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(54) Title: CANDIDATUS LIBERIBACTER PLANT DISEASE CONTROL BY APPLICATION OF GLYPHOSATE

(57) Abstract: Disclosed herein are methods of treating plants infected with huanglongbing disease or prophylaxis against huanglongbing disease. The methods herein involve administration of controlled concentrations of glyphosphate sufficient to kill *Candidatus* Liberibacter without diminishing the health of the plant.

CANDIDATUS LIBERIBACTER PLANT DISEASE CONTROL BY APPLICATION OF GLYPHOSATE

FIELD

[0001] This invention relates to a method for treatment and prophylaxis of Huanglongbing disease and diseases caused by *Candidatus* Liberibacter species in plants and in particular citrus plants.

BACKGROUND

[0002] Glyphosate (N-(phosphonomethyl)glycine) is a nonselective herbicide known to affect over 150 species of weeds, including mono and dicotyledonous plants of annual or perennial cycles. It is used worldwide to control weeds in many crops and is usually applied three or more times per year, however the manufacturer's recommendations specify not to contact the green parts of non-target plants with the sprayed composition in order to avoid toxicity to those plants. Non-target injury from herbicide drift is a concern, as it is for any other herbicide. Glyphosate drift can cause permanent damage in some crops.

[0003] The herbicide glyphosate inhibits the enzyme 5-enolpyruvoylshikimate-3-phosphate synthase (EPSPs, EC 2.5.1.19) and therefore affects the shikimic acid pathway in which the aromatic amino acids phenylalanine, tyrosine and tryptophan are synthesized. Glyphosate causes growth retardation, chlorosis and necrosis, especially in young plant tissues.

[0004] Huanglongbing disease (HLB), also sometimes referred to as citrus greening disease, yellow shoot, or yellow dragon, is a major bacterial disease of citrus crops and can be found in Asia, in the Americas and in Africa. It has been spreading worldwide, resulting in economic loss. HLB currently is the most economically devastating disease of citrus worldwide and no established cure is available. All commercial citrus varieties currently available are susceptible to HLB and the citrus industries in affected areas have suffered a decline in both production and profit. In Florida, HLB is now present in all commercial citrus-producing counties and is negatively affecting the $9 billion citrus industry at a rapid pace. It was estimated that HLB has played a key role in the loss of about 100,000 citrus acres since 2007 in Florida and has cost

Florida's economy approximately $3.6 billion in lost revenues since 2006 (Gottwald, 2010; Wang and Trivedi, 2013). Other plants can be affected by *Ca.* Liberibacter species. For example, tomato and potato can be affected by diseases caused by *Ca.* Liberibacter solanacearum.

[0005] Citrus HLB is associated with a phloem-limited fastidious α-proteobacterium belonging to the 'Candidatus' genus Liberibacter, formerly known as Liberobacter. Currently, three species of '*Ca.* Liberibacter' have been identified to cause HLB disease: '*Ca.* L. asiaticus' (Las), '*Ca.* L. africanus', and '*Ca.* L. americanus' (Gottwald, 2010). These bacteria have not been cultivated in pure culture.

[0006] The HLB pathogen is mainly spread by the insect (psyllid) vector *Diaphorina citri* in the field. There are two psyllid species transmitting Liberibacters: Asian citrus psyllid (*Diaphorina citri*) in Asia and the Americas (Bové, 2006; Halbert, 2005; Teixeira et al., 2005) and African citrus psyllid (*Trioza erytreae*) in Africa (Bové, 2006). Las and Asian citrus psyllid are the most prevalent and important throughout HLB-affected citrus-growing areas worldwide (Bové, 2006). Las propagates in the phloem of the host plants, resulting in die-back, small leaves, yellow shoots, blotchy mottles on leaves, corky veins, malformed and discolored fruit, aborted seed, premature fruit drop, root loss, and eventually tree death (Bové, 2006; Gottwald et al., 2007; Wang and Trivedi, 2013). The life span for the profitable productivity of infected citrus trees is dramatically shortened as the disease severity increases and the yield is significantly reduced while the tree is still alive (Gottwald et al., 2007). The understanding of virulence mechanism of the bacterial pathogen is limited, due to the difficulty in culturing Las. So far, most molecular insights of the HLB biology and Las pathogenicity are derived from the genome sequences of Las and other related Liberibacters (Duan et al., 2009; Lin et al., 2011; Leonard et al., 2012; Wulff et al., 2014).

[0007] Particularly sensitive citrus includes *Citrus halimii*, 'Nules' clementine mandarin, Valencia sweet orange, 'Madam Vinous' sweet orange, 'Duncan' grapefruit, 'Ruby' red grapefruit, and 'Minneola' tangelo, however, any Citrus species is vulnerable to HLB. In addition, some related plants in the genus Rutaceae, and other plants may become infected with *Ca.* Liberibacter species. Those of skill in the art are able to test for infection by *Ca.* Liberibacter, and therefore are able to determine which plants suffer from HLB or *Ca.* Liberibacter infection.

[0008] Current methods in use for HLB control include the use of HLB-free citrus seedlings, destruction of infected trees, and application of insecticides such as aldicarb (Temik®) or imidacloprid (Admire®). These insecticides are aimed at controlling psyllids, the insect vector for the disease, although it is not known if insecticides have a direct effect on the spread of HLB. These insecticide treatments do not reduce disease in trees already infected, in any case. An integrated control program has been recommended for HLB in commercial orchards by the United Nations Development Program, Food and Agriculture Organization (UNDP, FAO) Southeastern Asian citrus rehabilitation project (Aubert,1990). The program highlights controlling psyllid vectors with insecticides, reducing inoculum through removal of HLB-symptomatic trees, propagating and using pathogen-free budwood and nursery trees. In Florida, foliar nutrition programs coupled with vector control are often used to slow down the spread of HLB and reduce devastating effects of the disease (Gottwald, 2010). These control practices have shown limited effect for preventing the further spread of HLB. Other than destruction and removal of diseased trees, there is no effective control for HLB in infected trees, and there is no known cure for HLB. New and improved treatments for citrus (and other) HLB disease therefore are needed in the art.

