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Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3083436 A1 2019/06/06

(21) 3 083 436

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2018/11/28
(87) Date publication PCT/PCT Publication Date: 2019/06/06
(85) Entrée phase nationale/National Entry: 2020/05/25
(86) N° demande PCT/PCT Application No.: IL 2018/051302
(87) N° publication PCT/PCT Publication No.: 2019/106667
(30) Priorité/Priority: 2017/11/29 (US62/591,816)

(51) Cl.Int./Int.Cl. *A01H 1/02* (2006.01), *A01H 1/04* (2006.01), *A01H 1/06* (2006.01), *C12N 15/82* (2006.01)

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(54) Titre : COMPOSITIONS, KITS ET PROCEDES DE LUTTE CONTRE LES MAUVAISES HERBES DU GENRE AMARANTHUS

(54) Title: COMPOSITIONS, KITS AND METHODS FOR CONTROLLING WEED OF THE AMARANTHUS GENUS

(57) Abrégé/Abstract:
A method of producing pollen that reduces fitness of at least one Amaranthus species of interest is provided. The method comprises treating the pollen of plants of an Amaranthus species of interest with an irradiation regimen selected from the group consisting of: (i) X-ray radiation at an irradiation dose of 20-1600 Gy; (ii) gamma radiation at an irradiation dose of 20-2000 Gy; (iii) particle radiation; and (iv) UV-C radiation at an irradiation dose of IOOµl/cm^2 -50J/cm^2, with the proviso that when the irradiation is X-ray the irradiation dose is not 300 Gy and wherein when the irradiation is gamma irradiation the irradiation dose is not 100, 300 and 500 G, and wherein when said radiation is UV-C the dose radiation is not 2 J/cm^2.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 June 2019 (06.06.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/106667 A1

(51) International Patent Classification:
A01H 1/02 (2006.01) *A01H 1/06* (2006.01)
A01H 1/04 (2006.01) *C12N 15/82* (2006.01)

(21) International Application Number:
PCT/IL2018/051302

(22) International Filing Date:
28 November 2018 (28.11.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/591,816 29 November 2017 (29.11.2017) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*

WO 2019/106667 A1

(54) Title: COMPOSITIONS, KITS AND METHODS FOR CONTROLLING WEED OF THE AMARANTHUS GENUS

(57) Abstract: A method of producing pollen that reduces fitness of at least one Amaranthus species of interest is provided. The method comprises treating the pollen of plants of an Amaranthus species of interest with an irradiation regimen selected from the group consisting of: (i) X-ray radiation at an irradiation dose of 20-1600 Gy; (ii) gamma radiation at an irradiation dose of 20-2000 Gy; (iii) particle radiation; and (iv) UV-C radiation at an irradiation dose of IOOμI/cm^2 -50J/cm^2, with the proviso that when the irradiation is X-ray the irradiation dose is not 300 Gy and wherein when the irradiation is gamma irradiation the irradiation dose is not 100, 300 and 500 G, and wherein when said radiation is UV-C the dose radiation is not 2 J/cm^2.

COMPOSITIONS, KITS AND METHODS FOR CONTROLLING WEED OF THE AMARANTHUS GENUS

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions, kits and methods for controlling weed of the Amaranthus genus.

Weeds have been the major biotic cause of crop yield loses since the origins of agriculture. The potential of weed damages is estimated as 34 % loss of crop yield, on average, world-wide [Oerke, E-C., 2006]. In the USA alone, the annual cost of crop losses due to weeds is greater than 26 billion USD [Pimentel D et al., 2000]. Furthermore according to the Weed Science Society of America Weeds are estimated to cause more than 40 billion USD in annual global losses [wssa(dot)net/wssa/weed/biological-control/]. Weeds are thus a major threat to food security [Delye et al., 2013].

Herbicides are the most commonly used and effective weed control tools. Due to the intense selection pressure exerted by herbicides, herbicide resistance is constantly growing and as of 2016 there are over 470 weed biotypes currently identified as being herbicide resistant to one or more herbicides by The International Survey of Herbicide Resistant Weeds (weedscience(dot)org/).

Weeds, like other plants, have several sexual reproduction mechanisms: self-pollination, cross-pollination, or both. Self-pollination describes pollination using pollen from one flower that is transferred to the same or another flower of the same plant. Cross- pollination describes pollination using pollen delivered from a flower of a different plant. Weeds rely on wind, or animals such as bees and other insects to pollinate them.

Since the 1940's the use of sterile organisms has been reported for use in order to reduce pest population and the success of these methods was demonstrated in many cases such as the tsetse fly [Klassen& Curtis, 2005], melon fly [Yosiakiet al. 2003] and Sweet potato weevil [Kohama et al., 2003].

Planting in the field plants producing sterile pollen for the production of infertile seeds was mentioned but immediately over-ruled due to practical, regulatory and economic reasons. (quora(dot)com/Why-dont-they-genetically-modify-weeds-instead-of-crops).

Therefore, there still exists a need for biological weed control.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of producing pollen that reduces fitness of at least one Amaranthus species of interest, the method comprising treating the pollen of plants of an Amaranthus species of interest with an irradiation regimen selected from the group consisting of:

(i) X-ray radiation at an irradiation dose of 20-1600 Gy;

(ii) gamma radiation at an irradiation dose of 20-2000 Gy;

(iii) particle radiation; and

(iv) UV-C radiation at an irradiation dose of 100μJ/cm^2 -50J/cm^2, with the proviso that when the irradiation is X-ray the irradiation dose is not 300 Gy and wherein when the irradiation is gamma irradiation the irradiation dose is not 100, 300 and 500 Gy and wherein when said radiation is UV-C the dose radiation is not 2 J/cm^2.

According to some embodiments of the invention, the particle irradiation dose is 20 – 5000 Gy.

According to some embodiments of the invention, the pollen is a harvested pollen.

According to some embodiments of the invention, the pollen is a non-harvested pollen.

According to some embodiments of the invention, the method further comprises harvesting the pollen following the treating.

According to some embodiments of the invention, the Amaranthus species of interest comprise only male plants.

According to some embodiments of the invention, the plants are grown in a large scale setting.

According to some embodiments of the invention, the large scale setting essentially does not comprise crops.

According to an aspect of some embodiments of the present invention there is provided a harvested pollen obtainable according to the method as described herein.

According to an aspect of some embodiments of the present invention there is provided a ethod of Amaranthus control, the method comprising artificially pollinating a Amaranthus species of interest with the pollen as described herein.

According to some embodiments of the invention, the pollen and the Amaranthus species of interest are of the same species.

According to some embodiments of the invention, the pollen and the Amaranthus species of interest are of different species.

According to some embodiments of the invention, the artificially pollinating is effected in a large scale setting.

According to some embodiments of the invention, the pollen is herbicide resistant.

According to some embodiments of the invention, the pollen is coated with the herbicide.

According to some embodiments of the invention, the artificially pollinating results in reduced average seed weight of at least 1.2 lower than that of the average seed weight of a plant of the same developmental stage and of the same species fertilized by control pollen.

According to an aspect of some embodiments of the present invention there is provided a method of producing pollen for use in artificial pollination, the method comprising:

(a) providing the pollen as described herein; and

(b) treating the pollen for use in artificial pollination.

According to an aspect of some embodiments of the present invention there is provided a composition-of-matter comprising the pollen as described herein, the pollen having been treated for use in artificial pollination.

According to an aspect of some embodiments of the present invention there is provided a kit comprising a plurality of packaging means, each packaging different species of pollen wherein at least one of the different species of pollen is the pollen as described herein or the treated pollen as described herein.

According to some embodiments of the invention, all of the different species of pollen are of the Amaranthus genus.

According to some embodiments of the invention, a portion of the different species of pollen are of the Amaranthus genus.

According to some embodiments of the invention, a treatment of the treated pollen is selected from the group consisting of coating, priming, formulating, solvent solubilizing, chemical treatment, drying, heating, cooling and irradiating.

According to some embodiments of the invention, the Amaranthus species of interest is selected from the group consisting of a biotic stress or abiotic stress resistant Amaranthus.

According to some embodiments of the invention, the Amaranthus species of interest is a herbicide resistant Amaranthus.

According to some embodiments of the invention, the pollen is of an herbicide susceptible Amaranthus.

According to some embodiments of the invention, the herbicide susceptible Amaranthus is susceptible to a plurality of herbicides.

According to some embodiments of the invention, the pollen reduces productiveness of the Amaranthus species of interest.

According to some embodiments of the invention, reduction in the productiveness is manifested by:

(i) inability to develop an embryo;

(ii) embryo abortion;

(iii) seed non-viability;

(iv) seed that cannot fully develop; and/or

(v) seed that is unable to germinate; and/or

(vi) reduced or no seed set.

According to some embodiments of the invention, the pollen is non-genetically modified pollen.

According to some embodiments of the invention, the non-genetically modified pollen is produced from a plant having an imbalanced chromosome number.

According to some embodiments of the invention, the pollen is genetically modified pollen.

According to some embodiments of the invention, the composition or kit further comprises at least one agent selected from the group consisting of an agricultural acceptable carrier, a fertilizer, a herbicide, an insecticide, a miticide, a fungicide, a pesticide, a growth regulator, a chemosterilant, a semiochemical, a pheromone and a feeding stimulant.

According to some embodiments of the invention, the at least one Amaranthus species of interest comprises a plurality of Amaranthus species of interest.

According to some embodiments of the invention, the Amaranthus species of interest is A. palmeri.

According to some embodiments of the invention, the Amaranthus species of interest is A. tuberculatus.

According to some embodiments of the invention, the irradiation is X-ray with an irradiation dose which is not 300 Gy.

According to some embodiments of the invention, the irradiation is gamma irradiation with an irradiation dose which is not 100, 300 and 500 Gy.

According to some embodiments of the invention, the irradiation is UV-C irradiation with an irradiation dose which is not 2 J/cm^2.

According to some embodiments of the invention, the Amaranthus species is A. palmeri and the X-ray irradiation dose is of 50-350 Gy.

According to some embodiments of the invention, the Amaranthus species is A. tuberculatos and the X-ray irradiation dose is of 20-200 Gy.

According to some embodiments of the invention, the X-ray irradiation dose is 20-500 Gy.

According to some embodiments of the invention, the Amaranthus species is A. palmeri and the gamma irradiation dose is of 200-1200 Gy.

