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(54) Title: SYNERGISTIC METHODS OF USING BENZOXABOROLE COMPOUNDS AND PRESERVATIVE GASES AS AN ANTIMICROBIAL FOR CROPS

(57) Abstract: The present disclosure relates to methods for using benzoxaborole compounds in combination with preservative gases, such as carbon dioxide or sulfur dioxide, as an antimicrobial that synergistically controls pathogens of agricultural crops.

SYNERGISTIC METHODS OF USING BENZOXABOROLE COMPOUNDS AND PRESERVATIVE GASES AS AN ANTIMICROBIAL FOR CROPS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 USC § 119(e) of U.S. Provisional Patent Application Serial No. 62/304,636, filed on March 7, 2016, the entire disclosure of which is incorporated herein by reference.

FIELD OF THE PRESENT APPLICATION

The present application relates to methods for using benzoxaborole compounds with preservative gases, such as carbon dioxide or sulfur dioxide, as an antimicrobial to control or inhibit pathogens of agricultural crops.

BACKGROUND

Benzoxaborole is a drug known to be effective in treating eukaryotic fungal and parasitic infections. For example, benzoxaborole is used to treat fungal conditions affecting the toenails and fingernails of humans, such as Onychomycosis. Benzoxaborole is also known to be an effective treatment of Human African Trypanosomiasis, commonly called African Sleeping Sickness, which is caused by *T. brucei* parasites that infect thousands of people annually in sub-Saharan Africa.

One mechanism by which benzoxaborole has been shown to exhibit antimicrobial effects is by blocking or inhibiting protein synthesis in fungi. Benzoxaborole has also been shown to block fungal cytoplasmic leucyl-tRNA synthetase (LeuRS) to exhibit antimicrobial effects. Additional mechanisms of action by which benzoxaborole acts as an antibacterial, an antifungal, or an antimicrobial are not yet understood.

Benzoxaborole has also been shown to have antimicrobial effects in plants. For example, a benzoxaborole compound was proven to be effective as a volatile plant fungicide. The present disclosure describes methods of using benzoxaborole compounds combined with preservative gases, such as carbon dioxide (CO_2) and sulfur dioxide (SO_2), to inhibit plant pathogens. More specifically, the present disclosure provides methods of using benzoxaboroles combined with CO_2 or SO_2 to provide synergistic antimicrobial protection to plants and plant parts that is advantageous to other previously described antimicrobial treatments of plants.

SUMMARY OF THE INVENTION

The present disclosure provides a method of treating plants with an antimicrobial agent. The method comprises placing one or more plants in a chamber, and sealing the chamber. The method also comprises adding a benzoxaborole compound to the sealed chamber. The method further comprises adding a preservative gas to the sealed chamber, wherein the benzoxaborole compound and the preservative gas combine in the chamber to form a treatment. Finally, the method provides for administering the treatment to the plants, and then, unsealing the chamber.

In the method described herein, the one or more plants may be a strawberry or a grape. In addition, the benzoxaborole compound of the present method may be Compound A, Compound B, and/or Compound C. Finally, in the present method of treating plants, the preservative gas may be CO_2. All embodiments, features, elements, or limitations of the methods described herein are combinable with other embodiments, features, elements, or limitations of the methods described herein.

DETAILED DESCRIPTION

The following numbered embodiments are contemplated and are non-limiting:

1. A method of treating plants with an antimicrobial agent comprising:
placing one or more plants or plant parts in a chamber,
sealing the chamber,
adding a benzoxaborole compound to the chamber,
adding a preservative gas to the chamber,
wherein the benzoxaborole compound and the preservative gas combine to form an antimicrobial treatment,
administering the antimicrobial treatment to the one or more plants or plant parts in the sealed chamber, and
unsealing the chamber.

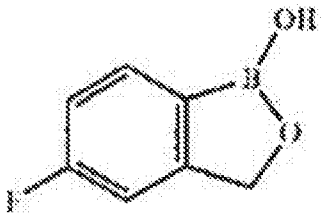
2. The method of clause 1 or clause 2, wherein the one or more plants or plant parts is a soft fruit.

3. The method of clause 1, wherein the preservative gas is CO_2 or SO_2.

4. The method of clause 2 or clause 3, wherein the soft fruit is selected from the group consisting of a strawberry, a raspberry, a blackberry, and a grape.

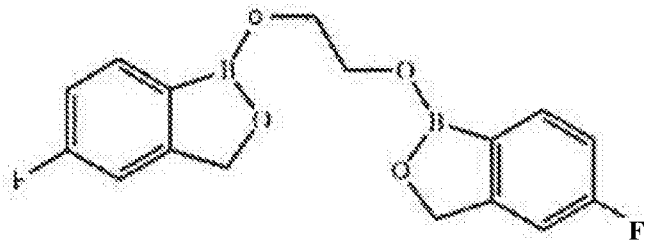
5. The method of any one of clauses 1 to 4, wherein the benzoxaborole compound is selected from the group consisting of Compound A, Compound B, Compound C, and combinations thereof.

6. The method of any one of clauses 1 to 5, wherein the benzoxaborole compound is Compound A having the structure:



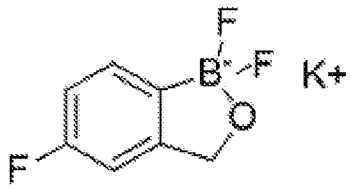
or an analog or a derivative thereof.

7. The method of any one of clauses 1 to 5, wherein the benzoxaborole compound is Compound B having the structure:



or an analog or a derivative thereof.

8. The method of any one of clauses 1 to 5, wherein the benzoxaborole compound is Compound C having the structure:



or an analog or a derivative thereof.

9. The method of any one of clauses 1 to 8, wherein the benzoxaborole compound is in the form of a liquid, a gas, a fog, or a solid.

10. The method of clause 9, wherein the solid benzoxaborole compound is a powder.

11. The method of any one of clauses 1 to 10, wherein the preservative gas is CO_2.

12. The method of any one of clauses 1 to 10, wherein the preservative gas is SO_2.

13. The method of any one of clauses 1 to 11, wherein the CO_2 concentration of the antimicrobial treatment ranges from about 4% to about 20%.

14. The method of any one of clauses 1 to 10 and 12, wherein the SO_2 concentration of the antimicrobial treatment ranges from about 0.001% to about 1%.

15. The method of any one of clauses 1 to 11 and 13, wherein the CO_2 concentration of the antimicrobial treatment is about 12%.

16. The method of any one of clauses 1 to 15, wherein the antimicrobial treatment is in the form of a spray, a mist, a gel, a thermal fog, a non-thermal fog, a dip, a drench, a vapor, a gas, or sublimation.

17. The method of any one of clauses 1 to 16, wherein administering the antimicrobial treatment comprises release of the antimicrobial treatment from a material selected from the group consisting of a sachet, a synthetic film, a natural film, a liner, a gas-releasing generator, a compressed gas cylinder, a non-compressed gas cylinder, a cylinder comprising dissolved Supercritical CO_2, and a droplet inside a box.

18. The method of any one of clauses 1 to 17, wherein the antimicrobial treatment further comprises a component selected from the group consisting of 1-methylcylopropene, adjuvants, and pesticides.

19. The method of any one of clauses 1 to 18, wherein the antimicrobial treatment further comprises a treatment carrier.

20. The method of clause 19, wherein the treatment carrier comprises a liquid, a gas, a solution, a solvent, and a chemical.

21. The method of clause 19 or clause 20, wherein the treatment carrier is Supercritical CO_2.

22. The method of clause 19 or clause 20, wherein the treatment carrier is selected from the group consisting of water, saline, a buffer, a solution, and a solvent.