SUMMARY

[0009] It has now been found that glyphosate kills *Candidatus* Liberibacter species, including L. crescens which is similar to L. asiaticus (causal bacteria for HLB) and is used as a surrogate for screening because it can be cultured in the laboratory. Therefore, the present invention provides a method for treatment and prophylaxis of HLB in citrus. Specifically, the invention primarily relates to a method of treatment or prophylaxis of huanglongbing disease of citrus, comprising administering to a citrus plant in need thereof a composition containing glyphosate. The inventive methods also can be used to treat *Ca.* Liberibacter diseases in other plants, such as glyphosate resistant tomato and potato.

BRIEF DESCRIPTION OF THE FIGURES

[0010] FIG 1 is a bar graph showing live cell (Liberibacter crescens) fluorescence intensity for the indicated glyphosate concentrations in the indicated media in culture.

[0011] FIG 2 is a bar graph showing the percentage of Liberibacter crescens viable after 3 days incubation, normalized to control, in the indicated media in culture.

[0012] FIG 3 is a bar graph showing live cell (Liberibacter crescens) fluorescence intensity for the indicated glyphosate concentrations in the indicated media in culture.

[0013] FIG 4 is a bar graph showing the percentage of Liberibacter crescens viable after 3 days incubation, normalized to control, in the indicated media in culture.

DETAILED DESCRIPTION

[0014] 1. Introduction

[0015] The invention is described herein with reference to specific embodiments. However, various modifications and changes can be made to the invention without departing from its broader spirit and scope. The specification and drawings therefore are to be regarded as illustrative rather than restrictive. Throughout this specification and the claims, unless the context requires otherwise, the word "comprise" and its variations, such as "comprises" and "comprising," are used to imply the inclusion of a stated item, element or step or group of items, elements or steps but not the exclusion of any other item, element or step or group of items, elements or steps. Furthermore, the indefinite article "a" or "an" is meant to indicate one or more of the item, element or step modified by the article.

[0016] All technical and scientific terms used herein, unless defined herein, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. The techniques employed herein are also those that are known to one of ordinary skill in the art, unless stated otherwise.

[0017] 2. Definitions

[0018] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although various methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. However, the skilled artisan understands that the methods and materials used and described are examples and

may not be the only ones suitable for use in the invention. Moreover, as measurements are subject to inherent variability, any temperature, weight, volume, time interval, pH, salinity, molarity or molality, range, concentration and any other measurements, quantities or numerical expressions given herein are intended to be approximate and not exact or critical figures unless expressly stated to the contrary.

[0019] The term "about," as used herein, means plus or minus 20 percent of the recited value, so that, for example, "about 0.125" means 0.125 ±0.025, and "about 1.0" means 1.0 ±0.2.

[0020] The term "citrus plant," as used herein, refers to a mature plant, seed, cutting, embryo, seedling, and/or sapling, and the like of any citrus variety.

[0021] The term "Citrus" or "citrus," as used herein, refers to any plant of the genus *Citrus*, family Rutaceae, and includes *Citrus maxima* (Pomelo), *Citrus medica* (Citron), *Citrus micrantha* (Papeda), *Citrus reticulata* (Mandarin orange), *Citrus trifolata* (trifoliate orange), *Citrus japonica* (kumquat), *Citrus australasica* (Australian Finger Lime), *Citrus australis* (Australian Round lime), *Citrus glauca* (Australian Desert Lime), *Citrus garrawayae* (Mount White Lime), *Citrus gracilis* (Kakadu Lime or Humpty Doo Lime), *Citrus inodora* (Russel River Lime), *Citrus warburgiana* (New Guinea Wild Lime), *Citrus wintersii* (Brown River Finger Lime), *Citrus halimii* (*limau kadangsa*, *limau kedut kera*) *Citrus indica* (Indian wild orange), *Citrus macroptera*, and *Citrus latipes*. Hybrids also are included in this definition, for example *Citrus* × *aurantiifolia* (Key lime), *Citrus* × *aurantium* (Bitter orange), *Citrus* × *latifolia* (Persian lime), *Citrus* × *limon* (Lemon), C*itrus* × *limonia* (Rangpur), *Citrus* × *paradisi* (Grapefruit), *Citrus* × *sinensis* (Sweet orange), C*itrus* × *tangerina* (Tangerine), *Poncirus trifoliata* x *C. sinensis* (Carrizo citrange), *C. paradisi* "Duncan" grapefruit x *Pondirus trifoliate* (Swingle citrumelo), and any other known species or hybrid of genus *Citrus*. Citrus known by their common names include, Imperial lemon, tangelo, orangelo, tangor, kinnow, kiyomi, Minneola tangelo, oroblanco, sweet orange, ugli, Buddha's hand, citron, lemon, orange, bergamot orange, bitter orange, blood orange, calamondin, clementine, grapefruit, Meyer lemon, Rangpur, tangerine, and yuzu, and these also are included in the definition of citrus or Citrus.

[0022] The term "Huanglongbing disease," as used herein, is a disease of plants caused by microorganisms of the *Candidatus* genus Liberibacter, such as L. asiaticus, L. africanus, and L. americanus. This disease, for example, can be found in citrus plants, or other plants in the genus *Rutaceae*. Symptoms of Huanglongbing disease include one or more of yellow shoots and

mottling of the plant leaves, occasionally with thickening of the leaves, reduced fruit size, fruit greening, premature dropping of fruit from the plant, low fruit soluble acid content, fruit with a bitter or salty taste, or death of the plant. The term "treating" or "treatment," or its cognates, as used herein indicates any process or method which cures, diminishes, ameliorates, or slows the progress of the disease or disease symptoms. Thus, treatment includes reducing bacterial titer in plant tissues or appearance of disease symptoms relative to controls which have not undergone treatment.

