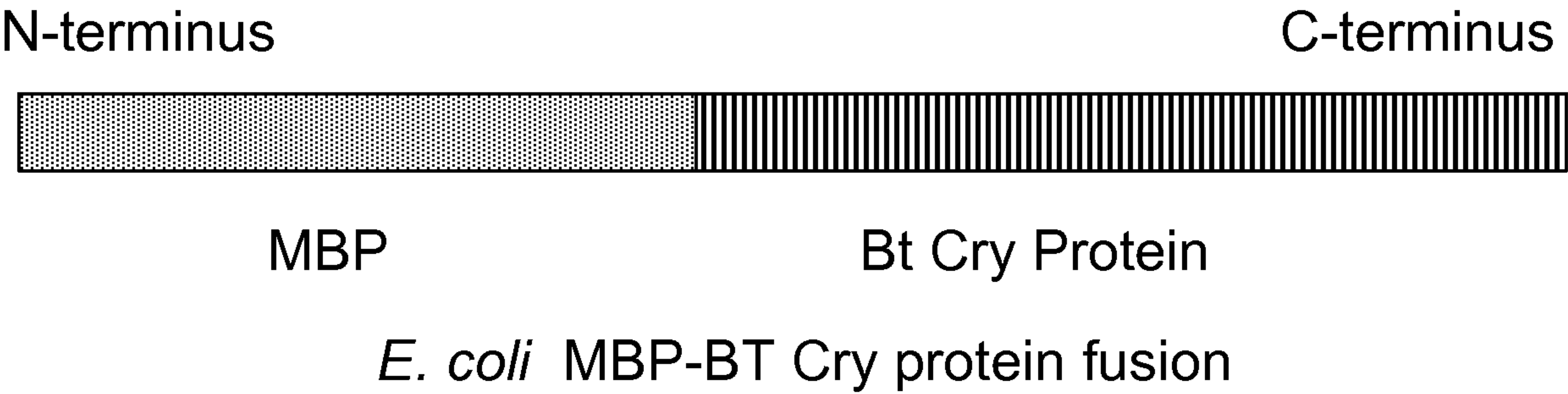
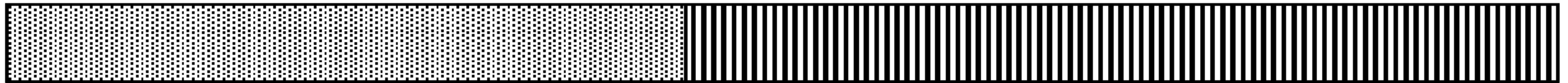


Fig. 1

N-terminus

C-terminus



MBP

Bt Cry Protein

E. coli MBP-BT Cry protein fusion