



US 20090282588A1

(19) **United States**

(12) **Patent Application Publication**  
**Scheer et al.**

(10) **Pub. No.: US 2009/0282588 A1**

(43) **Pub. Date: Nov. 12, 2009**

(54) **METHODS FOR REDUCING LEAF  
SENESCENCE USING METHYL  
DIHYDROJASMONATE**

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(21) Appl. No.: **12/424,790**

(22) Filed: **Apr. 16, 2009**

**Related U.S. Application Data**

(60) Provisional application No. 61/050,676, filed on May  
6, 2008.

**Publication Classification**

(51) **Int. Cl.**  
**A01H 5/00** (2006.01)  
**A01N 37/06** (2006.01)  
**A01H 5/12** (2006.01)

(52) **U.S. Cl.** ..... **800/323; 504/313; 800/298**

(57) **ABSTRACT**

Methods for reducing leaf senescence in plants or portions thereof by treating them with methyl dihydrojasmonate are disclosed. The methyl dihydrojasmonate may be in the form of an aqueous foliar spray, which may also include additives such as wetting agents, adjuvants, emulsifiers, dispersants, spreaders, surfactants, anchorage, disintegrants, and plant nutrients.

# METHODS FOR REDUCING LEAF SENESCENCE USING METHYL DIHYDROJASMONATE

## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to, and the benefit of, U.S. Provisional Patent Application No. 61/050,676, filed May 6, 2008, the contents of which are incorporated by reference in their entirety.

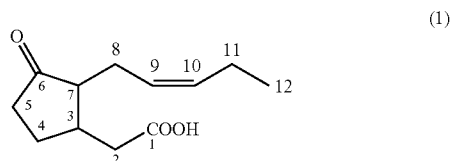
## BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] Generally speaking, the invention relates to the field of plant biology, and more particularly, to methods for reducing leaf senescence using methyl dihydrojasmonate.

[0004] 2. Description of Related Art

[0005] The jasmonates are a family of compounds related to jasmonic acid, 2-(3-oxo-2-(pent-2-enyl)cyclopentyl)acetic acid, the structure of which is shown below in Formula (1):



[0006] Jasmonates have been implicated in regulating a number of events in plant growth and development, as well as numerous types of plant responses to stressors. Osmotic stress or desiccation, touch, elicitation, wounding and pathogen and insect attack are all generally accompanied by increases in endogenous levels of jasmonates. Jasmonates are also widely used as flavoring and fragrance compounds because of their strong odor and taste characteristics.

[0007] In a number of studies, jasmonates have also been shown to promote leaf senescence, the process by which plant leaves age and ultimately die. Specifically, an extensive body of literature indicates that application of jasmonates, such as jasmonic acid (JA) and methyl jasmonate (MJ), promotes or accelerates leaf senescence in intact as well as excised plant leaves in such diverse plants as corn, rice, wheat, oat, barley, arabidopsis, sunflower, and zucchini (see, e.g., He et al., *Plant Physiology* 128 (2002), pp. 876-884; Ueda and Kato, *Plant Physiology* 66 (1980), pp. 246-249; Chou and Kao, *Plant Physiology* 99 (1992), pp. 1693-1694).

[0008] Aside from the purely biological implications of leaf senescence, the chlorosis (i.e., yellowing) and necrosis (i.e., tissue death) of senescing plant leaves can have commercial and practical implications. Senescing leaves may be unattractive, and may detract significantly from both the perceived health and the commercial value of a plant or a crop of plants. The problem may be particularly acute where plants or portions thereof are grown and harvested primarily for their aesthetic properties, as is the case with roses. Reducing the levels of senescing leaves may also substantially improve the

value of crops grown for food, particularly where the leaves of the plant are the edible portion (e.g., spinach).