23. The method of any one of clauses 1 to 22, wherein the antimicrobial treatment is effective against plant pathogens.

24. The method of clause 23, wherein the plant pathogens are fungal pathogens.

25. The method of clause 23 or clause 24, wherein the plant pathogens are selected from the group consisting of *Acremonium* spp., *Albugo* spp., *Alternaria* spp., *Ascochyta* spp., *Aspergillus* spp., *Botryodiplodia* spp., *Botryospheria* spp., *Botrytis* spp., *Byssochlamys* spp., *Candida* spp., *Cephalosporium* spp., *Ceratocystis* spp., *Cercospora* spp., *Chalara* spp., *Cladosporium* spp., *Colletotrichum* spp., *Cryptosporiopsis* spp., *Cylindrocarpon* spp., *Debaryomyces* spp., *Diaporthe* spp., *Didymella* spp., *Diplodia* spp., *Dothiorella* spp., *Elsinoe* spp., *Fusarium* spp., *Geotrichum* spp., *Gloeosporium* spp., *Glomerella* spp., *Helminthosporium* spp., *Khuskia* spp., *Lasiodiplodia* spp., *Macrophoma* spp., *Macrophomina* spp., *Microdochium* spp., *Monilinia* spp., *Monilochaethes* spp., *Mucor* spp., *Mycocentrospora* spp., *Mycosphaerella*

spp., *Nectria* spp., *Neofabraea* spp., *Nigrospora* spp., *Penicillium* spp., *Peronophythora* spp., *Peronospora* spp., *Pestalotiopsis* spp., *Pezicula* spp., *Phacidiopycnis* spp., *Phoma* spp., *Phomopsis* spp., *Phyllosticta* spp., *Phytophthora* spp., *Polyscytalum* spp., *Pseudocercospora* spp., *Pyricularia* spp., *Pythium* spp., *Rhizoctonia* spp., *Rhizopus* spp., *Sclerotium* spp., *Sclerotinia* spp., *Septoria* spp., *Sphaceloma* spp., *Sphaeropsis* spp., *Stemphyllium* spp., *Stilbella* spp., *Thielaviopsis* spp., *Thyronectria* spp., *Trachysphaera* spp., *Uromyces* spp., *Ustilago* spp., *Venturia* spp., and *Verticillium* spp., and bacterial pathogens, such as *Bacillus* spp., *Campylobacter* spp., *Clavibacter* spp., *Clostridium* spp., *Erwinia* spp., *Escherichia* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Listeria* spp., *Pantoea* spp., *Pectobacterium* spp., *Pseudomonas* spp., *Ralstonia* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Vibrio* spp., *Xanthomonas* spp., and *Yersinia* spp.

26. The method of any one of clauses 23 to 25, wherein the plant pathogens are selected from the group consisting of *Botrytis cinerea*, *Mucor piriformis, Fusarium sambucinum, Aspergillus brasiliensis,* and *Peniciliium expansum.*

27. The method of any one of clauses 1 to 26, wherein the chamber is sealed.

28. The method of any one of clauses 1 to 27, wherein the temperature of the chamber ranges from 1°C to 25°C.

29. The method of any one of clauses 1 to 28, wherein the chamber is air-tight.

30. The method of any one of clauses 1 to 29, wherein the chamber is semipermeable or impermeable.

31. The method of any one of clauses 1 to 30, wherein the chamber is made of a material selected from the group consisting of plastic, glass, cellulosic material, and cement.

32. The method of any one of clauses 1 to 31, wherein the chamber comprises a port, an outlet, or both.

33. The method of any one of clauses 1 to 32, wherein the chamber may have a volume ranging from about 10 L to about 50 L.

34. The method of any one of clauses 1 to 32, wherein the chamber may have a volume ranging from about 0.5 cm^3 to about 150 cm^3.

35. The method of any one of clauses 1 to 32, wherein the chamber may have a volume ranging from about 100 cm^3 to about 10,000 cm^3.

36. The method of any one of clauses 1 to 35, wherein the one or more plants or plant parts are manually or robotically placed in the chamber.

37. The method of any one of clauses 1 to 36, wherein the one or more plants or plant parts are treated for an initial time period ranging from about 12 hours to about 5 days.

38. The method of any one of clauses 1 to 37, wherein the antimicrobial treatment concentration ranges from about 0.0001 mg/L to about 0.5 mg/L.

39. The method of any one of clauses 1 to 38, wherein the time to administer the antimicrobial treatment ranges from about 3 seconds to about 2 hours.

40. The method of any one of clauses 1 to 39, wherein the method results in synergistic inhibition of plant pathogens on the one or more treated plants or plant parts.

The term "plant(s)" and "plant parts" include, but are not limited to, plant cells and plant tissues, such as leaves, calli, stems, roots, fruits, vegetables, flowers or flower parts, pollen, egg cells, zygotes, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant. A class of plants that may be used in the present invention is generally as broad as the class of higher and lower plants including, but not limited to, dicotyledonous plants, monocotyledonous plants, and all horticultural crops.

Horticultural crops, include, but are not limited to, vegetable crops, fruit crops, edible nuts, flowers and ornamental crops, nursery crops, aromatic crops, and medicinal crops. More specifically, horticultural crops of the present disclosure include, but are not limited to, fruits (e.g., grape, apple, pear, and persimmon) and berries (e.g., strawberries, blackberries, blueberries, and raspberries).

The phrase "preservative gases" refers to chemicals in their gaseous form that acts as a preservative of the plants and plant parts of the present invention. For example, the preservative gases of the present application help maintain the appearance, freshness, flavor, and prevents rotting of the plants and plant parts of the present invention. Exemplary preservative gases of the present invention include, but are not limited to carbon dioxide (CO_2) and sulfur dioxide (SO_2).

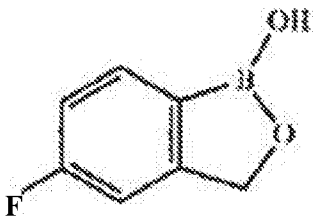
The phrase "Supercritical CO_2" refers to a solvent wherein the fluid state of carbon dioxide (CO_2) is held at or above its critical temperature and critical pressure. Typically, CO_2 at or above its critical temperature and critical pressure can adopt properties that are between a gas and a liquid. More specifically, the CO_2 may behave as a supercritical fluid at or above its critical temperature and critical pressure, such that the CO_2 can fill a container like a gas, but has a density like a liquid.

COMPOUNDS AND COMPONENTS OF THE PRESENT METHODS

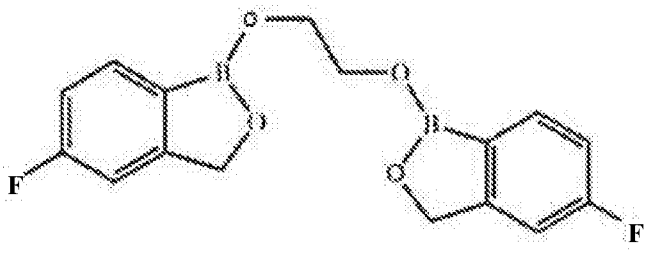
The methods of the present disclosure are directed to using benzoxaborole compounds in combination with preservative gases or chemicals as an antimicrobial to treat plants or plant pants. The methods of the present disclosure to treat plant or plant parts comprise, consist essentially of, or consist of benzoxaborole compounds.

Exemplary embodiments of the compounds of the present disclosure comprise Compounds A, B, and C, which may encompass diastereomers and enantiomers of the illustrative compounds. Enantiomers are defined as one of a pair of molecular entities which are mirror images of each other and non-superimposable. Diastereomers or diastereoisomers are defined as stereoisomers other than enantiomers. Diastereomers or diastereoisomers are stereoisomers not related as mirror images. Diastereoisomers are characterized by differences in physical properties.

One exemplary embodiment of a benzoxaborole compound of the present method is Compound A:

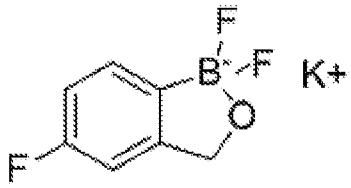


or an analog or derivative thereof. An additional illustrative embodiment of a benzoxaborole compound of the present method is Compound B:



or an analog or derivative thereof.

Another exemplary embodiment of a benzoxaborole compound of the present method is Compound C, which is a salt version of Compounds A and/or B:



or an analog or derivative thereof.

