# COMPOSITIONS AND RELATED METHODS FOR AGRICULTURE

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 62/703,304, filed July 25, 2018, the contents of which are incorporated herein by reference in their entirety.

## SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 17, 2019, is named 51215-012WO2_Sequence_Listing_07.17.19_ST25 and is 60,129 bytes in size.

## BACKGROUND

Plant pests, including insect pests, are pervasive in the human environment.

## SUMMARY

In a first example, the disclosure comprises a method for decreasing colonization by a bacterium of a gut of a stink bug, the method comprising (a) providing a composition comprising vanillin or an analog thereof; and (b) delivering said composition to an egg from which the stink bug will hatch, whereby colonization by the bacterium within the gut of the stink bug hatched from the egg treated with the composition is decreased relative to a stink bug hatched from an untreated egg.

In one aspect, the present disclosure refers to a method for decreasing colonization by a bacterium of a gut of a stink bug, the method comprising:

(a) providing a composition comprising vanillin or an analog thereof; and

(b) delivering said composition to a stink bug egg, wherein the gut of the stink bug hatched from the egg has decreased colonization by the bacterium relative to the gut of a stink bug hatched from an untreated egg.

In some examples, the composition is delivered to an egg mass of a stink bug. In some examples, the decrease in colonization by the bacterium decreases the fitness of the stink bug, e.g., decreases reproductive ability, survival, rate of development, number of eggs, number of hatched eggs, adult emergence rate, body length, body width, body mass, or cuticle thickness.

In some examples, the colonization is in the v4 region of the gut. In some examples, colonization by the bacterium of the v4 region of the gut is decreased by at least 5%. In some examples, the size of the v4 region of the gut is decreased.

In some examples, the stink bug is a *Halyomorpha* species (e.g., *Halyomorpha halys*), a *Nezara* species, an *Oebalus* species, a *Chinavia* species, an *Euthyrhynchus* species, an *Euschistus* species, an *Alcaeorrhynchus* species, or a *Podisus* species.

In some examples, the bacterium is an endosymbiont, e.g., an endosymbiont is of the genus *Pantoea.* In some examples, the endosymbiont is *Candidatus Pantoea carbekii.*

In some examples, the composition is a liquid, a solid, an aerosol, a paste, a gel, or a gas composition. In some examples, the composition is delivered as a spray. In some examples, the composition comprises an agriculturally acceptable carrier. In some examples, the composition comprises a wetting solution.

Disclosed herein are compositions and methods for altering the fitness of insects for agriculture or commerce, wherein the composition includes a bacterial colonization-disrupting agent (e.g., an agent (e.g., a lipopolysaccharide (LPS) synthesis inhibitor or a polyhydroxyalkanoate (PHA) synthesis inhibitor) that decreases colonization of a bacteria (e.g., an endosymbiotic bacteria) in the gut of the insect.

In one example, provided herein is a method of altering the fitness of an insect comprising delivering to the insect an effective amount of a composition including a bacterial colonization-disrupting agent. In some examples, the method includes decreasing the fitness of the insect delivered the bacterial colonization-disrupting agent. Alternatively, in some examples, the method includes increasing the fitness of the insect delivered the bacterial colonization-disrupting agent.

In another example, provided herein is a method of decreasing bacterial colonization in the gut of an insect including delivering to the insect an effective amount of a composition including a bacterial colonization-disrupting agent.

In some examples of the methods herein, the bacterial colonization-disrupting agent is an inhibitor of bacterial metabolism. In some examples, the bacterial colonization-disrupting agent is a polyhydroxyalkanoate (PHA) synthesis inhibitor.

In another example, provided herein is a method of altering the fitness of an insect including delivering to the insect an effective amount of a composition including a PHA synthesis inhibitor. In some examples, the method includes decreasing the fitness of the insect delivered the PHA inhibitor. In some examples, the method includes increasing the fitness of the insect delivered the PHA inhibitor. In some examples, the PHA synthesis inhibitor is vanillin or an analog thereof. In some examples, the PHA synthesis inhibitor is one or more compounds in Table 1. In some examples, the PHA synthesis inhibitor is levulinic acid or an analog thereof. In some examples, the PHA synthesis inhibitor is acrylic acid or an analog thereof. In some examples, the PHA synthesis inhibitor is 2-bromooctanoic acid or an analog thereof.

In some examples of the methods herein, the bacterial colonization-disrupting agent is an inhibitor of cell envelope biogenesis (e.g., biogenesis of the membrane(s) or other structures that surround and protect the bacterial cytoplasm, e.g., cell wall, inner membrane, and outer membrane). In some examples of the methods herein, the bacterial colonization-disrupting agent is a lipopolysaccharide (LPS) synthesis inhibitor.

