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(71) Applicant: **MONSANTO TECHNOLOGY LLC** [US/US]; 800 North Lindbergh Blvd., St. Louis, MO 63167 (US).

(72) Inventors: **HEMMES, Johannes, C.**; 800 North Lindbergh Blvd., St. Louis, MO 63167 (US). **JIA, Lijie**; 800 North Lindbergh Blvd., St. Louis, MO 63167 (US).

(74) Agent: **HANSON, Robert, E.**; Dentons US LLP, 7800 Willis Tower, 233 South Wacker Dr., Chicago, IL 60606 (US).

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(54) Title: METHODS AND COMPOSITIONS FOR CONTROLLING PLANT VIRAL INFECTION

(57) Abstract: The present invention provides methods for topical treatment and prevention of Tospovirus and/or Geminivirus disease in plants. The invention further provides compositions for treatment of Tospovirus and/or Geminivirus disease in plants, and methods for reducing expression of a Tospovirus and/or Geminivirus gene and for identifying polynucleotides useful in modulating gene expression in plant viruses.

TITLE OF THE INVENTION

METHODS AND COMPOSITIONS FOR CONTROLLING PLANT VIRAL INFECTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 61/714,733, filed October 16, 2012, and U.S. Provisional Patent Application No. 61/786,032, filed March 14, 2013, which are incorporated herein by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

The sequence listing that is contained in the file named "MONS317WOsequencelisting.txt", which is 251 kilobytes as measured in Microsoft Windows operating system and was created on October 11, 2013, is filed electronically herewith and incorporated herein by reference.

FIELD OF THE INVENTION

The methods and compositions generally relate to the field of plant disease control. More specifically, the invention relates to methods and compositions for treating or preventing symptoms associated with plant Tospovirus or Geminivirus infection.

BACKGROUND OF THE INVENTION

Plant viruses of the genus Tospovirus and Geminivirus are economically important, causing reduced vegetative output and death of infected plants. Growers seeking to protect their crops from tospoviruses have traditionally attempted to guard their crops from the insect vectors, either with insecticide application, or with reflective mulches or plastic covers. Because these strategies have had limited success, and are expensive and labor intensive, alternative strategies for controlling Tospovirus and Geminivirus infection are needed.

SUMMARY OF THE INVENTION

The embodiments described herein relate to methods and compositions for the prevention or treatment of viral infection in a plant comprising the topical

administration to a plant of a polynucleotide comprising at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a viral gene. The polynucleotide may be single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), or double-stranded RNA (dsRNA).

In one aspect, the invention provides a method of treatment or prevention of a Tospovirus infection in a plant comprising: topically applying to said plant a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein the symptoms of viral infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In some embodiments, the antisense single-stranded DNA polynucleotide comprises at least 18 contiguous nucleotides that are essentially complementary to a sequence selected from the group consisting of SEQ ID NOs:13-46. In one embodiment, the transfer agent is an organosilicone surfactant composition or compound contained therein. In another embodiment, the composition comprises more than one antisense single-stranded DNA polynucleotide complementary to all or a portion of an essential Tospovirus gene sequence, an RNA transcript of said essential Tospovirus gene sequence, or a fragment thereof. In another embodiment, the antisense single-stranded DNA polynucleotide is selected from the group consisting of SEQ NOs:1-12 or a fragment thereof. In another embodiment, the Tospovirus is selected from the group consisting of bean necrotic mosaic virus, Capsicum chlorosis virus, groundnut bud necrosis virus, groundnut ringspot virus, groundnut yellow spot virus, impatiens necrotic spot virus, iris yellow spot viris, melon yellow spot virus, peanut bud necrosis virus, peanut yellow spot virus, soybean vein necrosis-associated virus, tomato chlorotic spot virus, tomato necrotic ringspot virus, tomato spotted wilt virus, tomato zonate spot virus, watermelon bud necrosis virus, watermelon silver mottle virus, and zucchini lethal chlorosis virus. In another embodiment, the essential Tospovirus gene is selected from the group consisting of nucleocapsid gene (N), coat protein gene (CP), virulence factors NSm and NSs, and RNA-dependent RNA polymerase L segment (RdRp/L segment). In another embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46. In another embodiment,

composition is topically applied by spraying, dusting, or is applied to the plant surface as matrix-encapsulated DNA.

In another aspect, the invention provides a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In some embodiments, the essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46, or the transfer agent is an organosilicone composition, or the antisense single-stranded DNA polynucleotide is selected from the group consisting of SEQ ID NOs:1-12.

In another aspect, the invention provides a method of reducing expression of an essential Tospovirus gene comprising contacting a Tospovirus particle with a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential gene sequence in said Tospovirus or an RNA transcript thereof, wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46. In another embodiment, the transfer agent is an organosilicone compound. In another embodiment, the antisense single-stranded DNA polynucleotide is selected from the group consisting of SEQ ID NOs:1-12 or fragment thereof.

In another aspect, the invention provides a method of identifying antisense single-stranded DNA polynucleotides useful in modulating Tospovirus gene expression when topically treating a plant comprising: a) providing a plurality of antisense single-stranded DNA polynucleotides that comprise a region complementary to all or a part of an essential Tospovirus gene or RNA transcript thereof; b) topically treating said plant with one or more of said antisense single-stranded DNA polynucleotides and a transfer agent; c) analyzing said plant or extract

for modulation of symptoms of Tospovirus infection; and d) selecting an antisense single-stranded DNA polynucleotide capable of modulating the symptoms or occurrence of Tospovirus infection. In an embodiment, the transfer agent is an organosilicone compound.

In another aspect, the invention provides an agricultural chemical composition comprising an admixture of an antisense single-stranded DNA polynucleotide and a pesticide, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In an embodiment, the pesticide is selected from the group consisting of anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides.

In another aspect, the invention provides a method of treatment or prevention of a Tospovirus infection in a plant comprising: topically applying to said plant a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA comprises a polynucleotide that is essentially complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein the symptoms of viral infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In some embodiments, the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs:13-46. In one embodiment, transfer agent is an organosilicone surfactant composition or compound contained therein. In another embodiment, the composition comprises more than one double-stranded RNA comprising a polynucleotide that is complementary to all or a portion of an essential Tospovirus gene sequence, an RNA transcript of said essential Tospovirus gene sequence, or a fragment thereof. In another embodiment, the double-stranded RNA polynucleotide comprises a polynucleotide that is essentially

identical or essentially complementary to a nucleotide sequence as set forth in SEQ NOs:47-103, 448-483, or a fragment thereof. In some embodiments, the antisense polynucleotide of the dsRNA comprises a two (2) nucleotide overhang on the 3' end that is complementary to the target gene. In another embodiment, the Tospovirus is selected from the group consisting of bean necrotic mosaic virus, Capsicum chlorosis virus, groundnut bud necrosis virus, groundnut ringspot virus, groundnut yellow spot virus, impatiens necrotic spot virus, iris yellow spot viris, melon yellow spot virus, peanut bud necrosis virus, peanut yellow spot virus, soybean vein necrosis-associated virus, tomato chlorotic spot virus, tomato necrotic ringspot virus, tomato spotted wilt virus, tomato zonate spot virus, watermelon bud necrosis virus, watermelon silver mottle virus, and zucchini lethal chlorosis virus. In another embodiment, the essential Tospovirus gene is selected from the group consisting of nucleocapsid gene (N), coat protein gene (CP), virulence factors NSm and NSs, and RNA-dependent RNA polymerase L segment (RdRp/L segment). In another embodiment, the essential Tospovirus gene is selected from the group consisting of SEQ ID NOs:13-46. In another embodiment, the composition is topically applied by spraying, dusting, or is applied to the plant surface as matrix-encapsulated RNA.

In another aspect, the invention provides a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46. In another embodiment, the transfer agent is an organosilicone composition. In another embodiment, the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to a nucleotide sequence selected from the group consisting of SEQ NOs:47-103 and 448-483. In some embodiments, the antisense polynucleotide of the dsRNA comprises a two (2) nucleotide overhang on the 3' end that is complementary to the target gene.

In another aspect, the invention provides a method of reducing expression of an essential Tospovirus gene comprising contacting a Tospovirus particle with a

composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA comprises a polynucleotide that is complementary to all or a portion of an essential gene sequence in said Tospovirus or an RNA transcript thereof, wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46. In another embodiment, the transfer agent is an organosilicone compound. In another embodiment, the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to a nucleotide sequence selected from the group consisting of SEQ ID NOs:47-103, 448-483, or fragment thereof. In some embodiments, the antisense polynucleotide of the dsRNA comprises a two (2) nucleotide overhang on the 3' end that is complementary to the target gene.

In another aspect, the invention provides a method of identifying a double-stranded RNA polynucleotide useful in modulating Tospovirus gene expression when topically treating a plant comprising: a) providing a plurality of double-stranded RNA polynucleotides that comprise a region complementary to all or a part of an essential Tospovirus gene or RNA transcript thereof; b) topically treating said plant with one or more of said double-stranded RNA polynucleotides and a transfer agent; c) analyzing said plant or extract for modulation of symptoms of Tospovirus infection; and d) selecting a double-stranded RNA polynucleotide capable of modulating the symptoms or occurrence of Tospovirus infection. In one embodiment, the transfer agent is an organosilicone compound. In some embodiments, the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 contiguos nucleotides of a sequence selected from the group consisting of SEQ ID NOs:13-46.

In another aspect, the invention provides an agricultural chemical composition comprising an admixture of a double-stranded RNA polynucleotide and a pesticide, wherein said double-stranded RNA comprises a polynucleotide that is essentially complementary to all or a portion of an essential Tospovirus gene sequence or RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said

composition when grown under the same conditions. In one embodiment, the pesticide is selected from the group consisting of anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides.

In still another aspect, the invention provides a method of treatment or prevention of a Geminivirus infection in a plant comprising: topically applying to said plant a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA comprises a polynucleotide that is complementary to all or a portion of an essential Geminivirus gene sequence, or an RNA transcript thereof, wherein the symptoms of viral infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the transfer agent is an organosilicone surfactant composition or compound contained therein. In another embodiment, the composition comprises more than one double-stranded RNA comprising a polynucleotide that is essentially complementary to all or a portion of an essential Geminivirus gene sequence, an RNA transcript of said essential Geminivirus gene sequence, or a fragment thereof. In another embodiment, the double-stranded RNA comprises a polynucleotide that is essentially identical or esstentially complementary to at least 18 nucleotides of a sequence selected from the group consisting of SEQ NOs:104-268 or a fragment thereof. In another embodiment, the Geminivirus is selected from the group consisting of Barley yellow dwarf virus, Cucumber mosaic virus, Pepino mosaic virus, Cotton curl leaf virus, Tomato yellow leaf curl virus, Tomato golden mosaic virus, Potato yellow mosaic virus, Pepper leaf curl virus, Bean golden mosaic virus, Bean golden mosaic virus, Tomato mottle virus. In still another aspect, the essential Geminivirus gene is selected from the group consisting of nucleocapsid gene (N), a coat protein gene (CP), virulence factors NSm and NSs, and RNA-dependent RNA polymerase L segment (RdRp/L segment), a silencing suppressor gene, movement protein (MP), Nia, CP-N, a triple gene block, CP-P3, MP-P4, C2, and AC2. In another embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:269-447. In another embodiment, the composition is topically applied by spraying, dusting, or is applied to the plant surface as matrix-encapsulated RNA.

In another aspect, the invention provides a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA comprises a polynucleotide that is essentially complementary to all or a portion of an essential Geminivirus gene sequence, such as one set forth as SEQ ID NOs:104-268, 269-447, or an RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:269-447. In another embodiment, the transfer agent is an organosilicone composition. In another embodiment, the double-stranded RNA polynucleotide is selected from the group consisting of SEQ NOs:104-268.

In another aspect, a method of reducing expression of an essential Geminivirus gene comprising contacting a Geminivirus particle with a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA comprises a polynucleotide that is essentially complementary to all or a portion of an essential gene sequence in said Geminivirus or an RNA transcript thereof, wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:269-447. In another embodiment, the transfer agent is an organosilicone compound. In another embodiment, the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 nucleotides of a sequence selected from the group consisting of SEQ NOs:104-268 or fragment thereof.

In still another aspect, the invention provides a method of identifying a double-stranded RNA polynucleotide useful in modulating Geminivirus gene expression when topically treating a plant comprising: a) providing a plurality of double-stranded RNA polynucleotides that comprise a region complementary to all or a part of an essential Geminivirus gene or RNA transcript thereof; b) topically treating said plant with one or more of said double-stranded RNA polynucleotides and a transfer agent; c) analyzing said plant or extract for modulation of symptoms of

Geminivirus infection; and d) selecting a double-stranded RNA polynucleotide capable of modulating the symptoms or occurrence of Geminivirus infection. In one embodiment, the transfer agent is an organosilicone compound. In some embodiments, the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 contiguos nucleotides of a sequence selected from the group consisting of SEQ ID NOs:269-447. In some embodiments, the Geminivirus is Cucumber Mosiac Virus and the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 contiguos nucleotides of a sequence selected from the group consisting of SEQ ID NOs:269-316. In some embodiments, the Geminivirus is Pepino Mosaic Virus and the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 contiguos nucleotides of a sequence selected from the group consisting of SEQ ID NOs:317-349. In some embodiments, the Geminivirus is Tomato Yellow Curl Leaf Virus and the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 contiguos nucleotides of a sequence selected from the group consisting of SEQ ID NOs:386-421. In some embodiments, the Gemini virus is Cotton Leaf Curl Virus and the double-stranded RNA comprises a polynucleotide that is essentially identical or essentially complementary to at least 18 contiguos nucleotides of a sequence selected from the group consisting of SEQ ID NOs:422-441.

In another aspect, the invention provides an agricultural chemical composition comprising an admixture of a double-stranded RNA polynucleotide and a pesticide, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Geminivirus gene sequence or RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the pesticide is selected from the group consisting of anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides.

In one aspect, the invention provides a method of treatment or prevention of a Geminivirus infection in a plant comprising: topically applying to said plant a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Geminivirus gene sequence or an RNA transcript thereof, wherein the symptoms of viral infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In some embodiments, the antisense single-stranded DNA polynucleotide comprises at least 18 contiguous nucleotides that are essentially complementary a sequence selected from the group consisting of SEQ ID NOs:104-268. In some embodiments, the antisense single-stranded DNA polynucleotide comprises at least 18 contiguous nucleotides that are essentially complementary a sequence selected from the group consisting of SEQ ID NOs:269-447. In one embodiment, the transfer agent is an organosilicone surfactant composition or compound contained therein. In another embodiment, the composition comprises more than one antisense single-stranded DNA polynucleotide complementary to all or a portion of an essential Geminivirus gene sequence, an RNA transcript of said essential Geminivirus gene sequence, or a fragment thereof. In another embodiment, the Geminivirus is selected from the group consisting of Barley yellow dwarf virus, Cucumber mosaic virus, Pepino mosaic virus, Cotton curl leaf virus, Tomato yellow leaf curl virus, Tomato golden mosaic virus, Potato yellow mosaic virus, Pepper leaf curl virus, Bean golden mosaic virus, Bean golden mosaic virus, and Tomato mottle virus. In still another aspect, the essential Geminivirus gene is selected from the group consisting of nucleocapsid gene (N), a coat protein gene (CP), virulence factors NSm and NSs, and RNA-dependent RNA polymerase L segment (RdRp/L segment), a silencing suppressor gene, movement protein (MP), Nia, CP-N, a triple gene block, CP-P3, MP-P4, C2, and AC2. In another embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:269-447. In another embodiment, the composition is topically applied by spraying, dusting, or is applied to the plant surface as matrix-encapsulated RNA.

In another aspect, the invention provides a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of

an essential Geminivirus gene sequence or an RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In some embodiments, the essential gene sequence is selected from the group consisting of SEQ ID NOs:104-447, or the transfer agent is an organosilicone composition.

In another aspect, the invention provides a method of reducing expression of an essential Geminivirus gene comprising contacting a Geminivirus particle with a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential gene sequence in said Geminivirus or an RNA transcript thereof, wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In one embodiment, the essential gene sequence is selected from the group consisting of SEQ ID NOs:104-447. In another embodiment, the transfer agent is an organosilicone compound.

In another aspect, the invention provides a method of identifying antisense single-stranded DNA polynucleotides useful in modulating Geminivirus gene expression when topically treating a plant comprising: a) providing a plurality of antisense single-stranded DNA polynucleotides that comprise a region complementary to all or a part of an essential Geminivirus gene or RNA transcript thereof; b) topically treating said plant with one or more of said antisense single-stranded DNA polynucleotides and a transfer agent; c) analyzing said plant or extract for modulation of symptoms of Geminivirus infection; and d) selecting an antisense single-stranded DNA polynucleotide capable of modulating the symptoms or occurrence of Geminivirus infection. In an embodiment, the transfer agent is an organosilicone compound. In some embodiments, the antisense single-stranded DNA is essentially complementary to at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs:269-447. In some embodiments, the Geminivirus is Cucumber mosaic virus and the antisense single-stranded DNA is essentially complementary to at least 18 contiguous nucleotides of a sequence

selected from the group consisting of SEQ ID NOs:269-316. In some embodiments, the Geminivirus is Pepino mosaic virus and the antisense single-stranded DNA is essentially complementary to at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs:317-349. In some embodiments, the Geminivirus is Tomato yellow leaf curl virus and the antisense single-stranded DNA is essentially complementary to at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs:386-421. In some embodiments, the Geminivirus is Cotton leaf curl virus and the antisense single-stranded DNA is essentially complementary to at least 18 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs:422-441.

In another aspect, the invention provides an agricultural chemical composition comprising an admixture of an antisense single-stranded DNA polynucleotide and a pesticide, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Geminivirus gene sequence or RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions. In an embodiment, the pesticide is selected from the group consisting of anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the function of the compositions and methods. The function may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein. The function can be more fully understood from the following description of the figures:

FIG. 1: Shows a graph depicting the results of topical treatment of lettuce (*SVR3606 L4*) plants with antisense single stranded (ss) DNA oligonucleotides

(oligos). Fresh weight aerial tissue (in grams) was plotted against treatments performed at -1 Day infection, 0 Day Infection and +1 Day Infection.

FIG. 2: Shows symptom development on lettuce (*SVR3606 L4*) plants 18 days after virus inoculation. (A) Plants on the right were sprayed with antisense ssDNA oligos at 20 psi using an airbrush several hours after virus inoculation. Left side shows control plants inoculated with impatiens necrotic spotted virus (INSV) only. Leaves were punctured with a hole puncture for ELISA analysis. (B) Graph depicting the results of visual scoring for INSV symptom development in null treated or antisense ssDNA treated plants.

FIG. 3: Shows a graph of the results of ELISA analysis of the effects of topical treatment with antisense ssDNA on reduction of virus accumulation in lettuce leaves. The unit of measure is protein absorbance at optical density (OD) of 450 nm. Circles represent data points collected from the control plants (virus only, no polynucleotide). Triangles represent data points collected from plants treated with a mixture of antisense ssDNA oligos (SEQ ID NO:1 and SEQ ID NO:2).

FIG. 4: Panels A, B, and D show graphs depicting the optical density (OD 450 nm) of extracts of lettuce plants at day 5 (A), day 8 (B), and day 14 (D) after treatment with antisense ssDNA oligos. (C) Shows a graph depicting the results of visual assessment of plants at day 13 after treatment with antisense ssDNA oligos.

FIG. 5: Shows results of the effects of topical treatment with antisense ssDNA oligos on lettuce plants. Panels A and B show the OD 450 nm ELISA data at 5 and 14 days after treatment, respectively. Panel C shows a graph of the mean effective yield of photosystem II (PSII) determined by a portable chlorophyll fluorometer at day 21 after treatment with antisense ssDNA oligos. Panel D shows a graph of the fresh weight aerial tissue (in grams) for null or antisense ssDNA treated plants at day 21 after treatment.

FIG. 6: Shows a field trial planting scheme and day 60 photo in which tomato and pepper plants were topically treated with antisense ssDNA oligos against tomato spotted wilt virus (TSWV).

FIG. 7: Shows tomato plants both untreated (circled) and topically treated with antisense ssDNA oligos against TSWV.

FIG. 8: Shows graphs of the results of the effects of treatment of tomato plants with antisense ssDNA oligos. Panels A, B, and D show graphs depicting the OD 450 nm ELISA data for plants treated with buffer only or sprayed once or twice with antisense ssDNA oligonucleotides at 15 (A), 60 (B), and 78 (D) days post-treatment. Panel C shows a graph depicting the results of visual scoring of the tomato plants for symptoms at day 78 post-treatment.

FIG. 9: Shows graphs of the results of the effects of treatment of pepper plants with antisense ssDNA oligos. Panels A, B, and D show graphs depicting the OD 450 nm ELISA data for pepper plants treated with buffer only or sprayed once or twice with antisense ssDNA oligonucleotides at 15 (A), 60 (B), and 78 (D) days post-treatment. Panel C shows a graph depicting the results of visual scoring of the pepper plants for symptoms at day 78 post-treatment.

FIG. 10: Shows a graph of the effects of oligo treatment on reduction of virus accumulation in pepper leaves. The OD 450 nm was measured to assess the amount of virus present. The dots represent data points collected from the control plants (virus only, no oligo treatment). Diamonds (SEQ ID NOs:5-8) and triangles (SEQ ID NOs:9-12) represent data points collected from samples topically treated with the antisense ssDNA oligonucleotide solution. The left side shows data from inoculated leaves, and the right side shows data from systemic non-infected, non-oligo-treated leaves.

FIG. 11: Shows graphs of the results of the effects of oligo treatment on onion plants. Panel A shows a graph depicting the bulb diameter prior to treatment with topical oligonucleotides. Panel B shows a graph depicting the different bulb diameters in 4 different sections of the field. Panel C shows a graph depicting the bulb diameter after treatment with buffer or topical antisense ssDNA oligonucleotides. Panel D shows a graph depicting the OD 450 nm measurement for buffer and antisense ssDNA treated plants.

FIG. 12: Panel A shows a graph of the plant height for the different treatments. T25748, T25753, T25755, T25763, T25769, T25770, T25773, T25776, and T25778 are dsRNA triggers. Panel B shows a graph of the plant height for Healthy (uninfected), Virus infected but untreated, Virus infected buffer treated

(Buffer), Virus infected T25748 dsRNA trigger treated (T25748), and Virus infected T25773 dsRNA trigger treated (T25773) plants.

FIG. 13: Shows a graph of the plant height for the different treatments. T25748, T25755, T25763, T25769, T25770, T25772, T25775, and T25776 are dsRNA triggers.

DETAILED DESCRIPTION OF THE INVENTION

Provided are compositions and methods useful for treating or preventing viral infection in plants. Aspects of the methods and compositions disclosed herein can be applied to treat or prevent viral infection in plants in agronomic and other cultivated environments.

Several embodiments relate to methods and compositions for the prevention or treatment of Tospovirus infection in a plant comprising the topical administration of a polynucleotide comprising at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a Tospoviral gene. In some embodiments, the Tospoviral gene is selected from the group consisting of a nucleocapsid (N) gene, a suppressor (NSs) gene, a movement (NSm) gene, and a RNA dependent RNA polymerase (RdRp) gene. In some embodiments, methods and compositions for the prevention or treatment of Tospovirus infection in a plant comprising the topical administration of single-stranded (ss) DNA in antisense (as) orientation as set forth in SEQ ID NOs:1-12 (Tables 1-3) are provided. Also provided are methods and compositions for the prevention or treatment of Tospovirus infection in a plant comprising the topical administration of double-stranded (ds) RNA comprising a polynucleotide that is essentially identical or essentially complementary to a nucleotide sequence as set forth in SEQ ID NOs:47-103 (Table 5) or SEQ ID NOs:448-483 (Table 12). In some embodiments, the antisense polynucleotide of the dsRNA comprises a two (2) nucleotide overhang on the 3' end that is complementary to the target gene. In certain embodiments, the methods and compositions of the invention provide regulation, repression, or delay and/or modulation of symptoms or disease caused by Tospovirus.

Several embodiments relate to methods and compositions for the prevention or treatment of Geminivirus infection in a plant comprising the topical administration of a polynucleotide comprising at least 18 contiguous nucleotides that are essentially

identical or essentially complementary to a Geminiviral gene. In some embodiments, the Geminiviral gene is selected from the group consisting of a coat protien (CP) gene, a silencing suppressor gene, and a movement gene. Also provided are methods and compositions for the prevention or treatment of Geminivirus infection in a plant comprising the topical administration of dsRNA comprising a polynucleotide that is essentially identical or essentially complementary to a nucleotide sequence as set forth in SEQ ID NOs:104-268 (Table 6). Aspects of the methods and compositions can be applied to manage plant viral diseases in agronomic and other cultivated environments.

Compositions of the present invention may include ssDNA, dsDNA, ssRNA, or dsRNA polynucleotides and/or ssDNA, dsDNA, ssRNA, or dsRNA oligonucleotides designed to target single or multiple viral genes, or multiple segments of one or more viral genes, such as genes from a Tospovirus or other plant disease, including, but not limited to the viral gene sequences set forth in SEQ ID NOs:1-46 (Tables 1-4). In another embodiment, such polynucleotides and oligonucleotides may be designed to target single or multiple viral genes, or multiple segments of one or more viral genes, such as genes from a Geminivirus, including, but not limited to the viral gene sequences set forth in SEQ ID NOs:269-447 (Tables 7-11). In an embodiment, any viral gene from any plant virus may be targeted by compositions of the present invention. The target gene may include multiple consecutive segments of a target gene, multiple non-consecutive segments of a target gene, multiple alleles of a target gene, or multiple target genes from one or more Tospovirus species. In some embodiments, the polynucleotides or oligonucleotides are essentially identical or essentially complementary to a consensus nucleotide sequence.

Polynucleotides of the invention may be complementary to all or a portion of a viral gene sequence, including a promoter, intron, coding sequence, exon, 5' untranslated region, and 3' untranslated region. Compositions of the present invention further comprise a transfer agent that facilitates delivery of the polynucleotide of the invention to a plant, and may include solvents, diluents, a pesticide that complements the action of the polynucleotide, a herbicide or additional pesticides or that provides an additional mode of action different from the

polynucleotide, various salts or stabilizing agents that enhance the utility of the composition as an admixture of the components of the composition.

In certain aspects, methods of the invention may include one or more applications of a polynucleotide composition and one or more applications of a transfer agent for conditioning of a plant or plant virus to permeation by polynucleotides or activity or stability of the polynucleotides. When the agent for conditioning to permeation is an organosilicone composition or compound contained therein, the polynucleotide molecules may be ssDNA, dsDNA, ssRNA, or dsRNA oligonucleotides; or ssDNA, dsDNA, ssRNA, or dsRNA polynucleotides, chemically modified DNA oligonucleotides or polynucleotides, or mixtures thereof.

In one embodiment, the present invention provides a method for controlling Tospovirus or Geminivirus infection of a plant including treatment of the plant with at least a first antisense ssDNA complementary to all or a portion of a target viral gene, wherein the polynucleotide molecules are capable of modulation of the target gene and controlling Tospovirus or Geminivirus infection. In another embodiment, the present invention provides a method for controlling Tospovirus or Geminivirus infection of a plant including treatment of the plant with at least a first antisense dsDNA complementary to all or a portion of a target viral gene, wherein the polynucleotide molecules are capable of modulation of the target gene and controlling Tospovirus or Geminivirus infection. In another embodiment, the invention provides a method for controlling Tospovirus or Geminivirus infection of a plant including treatment of the plant with at least a first dsRNA complementary to all or a portion of a target viral gene, wherein the polynucleotide molecules are capable of modulation of the target gene and controlling Tospovirus or Geminivirus infection.

In certain embodiments, a conditioning step to increase permeability of a plant to the polynucleotide may be included. The conditioning and polynucleotide application can be performed separately or in a single step. When the conditioning and polynucleotide application are performed in separate steps, the conditioning can precede or can follow the polynucleotide application within minutes, hours, or days. In some embodiments, more than one conditioning step or more than one polynucleotide molecule application can be performed on the same plant.