Compounds A, B, and/or C may be used individually or as a mixture or combination. The benzoxaborole compounds may also be used in combination with preservative gases or

chemicals to form a benzoxaborole treatment. The benzoxaborole treatment provides antimicrobial protection to plants or plant parts when administered, applied, or exposed to plants or plant parts.

Benzoxaborole Compounds A, B, and/or C may be used in any form, including, but not limited to, a liquid, a solid (e.g., a powder), or a gaseous composition. In particular, the present method provides application of a benzoxaborole compound as, for example, a spray, a mist, a gel, a thermal and non-thermal fog, a dip or a drench, or via sublimation, a vapor, or a gas. Additional examples of benzoxaborole treatment administration include, but are not limited to, release from a sachet, a synthetic or natural film, a liner or other packaging materials, a gas-releasing generator, compressed or non-compressed gas cylinder, dissolved in Supercritical CO_2 within a cylinder, a droplet inside a box, or other similar methods as described in U.S. Patent Nos. 8,669,207, 9,138,001, and 9,138,001, and U.S. Patent Publication No. 2014/0349853, which are incorporated herein by reference.

The active compounds may be applied to plants or plant parts in a volume of a chamber. A chamber of the present disclosure may be any sealable container from which a gas or chemical cannot easily escape once it has been introduced to the sealed chamber. For example, a chamber may be made of plastic, glass, or any other semipermeable or impermeable material.

The chamber may have a port (e.g., a bulkhead septum port) for the introduction of the chemical treatment, either as a gas or liquid (e.g., water or solvent-based solution containing the product) or a fog. The chamber may also have an outlet to vent or remove the unused portion of the treatment carrier.

Carriers of the present disclosure may be combined with active benzoxaborole compounds to form a benzoxaborole treatment. Treatment carriers of the present disclosure may comprise gases, solutions, solvents, or chemicals. For example, a liquid carrier of the present disclosure may comprise water, buffer, saline solution, a solvent, etc. Illustrative gas carriers for the present invention are Supercritical CO_2 and/or CO_2 contained in a steel cylinder.

A chamber of the present disclosure may be any container or material from which a gas or chemical can be introduced to a food product in the chamber. For example, a chamber may be made of plastic, glass, cellulosic material, cement, or any other semipermeable or impermeable material.

The chamber may be of any size that is large enough to hold plants and plant parts to be treated. For example, an exemplary chamber may have a volume of about 10 liters (L) to about 50 L, from about 20 L to about 40 L, from about 25 L to about 50 L, from about 30 L to about 40 L, from about 35 L to about 40 L, and at about 35 L or about 36 L. In addition, an

illustrative chamber may be a pallet that may have a size, sealed or unsealed ranging from 0.5 cubic meters to about 150 cubic meters.

In addition, an exemplary chamber of the present invention may be a large storage room (e.g., a gymnasium) having a headspace of several hundred to several thousand cubic meters. Thus, an exemplary chamber of the present invention may have a headspace size ranging from 100 to about 10,000 cubic meters, from about 100 to about 8000 cubic meters, from about 100 to about 7500 cubic meters, from about 100 to about 5000 cubic meters, from about 200 to about 3000 cubic meters, and about 1000 cubic meters.

Gaseous benzoxaborole treatments may be applied to the plants or plants parts at a concentration that is applied over approximately 3 to about 5 seconds to about two hours. The gas chemical treatment concentration may be reported as the amount (milligrams, mg) of active ingredient (i.e., benzoxaborole compound) per volume (liter, L) of chamber headspace or amount (milligrams, mg) of active ingredient (i.e. benzoxaborole compound) per mass (kilogram, kg) of crop.

For example, the rate that the benzoxaborole treatment may be effectively applied in a chamber and/or to plants may range from 0.001 mg/L to 0.5 mg/L. For example, the rate of the benzoxaborole treatment may be from about 0.002 mg/L to about 0.2 mg/L, 0.002 mg/L to about 0.14 mg/L, 0.002 mg/L to about 0.035 mg/L, 0.002 mg/L to about 0.0088 mg/L, 0.002 mg/L to about 0.044 mg/L, from about 0.004 mg/L to about 0.15 mg/L, from about 0.0044 mg/L to about 0.14 mg/L, from about 0.0044 mg/L to about 0.0088 mg/L, from about 0.0044 mg/L to about 0.035 mg/L, from about 0.0088 mg/L to about 0.14 mg/L, from about 0.035 mg/L to about 0.14 mg/L, from about 0.0088 mg/L to about 0.035 mg/L, from about 0.001 mg/L to about 0.2 mg/L, from about 0.001 mg/L to about 0.14 mg/L, from about 0.001 mg/L to about 0.0088 mg/L, from about 0.001 mg/L to about 0.0044 mg/L, and at about 0.0044 mg/L, about 0.0088 mg/L, about 0.035mg/L, and about 0.14 mg/L.

Exemplary preservative gases of the method described herein include, but are not limited to, carbon dioxide (CO_2) and sulfur dioxide (SO_2). Additional chemicals that may be combined with benzoxaborole compound in the present disclosure include, but are not limited to, 1-methylcyclopropene, adjuvant(s), and commercial pesticides. Further chemicals that may be used in the present method include some that have been federally recognized. For example, Food, Drug and Cosmetic Act § § 201 and 409 Generally Recognized As Safe (GRAS) compounds and Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) § 25(b) chemicals, including eugenol, clove, thyme or mint oils), natural compounds, or compounds derived from natural sources may also be used in the present method.

While any concentration of preservative gas or chemical that provides the antimicrobial effect described herein may be utilized, a percent of CO_2 gas or chemical that may be used in the present method includes, but is not limited to, from about 4% to about 20%, from about 5% to about 18%, from about 6% to about 17%, from about 7% to about 15%, from about 8% to about 14%, from about 8% to about 13%, from about 8% to about 12%, from about 5% to about 14%, from about 6% to about 13%, from about 7% to about 13%, and at about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, and about 14%.

A percent of SO_2 gas or chemical that may be used in the present method includes, but is not limited to, from about 0.001% to about 1%, from about 0.005% to about 1%, from about 0.01% to about 1%, from about 0.05% to about 1%, and from about 0.1% to about 1%.

Any plants or plant parts, plant cells, or plant tissues may be treated using the present method. A class of plants that may be treated in the present invention is generally as broad as horticultural crops. Horticultural crops, include, but are not limited to, vegetable crops, fruit crops, edible nuts, flowers and ornamental crops, nursery crops, aromatic crops, and medicinal crops. More specifically, fruits (e.g., grape, apple, pear, and persimmon) and berries (e.g., strawberries, blackberries, blueberries, and raspberries) are plants encompassed by the present disclosure. It should be noted that any species of berries or fruits may be used in the present invention (e.g., Table grapes).

Plants and agricultural crops after harvest may be used in the method of the present application. For example, exemplary plants of the present invention include post-harvest plants and crops during field packing, palletization, in-box, during storage, and throughout the distribution network. Further, plants being transported by any mode, including, but not limited to local vehicles, transport trailers, marine containers, aircraft containers, etc. may be treated using the method described herein. For example, minimally-processed packaged products (e.g., packaged vegetables or fruits) may also be treated with the method described herein.