In another example, provided herein is a method of altering the fitness of an insect including delivering to the insect an LPS synthesis inhibitor. In some examples, the method includes decreasing the fitness of the insect delivered the LPS synthesis inhibitor. In some examples, the method includes increasing the fitness of the insect delivered the LPS synthesis inhibitor.

In some examples, the LPS synthesis inhibitor is an inhibitor of core oligosaccharide synthesis in the bacteria. In some examples, the LPS synthesis inhibitor inhibits an enzyme involved in core oligosaccharide synthesis in the bacteria. In some examples, the enzyme has at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to a polypeptide having the amino acid sequence of WaaA, WaaC, WaaF, or WaaG. In some examples, the LPS synthesis inhibitor (e.g., the inhibitor of an enzyme involved in LPS synthesis) is a sugar. In some examples, the sugar is ADP-2-fluoroheptose (AFH). In some examples, the sugar is 2-aryl-5-methyl-4-(5-aryl-furan-2-yl-methylene)-2,4-dihydro-pyrazol-3-ones (DHPO). In some examples, the sugar is AFH and DHPO. In some examples, the sugar is one or more compounds in Table 7.

In some examples, the LPS synthesis inhibitor inhibits expression of a gene involved in core oligosaccharide synthesis in the bacteria. In some examples, the gene has at least 40%, 50%, 60%,

70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to a polynucleotide having the nucleotide sequence of *waaA, waaC, waaF,* or *waaG*.

In some examples, the bacterial colonization-disrupting agent is an inhibitor of bacterial cell wall biogenesis. In some examples, the inhibitor of bacterial cell wall biogenesis is an inhibitor of undecaprenyl pyrophosphate phosphatase (UppP), e.g., bacitracin.

In some examples, the bacterial colonization-disrupting agent is an inhibitor of flagellar function, e.g., cellulose.

In some examples of the methods herein, the insect is a plant pest. In some examples, the plant pest is of the order Coleoptera, Diptera, Hemiptera, Lepidoptera, Orthoptera, Thysanoptera, or Acarina. In some examples, the insect is a stink bug, bean bug, beetle, weevil, fly, aphid, whitefly, leafhopper, scale, moth, butterfly, grasshopper, cricket, thrip, or mite. In some examples, the insect is of the genus *Riptortus*. In some examples, the insect is of the genus *Halyomorpha*.

In some examples of the methods herein, the insect is a vector of an animal pathogen and/or a human pathogen. In some examples, the insect is a mosquito, a midge, a louse, a sandfly, a tick, a triatomine bug, a tsetse fly, or flea.

In some examples of the methods herein, the bacteria is an endosymbiotic bacteria. In some examples, the endosymbiont resides in the gut of the insect. In some examples, the bacteria resides in a specialized cell or a specialized organ in the gut of the insect. In some examples, the specialized organ is a midgut crypt or a bacteriome. In some examples, the specialized cell is a bacteriocyte. In some examples, the endosymbiotic bacteria is of the genus *Burkholderia*. In some examples, the endosymbiotic bacteria is of the genus *Pantoea*.

In some examples of the methods herein, the method is effective to decrease the fitness of the insect relative to an untreated insect. In some examples, the decrease in fitness of the insect is a decrease (e.g., by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in reproductive ability, survival, rate of development, number of hatched eggs, adult emergence rate, body length, or weight relative to an untreated insect.

In some examples, the method is effective to decrease bacterial colonization (e.g., by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in the gut of the insect relative to an untreated insect.

In some examples, the method is effective to inhibit a physical interaction between the bacteria and the gut of the insect.

In some examples of the methods herein, the composition is delivered to the insect to at least one habitat where the insect grows, lives, or reproduces.

In some examples of the methods herein, the composition is a liquid, a solid, an aerosol, a paste, a gel, or a gas composition.

In some examples of the methods herein, the composition is delivered as an insect comestible composition for ingestion by the insect.

In some examples of the methods herein, the composition is delivered to the insect by ingestion, infusion, injection, or spraying. In some examples, the composition is delivered to eggs of the insect.

In some examples of the methods herein, the composition includes an agriculturally acceptable carrier.

In yet another example, provided herein is a modified insect produced by a method including contacting the insect with a composition including a bacterial colonization-disrupting agent in accordance with any of the methods herein.

In a further example, provided herein is a screening assay to identify a bacterial colonization-disrupting agent, including the steps of (a) exposing a target insect to one or more agents; and (b) identifying an agent that (i) decreases the fitness of the target insect (e.g., by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%), and (ii) inhibits colonization of a bacterium in the gut of the target insect (e.g., by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%).

In some examples of the assay herein, the decrease in fitness is decreased survival of the target insect. In some examples, the decrease in fitness is a decrease in reproductive ability, survival, rate of development, number of hatched eggs, adult emergence rate, body length, or body mass.

In some examples, the agent is effective to inhibit a physical interaction between the bacteria and the gut of the insect.