## SUMMARY OF THE INVENTION

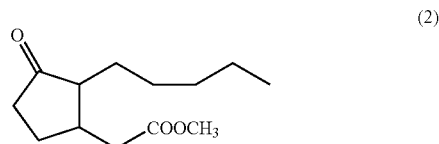
[0009] One aspect of the invention relates to a method of reducing leaf senescence in a plant by treating the plant with an effective amount of methyl dihydrojasmonate. The methyl dihydrojasmonate may be in a variety of different forms, including emulsions, suspensions, powders, hydrates, solutions, granules, pastes, aerosols, and volatile formulations, and other additives and compounds may be included in the formulation. In one embodiment, the methyl dihydrojasmonate may be in the form of an aqueous solution, accompanied by a surfactant and an oil. The MDHJ may also be provided or co-administered with plant nutrients. After treatment, in some embodiments, the plant, or a portion thereof, may be harvested.

[0010] Another aspect of the invention relates to plants and portions thereof exhibiting reduced leaf senescence. The plants are prepared by a process comprising treating the plants with an effective amount of methyl dihydrojasmonate.

[0011] Other aspects, features, and advantages of the invention will be set forth in the description that follows.

## DETAILED DESCRIPTION

[0012] The present inventors have found that 9,10-dihydromethyl jasmonate, also called methyl dihydrojasmonate (MDHJ), the general structure of which is given below in Formula (2):



is surprisingly effective in reducing naturally occurring leaf senescence when administered to a plant in an effective amount. This effect is contrary to what would be expected from the literature on jasmonate effects in leaf senescence.

[0013] Plants to which MDHJ may be applied to reduce leaf senescence include, but are not limited to, angiosperms, gymnosperms, monocots, dicots, roses, tomatoes, crop plants, ornamental plants, turf plants, shrubs, trees, exotic plants, house plants, and native plants in cultivated or natural environments. MDHJ may also be applied to plants grown for food, particularly where the leaves are the edible or commercially desirable portion of the plant. MDHJ has been found to be particularly efficacious in roses and spinach.

[0014] The MDHJ may be applied alone or in a formulation comprising other elements, compounds, or substances. Some examples of other compounds that may be included in the formulation include wetting agents, adjuvants, emulsifiers, dispersants, spreaders, stickers, pastes, anchorage agents, fixatives, extenders, coating agents, buffering agents, plant nutrients, absorptive additives, and disintegrants. The formulation may also include acids, bases, or other compounds that adjust or maintain the final pH of the formulation in order to increase solubility of certain compounds in the formulation or for other reasons. Those of skill in the art will recognize that

a single ingredient may perform multiple functions, and may thus be classified or grouped in different ways.

**[0015]** Particular examples of formulation ingredients include ionic, non-ionic, and zwitterionic surfactants, such as TRITON® X-100, TRITON® X-114, NP-40, SILWET, and sodium dodecyl sulfate; alcohols; synthetic or natural oils, such as castor oil, canola (rapeseed) oil, and soybean oil; soaps; and adjuvants derived from natural sources, such as lecithin, saponin, cocodiethanolamide, and extracts from yucca, coconut, and pine. Additionally, for example, citric acid may be used to acidify a formulation, and compounds such as dipotassium phosphate, calcium carbonate, and potassium silicate may be used to raise the pH.

**[0016]** In some embodiments, it may be beneficial to use ingredients that are high in compounds that play a role in the octadecanoic pathway. For example, canola oil is high in linoleic and linolenic acids, compounds that play a role in the octadecanoic pathway. Soaps of linoleic, linolenic, and cis-7,10,13-hexadecatrienoic acids may also be desirable formulation ingredients in some embodiments.

**[0017]** An MDHJ formulation used in embodiments of the invention may also include fixative and extender compounds, in order to reduce volatility and evaporation of the active ingredient or ingredients, so as to increase exposure of the plant to the active ingredient. Exemplary fixatives include canola oil, castor oil, benzoyl benzoate, benzyl salicylate and synthetic musks, and sandalwood. Gums, waxes, and other carbohydrates, such as carnauba wax, carob gum, dextrins, dextrose, gellan gum, guar gum, paraffin wax, sorbitol, xanthan gum, polyvinylpyrrolidone, and glycerin, may also be used as fixatives.