[0023] The term "applying," "application," "administering," "administration," and all their cognates, as used herein, refers to any method for contacting the plant with the glyphosate compositions discussed herein. Administration generally is achieved by application of the glyphosate, in a vehicle compatible with the plant to be treated (i.e., a botanically compatible vehicle or carrier), such as an aqueous vehicle, to the plant. Any application means can be used, however preferred application is foliar spraying. Other methods include application to the soil surrounding the plant, by injection, soaking or spraying, so that the applied glyphosate compound preferably comes into contact with the phloem of the plant.

[0024] The term "plant in need thereof," as used herein, means any plant which is healthy or which has been diagnosed with a plant disease or symptoms thereof, or which is susceptible to a plant disease, or may be exposed to a plant disease or carrier thereof.

[0025] The term "population of plants," as used herein, refers to a group of plants, all of the same species or all citrus plants, that inhabit a particular area at the same time. Therefore, the plants in a nursery, a grove, a farm, and the like are considered a population.

[0026] The term "effective amount" or "therapeutically effective amount," as used herein, with respect to treatment means any amount of the glyphosate compound or a composition containing this compound, which reduces the symptoms of HLB disease in a citrus plant or population of citrus plants, reduces the amount of pathogenic bacteria in a citrus plant or population of citrus plants, improves health, growth or productivity of the plant, or which reduces the effects, titer or symptoms of the plant disease, or prevents worsening of the plant disease, symptoms or infection of the plant. This term includes an amount effective to increase seed germination of a plant or a plant population, to increase the speed of seed germination of a plant or a plant population, to increase growth rates of a plant or a plant population, to increase crop yield of a plant or plant population, increase crop quality in a plant or plant population, reduce the plant pathogen titer, to

inhibit plant pathogen growth, to reduce the percent of infected plants in a plant population, to reduce the percent of plants showing disease symptoms in a plant or plant population, to reduce the disease symptom severity rating or damage rating of a plant or plant population, to reduce average pathogen population or titer in a plant or plant population by about 2%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, or more, compared to plants or a plant population not treated with the active ingredient.

[0027] The term "treating," "treatment," and all their cognates, as used herein, refers to any application or administration to a plant, the soil surrounding the plant, the water applied to the plant, or the hydroponic system in which the plant is grown, which is intended to improve the health, growth or productivity of a plant, particularly a crop plant, that is affected by HLB or symptoms of HLB. For example, a treatment intended to increase the health or growth or a crop plant, increase crop yield of a plant or population of plants is contemplated as part of this definition, as well as treatment intended to improve disease symptoms or pathogen titer in the plant.

[0028] The term "reduction of disease symptoms," as used herein, refers to a measurable decrease in the number or severity of disease symptoms.

[0029] The term "effective amount" or "therapeutically effective amount," as used herein, with respect to prophylaxis, means any amount of the glyphosate compound or a composition containing this compound, which reduces the likelihood of HLB disease in a citrus plant or a population of citrus plants, reduces the likelihood of any HLB disease symptom to a citrus plant or a population of citrus plants, reduces the spread of the disease to a citrus plant or a population of citrus plants. This term includes an amount effective to reduce the chance of HLB disease infection or to produce improved resistance to disease in a citrus plant or a population of citrus plants by about 2%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, or more, compared to plants or a plant population not treated with the active ingredient.

[0030] The term "prophylactic," "prophylaxis," and all their cognates, as used herein, refers to any application or administration to a plant, the soil surrounding the plant, the water applied to the plant, or the hydroponic system in which the plant is grown, which is intended to improve the health, growth or productivity of a plant, particularly a crop plant, that can be affected by HLB or

symptoms of HLB, including citrus plants that are not currently affected by HLB. For example, a prophylactic application intended to increase the health or growth or a crop plant, increase crop yield of a plant or population of plants, or intended to prevent or decrease the likelihood of infection with HLB disease is contemplated as part of this definition.

[0031] The term "improved resistance to disease," as used herein, refers to an increase of plant defense in a healthy plant or a decrease in disease severity in a plant or in a population of plants, or in the number of diseased plants or plants exhibiting plant disease symptoms in a plant population. The term "plant disease symptoms," as used herein, refers to any symptom of disease, including the detectable presence of a known plant pathogen, or the presence of rot, mottling, galls, discoloration such as yellowing or browning, fruit greening, stunted growth, plant death, cellular death, cell wall breakdown, and/or the presence of spots, lesions, dieback, wilting, dwarfing, Witch's broom and/or knots.

[0032] The term "botanically acceptable carrier/vehicle" or "botanically compatible carrier/vehicle," as used herein, refers to any non-naturally occurring vehicle, in liquid, solid or gaseous form which is compatible with use on a living plant and is convenient to contain a substance or substances for application of the substance or substances to the plant, its leaves or root system, its seeds, the soil surrounding the plant, or for injection into the trunk, or any known method of application of a compound to a living plant, preferably a crop plant, for example a citrus tree, citrus seedling, and the like. Useful vehicles can include any known in the art, for example liquid vehicles, including aqueous vehicles, such as water, solid vehicles such as powders, granules or dusts, or gaseous vehicles such as air or vapor. Any vehicle which can be used with known devices for soaking, drenching, injecting into the soil or the plant, spraying, dusting, or any known method for applying a compound to a plant, is contemplated for use with embodiments of the invention. Typical carriers and vehicles contain inert ingredients such as fillers, bulking agents, buffers, preservatives, anti-caking agents, pH modifiers, surfactants, soil wetting agents, adjuvants, and the like. Suitable carriers and vehicles within this definition also can contain additional active ingredients such as plant defense inducer compounds, nutritional elements, fertilizers, pesticides, and the like.