According to some embodiments of the invention, the Amaranthus species is A. tuberculatos and the gamma irradiation dose is of 50-600 Gy.

According to some embodiments of the invention, the gamma irradiation dose is 50-1500 Gy.

According to some embodiments of the invention, the particle irradiation dose is 20-5000 Gy.

According to some embodiments of the invention, the UV-C irradiation dose is 1 mJ/cm^2-10 J/cm^2.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is a graph showing that the weight of seed obtained by artificial pollination is equivalent to that of seeds collected from the field or obtained by natural pollination.

FIG. 2 is an image showing inhibition of seed development demonstrated by comparing the appearance of random assortment of seeds generated by artificial pollination with X-ray irradiated pollen vs. non-irradiated pollen.

FIG. 3 is an image showing inhibition of seed development demonstrated by comparing the appearance of random assortment of seeds generated by artificial pollination with X-ray irradiated pollen vs. non-irradiated pollen.

FIG. 4 is an image showing inhibition of seed development demonstrated by comparing the appearance of random assortment of seeds generated by artificial pollination with gamma irradiated pollen vs. non-irradiated pollen. A dose response is demonstrated.

FIG. 5 an image showing inhibition of seed development demonstrated by comparing the appearance of random assortment of seeds generated by artificial pollination with gamma irradiated pollen vs. non-irradiated pollen. A dose response is demonstrated.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions, kits and methods for controlling weed of the Amaranthus genus.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Weeds are plants that are unwanted in any particular environment. They compete with cultivated plants in an agronomic environment and also serve as hosts for crop diseases and insect pests. The losses caused by weeds in agricultural production environments include decreases in crop yield, reduced crop quality, increased irrigation costs, increased harvesting costs, reduced land value, injury to livestock, and crop damage from insects and diseases harbored by the weeds.

The use of herbicides and other chemicals to control weed has generated environmental concern.

Whilst conceiving the present invention, the present inventors have devised a novel approach for the biological control of weeds. The approach is based on producing weed pollen that when artificially applied to the invasive weed out-competes with native fertilization and causes reduction in fitness of the weed. Thus, the present teachings provide for products and methods which are highly efficient, environmentally safe and that can be successfully applied as a practical and economically affordable weed control in plethora of settings.

Thus, according to an aspect of the invention there is provided a method of weed control. The method comprises artificially pollinating at least one weed species of interest with pollen of the same species that reduces fitness of the at least one weed species of interest.

As used herein the term "weed species of interest" refers to a wild plant growing where it is not wanted and that may be in competition with cultivated plants of interest (i.e., crop-desirable plants). Weeds are typically characterized by rapid growth and/or ease of germination, and/or competition with crops for space, light, water and nutrients. According to some embodiments of the invention, the weed species of interest is traditionally non-cultivated.

According to a specific embodiment, the weed is of the Amaranthus genus.

The Amaranthus genus, collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial plants.

According to a specific embodiment, the weed is of the Amaranthus selected from the group consisting of:

redroot pigweed (A. retroflexus)

smooth pigweed (A. hybridus)

Powell amaranth (A. powelii)

Palmer amaranth (A. palmeri)

spiny amaranth (A. spinosus)

tumble pigweed (A. albus)

prostrate pigweed (A. blitoides)

waterhemp (A. tuberculatus = A. rudis or A. rudis Sauer)

According to a specific embodiment, the pollen is of A. Palmeri.

According to a specific embodiment, the pollen is of A. tuberculatus.

It will be appreciated that plants of the Amaranthus genus can fertilize cross-species. Hence the present teachings relate to mono-species pollen or heterospecies pollen i.e., pollen of two Amaranthus species e.g., A. palmeri and A. tuberculatus.

Any reference to a weed is meant to refer to an Amaranthus species of interest.

Different weed may have different growth habits and therefore specific weeds usually characterize a certain crop in given growth conditions.

According to a specific embodiment, the weed is a herbicide resistant weed.

According to a specific embodiment, weed is defined as herbicide resistant when it meets the Weed Science Society of America (WSSA) definition of resistance.

Accordingly, WSSA defines herbicide resistance as "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type.

Alternatively, herbicide resistance is defined as "The evolved capacity of a previously herbicide-susceptible weed population to withstand an herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation" (Source: Heap and Lebaron. 2001 in Herbicide Resistance and World Grains).

As used herein the phrase "weed control" refers to suppressing growth and optionally spread of a population of at least one weed species of interest and even reducing the size of the population in a given growth area.

According to a specific embodiment, the growth area is an urban area, e.g., golf courses, athletic fields, parks, cemeteries, roadsides, home gardens/lawns and the like.

According to an additional or alternative embodiment, the growth area is a rural area.

According to an additional or an alternative embodiment, the growth area is an agricultural growth area e.g., open field, greenhouse, plantation, vineyard, orchard and the like.

As mentioned, weed control according to the present teachings is effected by reducing fitness of the at least one weed species of interest.

As used herein "fitness" refers to the relative ability of the weed species of interest to develop, reproduce or propagate and transmit its genes to the next generation. As used herein "relative" means in comparison to a weed of the same species not having been artificially pollinated with the pollen of the invention and grown under the same conditions.

It will be appreciated that the effect of pollen treatment according to the present teachings is typically manifested in the first generation after fertilization.

The fitness may be affected by reduction in productiveness, propagation, fertility, fecundity, biomass, biotic stress tolerance, abiotic stress tolerance and/or herbicide resistance.

As used herein "productivity" refers to the potential rate of incorporation or generation of energy or organic matter by an individual, population or trophic unit per unit time per unit area or volume; rate of carbon fixation.

As used herein "fecundity" refers to the potential reproductive capacity of an organism or population, measured by the number of gametes.

According to a specific embodiment, the pollen affects any stage of seed development or germination.

According to a specific embodiment, the reduction in productiveness is manifested by at least one of:

(i) inability to develop an embryo;

(ii) embryo abortion;

(iii) seed non-viability;

(iv) seed that cannot fully develop; and/or

(v) seed that is unable to germinate (e.g., reduced germination by at least 70 %, 80 %, 85 %, 90 %, or even 100 % as compared to seed produced from a control plant that was not subjected to fertilization by the pollen of the invention); and/or

(vi) reduced or no seed set.

It will be appreciated that when pollen reduces the productiveness, fertility, propagation ability or fecundity of the weed in the next generation it may be referred to by the skilled artisan as sterile pollen, though it fertilizes the weed of interest. Hence, sterile pollen as used herein is still able to fertilize but typically leads to seed developmental arrest or seed abortion.

According to a specific embodiment, the reduction in fitness is by at least 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 75 %, 80 %, 85 %, 90 %, 92 %, 95 %, 97 % or even 100 %, within first generation after fertilization and optionally second generation after fertilization and optionally third generation after fertilization.

According to a specific embodiment, the reduction in fitness is by at least 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 75 %, 80 %, 85 %, 90 %, 92 %, 95 %, 97 % or even 100 %, within first generation after fertilization.

According to a specific embodiment, reduced fitness results from reduction in tolerance to biotic or abiotic conditions e.g., herbicide resistance.

Non-limiting examples of abiotic stress conditions include, salinity, osmotic stress, drought, water deprivation, excess of water (e.g., flood, waterlogging), etiolation, low temperature (e.g., cold stress), high temperature, heavy metal toxicity, anaerobiosis, nutrient deficiency (e.g., nitrogen deficiency or nitrogen limitation), nutrient excess, atmospheric pollution, herbicide, pesticide and UV irradiation.

Biotic stress is stress that occurs as a result of damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants.

Examples of herbicides which are contemplated according to the present teachings, include, but are not limited to, ACCase inhibitors, ALS inhibitors, Photosystem II inhibitors, PSII inhibitor (Ureas and amides), PSII inhibitors (Nitriles), PSI Electron Diverter, PPO inhibitors, Carotenoid biosynthesis inhibitors, HPPD inhibitors, Carotenoid biosynthesis (unknown target), EPSP synthase inhibitors, Glutamine synthase inhibitors, DHP synthase inhibitors, Microtubule inhibitors, Mitosis inhibitors, Long chain fatty acid inhibitors, Cellulose inhibitors, Uncouplers, Lipid Inhibitors (thiocarbamates), Synthetic Auxins, Auxin transport

inhibitors, Cell elongation inhibitors, Antimicrotubule mitotic disrupter, Nucleic acid inhibitors or any other form of herbicide site of action.

As used herein "pollen" refers to pollen that is able to fertilize the weed species of interest and therefore competes with native pollination.

Alternatively, when native pollen competition does not exist, or very low levels of native pollen are present, pollination by the designed pollen inhibits apomixis of weeds and by this reduces their quantities as well [Ribeiro et al. 2012].

According to a specific embodiment, the pollen is of the same species as of the target weed (e.g., invasive, aggressive weed).

According to a specific embodiment, the pollen exhibits susceptibility to a single growth condition e.g., herbicide, temperature.

According to a specific embodiment, the pollen exhibits susceptibility to multiple growth conditions e.g., different herbicides (see Example 9).

According to a specific embodiment, the pollen is non-genetically modified.

According to a specific embodiment, there is provided a method of producing pollen that reduces fitness of at least one weed species of interest, the method comprising treating the weed species of interest (e.g., seeds, seedlings, tissue/cells) or pollen thereof with an agent that reduces fitness.

When needed (such as when treating that weed (e.g., seeds, seedlings, tissue/cells) the method further comprises growing or regenerating the plant so as to produce pollen.

According to a specific embodiment, the method comprises harvesting pollen from the weed species of interest following treating with the agent that reduces the fitness.

It will be appreciated that the pollen may be first harvested and then treated with the agent (e.g., radiation) that reduces the fitness of the weed species of interest.

According to a specific embodiment, treatment of the pollen is with an irradiation regimen selected from the group consisting of:

(i) X-ray radiation at an irradiation dose of 20-1600 Gy. Examples include but are not limited to, 20-1000 Gy, 20-900 Gy, 20-800 Gy, 20-700 Gy, 20-600 Gy, 20-500 Gy, 20-400 Gy, 20-300 Gy, 20-200 Gy, 20-100 Gy, 50-1600 Gy, 50-1400 Gy, 50-1200 Gy, 50-1000 Gy, 50-900 Gy, 50-800 Gy, 50-700 Gy, 50-600 Gy, 50-550 Gy, 50-500 Gy, 50-400 Gy, 50-350 Gy, 50-300 Gy, 50-200 Gy, 50-150 Gy, 50-100 Gy, 100-1600 Gy, 100-1500 Gy, 100-1400 Gy, 100-1300 Gy, 100-800 1200, 100-1000 Gy, 100-900 Gy, 100-800 Gy, 100-700 Gy, 100-600 Gy, 100-500 Gy, 100-400 Gy, 100-300 Gy, 100-200 Gy, 300-800 Gy, 300-700 Gy, 300-500 Gy, 50-600 Gy, 50-500 Gy, 50-400 Gy, 50-300 Gy, 50-200 Gy, 500-800 Gy, 500-1000 Gy.