In specific embodiments of the method, a polynucleotide of the invention can be cloned or identified from (a) coding (protein-encoding), (b) non-coding (promoter and other gene related molecules), or (c) both coding and non-coding parts of the target viral gene. Non-coding parts may include DNA, such as promoter regions or an RNA transcribed by the DNA that provides RNA regulatory molecules, including but not limited to: introns, cis-acting regulatory RNA elements, 5' or 3' untranslated regions, and microRNAs (miRNA), *trans*-acting siRNAs, natural antisense siRNAs, and other small RNAs with regulatory function or RNAs having structural or enzymatic function including but not limited to: ribozymes, ribosomal RNAs, t-RNAs, aptamers, and riboswitches.

As used herein, "Tospovirus" refers to a virus from the genus Tospovirus, which may include bean necrotic mosaic virus, Capsicum chlorosis virus, groundnut bud necrosis virus, groundnut ringspot virus, groundnut yellow spot virus, impatiens necrotic spot virus, iris yellow spot viris, melon yellow spot virus, peanut bud necrosis virus, peanut yellow spot virus, soybean vein necrosis-associated virus, tomato chlorotic spot virus, tomato necrotic ringspot virus, tomato spotted wilt virus, tomato zonate spot virus, watermelon bud necrosis virus, watermelon silver mottle virus, or zucchini lethal chlorosis virus.

As used herein, a "Geminivirus" refers to a virus from the Geminiviridae Family of plant viruses. A Geminivirus may include, but is not limited to, Barley yellow dwarf virus (BYDW), Cucumber mosaic virus (CMV), Pepino mosaic virus (PepMV), Cotton curl leaf virus (CuCLV), Tomato yellow leaf curl virus (TYLCV), Tomato golden mosaic virus, Potato yellow mosaic virus, Pepper leaf curl virus (PepLCV), Bean golden mosaic virus (BGMV-PR), Bean golden mosaic virus (BGMV-DR), Tomato mottle virus (TMV), and the like.

The DNA or RNA polynucleotide compositions of the present invention are useful in compositions, such as liquids that comprise DNA or RNA polynucleotide molecules, alone or in combination with other components either in the same liquid or in separately applied liquids that provide a transfer agent. As used herein, a transfer agent is an agent that, when combined with a polynucleotide in a composition that is topically applied to a target plant surface facilitates the use of the polynucleotide in controlling a Tospovirus or Geminivirus. In one embodiment, the transfer agent enhances the ability of the polynucleotide to enter a plant cell. In

certain embodiments, a transfer agent is therefore an agent that conditions the surface of plant tissue, *e. g.*, leaves, stems, roots, flowers, or fruits, to permeation by the polynucleotide molecules into plant cells. The transfer of polynucleotides into plant cells can be facilitated by the prior or contemporaneous application of a polynucleotide-transferring agent to the plant tissue. In some embodiments the transferring agent is applied subsequent to the application of the polynucleotide composition. The polynucleotide transfer agent enables a pathway for polynucleotides through cuticle wax barriers, stomata and/or cell wall or membrane barriers into plant cells. Suitable transfer agents to facilitate transfer of the polynucleotide into a plant cell include agents that increase permeability of the exterior of the plant or that increase permeability of plant cells to oligonucleotides or polynucleotides. Such agents to facilitate transfer of the composition into a plant cell include a chemical agent, or a physical agent, or combinations thereof. Chemical agents for conditioning or transfer include (a) surfactants, (b) an organic solvent or an aqueous solution or aqueous mixtures of organic solvents, (c) oxidizing agents, (d) acids, (e) bases, (f) oils, (g) enzymes, or combinations thereof. Embodiments of the method can optionally include an incubation step, a neutralization step (*e.g.*, to neutralize an acid, base, or oxidizing agent, or to inactivate an enzyme), a rinsing step, or combinations thereof.

Embodiments of agents or treatments for conditioning of a plant to permeation by polynucleotides include emulsions, reverse emulsions, liposomes, and other micellar-like compositions. Embodiments of agents or treatments for conditioning of a plant to permeation by polynucleotides include counter-ions or other molecules that are known to associate with nucleic acid molecules, *e. g.*, inorganic ammonium ions, alkyl ammonium ions, lithium ions, polyamines such as spermine, spermidine, or putrescine, and other cations. Organic solvents useful in conditioning a plant to permeation by polynucleotides include DMSO, DMF, pyridine, *N*-pyrrolidine, hexamethylphosphoramide, acetonitrile, dioxane, polypropylene glycol, other solvents miscible with water or that will dissolve phosphonucleotides in non-aqueous systems (such as is used in synthetic reactions). Naturally derived or synthetic oils with or without surfactants or emulsifiers can be used, *e.g.*, plant-sourced oils, crop oils (such as those listed in the 9th Compendium of Herbicide Adjuvants, publicly available on the worldwide web (internet) at herbicide.adjuvants.com can be used, *e.g.*, paraffinic

oils, polyol fatty acid esters, or oils with short-chain molecules modified with amides or polyamines such as polyethyleneimine or *N*-pyrrolidine. Transfer agents include, but are not limited to, organosilicone preparations.

In certain embodiments, an organosilicone preparation that comprises an organosilicone compound comprising a trisiloxane head group is used in the methods and compositions provided herein. In certain embodiments, an organosilicone preparation that comprises an organosilicone compound comprising a heptamethyltrisiloxane head group is used in the methods and compositions provided herein. In certain embodiments of the methods and compositions provided herein, a composition that comprises a polynucleotide molecule and one or more effective organosilicone compound in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) is used or provided.

Organosilicone preparations used in the methods and compositions provided herein can comprise one or more effective organosilicone compounds. As used herein, the phrase "effective organosilicone compound" is used to describe any organosilicone compound that is found in an organosilicone preparation that enables a polynucleotide to enter a plant cell. In certain embodiments, an effective organosilicone compound can enable a polynucleotide to enter a plant cell in a manner permitting a polynucleotide mediated suppression of a target gene expression in the plant cell. In general, effective organosilicone compounds include, but are not limited to, compounds that can comprise: i) a trisiloxane head group that is covalently linked to, ii) an alkyl linker including, but not limited to, an n-propyl linker, that is covalently linked to, iii) a poly glycol chain, that is covalently linked to, iv) a terminal group. Trisiloxane head groups of such effective organosilicone compounds include, but are not limited to, heptamethyltrisiloxane. Alkyl linkers can include, but are not limited to, an n-propyl linker. Poly glycol chains include, but are not limited to, polyethylene glycol or polypropylene glycol. Poly glycol chains can comprise a mixture that provides an average chain length "n" of about "7.5." In certain embodiments, the average chain length "n" can vary from about 5 to about 14. Terminal groups can include, but are not limited to, alkyl groups such as a methyl

group. Effective organosilicone compounds are believed to include, but are not limited to, trisiloxane ethoxylate surfactants or polyalkylene oxide modified heptamethyl trisiloxane.

In certain embodiments, an organosilicone preparation that is commercially available as Silwet® L-77 surfactant having CAS Number 27306-78-1 and EPA Number: CAL.REG.NO. 5905-50073-AA, and currently available from Momentive Performance Materials, Albany, New York can be used to prepare a polynucleotide composition. In certain embodiments where a Silwet L-77 organosilicone preparation is used as a pre-spray treatment of plant leaves or other plant surfaces, freshly made concentrations in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) are efficacious in preparing a leaf or other plant surface for transfer of polynucleotide molecules into plant cells from a topical application on the surface. In certain embodiments of the methods and compositions provided herein, a composition that comprises a polynucleotide molecule and an organosilicone preparation comprising Silwet L-77 in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) is used or provided.

In certain embodiments, any of the commercially available organosilicone preparations provided such as the following Breakthru S 321, Breakthru S 200 Cat# 67674-67-3, Breakthru OE 441 Cat#68937-55-3, Breakthru S 278 Cat #27306-78-1, Breakthru S 243, Breakthru S 233 Cat#134180-76-0, available from manufacturer Evonik Goldschmidt (Germany), Silwet® HS 429, Silwet® HS 312, Silwet® HS 508, Silwet® HS 604 (Momentive Performance Materials, Albany, New York) can be used as transfer agents in a polynucleotide composition. In certain embodiments where an organosilicone preparation is used as a pre-spray treatment of plant leaves or other surfaces, freshly made concentrations in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5,

0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) are efficacious in preparing a leaf or other plant surface for transfer of polynucleotide molecules into plant cells from a topical application on the surface. In certain embodiments of the methods and compositions provided herein, a composition that comprises a polynucleotide molecule and an organosilicone preparation in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) is used or provided.

Delivery of a polynucleotide according to the invention can be accomplished by a variety of methods including, without limitation, (1) loading liposomes with a ssDNA, dsDNA, ssRNA, or dsRNA molecule provided herein and (2) complexing a ssDNA, dsDNA, ssRNA, or dsRNA molecule with lipids or liposomes to form nucleic acid-lipid or nucleic acid-liposome complexes. The liposome can be composed of cationic and neutral lipids commonly used to transfect cells *in vitro*. Cationic lipids can complex (*e.g.*, charge-associate) with negatively charged, nucleic acids to form liposomes. Examples of cationic liposomes include, without limitation, lipofectin, lipofectamine, lipofectace, and DOTAP. Procedures for forming liposomes are well known in the art. Liposome compositions can be formed, for example, from phosphatidylcholine, dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidyl glycerol, dioleoyl phosphatidylethanolamine or liposomes comprising dihydrosphingomyelin (DHSM). Numerous lipophilic agents are commercially available, including Lipofectin® (Invitrogen/Life Technologies, Carlsbad, Calif.) and Effectene™ (Qiagen, Valencia, Calif.). In addition, systemic delivery methods can be optimized using commercially available cationic lipids such as DDAB or DOTAP, each of which can be mixed with a neutral lipid such as DOPE or cholesterol. In some eases, liposomes such as those described by Templeton *et al.* (*Nature Biotechnology*, 15:647-652, 1997) can be used. In other embodiments, polycations such as polyethyleneimine can be used to achieve delivery *in vivo* and *ex vivo* (Boletta *et al.*, *J. Am Soc. Nephrol.* 7:1728, 1996). Additional information regarding the use of liposomes to deliver nucleic acids can be found in U.S. Pat. No. 6,271,359, PCT Publication WO 96/40964 and Morrissey *et al.* (*Nat Biotechnol.* 23(8):1002-7, 2005).

The following definitions and methods are provided to guide those of ordinary skill in the art. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Where a term is provided in the singular, the inventors also contemplate aspects described by the plural of that term.

By "non-transcribable" polynucleotides is meant that the polynucleotides do not comprise a complete polymerase II transcription unit.

As used herein "solution" refers to homogeneous mixtures and non-homogeneous mixtures such as suspensions, colloids, micelles, and emulsions.

A "trigger" or "trigger polynucleotide" is a DNA polynucleotide molecule that is homologous or complementary to a target gene polynucleotide. The trigger polynucleotide molecules modulate expression of the target gene when topically applied to a plant surface with a transfer agent, whereby a virus-infected plant that is treated with said composition is able to sustain its growth or development or reproductive ability, or said plant is less sensitive to a virus as a result of said polynucleotide-containing composition relative to a plant not treated with a composition containing the trigger molecule. A plant treated with such a composition may be resistant to viral expression as a result of said polynucleotide-containing composition relative to a plant not treated with a composition containing the trigger molecule. Trigger polynucleotides disclosed herein may be generally described in relation to the target gene sequence in an antisense (complementary) or sense orientation as ssDNA, dsDNA, ssRNA, or dsRNA molecules or nucleotide variants and modified nucleotides thereof depending on the various regions of a gene being targeted.

It is contemplated that the composition may contain multiple DNA or RNA polynucleotides and/or pesticides that include, but are not limited to, anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides. Essential genes are genes in a plant that provide key enzymes or other proteins, for example, a biosynthetic enzyme, metabolizing enzyme, receptor, signal transduction protein, structural gene product, transcription factor, or transport protein; or regulating RNAs, such as, microRNAs,

that are essential to the growth or survival of the organism or cell or involved in the normal growth and development of the plant (Meinke *et al.*, *Trends Plant Sci.* 2008:13(9):483-91). Essential genes in a virus may include genes responsible for capsid production, virus assembly, infectivity, budding, and the like. The suppression of an essential gene in a virus affects the function of a gene product that enables viral infection in a plant. The compositions may include various trigger DNA or RNA polynucleotides that modulate the expression of an essential gene in a Tospovirus.

As used herein, the term "DNA," "DNA molecule," or "DNA polynucleotide molecule" refers to a ssDNA or dsDNA molecule of genomic or synthetic origin, such as a polymer of deoxyribonucleotide bases or a DNA polynucleotide molecule. As used herein, the term "DNA sequence," "DNA nucleotide sequence," or "DNA polynucleotide sequence" refers to the nucleotide sequence of a DNA molecule. Unless otherwise stated, nucleotide sequences in the text of this specification are given, when read from left to right, in the 5' to 3' direction. The nomenclature used herein is that required by Title 37 of the United States Code of Federal Regulations § 1.822 and set forth in the tables in WIPO Standard ST.25 (1998), Appendix 2, Tables 1 and 3.

As used herein, the term "RNA," "RNA molecule," or "RNA polynucleotide molecule" refers to a ssRNA or dsRNA molecule of genomic or synthetic origin, such as a polymer of ribonucleotide bases or an RNA polynucleotide molecule. As used herein, the term "RNA sequence," "RNA nucleotide sequence," or "RNA polynucleotide sequence" refers to the nucleotide sequence of an RNA molecule. Unless otherwise stated, nucleotide sequences in the text of this specification are given, when read from left to right, in the 5' to 3' direction. The nomenclature used herein is that required by Title 37 of the United States Code of Federal Regulations § 1.822 and set forth in the tables in WIPO Standard ST.25 (1998), Appendix 2, Tables 1 and 3.

As used herein, "polynucleotide" refers to a DNA or RNA molecule containing multiple nucleotides and generally also refers to "oligonucleotides" (a polynucleotide molecule of typically 50 or fewer nucleotides in length). Embodiments include compositions including oligonucleotides having a length of 18-25 nucleotides (18-mers, 19-mers, 20-mers, 21-mers, 22-mers, 23-mers, 24-mers, or 25-mers), for example, oligonucleotides as set forth by SEQ ID NOs:1-12, 47-268,

and 448-483 or fragments thereof. A target gene comprises any polynucleotide molecule in a plant cell or fragment thereof for which the modulation of the expression of the target gene is provided by the methods and compositions. A gene has noncoding genetic elements (components) that provide for the function of the gene, these elements are polynucleotides that provide gene expression regulation, such as, a promoter, an enhancer, a 5' untranslated region, intron regions, and a 3' untranslated region. Oligonucleotides and polynucleotides can be made to any of the genetic elements of a gene and to polynucleotides spanning the junction region of a genetic element, such as, an intron and exon, the junction region of a promoter and a transcribed region, the junction region of a 5' leader and a coding sequence, the junction of a 3' untranslated region and a coding sequence.

Polynucleotide compositions used in the various embodiments include compositions including oligonucleotides or polynucleotides, or a mixture of both, of DNA or RNA, or chemically modified oligonucleotides or polynucleotides or a mixture thereof. In some embodiments, the polynucleotide includes chemically modified nucleotides. Examples of chemically modified oligonucleotides or polynucleotides are well known in the art; see, for example, US Patent Publication 20110171287, US Patent Publication 20110171176, and US Patent Publication 20110152353, US Patent Publication, 20110152346, US Patent Publication 20110160082, herein incorporated in its entirety by reference hereto. For example, including, but not limited to, the naturally occurring phosphodiester backbone of an oligonucleotide or polynucleotide can be partially or completely modified with phosphorothioate, phosphorodithioate, or methylphosphonate internucleotide linkage modifications, modified nucleoside bases or modified sugars can be used in oligonucleotide or polynucleotide synthesis, and oligonucleotides or polynucleotides can be labeled with a fluorescent moiety (for example, fluorescein or rhodamine) or other label (for example, biotin).

The term "gene" refers to components that comprise chromosomal DNA, RNA, plasmid DNA, cDNA, intron and exon DNA, artificial DNA polynucleotide, or other DNA that encodes a peptide, polypeptide, protein, or RNA transcript molecule, and the genetic elements flanking the coding sequence that are involved in the regulation of expression, such as, promoter regions, 5' leader regions, 3' untranslated region that may exist as native genes or transgenes in a plant genome. The gene or a

fragment thereof is isolated and subjected to polynucleotide sequencing methods that determines the order of the nucleotides that comprise the gene. Any of the components of the gene are potential targets for a trigger oligonucleotide and polynucleotides.

The trigger polynucleotide molecules are designed to modulate expression by inducing regulation or suppression of a viral gene and are designed to have a nucleotide sequence essentially identical or essentially complementary to the nucleotide sequence of a viral gene or to the sequence of RNA transcribed from a viral gene of a plant, the sequence thereof determined by isolating the gene or a fragment of the gene from the plant, which can be coding sequence or non-coding sequence. Effective molecules that modulate expression are referred to as "a trigger molecule, or trigger polynucleotide". By "essentially identical" or "essentially complementary" is meant that the trigger polynucleotides (or at least a portion of a polynucleotide) are designed to hybridize to the endogenous gene noncoding sequence or to RNA transcribed (known as messenger RNA or an RNA transcript) from the endogenous gene to effect regulation or suppression of expression of the endogenous gene. Trigger molecules are identified by "tiling" the gene targets with partially overlapping probes or non-overlapping probes of antisense polynucleotides that are essentially identical or essentially complementary to the nucleotide sequence of an endogenous gene. Multiple target sequences can be aligned and sequence regions with homology in common, according to the methods, are identified as potential trigger molecules for the multiple targets. Multiple trigger molecules of various lengths, for example 18-25 nucleotides, 26-50 nucleotides, 51-100 nucleotides, 101-200 nucleotides, 201-300 nucleotides or more can be pooled into a few treatments in order to investigate polynucleotide molecules that cover a portion of a gene sequence (for example, a portion of a coding versus a portion of a noncoding region, or a 5' versus a 3' portion of a gene) or an entire gene sequence including coding and noncoding regions of a target gene. Polynucleotide molecules of the pooled trigger molecules can be divided into smaller pools or single molecules in order to identify trigger molecules that provide the desired effect.

The target gene ssDNA polynucleotide molecules, including SEQ ID NOs:1-12, or dsRNA molecules, including SEQ ID NOs:47-268 and 448-483 may be sequenced by any number of available methods and equipment known in the art.

Some of the sequencing technologies are available commercially, such as the sequencing-by-hybridization platform from Affymetrix Inc. (Sunnyvale, Calif.) and the sequencing-by-synthesis platforms from 454 Life Sciences (Bradford, Conn.), Illumina/Solexa (Hayward, Calif.) and Helicos Biosciences (Cambridge, Mass.), and the sequencing-by-ligation platform from Applied Biosystems (Foster City, Calif.). In addition to the single molecule sequencing performed using sequencing-by-synthesis of Helicos Biosciences, other single molecule sequencing technologies are encompassed and include the SMRT™ technology of Pacific Biosciences, the Ion Torrent™ technology, and nanopore sequencing being developed for example, by Oxford Nanopore Technologies. A viral target gene comprising DNA or RNA can be isolated using primers or probes essentially complementary or essentially homologous to the target gene or a fragment thereof. A polymerase chain reaction (PCR) gene fragment can be produced using primers essentially complementary or essentially homologous to a viral gene or a fragment thereof that is useful to isolate a viral gene from a plant genome. Various sequence capture technologies can be used to isolate additional target gene sequences, for example, including but not limited to Roche NimbleGen® (Madison, WI) and Streptavdin-coupled Dynabeads® (Life Technologies, Grand Island, NY) and US20110015084, herein incorporated by reference in its entirety.

Embodiments of functional single-stranded or double-stranded polynucleotides have sequence complementarity that need not be 100 percent, but is at least sufficient to permit hybridization to RNA transcribed from the target gene or DNA of the target gene to form a duplex to permit a gene silencing mechanism. Thus, in embodiments, a polynucleotide fragment is designed to be complementary to all or a portion of an essential target Tospovirus or Geminivirus gene sequence. For instance, the fragment may be essentially identical or essentially complementary to a sequence of 18 or more contiguous nucleotides in either the target viral gene sequence or messenger RNA transcribed from the target gene. By "essentially identical" is meant having 100 percent sequence identity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence identity when compared to the sequence of 18 or more contiguous nucleotides in either the target gene or RNA transcribed from the target gene; by "essentially complementary" is meant having 100 percent sequence complementarity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91,

92, 93, 94, 95, 96, 97, 98, or 99 percent sequence complementarity when compared to the sequence of 18 or more contiguous nucleotides in either the target gene or RNA transcribed from the target gene. In some embodiments, polynucleotide molecules are designed to have 100 percent sequence identity with or complementarity to one allele or one family member of a given target gene (coding or non-coding sequence of a gene); in other embodiments the polynucleotide molecules are designed to have 100 percent sequence identity with or complementarity to multiple alleles or family members of a given target gene.

"Identity" refers to the degree of similarity between two polynucleic acid or protein sequences. An alignment of the two sequences is performed by a suitable computer program. A widely used and accepted computer program for performing sequence alignments is CLUSTALW v1.6 (Thompson, *et al. Nucl. Acids Res.*, 22: 4673-4680, 1994). The number of matching bases or amino acids is divided by the total number of bases or amino acids, and multiplied by 100 to obtain a percent identity. For example, if two 580 base pair sequences had 145 matched bases, they would be 25 percent identical. If the two compared sequences are of different lengths, the number of matches is divided by the shorter of the two lengths. For example, if there are 100 matched amino acids between a 200 and a 400 amino acid protein, they are 50 percent identical with respect to the shorter sequence. If the shorter sequence is less than 150 bases or 50 amino acids in length, the number of matches are divided by 150 (for nucleic acid bases) or 50 (for amino acids), and multiplied by 100 to obtain a percent identity.

Trigger molecules for specific viral gene family members can be identified from coding and/or non-coding sequences of gene families of a plant virus or multiple plant viruses, by aligning and selecting 200-300 polynucleotide fragments from the least homologous regions among the aligned sequences and evaluated using topically applied polynucleotides (antisense ssDNA or dsRNA) to determine their relative effectiveness in providing the anti-viral phenotype. In some embodiments, the viral gene family is Tospovirus and the sequences are selected from SEQ ID NOs:13-46. In some embodiments, the viral gene family is Cucumber mosaic virus and the sequences are selected from SEQ ID NOs:269-316. In some embodiments, the viral gene family is Pepino mosaic virus and the sequences are selected from SEQ ID NOs:317-349. In some embodiments, the viral gene family is Barley yellow dwarf

virus and the sequences are selected from SEQ ID NOs:350-385. In some embodiments, the viral gene family is Tomato yellow leaf curl virus and the sequences are selected from SEQ ID NOs:386-421. In some embodiments, the viral gene family is Cotton leaf curl virus and the sequences are selected from SEQ ID NOs:422-441. The effective segments are further subdivided into 50-60 polynucleotide fragments, prioritized by least homology, and reevaluated using topically applied polynucleotides. The effective 50-60 polynucleotide fragments are subdivided into 19-30 polynucleotide fragments, prioritized by least homology, and again evaluated for induction of the anti-viral phenotype. Once relative effectiveness is determined, the fragments are utilized singly, or again evaluated in combination with one or more other fragments to determine the trigger composition or mixture of trigger polynucleotides for providing the anti-viral phenotype.

Trigger molecules for broad anti-viral activity can be identified from coding and/or non-coding sequences of gene families of a plant virus or multiple plants viruses, by aligning and selecting 200-300 polynucleotide fragments from the most homologous regions amongst the aligned sequences and evaluated using topically applied polynucleotides (antisense ssDNA or dsRNA) to determine their relative effectiveness in inducing the anti-viral phenotype. In some embodiments, the viral gene family is Tospovirus and the sequences are selected from SEQ ID NOs:13-46. In some embodiments, the viral gene family is Cucumber mosaic virus and the sequences are selected from SEQ ID NOs:269-316. In some embodiments, the viral gene family is Pepino mosaic virus and the sequences are selected from SEQ ID NOs:317-349. In some embodiments, the viral gene family is Barley yellow dwarf virus and the sequences are selected from SEQ ID NOs:350-385. In some embodiments, the viral gene family is Tomato yellow leaf curl virus and the sequences are selected from SEQ ID NOs:386-421. In some embodiments, the viral gene family is Cotton leaf curl virus and the sequences are selected from SEQ ID NOs:422-441. The effective segments are subdivided into 50-60 polynucleotide fragments, prioritized by most homology, and reevaluated using topically applied polynucleotides. The effective 50-60 polynucleotide fragments are subdivided into 19-30 polynucleotide fragments, prioritized by most homology, and again evaluated for induction of the anti-viral phenotype. Once relative effectiveness is determined, the fragments may be utilized singly, or in combination with one or more other

fragments to determine the trigger composition or mixture of trigger polynucleotides for providing the anti-viral phenotype.

Methods of making polynucleotides are well known in the art. Chemical synthesis, *in vivo* synthesis and *in vitro* synthesis methods and compositions are known in the art and include various viral elements, microbial cells, modified polymerases, and modified nucleotides. Commercial preparation of oligonucleotides often provides two deoxyribonucleotides on the 3' end of the sense strand. Long polynucleotide molecules can be synthesized from commercially available kits. Long polynucleotide molecules can also be assembled from multiple DNA fragments. In some embodiments design parameters such as Reynolds score (Reynolds *et al. Nature Biotechnology* 22, 326-330 (2004), Tuschl rules (Pei and Tuschl, *Nature Methods* 3(9):670-676, 2006), i-score (*Nucleic Acids Res* 35:e123, 2007), i-Score Designer tool and associated algorithms (*Nucleic Acids Res* 32:936-948, 2004. *Biochem Biophys Res Commun* 316:1050-1058, 2004, *Nucleic Acids Res* 32:893-901, 2004, *Cell Cycle* 3:790-5, 2004, *Nat Biotechnol* 23:995-1001, 2005, *Nucleic Acids Res* 35:e27, 2007, *BMC Bioinformatics* 7:520, 2006, *Nucleic Acids Res* 35:e123, 2007, *Nat Biotechnol* 22:326-330, 2004) are known in the art and may be used in selecting polynucleotide sequences effective in gene silencing. In some embodiments the sequence of a polynucleotide is screened against the genomic DNA of the intended plant to minimize unintentional silencing of other genes.

Ligands can be tethered to a ssDNA or dsRNA polynucleotide. Ligands in general can include modifiers, *e.g.*, for enhancing uptake; diagnostic compounds or reporter groups *e.g.*, for monitoring distribution; cross-linking agents; nuclease-resistance conferring moieties; and natural or unusual nucleobases. General examples include lipophiles, lipids (*e.g.*, cholesterol, a bile acid, or a fatty acid (*e.g.*, lithocholic-oleyl, lauroyl, docosnyl, stearoyl, palmitoyl, myristoyl oleoyl, linoleoyl), steroids (*e.g.*, uvaol, hecigenin, diosgenin), terpenes (*e.g.*, triterpenes, *e.g.*, sarsasapogenin, Friedelin, epifriedelanol derivatized lithocholic acid), vitamins (*e.g.*, folic acid, vitamin A, biotin, pyridoxal), carbohydrates, proteins, protein binding agents, integrin targeting molecules, polycationics, peptides, polyamines, and peptide mimics. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, polyethylene glycol (PEG), PEG-40K, PEG-20K and PEG-5K. Other examples of ligands include lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane

acetic acid, 1-pyrene butyric acid, dihydrotestosterone, glycerol (*e.g.*, esters and ethers thereof, *e.g.*, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl; *e.g.*, lauroyl, docosnyl, stearoyl, oleoyl, linoleoyl 1,3-bis-O(hexadecyl)glycerol, 1,3-bis-O(octaadecyl)glycerol), geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dodecanoyl, lithocholyl, 5β-cholanyl, N,N-distearyl-lithocholamide, 1,2-di-O-stearoylglyceride, dimethoxytrityl, or phenoxazine) and PEG (*e.g.*, PEG-5K, PEG-20K, PEG-40K). Preferred lipophilic moieties include lipid, cholesterols, oleyl, retinyl, or cholesteryl residues.