3. Exemplary Embodiments

[0033] Glyphosate (N-(phosphonomethyl)glycine) is a broad spectrum systemic

organophosphate herbicide and crop desiccant used to kill weeds, especially weeds that compete with crops and reduce crop yield. It is the active ingredient in the popular herbicide composition ROUNDUP (Montanso®). ROUNDUP and other glyphosate herbicides generally are used for destruction of weeds by application to genetically engineered crops with altered DNA that allows them to withstand the glyphosate herbicide. These crops are commonly referred to as "ROUNDUP Ready." Any glyphosate compound or composition containing glyphosate is contemplated for use with the invention.

[0034] In brief, the work pertaining to this disclosure has shown that glyphosate kills *L. crescens*, a model for species causing HLB disease, at a concentration less than that which kills or seriously harms citrus. Citrus plants are relatively refractory to the broad spectrum herbicide, glyphosate. Thus, aspects of the invention pertain to a method of treatment and/or prophylaxis of HLB disease using application(s) of glyphosate in plants which either have or are subject to infection with HLB disease and other diseases related to *Ca.* Liberibacter. The glyphosate treatments could also be used to treat other plant diseases caused by *Ca.* Liberibacter, such as diseases of tomato and potato which are caused by *Ca.* Liberibacter species. For these plants, which are more susceptible to glyphosate, glyphosate-resistant or ROUNDUP Ready™ plants would be necessary in using the inventive methods.

[0035] ROUNDUP or other glyphosate formulations can be used at low concentrations (about 0.5mM to about 50mM to kill these bacteria without killing citrus, which is counterintuitive and runs against the common wisdom, which indicates that one should avoid contacting glyphosate with crop plants, particularly those which are not ROUNDUP Ready™. At these very low concentrations, glyphosate is able to kill the disease bacteria while sparing the plant. In certain method embodiments of the invention, the citrus plant is healthy. In optional embodiments, the citrus plant has been previous modified to be resistant to glyphosphate. In other method embodiments, the citrus plant is affected by HLB disease or HLB disease symptoms.

[0036] The product to be applied to plants for control of citrus greening disease preferably is any glyphosate formulation suitable as a foliar spray for citrus. The glyphosate dose used for application to the plants preferably is low enough to be well-tolerated by citrus but high enough to enter phloem in a high enough amount to kill the *Ca.* Liberibacter pathogen. Such lower concentration glyphosate formulations also can be used on glyphosate-resistant lines of tomato and potato as a treatment for or for prophylaxis of diseases caused by *Ca.* Liberibacter

solanacearum in these plants. Persons of skill are able to determine doses of glyphosate to be applied to crop plants, whether glyphosate resistant or not.

[0037] The citrus for use with the invention advantageously can be selected from the group consisting of *Citrus maxima* (Pomelo), *Citrus medica* (Citron), *Citrus micrantha* (Papeda), *Citrus reticulata* (Mandarin orange), *Citrus paradisi* (grapefruit), *Citrus trifolata* (trifoliate orange), *Citrus japonica* (kumquat), *Citrus australasica* (Australian Finger Lime), *Citrus australis* (Australian Round lime), *Citrus glauca* (Australian Desert Lime), *Citrus garrawayae* (Mount White Lime), *Citrus gracilis* (Kakadu Lime or Humpty Doo Lime), *Citrus inodora* (Russel River Lime), *Citrus warburgiana* (New Guinea Wild Lime), *Citrus wintersii* (Brown River Finger Lime), *Citrus halimii* (*limau kadangsa*, *limau kedut kera*) *Citrus indica* (Indian wild orange), *Citrus macroptera*, and *Citrus latipes*, *Citrus* × *aurantiifolia* (Key lime), *Citrus* × *aurantium* (Bitter orange), *Citrus* × *latifolia* (Persian lime), *Citrus* × *limon* (Lemon), C*itrus* × *limonia* (Rangpur), *Citrus* × *sinensis* (Sweet orange), C*itrus* × *tangerina* (Tangerine), Imperial lemon, tangelo, orangelo, tangor, kinnow, kiyomi, Minneola tangelo, oroblanco, sweet orange, ugli, Buddha's hand, citron, lemon, orange, bergamot orange, bitter orange, blood orange, calamondin, clementine, grapefruit, Meyer lemon, Rangpur, tangerine, and yuzu.

[0038] Glyphosate-containing compositions according to embodiments of the invention preferably include a botanically acceptable vehicle or carrier, preferably a liquid, aqueous vehicle or other carrier such as water, and glyphosate. Typically, the composition has a concentration of 0.5mM to 850.5mM glyphosate, and more typically about 35-50 mM. In a specific embodiment, the composition contains 42.5 mM glyphosate. The composition may be formulated as an emulsifiable concentrate(s), suspension concentrate(s), directly sprayable or dilutable solution(s), coatable paste(s), dilute emulsion(s), wettable powder(s), soluble powder(s), dispersible powder(s), dust(s), granule(s) or capsule(s), or any known or available composition.

[0039] When applying the glyphosate composition to citrus plants for control, treatment or prophylaxis of HLB disease, the glyphosate is provided to the plant at a concentration of about 0.5mM to about 100mM, and in a specific embodiment at about 35-50 mM.

[0040] The composition optionally also can include a botanically acceptable carrier that contains or is blended with additional active ingredients and/or additional inert ingredients. Active ingredients which can be included in the carrier formulation can be selected from any combination of pesticides, herbicides, plant nutritional compositions such as fertilizers, and the like. Plant inducer compounds such as salicylic acid or β-aminobutyric acid (BABA) also can be included in the compositions. Additional active ingredients can be administered simultaneously with the glyphosate compositions described here, in the same composition, or in separate compositions, or can be administered sequentially.