**[0018]** Absorptive additives may also be included for extending the release and exposure time. Exemplary absorptive additives include, but are not limited to, silica gel; precipitated crystalline-free silica gel; amorphous, fumed, crystalline-free silica; amorphous, precipitated gel silica; silica hydrate; vitreous silica; silicic acid; and silicon dioxide.

**[0019]** Alone or in combination with other ingredients, the MDHJ may be delivered in the form of emulsions, suspensions, powders, hydrates, solutions, granules, pastes, aerosols, and volatile formulations. If MDHJ is delivered in the form of a solution, it may be in solution with any compatible solvent, including aqueous (water) solutions, alcohol (e.g., ethanol) solutions, or in combinations of solvents (e.g., water/ethanol.) In general, a “compatible solvent,” as the term is used here, refers to any solvent in which MDHJ is at least slightly soluble and which is not phytotoxic in the amounts or concentrations used to apply the MDHJ.

**[0020]** Forms of MDHJ may be adapted for application to the plant's foliage, roots, stems, flowers, or any other portion of the plant that is capable of absorbing it. Particularly advantageous forms include foliar sprays, root solutions, and pellet-based root preparations. As a root solution or preparation, jasmonates such as MDHJ may be formulated and applied to plants grown in soil, non-soil, artificial growing media, and/or hydroponic systems. In some embodiments, the MDHJ formulations may be combined with other active compounds that can be administered in the same fashion as the MDHJ formulation. Examples include fertilizers, seaweed, kelp, humic acid, and microbes. An MDHJ foliar spray may be combined with a foliar fertilizer, and a root solution may be combined with a fertilizer that is applied to the roots. Specific fertilizer and plant nutrient elements include, but are not limited to, nitrogen, potassium, phosphorus, calcium, mag-

nesium, which may be compounded in any known manner so as to be absorbable by the plant. For example, plant nutrients may include monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and magnesium sulfate ( $\text{MgSO}_4$ ).

**[0021]** As was noted above, the MDHJ would be applied in an “effective amount” to reduce leaf senescence. For purposes of this description, an effective amount of MDHJ is any amount of MDHJ that produces a noticeable decrease in leaf senescence as compared with untreated plants.

**[0022]** Effective amounts of MDHJ will vary from species to species and cultivar to cultivar, and will depend on the manner of application, the environmental conditions around the plant or plants, the form in which the MDHJ is administered, and the nature and type of additive compounds, if any, present in the formulation with the MDHJ. For example, if an MDHJ formulation is applied over a substantial portion of a plant's foliage, or is applied using a formulation that includes wetting agents, fixatives, and/or other additives intended to increase the level of exposure of the plant to the MDHJ, the formulation itself may contain a smaller amount or lower concentration of MDHJ than if an MDHJ formulation is applied over only a small portion of a plant's foliage, or without additives intended to increase the plant's exposure to the MDHJ. Similarly, if the MDHJ is administered in a form that tends to dwell on the plant's foliage, or in proximity to another part of the plant, then it may be administered in a lower concentration or amount.

**[0023]** As one example, an effective amount of MDHJ for reducing leaf senescence may comprise an aqueous solution with an MDHJ concentration in the range from about 0.15 mM to about 6 mM, inclusive. However, for some purposes, and in some species, concentrations up to about 10 mM may be used. As those of skill in the art will realize, in general, MDHJ may be used in even higher concentrations for some applications, provided that the total dose of MDHJ that is absorbed by the plant is not phytotoxic. Similarly, lower concentrations may be adequate in some situations, for example, in an enclosed environment or greenhouse.