The method of the invention may be applied to plants that are or are not transgenic. Non-limiting examples of transgenic plants include those that comprise one or more transgene conferring a trait selected from the group consisting of insect resistance, pesticide resistance, enhanced shelf life, fruit coloring, fruit ripening, fruit sweetness, nutritional value, and the like.

In specific embodiments of the invention, a plant disease control composition as provided herein may further be provided in a composition formulated for application to a plant that comprises at least one other active ingredient. Examples of such active ingredients may include, but are not limited to, an insecticidal protein such as a patatin, a *Bacillus thuringiensis* insecticidal protein, a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Bacillus laterosporous* insecticidal protein, and a *Bacillus sphearicus* insecticidal protein. In another non-limiting example, such an active ingredient is a herbicide, such as one or more of acetochlor, acifluorfen, acifluorfen-sodium, aclonifen, acrolein, alachlor, alloxydim, allyl alcohol, ametryn, amicarbazone, amidosulfuron, aminopyralid, amitrole, ammonium sulfamate, anilofos, asulam, atraton, atrazine, azimsulfuron, BCPC, beflubutamid, benazolin, benfluralin, benfuresate, bensulfuron, bensulfuron-methyl, bensulide, bentazone, benzfendizone, benzobicyclon, benzofenap, bifenox, bilanafos, bispyribac, bispyribac-sodium, borax, bromacil, bromobutide, bromoxynil, butachlor, butafenacil, butamifos, butralin, butroxydim, butylate, cacodylic acid, calcium chlorate, cafenstrole, carbetamide, carfentrazone, carfentrazone-ethyl, CDEA, CEPC, chlorflurenol, chlorflurenol-methyl, chloridazon, chlorimuron, chlorimuron-ethyl, chloroacetic acid, chlorotoluron, chlorpropham, chlorsulfuron, chlorthal, chlorthal-dimethyl, cinidon-ethyl,

cinmethylin, cinosulfuron, cisanilide, clethodim, clodinafop, clodinafop-propargyl, clomazone, clomeprop, clopyralid, cloransulam, cloransulam-methyl, CMA, 4-CPB, CPMF, 4-CPP, CPPC, cresol, cumyluron, cyanamide, cyanazine, cycloate, cyclosulfamuron, cycloxydim, cyhalofop, cyhalofop-butyl, 2,4-D, 3,4-DA, daimuron, dalapon, dazomet, 2,4-DB, 3,4-DB, 2,4-DEB, desmedipham, dicamba, dichlobenil, ortho-dichlorobenzene, para-dichlorobenzene, dichlorprop, dichlorprop-P, diclofop, diclofop-methyl, diclosulam, difenzoquat, difenzoquat metilsulfate, diflufenican, diflufenzopyr, dimefuron, dimepiperate, dimethachlor, dimethametryn, dimethenamid, dimethenamid-P, dimethipin, dimethylarsinic acid, dinitramine, dinoterb, diphenamid, diquat, diquat dibromide, dithiopyr, diuron, DNOC, 3,4-DP, DSMA, EBEP, endothal, EPTC, esprocarb, ethalfluralin, ethametsulfuron, ethametsulfuron-methyl, ethofumesate, ethoxyfen, ethoxysulfuron, etobenzanid, fenoxaprop-P, fenoxaprop-P-ethyl, fentrazamide, ferrous sulfate, flamprop-M, flazasulfuron, florasulam, fluazifop, fluazifop-butyl, fluazifop-P, fluazifop-P-butyl, flucarbazone, flucarbazone-sodium, flucetosulfuron, fluchloralin, flufenacet, flufenpyr, flufenpyr-ethyl, flumetsulam, flumiclorac, flumiclorac-pentyl, flumioxazin, fluometuron, fluoroglycofen, fluoroglycofen-ethyl, flupropanate, flupyrsulfuron, flupyrsulfuron-methyl-sodium, flurenol, fluridone, fluorochloridone, fluoroxypyr, flurtamone, fluthiacet, fluthiacet-methyl, fomesafen, foramsulfuron, fosamine, glufosinate, glufosinate-ammonium, glyphosate, halosulfuron, halosulfuron-methyl, haloxyfop, haloxyfop-P, HC-252, hexazinone, imazamethabenz, imazamethabenz-methyl, imazamox, imazapic, imazapyr, imazaquin, imazethapyr, imazosulfuron, indanofan, iodomethane, iodosulfuron, iodosulfuron-methyl-sodium, ioxynil, isoproturon, isouron, isoxaben, isoxachlortole, isoxaflutole, karbutilate, lactofen, lenacil, linuron, MAA, MAMA, MCPA, MCPA-thioethyl, MCPB, mecoprop, mecoprop-P, mefenacet, mefluidide, mesosulfuron, mesosulfuron-methyl, mesotrione, metam, metamifop, metamitron, metazachlor, methabenzthiazuron, methylarsonic acid, methyldymron, methyl isothiocyanate, metobenzuron, metolachlor, S-metolachlor, metosulam, metoxuron, metribuzin, metsulfuron, metsulfuron-methyl, MK-66, molinate, monolinuron, MSMA, naproanilide, napropamide, naptalam, neburon, nicosulfuron, nonanoic acid, norflurazon, oleic acid (fatty acids), orbencarb, orthosulfamuron, oryzalin, oxadiargyl, oxadiazon, oxasulfuron, oxaziclomefone, oxyfluorfen, paraquat, paraquat dichloride, pebulate, pendimethalin, penoxsulam,

pentachlorophenol, pentanochlor, pentoxazone, pethoxamid, petrolium oils, phenmedipham, phenmedipham-ethyl, picloram, picolinafen, pinoxaden, piperophos, potassium arsenite, potassium azide, pretilachlor, primisulfuron, primisulfuron-methyl, prodiamine, profluazol, profoxydim, prometon, prometryn, propachlor, propanil, propaquizafop, propazine, propham, propisochlor, propoxycarbazone, propoxycarbazone-sodium, propyzamide, prosulfocarb, prosulfuron, pyraclonil, pyraflufen, pyraflufen-ethyl, pyrazolynate, pyrazosulfuron, pyrazosulfuron-ethyl, pyrazoxyfen, pyribenzoxim, pyributicarb, pyridafol, pyridate, pyriftalid, pyriminobac, pyriminobac-methyl, pyrimisulfan, pyrithiobac, pyrithiobac-sodium, quinclorac, quinmerac, quinoclamine, quizalofop, quizalofop-P, rimsulfuron, sethoxydim, siduron, simazine, simetryn, SMA, sodium arsenite, sodium azide, sodium chlorate, sulcotrione, sulfentrazone, sulfometuron, sulfometuron-methyl, sulfosate, sulfosulfuron, sulfuric acid, tar oils, 2,3,6-TBA, TCA, TCA-sodium, tebuthiuron, tepraloxydim, terbacil, terbumeton, terbuthylazine, terbutryn, thenylchlor, thiazopyr, thifensulfuron, thifensulfuron-methyl, thiobencarb, tiocarbazil, topramezone, tralkoxydim, tri-allate, triasulfuron, triaziflam, tribenuron, tribenuron-methyl, tricamba, triclopyr, trietazine, trifloxysulfuron, trifloxysulfuron-sodium, trifluralin, triflusulfuron, triflusulfuron-methyl, trihydroxytriazine, tritosulfuron, [3-[2-chloro-4-fluoro-5-(-methyl-6-trifluoromethyl-2,4-dioxo-,2,3,4-t- etrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetic acid ethyl ester (CAS RN 353292-3-6), 4-[(4,5-dihydro-3-methoxy-4-methyl-5-oxo)-H-,2,4-triazolylcarbonyl-sulfamoyl]-5-methyl-thiophene-3-carboxylic acid (BAY636), BAY747 (CAS RN 33504-84-2), topramezone (CAS RN 2063-68-8), 4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-(trifluoro-methyl)-3-pyridi- nyl]carbonyl]-bicyclo[3.2.]oct-3-en-2-one (CAS RN 35200-68-5), and 4-hydroxy-3-[[2-(3-methoxypropyl)-6-(difluoromethyl)-3-pyridinyl]carbon- yl]-bicyclo[3.2.]oct-3-en-2-one.

The trigger DNA or RNA polynucleotide and/or oligonucleotide molecule compositions are useful in compositions, such as liquids that comprise the polynucleotide molecules at low concentrations, alone or in combination with other components, for example one or more herbicide molecules, either in the same solution or in separately applied liquids that also provide a transfer agent. While there is no upper limit on the concentrations and dosages of polynucleotide molecules that can

useful in the methods, lower effective concentrations and dosages will generally be sought for efficiency. The concentrations can be adjusted in consideration of the volume of spray or treatment applied to plant leaves or other plant part surfaces, such as flower petals, stems, tubers, fruit, anthers, pollen, or seed. In one embodiment, a useful treatment for herbaceous plants using 25-mer oligonucleotide molecules is about 1 nanomole (nmol) of oligonucleotide molecules per plant, for example, from about 0.05 to 1 nmol per plant. Other embodiments for herbaceous plants include useful ranges of about 0.05 to about 100 nmol, or about 0.1 to about 20 nmol, or about 1 nmol to about 10 nmol of polynucleotides per plant. Very large plants, trees, or vines may require correspondingly larger amounts of polynucleotides. To illustrate embodiments, the factor 1X, when applied to oligonucleotide molecules is arbitrarily used to denote a treatment of 0.8 nmol of polynucleotide molecule per plant; 10X, 8 nmol of polynucleotide molecule per plant; and 100X, 80 nmol of polynucleotide molecule per plant.

An agronomic field in need of virus control may be treated by application of an agricultural chemical composition directly to the surface of the growing plants, such as by a spray. For example, the method is applied to control virus infection in a field of crop plants by spraying the field with the composition. The composition can be provided as a tank mix with one or more pesticidal or herbicidal chemicals to control pests and diseases of the crop plants in need of pest and disease control, a sequential treatment of components (generally the polynucleotide containing composition followed by the pesticide), or a simultaneous treatment or mixing of one or more of the components of the composition from separate containers. Treatment of the field can occur as often as needed to provide virus control and the components of the composition can be adjusted to target specific Tospoviruses or Geminiviruses through utilization of specific polynucleotides or polynucleotide compositions capable of selectively targeting the specific virus to be controlled. The composition can be applied at effective use rates according to the time of application to the field, for example, preplant, at planting, post planting, or post harvest. The polynucleotides of the composition can be applied at rates of 1 to 30 grams per acre depending on the number of trigger molecules needed for the scope of virus infection in the field.

Crop plants in which virus control may be needed include but are not limited to corn, soybean, cotton, canola, sugar beet, alfalfa, sugarcane, rice, barley, and

wheat; vegetable plants including, but not limited to, tomato, sweet pepper, hot pepper, melon, watermelon, cucumber, zucchini, eggplant, cauliflower, broccoli, lettuce, spinach, onion, peas, carrots, sweet corn, Chinese cabbage, leek, fennel, pumpkin, squash or gourd, radish, potato, Brussels sprouts, tomatillo, peanut, garden beans, dry beans, or okra; culinary plants including, but not limited to, basil, parsley, coffee, or tea; or fruit plants including but not limited to apple, pear, cherry, peach, plum, apricot, banana, plantain, table grape, wine grape, citrus, avocado, mango, or berry; a tree grown for ornamental or commercial use, including, but not limited to, a fruit or nut tree; ornamental plant (*e.g.*, an ornamental flowering plant or shrub or turf grass), such as iris and impatiens. The methods and compositions provided herein can also be applied to plants produced by a cutting, cloning, or grafting process (*i.e.*, a plant not grown from a seed) including fruit trees and plants that include, but are not limited to, avocados, tomatoes, eggplant, cucumber, melons, watermelons, and grapes, as well as various ornamental plants.

The trigger polynucleotide compositions may also be used as mixtures with various agricultural chemicals and/or insecticides, miticides and fungicides, pesticidal and biopesticidal agents. Examples include, but are not limited to, azinphos-methyl, acephate, isoxathion, isofenphos, ethion, etrimfos, oxydemeton-methyl, oxydeprofos, quinalphos, chlorpyrifos, chlorpyrifos-methyl, chlorfenvinphos, cyanophos, dioxabenzofos, dichlorvos, disulfoton, dimethylvinphos, dimethoate, sulprofos, diazinon, thiometon, tetrachlorvinphos, temephos, tebupirimfos, terbufos, naled, vamidothion, pyraclofos, pyridafenthion, pirimiphos-methyl, fenitrothion, fenthion, phenthoate, flupyrazophos, prothiofos, propaphos, profenofos, phoxime, phosalone, phosmet, formothion, phorate, malathion, mecarbam, mesulfenfos, methamidophos, methidathion, parathion, methyl parathion, monocrotophos, trichlorphon, EPN, isazophos, isamidofos, cadusafos, diamidaphos, dichlofenthion, thionazin, fenamiphos, fosthiazate, fosthietan, phosphocarb, DSP, ethoprophos, alanycarb, aldicarb, isoprocarb, ethiofencarb, carbaryl, carbosulfan, xylylcarb, thiodicarb, pirimicarb, fenobucarb, furathiocarb, propoxur, bendiocarb, benfuracarb, methomyl, metolcarb, XMC, carbofuran, aldoxycarb, oxamyl, acrinathrin, allethrin, esfenvalerate, empenthrin, cycloprothrin, cyhalothrin, gamma-cyhalothrin, lambda-cyhalothrin, cyfluthrin, beta-cyfluthrin, cypermethrin, alpha-cypermethrin, zeta-cypermethrin, silafluofen, tetramethrin, tefluthrin, deltamethrin, tralomethrin,

bifenthrin, phenothrin, fenvalerate, fenpropathrin, furamethrin, prallethrin, flucythrinate, fluvalinate, flubrocythrinate, permethrin, resmethrin, ethofenprox, cartap, thiocyclam, bensultap, acetamiprid, imidacloprid, clothianidin, dinotefuran, thiacloprid, thiamethoxam, nitenpyram, chlorfluazuron, diflubenzuron, teflubenzuron, triflumuron, novaluron, noviflumuron, bistrifluoron, fluazuron, flucycloxuron, flufenoxuron, hexaflumuron, lufenuron, chromafenozide, tebufenozide, halofenozide, methoxyfenozide, diofenolan, cyromazine, pyriproxyfen, buprofezin, methoprene, hydroprene, kinoprene, triazamate, endosulfan, chlorfenson, chlorobenzilate, dicofol, bromopropylate, acetoprole, fipronil, ethiprole, pyrethrin, rotenone, nicotine sulphate, BT (Bacillus Thuringiensis) agent, spinosad, abamectin, acequinocyl, amidoflumet, amitraz, etoxazole, chinomethionat, clofentezine, fenbutatin oxide, dienochlor, cyhexatin, spirodiclofen, spiromesifen, tetradifon, tebufenpyrad, binapacryl, bifenazate, pyridaben, pyrimidifen, fenazaquin, fenothiocarb, fenpyroximate, fluacrypyrim, fluazinam, flufenzin, hexythiazox, propargite, benzomate, polynactin complex, milbemectin, lufenuron, mecarbam, methiocarb, mevinphos, halfenprox, azadirachtin, diafenthiuron, indoxacarb, emamectin benzoate, potassium oleate, sodium oleate, chlorfenapyr, tolfenpyrad, pymetrozine, fenoxycarb, hydramethylnon, hydroxy propyl starch, pyridalyl, flufenerim, flubendiamide, flonicamid, metaflumizole, lepimectin, TPIC, albendazole, oxibendazole, oxfendazole, trichlamide, fensulfothion, fenbendazole, levamisole hydrochloride, morantel tartrate, dazomet, metam-sodium, triadimefon, hexaconazole, propiconazole, ipconazole, prochloraz, triflumizole, tebuconazole, epoxiconazole, difenoconazole, flusilazole, triadimenol, cyproconazole, metconazole, fluquinconazole, bitertanol, tetraconazole, triticonazole, flutriafol, penconazole, diniconazole, fenbuconazole, bromuconazole, imibenconazole, simeconazole, myclobutanil, hymexazole, imazalil, furametpyr, thifluzamide, etridiazole, oxpoconazole, oxpoconazole fumarate, pefurazoate, prothioconazole, pyrifenox, fenarimol, nuarimol, bupirimate, mepanipyrim, cyprodinil, pyrimethanil, metalaxyl, mefenoxam, oxadixyl, benalaxyl, thiophanate, thiophanate-methyl, benomyl, carbendazim, fuberidazole, thiabendazole, manzeb, propineb, zineb, metiram, maneb, ziram, thiuram, chlorothalonil, ethaboxam, oxycarboxin, carboxin, flutolanil, silthiofam, mepronil, dimethomorph, fenpropidin, fenpropimorph, spiroxamine, tridemorph, dodemorph, flumorph, azoxystrobin, kresoxim-methyl, metominostrobin, orysastrobin, fluoxastrobin, trifloxystrobin, dimoxystrobin, pyraclostrobin, picoxystrobin, iprodione, procymidone, vinclozolin,

chlozolinate, flusulfamide, dazomet, methyl isothiocyanate, chloropicrin, methasulfocarb, hydroxyisoxazole, potassium hydroxyisoxazole, echlomezol, D-D, carbam, basic copper chloride, basic copper sulfate, copper nonylphenolsulfonate, oxine copper, DBEDC, anhydrous copper sulfate, copper sulfate pentahydrate, cupric hydroxide, inorganic sulfur, wettable sulfur, lime sulfur, zinc sulfate, fentin, sodium hydrogen carbonate, potassium hydrogen carbonate, sodium hypochlorite, silver, edifenphos, tolclofos-methyl, fosetyl, iprobenfos, dinocap, pyrazophos, carpropamid, fthalide, tricyclazole, pyroquilon, diclocymet, fenoxanil, kasugamycin, validamycin, polyoxins, blasticiden S, oxytetracycline, mildiomycin, streptomycin, rape seed oil, machine oil, benthiavalicarbisopropyl, iprovalicarb, propamocarb, diethofencarb, fluoroimide, fludioxanil, fenpiclonil, quinoxyfen, oxolinic acid, chlorothalonil, captan, folpet, probenazole, acibenzolar-S-methyl, tiadinil, cyflufenamid, fenhexamid, diflumetorim, metrafenone, picobenzamide, proquinazid, famoxadone, cyazofamid, fenamidone, zoxamide, boscalid, cymoxanil, dithianon, fluazinam, dichlofluanide, triforine, isoprothiolane, ferimzone, diclomezine, tecloftalam, pencycuron, chinomethionat, iminoctadine acetate, iminoctadine albesilate, ambam, polycarbamate, thiadiazine, chloroneb, nickel dimethyldithiocarbamate, guazatine, dodecylguanidine-acetate, quintozene, tolylfluanid, anilazine, nitrothalisopropyl, fenitropan, dimethirimol, benthiazole, harpin protein, flumetover, mandipropamide and penthiopyrad.

All publications, patents, 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.

The following Examples are presented for the purposes of illustration and should not be construed as limitations. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed herein and still obtain a like or similar result without departing from the spirit and scope.

Example 1

Topical application of antisense ssDNA oligonuceotides to lettuce plants for control of impatiens necrotic spotted virus (INSV)

Single-stranded DNA (ssDNA) fragments in antisense (as) orientation were identified and mixed with a transfer agent and other components. This composition was topically applied to lettuce plants to effect repression of the target INSV nucleocapsid (N) gene to reduce or eliminate symptoms of viral infection in the plants. The procedure was as follows.

Growing lettuce plants (*Lactuca sativa, c.v. SVR3606-L4*) were topically treated with a composition for inducing suppression of a target gene in a plant. The composition included: (a) an agent to enable permeation of the polynucleotides into the plant, and (b) at least one polynucleotide strand including at least one segment of 17-25 contiguous nucleotides of the target gene in antisense orientation. Lettuce plants were topically treated with an adjuvant solution comprising antisense ssDNA, essentially homologous or essentially complementary to the INSV N protein coding sequence. Plants were grown and treated in growth chambers [22°C, 8 hour light (~50 µmol), 16 hour dark cycles].

Lettuce plants were germinated for approximately 16-21 days prior to assay. Single leaves of lettuce plants (40 plants total) were infected with approximately 200 nanograms (100 ng/µL in phosphate buffer) of INSV virus. Approximately 3 hours after virus infection, 20 plants were sprayed with a mixture of oligonucleotides in solution (SEQ ID NO:1 and SEQ ID NO:2, mixed together) using an airbrush at 20 psi. The sequences of the antisense ssDNA oligonucleotides are listed in Table 1. The remaining 20 plants were not treated with oligonucleotides and served as the control.

The final concentration of each oligonucleotide or polynucleotide was 20 nMoles for ssDNA (in 0.1% Silwet L-77, 2% ammonium sulfate, 5 mM sodium phosphate buffer, pH 6.8) unless otherwise stated. The spray solution was applied to the plant to provide a total of 200-300 µL volume. The fresh weight of aerial tissue was measured (see FIG. 1).

TABLE 1. The sequence of antisense ssDNA oligonucleotides directed to INSV nucleocapsid gene N.

SEQ ID NO	Sequence (5'-3')	Length	Virus	Target
1	GCTATAAACAGCCTTCCAAGTCA	23	INSV	Nucleocapsid Gene (N)
2	GTCATTAAGAGTGCTGACTTCAC	23	INSV	Nucleocapsid Gene (N)

Example 2

Quantification of virus using ELISA

Leaf punctures harvested from untreated or treated plants lettuce plants (FIG. 2) as described in Example 1 were crushed in antigen buffer using a mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 5 minutes at 4°C. The supernatant was extracted and subjected to indirect-ELISA against the anti-INSV N protein.

As shown in FIG. 3, circles represent a readout of INSV N protein in individual leaf punches collected from the control plants (virus only, no polynucleotide). Triangles represent a readout of INSV N protein in individual leaf punches collected from plants treated with a mixture of antisense ssDNA oligonucleotides (SEQ ID NO:1 and SEQ ID NO:2). Approximately 65% of the oligo-treated plants exhibited OD_{405} values of 0.2 or lower, and 100% of the control plants exhibited an OD_{405} value of 1 or higher. FIG. 4 and FIG. 5 show optical density (OD) and visual assessment of extracts of lettuce plants after treatment with antisense ssDNA oligos.

Example 3

Topical application of antisense ssDNA oligonucleotides to lettuce plants after virus treatment improves photosystem II function

In this example, lettuce plants that were untreated (null) or that had been infected with INSV virus and treated with ss antisense oligonucleotides were measured using a portable chlorophyll fluorometer (PAM-2500). This measurement gives an effective yield of photosystem II (PSII) function, a measure of overall yield.

A group of six randomly picked non-treated and six randomly picked treated plants were measured at leaf number 2, 4, 6 and 8. The leaf number is indicative of the age of the lettuce head with the youngest leaf (leaf 2) being inside the forming lettuce head and the oldest leaf (leaf 8) located on the outside of the forming lettuce head. Plants treated with ss antisense DNA oligos exhibited the most protection on the outer leaves compared to untreated (null) plants.

Example 4

Topical application of antisense ssDNA oligonucleotides to tomato and pepper plants for control of tomato spotted wilt virus (TSWV)

Single-stranded or double-stranded DNA or RNA fragments in sense or antisense orientation, or both, were identified and mixed with a transfer agent and other components. This composition was topically applied to tomato plants to effect expression of the target TSWV nucleocapsid or capsid genes to reduce or eliminate symptoms of viral infection in the plants. The procedure was as follows.

Tomato plants (*Solanum lycopersicum* HP375) and pepper plants (*c.v. Yolo Wonder B*) were grown in a cage outdoors. Pepper plants infected with TSWV, a negative-sense RNA virus, were transplanted from a breeder's infected pepper field in the center of the rows containing either tomato or pepper plants. Any subsequent infection was due to thrips transmiting TSWV from the infected center plants, thus mimicking a natural TSWV infection (see FIG. 6). Topical treatment with a mixture of at least one polynucleotide strand including at least one segment of 17-25 contiguous nucleotides of the target gene in either antisense or sense orientation was performed. Plants were treated with a topically applied adjuvant solution of trigger molecules comprising ssDNA oligonucleotides essentially homologous or essentially complementary to the TSWV nucleocapsid coding sequence. The sequence of the trigger molecule used in each treatment is shown in Table 2.

TABLE 2. The sequence of antisense ssDNA oligonucleotides directed to TSWV nucleocapsid gene N.

SEQ ID NO	Sequence (5'-3')	Length	Virus	Target
3	CATCTCAAAGCTATCAACTGAA	22	TSWV	Nucleocapsid gene (N)
4	TGATCTTCATTCATTTCAAATG	22	TSWV	Nucleocapsid gene (N)

Plants at the 2-5 fully expanded leaf stage were used in these assays. Seven or 8 plants were treated as control (virus infection only) and 7 or 8 plants were treated with polynucleotides. Two fully expanded leaves per plant were treated with the polynucleotide/Silwet L-77 solution. The final concentration for each oligonucleotide or polynucleotide was 10 nmoles for ssDNA (in 0.1% Silwet L-77, 2% ammonium sulfate, 5 mM sodium phosphate buffer, pH 6.8) unless otherwise stated. Twenty microliters of the solution was applied to the top surface of each of the two leaves to provide a total of 40 µL for each plant. FIG. 7 shows tomato plants both untreated (circled) and topically treated with antisense ssDNA oligos against TSWV, while FIGS. 8 and 9 show the results of the topical treatment of tomato and pepper plants, respectively.

Example 5

Topical application of antisense ssDNA oligonucleotides to pepper plants for control of cucumber mosaic virus (CMV)

In this example, growing pepper plants *(c.v. Yolo Wonder B)* were inoculated with cucumber mosaic virus (CMV), a positive strand RNA virus, and the plants were separated into two groups. The experimental group was then topically treated with a mixture of at least one polynucleotide strand including at least one segment of 17-25 contiguous nucleotides of the target gene in either antisense or sense orientation. The trigger molecules in the topical adjuvant solution comprised dsRNA and ssDNA essentially homologous or essentially complementary to the CMV capsid coding sequence. The sequences of the trigger molecules used in each treatment are shown in Table 3.

TABLE 3. The sequence of antisense ssDNA oligonucleotides directed to CMV coat protein (CP).

SEQ ID NO	Sequence (5'-3')	Length	Virus	Target
5	AGACGTGGGAATGCGTTGGTG	21	CMV	Coat Protein (CP)
6	CTCGACGTCAACATGAAGTAC	21	CMV	Coat Protein (CP)
7	GCTTGGACTCCAGATGCAGCA	21	CMV	Coat Protein (CP)
8	TACTGATAAACCAGTACCGGT	21	CMV	Coat Protein (CP)
9	CGAATTTGAATGCGCGAAACA	21	CMV	Coat Protein (CP)
10	AGTTTCTTGTCATATTCTGTG	21	CMV	Coat Protein (CP)
11	GACGACCAGCTGCCAACGTCT	21	CMV	Coat Protein (CP)
12	TATTAAGTCGCGAAAGCTGCT	21	CMV	Coat Protein (CP)

Pepper plants at the 2-5 fully expanded leaf stage were used in the assays. Seven or 8 plants were used as the control (virus infection only) and 7 or 8 plants were treated with virus followed by a polynucleotide trigger solution. Two fully expanded leaves per plant were treated with the polynucleotide/Silwet L-77 solution. One set of plants was treated with a mixture of polynucleotides comprising SEQ ID NOs:5-8 and another set of plants was treated with a mixture of polynucleotides comprising SEQ ID NOs:9-12. The final concentration for each oligonucleotide or polynucleotide was 5 nmol for ssDNA (in 0.1% Silwet L-77, 2% ammonium sulfate, 5 mM sodium phosphate buffer, pH 6.8) unless otherwise stated. Twenty microliters of the solution was applied to the top surface of each of the two leaves to provide a total of 40 μL for each plant.