[0041] Inert ingredients which can be included in the carrier formulation can be selected from any compounds to aid in the physical or chemical properties of the composition. Such inert ingredients can be selected from buffers, salts, ions bulking agents, colorants, pigments, dyes, fillers, wetting agents, dispersants, emulsifiers, penetrants, preservatives, antifreezes, evaporation inhibitors, nutrient compounds, anti-caking agents, defoamers, antioxidants, and the like.

[0042] Persons of skill are aware of various methods to apply compounds such as herbicides, including glyphosate, to plants for surface application or for uptake, and any of these methods are contemplated for use in this invention. Methods of administration to plants include, by way of non-limiting example, application to any part of the plant, by inclusion in irrigation water, by injection to the plant or to the soil surrounding the plant, or by exposure of the root system to aqueous solutions containing the compounds, by use in hydroponic or aeroponic systems, by seed treatment, by exposure of cuttings of citrus plants used for grafting to aqueous solutions containing the compounds, by application to the roots, stems or leaves, by application to the plant interior, or any part of the plant to be treated. Any means known to those of skill in the art is contemplated

[0043] Application of the glyphosate compound or composition according to the invention can be performed in a nursery setting, a greenhouse, hydroponics facility, or in the field, or any setting where it is desirable to treat plants which have been or can become exposed to a plant disease, such as HLB or *Ca.* Liberibacter infection. The methods of this invention can be used for treatment or prophylaxis of infection with HLB. Thus, any plant in need, in the context of this invention, includes any plant susceptible to or infected with HLB, in the judgement of the person of skill in this and related arts.

[0044] Application to hydroponic or culture media preferably is performed as follows, however any method known in the art can be used. A solution or vehicle containing glyphosate at a concentration of about 42.5 mM to about 850.5 mM can be added into the hydroponic or culture media at final concentrations suitable for plant growth and development. The concentrations, and volumes may change depending on the plant, and can be determined by one of skill in the art.

[0045] Application to seeds is preferably accomplished as follows, however any method known in the art can be used. Seeds may be treated or dressed prior to planting, by soaking the seeds in a solution containing glyphosate at a concentration of about 42.5 mM to about 850.5 mM over a period of minutes or hours.

[0046] Application to the stems or leaves of the plant preferably is performed by spraying or other direct application to the desired area of the plant, however any method known in the art can be used. A solution or vehicle containing glyphosate at a concentration of about 0.5mM to about 42.5 mM may be applied with a sprayer to the stems or leaves until runoff to ensure complete coverage, and repeat three or four times in a growing season. The concentrations, volumes and repeat treatments may change depending on the plant and can be determined by one of skill in the art.

[0047] The inventors here have been able to show that glyphosate is able to inhibit or kill *Ca*. Liberibacter crescens at concentrations of glyphosate that are not harmful to citrus plants or to glyphosate-resistant crop plants, therefore producing a method suitable for treatment and prophylaxis of crop diseases caused by *Ca*. Liberibacter species in citrus and any other plants so affected by these bacterial diseases. This is a new approach (using a herbicide used to treat a plant disease) to solving the citrus greening problem, which is simple and relatively inexpensive. Glyphosate is already registered for use on citrus. Adapting that registration for the treatment of citrus greening disease should not be expensive or time consuming.

[0048] From the data presented here, it is apparent that 25 mM concentrations in culture are sufficient to reach the minimum inhibitory concentration$_{50}$ (MIC_{50}) even in media rich in amino acids. Lower concentrations of glyphosate are necessary to reach the MIC_{50} in M17 medium (with phloem levels of aromatics) in these experiments. It is possible that these effects in these results could be inflated because *L. crescens* wasn't growing optimally in this media prior to the addition of glyphosate.

4. Examples

[0049] This invention is not limited to the particular processes, compositions, or methodologies described, as these may vary. The terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein, are incorporated by reference in their entirety; nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0050] Example 1:

[0051] FIGs. 1-4 present data showing that glyphosate is able to significantly inhibit Liberibacter crescens in culture. The effects are particularly noticeable in the M17 phloem medium, a defined medium that contains the same concentrations of aromatic amino acids found in citrus phloem. The M17 medium lacks those amino acids entirely. M15 and HiG media have high concentrations of the aromatic amino acids. The initial pH of the media was 5.92 in each case and was maintained in the controls after 4 days incubation.

[0052] While a number of embodiments of the present invention have been shown and described herein in the present context, such embodiments are provided by way of example only, and not of limitation. Numerous variations, changes and substitutions will occur to those of skill in the art without materially departing from the invention herein. Any means-plus-function and step-plus-function clauses are intended to cover the structures and acts, respectively, described herein as performing the recited function and not only structural equivalents or act equivalents, but also equivalent structures or equivalent acts, respectively. Accordingly, all such modifications are intended to be included within the scope of this invention as defined in the following claims, in accordance with relevant law as to their interpretation.

REFERENCES

[0053] All references listed below and throughout the specification are hereby incorporated by reference in their entirety.

1. United States Patent Publication No. 2017-0044560A1 to Forrest Innovations Ltd. (15/306095).
2. Chinese Patent No. 04397B to South China Agricultural University.
3. Chinese Patent Application No. CN101589725A to Institute of Botany Protection, Guangxi Zhuang Autonomous Region Academy of Agricultural Sciences.
4. Johal and Huber, Eur. J. Agronomy, 31(3):144-152, 2009.
5. Huber and Jones, Plant and Soil, 368(1-2):73-85, 2012.
6. Barriuso et al., Env. Microbiol., 12(4):1021-1030, 2010.
7. Gravena et al., Can. J. Plant Sci., 92:119-127, 2012.
8. Gravena et al., Pest Manag. Sci., 65:420-425, 2009.

CLAIMS

1. A method of treatment or prophylaxis of huanglongbing disease of citrus, comprising administering to a citrus plant in need thereof a composition comprising glyphosate.