METHODS OF USING BENZOXABOROLE COMPOUNDS

The present disclosure is directed to methods of providing antimicrobial protection to plants from plant pathogens. More specifically, fungal plant pathogens may be treated, prevented, or eradicated by the method described herein. Exemplary, pathogens encompassed by the present disclosure include, but are not limited to, *Botrytis cinerea*, *Mucor piriformis*, *Fusarium sambucinum*, *Aspergillus brasiliensis*, and *Peniciliium expansum*. Additional pathogens encompassed by the present invention include, but are not limited to *Acremonium* spp., *Albugo* spp., *Alternaria* spp., *Ascochyta* spp., *Aspergillus* spp., *Botryodiplodia* spp., *Botryospheria* spp., *Botrytis* spp., *Byssochlamys* spp., *Candida* spp., *Cephalosporium* spp.,

Ceratocystis spp., *Cercospora* spp., *Chalara* spp., *Cladosporium* spp., *Colletotrichum* spp., *Cryptosporiopsis* spp., *Cylindrocarpon* spp., *Debaryomyces* spp., *Diaporthe* spp., *Didymella* spp., *Diplodia* spp., *Dothiorella* spp., *Elsinoe* spp., *Fusarium* spp., *Geotrichum* spp., *Gloeosporium* spp., *Glomerella* spp., *Helminthosporium* spp., *Khuskia* spp., *Lasiodiplodia* spp., *Macrophoma* spp., *Macrophomina* spp., *Microdochium* spp., *Monilinia* spp., *Monilochaethes* spp., *Mucor* spp., *Mycocentrospora* spp., *Mycosphaerella* spp., *Nectria* spp., *Neofabraea* spp., *Nigrospora* spp., *Penicillium* spp., *Peronophythora* spp., *Peronospora* spp., *Pestalotiopsis* spp., *Pezicula* spp., *Phacidiopycnis* spp., *Phoma* spp., *Phomopsis* spp., *Phyllosticta* spp., *Phytophthora* spp., *Polyscytalum* spp., *Pseudocercospora* spp., *Pyricularia* spp., *Pythium* spp., *Rhizoctonia* spp., *Rhizopus* spp., *Sclerotium* spp., *Sclerotinia* spp., *Septoria* spp., *Sphaceloma* spp., *Sphaeropsis* spp., *Stemphyllium* spp., *Stilbella* spp., *Thielaviopsis* spp., *Thyronectria* spp., *Trachysphaera* spp., *Uromyces* spp., *Ustilago* spp., *Venturia* spp., and *Verticillium* spp., and bacterial pathogens, such as *Bacillus* spp., *Campylobacter* spp., *Clavibacter* spp., *Clostridium* spp., *Erwinia* spp., *Escherichia* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Listeria* spp., *Pantoea* spp., *Pectobacterium* spp., *Pseudomonas* spp., *Ralstonia* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Vibrio* spp., *Xanthomonas* spp., and *Yersinia* spp.

The benzoxaborole and preservative gas treatments may be applied to the plants or plants parts inside of a container or chamber. The chamber may be open or closed/sealed during application of the benzoxaborole and preservative gas treatment. Typically, the plants or plant parts are manually or robotically placed in the chamber, and the chamber is then sealed. The benzoxaborole and preservative gas treatment is then applied to the sealed chamber comprising the plants or plant parts via the port (e.g., a bulkhead septum port).

The benzoxaborole and preservative gas treatments are applied to the sealed chamber for an initial time period. For example, the plants may be exposed to the treatments in the chamber or sealed container for any initial time period. An illustrative initial time period may range from about 12 hours to about 5 days (120 hours), from about 1 day to about 4 days, from about 2 days to about 3.5 days, from about 2 days to about 3.5 days, and at about 3.5 days. The temperature of the sealed chamber can range from about 1°C to about 25°C.

After expiration of the treatment time period, inhibition of plant pathogens may be assessed. For example, *in vitro* samples may have the growth of the pathogen on agar or in media assessed, evaluated, and compared to a control sample where no benzoxaborole or preservative gas treatment was administered or different treatment conditions were applied. Similarly, *in vivo* samples may have the severity and incidence of fungal or bacterial disease assessed, evaluated, and compared to a control sample where no benzoxaborole or preservative gas treatment was administered or different treatment conditions were applied.

Synergy of benzoxaborole compounds combined with a gas and/or a chemical to form a benzoxaborole treatment was determined using the Colby equation, E = x + y - x * y/100, where:

- E = Expected efficacy expressed in percent (%) of untreated control, when using a mixture of the active Compounds A and B at the concentrations a and b, respectively.
- x = Efficacy expressed in % of the untreated control, when using the active compound A at the concentration a
- y = Efficacy, expressed in % of the untreated control, when using the active compound B at the concentration b

EXAMPLES

Illustrative embodiments of the methods of the present disclosure are provided herein by way of examples. While the concepts and technology of the present disclosure are susceptible to broad application, various modifications, and alternative forms, specific embodiments will be described here in detail. It should be understood, however, that there is no intent to limit the concepts of the present disclosure to the particular forms disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives consistent with the present disclosure and the appended claims.

The following experiments were used to determine synergy between benzoxaborole Compounds A, B, and/or C and preservative gases, such as carbon dioxide (CO_2) and sulfur dioxide (SO_2). Specific rates or any other unique conditions are noted within the corresponding results tables. Each experiment comprised an air-tight 36 liter (L) chamber (Fisher Scientific, P/N: 08-642-23C), fitted with a bulkhead septum port (Swagelok, P/N: SS-400-61, Solon, OH) used for treatment delivery, as well as for monitoring (using needle extraction) carbon dioxide (CO_2), sulfur dioxide (SO_2), oxygen (O_2) or other headspace samples. A variable speed fan (Thermaltake, Mobile Fan II, P/N: A1888, Taipei City, Taiwan) set to low was also used to circulate the headspace atmosphere.

In Vitro and *In Vivo* Experiments

In vitro and *in vivo* experiments were performed concurrently in triple replicates (i.e., triplicate) unless otherwise noted. For *in vitro* experiments, a single 6-well microtiter plate or three, 10-cm petri plates each containing half strength Potato Dextrose Agar were prepared. Each well or plate was inoculated with 1 microliter (µL) of $1x10^5$ spores/ml of the appropriate pathogenic spore suspension (e.g., *Botrytis cinerea,* BOTRCI; *Penicillium expansum,* PENIEX; *Mucor piriformis,* MUCOPI; *Fusarium sambucinum* FUSASA; or *Aspergillus*

brasiliensis, ASPEBR). The inoculated microtiter or petri plates were then sealed with a breathable film (AeraSeal; P/N: B- 100, Excel Scientific, Victorville, CA).

For *in vivo* experiments, eight strawberry fruits per repetition were surface sterilized with a 70% ethanol solution. After sterilization, the strawberries were rinsed twice with deionized water. Each strawberry fruit was wounded using a T15 screwdriver tip to a uniform depth of eight mm (8mm). Each fruit wound was inoculated with 20 µL of $1x10^5$ spores/ml pathogen spore suspension of *B. cinerea* or *M. piriformis.* The strawberries were then placed with the stem facing downwards inside a 1-pound strawberry clamshell.

Similarly, table grape fruits were also tested *in vivo.* More specifically, 16-20 table grapes per repetition were washed and inoculated with pathogens (as described above for the strawberries). However, an ethanol-sterilized push pin, rather than a T15 screwdriver, was used to wound the stem-end of the grapes. The grapes were inoculated in the wound, and then arranged inside a 1-pint clamshell.

For treatment, the inoculated agar plates and clamshells containing fruits (e.g., strawberries and grapes) were placed inside a 36 L experimental chamber unit, which was then sealed prior to commencement of compound treatments. The chamber remained sealed throughout the duration of the experiment except for addition of treatment to the chamber, and post-treatment ventilation.

Benzoxaborole Compound and Preservative Gas Treatment of Agar Plates (*In Vitro*) and Fruits

Benzoxaborole Compound A or Compound B was dissolved in acetone and dispensed onto a 60 mm Whatman #1 filter paper disk using a pipette. The acetone was permitted to evaporate for five minutes before the disk was placed on a watch glass in each chamber.

A 12% CO_2 headspace treatment was then established in the chamber by permitting 10 grams (g) of crushed dry ice placed in a weigh boat in the chamber to evaporate in the sealed chamber. However, the bulkhead port of the chamber was loosened to permit the release of internal pressure. Optionally, a fan was used during the initial hours of treatment (i.e., up to about 6 hours of treatment) to ensure uniform treatment exposure of fruits and plates.

Untreated controls and CO_2 treated chambers contained an acetone-treated disk with similar evaporation for five minutes. CO_2 and O_2 levels in the chamber were monitored using a handheld gas detector (Dansensor Checkpoint II; P/N 600111, Ringsted, Denmark). Temperature and relative humidity were also monitored within the chamber using data loggers (Onset HOBO; P/N U12-013, Bourne, MA).