In some examples of the assay herein, the bacteria is an endosymbiotic bacteria. In some examples, the endosymbiotic bacteria resides in the gut of the insect. In some examples, the bacteria resides in a specialized cell or a specialized organ in the gut of the insect. In some examples, the specialized organ is a midgut crypt or a bacteriome. In some examples, the specialized cell is a bacteriocyte. In some examples, the bacterium is of the genus *Burkholderia*. In some examples, the bacterium is of the genus *Pantoea*.

In some examples of the assay herein, the bacterial colonization-disrupting agent is a PHA synthesis inhibitor.

In some examples of the assay herein, the bacterial colonization-disrupting agent is an LPS synthesis inhibitor.

In some examples of the assay herein, the insect is a plant pest. In some examples, the plant pest is of the order *Coleoptera, Diptera, Hemiptera, Lepidoptera, Orthoptera, Thysanoptera,* or *Acarina*.

In some examples of the assay herein, the insect is a vector of an animal pathogen and/or a human pathogen. In some examples, the insect is a mosquito, a midge, a louse, a sandfly, a tick, a triatomine bug, a tsetse fly, or flea.

In another example, provided herein is a modified insect produced by a method including contacting the insect with a composition including a bacterial colonization-disrupting agent identified by the screening assay herein.

In yet another example, provided herein is a method of decreasing the fitness of an insect including delivering to the insect an effective amount of a composition including a bacterial colonization-disrupting agent identified by the screening assay herein.

In a further example, provided here in is a composition including a bacterial colonization-disrupting agent and a carrier, wherein the composition is formulated for delivery to an insect, or a habitat thereof.

In some examples of the composition herein, the bacterial colonization-disrupting agent is a polyhydroxyalkanoate (PHA) synthesis inhibitor. In some examples, the PHA synthesis inhibitor is vanillin or an analog thereof. In some examples, the PHA synthesis inhibitor is one or more compounds in Table 1. In some examples, the PHA synthesis inhibitor is levulinic acid or an analog thereof. In some examples, the PHA synthesis inhibitor is acrylic acid or an analog thereof. In some examples, the PHA synthesis inhibitor is 2-bromooctanoic acid or an analog thereof.

In some examples of the composition herein, the bacterial colonization-disrupting agent is an inhibitor of bacterial cell envelope biogenesis. In some examples, the inhibitor of bacterial cell envelope biogenesis is a lipopolysaccharide (LPS) synthesis inhibitor. In some examples, the LPS synthesis inhibitor is an inhibitor of core oligosaccharide synthesis in the bacteria. In some examples, the LPS synthesis inhibitor inhibits an enzyme involved in core oligosaccharide synthesis in the bacteria. In some examples, the enzyme has at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to a polypeptide having the amino acid sequence of WaaA, WaaC, WaaF, or WaaG. In some examples, the LPS synthesis inhibitor (e.g., the inhibitor of an enzyme involved in LPS synthesis) is a sugar. In some examples, the sugar is ADP-2-fluoroheptose (AFH). In some examples, the sugar is 2-aryl-5-methyl-4-(5-aryl-furan-2-yl-methylene)-2,4-dihydro-pyrazol-3-ones (DHPO). In some examples, the sugar is AFH and DHPO. In some examples, the sugar is one or more compounds in Table 7.

In some examples, the LPS synthesis inhibitor inhibits expression of a gene involved in core oligosaccharide synthesis in the bacteria. In some examples, the gene has at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to a polynucleotide having the nucleotide sequence of *waaA, waaC, waaF,* or *waaG*.

In some examples, the bacterial colonization-disrupting agent is an inhibitor of bacterial cell wall biogenesis. In some examples, the inhibitor of bacterial cell wall biogenesis is an inhibitor of undecaprenyl pyrophosphate phosphatase (UppP), e.g., bacitracin.

In some examples, the bacterial colonization-disrupting agent is an inhibitor of flagellar function, e.g., cellulose.

In some examples of the composition herein, the bacterial colonization-disrupting agent is at least 0.1%, 0.2%, 0.4%, 0.5%, 0.8%, 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the composition. In some examples, the carrier is a liquid, a solid, an aerosol, a paste, a gel, or a gas composition. In some examples, the carrier is a sugar syrup, corn syrup, or honey. In some examples, the carrier is a nanoparticle or lipid membrane.

In some examples of the composition herein, the composition is formulated for delivery to the insect, for example, by ingestion, infusion, injection, spraying, smoking, or fogging. In some examples, the composition is formulated for delivery to at least one habitat, for example, where the insect grows, lives, reproduces, or feeds. In some examples, the composition is formulated for delivery to a plant ingested by the insect. In another example, provided herein is a modified plant or part thereof including a bacterial colonization-disrupting agent, wherein the plant or part thereof is ingested by an insect. In some examples, the plant is genetically engineered to produce the bacterial colonization-disrupting agent, e.g., by expression from a heterologous genetic construct.