COMPOSITIONS AND RELATED METHODS FOR MODULATING ENDOSYMBIONTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/463,451, filed on February 24, 2017, and U.S. Provisional Application No. 62/583,990, filed on November 9, 2017, the contents of which are hereby incorporated herein by reference in their entireties.

BACKGROUND

Invertebrate organisms (e.g., insects, mollusks, or nematodes) are pervasive in the human environment. In some instances, invertebrates serve beneficial roles, such as nematodes or arthropods utilized in agriculture for pollination efforts and pest control or in commerce for the production of commercial products (e.g., honey or silk). In other instances, invertebrates can be detrimental, including some species of mollusks (e.g., snails and slugs), nematodes, or insects that can be serious crop pests or carriers of disease. Thus, there is need in the art for methods and compositions to modulate the fitness of invertebrates that are important in agriculture, commerce, or public health.

SUMMARY

Disclosed herein are compositions and methods for modulating the fitness of invertebrates, including insects, nematodes, or mollusks, by altering the interactions between the host and one or more microorganisms resident in the host.

In one example, provided herein is a method for decreasing the fitness of an insect, the method including delivering to the insect an effective amount of a polynucleotide that includes a dsRNA that decreases expression of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene in the insect relative to an insect that has not been administered the dsRNA.

In one aspect, the present disclosure refers to a method for decreasing the fitness of an insect of the order Hemiptera, the method comprising delivering to the insect an effective amount of a polynucleotide comprising a dsRNA that decreases expression of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene in the insect relative to an insect that has not been administered the dsRNA, wherein the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein).

In some examples, the gene encodes a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), or imd (immune deficiency protein). In some examples, the gene encodes a protein having at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% amino acid sequence identity to a protein listed in Table 5, Table 8, or Table 9. In some examples, the gene encodes a functional homolog of a protein listed in Table 5, Table 8, or Table 9. For example, the gene may encode a cactus-like protein in aphids (e.g., any one of the proteins described by GenBank Accession Nos: XP_022175228.1, XP_016656687.1, NP_001156668.1, XP_008179071.1, or XP_016656686.1, the associated amino acid and nucleotide sequences of which are incorporated by reference).

In some examples, the dsRNA is complementary to 10 to 30 nucleotides of the gene in the insect (e.g., 10 to 30 nucleotides, 12 to 28 nucleotides, 14 to 26 nucleotides, 16 to 24 nucleotides, 14 to 22 nucleotides, or 18 to 20 nucleotides). In some examples, the dsRNA is complementary to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides of the gene in the insect. In some examples, the entire length of the dsRNA is complementary to the gene. In alternative examples, only a portion of the dsRNA is complementary to the gene.

In some examples, the polynucleotide is formulated with an agriculturally acceptable carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas composition.

In some examples, the insect is an aphid.

In another aspect, the present disclosure refers to a composition comprising a polynucleotide comprising a dsRNA formulated for delivery to an insect of the order Hemiptera, wherein the dsRNA is complementary to 15 to 30 nucleotides of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene, wherein the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein). Provided herein is a composition including a polynucleotide that includes a dsRNA formulated for delivery to an insect, wherein the dsRNA is complementary to 15 to 30 nucleotides of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene. In some examples, the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein). In some examples, the gene encodes a protein having at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% amino acid sequence identity to a protein listed in Table 5, Table 8, or Table 9. In some examples, the gene encodes a functional homolog of a protein listed in Table 5, Table 8, or Table 9. For example, the gene may encode a cactus-like protein in aphids (e.g., any one of the proteins described by GenBank Accession Nos: XP_022175228.1, XP_016656687.1, NP_001156668.1, XP_008179071.1, or XP_016656686.1, the associated amino acid and nucleotide sequences of which are incorporated by reference). In some examples, the dsRNA is complementary to 10 to 30 nucleotides of the gene in the insect (e.g., 10 to 30 nucleotides, 12 to 28 nucleotides, 14 to 26 nucleotides, 16 to 24 nucleotides, 14 to 22 nucleotides, or 18 to 20 nucleotides). In some examples, the dsRNA is complementary to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides of the gene in the insect. In some examples, the entire length of the dsRNA is complementary to the gene. In alternative examples, only a portion of the dsRNA is complementary to the gene.

In a further aspect, the present disclosure refers to a plant comprising a topical application of the compositions described herein.