The chamber for all *in vitro* and *in vivo* experiments was held at room temperature (i.e., from about 21°C to about 23°C) typically for about 3.5 days (i.e., about 84 hours) of treatment exposure. Chambers were then vented. After an additional 2-3 days with the chamber at room

temperature, post-treatment radial growth (in mm) of each *in vitro* pathogen (see Examples 1-7, Tables 1-7) was measured on the inoculated plates using electronic calipers (Mitutoyo, P/N: CD-6-CX, Aurora, IL). In addition, the point of inoculation on fruits was assessed for indication of *in vivo* disease incidence (e.g., yes or no) and severity of disease incidence (see Examples 8-16, Tables 8-16). Disease severity was rated on a scale ranging from 0 to 4, where "0" indicated no disease severity, "1" indicated minimal disease severity, "2" indicated medium disease severity, "3" indicated high disease severity, and "4" indicated exceptionally high disease severity.

Synergy was determined using the following Colby equation,

$E = x + y - x * y/100$, where:

- E = Expected efficacy expressed in percent (%) of untreated control, when using a mixture of the active Compounds A and B at the concentrations a and b, respectively.
- x = Efficacy expressed in % of the untreated control, when using the active compound A at the concentration a
- y = Efficacy, expressed in % of the untreated control, when using the active compound B at the concentration b

Example 1: Synergistic Inhibition of BOTRCI and PENIEX pathogens by Benzoxaborole and CO_2 Treatment of Agar Plates (*In Vitro*)

Table 1 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on agar plates. As expected, the plates treated with the control (i.e., air) showed the highest pathogenic growth of 34 mm of BOTRCI and 22.6 mm of PENIEX. Methods of treating plant pathogens were used on agar plates growing BOTRCI and PENIEX and treated with 12% CO_2 only had 28 mm and 18.6 mm of pathogenic growth, respectively. Plates growing BOTRCI and PENIEX and treated with 0.14 mg/L of benzoxaborole Compound A only had 16.3 mm and 17 mm of pathogenic growth, respectively. However, the plates treated with both benzoxaborole Compound A and 12% CO_2 showed no growth and 3.3 mm of growth for BOTRCI and PENIEX pathogens, respectively. The Colby calculations of 165.4 and 225 indicated that the benzoxaborole treatment of Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI and PENIEX *in vitro* growth, respectively.

Table 1: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vitro* fungal growth of *Botrytis cinerea* (BOTRCI) and *Penicillium expansum* (PENIEX) when evaluated 2 days after treatment completion. [a, b, c, d]

Cmpd	Rate (mg/L)	Pathogen	Growth (diameter, mm)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	BOTRCI	34.0	16.3	28.0	0.0	165.4	Synergistic
A	0.14	PENIEX	22.6	17.0	18.6	3.3	225.0	Synergistic

[a] Assay was performed using a 6-well microtiter plate
[b] Experiment was performed with 4 replicates
[c] Fan was used for first 6 hours of treatment

Example 2: Synergistic Inhibition of PENIEX pathogen by Benzoxaborole and CO_2 Treatment of Agar Plates (*In Vitro*)

Table 2 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on agar plates. As expected, the plate treated with the control (i.e., air) showed the highest pathogenic growth of 24.6 mm of PENIEX. Methods of treating plant pathogens were used on agar plates growing PENIEX and treated with 12% CO_2 only had 21.3 mm of pathogenic growth. Plates growing PENIEX and treated with 0.14 mg/L of benzoxaborole Compound A only had 9.7 mm of pathogenic growth. However, the plates treated with both benzoxaborole Compound A and 12% CO_2 showed 5.2 mm of growth for PENIEX pathogens. The Colby calculation of 119.7 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit PENIEX growth *in vitro*.

Table 2: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vitro* fungal growth of *Penicillium expansum* (PENIEX) when evaluated 2 days after completion of treatment. [a, b, c]

Cmpd	Rate (mg/L)	Pathogen	Growth (diameter, mm)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	PENIEX	24.6	9.7	21.3	5.2	119.7	Synergistic

[a] Assay was performed using a 6-well microtiter plate
[b] Fan was used for first 6 hours of treatment
[c] Additional water vapor was added to each chamber to achieve >90% R.H.

Example 3: Synergistic Inhibition of BOTRCI and MUCOPI pathogens by Benzoxaborole and CO_2 Treatment of Agar Plates (*In Vitro*)

Table 3 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on agar plates. As expected, the plate treated with the control (i.e., air)

showed the highest pathogenic growth of 35 mm for both BOTRCI and MUCOPI. Methods of treating plant pathogens were used on agar plates growing BOTRCI and MUCOPI and treated with 12% CO_2 only had 30.9 mm and 35 mm of pathogenic growth, respectively. Plates growing BOTRCI and MUCOPI and treated with 0.14 mg/L of benzoxaborole Compound A only had 5.5 mm and 22.3 mm of pathogenic growth, respectively. However, the plates treated with both benzoxaborole Compound A and 12% CO_2 showed 4.5 mm and 11 mm of growth for BOTRCI and MUCOPI pathogens, respectively. The Colby calculations of 101.2 and 189 indicated that the benzoxaborole treatment of Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI and PENIEX *in vitro* growth, respectively.

Table 3: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vitro* fungal growth of *Mucor piriformis* (MUCOPI) and *Botrytis cinerea* (BOTRCI) when evaluated 2 days after completion of treatment.[a, b, c]

Cmpd	Rate (mg/L)	Pathogen	Growth (diameter, mm)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	MUCOPI	35.0	22.3	35.0	11.0	189.0	Synergistic
A	0.14	BOTRCI	35.0	5.5	30.9	4.5	101.2	Synergistic

[a] Assay was performed using a 6-well microtiter plate
[b] Fan was used for first 6 hours of treatment
[c] Additional water vapor was added to each chamber to achieve >90% R.H.

Example 4: Synergistic Inhibition of BOTRCI and PENIEX pathogens by Benzoxaborole and CO_2 Treatment of Agar Plates (*In Vitro*)

Table 4 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on agar plates. As expected, the plate treated with the control (i.e., air) showed the highest pathogenic growth of 35 mm of BOTRCI and 25.3 mm of PENIEX. Methods of treating plant pathogens were used on agar plates growing BOTRCI and PENIEX and treated with 12% CO_2 only had 33.4 mm and 21.2 mm of pathogenic growth, respectively. Plates growing BOTRCI and PENIEX and treated with 0.14 mg/L of benzoxaborole Compound A only had 1.5 mm and 6.4 mm of pathogenic growth, respectively. However, the plates treated with both benzoxaborole Compound A and 12% CO_2 showed no growth and 0.5 mm of growth for BOTRCI and PENIEX pathogens, respectively. The Colby calculations of 104.3 and 124.4, respectively, indicated that the benzoxaborole treatment of Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI and PENIEX growth *in vitro*.

Table 4: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vitro* fungal growth of *Botrytis cinerea* (BOTRCI) and *Penicillium expansum* (PENIEX) when evaluated 2 days after treatment completion.[a, b]

Cmpd	Rate (mg/L)	Pathogen	Growth (diameter, mm)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	BOTRCI	35.0	1.5	33.4	0.0	104.3	Synergistic
A	0.14	PENIEX	25.3	6.4	21.2	0.5	124.4	Synergistic

[a] Assay was performed using a 6-well microtiter plate

[b] Fan was used for first 6 hours of treatment

Example 5: Synergistic Inhibition of BOTRCI, PENIEX, FUSASA, and ASPEBR pathogens by Benzoxaborole and CO_2 Treatment of Agar Plates (*In Vitro*)

Table 5 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on agar plates. As expected, the plate treated with the control (i.e., air) showed the highest pathogenic growth of 32.9 mm of BOTRCI, 22.7 mm of PENSIEX, 33.9 mm of FUSASA, and 29.6 mm of ASPEBR. Methods of treating plant pathogens were used on agar plates growing BOTRCI, PENIEX, FUSASA, and ASPEBR and treated with 12% CO_2 only had 35 mm, 19.9 mm, 33.9 mm, and 26.2 mm of pathogenic growth, respectively. Plates growing BOTRCI, PENIEX, FUSASA, and ASPEBR and treated with 0.14 mg/L of benzoxaborole Compound A only had 3.6 mm, 10.2 mm, 5.0 mm, and 2.6 mm of pathogenic growth, respectively. However, the plates treated with both benzoxaborole Compound A and 12% CO_2 showed 3 mm, 8.6 mm, 3.8 mm, and no growth of BOTRCI, PENIEX, FUSASA, and ASPEBR pathogens, respectively. The Colby calculations of 102.9, 102.5, 104.0, and 108.3 indicated that the benzoxaborole treatment of Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI, PENIEX, FUSASA, and ASPEBR *in vitro* growth, respectively.