According to a specific embodiment, the Amaranthus species is A. palmeri subjected to a X-ray irradiation dose of 50-350 Gy.

According to a specific embodiment, the Amaranthus species is A. tuberculatus subjected to a X-ray irradiation dose of 20-200 Gy.

According to a specific embodiment, the X-ray irradiation dose is 20-500 Gy.

(ii) gamma radiation at an irradiation dose of 20-2000 Gy. Examples include but are not limited to, 100-2000 Gy, 100-1500 Gy, 20-1500 Gy, 20-1000 Gy, 20-900 Gy, 20-800 Gy, 20-700 Gy, 20-600 Gy, 20-500 Gy, 20-400 Gy, 20-300 Gy, 20-200 Gy, 20-100 Gy, 100-1600 Gy, 100-1500 Gy, 100-1400 Gy, 100-1300 Gy, 100-800 1200, 100-1000 Gy, 100-900 Gy, 100-800 Gy, 100-700 Gy, 100-600 Gy, 100-500 Gy, 100-400 Gy, 100-300 Gy, 100-200 Gy, 200-2000 Gy, 200-1800 Gy, 200-1600 Gy, 200-1200 Gy, 200-1000 Gy, 200-800 Gy, 200-600 Gy, 200-400 Gy, 300-800 Gy, 300-700 Gy, 300-500 Gy, 50-600 Gy, 50-500 Gy, 50-400 Gy, 50-300 Gy, 50-200 Gy, 500-800 Gy, 500-1000 Gy.

According to a specific embodiment, the Amaranthus species is A. palmerisubjected to a gamma irradiation dose of 200-1200 Gy.

According to a specific embodiment, the Amaranthus species is A. tuberculatus subjected to a gamma irradiation dose of 50-600 Gy.

According to a specific embodiment, the gamma irradiation dose is 50-1500 Gy.

(iii) Particle irradiation such as alpha, beta or other accelerated particle at an irradiation dose of 20-5000 Gy produced from a particle accelerator such as a linear accelerator;

Examples include but are not limited to, 20-5000 Gy, 100-5000 Gy, 100-4000 Gy, 100-3000 Gy, 100-2000 Gy, 100-1500 Gy, 20-1500 Gy, 20-1000 Gy, 20-900 Gy, 20-800 Gy, 20-700 Gy, 20-600 Gy, 20-500 Gy, 20-400 Gy, 20-300 Gy, 20-200 Gy, 20-100 Gy, 50-5000 Gy, 50-3000 Gy, 50-2000 Gy, 50-1000 Gy, 50-900 Gy, 50-800 Gy, 50-700 Gy, 50-600 Gy, 50-500 Gy, 50-400 Gy, 50-300 Gy, 50-200 Gy, 50-100 Gy, 100-1600 Gy, 100-1500 Gy, 100-1400 Gy, 100-1300 Gy, 100-800 1200, 100-1000 Gy, 100-900 Gy, 100-800 Gy, 100-700 Gy, 100-600 Gy, 100-500 Gy, 100-400 Gy, 100-300 Gy, 100-200 Gy, 300-800 Gy, 300-700 Gy, 300-500 Gy, 50-600 Gy, 50-500 Gy, 50-400 Gy, 50-300 Gy, 50-200 Gy, 500-800 Gy, 500-1000 Gy;

According to a specific embodiment the irradiation dose is 20 – 5000 Gy.

(iiii) UV-C radiation at an irradiation at a dose of 100 $\mu J/cm^2$ -50 J/cm^2.

Examples include, but are not limited to, 100 $\mu J/cm^2$ – 50 J/cm^2, 1 mJ/cm^2-10 J/cm^2, 200 $\mu J/cm^2$ – 10 J/cm^2, 500 $\mu J/cm^2$ – 10 J/cm^2, 1 mJ/cm^2 – 10 J/cm^2, 1 5 mJ//cm^2 – 10 J/cm^2, 10 mJ/cm^2 – 10 J/cm^2, 20 mJ/cm^2 – 10 J/cm^2, 50 mJ/cm^2 – 10 J/cm^2, 100 mJ/cm^2 – 10 J/cm^2, 200 mJ/cm^2 – 10 J/cm^2, 300 mJ/cm^2 – 10 J/cm^2, 400 mJ/cm^2 – 10 J/cm^2, 500 mJ/cm^2 – 10 J/cm^2, 600

mJ/cm^2 – 10 J/cm^2, 700 mJ/cm^2 – 10 J/cm^2, 800 mJ/cm^2 – 10 J/cm^2, 900 mJ/cm^2 – 10 J/cm^2, 1 J/cm^2 – 10 J/cm^2, 2 J/cm^2 – 10 J/cm^2, 5 J/cm^2 – 10 J/cm^2.

According to a specific embodiment, the dose irradiation is 1 mJ/cm^2-10 J/cm^2.

According to a specific embodiment, when said radiation is UV-C the dose radiation is not 2 J/cm^2.

It will be appreciated by the skilled artisan that the irradiation duration depends on the dose rate that the machine delivers to the treated sample. This parameter is dependent on various variables such as beam energy, distance between beam source and sample and filter that is used and are well known the artisan in the relevant field. For example, X-ray machine X-rad 320 without any filtration with source to sample distance (SSD) of 50 cm at 320 kV will deliver to the sample ~15 Gy/min, with filtration of 2 mm Aluminum or 1 mm Copper will deliver to the sample 3 Gy/min and with filter of 4 mm Copper will deliver 1 Gy/min. It is possible to increase the dose absorbed by the sample by decreasing the SSD thus, by changing SSD from 50 cm to 30 cm with filter of ~1 mmCu the sample will absorb ~8 Gy/min (instead of 3Gy/min).

It is also possible to change the beam energy, for example, X-rad 160 machine will deliver to the sample more than 60 Gy/min at energy of 160 kV, 19 mA at SSD of 30 cm without any filtration and more than 6.5 Gy/min with filter of 2 mm Aluminum.

As duration depends on the dose rate, a dose of 20-1600 Gy can be achieved by 1 Gy/min up to 60G y/min. Therefore, it can range from 20 seconds to hours. According to a specific embodiment, X-rad 320 is used with 3 Gy/min (320kV,50cm SSD, filter=2mm Al). Accordingly radiation time can range from ~7 minutes to 9 hours.

According to a specific embodiment the radiation is gamma radiation for which various machines can be employed based on e.g., Cesium-137, Cobalt-60 or Iridium-192. The dose rate can vary from 1-300 Gy/min. According to a specific embodiment a BIOBEAM GM 8000 device is used with Cs137 that generates 2.8Gy/min. Therefore, irradiation duration can vary from 7 minutes (=20Gy) to ~12 hours (2000Gy).

According to a specific embodiment, in the case of A. palmeri, when the irradiation is X-ray, the irradiation dose is not 300 Gy and when the irradiation is gamma irradiation the irradiation dose is not 100, 300 and 500 Gy.

As mentioned the pollen may be a harvested pollen (harvested prior to treating with the irradiation).

Alternatively, the pollen is a non-harvested pollen (e.g., on a whole plant). In such an embodiment, the pollen is harvested following treating.

There are various methods to achieve ionizing radiation. Sources of radiation include radioactive isotypes, particle accelerators and X–ray tubes.

Standard X-ray machines include superficial x-ray machines and orthovoltage X-ray machines. Examples include but are not limited to X-rad 160/225/320/350/400/450 series that the dose rate that they deliver can vary greatly and can range between 1-60Gy/min, MultiRad 160/225/350 that can range between 16-300 Gy/min, CellRad that can range between 8-45 Gy/min or RAD source machines (examples include but are not limited to RS420/RS1300/RS1800/RS2000/RS2400/RS3400).

Gamma machines include various radioactive sources that can be Caesium-137, Cobalt-60 or Iridium-192. Examples of Caesium-137 Gamma radiation devices include, but are not limited to, BIOBEAM GM 2000/3000/8000 that generates between 2.5-5 Gy/min or Gammacell 1000 Elite / 3000 Elan that generate between 3.5-14Gy/min. Additional irradiators are particle accelerators such as Electrostatic particle accelerators and Electrodynamic (electromagnetic) particle accelerators such as Magnetic Induction Accelerators (such as Linear Induction Accelerators or Betatrons), Linear accelerators, Circular or cyclic RF accelerators (such as Cyclotrons, Synchrocyclotrons and isochronous cyclotrons Synchrotrons, Electron synchrotrons, Storage rings, Synchrotron radiation sources

or FFAG accelerators).

An example of a cyclic accelerator is the linac. Other examples include, but are not limited to, microtrons, betatrons and cyclotrons. More exotic particles, such as protons, neutrons, heavy ions and negative π mesons, all produced by special accelerators, may be also used. Various types of linac accelerators are available: some provide X rays only in the low megavoltage range (4 or 6 MV), while others provide both X rays and electrons at various megavoltage energies. A typical modern high-energy linac will provide two photon energies (6 and 18 MV) and several electron energies (e.g. 6, 9, 12, 16 and 22 MeV) (Radiation Oncology Physics: A Handbook for Teachers and Students E.B. PODGORSAK).

Typical UV irradiation can be achieved by UV crosslinkers. UVC irradiators include, but are not limited to, Mercury-based lamps that emit UV light at the 253.7 nm line, Ultraviolet Light Emitting Diodes (UV-C LED) lamps that emit UV light at selectable wavelengths between 255 and 280 nm, Pulsed-xenon lamps emit UV light across the entire UV spectrum with a peak emission near 230nm.

Following are non-limiting examples of commercial means for executing embodiments of the invention, though custom-made machines are also contemplated herein.