As shown in FIG. 10, circles represent data points collected from the control plants (virus only, no oligo treatment). Diamonds (SEQ ID NOs:5-8) and triangles (SEQ ID NOs:9-12) represent data points collected from samples topically treated with the antisense ssDNA oligonucleotide solution. The left part shows data from inoculated leaves, and the right part shows data from systemic non-infected, non-oligo-treated leaves.

Example 6

Topical application of antisense ssDNA oligonucleotides to onion plants for control of iris yellow spot virus (IYSV)

In this example, growing onion plants were inoculated with iris yellow spot virus (IYSV), and the plants were separated into two groups (31 plants per group). The experimental group was then topically treated with a mixture of at least one polynucleotide strand including at least one segment of 17-25 contiguous nucleotides of the target gene in antisense orientation. The trigger molecules in the topical adjuvant solution comprised ssDNA essentially homologous or essentially complementary to an IYSV coding sequence. The results of treatment of onion plants with antisense ssDNA are shown in FIG. 11.

Example 7

Topical application of polynucleotide triggers for control of commercially relevant Tospovirus isolates

In Table 4 of this example, the sequences of genes of Tospovirus isolates considered to be commercially relevant because of yield losses in tomato, pepper, potato, or soybean were identified and constitute SEQ ID NOs:13-46.

A computer alignment was used to identify highly conserved areas within the Nucleocapsid (N), Silencing Suppressor (NSs), Movement (NSm), and RNA-dependent RNA polymerase genes (SEQ ID NOs:47-103 in Table 5) to serve as candidates for antisense ssDNA or dsRNA polynucleotides homologous to the gene sequence for topical application treatment to control Tospovirus infection (Table 5). These polynucleotides can be tested on Tospovirus-infected tomato plants to control viral infection.

TABLE 4. RNA Sequences of Tospoviruses.

SEQ ID NO:	Species	Gene	Host	Isolate	Accession No.
13	Groundnut ringspot virus isolate	N	Florida tomato	FL, USA	HQ634665.1
14	Groundnut ringspot and Tomato chlorotic spot virus reassortant	N	*Solanum lycopersicum*	FL, USA	gi|332290587
15	Tomato spotted wilt virus	N	*Eustoma grandiflorum*	USA	HQ655877.1
16	Tomato spotted wilt virus	N	Pepper	Brazil	DQ915948.1
17	Tomato spotted wilt virus	N	Potato	NC, USA	AY856344
18	Tomato chlorotic spot virus	N	Florida tomato	FL, USA	HQ634664.1
19	Tomato chlorotic spot virus	N		FL, USA	JX244198.1
20	Tomato chlorotic spot virus	N		FL, USA	JX244196
21	Tomato spotted wilt virus	N	*Solanum lycopersicum*	FL, USA	HQ634670
22	Tomato spotted wilt virus	N	*Solanum lycopersicum*	FL, USA	HQ634668.1
23	Tomato spotted wilt virus	N	*Solanum lycopersicum*	FL, USA	HQ634669.1
24	Tomato spotted wilt virus	N	*Solanum lycopersicum*	FL, USA	HQ634667.1

25	Groundnut ringspot virus isolate	NSm	Florida tomato	FL, USA	HQ634675.1
26	Groundnut ringspot virus isolate	NSm	*Glycine max*	S.A	HQ634674
27	Tomato spotted wilt virus	NSm		USA	NC_002050
28	Tomato chlorotic spot virus	NSm	Florida tomato	FL, USA	HQ634671.1
29	Tomato chlorotic spot virus	NSm	*Solanum lycopersicum*	FL, USA	JX244201.1
30	Tomato spotted wilt virus	NSm	*Solanum lycopersicum*	FL, USA	HQ634676.1
31	Tomato spotted wilt virus	NSm	*Solanum lycopersicum*	FL, USA	AY956380
32	Groundnut ringspot and Tomato chlorotic spot virus reassortant	NSs	*Solanum lycopersicum*	FL, USA	gi|332290587
33	Groundnut ringspot virus isolate	NSs	Groundnut	S.A	JN571117
34	Tomato spotted wilt virus	NSs	*Solanum lycopersicum*	USA	FR693044
35	Tomato spotted wilt virus	NSs	Pepper	Brazil	D00645.1
36	Tomato spotted wilt virus	NSs		USA	AF020659.1
37	Tomato spotted wilt virus	NSs		USA	AF020659
38	Groundnut ringspot virus isolate	RdRp/ L segment	Florida tomato	FL, USA	HQ634677.1

39	Groundnut ringspot virus isolate	RdRp/ L segment	Florida tomato	FL 34945, USA 95/0188	HQ634679.1
40	Groundnut ringspot virus isolate	RdRp/ L segment	Florida tomato	FL, USA 95/0137	HQ634678.1
41	Tomato spotted wilt virus	RdRp/ L segment	strain="BR-01 (CNPH1	Brazil	NC_002052
42	Tomato chlorotic spot virus	RdRp/ L segment	Florida tomato	FL, USA	HQ634680.1
43	Tomato chlorotic spot virus	RdRp/ L segment	*Solanum lycopersicum*	Brazil	HQ700667.1
44	Tomato chlorotic spot virus	RdRp/ L segment	*Solanum lycopersicum*	FL, USA	JX244205.1
45	Tomato chlorotic spot virus	RdRp/ L segment	*Solanum lycopersicum*	FL, USA	JX244203
46	Tomato chlorotic spot virus	RdRp/ L segment	*Solanum lycopersicum*	USA	FR692596

TABLE 5. The sequence of dsRNA oligonucleotides directed to Tospoviruses.

SEQ ID NO:	Type	Length	Gene, Virus, Description
47	dsRNA	101	N gene, Groundnut ringspot virus
48	dsRNA	47	N gene, Groundnut ringspot virus, 2NT overhangs at 3'
49	dsRNA	47	N gene, Groundnut ringspot virus, 2NT overhangs at 3'
50	dsRNA	47	N gene, Groundnut ringspot virus , 2NT overhangs at 3'

51	dsRNA	47	N gene, Groundnut ringspot virus , 2NT overhangs at 3'
52	dsRNA	100	N gene, Tomato spotted wilt virus
53	dsRNA	47	N gene, Tomato spotted wilt virus, 2NT overhangs at 3'
54	dsRNA	51	N gene, Tomato spotted wilt virus, 2NT overhangs at 3'
55	dsRNA	51	N gene, Tomato spotted wilt virus, 2NT overhangs at 3'
56	dsRNA	100	N gene, Tomato chlorotic spot virus
57	dsRNA	47	N gene, Tomato chlorotic spot virus, 2NT overhangs at 3'
58	dsRNA	47	N gene, Tomato chlorotic spot virus, 2NT overhangs at 3'
59	dsRNA	47	N gene, Tomato chlorotic spot virus, 2NT overhangs at 3'
60	dsRNA	47	N gene, Tomato chlorotic spot virus, 2NT overhangs at 3'
61	dsRNA	47	N gene, Tomato chlorotic spot virus, 2NT overhangs at 3'
62	dsRNA	100	NSm, Groundnut ringspot virus +TCSV
63	dsRNA	47	NSm, Groundnut ringspot virus +TCSV, 2NT overhangs at 3'
64	dsRNA	47	NSm, Groundnut ringspot virus ; long stretches of A/T's, 2NT overhangs at 3'
65	dsRNA	47	NSm, Groundnut ringspot virus +TCSV, 2NT overhangs at 3'
66	dsRNA	201	NSm, Tomato chlorotic spot virus +GRV
67	dsRNA	47	NSm, Tomato chlorotic spot virus+GRV, 2NT overhangs at 3'
68	dsRNA	23	NSm, Tomato chlorotic spot virus+GRV
69	dsRNA	51	NSm, Tomato chlorotic spot virus+GRV, 2NT overhangs at 3'
70	dsRNA	150	NSm, Tomato spotted wilt virus
71	dsRNA	47	NSm, Tomato spotted wilt virus, 2NT overhangs at 3'

72	dsRNA	47	NSm, Tomato spotted wilt virus, 2NT overhangs at 3'
73	dsRNA	47	NSm, Tomato spotted wilt virus, 2NT overhangs at 3'
74	dsRNA	100	NSs, Tomato spotted wilt virus
75	dsRNA	47	NSs, Tomato spotted wilt virus, 2NT overhangs at 3'
76	dsRNA	47	NSs, Tomato spotted wilt virus, 2NT overhangs at 3'
77	dsRNA	47	NSs, Tomato spotted wilt virus, 2NT overhangs at 3'
78	dsRNA	47	NSs, Tomato spotted wilt virus, 2NT overhangs at 3'
79	dsRNA	201	RdRp, Groundnut ringspot virus isolate
80	dsRNA	47	RdRp, Groundnut ringspot virus isolate, 2NT overhangs at 3'
81	dsRNA	47	RdRp, Groundnut ringspot virus isolate, 2NT overhangs at 3'
82	dsRNA	47	RdRp, Groundnut ringspot virus isolate, 2NT overhangs at 3'
83	dsRNA	201	RdRp, Tomato spotted wilt virus
84	dsRNA	47	RdRp, Tomato spotted wilt virus, 2NT overhangs at 3'
85	dsRNA	47	RdRp, Tomato spotted wilt virus, 2NT overhangs at 3'
86	dsRNA	47	RdRp, Tomato spotted wilt virus, 2NT overhangs at 3'
87	dsRNA	201	RdRp, Tomato chlorotic spot virus
88	dsRNA	47	RdRp, Tomato chlorotic spot virus, 2NT overhangs at 3'
89	dsRNA	47	RdRp, Tomato chlorotic spot virus, 2NT overhangs at 3'
90	dsRNA	47	RdRp, Tomato chlorotic spot virus, 2NT overhangs at 3'
91	dsRNA	100	Nsm, Tomato chlorotic spot virus
92	dsRNA	47	Nsm, Tomato chlorotic spot virus, 2NT overhangs at 3'
93	dsRNA	47	Nsm, Tomato chlorotic spot virus, long stretches of T's, 2NT overhangs at 3'

94	dsRNA	47	Nsm, Tomato chlorotic spot virus, 2NT overhangs at 3'
95	dsRNA	47	Nsm, Tomato chlorotic spot virus, 2NT overhangs at 3'
96	dsRNA	47	Nsm, Tomato chlorotic spot virus, 2NT overhangs at 3'
97	dsRNA	47	Nsm, Tomato chlorotic spot virus, 2NT overhangs at 3'
98	dsRNA	47	Nsm, Tomato chlorotic spot virus, 2NT overhangs at 3'
99	dsRNA	201	NSs, Groundnut ringspot and Tomato chlorotic spot virus reassortant
100	dsRNA	47	NSs, Groundnut ringspot and Tomato chlorotic spot virus reassortant, 2NT overhangs at 3'
101	dsRNA	47	NSs, Groundnut ringspot and Tomato chlorotic spot virus reassortant, 2NT overhangs at 3'
102	dsRNA	47	NSs, Groundnut ringspot and Tomato chlorotic spot virus reassortant, 2NT overhangs at 3'
103	dsRNA	47	NSs, Groundnut ringspot and Tomato chlorotic spot virus reassortant, 2NT overhangs at 3'

Example 8

Topical application of polynucleotide triggers for control of other commercially relevant plant viruses in agriculture

In Table 6 of this example, a commonly used computer algorithm was used to identify highly conserved regions in the coat protein (CP), Movement Protein (MP), and Silencing Suppressor protein, of plant virus isolates that are commercially relevant in agriculture. These viruses may be of different families, such as Geminiviruses (*i.e.*, Cotton leaf curl virus, Barley yellow dwarf virus), or Bromoviruses (*i.e.*, CMV), or Potexviruses (*i.e.*, PepMV). The triggers identified in Table 6 constitue SEQ ID NOs:104-268 and can be topically applied with a transfer agent to tomato, or pepper plants to test the efficacy against infection by the respective viruses.

TABLE 6. The sequence of dsRNA oligonucleotides directed to viruses of commercial relevance.

SEQ ID NO:	Type	Length	Alias
104	dsRNA	150	BYD_CP
105	dsRNA	150	BYD_CP
106	dsRNA	25	BYD_CP_Conserved_across_strains_Overhangs
107	dsRNA	140	BYD_CP_Conserved_across_Strains
108	dsRNA	25	BYD_CP_overhangs
109	dsRNA	21	BYD_CP_overhangs
110	dsRNA	150	BYD_MP_Conserved_Across_Strains_Blunt
111	dsRNA	22	BYD_MP
112	dsRNA	25	BYD_MP
113	dsRNA	150	BYD_MP
114	dsRNA	25	BYD_MP
115	dsRNA	25	BYD_MP
116	dsRNA	150	BYD__Silencing_Suppressor
117	dsRNA	25	BYD__Silencing_Suppressor
118	dsRNA	21	BYD__Silencing_Suppressor_Blunt
119	dsRNA	25	BYD__Silencing_Suppressor_Overhang
120	dsRNA	150	CMV_CP
121	dsRNA	25	CMV_CP_Overhang_Conserved_Across_Strains
122	dsRNA	25	CMV_CP_Overhang_Conserved_Across_Strains
123	dsRNA	25	CMV_CP_Conserved_Across_Strains
124	dsRNA	150	CMV_CP
125	dsRNA	150	CMV_Silencing_Suppressor_Overhangs_Semi-Conserved_Across_Strains
126	dsRNA	25	CMV__Silencing_Suppressor

127	dsRNA	25	CMV__Silencing_Suppressor_Overhangs_Conserved_Across_Strains
128	dsRNA	25	CMV__Silencing_Suppressor_Overhangs_Conserved_Across_Strains
129	dsRNA	21	CMV__Silencing_Suppressor_Overhangs
130	dsRNA	25	CMV_MP_Overhangs_Semi-Conserved_Across_Strains
131	dsRNA	21	CMV_MP_Overhangs
132	dsRNA	21	CMV_MP_Overhangs
133	dsRNA	21	CMV_MP_Overhangs
134	dsRNA	21	CMV_MP_Overhangs_Semi-Conserved_Across_Strains
135	dsRNA	21	CMV_MP_Overhangs_Conserved_Across_Strains
136	dsRNA	21	CMV_MP_Overhangs_Conserved_Across_Strains
137	dsRNA	21	CMV_MP_Overhangs_Conserved_Across_Strains
138	dsRNA	21	CMV_MP_Overhangs
139	dsRNA	150	CMV_MP_Overhangs
140	dsRNA	150	CMV_MP_Overhangs
141	dsRNA	25	CMV_MP_Overhangs
142	dsRNA	25	CMV_MP_Overhangs
143	dsRNA	25	CMV_MP_Overhangs
144	dsRNA	25	CMV_MP_Overhangs
145	dsRNA	21	CMV_MP_Overhangs
146	dsRNA	150	PepMV_CP
147	dsRNA	25	PepMV_CP_Overhangs_Semi_Conserved_Across_Strains
148	dsRNA	25	PepMV_CP_Overhangs_Semi_Conserved_Across_Strains
149	dsRNA	25	PepMV_CP_Overhangs_Semi_Conserved_Across_Strains
150	dsRNA	21	PepMV_CP
151	dsRNA	21	PepMV_CP
152	dsRNA	21	PepMV_CP

153	dsRNA	150	PepMV_CP
154	dsRNA	150	PepMV_MP
155	dsRNA	150	PepMV_MP_Triple Gene Block1_
156	dsRNA	25	PepMV_MP_Triple Gene Block1_Overhangs_Conserved_Across_Strains
157	dsRNA	21	PepMV_MP_Triple Gene Block1_Overhangs_Conserved_Across_Strains
158	dsRNA	21	PepMV_MP_Triple Gene Block1_Overhangs_Conserved_Across_Strains
159	dsRNA	21	PepMV_MP_Triple Gene Block1_Overhangs_Conserved_Across_Strains
160	dsRNA	21	PepMV_MP_Triple Gene Block1_Overhangs_Conserved_Across_Strains
161	dsRNA	21	PepMV_MP_Triple Gene Block1_Overhangs_Conserved_Across_Strains
162	dsRNA	21	PepMV_MP_Triple Gene Block1_Overhangs_Conserved_Across_Strains
163	dsRNA	150	PepMV_MP_Triple Gene Block2
164	dsRNA	21	PepMV_MP_Triple Gene Block2_Overhangs_Conserved_Across_Strains
165	dsRNA	21	PepMV_MP_Triple Gene Block2_Overhangs_Conserved_Across_Strains
166	dsRNA	21	PepMV_MP_Triple Gene Block2_Overhangs_Conserved_Across_Strains
167	dsRNA	21	PepMV_MP_Triple Gene Block2_Overhangs_Conserved_Across_Strains
168	dsRNA	21	PepMV_MP_Triple Gene Block2_Overhangs_Conserved_Across_Strains
169	dsRNA	150	PepMV_MP_Triple Gene Block2
170	dsRNA	150	PepMV_MP_Triple Gene Block3
171	dsRNA	21	PepMV_MP_Triple Gene Block3_Overhangs
172	dsRNA	21	PepMV_MP_Triple Gene Block3_Overhangs
173	dsRNA	21	PepMV_MP_Triple Gene Block3_Overhangs
174	dsRNA	21	PepMV_MP_Triple Gene Block3_Overhangs
175	dsRNA	150	PepMV_MP_Triple Gene Block3_Overhangs
176	dsRNA	21	PepMV_MP_Triple Gene Block3_Overhangs
177	dsRNA	150	PepMV_MP_Triple Gene Block3
178	dsRNA	150	CuCLV_CP_Overhangs_Conserved_across_Strains

179	dsRNA	21	CuCLV_CP_Overhangs_Conserved_across_Strains
180	dsRNA	21	CuCLV_CP_Overhangs_Conserved_across_Strains
181	dsRNA	21	CuCLV_CP_Overhangs_Conserved_across_Strains
182	dsRNA	21	CuCLV_CP_Overhangs_Conserved_across_Strains
183	dsRNA	25	CuCLV_CP_Overhangs_Conserved_across_Strains
184	dsRNA	21	CuCLV_CP_Overhangs_Conserved_across_Strains
185	dsRNA	21	CuCLV_CP_Overhangs_Conserved_across_Strains
186	dsRNA	25	CuCLV_CP_Overhangs_Conserved_across_Strains
187	dsRNA	21	CuCLV_CP_Overhangs_Conserved_across_Strains
188	dsRNA	150	CuCLV_Silencing Suppressor
189	dsRNA	21	CuCLV_Silencing Suppressor_Overhangs
190	dsRNA	21	CuCLV_Silencing Suppressor_Overhangs
191	dsRNA	21	CuCLV_Silencing Suppressor_Overhangs
192	dsRNA	21	CuCLV_Silencing Suppressor_Overhangs
193	dsRNA	21	CuCLV_Silencing Suppressor_Overhangs
194	dsRNA	21	CuCLV_Silencing Suppressor_Overhangs
195	dsRNA	21	CuCLV_Silencing Suppressor_Overhangs
196	dsRNA	150	CuCLV_MP_Overhang_Conserved_Across_Strains
197	dsRNA	21	CuCLV_MP_Overhang
198	dsRNA	21	CuCLV_MP_Overhang
199	dsRNA	21	CuCLV_MP_Overhang_Conserved_Across_Strains
200	dsRNA	21	CuCLV_MP_Overhang_Conserved_Across_Strains
201	dsRNA	21	CuCLV_MP_Overhang_Conserved_Across_Strains
202	dsRNA	21	CuCLV_MP_Overhang_Conserved_Across_Strains
203	dsRNA	21	CuCLV_MP_Overhang_Conserved_Across_Strains
204	dsRNA	25	CuCLV_MP_Overhang_Conserved_Across_Strains

205	dsRNA	150	TYLCV_CP
206	dsRNA	21	TYLCV_CP_Overhangs
207	dsRNA	21	TYLCV_CP_Overhangs
208	dsRNA	21	TYLCV_CP_Overhangs
209	dsRNA	21	TYLCV_CP_Overhangs
210	dsRNA	21	TYLCV_CP_Overhangs
211	dsRNA	21	TYLCV_CP_Overhangs
212	dsRNA	21	TYLCV_CP_Overhangs
213	dsRNA	150	TYLCV_CP
214	dsRNA	150	TYLCV_CP
215	dsRNA	21	TYLCV_CP_Overhangs
216	dsRNA	150	TYLCV_MP
217	dsRNA	21	TYLCV_MP_Overhangs_Conserved
218	dsRNA	21	TYLCV_MP_Overhangs_Conserved
219	dsRNA	21	TYLCV_MP_Overhangs_Conserved
220	dsRNA	21	TYLCV_MP_Overhangs_Conserved
221	dsRNA	21	TYLCV_MP_Overhangs_Conserved
222	dsRNA	21	TYLCV_MP_Overhangs_Conserved
223	dsRNA	21	TYLCV_MP_Overhangs_Conserved
224	dsRNA	150	TYLCV_Silencing Suppressor_C2
225	dsRNA	21	TYLCV_Silencing Suppressor_C2_Overhangs
226	dsRNA	21	TYLCV_Silencing Suppressor_C2_Overhangs
227	dsRNA	21	TYLCV_Silencing Suppressor_C2_Overhangs
228	dsRNA	21	TYLCV_Silencing Suppressor_C2_Overhangs
229	dsRNA	21	TYLCV_Silencing Suppressor_C2_Overhangs
230	dsRNA	21	TYLCV_Silencing Suppressor_C2_Overhangs

231	dsRNA	21	TYLCV_Silencing Suppressor_C2_Overhangs
232	dsRNA	150	TYLCV_Silencing Suppressor_C2
233	dsRNA	150	WSMV_CP
234	dsRNA	21	WSMV_CP_Overhangs
235	dsRNA	21	WSMV_CP_Overhangs
236	dsRNA	21	WSMV_CP_Overhangs
237	dsRNA	21	WSMV_CP_Overhangs
238	dsRNA	21	WSMV_CP_Overhangs
239	dsRNA	21	WSMV_CP_Overhangs
240	dsRNA	21	WSMV_CP_Overhangs
241	dsRNA	150	WSMV_CP
242	dsRNA	150	WSMV_CP
243	dsRNA	21	WSMV_CP_Overhangs
244	dsRNA	21	WSMV_CP_Overhangs
245	dsRNA	21	WSMV_CP_Overhangs
246	dsRNA	21	WSMV_CP_Overhangs
247	dsRNA	21	WSMV_CP_Overhangs
248	dsRNA	21	WSMV_CP_Overhangs
249	dsRNA	21	WSMV_CP_Overhangs
250	dsRNA	25	WSMV_CP_Blunt
251	dsRNA	150	WSMV_Nia_Vpg
252	dsRNA	21	WSMV_Nia_Vpg_Overhang
253	dsRNA	21	WSMV_Nia_Vpg_Overhang
254	dsRNA	21	WSMV_Nia_Vpg_Overhang
255	dsRNA	21	WSMV_Nia_Vpg_Overhang
256	dsRNA	150	WSMV_Nia_Vpg

257	dsRNA	25	WSMV_Nia_Vpg_Overhang
258	dsRNA	21	WSMV_Nia_Vpg_Overhang
259	dsRNA	150	WSMV_Nia_Pro_Overhang
260	dsRNA	21	WSMV_Nia_Pro_Overhang
261	dsRNA	21	WSMV_Nia_Pro_Overhang
262	dsRNA	150	WSMV_Nia_Pro_Overhang
263	dsRNA	21	WSMV_Nia_Pro_Overhang
264	dsRNA	150	WSMV_Nia_Pro
265	dsRNA	21	WSMV_Nia_Pro_Overhang
266	dsRNA	25	WSMV_Nia_Pro_Overhang
267	dsRNA	21	WSMV_Nia_Pro_Overhang
268	dsRNA	21	WSMV_Nia_Pro_Overhang

Example 9

Topical application of polynucleotide triggers for control of Cucumber mosaic virus

In this example, the sequences of the Coat Protein (CM) Movement Protein (MP) or Silencing Suppressor (S) for different Cucumber Mosaic Viruses were identified and can be seen in Table 7. Topical application of ss antisense DNA or dsRNA sequences derived from the listed sequences (SEQ ID NOs:269-316) will be performed in pepper plants infected by Cucumber Mosaic Virus (CMV) using a transfer reagent and the plants will be scored by ELISA analysis and visual assessment for reduction of symptoms.

TABLE 7. Sequences of target genes in Cucumber Mosaic Virus (CMV).

SEQ ID NO:	Sequence ID	Host	Strain	Isolate	Gene
269	CMV				CP -N Gene
270	AB004780		KM	Japan	CP -N Gene
271	D10538		Fny	USA (NY)	CP -N Gene
272	D00462		C	USA (NY)	CP -N Gene
273	L36251		Kor	Korea	CP -N Gene
274	U66094		Sny	Israel	CP -N Gene
275	U22821		Ny	Australia	CP -N Gene
276	D28487		FT	Japan	CP -N Gene
277	D10544		FC	USA	CP -N Gene
278	AJ890464	Oriental Lily (Expression)	OL	India	CP -N Gene
279	AJ831578		Ll	India	CP -N Gene
280	AJ890465		Lt	India	CP -N Gene
281	D42079		C7-2	Japan	CP -N Gene
282	AJ271416		2A1-A	USA	CP -N Gene
283	AF013291		As	Korea	CP -N Gene
284	Y16926		Tfn	Italy	CP -N Gene
285	AB042294		IA-3a	Japan	CP -N Gene
286	D28780		NT9	Taiwan	CP -N Gene
287	U31220	Banana in Hawaii	Oahu	USA	CP -N Gene
288	D49496		Tai	Taiwan	CP -N Gene
289	X89652		Phym	India	CP -N Gene
290	AF281864		D	India	CP -N Gene
291	AF350450		H	India	CP -N Gene

292	L15336		Trk7	Hungary	CP -N Gene
293	M21464		Q	Australia	CP -N Gene
294	AF063610		S	USA	CP -N Gene
295	AF127976		LS	USA	CP -N Gene
296	U10923	Spinacia oleracea	SP103	USA	CP -N Gene
297	AB006813		m2	Japan	CP -N Gene
298	U22822		Sn	Australia	CP -N Gene
299	L40953		Wem	Unknown	CP -N Gene
300	AJ585086		AL	India	CP -N Gene
301	FN555197	*Capsicum* sp	AN	India	Supressor Gene - 2b
302	FN555198	*Capsicum* sp	CN04	China	Supressor Gene - 2b
303	FN555199.1	*Capsicum* sp	KS44	Thailand	Supressor Gene - 2b
304	FN555200	*Capsicum* sp	P522	China	Supressor Gene - 2b
305			P3613	China	Supressor Gene - 2b
306	HQ916353	Oilseed pumpkin			Supressor Gene - 2b
307	aj517801	*Raphanus sativus*			Supressor Gene - 2b
308	ay827561	Paprika			Supressor Gene - 2b
309	jq074218	*Solanum lycopersicum*			Supressor Gene - 2b
310	EU432184.1		CMV-NEP		MP
311	EU432178.1		CMV-ANC		MP
312	JF918963.1				MP
313	JN593375.1			Italy	MP
314	EU414791.1	tobacco	CMV-RZ	China	MP
315	JF918961.1		N1-03	USA: Ohio	MP
316	JN593378		PhA_Italy	Italy	MP

Example 10

Topical application of polynucleotide triggers for control of Pepino mosaic virus infection

In this example the sequences of the Coat Protein (CM) and Movement Protein (MP) for different Pepino Mosaic Virus isolates were identified and can be seen in Table 8. Topical application of ss antisense DNA or dsRNA sequences derived from the listed sequences (SEQ ID NOs:317-349) will be performed in tomato plants infected by Pepino Mosaic Virus (PepMV) using a transfer reagent and the plants will be scored by ELISA analysis and visual assessment for reduction of symptoms.