**[0024]** One example of an aqueous MDHJ foliar spray formulation is given below in Table 1:

TABLE 1

Exemplary aqueous MDHJ Foliar Spray Formulation.				
Ingredient	g/L	ml/L	% by weight	% by volume
Water	993.411	993.411	99.3201%	99.341%
Methyl Dihydrojasmonate (1.5 mM)	0.339	0.339	0.0339%	0.034%
Canola Oil	4.600	5.000	0.4599%	0.500%
Triton X-100	1.325	1.250	0.1325%	0.125%
$\text{KH}_2\text{PO}_4$ - 2 mM	0.272		0.0272%	
$\text{MgSO}_4$ - 0.8 mM	0.197		0.0197%	
Citric Acid - 0.347 mM	0.067		0.0067%	
Total	1000.211	1000.000	1.000	1.000

**[0025]** In addition to liquid and aqueous preparations, MDHJ may be formulated for use in a slow-release application and provided in a granular- or pellet-based form, including fertilizer and/or pesticide components. MDHJ may be present in those formulations in weight/weight ratios of MDHJ to other ingredients in the range of 0.008% to 0.8%, and in some cases an effective ratio could be greater than

1.0% or less than 0.008%. Other inert or nutritive ingredients included in the pellets or granules can include binding agents and polymers, such as polysaccharides and polyvinylpyrrolidone, at 5-95%, a surfactant at 0.001-10%, and other absorptive ingredients, such as acrylamide and acrylamide polymers.

**[0026]** Formulations including MDHJ may be applied once or repeatedly, depending on the circumstances and the type of formulation, to treat a plant. For example, MDHJ formulations according to embodiments of the invention may be applied to the roots, foliage or some other part of a plant once or, alternatively, two or more times at defined intervals of time, such as every 2-14 days, every 30 days, or 1-2 times per month. The intervals at which the MDHJ is applied may vary. A plant may be treated with MDHJ whether or not it has senescing leaves at the time of treatment. Additionally, plants may be treated with MDHJ for purposes of reducing leaf senescence whether they are healthy or not. (For example, work by the present inventors has also shown that MDHJ is effective in reducing biotic attack and disease in plants; see, for example, U.S. Patent Application Publication No. 2009/0082453, the contents of which are incorporated by reference herein in their entirety. Therefore, a plant may be treated with MDHJ for multiple reasons.)

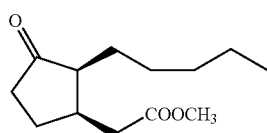
**[0027]** Among other factors, the environmental conditions around the plant or plants may influence the manner in which the MDHJ is applied or its frequency. For example, if the plants are field-grown or otherwise exposed to the elements, rain showers, excessive wind gusts, or other environmental factors shortly after an application, it may be desirable to reapply it. Under some circumstances, a more dilute formulation or solution may be used if repeated applications are to be performed.

**[0028]** Optionally, in at least some embodiments, a plant treated with MDHJ, or a portion thereof, may be harvested some time after the plant is treated with MDHJ. Harvesting may occur shortly after (e.g., several days after) treatment, or it may occur after sustained, relatively long-term treatment with MDHJ (e.g., several weeks or several months of treatment at regular intervals).

**[0029]** Treatment with MDHJ may reduce the levels of chlorosis and necrosis seen in plant leaves, and/or it may reduce the total number of senescing leaves, thus potentially improving the appearance of the plant and, consequently, its perceived health and/or commercial value.

#### EXAMPLES

**[0030]** The following examples serve to illustrate the efficacy of MDHJ in reducing leaf senescence. Unless otherwise noted, in the following examples, the MDHJ was obtained from Bedoukian Research, Inc. (Danbury, Conn, United States; product no. 398E). As supplied, the MDHJ solution was specified as having a minimum purity of 92.5%, of which 25-40% was the "epi" or "cis" isomer of MDHJ, shown as Formula (3) below:



(3)

**[0031]** Unless otherwise noted, percentages, for example, percentages of additional or inert formulation ingredients, are given as percentages by volume.