X-ray machines:

Vendor: Precision X-Ray

Table A

Machine type: X-RAD	Output Voltage		SSD (Source to sample distance)	Filter type + width	i.e Gy/min
X-RAD 160 series	5KV – 160 KV in 0.1 KV increments		10 to 100cm	-No filter - 2mm Al	>60 Gy/min at 160KV, 19mA, 30 cm SSD >6.5 Gy/min at 160KV, 19mA, 30cm SSD, (Filter = 2mm Al)
X-RAD 225 series					
X-RAD iR225	7.5 KV – 225 KV in 0.1 KV increments	0.1 mA to 45 mA in 0.01 mA increments	10 to 95 cm	-No filter - 2mm Al	- 12 Gy/min at 225 KV, 13.3 mA, 30 cm SSD - 6.4 Gy/min at 225 KV, 19 mA, 30cm SSD, (Filter = 2 mm Al)
X-RAD 225	5KV – 225 KV in 0.1 KV increments	0.1mA to 45 mA in 0.01 mA increments	15cm to 63cm	-No filter - 2mm Al	Raw Beam: >60 Gy/min at 225KV, 19mA, 30 cm SSD Filtered Beam: >7.5 Gy/min at 225KV, 19mA, 30cm SSD, (Filter = 2mm Al)
X-RAD 225HP	5 – 225KV	0.5mA to 45mA in 0.01 mA increments	15cm to 63cm	-No filter - 2mm Al	
X-RAD 225XL	5 – 225kV in 0.1 kV increments	0.5mA to 30mA in 0.01 mA increments	15cm to 100cm	-No filter - 2mm Al	
X-RAD 320 series					
X-RAD 320	5KV – 320 KV in 0.1 KV increments	0.5mA to 45 mA in 0.01 mA increments	20cm to 90cm	-No filter - 1mm Cu - 4mm Cu	3 Gy/min at 320KV, 12.5mA, 50cm SSD, (HVL≈1mm Cu) >15 Gy/min at 320KV, 12.5mA, 50cm SSD

X-RAD 320Dx	5KV – 320 KV in 0.1 KV increments	0.5mA to 45 mA in 0.01 mA increments	20cm to 90cm	-No filter - 1mm Cu - 4mm Cu	Same as above
X-RAD 320ix	5KV – 320 KV in 0.1 KV increments	0.5mA to 45 mA in 0.01 mA increments	20cm to 90cm	-No filter - 1mm Cu - 4mm Cu	Same as above
X-RAD 350 series	5 – 350 kV in 0.1 kV increments	0.5mA to 45 mA in 0.01 mA increments		-No filter - 1.2mm Cu - 4mm Cu	3Gy/min at 350 kV, 11.4 mA, 50 cm SSD, (HVL = 1.2mm Cu) >1Gy/min at 350 kV, 11.4 mA, 50 cm SSD, (HVL = 4.0 mm Cu) >15 Gy/min at 350 kV, 11.4 mA, 50 cm SSD, (unfiltered)
X-RAD 400/450 series	5KV – 450 KV in 0.1 KV increments	0.5mA to 45 mA in 0.01 mA increments	20cm to 100cm	- 4mm Cu	>4Gy/min at 50cm SSD (HVL=4mm Cu)

*Al = Aluminum, Cu = Copper

Other machines are available from RAD Source www(dot)radsource(dot)com(dot) Examples include, but are not limited to:

RS 3400

1. ~25 Gy Central Dose
2. 15 Gy/min /25 Gy central/ 50 Gy max

4 pi emitter

RS 2000

Available in 160 kV and 225 kV (Custom Built X-ray Irradiators with 350 kV are available).

Excellent for small animals irradiation with as doses rates ~1.2Gy/min (120 rads/min)

3 mm cooper filter.

160kv AT 225 kV

Other dose rates: for cells: > 5 Gy/min (500 rads/min) filtered and up to 17 Gy/min unfiltered

RS 1800

Operates at 160 kV and 12.5 mA (2,000 watts)

RS 5000

utilizes MULTIPLE 4pi emitters to achieve dose rates up to 120 Gy/min to a 500mL canister

RS1300

4 pi X-ray Emitter (also described in U.S. Patent No.7346147)

~70 Gy/min for product density of 1.0 g/ml (3" diameter canister)

RS 2400 featuring the 4 pi X-ray Tube

Single 4pi Au target X-ray Tube

Dose Rate: 420,000 rad/h (4.2 kGy/h) based on product density

RS 420

Faxitron **www(dot)faxitron(dot)com/** www(dot)faxitron(dot)com/application/biological-irradiation/

Tables B-H provide the specification for some commercially available irradiation devices that can be used in implementing the teachings of some embodiments of the invention.

Table B

Specifications	MultiRad 160	MultiRad 225	MultiRad 350
Energy range	up to 160kV	up to 225kV	up to 350kV
Tube current at max voltage	25mA	17.8mA	11.4mA
System power	4000W	4000W	4000W
Dose rate at max kVp & mA	Up to: 300Gy/min (unfiltered) Up to: 32Gy/min (2mm Al) Up to: 16Gy/min (0.3mm Cu)	Up to: 265Gy/min (unfiltered) Up to: 42Gy/min (2mm Al) Up to: 25Gy/min (0.3mm Cu)	Up to: 140Gy/min (unfiltered) Up to: 40Gy/min (2mm Cu Al) Up to: 16.5Gy/min (4.0mm Cu HVL)
Focal spot size	5.5mm 1.2mm for imaging (<0.5 IEC)	5.5mm 1.2mm for imaging (<0.5 IEC)	8mm
Inherent filtration	0.8mm Be	1.2mm Be	3mm Be
Beam angle	40° divergence	40° divergence	40° divergence
Beam coverage	9cm - 40cm diameter	9cm - 40cm diameter	9cm - 40cm diameter
Source to sample distance	13cm - 65cm	13cm - 65cm	13cm - 65cm
Exposure time	Programmable or continuous	Programmable or continuous	Programmable or continuous
Power requirements	220 VAC +/- 10 %, 50/60Hz, single phase, 7.5kVA	220 VAC +/- 10 %, 50/60Hz, single phase, 7.5kVA	220 VAC +/- 10 %, 50/60Hz, single phase, 7.5kVA
Cooling	Integrated closed-loop heat exchanger	Integrated closed-loop heat exchanger	Integrated closed-loop heat exchanger
Specimen turntable	Electrically-operated, 2 RPM, with integrated dosimeter	Electrically-operated, 2 RPM, with integrated dosimeter	Electrically-operated, 2 RPM, with integrated dosimeter
External dimensions	74" H x 43" W x 35" D (188cm x 108cm x 88cm)	74" H x 43" W x 35" D (188cm x 108cm x 88cm)	74" H x 43" W x 35" D (188cm x 108cm x 88cm)
Chamber dimensions	23" H x 16" W x 17" D (58cm x 41cm x 43cm)	23" H x 16" W x 17" D (58cm x 41cm x 43cm)	23" H x 16" W x 17" D (58cm x 41cm x 43cm)
Weight	2120lbs (960kg)	2550lbs (1160kg)	3470lbs (1575kg)

Table C

Specifications

Energy range	10 - 130kV
Tube current	0.1 - 5mA
Tube power	650W
Dose rate (130kVp, 5.0mA)	Up to >45 Gy/min (unfiltered) Up to >8 Gy/min (0.5mm Al)
Focal spot size	1.0 x 1.4mm
Inherent filtration	1.6mm Be
Beam angle	40° divergence
Beam coverage	9cm - 27cm diameter
Source to sample distance	13cm - 38cm
Exposure time	5 sec to 180 min (1 sec increments)
Power requirements	100 - 230VAC +/- 10%, 50 - 60Hz
Cooling	Integrated closed-loop heat exchanger
Specimen turntable	Electrically operated, 2 RPM, with integrated dosimeter
External dimensions	30" H x 21" W x 24" D (77cm x 53cm x 61cm)
Chamber dimensions	14" H x 12" W x 12" D (37cm x 30cm x 32cm)
Weight	460lbs (210kg)
Shipping weight	540lbs (245kg)

Table D

Faxitron® Cabinet X-ray System Model 43855C
SPECIFICATIONS

X-ray Sources:

There are five X-ray sources offered with the Faxitron Model 43855C. The system comes standard with a 110kVp maximum source.

Standard Source

- **Energy Range** - 10 - 110 kVp
- **Tube Current** - 3.0 mA fixed*
- **Focal Spot** - 0.5 mm, nominal
- **X-Ray Tube** - Stationary anode, glass tube with beryllium window (0.76mm thick)
- **Beam Angle** - 30° divergence

Option A04

- **Energy Range**- 10 - 130 kVp
- **Tube Current** - 3.0 mA fixed*
- **Focal Spot** - 0.5 mm, nominal
- **X-Ray Tube** - Stationary anode, glass tube with beryllium window (0.76mm thick)
- **Beam Angle** - 30° divergence

Option A05

- **Energy Range**- 10 - 150kVp
- **Tube Current** - 3.0 mA fixed*
- **Focal Spot** - 1.5 mm, nominal
- **X-Ray Tube** - Stationary anode, glass tube with beryllium window (0.76mm thick)
- **Beam Angle** - 40° divergence

Option M110

- **Energy Range** - 10 - 110 kVp
- **Tube Current** - 300 µA fixed*
- **Focal Spot** - 50 µm
- **X-Ray Tube** - Stationary anode glass tube with beryllium window (0.76mm thick)
- **Beam Angle** - 30° divergence

Option M130

- **Energy Range** - 10 - 130 kVp
- **Tube Current** - 300 µA fixed*
- **Focal Spot** - 50 µm
- **X-Ray Tube** - Stationary anode glass tube with beryllium window (0.76mm thick)

Max C. Meiste AG, Morgental 31, CH-8128 Zumikon

Beam Angle - 30° divergence

X-ray generators are also available from Kimtron www(dot)kimtron(dot)com/products/

Table E

Polaris® Generator Specifications

Parameters	160kV	225kV	320kV	450kV
Output				
DC Output Voltage	0-160kV	0-225kV	0+-160kV	0+-225kV
Max Output Current	30mA	30mA	30mA	30mA
Max Output Power	3kW	3kW	4.2kW	4.2kW
Polarity	Negative	Negative	Bi-Polar	Bi-Polar

*All high voltage connectors are tapered with flanged fittings 160, 320, 450 or 600kv

Other X-ray generators are available from Xstrahl. For example, XenX:

xstrahl(dot)com/life-science-systems/xenx/

Treatment distances: 30-38cm or 80cm FSD

Maximum Field Size: 18 cm circle at 35 cm FSD

Tube Voltage: 20-220 kV

Tube Current: 0-25 mA

XSTRAHL CABINET IRRADIATORS: CIX2, CIX3, CIXD

RS225 (Voltage Up to 220kV Current 1.0mA to 30mA) and RS320 (Voltage Up to 300kV Current Up to 30mA)

CIXD

Tube Voltage: 20-220 kV

Tube Current: 0-25 mA

Gamma radiation machines:

Examples of Gamma radiation machines include, but are not limited to:

BIOBEAM GM 2000/3000/8000 – Radionuclide source: Cs-(137).