TABLE 8. Sequences of target genes in Pepino Mosaic Virus (PepMV).

SEQ ID NO:	Sequence ID	Host	Strain	Isolate	Gene	Length
317	Orignal_file				CP	714
318	FJ820177.1	*Solanum lycopersicum*			CP	714
319	FJ820182.1	*Solanum lycopersicum*			CP	597
320	FJ384784.1	*Lycopersicon esculentum*			CP	702
321	FN429033	*Solanum lycopersicum*	PV-0554		CP	693
322	AM040187	*Lycopersicon esculentum*	Mu 04.12		CP	488
323	FJ263316.1	*Solanum lycopersicum*	PMU05/5	Spain	MP; Triple Gene Block1	708
324	FJ263326.1	*Solanum lycopersicum*	PMU08/47	Spain	MP; Triple Gene Block1	705
325	GQ438737.1	*Solanum lycopersicum*	Al 2-01	Spain	MP; Triple Gene Block1	705
326	FJ263325.1	*Solanum lycopersicum*	PMU08/42	Spain	MP; Triple Gene Block1	705
327	FJ384784.1	*Lycopersicon esculentum*	isolate 4988	Spain	MP; Triple Gene Block1	705
328	AM041982.1	*Lycopersicon esculentum*	isolate 1	Spain:Murcia	MP; Triple Gene Block1	705
329	AM041968	*Lycopersicon esculentum*	isolate 1	Spain:Murcia	MP; Triple Gene Block1	705
330	AM041967.1	*Lycopersicon esculentum*	isolate 1	Spain:Murcia	MP; Triple Gene Block1	705
331	AM041956.1	*Lycopersicon esculentum*	Mu 03.2	Spain:Murcia	MP; Triple Gene Block1	705
332	AM041955.1	*Lycopersicon esculentum*	Mu 03.1	Spain:Murcia	MP; Triple Gene Block1	705
333	AM041952.1	*Lycopersicon esculentum*	Al 01.1	Spain:Alicante	MP; Triple Gene Block1	706
334	FJ263323.1	*Solanum lycopersicum*	PMU08/38	Spain	MP; Triple gene block protein 2 (TGBp2)	372
335	FJ263322.1	*Solanum lycopersicum*	PMU07/36	Spain	MP; Triple gene block protein 2 (TGBp2)	372
336	FJ820184.1	*Solanum lycopersicum*	virus isolate 4911	Spain	MP; Triple gene block protein 2 (TGBp2)	373

337	FJ820181	*Solanum lycopersicum*	isolate 7156	Spain	MP; Triple gene block protein 2 (TGBp2)	373
338	FJ820176	*Solanum lycopersicum*	isolate 5577	Spain	MP; Triple gene block protein 2 (TGBp2)	373
339	FJ820174.1	*Solanum lycopersicum*	isolate 4983	Spain	MP; Triple gene block protein 2 (TGBp2)	372
340	GU130080.1	*Solanum lycopersicum*	isolate CI-05	Spain	MP; Triple gene block protein 2 (TGBp2)	372
341	GQ438737.1	*Solanum lycopersicum*	Al 2-01	Spain	MP; Triple gene block protein 2 (TGBp2)	372
342	FJ263320.1	*Solanum lycopersicum*	PMU07/27	Spain	MP; Triple gene block protein 2 (TGBp2)	372
343	FJ263317.1	*Solanum lycopersicum*	PMU06/17a	Spain	MP; Triple gene block protein 2 (TGBp2)	372
344	AM041992.1	*Lycopersicon esculentum*	isolate 1	Spain	MP; Triple gene block protein 2 (TGBp2)	372
345	FJ820184.1	*Solanum lycopersicum*	isolate 4911	Spain	MP; Triple gene block protein 3	255
346	FJ263325	*Solanum lycopersicum*	PMU08/42	Spain	MP; Triple gene block protein 3	255
347	FJ820174	*Solanum lycopersicum*	isolate 4983	Spain	MP; Triple gene block protein 3	255
348	FJ820173.1	*Solanum lycopersicum*	isolate 4910-10	Spain	MP; Triple gene block protein 3	255
349	GQ438737.1	*Solanum lycopersicum*	Al 2-01	Spain	MP; Triple gene block protein 3	715

Example 11

Topical application of polynucleotide triggers for control of infection by Barley yellow dwarf virus (BYDV)

In this example, the sequences of the Coat Protein (CM), Movement Protein (MP), and Silencing Suppressor (SS) for different Barley yellow dwarf virus isolates were identified and are set forth in Table 9. Topical application of antisense ssDNA or dsRNA sequences derived from the listed sequences (SEQ ID NOs:350-385) can be performed in barley plants infected by BYDV using a transfer reagent and the plants can be scored by ELISA analysis and visual assessment for reduction of symptoms.

TABLE 9. Sequences of target genes in Barley Yellow Dwarf Virus (BYDV).

SEQ ID NO:	Sequence ID	Strain	Isolate	Gene	Length
350	Orignal_file			CP-P3 and MP P4 (overlap)	603
351	BYDPCT			CP	605
352	JX402456.1	B-Keb	Tunisia: Kebili	CP - P3, Partial CDS	531
353	JX402454.1	B-Bej2	Tunisia: Beja	CP - P3, Partial CDS	532
354	HM488005		Jordan	CP - P3, Partial CDS	139
355	EF408184.1	MAV LMB2a		CP - P3, Partial CDS	593
356	EU332334.1	PAV isolate 06WH1		CP - P3, Partial CDS	600
357	EU332332.1	PAV isolate 06KM14		CP - P3, Partial CDS	603
358	EU332330.1	PAV isolate 05ZZ12		CP - P3, Partial CDS	600
359	EU332328.1	PAV isolate 05ZZ9		CP - P3, Partial CDS	600
360	EU332326.1	PAV isolate 05ZZ6		CP - P3, Partial CDS	600
361	EU332320.1	PAV isolate 05ZZ1		CP - P3, Partial CDS	600
362	HM488005.1	SGV		CP - P3, Partial CDS	139
363	GU002361	BYDV-MAV-OA1	New Zealand: Lincoln	CP - P3, Partial CDS	501
364	GU002328	BYDV-PAV-OA4	New Zealand: Lincoln	CP - P3, Partial CDS	502
365	GU002324.1	BYDV-PAS-DC2	New Zealand: Lincoln	CP - P3, Partial CDS	412
366	GU002322.1	BYDV-MAV-WC5	New Zealand: Lincoln	CP - P3, Partial CDS	412
367	GU002360.1	BYDV-MAV-O1LU	New Zealand: Lincoln	CP - P3, Partial CDS	502
368	GU002329.1	BYDV-PAV-PC3	New Zealand: Lincoln	CP - P3, Partial CDS	490
369	GU002325.1	BYDV-PAV-327		CP - P3, Partial CDS	502
370	EF408184.1			CP - P3, Partial CDS	593

371	EF408180.1	isolate MAV SI-o4		CP - P3, Partial CDS	593
372	AF235167.1			CP - P3, Partial CDS	603
373	ABR26505			CP - P3, Partial CDS	596
374	AAZ93695.	UCD2-PAV	USA:California	MP-P4	462
375	EF408167.1	PAV sim10-2	New Zealand:Coromandel	MP-P4	462
376	EF408166.1	PAV sim10-1	New Zealand:Coromandel	MP-P4	462
377	AY855920.1	PAV-CN	China	MP-P4	462
378	GU002330.1	BYDV-PAV-WC2	New Zealand:Lincoln	MP-P4	400
379	X07653.1			Silencing suppressor, P6	192
380	EF521828.1			Silencing suppressor, P6	126
381	AJ007492.1			Silencing suppressor, P6	129
382	EU332332.1	05GG2	China:Gangu	Silencing suppressor, P6	129
383	EF521850.1	PAV isolate 064	USA:Alaska	Silencing suppressor, P6	120
384	EU332335.1		China:Zhengzhou	Silencing suppressor, P6	123
385	EF521849.1l	PAV 0102	USA:California	Silencing suppressor, P6	87

Example 12

Topical application of polynucleotide triggers for control of infection by Tomato yellow leaf curl virus (TYLCV)

In this example, the sequences of the Coat Protein (CM), Movement Protein (MP), and Complement (C2) protein for different Tomato yellow leaf curl virus isolates were identified and are set forth in Table 10. Topical application of antisense ssDNA or dsRNA sequences derived from the listed sequences (SEQ ID NOs:386-421) can be performed in tomato plants infected by TYLCV using a transfer reagent and the plants scored by ELISA analysis and visual assessment for reduction of symptoms.

TABLE 10. Sequences of target genes in Tomato Yellow Curl Leaf Virus (TYCLV).

SEQ ID NO:	Sequence ID	Host	Strain	Isolate	Gene	Note
386	AJ519441.1				CP	
387	JX075187.1			South Korea	CP	
388	HM856915.1				CP	
389	HM856913.1|				CP	
390	EF210554.1			Arizona	CP	
391	AB116631.1	*Stellaria aquatica*	TYLCV-IL[JR:Mis:Ste]	Japan	CP	
392	L27708.1		Almeria	Spain	CP	
393	X15656.1				CP	
394	X61153.1				CP	
395	X76319.1				CP	
396	GU723744.1			Thailand	CP	
397	EF110890.1	*Lycopersicon esculentum*		USA: Texas	CP	
398	HE603246.1	*Solanum lycopersicum*	New Caledonia:Ouvea:2010	Israel	MP	
399	HM448447.1	*Solanum lycopersicum*		Mauritius	MP	
400	EU143754.1	Squash		Jordan	MP	
401	AJ842308.1			Saint Gilles	MP	
402	AJ842307.1			Saint Gilles	MP	
403	EU143745.1	Cucumber		Jordan	MP	
404	AM409201.1	*Solanum lycopersicum*		Reunion:Saint-Gilles les Hauts	MP	
405	JX456639.1		KYCTo18	China	MP	

406	JN183880.1		Andong 2	South Korea: Andong	MP	
407	FR851297.1			Israel	MP	
408	HM856914.1			Gwangyang 6	MP	
409	HM856912.1			South Korea: Gunwi	MP	
410	GU348995.1	*Solanum lycopersicum*		China: Hebei	MP	
411	EF490995.1	*Solanum lycopersicum*		Martinique	MP	
412	EF110890.1	*Lycopersicon esculentum*		USA: Texas		
413	DQ144621.1	*Lycopersicon esculentum*		Italy: Sicily	C2	Complement
414	AB116632	*Lycopersicon esculentum*		Japan	C2	Complement
415	AB110218.1			Israel	C2	Complement
416	GU325634.1	*Lycopersicon esculentum*		South Korea: Boseong	C2	Complement
417	EU143745.1	Cucumber		Jordan: Homrat Al-Sahen	C2	Complement
418	GU178814	*Solanum lycopersicum*		Australia:Brisbane2:2006	C2	Complement
419	EF523478.1			Mexico	C2	Complement
420	EF433426.1	cucumber		Jordan	C2	Complement
421	EF110890	*Lycopersicon esculentum*		USA: Texas	C2	Complement

Example 13

Topical application of polynucleotide triggers for control of infection by Cotton leaf curl virus (CLCuV)

In this example the sequences of the Coat Protein (CM), Movement Protein (MP) and AC2 protein for different Cotton Leaf Curl Virus isolates were identified and can be seen in Table 11. Topical application of ss antisense DNA or dsRNA sequences derived from the listed sequences (SEQ ID NOs:422-447) will be performed in cotton plants infected by CLCuV using a transfer reagent and the plants will be scored by ELISA analysis and visual assessment for reduction of symptoms.

TABLE 11. Sequences of target genes in Cotton Leaf Curl Virus (CLCuV).

SEQ ID NO:	Sequence ID	Host	Species	Isolate	Gene	length
422	EF057791.1		Cotton leaf curl virus		CP	771
423	JN558352.1	papaya	Cotton leaf curl virus		CP	771
424	FJ218487.1	*Gossypium hirsutum*	Cotton leaf curl virus		CP	771
425	AF521594.1		Cotton leaf curl virus	India: Hisar	CP	771
426	AY765254		Cotton leaf curl virus	India: Sirsa, Haryana	CP	771
427	JX914662.1				CP	771
428	EF465535.1		*Hibiscus rosa-sinensis*		CP	771
429	FJ159268.1		*Hibiscus cannabinus*	Amadalavalasa:South India	CP	771
430	JX286658.1		*Hibiscus rosa-sinensis*	China	CP	772
431	JN968573.1		*Hibiscus rosa-sinensis*	China: Guangdong	CP	771
432	GU574208.1		Okra	China	CP	771
433	GU112008.1		*Abelmoschus esculentus* (okra)	India: Karnal, Haryana	CP	771
434	AJ002455.1				CP	771
435	AJ002455.1			Pakistan	CP	771
436	JX286660		*Hibiscus rosa-sinensis*	China	CP	771
437	HQ455367.1		*Hibiscus rosa-sinensis* (Rose Mallow)	China	CP	771
438	EU384573		*Gossypium hirsutum* subsp. *Latifolium*	Pakistan: Multan	CP	772
439	AJ002458.1		Cotton leaf curl Multan virus-[26]	Pakistan	CP	772
440	AY028808.1			India	MP	359
441	AF363011.1				MP	358
442	HM235774.1		*Gossypium hirsutum*	India	MP	358

443	AY028808.1		India	MP	357
444	AY146959.1		India	MP	358
445	AY146960.1			MP	357
446	AY146957.1		India: Sirsa	MP	367
447	HM037923.1	*Gossypium hirsutum*	Sirsa-Haryana-En(P)	AC2	454

Example 14

Topical application of dsRNA oligonucleotides to pepper plants for control of Tomato spotted wilt virus (TSWV)

In this example, growing pepper plants (*c.v. Yolo Wonder B*) were inoculated with tomato spotted wilt virus (TSWV), a negative strand ssRNA virus, and the plants were separated into different groups. The experimental group was topically treated with a liquid composition containing at least one dsRNA polynucleotide comprising an approximately 100 bp sequence that is homologous to a transcript of the nucleocapsid (N), suppressor (NSs) or movement (NSm) gene of TSWV and its complement. The sequences of the sense strand of the trigger molecules used in the experiments outlined in this Example are shown in Table 12.

Table 12. dsRNA polynucleotides directed to TSWV nucleocapsid (N), suppressor (NSs) or movement (NSm) gene transcripts.

SEQ ID NO	Trigger ID	Length	Virus	Target
448	T25748	99	TSWV	Nucleocapsid (N)
449	T25749	101	TSWV	Nucleocapsid (N)
450	T25750	101	TSWV	Nucleocapsid (N)
451	T25751	101	TSWV	Nucleocapsid (N)
452	T25752	101	TSWV	Nucleocapsid (N)
453	T25753	101	TSWV	Nucleocapsid (N)
454	T25754	108	TSWV	Nucleocapsid (N)
455	T25755	101	TSWV	Nucleocapsid (N)
456	T25756	97	TSWV	Nucleocapsid (N)
457	T25757	103	TSWV	Movement (NSm)
458	T25758	100	TSWV	Movement (NSm)

459	T25759	99	TSWV	Movement (NSm)
460	T25760	101	TSWV	Movement (NSm)
461	T25761	101	TSWV	Movement (NSm)
462	T25762	96	TSWV	Movement (NSm)
463	T25763	101	TSWV	Movement (NSm)
464	T25764	97	TSWV	Movement (NSm)
465	T25765	98	TSWV	Movement (NSm)
466	T25766	109	TSWV	Movement (NSm)
467	T25767	100	TSWV	Suppressor (NSs)
468	T25768	100	TSWV	Suppressor (NSs)
469	T25769	97	TSWV	Suppressor (NSs)
470	T25770	101	TSWV	Suppressor (NSs)
471	T25771	95	TSWV	Suppressor (NSs)
472	T25772	100	TSWV	Suppressor (NSs)
473	T25773	102	TSWV	Suppressor (NSs)
474	T25774	103	TSWV	Suppressor (NSs)
475	T25775	97	TSWV	Suppressor (NSs)
476	T25776	96	TSWV	Suppressor (NSs)
477	T25777	102	TSWV	Suppressor (NSs)
478	T25778	101	TSWV	Suppressor (NSs)
479	T25779	98	TSWV	Suppressor (NSs)
480	T25780	103	TSWV	Suppressor (NSs)

481	T25781	101	TSWV	Suppressor (NSs)
482	T25782	102	TSWV	Suppressor (NSs)
483	T34084	100	CMV	Coat Protein (CP)

Plants were sown in a growth chamber [22°C, 8 hour light (~50 µmol), 16 hour dark cycles] and transferred to a green house a couple of days before treatment. Pepper plants at the 2-5 fully expanded leaf stage were used in this assay. The experimental setup consisted of between 20-24 plants per treatment. Treatments consisted of: (a) healthy controls (no viral infection), (b) virus only control (no polynucleotide solution), (c) formulation only (no polynucleotides), or (d) experimental application with polynucleotide/Silwet L-77 trigger solution comprising a trigger molecule selected from the list of SEQ ID NOs:448-483 following virus infection. Virus infection was carried out using standard mechanical inoculation technique and using Tomato spotted wilt virus (TSWV) or Cucumber mosaic virus (CMV), a positive strand RNA virus unrelated to TSWV. The final concentration used for each dsRNA polynucleotide was between 14.2-15.15 pmol/plant (in 0.1% Silwet L-77, 2% ammonium sulfate, 5 mM sodium phosphate buffer, pH 6.8). One thousand micro-liters of the polynucleotide/Silwet L-77 solution was applied using an airbrush (Badger 200G) at 10 psi to each plant group. Plants were arranged in the greenhouse following a randomized complete block design and monitored visually for symptom development. Plant height and ELISA analysis were both carried out at 32 days post-infection (32 DPI). ELISA analysis was performed on supernatant extracts from control and systemic leaf tissue punctures using an antibody to TSWV nucleocapsid (N) protein. The experiment was repeated twice (see Tables 13-17).

Table 13. Experiment 1: Plant height measurements at 32DPI after treatment with dsRNA polynucleotides.

Treatment	Mean		Group				N	Std Dev
Healthy	39.9		A				24	5.4
T25748	33.4			B			24	10.0

T25773	32.9				BC		24	7.9
T25763	32.7				BC		24	8.7
T25769	32.5				BC		24	9.5
T25755	32.3				BC		24	7.8
T25776	32.3				BC		24	7.8
T25770	31.9				BC		24	8.8
T25778	31.7				BC		24	7.1
T25753	31.6				BC		24	9.9
Virus (TSWV)	31.3				BC		24	8.7
CMV	29.9				BC		24	7.5
Buffer (Formulation)	29.2					C	24	7.1

*Levels not connected by the same letter are significantly different.

Table 14. Experiment 1: Statistical analysis of best performing trigger sequences compared to controls.

Treatment	Mean	Std Deviation	Std Err
Healthy	39.9	5.4	1.10486
Virus (TSWV)	31.3	8.7	1.77702
Buffer (Formulation)	29.2	7.1	1.44554
T25748	33.4	10.0	2.05127
T25773	32.9	7.9	1.61158

Plants treated with polynucleotide trigger sequence T25748 corresponding to SEQ ID NO:448 in the TSWV Nucleocapsid (N) gene were significantly taller than plants treated with other polynucleotides. This is also shown in FIG. 12A and B which shows a graphical representation of these results.

Table 15. Experiment 1: ELISA analysis at 32 DPI after treatment with dsRNA polynucleotides.

Treatment	Mean	Std Err
Healthy	0.06	0.02
T25773	0.15	0.06
Virus (TSWV)	0.23	0.09
T25763	0.24	0.09
T25778	0.25	0.12
Buffer (Form.)	0.27	0.13
T25755	0.28	0.13
T25776	0.28	0.14
CMV	0.29	0.16
T25769	0.30	0.13
T25748	0.40	0.17
T25753	0.47	0.20
T25770	0.61	0.23

Table 16. Experiment 2: Plant height measurements at 32DPI after treatment with dsRNA polynucleotides.

Treatment	Mean		Group				N	Std Dev
Healthy	30.1		A				24	7.2
T25772	25.6			B			24	7.1
T25748	25.1				BC		24	7.0
T25769	24.8				BC		24	5.7
T25755	24.3				BC		24	8.0
T25775	24.2				BC		24	6.3
T25776A	23.9				BC		24	6.6
Virus	23.6				BC		24	6.2
T25763	23.3				BC		24	5.4
CMV	23.2				BC		24	7.1
T25770	23.1				BC		24	6.1
Buffer	22.6				BC		24	6.6
T25776B	22.0					C	24	6.6

*Levels not connected by the same letter are significantly different.

In this experiment treatment with trigger sequence T25748 (SEQ ID NO:448) was the best performer of the "BC" group. FIG. 13 shows a graphical display of the results of this experiment.

Table 17. Experiment 2: ELISA analysis at 32DPI after treatment with dsRNA polynucleotides.

Experiment 2		
Treatment	**Mean**	**StdErr**
T25776A	0.05	0.01
Healthy	0.06	0.01
T25776B	0.06	0.02
T25772	0.44	0.17
Virus (TSWV)	0.45	0.16
T25769	0.53	0.20
T25755	0.55	0.20
T25775	0.58	0.21
T25770	0.61	0.18
T25763	0.79	0.19
T25748	0.83	0.24
Buffer (Form.)	1.05	0.24
CMV	1.11	0.24
T25776	1.98	0.20

CLAIMS

Claim 1. A method of treatment or prevention of a Tospovirus infection in a plant comprising: topically applying to said plant a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein the symptoms of viral infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 2. The method of claim 1, wherein said transfer agent is an organosilicone surfactant composition or compound contained therein.

Claim 3. The method of claim 1, wherein said composition comprises more than one antisense single-stranded DNA polynucleotide complementary to all or a portion of an essential Tospovirus gene sequence, an RNA transcript of said essential Tospovirus gene sequence, or a fragment thereof.

Claim 4. The method of claim 1, wherein said antisense single-stranded DNA polynucleotide is selected from the group consisting of SEQ NO:1-12 or a fragment thereof.

Claim 5. The method of claim 1, wherein said Tospovirus is selected from the group consisting of bean necrotic mosaic virus, Capsicum chlorosis virus, groundnut bud necrosis virus, groundnut ringspot virus, groundnut yellow spot virus, impatiens necrotic spot virus, iris yellow spot viris, melon yellow spot virus, peanut bud necrosis virus, peanut yellow spot virus, soybean vein necrosis-associated virus, tomato chlorotic spot virus, tomato necrotic ringspot virus, tomato spotted wilt virus, tomato zonate spot virus, watermelon bud necrosis virus, watermelon silver mottle virus, and zucchini lethal chlorosis virus.

Claim 6. The method of claim 1, wherein said essential Tospovirus gene is selected from the group consisting of nucleocapsid gene (N), coat protein gene (CP), virulence factors NSm and NSs, and RNA-dependent RNA polymerase L segment (RdRp/L segment).

Claim 7. The method of claim 6, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46.

Claim 8. The method of claim 1, wherein said composition is topically applied by spraying, dusting, or is applied to the plant surface as matrix-encapsulated DNA.

Claim 9. A composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 10. The composition of claim 9, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46.

Claim 11. The composition of claim 9, wherein said transfer agent is an organosilicone composition.

Claim 12. The composition of claim 9, wherein said antisense single-stranded DNA polynucleotide is selected from the group consisting of SEQ ID NOs:1-12.

Claim 13. A method of reducing expression of an essential Tospovirus gene comprising contacting a Tospovirus particle with a composition comprising an antisense single-stranded DNA polynucleotide and a transfer agent, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential gene sequence in said Tospovirus or an RNA transcript thereof, wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 14. The method of claim 13, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46.

Claim 15. The method of claim 13, wherein said transfer agent is an organosilicone compound.

Claim 16. The method of claim 13, wherein said antisense single-stranded DNA polynucleotide is selected from the group consisting of SEQ ID NOs:1-12 or fragment thereof.

Claim 17. A method of identifying antisense single-stranded DNA polynucleotides useful in modulating Tospovirus gene expression when topically treating a plant comprising: a) providing a plurality of antisense single-stranded DNA polynucleotides that comprise a region complementary to all or a part of an essential Tospovirus gene or RNA transcript thereof; b) topically treating said plant with one or more of said antisense single-stranded DNA polynucleotides and a transfer agent; c) analyzing said plant or extract for modulation of symptoms of Tospovirus infection; and d) selecting an antisense single-stranded DNA polynucleotide capable of modulating the symptoms or occurrence of Tospovirus infection.

Claim 18. The method of claim 17, wherein said transfer agent is an organosilicone compound.

Claim 19. An agricultural chemical composition comprising an admixture of an antisense single-stranded DNA polynucleotide and a pesticide, wherein said antisense single-stranded DNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 20. The agricultural chemical composition of claim 19, wherein said pesticide is selected from the group consisting of anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides.

Claim 21. A method of treatment or prevention of a Tospovirus infection in a plant comprising: topically applying to said plant a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein the symptoms of viral infection

or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 22. The method of claim 21, wherein said transfer agent is an organosilicone surfactant composition or compound contained therein.

Claim 23. The method of claim 21, wherein said composition comprises more than one double-stranded RNA polynucleotide complementary to all or a portion of an essential Tospovirus gene sequence, an RNA transcript of said essential Tospovirus gene sequence, or a fragment thereof.

Claim 24. The method of claim 21, wherein said double-stranded RNA polynucleotide is selected from the group consisting of SEQ NO:47-103 or a fragment thereof.

Claim 25. The method of claim 21, wherein said Tospovirus is selected from the group consisting of bean necrotic mosaic virus, Capsicum chlorosis virus, groundnut bud necrosis virus, groundnut ringspot virus, groundnut yellow spot virus, impatiens necrotic spot virus, iris yellow spot viris, melon yellow spot virus, peanut bud necrosis virus, peanut yellow spot virus, soybean vein necrosis-associated virus, tomato chlorotic spot virus, tomato necrotic ringspot virus, tomato spotted wilt virus, tomato zonate spot virus, watermelon bud necrosis virus, watermelon silver mottle virus, and zucchini lethal chlorosis virus.

Claim 26. The method of claim 21, wherein said essential Tospovirus gene is selected from the group consisting of nucleocapsid gene (N), coat protein gene (CP), virulence factors NSm and NSs, and RNA-dependent RNA polymerase L segment (RdRp/L segment).

Claim 27. The method of claim 26, wherein said essential Tospovirus gene is selected from the group consisting of SEQ ID NOs:13-46.

Claim 28. The method of claim 21, wherein said composition is topically applied by spraying, dusting, or is applied to the plant surface as matrix-encapsulated RNA.

Claim 29. A composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or an RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the

symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 30. The composition of claim 29, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46.

Claim 31. The composition of claim 29, wherein said transfer agent is an organosilicone composition.

Claim 32. The composition of claim 29, wherein said double-stranded RNA polynucleotide is selected from the group consisting of SEQ NO:47-103.

Claim 33. A method of reducing expression of an essential Tospovirus gene comprising contacting a Tospovirus particle with a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential gene sequence in said Tospovirus or an RNA transcript thereof, wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 34. The method of claim 33, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:13-46.

Claim 35. The method of claim 33, wherein said transfer agent is an organosilicone compound.

Claim 36. The method of claim 33, wherein said double-stranded RNA polynucleotide is selected from the group consisting of SEQ ID NOs:47-103 or fragment thereof.

Claim 37. A method of identifying double-stranded RNA polynucleotide useful in modulating Tospovirus gene expression when topically treating a plant comprising: a) providing a plurality of double-stranded RNA polynucleotides that comprise a region complementary to all or a part of an essential Tospovirus gene or RNA transcript thereof; b) topically treating said plant with one or more of said double-stranded RNA polynucleotides and a transfer agent; c) analyzing said plant or extract for modulation of symptoms of Tospovirus infection; and d) selecting a double-

stranded RNA polynucleotide capable of modulating the symptoms or occurrence of Tospovirus infection.

Claim 38. The method of claim 37, wherein said transfer agent is an organosilicone compound.