In yet another aspect, the present disclosure refers to a transgenic plant cell having in its genome a recombinant DNA construct, wherein the recombinant DNA construct comprises a heterologous promoter operably linked to a DNA encoding a RNA comprising at least one double-stranded RNA region, at least one strand of which comprises a nucleotide sequence that is complementary to 15 to 30 nucleotides of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene, wherein the insect is of the order Hemiptera and wherein the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein). Provided herein is a transgenic plant cell having in its genome a recombinant DNA construct, wherein the recombinant DNA construct includes a heterologous promoter operably linked to a DNA encoding a RNA including at least one double-stranded RNA region, at least one strand of which includes a nucleotide sequence that is complementary to 15 to 30 nucleotides of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene. In some examples, the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein). In some examples, the gene encodes a protein having at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% amino acid sequence identity to a protein listed in Table 5, Table 8, or Table 9. In some examples, the gene encodes a functional homolog of a protein listed in Table 5, Table 8, or Table 9. For example, the gene may encode a cactus-like protein in aphids (e.g., any one of the proteins described by GenBank Accession Nos: XP_022175228.1, XP_016656687.1, NP_001156668.1, XP_008179071.1, or XP_016656686.1, the associated amino acid and nucleotide sequences of which are incorporated by reference). In some examples, the dsRNA is complementary to 10 to 30 nucleotides of the gene in the insect (e.g., 10 to

CLAIMS

1. A method for decreasing the fitness of an insect of the order Hemiptera, the method comprising delivering to the insect an effective amount of a polynucleotide comprising a dsRNA that decreases expression of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene in the insect relative to an insect that has not been administered the dsRNA, wherein the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein).

2. The method of claim 1, wherein the gene encodes a protein having at least 90% amino acid sequence identity, at least 95% amino acid sequence identity, or 100% amino acid sequence identity to a protein listed in Table 5, Table 8, or Table 9.

3. The method of claim 1 or 2, wherein the dsRNA is complementary to 10 to 30 nucleotides of the gene in the insect.

4. The method of any one of claims 1-3, wherein the method is effective to inhibit expression of the gene in the insect.

5. The method of any one of claims 1-4, wherein the method is effective to decrease the level, diversity, or metabolism of one or more microorganisms resident in the insect relative to an insect that has not been delivered the agent.

6. The method of claim 5, wherein the one or more microorganisms is a *Buchnera* spp.

7. The method of any one of claims 1-6, wherein the polynucleotide is delivered in a composition formulated for delivery to insects.

8. The method of any one of claims 1-7, wherein the delivery comprises delivering the polynucleotide to at least one habitat where the insect pest grows, lives, reproduces, feeds, or infests.

9. The method of any one of claims 1-8, wherein the delivery comprises spraying the polynucleotide on an agricultural crop.

10. The method of any one of claims 1-9, wherein the polynucleotide is delivered as an insect comestible composition for ingestion by the insect.

11. The method of any one of claims 1-10, wherein the polynucleotide is formulated with an agriculturally acceptable carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas composition.

12. The method of any one of claims 1-11, wherein the insect is an aphid.

13. A composition comprising a polynucleotide comprising a dsRNA formulated for delivery to an insect of the order Hemiptera, wherein the dsRNA is complementary to 15 to 30 nucleotides of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene, wherein the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein).

14. The composition of claim 13, wherein the gene encodes a protein having at least 90% amino acid sequence identity, 95% amino acid sequence identity, or 100% amino acid sequence identity to a protein listed in Table 5, Table 8, or Table 9.

15. A plant comprising a topical application of the composition of claim 13 or 14.

16. A transgenic plant cell having in its genome a recombinant DNA construct, wherein the recombinant DNA construct comprises a heterologous promoter operably linked to a DNA encoding a RNA comprising at least one double-stranded RNA region, at least one strand of which comprises a nucleotide sequence that is complementary to 15 to 30 nucleotides of an insect bacteriocyte regulatory gene or an insect immunoregulatory gene, wherein the insect is of the order Hemiptera and wherein the gene encodes a protein selected from the group consisting of a protein from the bacteriocyte-specific cysteine rich proteins BCR family, a protein from the secreted proteins SP family, BicD (Protein bicaudal D), Cact (cactus), DIF (Dorsal related immunity factor), Toll (Toll Interacting Protein), and imd (immune deficiency protein).

17. The transgenic plant of claim 16, wherein the gene encodes a protein having at least 90% amino acid sequence identity, 95% amino acid sequence identity, or 100% amino acid sequence identity to a protein listed in Table 5, Table 8, or Table 9.

18. The method of claim 1, wherein the gene is a Bcr1 gene, a Bcr4 gene, an ApGLNT1 gene, or a Cact gene.

19. The method of claim 18, where the dsRNA is delivered as a PNA.