Table F

	BIOBEAM GM 2000	BIOBEAM GM 3000	BIOBEAM GM 8000
Dose rate	2.5Gy/min	5Gy/min	5-2.6Gy/min

Table G

Gammacell® 1000 Elite / 3000 Elan - Radionuclide source: Cs-(137).

	Gammacell® 1000 Elite	Gammacell 3000 Elan
Dose rate	3.5, 7.6 or 14.3 Gy/min	4.5 or 8.7 Gy/min

Gammabeam™ X200 (GBX200) - Cobalt-60 capacity of 434 TBq (11,725 Ci) that can deliver a dose rate of 800 cGy/min at 50 centimeters from the source at maximum field size.

A list of Radionuclide sources for gamma radiation appears in Table H below.

Table H

Data from the U.S. NRC show that out of the thousands of manufactured and natural radionuclides, americium-241, cesium-137, cobalt-60, and iridium-192 account for nearly all (over 99 percent) of the Category 1 and 2 sources. The features of these and some other key radionuclide radiation sources are summarized in Table S-1.

TABLE S-1 Summary of Radionuclides in Category 1 and 2 Radiation Sources in the United States[a]

Radionuclide	Half-life	Radioactive Emissions and Energies	Typical Specific Activity (TBq/g) [Ci/g]	Total Activity in U.S. Inventory (TBq) [Ci]	Major Applications	Typical Activity (TBq) [Ci]	Physical or Chemical Form
Americium-241	432.2 y	α–5.64 MeV γ-60 keV, principal	0.13 [3.5]	240 [6,482]	Well logging	0.5-0.8 [13-22]	Pressed powder (americium oxide)
Californium-252	2.645 y	α–6.22 MeV, Fission fragments, neutrons, and gammas	20 [540]	0.26 [7]	Well logging	0.0004 [0.011]	Metal oxide
Cesium-137 (Ba-137m)	30.17 y	β-518 keV max with γ-662 keV (94.4% of decays) or β-1.18 MeV max	0.75 [20]	104,160 [2.8 million]	Self-contained irradiators Teletherapy Calibrators	75 [2,000] 50 [1,400] 15 [400]	Pressed powder (cesium chloride)
Cobalt-60	5.27 y	γ-1.173 and 1.333 MeV	3.7 [100] 11 [300]	7.32 million [198 million]	Panoramic irradiators Self-contained irradiators Teletherapy Industrial radiography	150,000 [4 million] 900 [24,000] 500 [14,000] 4 [100]	Metal slugs Metal pellets
Iridium-192	74 d	β-1.46 MeV max with 2.3 γ-380 keV average, 1.378 MeV max (0.04% of decays)	18.5 [500]	5,436 [146,922]	Industrial radiography	4 [100]	Metal
Plutonium-238	87.7 y	α–5.59 MeV, and γ-43 keV (30% of decays)	2.6 [70]	34.7 [937]	RTG Pacemakers (obsolete) Fixed gauges	10 [270] 0.1 [3] 0.75 [20]	Metal oxide
Selenium-75	119.8 d	γ-280 keV average, 800 keV max	20-45 [530-1200]	9.7 [261]	Industrial radiography	3 [75]	Elemental or metal compound
Strontium-90 (Yttrium-90)	28.9 y	β-546 keV	5.2 [140]	64,000 [1.73 million]	RTG	750 [20,000]	Metal oxide

[a] Nuclear decay data for this table and throughout the report are from Firestone and Shirley (1996).

UV machines:

Examples of UV radiation machines include, but are not limited to:

UV CROSS-LINKER CL-508 UVITEC Cambridge

UV Energy exposure: Min.0.025 Joules / Max. 99.99 Joules

UV exposure Time: Min.10 Seconds / Max.599 Minutes

Fisher Scientific™ UV Crosslinker AH

UVP CL-1000 and CX-2000 Crosslinkers: Maximum UV energy setting of 999,900 microjoules/cm2

Spectroline™ Microprocessor-Controlled UV Crosslinkers: 100µJ/cm^2 to 0.9999J/cm^2

BIO-LINK BLX: Energy – 0-99.99 Joules/cm^2 Exposure Time: Up to 999.9 minutes

Linear accelerators:

Examples of linear accelerators that can be used in accordance with some embodiment s of the invention include, but are not limited to:

Basic Varian 600CD/6EX

Basic Varian 21/23 Series

Elekta Precise Systems

Elekta Synergy Platforms

Siemens Primus

Siemens Oncor

TomoTherapy Machines

Varian Trilogy

Varian iX

Elekta Synergy

Elekta Infinity

Cyberknife G4 & VSI

Elekta Versa HD

CyberKnife VSI

Varian TrueBeam.

Varian 21/23 series with OBI and RapidArc

Varian Trilogy with RapidArc

Cyberknife M6

According to a specific embodiment, when the irradiation is X-ray the dose is not 300 Gy.

According to a specific embodiment, when the irradiation is gamma irradiation the dose is not 100, 300 and 500 Gy.

Examples of such treatments are provided in Examples 29 to 39 of the Examples section which follows.

Embodiments of the invention also refer to harvested pollen obtainable according to the method as described herein.

It will be appreciated that pollen obtained according to embodiments of the invention facilitate in fertilizing plants such that the aborted seeds per plant are uniform as manifested by a statistically significant average reduced weight that has a statistically significant reduced standard deviation as compared to naturally occurring aborted seeds per plant.

According to another specific embodiment, the average seed weight following pollen treatment at first generation is at least about 1.2 fold lower (e.g., 1.2-20, 1.2-15, 1.2-10, 1.2-8, 1.5-20, 1.5-15, 1.5-10, 1.5-8, 2-20, 2-15, 2-10, 2-8 fold lower) than that of an average seed of a control plant of the same developmental stage and of the same species fertilized by control pollen (not treated).

Additionally, the pollen is produced from a plant having an imbalanced chromosome number (genetic load) with the weed species of interest.

Thus, for example, when the weed of interest is diploid, the plant producing the pollen is treated with an agent rendering it polyploid, typically tetraploids are selected, such that upon fertilization with the diploid female plant an aborted or developmentally arrested, not viable seed set are created. Alternatively, a genomically imbalanced plant is produced which rarely produces a seed set.

According to a specific embodiment, the weed (or a regenerating part thereof or the pollen) is subjected to a polyploidization protocol using a polyploidy inducing agent, that produces plants which are able to cross but result in reduced productiveness,

Thus, according to some embodiments of the invention, the polyploid weed has a higher chromosome number than the wild type weed species (e.g., at least one chromosome set or portions thereof) such as for example two folds greater amount of genetic material (i.e., chromosomes) as compared to the wild type weed. Induction of polyploidy is typically performed by subjecting a weed tissue (e.g., seed) to a G2/M cycle inhibitor.

Typically, the G2/M cycle inhibitor comprises a microtubule polymerization inhibitor.

Examples of microtubule cycle inhibitors include, but are not limited to oryzalin, colchicine, colcemid, trifluralin, benzimidazole carbamates (e.g. nocodazole, oncodazole, mebendazole, R 17934, MBC), o-isopropyl N-phenyl carbamate, chloroisopropyl N-phenyl

carbamate, amiprophos-methyl, taxol, vinblastine, griseofulvin, caffeine, bis-ANS, maytansine, vinbalstine, vinblastine sulphate and podophyllotoxin.

According to a specific embodiment, the microtubule cycle inhibitor is colchicine.

Still alternatively or additionally, the weed may be selected producing pollen that reduces fitness of the weed species of interest by way of subjecting it to a mutagenizing agent and if needed further steps of breeding.

Thus, weed can be exposed to a mutagen or stress followed by selection for the desired phenotype (e.g., pollen sterility, herbicide susceptibility).

Examples of stress conditions which can be used according to some embodiments of the invention include, but are not limited to, X-ray radiation, gamma radiation, UV radiation or alkylating agents such as NEU, EMS, NMU and the like. The skilled artisan will know which agent to select.

According to a specific embodiment, the stress is selected from the group consisting of X-ray radiation, gamma radiation, UV radiation. Pollen of the weed can be treated with the agent that reduces the fitness (e.g., radiation) following harvest.

A specific description of such treatments are provided in Examples 19, 24, 25 and 26 of the Examples section which follows and should be considered as part of the specification.

Guidelines for plant mutagenesis are provided in K Lindsey Plant Tissue Culture Manual - Supplement 7: Fundamentals and Applications, 1991, which is hereby incorporated in its entirety.

Other mutagenizing agents include, but are not limited to, alpha radiation, beta radiation, neutron rays, heating, nucleases, free radicals such as but not limited to hydrogen peroxide, cross linking agents, alkylating agents, BOAA, DES, DMS, EI, ENH, MNH, NMH Nitrous acid, bisulfate, base analogs, hydroxyl amine, 2-Naphthylamine or alfatoxins.

Alternatively or additionally, the pollen may be genetically modified pollen (e.g., transgenic pollen, DNA-editing).

Thus, according to some embodiments of the invention the pollen of the invention confers reduced fitness by way of partial genome incompatibility, parthenocarpy, stenospermocarpy, reduced shattering, inhibition of seed dormancy, cleistogamy, induced triploidy, conditional lethality, male sterility, female sterility, inducible promoters, complete sterility by nonflowering, reduced biotic/abiotic stress tolerance. The skilled artisan will know which method to select.

According to a further aspect of the invention there is provided a method of producing pollen, the method comprising:

(a) growing weed producing pollen that reduces fitness of at least one weed species of interest; and

(b) harvesting said pollen.