Claim 39. An agricultural chemical composition comprising an admixture of a double-stranded RNA polynucleotide and a pesticide, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Tospovirus gene sequence or RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Tospovirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 40. The agricultural chemical composition of claim 39, wherein said pesticide is selected from the group consisting of anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides.

Claim 41. A method of treatment or prevention of a Geminivirus infection in a plant comprising: topically applying to said plant a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Geminivirus gene sequence, or an RNA transcript thereof, wherein the symptoms of viral infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 42. The method of claim 41, wherein said transfer agent is an organosilicone surfactant composition or compound contained therein.

Claim 43. The method of claim 41, wherein said composition comprises more than one double-stranded RNA polynucleotide complementary to all or a portion of an essential Geminivirus gene sequence, an RNA transcript of said essential Geminivirus gene sequence, or a fragment thereof.

Claim 44. The method of claim 41, wherein said double-stranded RNA polynucleotide is selected from the group consisting of SEQ NO:104-268 or a fragment thereof.

Claim 45. The method of claim 41, wherein said Geminivirus is selected from the group consisting of Barley yellow dwarf virus, Cucumber mosaic virus, Pepino mosaic virus, Cotton curl leaf virus, Tomato yellow leaf curl virus, Tomato golden mosaic virus, Potato yellow mosaic virus, Pepper leaf curl virus, Bean golden mosaic virus, Bean golden mosaic virus, Tomato mottle virus.

Claim 46. The method of claim 41, wherein said essential Geminivirus gene is selected from the group consisting of nucleocapsid gene (N), a coat protein gene (CP), virulence factors NSm and NSs, and RNA-dependent RNA polymerase L segment (RdRp/L segment), a silencing suppressor gene, movement protein (MP), Nia, CP-N, a triple gene block, CP-P3, MP-P4, C2, and AC2.

Claim 47. The method of claim 46, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:269-447.

Claim 48. The method of claim 41, wherein said composition is topically applied by spraying, dusting, or is applied to the plant surface as matrix-encapsulated RNA.

Claim 49. A composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Geminivirus gene sequence, such as one set forth as SEQ ID NOs:269-447, or an RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 50. The composition of claim 49, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:269-447.

Claim 51. The composition of claim 49, wherein said transfer agent is an organosilicone composition.

Claim 52. The composition of claim 49, wherein said double-stranded RNA polynucleotide is selected from the group consisting of SEQ NO:104-268.

Claim 53. A method of reducing expression of an essential Geminivirus gene comprising contacting a Geminivirus particle with a composition comprising a double-stranded RNA polynucleotide and a transfer agent, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential

gene sequence in said Geminivirus or an RNA transcript thereof, wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 54. The method of claim 53, wherein said essential gene sequence is selected from the group consisting of SEQ ID NOs:269-447.

Claim 55. The method of claim 53, wherein said transfer agent is an organosilicone compound.

Claim 56. The method of claim 53, wherein said double-stranded RNA polynucleotide is selected from the group consisting of SEQ NO:104-268 or fragment thereof.

Claim 57. A method of identifying double-stranded RNA polynucleotide useful in modulating Geminivirus gene expression when topically treating a plant comprising: a) providing a plurality of double-stranded RNA polynucleotides that comprise a region complementary to all or a part of an essential Geminivirus gene or RNA transcript thereof; b) topically treating said plant with one or more of said double-stranded RNA polynucleotides and a transfer agent; c) analyzing said plant or extract for modulation of symptoms of Geminivirus infection; and d) selecting a double-stranded RNA polynucleotide capable of modulating the symptoms or occurrence of Geminivirus infection.

Claim 58. The method of claim 57, wherein said transfer agent is an organosilicone compound.

Claim 59. An agricultural chemical composition comprising an admixture of a double-stranded RNA polynucleotide and a pesticide, wherein said double-stranded RNA polynucleotide is complementary to all or a portion of an essential Geminivirus gene sequence or RNA transcript thereof, wherein said composition is topically applied to a plant and wherein the symptoms of Geminivirus infection or development of symptoms are reduced or eliminated in said plant relative to a plant not treated with said composition when grown under the same conditions.

Claim 60. The agricultural chemical composition of claim 59, wherein said pesticide is selected from the group consisting of anti-viral compounds, insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants,

semiochemicals, repellents, attractants, pheromones, feeding stimulants, and biopesticides.

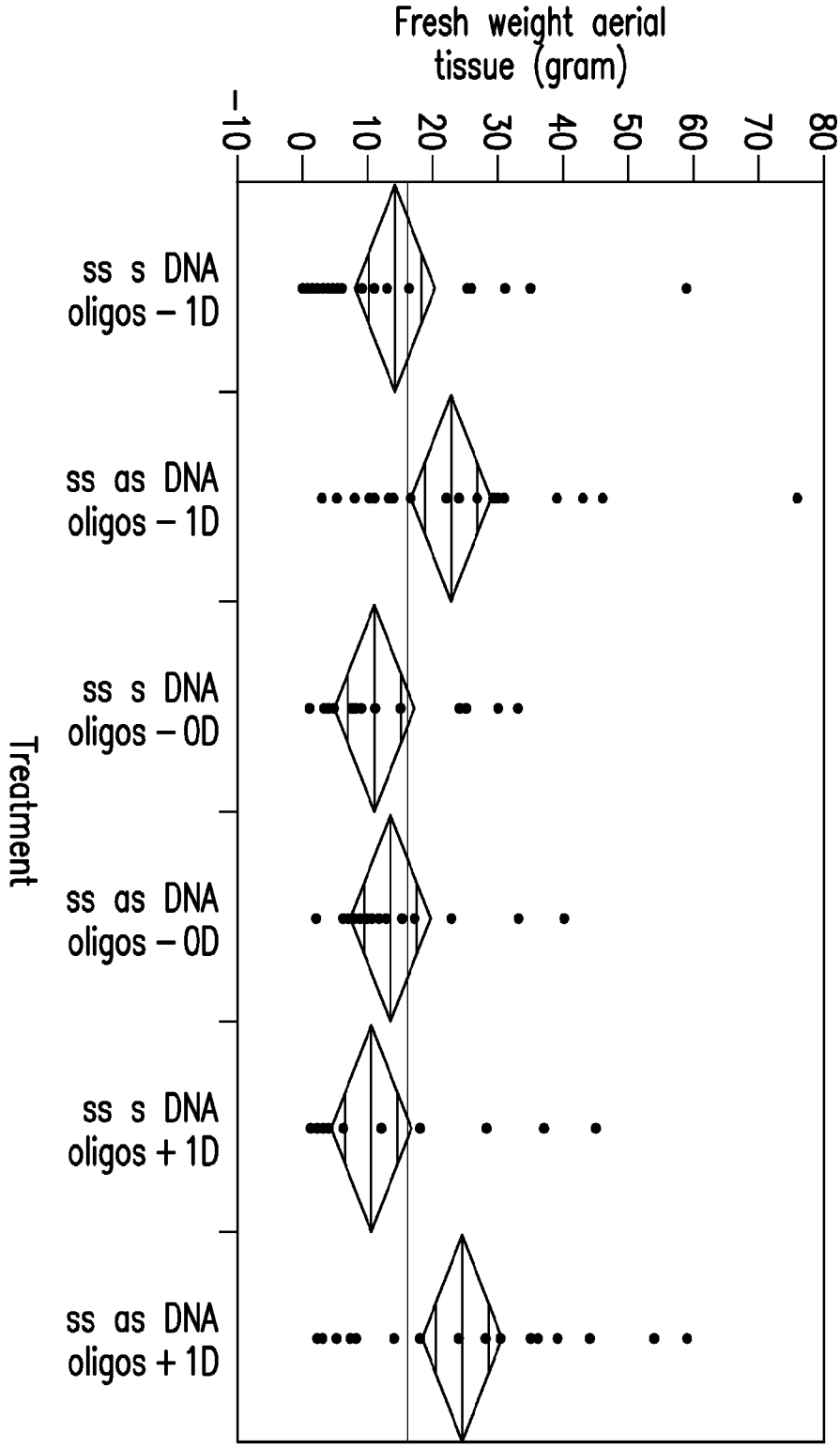
Fresh weight aerial tissue (gram)
-10
0
10
20
30
40
50
60
70
80
Treatment
ss s DNA oligos – 1D
ss as DNA oligos – 1D
ss s DNA oligos – 0D
ss as DNA oligos – 0D
ss s DNA oligos + 1D
ss as DNA oligos + 1D

FIG. 1

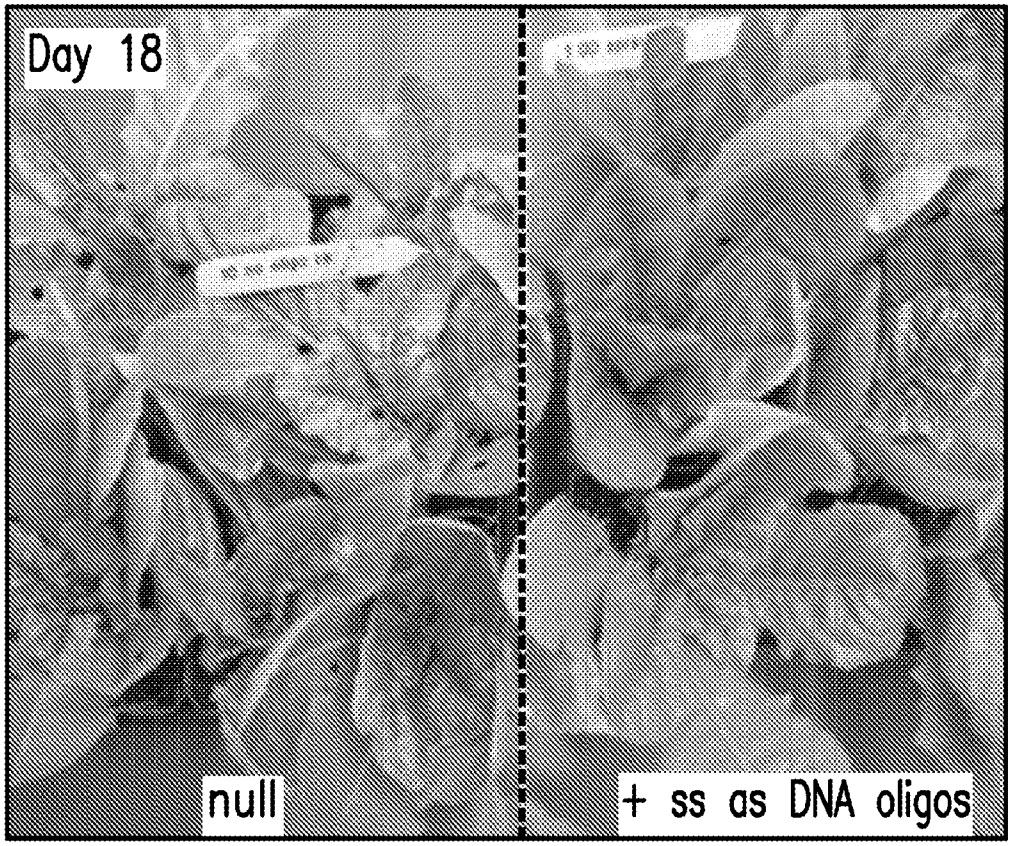
Day 18
null
+ ss as DNA oligos

FIG. 2A

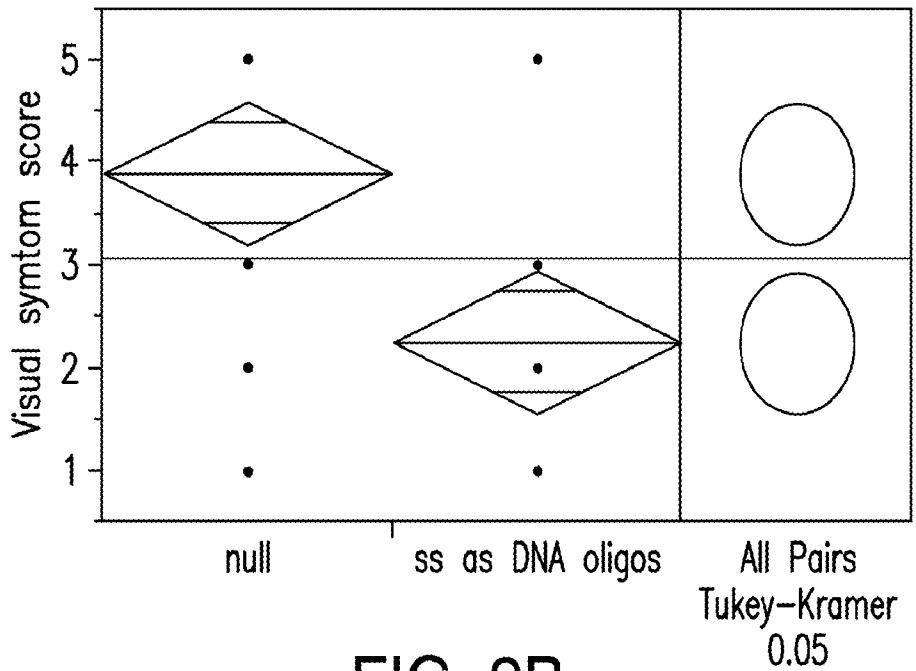
Visual symtom score
5
4
3
2
1
null
ss as DNA oligos
All Pairs
Tukey-Kramer
0.05

FIG. 2B

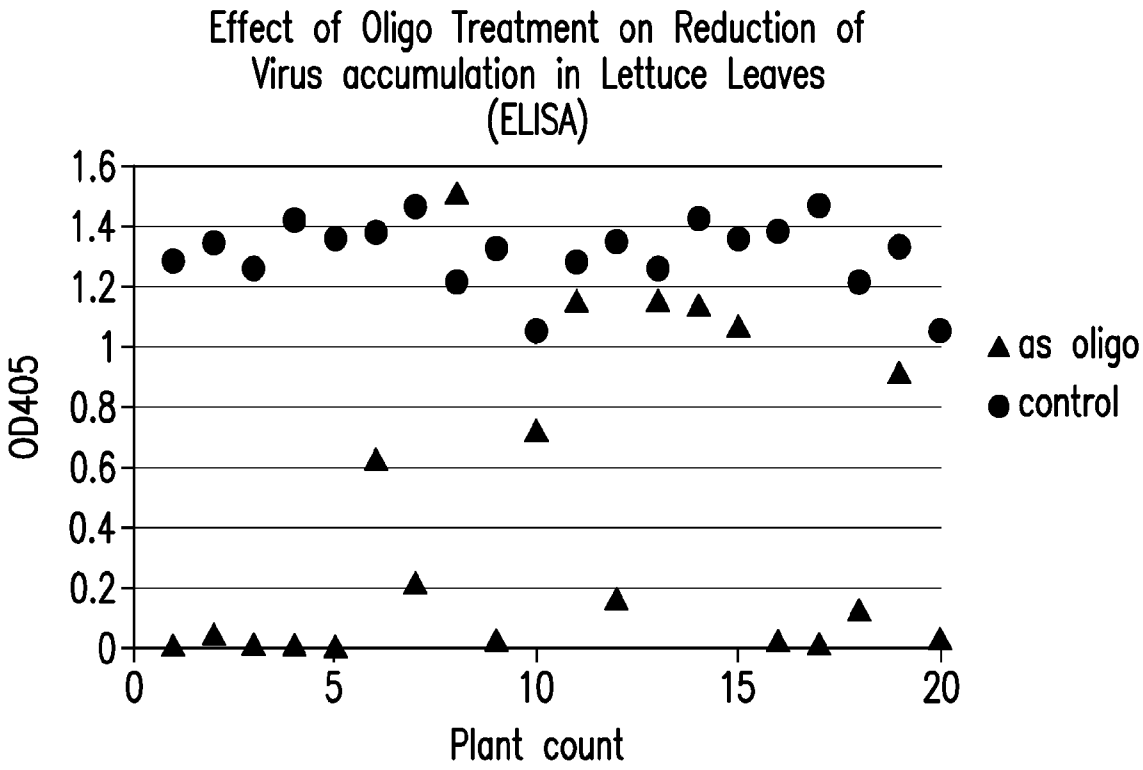
Effect of Oligo Treatment on Reduction of Virus accumulation in Lettuce Leaves (ELISA)
OD405
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
Plant count
as oligo
control

FIG. 3

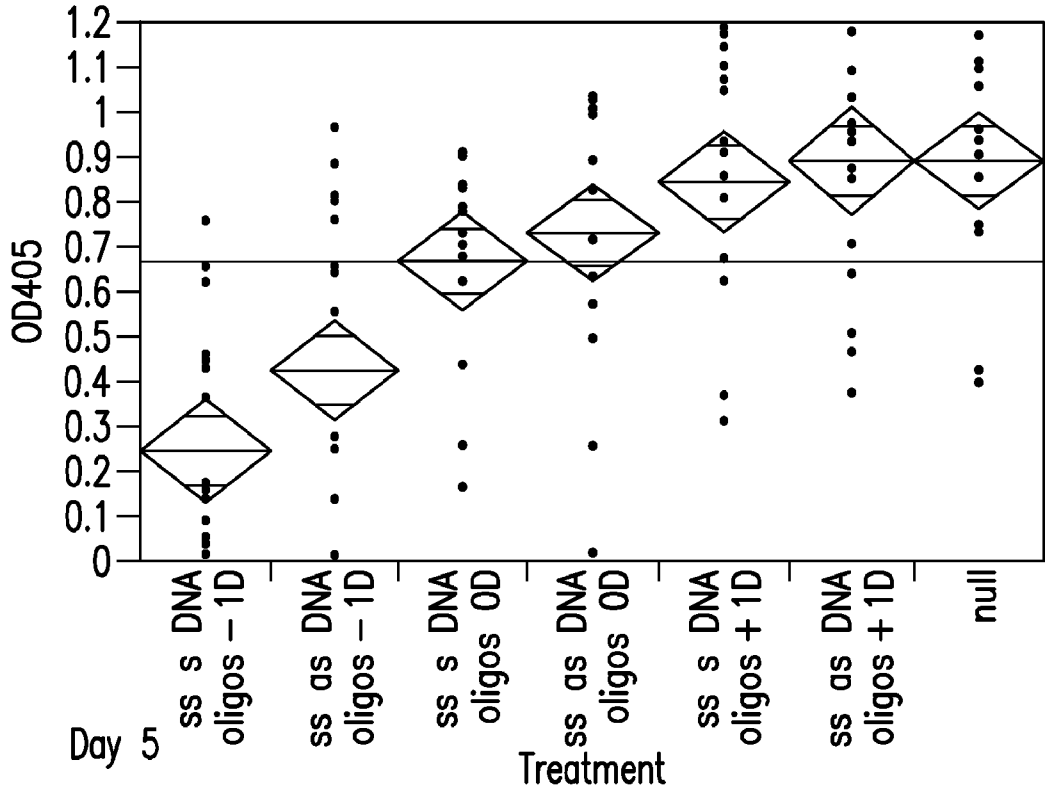
OD405
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Day 5
ss s DNA oligos −1D
ss as DNA oligos −1D
ss s DNA oligos 0D
ss as DNA oligos 0D
ss s DNA oligos +1D
ss as DNA oligos +1D
null
Treatment

FIG. 4A

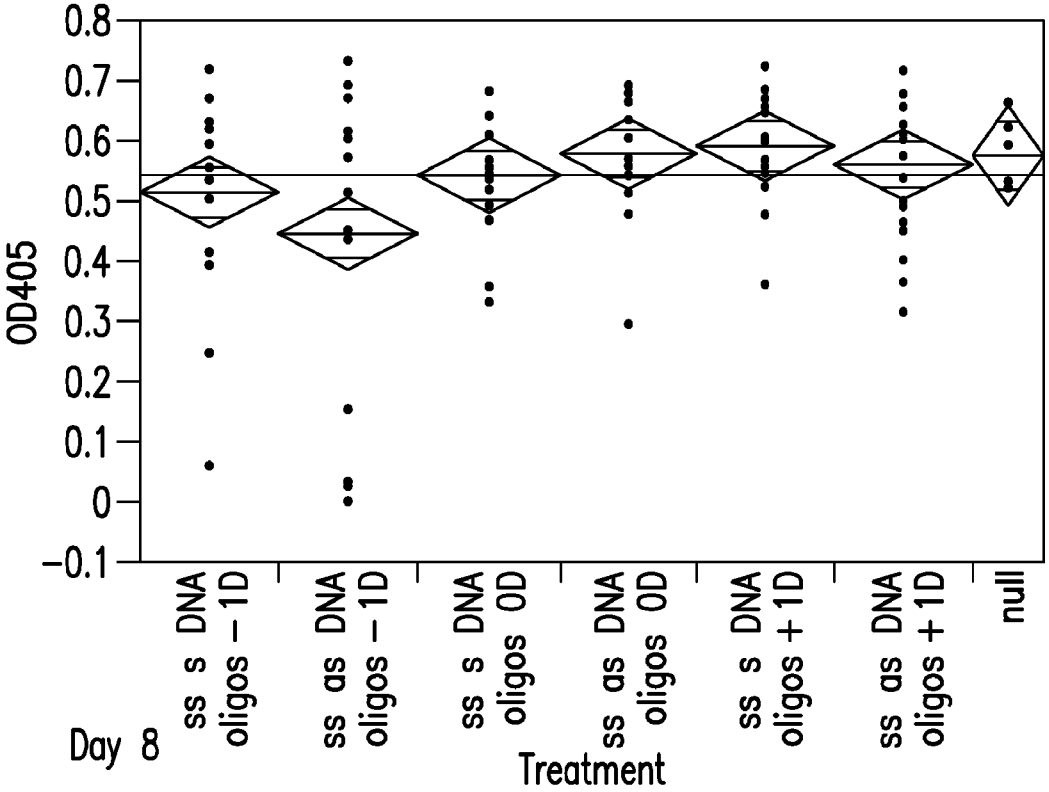
OD405
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
−0.1
Day 8
ss s DNA oligos −1D
ss as DNA oligos −1D
ss s DNA oligos 0D
ss as DNA oligos 0D
ss s DNA oligos +1D
ss as DNA oligos +1D
null
Treatment

FIG. 4B

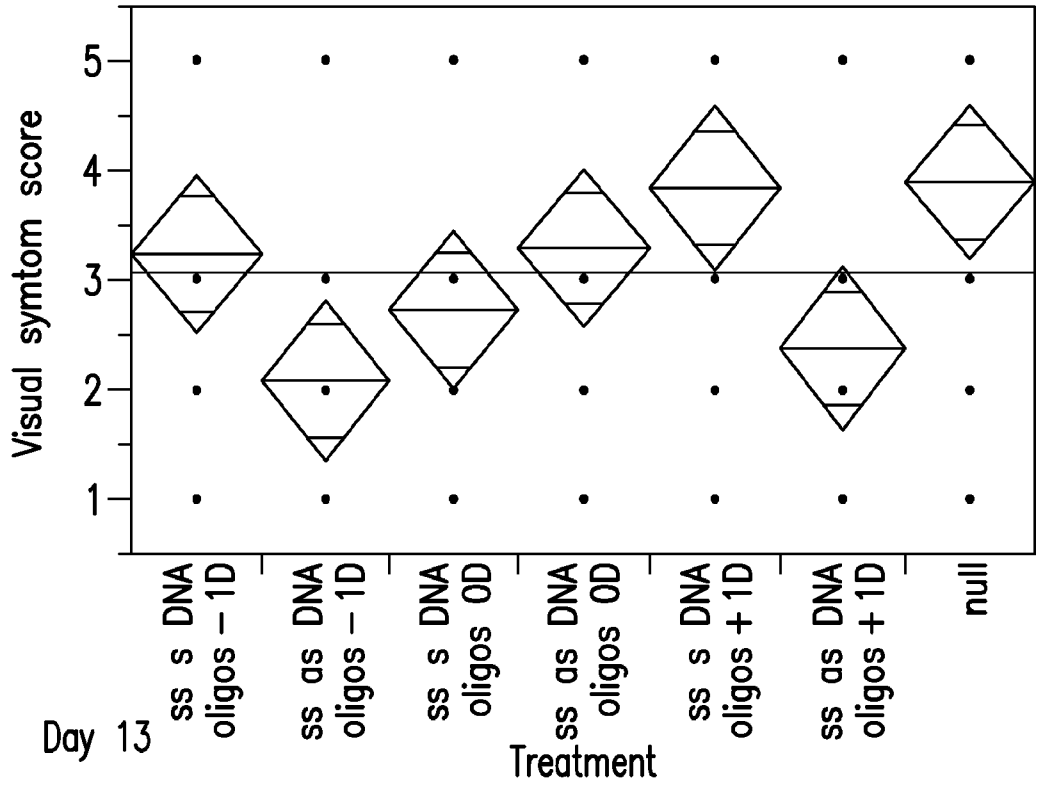
Visual symtom score
5
4
3
2
1
Day 13
ss s DNA oligos −1D
ss as DNA oligos −1D
ss s DNA oligos 0D
ss as DNA oligos 0D
ss s DNA oligos +1D
ss as DNA oligos +1D
null
Treatment

FIG. 4C

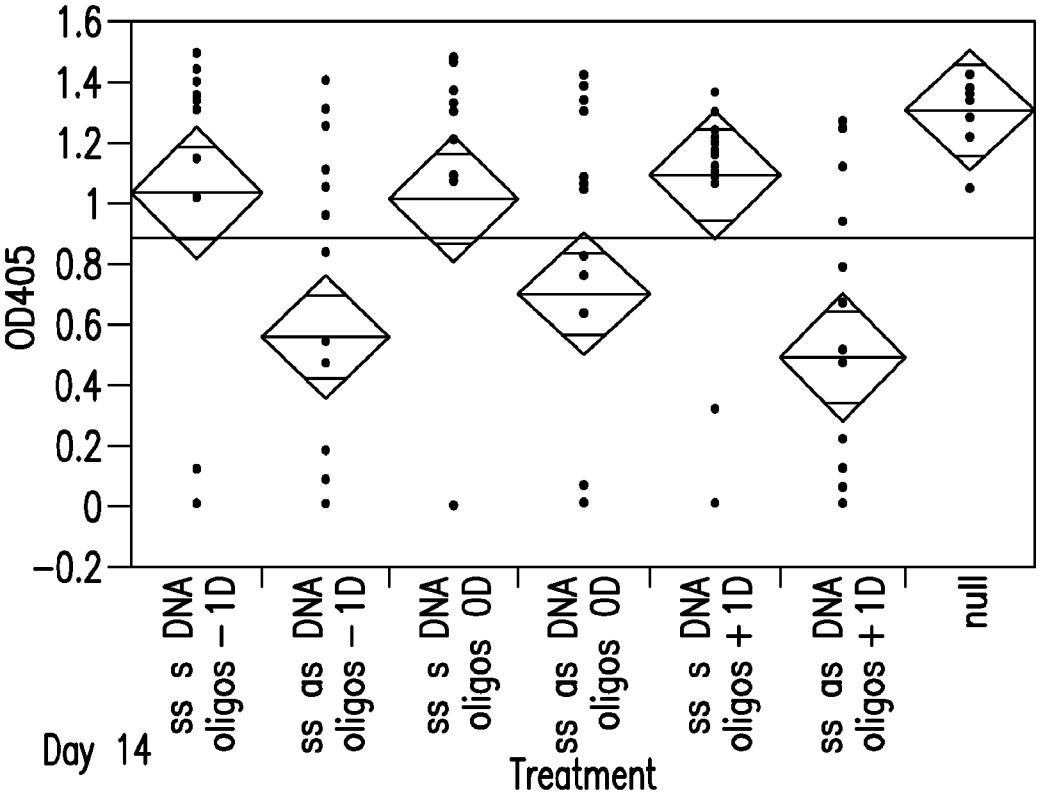
OD405
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
−0.2
Day 14
ss s DNA oligos −1D
ss as DNA oligos −1D
ss s DNA oligos 0D
ss as DNA oligos 0D
ss s DNA oligos +1D
ss as DNA oligos +1D
null
Treatment