#### Example 1

##### Foliar MDHJ Treatment Reduces Senescence in Miniature Roses

**[0032]** Potted miniature PARADE® roses were evaluated for the effect on leaf senescence of foliar spray treatment with an MDHJ formulation. One group of four plants acted as an untreated control group and did not receive any foliar spray. Another group of four plants was sprayed with a formulation comprising 1.5 mM MDHJ, 0.5% TRITON® X-100, and 0.125% canola oil. The treated plants were sprayed on six occasions, on days 1, 3, 12, 29, 31, and 58, by spraying foliage to the point of drip. The number of leaflets exhibiting senescence was analyzed 65 days after the first treatment. Senescence was indicated by chlorosis and necrosis affecting greater than 50% of an individual leaflet.

**[0033]** The results of Example 1 are shown in Table 2. Specifically, MDHJ treatment reduced leaf senescence normally associated with the end of the growing season. Sixty-five days after treatment, MDHJ-treated roses showed no senescing leaves, while untreated roses exhibited leaf senescence in up to 9.4% of their leaflets.

TABLE 2

Results of Example 1.		
	Plant	% Leaflets exhibiting senescence
Untreated Control	1	8.2%
	2	9.4%
	3	8.2%
	4	0.0%
MDHJ-Treated Plants	1	0.0%
	2	0.0%
	3	0.0%
	4	0.0%

#### Example 2

##### Foliar MDHJ Treatment Reduces Senescence in Yellow Dwarf Rose

**[0034]** Potted Yellow Dwarf roses were grouped into one of four groups, and each of the groups received one treatment. The four groups are set forth in Table 3 below. One group was an untreated control (UTC), while the other three groups were treated with aqueous solutions of MDHJ that differed only in the concentration of MDHJ. There were three plants in each group. Before the trial began, naturally senescing leaves were visible on all plants; these were removed at the time that the trial began. The rose leaves received one foliar spray treatment by applying spray until the point of drip. Evaluation for

leaf senescence, without any additional treatment, occurred on days 3, 7, 10, 14, and 31 by counting the number of leaves on each plant exhibiting senescence. Senescence was indicated by chlorosis and necrosis affecting greater than 50% of an individual leaf. After each evaluation, senescing leaves were removed.

TABLE 3

Treatment Groups for Example 2.	
Treatment Group	Formulation
1	Untreated Control Group
2	1.5 mM MDHJ + 0.125% TRITON ® X-100 + 0.5% Canola Oil
3	3.0 mM MDHJ + 0.125% TRITON ® X-100 + 0.5% Canola Oil
4	6.0 mM MDHJ + 0.125% TRITON ® X-100 + 0.5% Canola Oil

[0035] The results of Example 2 are shown in Table 4 below. In general, MDHJ treatment reduced the rate of naturally senescing leaves of Yellow Dwarf Rose compared to the untreated control group. In Table 4 below, treatment groups indicated by the same letter did not significantly differ ( $P=0.5$ , Student-Newman-Keuls).

TABLE 4

Results of Example 2.										
Day 3			Day 7			Day 10				
Mean			Mean			Mean		Day 14		Day 31
Treatment Group	No. Leaves		No. Leaves		No. Leaves		Mean No. Leaves		Mean No. Leaves	
1	28	a	2	a	7	a	13	a	28	a
2	27	a	1	b	5	ab	13	a	27	a
3	25	a	1	b	4	b	12	a	25	ab
4	23	a	2	a	5	ab	12	a	23	b

### Example 3

#### MDHJ Treatment Reduces Senescence in Spinach

[0036] Greenhouse-grown spinach seedlings, cultivar 'Merlo Nero,' were divided into two groups, with eight plants per group. One group was left untreated, while the other group was treated with 0.5 mM MDHJ in 0.1% ethanol by spraying leaves until the point of drip. Treatments occurred on days 1, 4 and 8 of the experiment. On day 6, senescence was noted on some leaves, defined by chlorosis and/or necrosis. Senescence began at the leaf tip and spread towards the petiole. On day 8, the number of plants exhibiting senescence was counted. All leaves from each plant were then harvested and combined for each treatment. The total number of senescing leaves were counted and taken as a percentage of the total number of leaves per treatment. Senescence was indicated by chlorosis and necrosis affecting greater than 50% of an individual leaf.