2. The method of claim 1, wherein the composition is administered at an amount that kills *Candidatus* Liberibacter but which does not kill the citrus plant.

3. The method of either of claim 1 or 2 wherein glyphosphate is administered to the citrus plant at a concentration of at least 0.5mM glyphosphate.

4. The method of claim 1, wherein glyphosphate is administered to the citrus plant at a concentration of no more than 850.5 mM glyphosphate.

5. The method of claim 1, wherein glyphosphate is administered to the citrus plant at a concentration range of 0.5mM to 850.5 mM glyphosphate.

6. The method of any of claims 1-5, wherein the composition further comprises a botanically acceptable carrier/vehicle.

7. The method of any of claims 1-6, wherein the composition is administered via foliar spray.

8. The method of any of claims 1-7, wherein glyphosphate is administered at a range of 0.5mM to 100mM.

9. The method of any of claims 1-8, wherein glyphosphate at a range of 35 to 50 mM.

10. An article of manufacture comprising a container and glyphosphate disposed within the container, wherein the glyphosphate is comprised of an aqueous solution comprising a concentration of glyphosphate of 850.5 mM or less.

11. The article of manufacture of claim 10, where the concentration of glyphosphate is 100mM or less.

12. The article of manufacture of claim 10, where the concentration of glyphosphate is in a range of 1-100mM.

FIG. 1

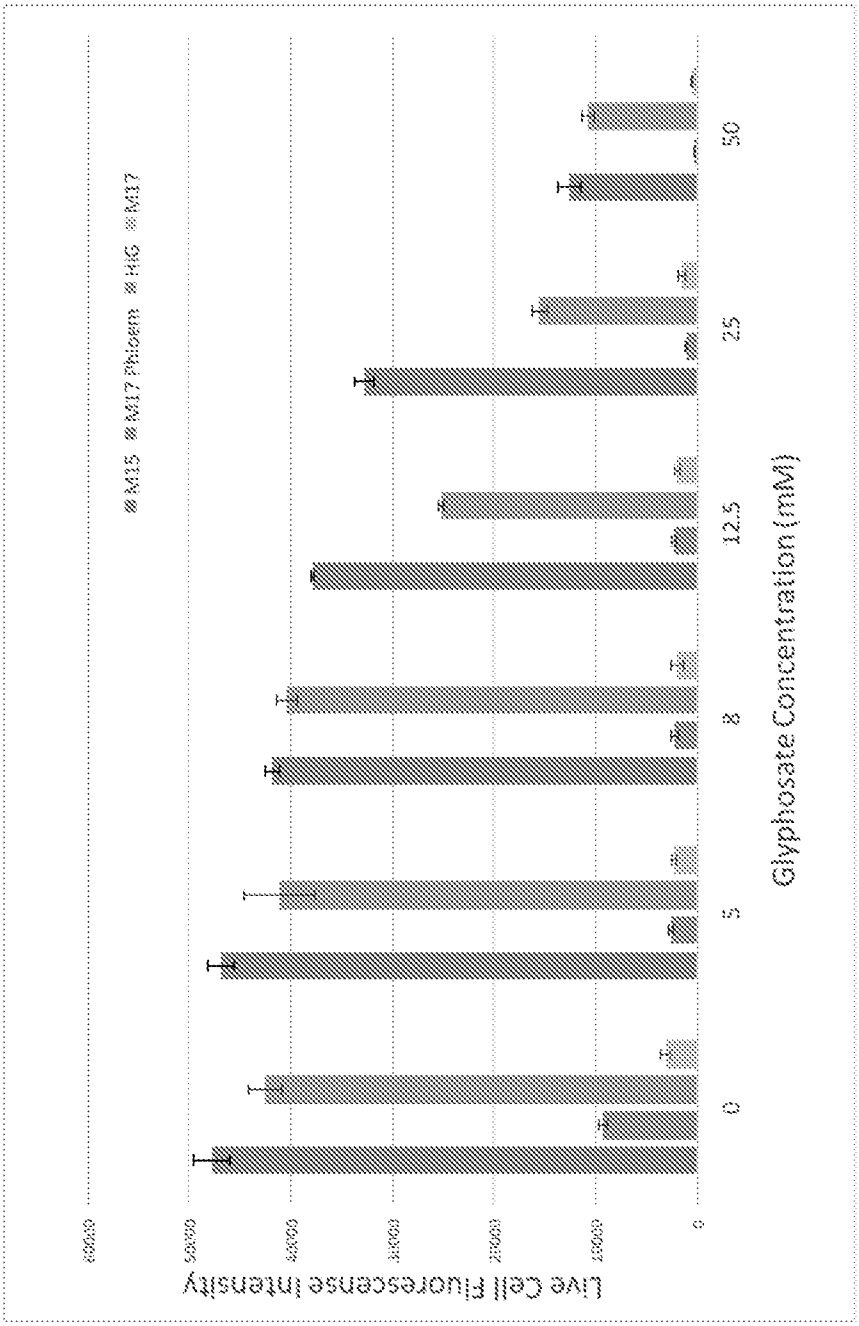

M15
M17 Rhizem
HG
M17
Live Cell Fluorescense Intensity
0
5
8
12.5
25
50
Glyphosate Concentration (mM)