FIG. 4D

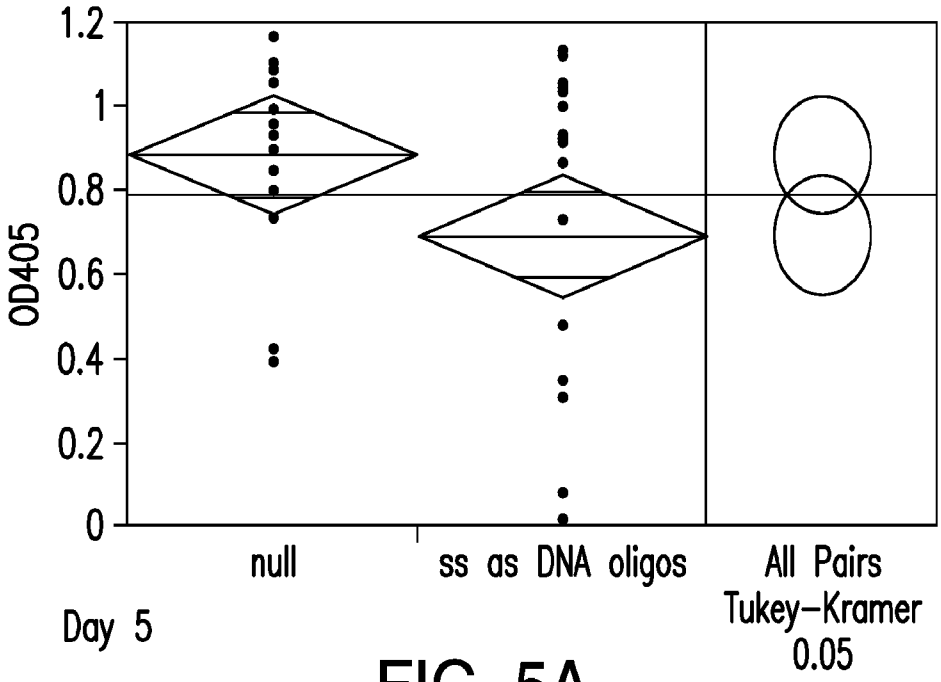
1.2
1
0.8
0.6
0.4
0.2
0
OD405
null
ss as DNA oligos
All Pairs
Tukey-Kramer
0.05
Day 5

FIG. 5A

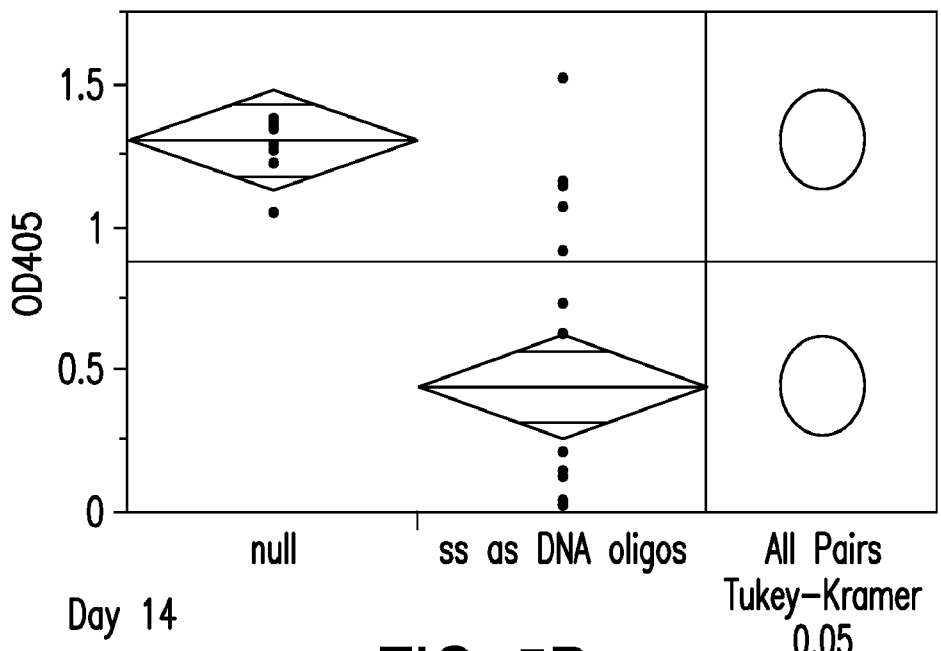
1.5
1
0.5
0
OD405
null
ss as DNA oligos
All Pairs
Tukey-Kramer
0.05
Day 14

FIG. 5B

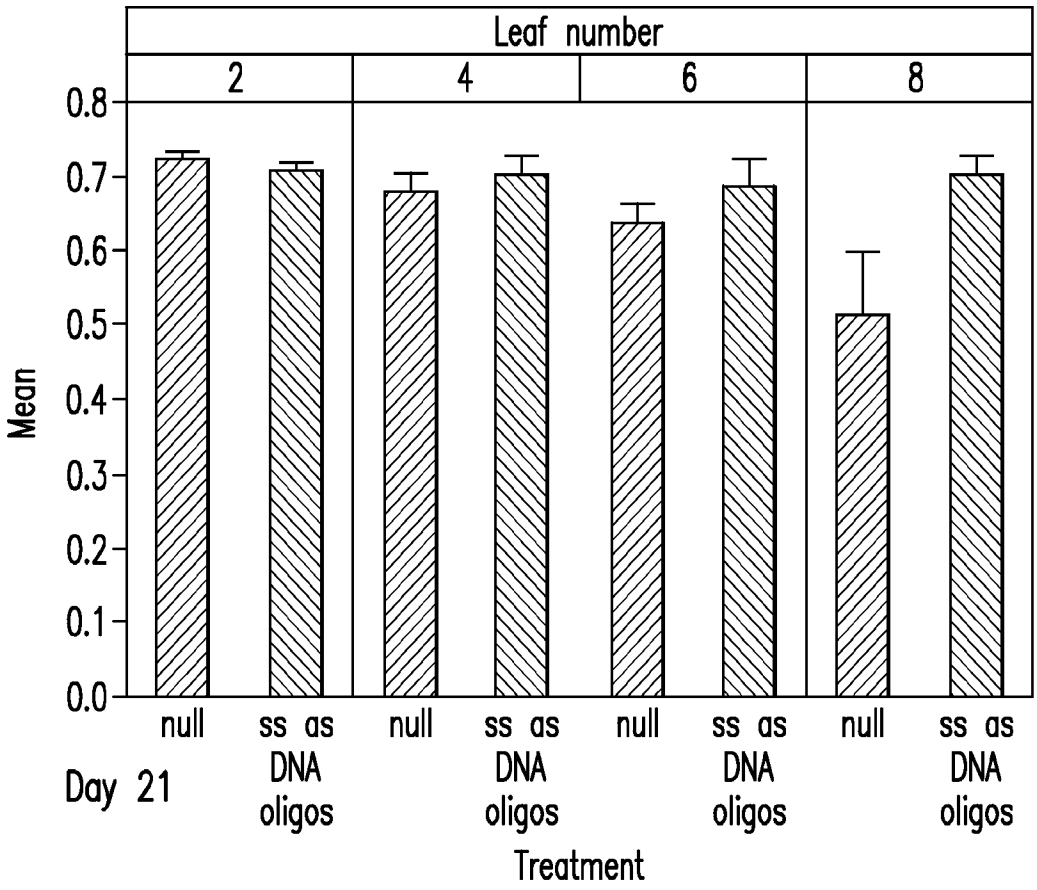
Leaf number
2
4
6
8
Mean
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
null
ss as DNA oligos
Day 21
Treatment

FIG. 5C

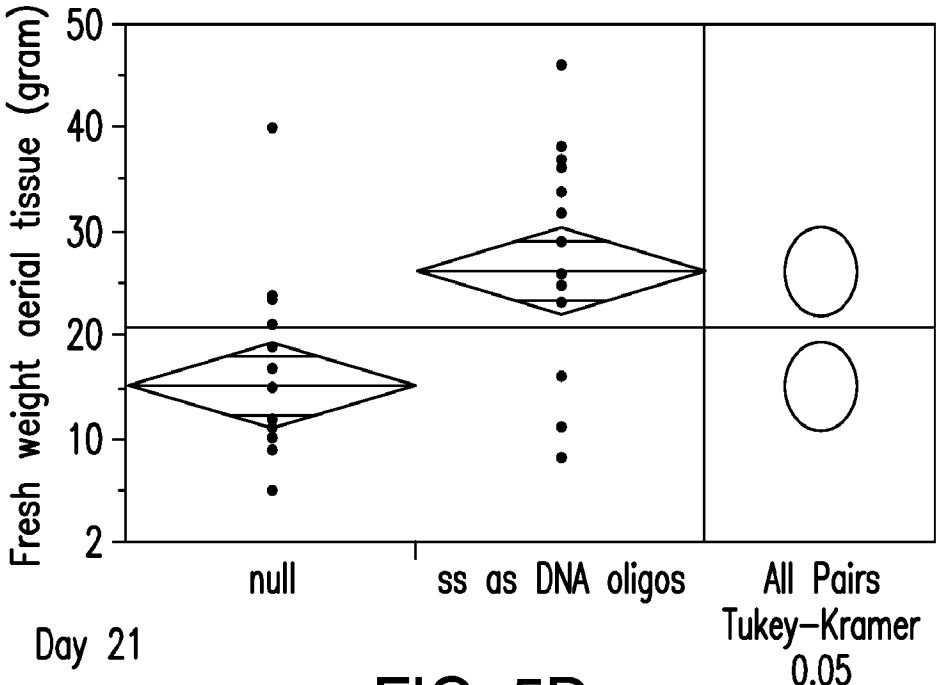
Fresh weight aerial tissue (gram)
2
10
20
30
40
50
null
ss as DNA oligos
All Pairs
Tukey–Kramer
0.05
Day 21

FIG. 5D

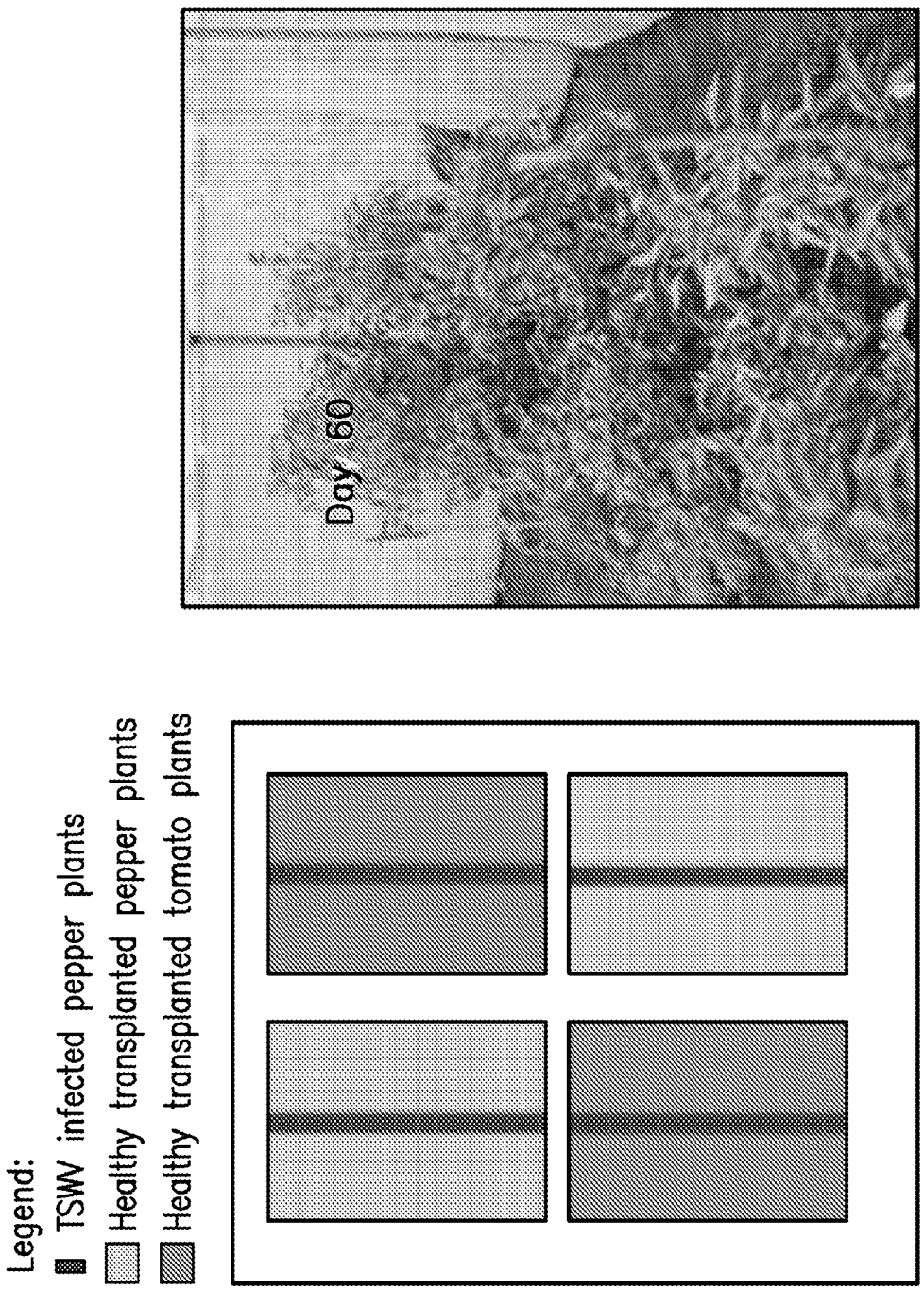
Day 60
Legend:
TSWV infected pepper plants
Healthy transplanted pepper plants
Healthy transplanted tomato plants

FIG. 6

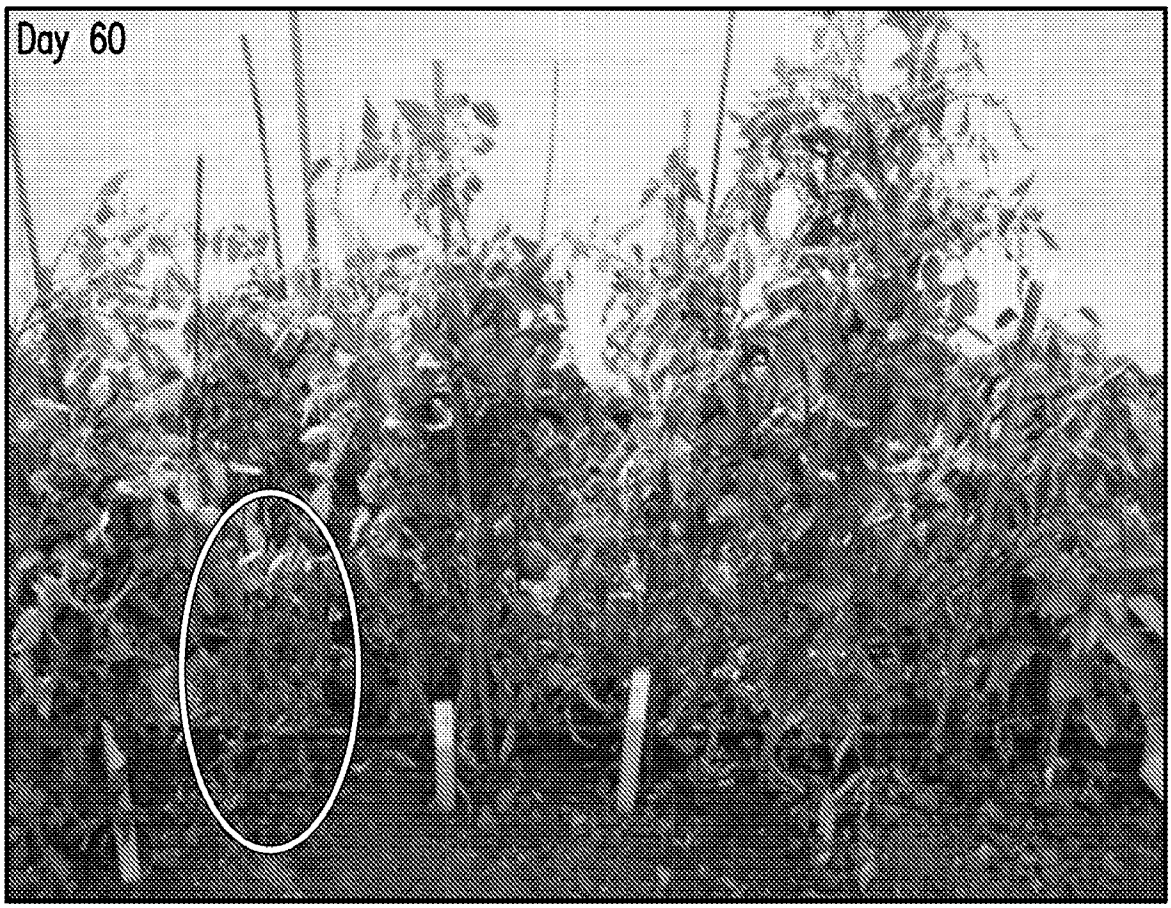

FIG. 7

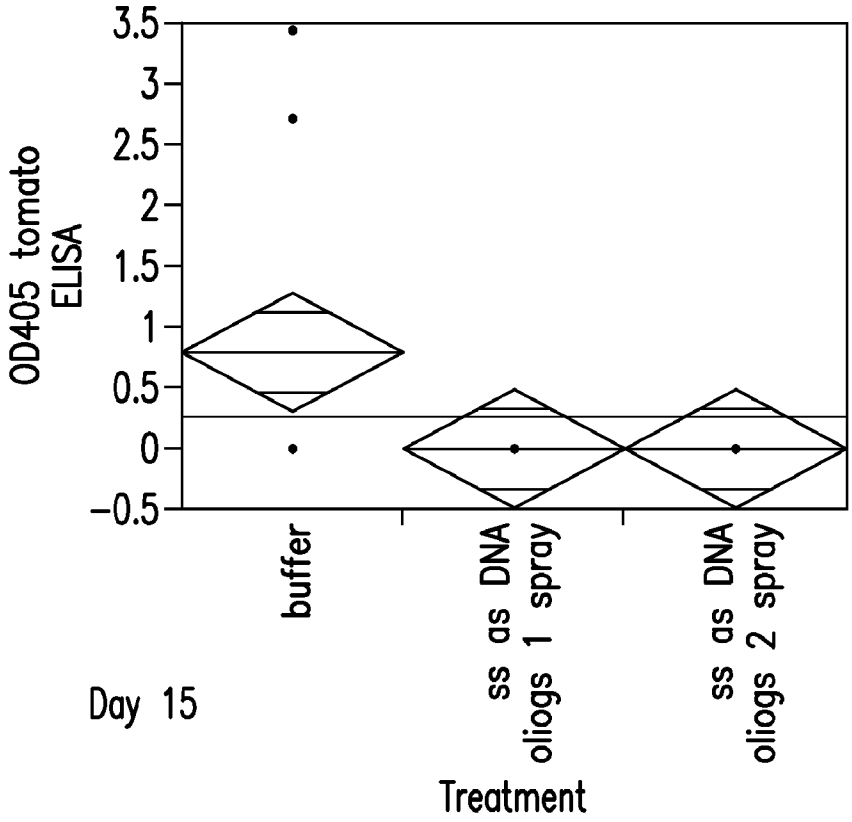
OD405 tomato ELISA
3.5
3
2.5
2
1.5
1
0.5
0
−0.5
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 15
Treatment

FIG. 8A

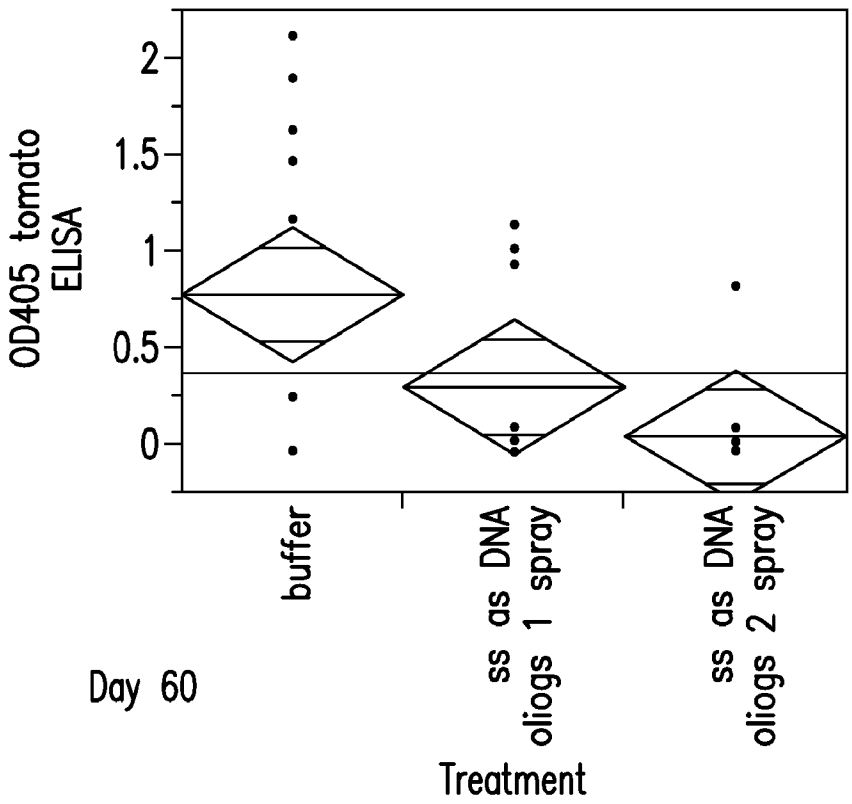
OD405 tomato ELISA
2
1.5
1
0.5
0
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 60
Treatment

FIG. 8B

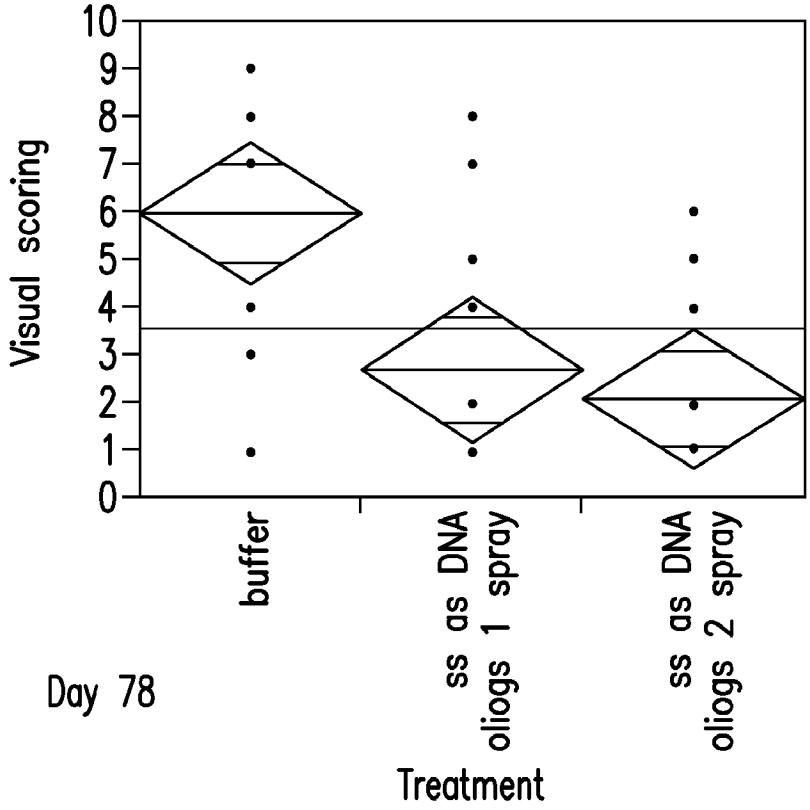
Visual scoring
10
9
8
7
6
5
4
3
2
1
0
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 78
Treatment

FIG. 8C

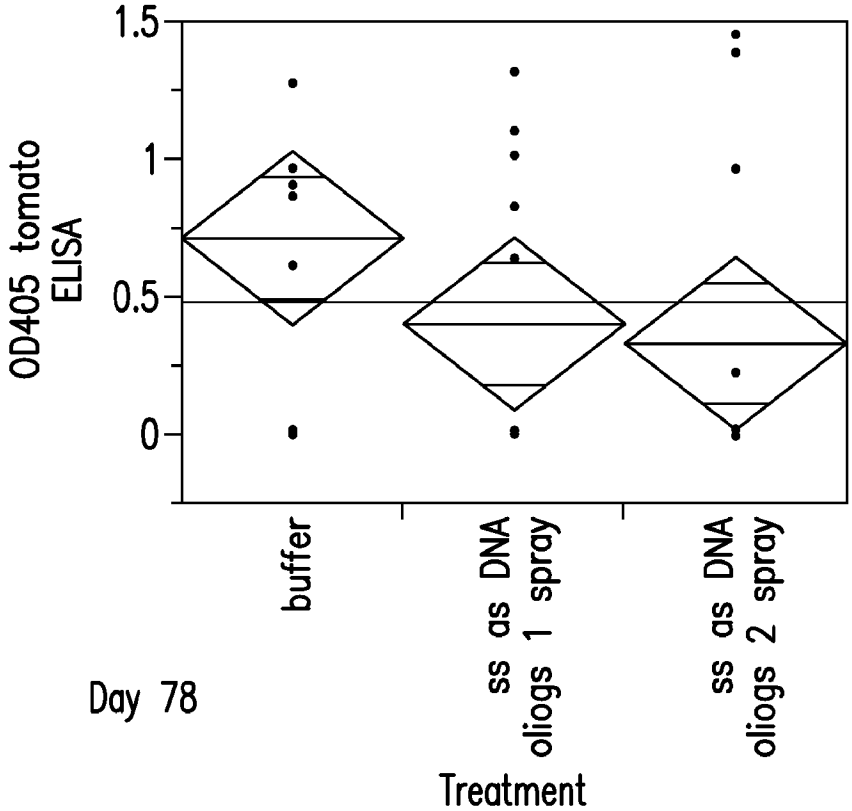
OD405 tomato ELISA
1.5
1
0.5
0
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 78
Treatment

FIG. 8D

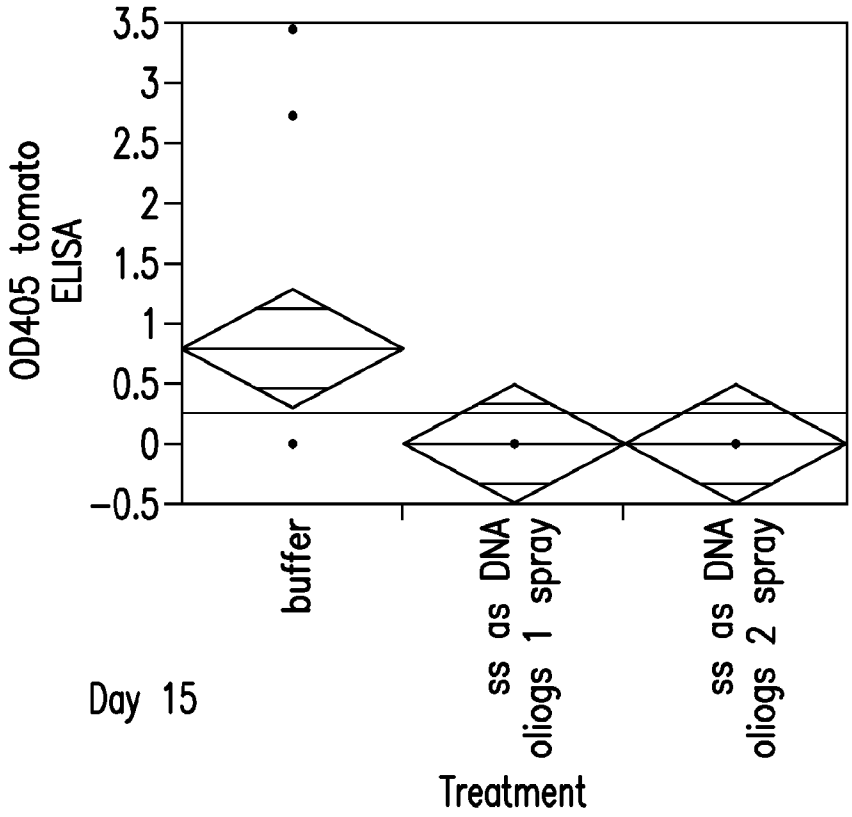
OD405 tomato ELISA
3.5
3
2.5
2
1.5
1
0.5
0
−0.5
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 15
Treatment