Plates growing BOTRCI, PENIEX, FUSASA, and ASPEBR and treated with 0.14 mg/L of benzoxaborole Compound B only had 3.5 mm, 7.7 mm, 3.6 mm, and 3.6 mm of pathogenic growth, respectively. However, plates treated with both benzoxaborole Compound B and 12% CO_2 showed 2.7 mmm, 6.4 mm, 3.4 mm, and no growth of BOTRCI, PENIEX, FUSASA, and ASPEBR pathogens, respectively. The Colby calculations of 103.4, 102.4, 100.4, and 112.0 indicated that the benzoxaborole treatment of Compound B and CO_2 was synergistic over the other treatments to inhibit BOTRCI, PENIEX, FUSASA, and ASPEBR *in vitro* growth, respectively.

Table 5: Synergistic effect of benzoxaborole Compounds A or B and CO_2 on inhibition of *in vitro* fungal growth of *Botrytis cinerea* (BOTRCI), *Penicillium expansum* (PENIEX), *Fusarium sambucinum* (FUSASA), and *Aspergillus brasiliensis* (ASPEBR) when evaluated 2 days after completion of treatment.[a, b, c]

Cmpd	Rate (mg/L)	Pathogen	Growth (diameter, mm)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	BOTRCI	32.9	3.6	35.0	3.0	102.9	Synergistic
A	0.14	PENIEX	22.7	10.2	19.9	8.6	102.5	Synergistic
A	0.14	FUSASA	33.9	5.0	33.9	3.8	104.0	Synergistic
A	0.14	ASPEBR	29.6	2.6	26.2	0.0	108.3	Synergistic
B	0.14	BOTRCI	32.9	3.5	35.0	2.7	103.4	Synergistic
B	0.14	PENIEX	22.7	7.7	19.9	6.4	102.4	Synergistic
B	0.14	FUSASA	33.9	3.6	33.9	3.4	100.4	Synergistic
B	0.14	ASPEBR	29.6	3.6	26.2	0.0	112.0	Synergistic

[a] Assay was performed using a 6-well microliter plate
[b] Fan was used for first 6 hours of treatment
[c] Additional water vapor was added to each chamber to achieve >90% R.H,

Example 6: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Agar Plates (*In Vitro*)

Table 6 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on agar plates. As expected, the plate treated with the control (i.e., air) showed the highest pathogenic growth of 85.0 mm of BOTRCI. Methods of treating plant pathogens were used on agar plates growing BOTRCI and treated with 12% CO_2 only had 78.9 mm of pathogenic growth. Plates growing BOTRCI and treated with 0.14 mg/L of benzoxaborole Compound A only had 13.2 mm of pathogenic growth. However, the plates treated with both benzoxaborole Compound A and 12% CO_2 showed 6.4 mm of growth for BOTRCI pathogens. The Colby calculation of 108.2 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vitro.*

Table 6: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vitro* fungal growth of *Botrytis cinerea* (BOTRCI) when evaluated 3 days after completion of treatment. [a, b]

Cmpd	Rate (mg/L)	Pathogen	Growth (diameter, mm)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	BOTRCI	85.0	13.2	78.9	6.4	108.2	Synergistic

[a] Assay was performed using a 10 cm petri plates
[b] Fan was run continuous during treatment

Example 7: Synergistic Inhibition of PENIEX pathogen by Benzoxaborole and CO_2 Treatment of Agar Plates (*In Vitro*)

Table 7 demonstrates that the present method may be used to inhibit growth of plant

pathogens inoculated on agar plates. As expected, the plate treated with the control (i.e., air) showed the highest pathogenic growth of 32.8 mm of PENIEX. Methods of treating plant pathogens were used on agar plates growing PENIEX and treated with 12% CO_2 only had 25.8 mm of pathogenic growth. Plates growing PENIEX and treated with benzoxaborole Compound A only had 10.6 mm of pathogenic growth. However, the plates treated with both benzoxaborole Compound A and 12% CO_2 showed 4.7 mm of growth for PENIEX pathogens. The Colby calculation of 114.7 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit PENIEX growth *in vitro.*

Table 7: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vitro* fungal growth of *Penicillium expansum* (PENIEX) when evaluated 3 days after completion of treatment. [a, b]

Cmpd	Rate (mg/L)	Pathogen	Growth (diameter, mm)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.035	PENIEX	32.8	10.6	25.8	4.7	114.7	Synergistic

[a] Assay was performed using a 10 cm Petri plates
[b] Fan was run continuous during treatment

Example 8: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 8 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as strawberries. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 4.0 mm of BOTRCI. Methods of treating plant pathogens were used on fruit growing BOTRCI and treated with 12% CO_2 only had 3.7 mm of pathogenic growth. Fruit growing BOTRCI and treated with 0.14 mg/L of benzoxaborole Compound A only had 2.1 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 only showed 0.4 mm of growth for BOTRCI pathogens. The Colby calculation of 176.2 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo.*

Table 8: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vivo* fungal growth of *Botrytis cinerea* (BOTRCI) on strawberries when evaluated 2 days after completion of treatment. [a, b, c]

Cmpd	Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	BOTRCI	4.0	2.1	3.7	0.4	176.2	Synergistic

[a] Experiment was performed with 4 replicates
[b] Fan was used for first 6 hours of treatment
[c] Additional water vapor was added to each chamber to achieve >90% R.H.

Example 9: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 9 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as strawberries. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 3.8 mm of BOTRCI. Methods of treating plant pathogens were used on fruit growing BOTRCI and treated with 12% CO_2 only had 3.5 mm of pathogenic growth. Fruit growing BOTRCI and treated with 0.14 mg/L of benzoxaborole Compound A only had 1.1 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 showed 0.8 mm of growth for BOTRCI pathogens. The Colby calculation of 107.6 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo*.

Table 9: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vivo* fungal growth of *Botrytiscinerea* (BOTRCI) on strawberries when evaluated 3 days after completion of treatment.[a, b]

Cmpd	Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd +12% CO_2		
A	0.14	BOTRCI	3.8	1.1	3.5	0.8	107.6	Synergistic

[a] Fan was used for first 6 hours of treatment

[b] Additional water vapor was added to each chamber to achieve >90% R H.

Example 10: Synergistic Inhibition of MUCOPI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 10 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as strawberries. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 3.6 mm of MUCOPI. Methods of treating plant pathogens were used on fruit growing MUCOPI and treated with 12% CO_2 only had 3.0 mm of pathogenic growth. Fruit growing MUCOPI and treated with 0.14 mg/L of benzoxaborole Compound A only had 0.5 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 showed 0.2 mm of growth for MUCOPI pathogens. The Colby calculation of 106.8 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit MUCOPI growth *in vivo*.

Table 10: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vivo* fungal growth of *Mucor piriformis* (MUCOPI) on strawberries when evaluated 2 days after completion of treatment.[a, b]

Cmpd	Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	MUCOPI	3.6	0.5	3.0	0.2	106.8	Synergistic

[a] Fan was used for first 6 hours of treatment
[b] Additional water vapor was added to each chamber to achieve >90% R H.