Thus the pollen product producing weed is grown in dedicated settings, e.g., open or closed settings, e.g., a greenhouse. According to a specific embodiment, the growth environment for the manufacture of the pollen does not include crop plants or the weed species of interest. For example, the growth area includes a herbicide susceptible weed variant but not a herbicide resistant weed variant (of the same species). Another example, the growth environment comprises a GM weed with a destructor gene said weed being fertile and producing pollen, but doesn't include the weed in which the destructor gene is expressed.

According to a specific embodiment, growing said weed producing pollen that reduces fitness is effected in a large scale setting (e.g., hundreds to thousands m^2).

According to some embodiments of the invention, the weed producing pollen comprises only male plants.

According to some embodiments of the invention, the weed producing pollen comprises only male plants.

Harvesting pollen is well known in the art. For example, by the use of paper bags (Example 1). Another example is taught in U.S. 20060053686, which is hereby incorporated by reference in its entirety.

Once pollen is obtained it can be stored for future use. Examples of storage conditions include, but are not; limited to, storage temperatures in Celsius degrees e.g., -196, -160, -130, -80, -20, -5, 0, 4, 20, 25, 30 or 35; percent of relative humidity e.g., 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100. Additionally, the pollen can be stored in light or dark.

Alternatively, the pollen product of the present teachings is subjected to a post harvest treatment.

Thus, according to an aspect of the invention there is provided a method of producing pollen for use in artificial pollination, the method comprising:

(a) obtaining pollen that reduces fitness of at least one weed species of interest, e.g., as described herein; and

(b) treating said pollen for use in artificial pollination.

Accordingly, there is provided a composition of matter comprising weed pollen that reduces fitness of at least one weed species of interest, said pollen having been treated for improving its use in artificial pollination.

Examples of such treatments include, but are not limited to coating, priming, formulating, chemical inducers, physical inducers [e.g., potential inducers include, but are not limited to, ethanol, hormones, steroids, (e.g., dexamethasone, glucocorticoid, estrogen, estradiol), salicylic acid, pesticides and metals such as copper, antibiotics such as but not limited to tetracycline, Ecdysone, ACEI, Benzothiadiazole and Safener, Tebufenozide or Methoxyfenozide], solvent solubilization, drying, heating, cooling and irradiating (e.g., gamma, UV, X-ray).

According to a specific embodiment, the pollen is resistant to a herbicide. In such a case the pollen may be coated with the herbicide so as to reduce competition with native pollen that is sensitive to the herbicide.

Additional ingredients and additives can be advantageously added to the pollen composition of the present invention and may further contain sugar, potassium, calcium, boron, and nitrates. These additives may promote pollen tube growth after pollen distribution on flowering plants.

In some embodiments, the pollen composition of the present invention contains dehydrated or partially dehydrated pollen.

Thus, the pollen composition may comprise a surfactant, a stabilizer, a buffer, a preservative, an antioxidant, an extender, a solvent, an emulsifier, an invert emulsifier, a spreader, a sticker, a penetrant, a foaming agent, an anti-foaming agent, a thickener, a safener, a compatibility agent, a crop oil concentrate, a viscosity regulator, a binder, a tacker, a drift control agent, a fertilizer, a timed-release coating, a water-resistant coating, an antibiotic, a fungicide, a nematicide, a herbicide or a pesticide.

Other ingredients and further description of the above ingredients is provided hereinbelow.

Under ordinary conditions of storage and use, the composition of the present invention may contain a preservative to prevent the growth of microorganisms.

The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, sorbic acid, and the like. Antioxidants may also be added to the pollen suspension to preserve the pollen from oxidative damage during storage. Suitable antioxidants include, for example, ascorbic acid, tocopherol, sulfites, metabisulfites such as potassium metabisulfite, butylhydroxytoluene, and butylhydroxyanisole.

Thus, pollen compositions that may also be used but not limited to mixtures with various agricultural chemicals and/or herbicides, insecticides, miticides and fungicides, pesticidal and biopesticidal agents, nematocides, bactericides, acaricides, growth regulators, chemosterilants,

semiochemicals, repellents, attractants, pheromones, feeding stimulants or other biologically active compounds all of which can be added to the pollen to form a multi- component composition giving an even broader spectrum of agricultural protection.

Thus in the artificial pollination method of the present invention can be applied together with the following herbicides but not limited to: ALS inhibitor herbicide, auxin-like herbicides, glyphosate, glufosinate, sulfonylureas, imidazolinones, bromoxynil, delapon, dicamba, cyclohezanedione, protoporphyrionogen oxidase inhibitors, 4-hydroxyphenyl-pyruvate-dioxygenase inhibitors herbicides.

In some embodiments, the pollen can be combined with appropriate solvents or surfactants to form a formulation. Formulations enable the uniform distribution of a relatively small amount of the pollen over a comparatively large growth area. In addition to providing the user with a form of a pollen that is easy to handle, formulating can enhance its fertilization activity, improve its ability to be applied to a plant, enable the combination of aqueous-soluble and organic-soluble compounds, improve its shelf-life, and protect it from adverse environmental conditions while in storage or transit.

Numerous formulations are known in the art and include, but are not limited to, solutions, soluble powders, emulsifiable concentrates, wettable powders, liquid flowables, and dry flowables. Formulations vary according to the solubility of the active or additional formulation ingredients in water, oil and organic solvents, and the manner the formulation is applied (i.e., dispersed in a carrier, such as water, or applied as a dry formulation).

Solution formulations are designed for those active ingredients that dissolve readily in water or other non-organic solvents such as methanol. The formulation is a liquid and comprises of the active ingredient and additives.

Suitable liquid carriers, such as solvents, may be organic or inorganic. Water is one example of an inorganic liquid carrier. Organic liquid carriers include vegetable oils and epoxidized vegetable oils, such as rape seed oil, castor oil, coconut oil, soybean oil and epoxidized rape seed oil, epoxidized castor oil, epoxidized coconut oil, epoxidized soybean oil, and other essential oils. Other organic liquid carriers include aromatic hydrocarbons, and partially hydrogenated aromatic hydrocarbons, such as alkylbenzenes containing 8 to 12 carbon atoms, including xylene mixtures, alkylated naphthalenes, or tetrahydronaphthalene. Aliphatic or cycloaliphatic hydrocarbons, such as paraffins or cyclohexane, and alcohols, such as ethanol, propanol or butanol, also are suitable organic carriers. Gums, resins, and rosins used in forest products applications and naval stores (and their derivatives) also may be used. Additionally, glycols, including ethers and esters, such as propylene glycol, dipropylene glycol ether,

diethylene glycol, 2-methoxyethanol, and 2-ethoxyethanol, and ketones, such as cyclohexanone, isophorone, and diacetone alcohol may be used. Strongly polar organic solvents include N-methylpyrrolid-2-one, dimethyl sulfoxide, and N,N-dimethylformamide.

Soluble powder formulations are similar to solutions in that, when mixed with water, they dissolve readily and form a true solution. Soluble powder formulations are dry and include the active ingredient and additives.

Emulsifiable concentrate formulations are liquids that contain the active ingredient, one or more solvents, and an emulsifier that allows mixing with a component in an organic liquid carrier. Formulations of this type are highly concentrated, relatively inexpensive per pound of active ingredient, and easy to handle, transport, and store. In addition, they require little agitation (will not settle out or separate) and are not abrasive to machinery or spraying equipment.

Wettable powders are dry, finely ground formulations in which the active ingredient is combined with a finely ground carrier (usually mineral clay), along with other ingredients to enhance the ability of the powder to suspend in water. Generally, the powder is mixed with water for application. Typical solid diluents are described in Watkins et al., Handbook of Insecticide Dust Diluents and Carriers, 2nd Ed., Dorland Books, Caldwell, N.J. The more absorptive diluents are preferred for wettable powders and the denser ones for dusts.

Liquid flowable formulations are made up of finely ground active ingredient suspended in a liquid. Dry flowable and water-dispersible granule formulations are much like wettable powders except that the active ingredient is formulated on a large particle (granule) instead of onto a ground powder.

The methods of making such formulations are well known. Solutions are prepared by simply mixing the ingredients. Fine, solid compositions are made by blending and, usually, grinding, as in a hammer or fluid energy mill. Suspensions are prepared by wet-milling (see, for example, U.S. Pat. No. 3,060,084).

The concentration of a pollen growth stimulating compound in a formulation may vary according to particular compositions and applications.

In some embodiments of the disclosure, inactive ingredients i.e., adjuvants) are added to pollen to improve the performance of the formulation. For example, in one embodiment of the disclosure, pollen is formulated with a surfactant. A surfactant (surface active agent) is a type of adjuvant formulated to improve the dispersing/emulsifying, absorbing, spreading, and sticking properties of a spray mixture. Surfactants can be divided into the following five groupings: (1) non-ionic surfactants, (2) crop oil concentrates, (3) nitrogen-surfactant blends, (4) esterified seed oils, and (5) organo-silicones.

Suitable surfactants may be nonionic, cationic, or anionic, depending on the nature of the compound used as an active ingredient. Surfactants may be mixed together in some embodiments of the disclosure. Nonionic surfactants include polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated or unsaturated fatty acids and alkylphenols. Fatty acid esters of polyoxyethylene sorbitan, such as polyoxyethylene sorbitan trioleate, also are suitable nonionic surfactants. Other suitable nonionic surfactants include water-soluble polyadducts of polyethylene oxide with polypropylene glycol, ethylenediaminopolypropylene glycol and alkylpolypropylene glycol. Particular nonionic surfactants include nonylphenol polyethoxyethanols, polyethoxylated castor oil, polyadducts of polypropylene and polyethylene oxide, tributylphenol polyethoxylate, polyethylene glycol and octylphenol polyethoxylate. Cationic surfactants include quaternary ammonium salts carrying, as N-substituents, an 8 to 22 carbon straight or branched chain alkyl radical.

The quaternary ammonium salts carrying may include additional substituents, such as unsubstituted or halogenated lower alkyl, benzyl, or hydroxy-lower alkyl radicals. Some such salts exist in the form of halides, methyl sulfates, and ethyl sulfates. Particular salts include stearyldimethylammonium chloride and benzyl bis(2-chloroethyl)ethylammonium bromide.