FIG. 9A

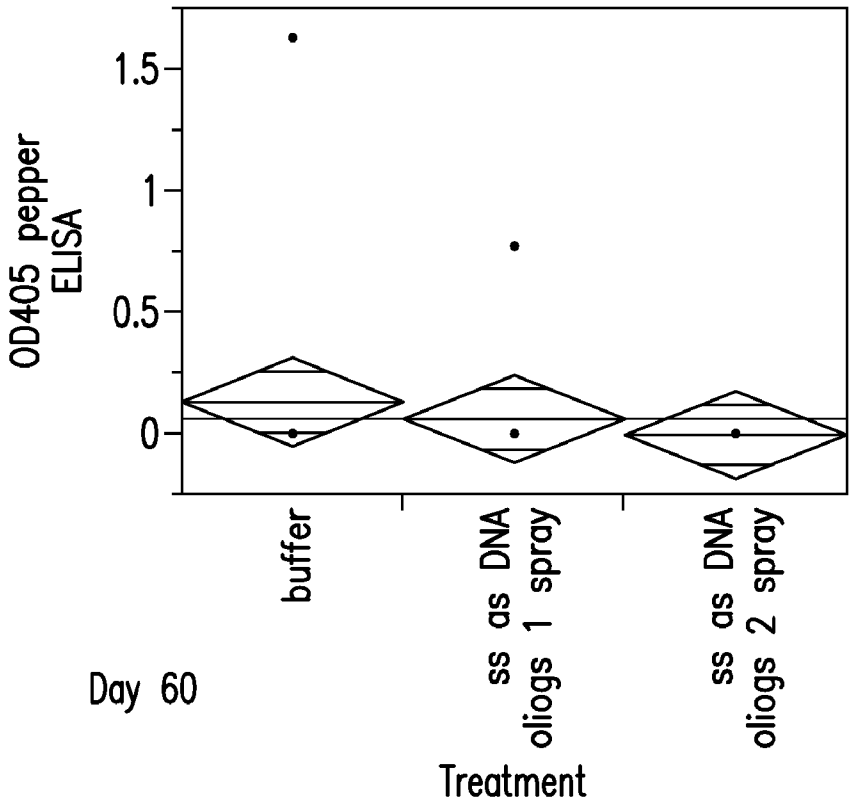
OD405 pepper ELISA
1.5
1
0.5
0
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 60
Treatment

FIG. 9B

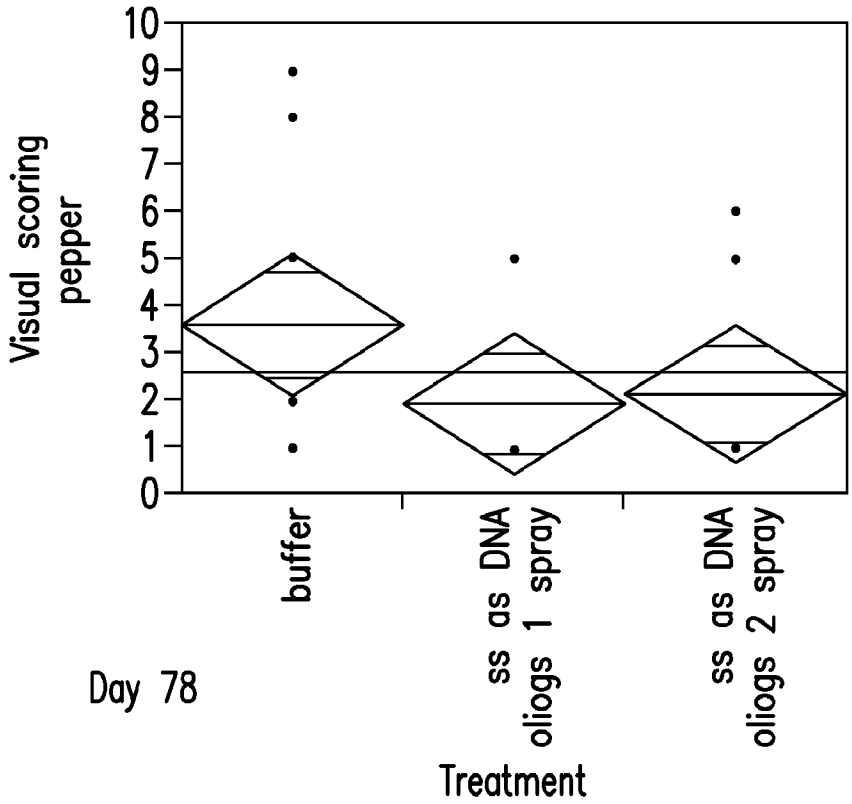
Visual scoring pepper
10
9
8
7
6
5
4
3
2
1
0
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 78
Treatment

FIG. 9C

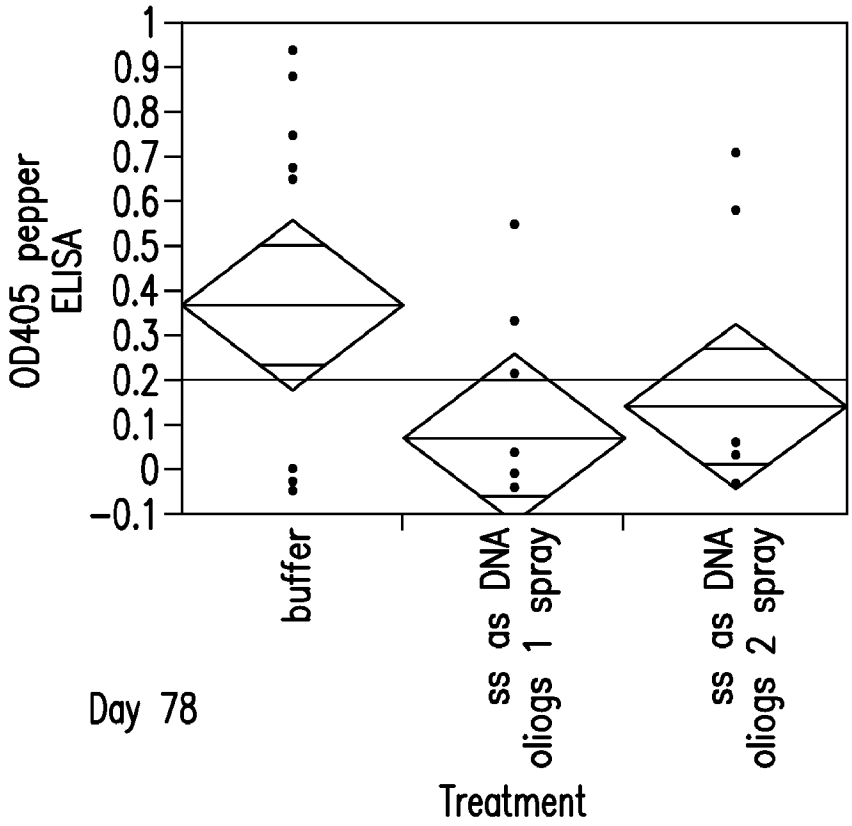
OD405 pepper ELISA
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
−0.1
buffer
ss as DNA oligos 1 spray
ss as DNA oligos 2 spray
Day 78
Treatment

FIG. 9D

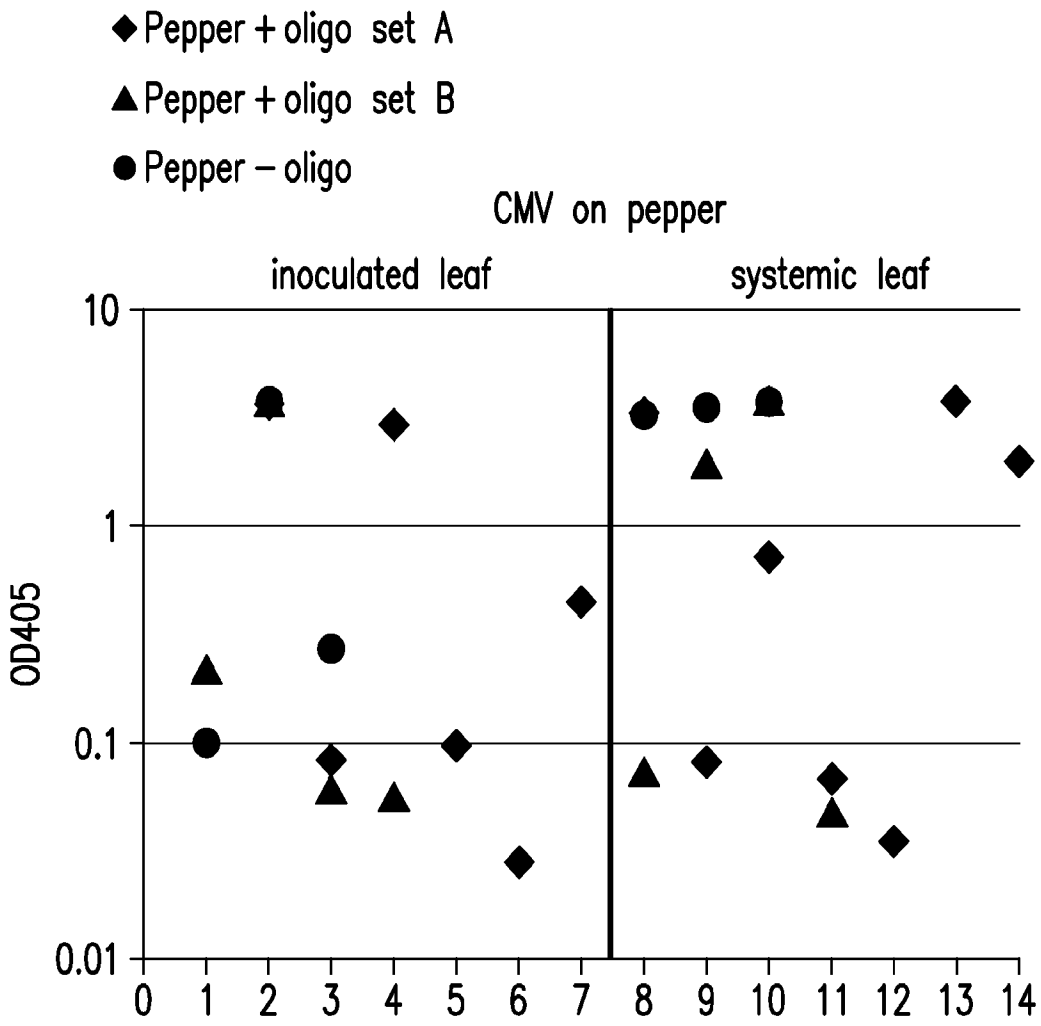
◆Pepper + oligo set A
▲Pepper + oligo set B
●Pepper – oligo
CMV on pepper
inoculated leaf
systemic leaf
OD405
10
1
0.1
0.01
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIG. 10

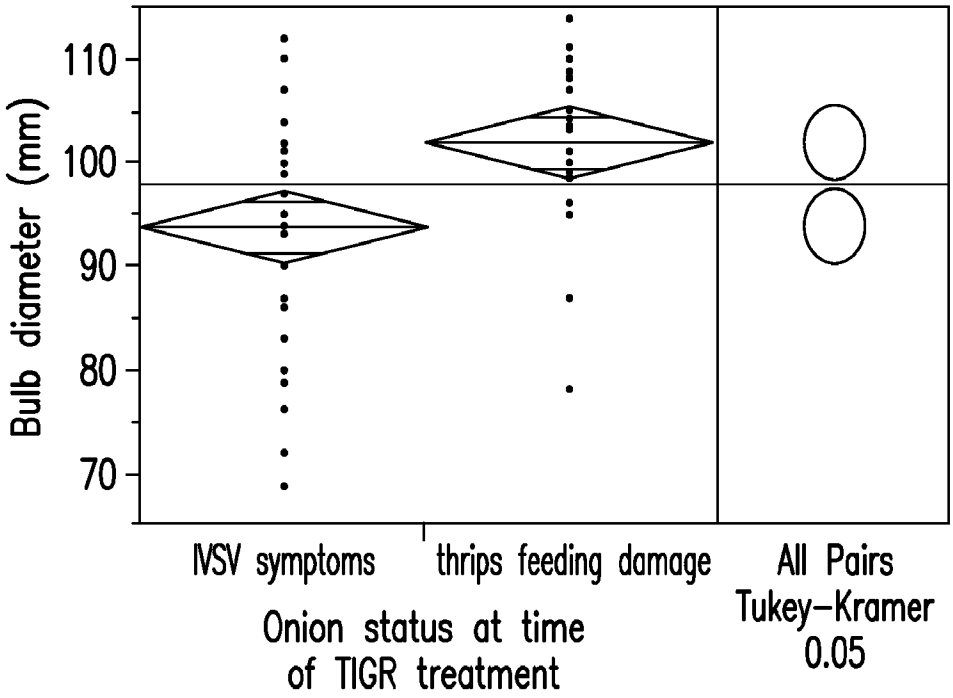

FIG. 11A

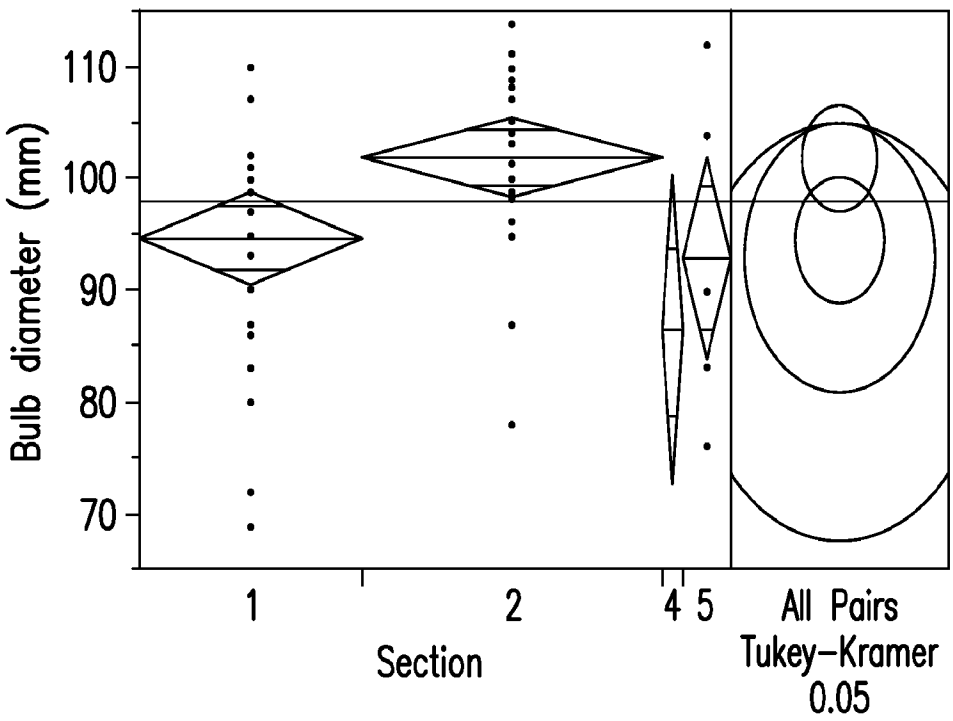

FIG. 11B

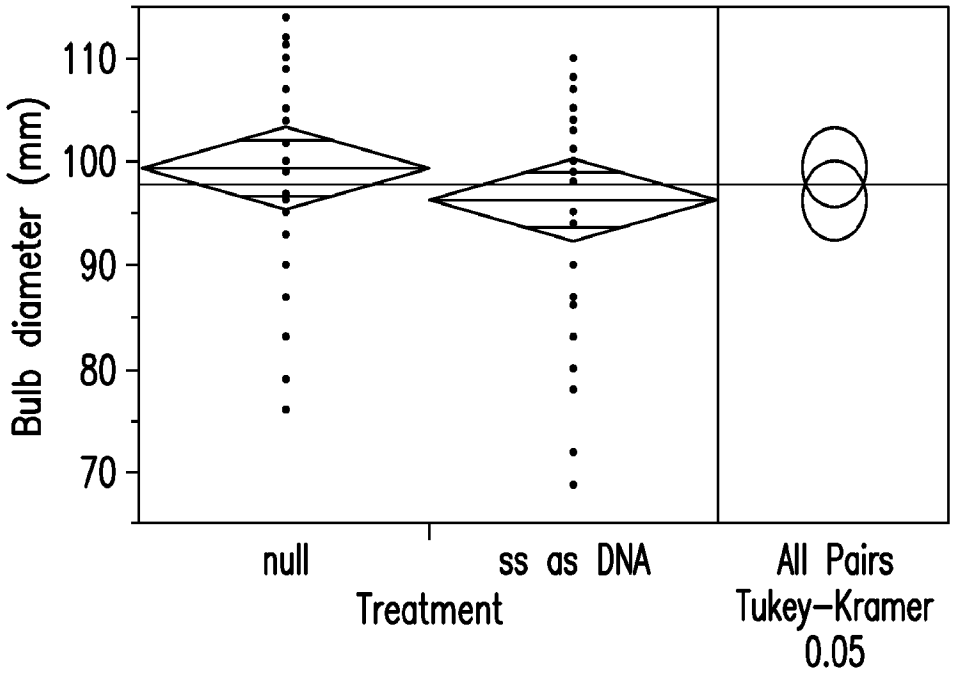

FIG. 11C

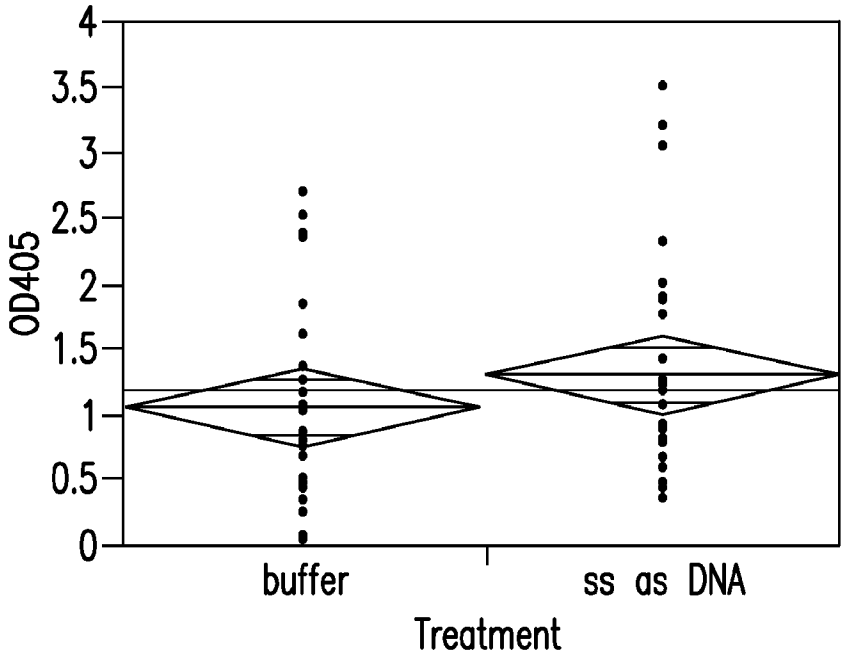

FIG. 11D

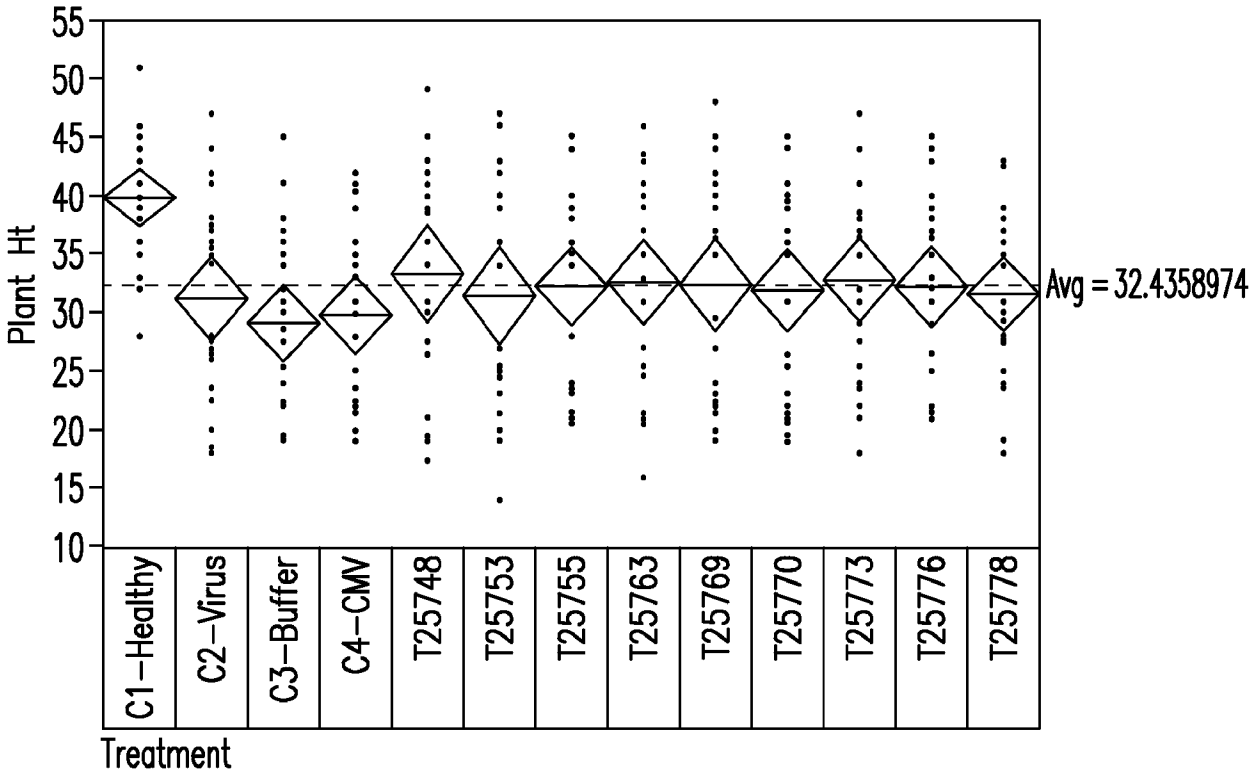

FIG. 12A

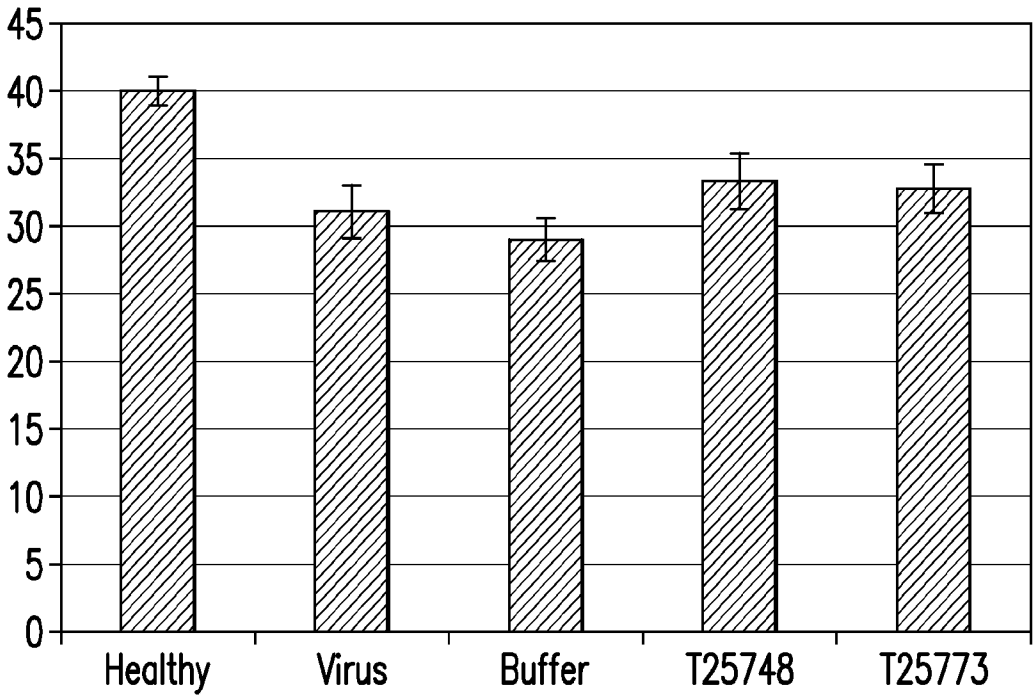

FIG. 12B

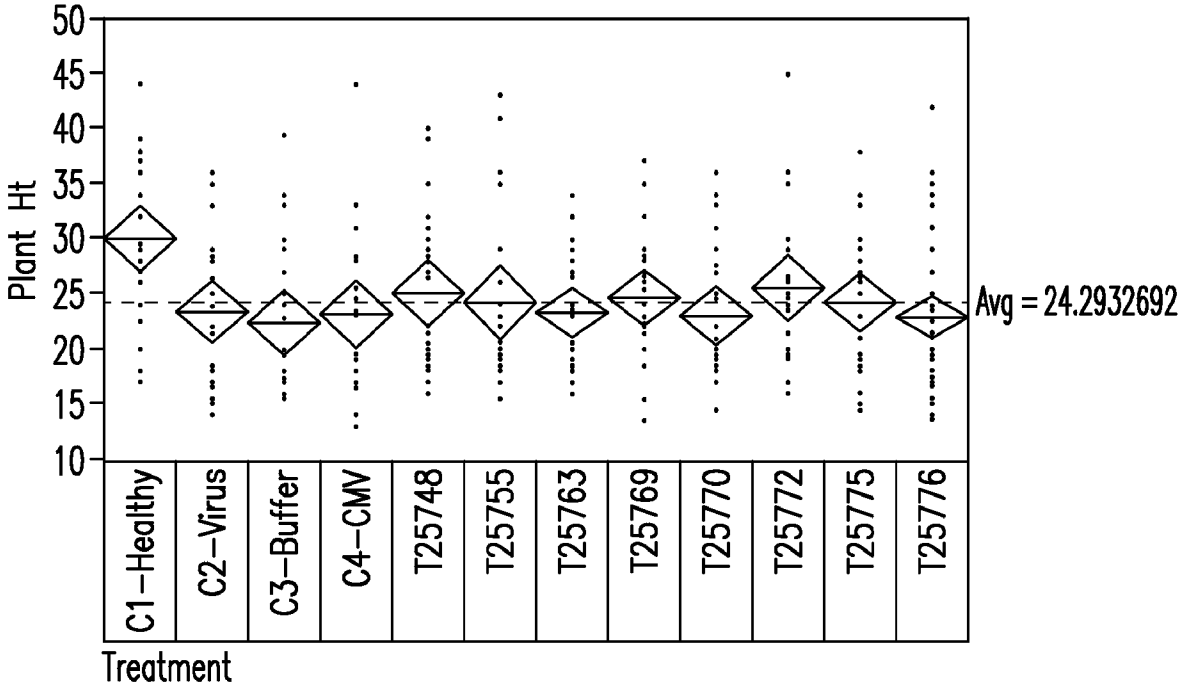
Plant Ht
50
45
40
35
30
25
20
15
10
Avg = 24.2932692
C1–Healthy
C2–Virus
C3–Buffer
C4–CMV
T25748
T25755
T25763
T25769
T25770
T25772
T25775
T25776
Treatment

FIG. 13