Example 11: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 11 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as strawberries. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 3.7 mm of BOTRCI. Methods of treating plant pathogens were used on fruit growing BOTRCI and treated with 12% CO_2 only had 2.7 mm of pathogenic growth. Fruit growing BOTRCI and treated with 0.14 mg/L of benzoxaborole Compound A only 1.4 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 showed 0.8 mm of growth for BOTRCI pathogens. The Colby calculation of 109.0 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo*.

Fruit growing BOTRCI and treated with benzoxaborole Compound B only had 0.9 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound B and 12% CO_2 showed 0.5 mm of growth for BOTRCI pathogens. The Colby calculation of 105.0 indicated that the benzoxaborole treatment with Compound B and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo*.

Table 11: Synergistic effect of benzoxaborole Compounds A or B and CO_2 on inhibition of *in vivo* fungal growth of *Botrytis cinerea* (BOTRCI) on strawberries when evaluated 2 days after completion of treatment. [a, b]

Cmpd Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
		Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A 0.14	BOTRCI	3.7	1.4	2.7	0.8	109.0	Synergistic
B 0.14	BOTRCI	3.7	0.9	2.7	0.5	105.0	Synergistic

[a] Fan was used for first 6 hours of treatment
[b] Additional water vapor was added to each chamber to achieve >90% R H.

Example 12: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 12 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as strawberries. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 3.6 mm of BOTRCI. Methods of treating plant pathogens were used on fruit growing BOTRCI and treated with 12% CO_2 only had 3.4 mm of pathogenic growth. Fruit growing BOTRCI and treated with 0.14 mg/L of benzoxaborole Compound A only 0.7 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 showed 0.4 mm of growth for BOTRCI pathogens. The Colby calculation of 107.9 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo.*

Fruit growing BOTRCI and treated with benzoxaborole Compound B only had 0.4 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound B and 12% CO_2 showed 0.3 mm of growth for BOTRCI pathogens. The Colby calculation of 100.9 indicated that the benzoxaborole treatment with Compound B and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo.*

Table 12: Synergistic effect of benzoxaborole Compounds A or B and CO_2 on inhibition of *in vivo* fungal growth of *Botrytis cinerea* (BOTRCI) on strawberries when evaluated 2 days after completion of treatment.[a, b]

Cmpd	Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.14	BOTRCI	3.6	0.7	3.4	0.4	107.9	Synergistic
B	0.14	BOTRCI	3.6	0.4	3.4	0.3	100.9	Synergistic

[a] Fan was used for first 6 hours of treatment

[b] Additional water vapor was added to each chamber to achieve >90% R.H.

Example 13: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 13 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as strawberries. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 3.4 mm of BOTRCI. Methods of treating plant pathogens were used on fruit growing BOTRCI and treated with 12% CO_2 only had 3.2 mm of pathogenic growth. Fruit growing BOTRCI and treated with 0.14 mg/L of benzoxaborole Compound A only had 2.4 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 only showed 1.4 mm of growth for BOTRCI pathogens. The Colby calculation of 163.4 indicated that the benzoxaborole treatment

with Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo.*

Table 13: Synergistic effect of benzoxaborole Compound A and CO_2 on the inhibition of *in vivo* fungal growth of *Botrytis cinerea* (BOTRCI) on strawberries when evaluated 3 days after completion of treatment. [a]

Cmpd Rate Pathogen (mg/L)	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
	Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A 0.14 BOTRCI	3.4	2.4	3.2	1.4	163.4	Synergistic

[a] Fan was run continuously during treatment

Example 14: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 14 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as strawberries. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 4.0 mm of BOTRCI. Methods of treating plant pathogens were used on fruit growing BOTRCI and treated with 12% CO_2 only had 3.9 mm of pathogenic growth. Fruit growing BOTRCI and treated with 0.0044 mg/L or 0.0088 mg/L benzoxaborole Compound A only had 3.4 mm and 3.3 mm of pathogenic growth, respectively. However, the fruit treated with 0.0044 mg/L or 0.0088 mg/L of benzoxaborole Compound A and 12% CO_2 only showed 3.2 mm and 0.6 mm of growth for BOTRCI pathogens, respectively. The Colby calculation of 114.8 and 502.5 indicated that the benzoxaborole treatment with 0.0044 mg/L or 0.0088 mg/L Compound A, respectively, combined with CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo.*

Table 14: Synergistic effect of benzoxaborole Compound A and CO_2 on the inhibition of *in vivo* fungal growth of *Botrytis cinerea* (BOTRCI) on strawberries when evaluated 3 days after completion of treatment. [a]

Cmpd	Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.0044	BOTRCI	4.0	3.4	3.9	3.2	114.8	Synergistic
A	0.0088	BOTRCI	4.0	3.3	3.9	0.6	502.5	Synergistic

[a] Fan was run continuous during treatment

Example 15: Synergistic Inhibition of BOTRCI pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 15 demonstrates that the present method may be used to inhibit growth of plant

pathogens inoculated on fruit, such as Table grapes. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 1.6 mm of BOTRCI. Methods of treating plant pathogens were used on fruit growing BOTRCI and treated with 12% CO_2 only had 1.4 mm of pathogenic growth. Fruit growing BOTRCI and treated with 0.035 mg/L of benzoxaborole Compound A only had 0.9 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 only showed 0.7 mm of growth for BOTRCI pathogens. The Colby calculation of 115.2 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit BOTRCI growth *in vivo*.

Table 15: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vivo* fungal growth of *Botrytis cinerea* (BOTRCI) on table grapes when evaluated 1 day after completion of treatment.[a]

Cmpd	Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd + 12% CO_2		
A	0.035	BOTRCI	1.6	0.9	1.4	0.7	115.2	Synergistic

[a] Fan was run continuous during treatment

Example 16: Synergistic Inhibition of PENIEX pathogen by Benzoxaborole and CO_2 Treatment of Fruit (*In Vivo*)

Table 16 demonstrates that the present method may be used to inhibit growth of plant pathogens inoculated on fruit, such as Table grapes. As expected, the fruit treated with the control (i.e., air) showed the highest pathogenic growth of 2.4 mm of PENIEX. Methods of treating plant pathogens were used on fruit growing PENIEX and treated with 12% CO_2 only had 1.9 mm of pathogenic growth. Fruit growing PENIEX and treated with 0.035 mg/L of benzoxaborole Compound A only had 1.8 mm of pathogenic growth. However, the fruit treated with both benzoxaborole Compound A and 12% CO_2 only showed 1.3 mm of growth for PENIEX pathogens. The Colby calculation of 108.2 indicated that the benzoxaborole treatment with Compound A and CO_2 was synergistic over the other treatments to inhibit PENIEX growth *in vivo*.

Table 16: Synergistic effect of benzoxaborole Compound A and CO_2 on inhibition of *in vivo* fungal growth of *Penicillium expansum* (PENIEX) on table grapes when evaluated 1 day after completion of treatment.[a]

Cmpd	Rate (mg/L)	Pathogen	Growth (Severity, 0 to 4)				Colby Calculation	Outcome
			Control (Air)	Cmpd	12% CO_2	Cmpd +12 CO_2		
A	0.035	PENIEX	2.4	1.8	1.9	1.3	108.2	Synergistic

[a] Fan was run continuous during treatment

The preceding description enables others skilled in the art to utilize the technology in various embodiments and with various modifications as are suited to the particular use contemplated. In accordance with the provisions of the patent statutes, the principles and modes of operation of this disclosure have been explained and illustrated in exemplary embodiments. Accordingly, the present invention is not limited to the particular embodiments described and/or exemplified herein.

It is intended that the scope of disclosure of the present technology be defined by the following claims. However, it must be understood that this disclosure may be practiced otherwise than is specifically explained and illustrated without departing from its spirit or scope. It should be understood by those skilled in the art that various alternatives to the embodiments described herein may be employed in practicing the claims without departing from the spirit and scope as defined in the following claims.

The scope of this disclosure should be determined, not only with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. It is anticipated and intended that future developments will occur in the arts discussed herein, and that the disclosed compositions and methods will be incorporated into such future examples.