Suitable anionic surfactants may be water-soluble soaps as well as water-soluble synthetic surface-active compounds. Suitable soaps include alkali metal salts, alkaline earth metal salts, and unsubstituted or substituted ammonium salts of higher fatty acids. Particular soaps include the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures. Synthetic anionic surfactants include fatty sulfonates, fatty sulfates, sulfonated benzimidazole derivatives, and alkylarylsulfonates. Particular synthetic anionic surfactants include the sodium or calcium salt of ligninsulfonic acid, of dodecyl sulfate, or of a mixture of fatty alcohol sulfates obtained from natural fatty acids. Additional examples include alkylarylsulfonates, such as sodium or calcium salts of dodecylbenzenesulfonic acid, or dibutylnaphthalenesulfonic acid. Corresponding phosphates for such anionic surfactants are also suitable.

Other adjuvants include carriers and additives, for example, wetting agents, such as anionic, cationic, nonionic, and amphoteric surfactants, buffers, stabilizers, preservatives, antioxidants, extenders, solvents, emulsifiers, invert emulsifiers, spreaders, stickers, penetrants, foaming agents, anti-foaming agents, thickeners, safeners, compatibility agents, crop oil concentrates, viscosity regulators, binders, tackers, drift control agents, or other chemical agents, such as fertilizers, antibiotics, fungicides, nematicides, or pesticides (others are described

hereinabove). Such carriers and additives may be used in solid, liquid, gas, or gel form, depending on the embodiment and its intended application.

As used herein "artificial pollination" is the application, by hand or dedicated machinery, of fertile stigmas with the pollen from plants with desired characteristics, as described herein.

Artificial pollination in the field can be achieved by pollen spraying, spreading, dispersing or any other method. The application itself will be performed by ground equipment, aircraft, unmanned aerial vehicles (UAV), remote-piloted vehicles(RPV), drones or specialized robots, special vehicles or tractors, animal assisted, specialized apparatus that is designed to spread boosts of pollen, specialized apparatus that combines ventilation and spraying of pollen to enhance recycling of pollen or any other application method or apparatus wherein application can be of a single dose, multiple doses, continuous, on an hourly/daily/weekly/monthly basis or any other application timing methodology.

Example 2 below (which is hereby incorporated into this section in its entirety) describes a number of embodiments for artificial pollination by hand, including:

(i) Direct application using paper bags;

(ii) Simple pollen dispersal above the female inflorescence (single application of total amount); or

(iii) Continuous pollen spraying above the female inflorescence.

It will be appreciated that at any time the weed of interest can be further treated with other weed control means. For example, the weed may be treated with a herbicide (which is usually applied at early stages of germination as opposed to the pollen which is applied at flowering). Thus a herbicide for instance can be applied prior to, concomitantly with or following pollen treatment.

Any of the pollen compositions described herein can be produced as a single species pollen with a single trait for reducing weed fitness, a single species pollen with a plurality of traits for reducing weed fitness (e.g., a number of different herbicide resistances or a number of sterility encoding mechanisms) all introduced into a single weed or to a plurality of weeds of the same species, a multispecies pollen with a single trait or a multispecies pollen with a plurality of said traits.

Thus, commercial products can be manufactured as kits whereby each pollen type is packed in a separate packaging means (e.g., bag), or two or more types of pollen are combined into a single composition and packed in a single packaging means (e.g., bag). The product may be accompanied by instructions for use, regulatory information, product description and the like.

The kit may also include in a separate packaging means other active ingredients such as at least one of a chemical inducer (as described above), herbicide, fertilizer, antibiotics and the like.

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of” means “including and limited to”.

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof. Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single

embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); “Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic

Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Pollen collection – Amaranthaceae, Poaceae, Asteraceae

Paper bags are used for pollen collection. Pollen is collected at morning (9:00 AM) by carefully inserting a male inflorescence into a paper bag and gently tapping the bag to release the pollen off the anthers. This collection process is repeated until pollen dust is visible inside the paper bags. Pollen grains are collected and pooled from multiple male plants. Each paper bag is weighed and the average pollen amount generated from a single male inflorescence and a single plant is calculated.

EXAMPLE 2

Calibration of pollen amounts needed for optimal pollination and comparison between different application methods for diecious species – Amaranthus palmeri, Amaranthus tuberculatus

The experiment compares three pollen doses under four different application methods each group contains three female plants that are pollinated. In addition, one group of female plants is not pollinated at all and is used as control for apomixis levels. In all cases female plants are kept isolated from male plants. The doses that are used are approximately equivalent to pollen harvested from 0.1, 1, 10 total pollen of male plants, respectively. The application methods compared are: (i) Direct application using paper bags, (ii) Simple pollen dispersal above the female inflorescence (single application of total amount) (iii) Simple pollen dispersal above the female inflorescence (4 applications in intervals of 2 days, each application of 0.25 of the total amount of pollen dose) (iv) Continuous pollen spraying above the female inflorescence for 1 hour (the overall dose applied is identical to other treatments).

Pollen application by paper bags is conducted as follows: four paper bags with pollen and one paper bag without pollen are put on each of five flowering spikes randomly chosen. The spikes are longer than the paper bags, therefore, a label is attached just below the paper bag to

mark the portion of the spike that is exposed to pollen. The paper bag with no pollen is used as a control.

Pollen application by simple pollen dispersal is conducted as follows: pollen is dispersed above the inflorescences of the female plants from 50 cm distance of the average female plant height. The pollen application process is repeated 4 times in application method iii.

Continuous pollen application by spraying is conducted from the same height as in application method ii for 1 hour.

14 days post pollination, seeds are harvested. In the paper bags method, the number of seeds per cm of spike is determined and in all other methods the number of seeds per female plant is determined.

Table 2

Application method	Amount of pollen applied (as estimated from N male plants)	Single dose/Multiple dose continuous application
Paper bags (i)	N=0.1	Single dose
Paper bags (i)	N=1	Single dose
Paper bags (i)	N=10	Single dose
Pollen dispersal (ii)	N=0.1	Single dose
Pollen dispersal (ii)	N=1	Single dose
Pollen dispersal (ii)	N=10	Single dose
Pollen dispersal (iii)	N=0.1	Multiple doses
Pollen dispersal (iii)	N=1	Multiple doses
Pollen dispersal (iii)	N=10	Multiple doses
Pollen spraying (iv)	N=0.1	Continuous
Pollen spraying (iv)	N=1	Continuous
Pollen spraying (iv)	N=10	Continuous

EXAMPLE 3

Calibration of pollen amounts needed for optimal pollination and comparison between different application methods for monocious species – Lolium rigidum, Ambrosia trifida, Ambrosia artemisiifolia and sorghum halepense

This example is conducted similarly to Example 2 but rather instead of using female plants, all the male inflorescence on the pollinated plants are covered by paper bags in order to avoid self-pollination.

EXAMPLE 4

Achieving enhanced susceptibility to acetolactate synthase (ALS) inhibitors or EPSP synthase inhibitors by pollen application in growth rooms in A.palmeri and A.tuberculatus

A. palmeri resistant to ALS inhibitors seeds (Horak MJ et al., 1997, Heap I, 2016) are germinated on soil and seedlings are transferred and transplanted into pots. When plants begin to flower, they are closely monitored daily to identify female plants at an early stage. Identified female plants are immediately transferred to another growth room to avoid being pollinated. Ten ALS resistant female plants are transferred into larger pots to allow full growth in size. 2 days after the transfer to large pots, female plants are divided into 2 groups of 5 female plants and each group is placed in a separate growth room having the same conditions and the plants continue to grow. At flowering time pollination procedure is conducted. In each separate room 5 female plants are pollinated by simple dispersal. In one room, the dispersed pollen was collected from males susceptible to ALS inhibitors (seeds obtained from Agriculture Research Service National Plant Germplasm System plant introduction as well as from various locations in Israel) and in the other room the dispersed pollen was collected from males resistant to ALS inhibitors. After 24 hours all the 10 female plants are transferred to the same room and seeds are harvested 14 days after the pollination event.

From each female plant, 100 seeds are taken and split into 2. Each set of 50 seeds are planted in trays of 15 by 15 cm. One tray is covered with a thin layer of soil before spraying the ALS inhibitor (ALS inhibitor - Atlantis, 2+10g/L OD, Bayer is sprayed according to manufacturer instructions – 25+120g/ha). Control trays are not sprayed. Emerging seedlings are counted 14 days after spraying. Emergence in control trays is used to estimate the potential total number of germinating seeds in sprayed trays of the same seed source. The proportion of resistance to ALS inhibitors is compared between the two progeny populations. The reduction in this proportion between the groups pollinated with resistant pollen and susceptible one reflects the effect of the susceptibility property that can be inherited by crossing these two specific susceptible and resistant varieties.

Table 3

Female plants	Pollen source	Resistance estimation in progeny (as calculated from the number of seedlings that emerge out of 50 following herbicide application)
5 resistant plants F_R	Pollen from resistant plants M_R	$N^R(F_R x M_R)$ – Number of resistant seedlings
5 resistant Plants F_R	Pollen from susceptible plants M_S	$N^R(F_R x M_s)$ – Number of resistant seedlings
Susceptibility inheritance = 1 - $N^R(F_R x M_s)$/ $N^R(F_R x M_R)$		

A similar experiment is conducted using seeds from A. palmeri resistant to EPSP synthase inhibitors seeds (Culpepper AS et al. 2006, Heap I, 2016) where EPSPS inhibitor is used for selection (EPSPS inhibitor -ROUNDUP, 360 g/l SL, MONSANTO is sprayed according to manufacturer instructions – 720 g/ha).

Separately, the experiment is repeated in an identical setup using A.tuberculatus resistant to ALS inhibitor seeds (Patzoldt WL et al., 2002, Heap I, 2016) or A.tuberculatus resistant to EPSP synthase inhibitors seeds (Vijay K. et al. 2013, Heap I, 2016). The source of susceptible seeds is from Agriculture Research Service National Plant Germplasm System plant introduction as well as from various locations in Israel.

EXAMPLE 5

Achieving enhanced susceptibility to ALS or EPSPS inhibitors by pollen application under competitive conditions in growth rooms in A. palmeri and A. tuberculatus

Palmeri plants resistant to ALS inhibitors or EPSPS inhibitors (seeds source same as in Example 4) are grown and the separation between female and male plants is conducted as described in Example 4. At flowering time, two plots are being established, each of size 4 x 4 m, each containing together 5 females and 4 males plants. Both plots contain only resistant plants (both female and males). The two plots are located in separate growth rooms in order to avoid pollen cross contamination.