FIG. 2

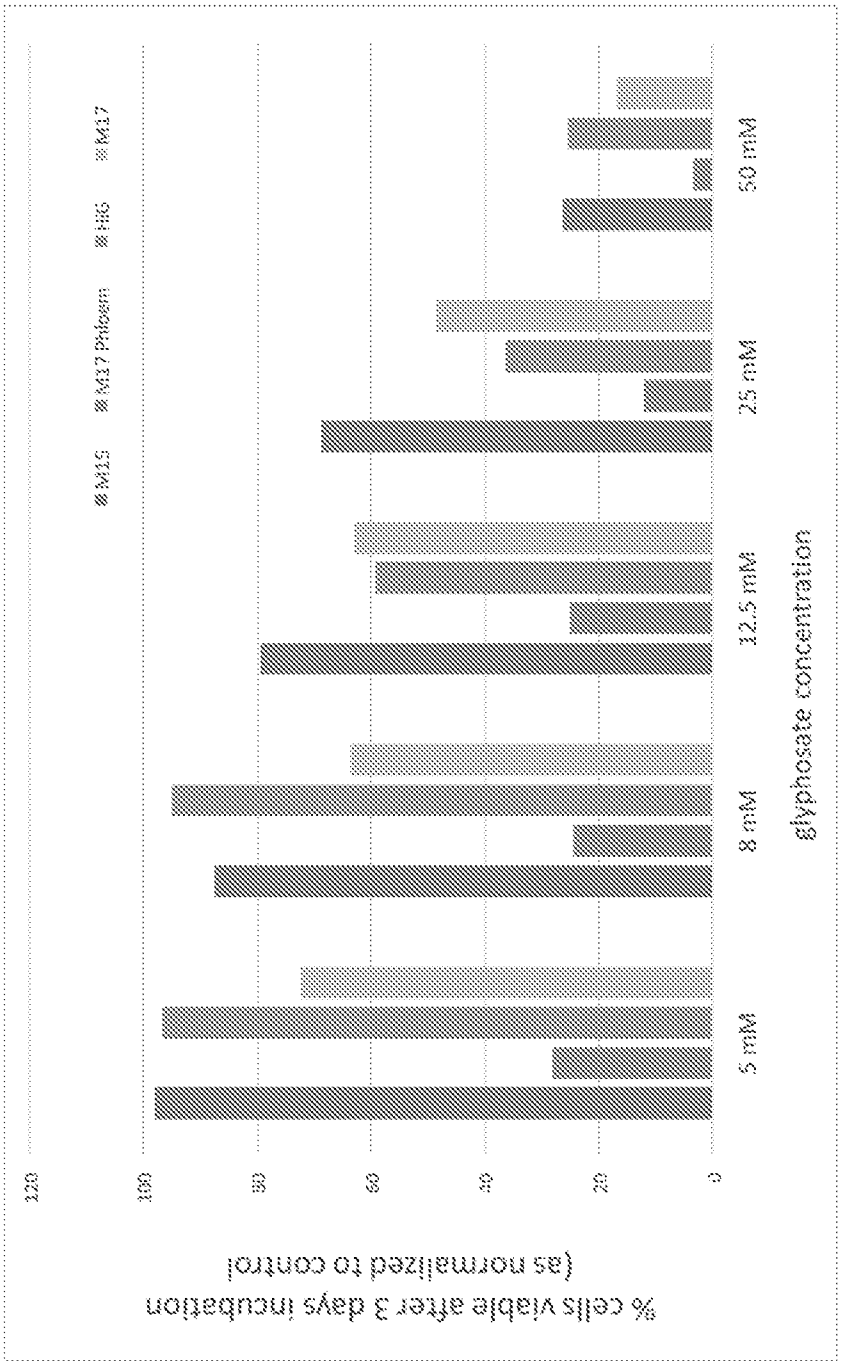

% cells viable after 3 days incubation
(as normalized to control
120
100
80
60
40
20
0
M15
M17 Phoem
H6
M17
5 mM
8 mM
12.5 mM
25 mM
50 mM
glyphosate concentration