[0037] In the untreated spinach group, 87.5% of the plants exhibited signs of senescence compared to 37.5% in the treated group. In the untreated group, 34.6% of the leaves exhibited senescence. In the treated group, 8.6% of the leaves showed senescence. The full results of Example 3 are set forth below in Table 5 below.

TABLE 5

Results of Example 3.		
	Untreated	Treated
% of Leaves Exhibiting Senescence	34.6%	8.6%
% of Plants Exhibiting Senescence	87.5%	37.5%
Number of Senescent Leaves	36	8
Total Number of Leaves	104	93
Number of Plants Exhibiting Chlorosis or Necrosis	7	3
Total Number of Plants	8	8

[0038] All references cited above are hereby incorporated by reference in their entireties.

[0039] While the invention has been described with respect to certain embodiments and examples, the description is intended to be illustrative, rather than limiting. Modifications and changes may be made within the scope of the invention, which is defined by the appended claims.

#### What is claimed is:

1. A method for reducing leaf senescence in a plant comprising treating the plant with an effective amount of methyl dihydrojasmonate.

2. The method of claim 1, further comprising, after treating the plant, harvesting the plant or a portion thereof.

3. The method of claim 1, wherein the plant comprises a rose plant or a spinach plant.

4. The method of claim 1, wherein treating the plant with an effective amount of methyl dihydrojasmonate comprises applying methyl dihydrojasmonate to the foliage of the plant one or more times.

5. The method of claim 4, wherein treating the plant with an effective amount of methyl dihydrojasmonate comprises applying methyl dihydrojasmonate to the foliage of the plant two or more times, the applications of methyl dihydrojasmonate being spaced from one another by a defined interval of time.

6. The method of claim 4, wherein the methyl dihydrojasmonate is in solution with a compatible solvent.

7. The method of claim 6, wherein the compatible solvent comprises water.

8. The method of claim 7, wherein the methyl dihydrojasmonate has a concentration in solution from about 0.15 mM to about 6 mM.

9. The method of claim 7, wherein the methyl dihydrojasmonate has a concentration in solution of about 0.5 mM.

10. The method of claim 7, wherein the methyl dihydrojasmonate has a concentration in solution of about 1.5 mM.

11. The method of claim 7, wherein the methyl dihydrojasmonate has a concentration in solution of about 6 mM.

12. The method of claim 6, wherein the solution further comprises one or more additives selected from the group consisting of wetting agents, adjuvants, emulsifiers, dispersants, spreaders, surfactants, anchorage, and disintegrants.

13. The method of claim 6, wherein the solution further comprises one or more plant nutrients.

14. The method of claim 6, wherein the solution further comprises a surfactant and an oil.

15. The method of claim 14, wherein the surfactant comprises TRITON® X-100 and the oil comprises canola (rape-seed) oil.

16. The method of claim 1, wherein the methyl dihydrojasmonate is in a form selected from the group consisting of

emulsion, suspension, powder, hydrate, solution, granules, paste, aerosol, and volatile formulation.

17. A method for reducing leaf senescence in a plant, comprising:

spraying the plant one or more times with a foliar spray comprising an amount of methyl dihydrojasmonate effective to reduce leaf senescence.

18. The method of claim 17, wherein the foliar spray is an aqueous foliar spray.

19. The method of claim 18, wherein the foliar spray further comprises one or more additives selected from the group consisting of wetting agents, adjuvants, emulsifiers, dispersants, spreaders, surfactants, anchorage, disintegrants, and plant nutrients.

20. The method of claim 17, wherein the foliar spray comprises methyl dihydrojasmonate, an oil, a surfactant, and optionally, one or more plant nutrients.

21. A plant or cut portion thereof having reduced leaf senescence, the plant produced by a process comprising:

treating the plant one or more times with a foliar spray comprising methyl dihydrojasmonate.

22. The plant of claim 21, wherein the plant is a rose plant or a spinach plant.

23. The plant of claim 21, wherein the process further comprises, after treating the plant, harvesting a portion of the plant.

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