Furthermore, all terms used in the claims are intended to be given their broadest reasonable constructions and their ordinary meanings as understood by those skilled in the art unless an explicit indication to the contrary is made herein. In particular, use of the singular articles such as "a," "the," "said," etc. should be read to recite one or more of the indicated elements unless a claim recites an explicit limitation to the contrary. It is intended that the following claims define the scope of the disclosure and that the technology within the scope of these claims and their equivalents be covered thereby. In sum, it should be understood that the disclosure is capable of modification and variation and is limited only by the following claims

WHAT IS CLAIMED IS:

1. A method of treating plants with an antimicrobial agent comprising:
placing one or more plants or plant parts in a chamber,
sealing the chamber,
adding a benzoxaborole compound to the chamber,
adding a preservative gas to the chamber,
wherein the benzoxaborole compound and the preservative gas combine to form an antimicrobial treatment,
administering the antimicrobial treatment to the one or more plants or plant parts in the sealed chamber, and
unsealing the chamber.

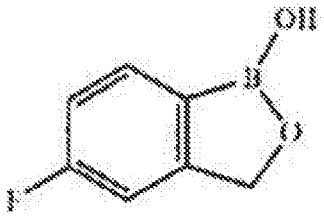
2. The method of claim 1, wherein the one or more plants or plant parts is a soft fruit.

3. The method of claim 1, wherein the preservative gas is CO_2 or SO_2.

4. The method of claim 2, wherein the soft fruit is selected from the group consisting of a strawberry, a raspberry, a blackberry, and a grape.

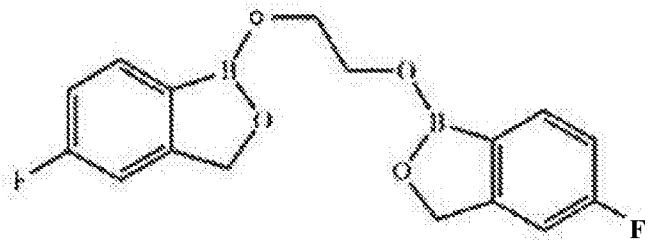
5. The method of claim 1, wherein the benzoxaborole compound is selected from the group consisting of Compound A, Compound B, Compound C, and combinations thereof.

6. The method of claim 5, wherein the benzoxaborole compound is Compound A having the structure:



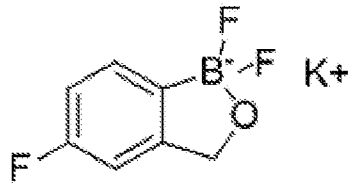
or an analog or a derivative thereof.

7. The method of claim 5, wherein the benzoxaborole compound is Compound B having the structure:



or an analog or a derivative thereof.

8. The method of claim 5, wherein the benzoxaborole compound is Compound C having the structure:



or an analog or a derivative thereof.

9. The method of claim 1, wherein the benzoxaborole compound is in the form of a liquid, a gas, a fog, or a solid.

10. The method of claim 9, wherein the solid benzoxaborole compound is a powder.

11. The method of claim 3, wherein the CO_2 concentration of the antimicrobial treatment ranges from about 4% to about 20%.

12. The method of claim 3, wherein the SO_2 concentration of the antimicrobial treatment ranges from about 0.001% to about 1%.

13. The method of claim 1, wherein the antimicrobial treatment is in the form of a spray, a mist, a gel, a thermal fog, a non-thermal fog, a dip, a drench, a vapor, a gas, or sublimation.

14. The method of claim 1, wherein the antimicrobial treatment further comprises a treatment carrier.

15. The method of claim 1, wherein the antimicrobial treatment is effective against plant pathogens.

16. The method of claim 15, wherein the plant pathogens are fungal pathogens.

17. The method of claim 16, wherein the fungal pathogens are selected from the group consisting of *Acremonium* spp., *Albugo* spp., *Alternaria* spp., *Ascochyta* spp., *Aspergillus* spp., *Botryodiplodia* spp., *Botryospheria* spp., *Botrytis* spp., *Byssochlamys* spp., *Candida* spp., *Cephalosporium* spp., *Ceratocystis* spp., *Cercospora* spp., *Chalara* spp., *Cladosporium* spp., *Colletotrichum* spp., *Cryptosporiopsis* spp., *Cylindrocarpon* spp., *Debaryomyces* spp., *Diaporthe* spp., *Didymella* spp., *Diplodia* spp., *Dothiorella* spp., *Elsinoe* spp., *Fusarium* spp., *Geotrichum* spp., *Gloeosporium* spp., *Glomerella* spp., *Helminthosporium* spp., *Khuskia* spp., *Lasiodiplodia* spp., *Macrophoma* spp., *Macrophomina* spp., *Microdochium* spp., *Monilinia* spp., *Monilochaethes* spp., *Mucor* spp., *Mycocentrospora* spp., *Mycosphaerella* spp., *Nectria* spp., *Neofabraea* spp., *Nigrospora* spp., *Penicillium* spp., *Peronophythora* spp., *Peronospora* spp., *Pestalotiopsis* spp., *Pezicula* spp., *Phacidiopycnis* spp., *Phoma* spp., *Phomopsis* spp., *Phyllosticta* spp., *Phytophthora* spp., *Polyscytalum* spp., *Pseudocercospora* spp., *Pyricularia* spp., *Pythium* spp., *Rhizoctonia* spp., *Rhizopus* spp., *Sclerotium* spp., *Sclerotinia* spp., *Septoria* spp., *Sphaceloma* spp., *Sphaeropsis* spp., *Stemphyllium* spp., *Stilbella* spp., *Thielaviopsis* spp., *Thyronectria* spp., *Trachysphaera* spp., *Uromyces* spp., *Ustilago* spp., *Venturia* spp., and *Verticillium* spp., and bacterial pathogens, such as *Bacillus* spp., *Campylobacter* spp., *Clavibacter* spp., *Clostridium* spp., *Erwinia* spp., *Escherichia* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Listeria* spp., *Pantoea* spp., *Pectobacterium* spp., *Pseudomonas* spp., *Ralstonia* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Vibrio* spp., *Xanthomonas* spp., and *Yersinia* spp.

18. The method of claim 17, wherein the fungal pathogens are selected from the group consisting of *Botrytis cinerea, Mucor piriformis, Fusarium sambucinum, Aspergillus brasiliensis,* and *Peniciliium expansum.*

19. The method of claim 16, wherein the antimicrobial treatment is a gas, and wherein the gas antimicrobial treatment concentration ranges from about 0.0001 mg/L to about 0.5 mg/L.

20. The method of claim 1, wherein the method results in synergistic inhibition of plant pathogens on the one or more treated plants or plant parts.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/20940

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 33/02, A01N 37/10 (2017.01)
CPC - A01N 43/54, A01N 43/12, A01N 37/44, A01N 35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0349853 A1 (Maclean et al.) 27 November 2014 (27.11.2014); para [0004]-[0005], [0021]-[0022], [0029], [0042], [0198], [0202], [0204], [0222], [0224]	1-2, 4-6, 8-10, 13-20 ---------- 3, 7, 11-12
Y	Dauthy "Fruit and vegetable processing: Chapter 5.3 Chemical preservation" FAO AGRICULTURAL SERVICES BULLETIN.1995, No.119 <http://www.fao.org/docrep/v5030e/V5030E00.htm#Contents>; pg. 2, para 13 - pg. 3, para 1-3, 6, 8	3, 11-12
Y	WO 2015/175157 A1 (DOW AGROSCIENCES LLC) 19 November 2015 (19.11.2015); para [0053]	7
A	Reddy et al. "1-MCP, a novel plant growth regulator for regulation of ripening" Agricultural & Horticultural Sciences. 2014, vol 2, pg. 146; pg. 146, para 1	1-20
A	US 2014/0259230 A1 (Bobbio et al.) 11 September 2014 (11.09.2014); entire document	1-20
A	US 4,421,774 A (Vidal et al.) 20 December 1983 (20.12.1983); entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April 2017	23 MAY 2017

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774