FIG. 3

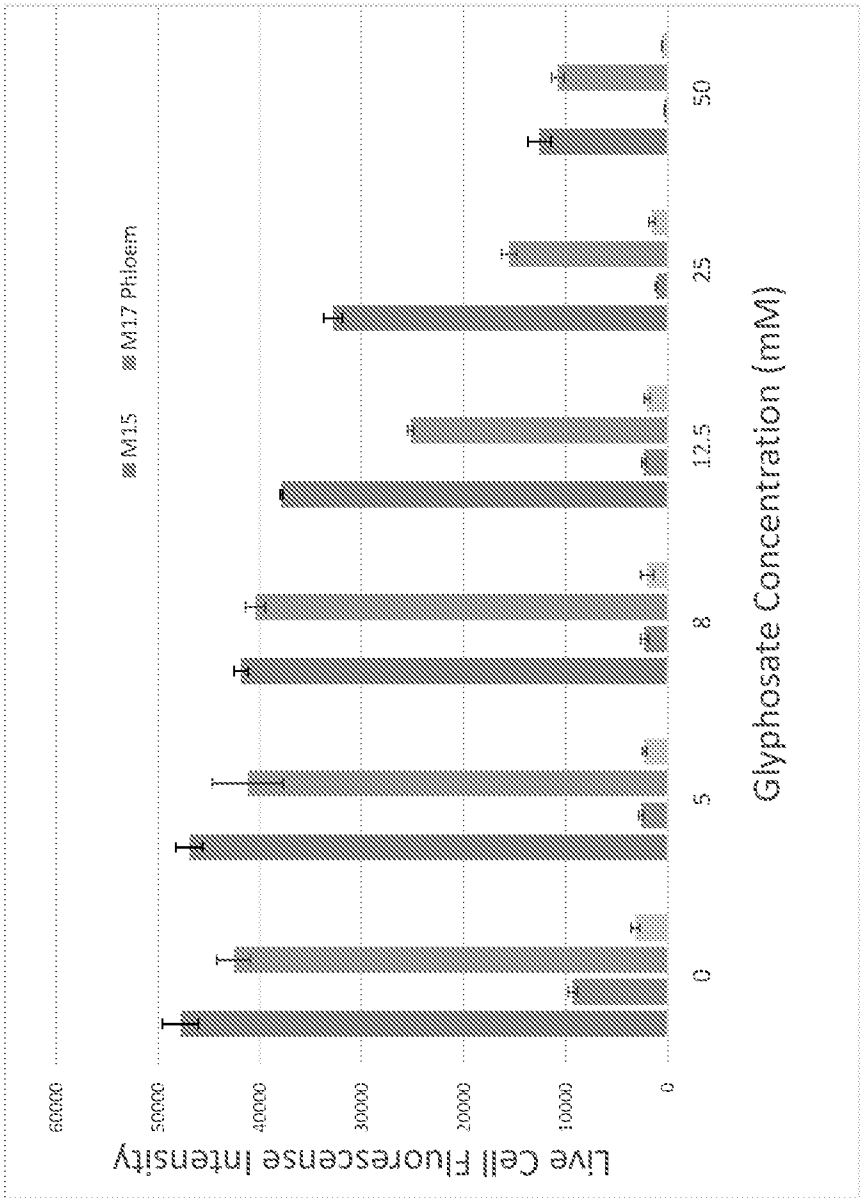
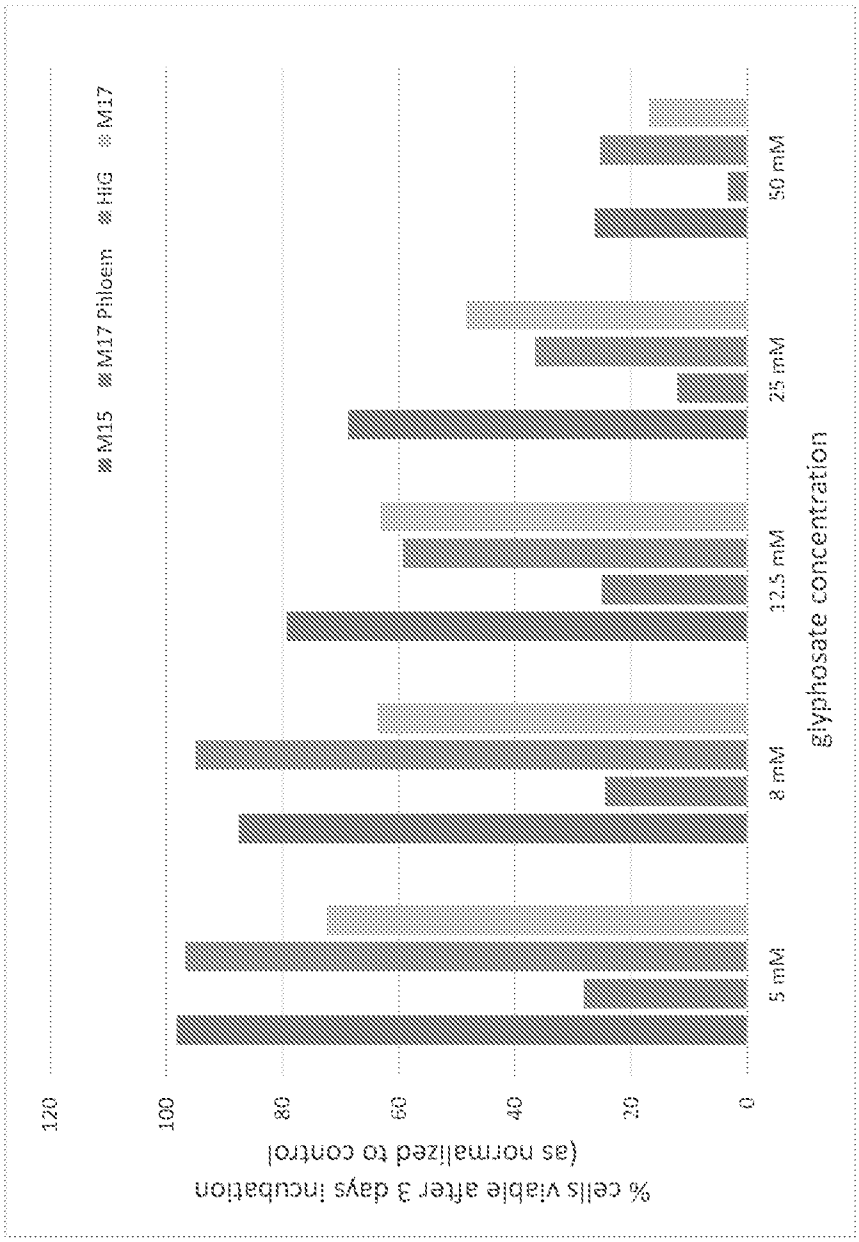

Live Cell Fluorescense Intensity
60000
50000
40000
30000
20000
10000
0
M15
M17 Ptiloem
0
5
8
12.5
25
50
Glyphosate Concentration (mM)

FIG. 4


M15
M17 Phloem
HIG
M17
% cells viable after 3 days incubation
(as normalized to control)
120
100
80
60
40
20
0
5 mM
8 mM
12.5 mM
25 mM
50 mM
glyphosate concentration

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/58956

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A01N 63/00; C05F 11/08; C05G 3/00 (2020.01)

CPC - A01N 63/00; A01N 63/04; C05F 11/00; C05F 11/08; C05G 3/00; C05G 3/0064; C09K 17/14

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	CN 101589725 A (Institute of Botany Protection Guangxi Zhuang) 02 December 2009 (02.12.2009); entire document, especially abstract, pg 2 para 1	1
X	US 2015/0126423 A1 (Monsanto Technology LLC) 07 May 2015 (07.05.2015); entire document, especially abstract, [0083] Table 12B	10-12
X	US 2017/0044560 A1 (Forrest Innovations Ltd.) 16 February 2017 (16.02.2017); entire document, especially abstract, [0226], [0229]	1-5

☐ 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
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"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
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"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 January 2020	06 FEB 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/58956

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-9
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.