Pollen harvested from susceptible male plants is being dispersed on one of the plots and plants continue to grow for 14 days and then harvested. From each female plant, 100 seeds are collected and split into 2 sets. Each set of 50 seeds is planted in trays of 15 x 15 cm. One tray is covered with a thin layer of soil before spraying the ALS inhibitor or EPSPS inhibitor.

Control trays are not sprayed. Emerging seedlings are counted 14 days after spraying. Emergence in control trays is used to estimate the potential total number of germinating seeds in sprayed trays of the same seed source.

The proportion of resistance to ALS inhibitors or EPSPS inhibitors is compared between the progeny population originated from the two plots with and without the additional susceptible pollen. The enhanced susceptibility to ALS inhibitors or EPSPS inhibitors between the plots with the artificial pollination relatively to the one without it shows the efficacy of the artificial pollination under competitive conditions.

Table 4

Female plants	Pollen source	Resistance estimation in progeny (as calculated from the number of seedlings emerge out of 50 following herbicide application)
5 resistant plants F_R	5 resistant plants M_R	$N^R(F_R x M_R)$ – Number of resistant seedlings
5 resistant Plants F_R	5 Resistant plants + pollen from susceptible plants $M_R + M_s$	$N^R(F_R x(M_R + M_s))$ - Number of resistant seedlings
Efficacy of the artificial pollination under competitive conditions = 1- $N^R(F_R x(M_R + M_s))/ N^R(F_R x M_R)$		

EXAMPLE 6

Achieving enhanced Lolium rigidum susceptibility to ALS/EPSPS inhibitor by pollen application in growth rooms

L. rigidum resistant to ALS inhibitor or EPSPS inhibitor seeds (Matzrafi M and Baruch R, 2015) are germinated on soil and seedlings are transferred and transplanted into pots. The experiment is conducted as described in Example 4.

EXAMPLE 7

Achieving enhanced Ambrosia artemisiifolia (common ragweed) susceptibility to ALS/EPSPS inhibitor by pollen application under competitive conditions in growth rooms

A. artemisiifolia resistant to EPSPS inhibitor seeds (Heap I, 2016) is germinated on soil and seedlings are transferred and transplanted into pots. Ten female plants are taken and divided into two groups of 5. Each group is placed in separate growth rooms with similar conditions to avoid cross-pollination. When plants begin to flower, one group is being artificially pollinated by dispersal of pollen harvested from male plants susceptible to EPSPS inhibitor while the other group is not artificially pollinated.

As the Ambrosia species is monoecious, the artificial pollination that is conducted here is under competitive conditions as native pollen exists at the flowering period. Seeds are harvested 14 days after the pollination event.

From each female plant, 100 seeds are collected and split into 2 sets. Each set of 50 seeds is planted in trays of 15 x 15 cm. One tray is covered with a thin layer of soil before spraying with ALS/EPSPS inhibitor. (ALS inhibitor - Atlantis, 2+10g/L OD, Bayer is sprayed according to manufacturer instructions – 25+120g/ha, EPSPS inhibitor - ROUNDUP, 360 g/l SL, MONSANTO is sprayed according to manufacturer instructions – 720 g/ha).

Control trays are not sprayed but are only covered with a thin layer of soil. Emerging seedlings are counted 14 days after spraying. Emergence in control trays is used to estimate the potential total number of germinating seeds in sprayed trays of the same seed source. The proportion of resistance to ALS/EPSPS inhibitor is compared between the two progeny

populations. The reduction in this proportion between the groups pollinated with susceptible pollen and the one not artificially pollinated reflects the efficacy of the pollination treatment in monoecious species such as ambrosia.

Table 5

# of plants	Pollen source (native/external)	Resistance estimation in progeny (as calculated from the number of seedlings emerge out of 50 following herbicide application)
5 resistant plants (R)	Native pollen only (R)	N^R(RxR) – Number of resistant seedlings
5 resistant Plants (R)	Native pollen (R) + external application (S)	N^R(Rx(R+S)) – Number of resistant seedlings
Efficacy of treatment for susceptibility inheritance = 1- N^R(Rx(R+S))/ N^R(RxR)		

EXAMPLE 8

Achieving enhanced Ambrosia trifida (giant ragweed) susceptibility to ALS/EPSPS inhibitor by pollen application under competitive conditions in growth rooms

Experiment is conducted and evaluated as described in Example 7 with Ambrosia trifida instead of Ambrosia artemisiifolia.

EXAMPLE 9

Generation and Evaluation of a "super herbicide sensitive" weed by breeding of A.palmeri, A.tuberculatus

To produce super herbicide sensitive pollen from A. Palmeri the following selection for highest sensitivity to various herbicides was performed:

1. A. Palmeri line with highest sensitivity to EPSP synthase inhibitors mode of action was first picked in the following way: application of EPSPS inhibitor at 0.125x, 0.25x, 0.5x, 1x and 2x, where x is the standard recommended levels of glyphosate. Clones of plants that died from 0.125x were allowed to produce seed and were further subjected to recurrent selection to generate the most sensitive plants (S lines), which died from 0.125x glyphosate.
2. A. Palmeri with highest sensitivity to ALS inhibitors mode of action was picked by application of ALS inhibitor at 0.125x, 0.25x, 0.5x, 1x and 2x, where x is the standard recommended levels of ALS inhibitor. Clones of plants that died from 0.125x were allowed to produce seed and were further subjected to recurrent selection to generate the most sensitive plants (S lines), which died from 0.125x ALS inhibitor.
3. A. Palmeri with highest sensitivity to Acetyl CoA Carboxylase (ACCase) inhibitors mode of action was picked by application of ACCase inhibitor at 0.125x, 0.25x, 0.5x,

1x and 2x, where x is the standard recommended levels of ACCase inhibitor. Clones of plants that died from 0.125x were allowed to produce seed and were further subjected to recurrent selection to generate the most sensitive plants (S lines), which died from 0.125x ACCase inhibitor.

The A. Palmeri lines obtained by the methods described herein may be further crossed by traditional breeding techniques to obtain a plant weed line that is "Super herbicide sensitive" to multiple modes of actions.

Evaluation of enhanced A. palmeri susceptibility to EPSP synthase inhibitors, ALS inhibitors and Acetyl CoA Carboxylase (ACCase) inhibitors by pollen application in growth rooms is conducted as described in Example 4 with the usage of multiple herbicides instead of one herbicide.

The same procedure to obtain "super herbicide sensitive" is done with A. tuberculatus.

EXAMPLE 10

Generation and evaluation of the sterility property of A. Palmeri or A. tuberculatus transformed with "terminator technology" genes

As previously described in U.S. Patent 5,925,808, 3 plasmids are being used for A. palmeri or A. tubercultus transformation.

1. a gene which expression results in an altered plant phenotype linked to a transiently active promoter, the gene and promoter being separated by a blocking sequence flanked on either side by specific excision sequences.

2. A second gene that encodes a recombinase specific for the specific excision sequences linked to a repressible promoter.

3. A third gene that encodes the repressor specific for the repressible promoter.

Plasmid sequences and procedures are used as described in U.S. 5,925,808, supra:

1. The death gene used is RIP (ribosomal inactivating protein, sequence of a complete RIP gene, saporin 6:GenBank ID SOSAP6, Accession No. X15655) or barnase (Genbank Accession M14442)

2. Construction of a CRE Gene under the control of a Tetracycline-derepressible 35S Promoter.

3. Third plasmid is Tet Repressor Gene Driven by a 35S Promoter.

The transiently active promoter in the first plasmid is replaced with A.palmeri promoter or A. tuberculatus that is expressed during embryogenesis, seed development or seed germination. A. palmeri or A. tuberculatus transformation is carried out as previously described

in Pal A., et al 2013. A stably transformed line that highly expresses the desired plasmids is picked for further stages.

Seeds from this A. Palmeri or A. tuberculatus line are split into two groups: one group is treated with tetracycline whereas the other group is left untreated. The plants are grown and identified males from each group are picked for the evaluation stage.

Evaluation of the efficiency of sterility in the transformed line is conducted in the following way: Two plots are being established at flowering time: 1. Containing 5 natural female A. palmeri or A. tuberculatus plants with 4 males from this transformed line that are not treated with tetracycline in the seed stage. 2. Containing 5 natural female A. palmeri or A. tuberculatus plants with 4 males from this genetically modified line that is treated with tetracycline in the seed stage. Plants continue to grow for 14 days and then seeds are being harvested. Two measures are being estimated: 1. Total count and weight of seeds produced from each female plant where the difference between the counts and weights between the two groups represent sterility efficiency. 2. From each female plant 50 seeds are taken and planted and the number of emerged seedlings is counted at the age of 14 days. The sterility efficiency is estimated from these two parameters.

Table 6

Female plants	Pollen source	Seeds count and weight	Seedling emergence estimation in progeny (as calculated from the number of seedlings emerge out of 50)
5 female plants F	5 males with the "terminator technology" without tetracycline treatment $M_{T\text{-}tet}$	$N_{seeds}(Fx\ M_{T\text{-}tet})$ – seed count $W_{seeds}(Fx\ M_{T\text{-}tet})$ - total seed weight	$N_{seedlings}(Fx\ M_{T\text{-}tet})$ - Number of seedlings
5 female plants F	5 males with the "terminator technology" with tetracycline treatment M_{T+tet}	$N_{seeds}(Fx\ M_{T+tet})$ – seed count $W_{seeds}(Fx\ M_{T+tet})$ - total seed weight	$N_{seedlings}$ $(Fx\ M_{T+tet})$ - Number of seedlings
Efficacy of Sterility by number of seeds or seedlings= $1 - (N(Fx\ M_{T+tet}) / N(Fx\ M_{T\text{-}tet}))$			

An alternative set of plasmids that are used are based on the Tet ON system in which the rtTA (reverse tetracycline controlled transactivator) protein is capable of binding the operator only if bound by a tetracycline and as a consequence activates transcription:

1. a gene which expression results in an altered plant phenotype linked to a transiently active promoter, the gene and promoter being separated by a blocking sequence flanked on either side by specific excision sequences.

2. A second gene that encodes a recombinase specific for the specific excision sequences linked to an operator that is upstream to the promoter and is responsive to an activator.

3. A third gene that encodes the activator specific for the operator in the second plasmid. Under one instance the activator can be regulated by an inducible promoter. Alternatively, the inducer can bind the activator protein eliciting a conformational change to its active form.

Plasmid sequences are:

1. The death gene used is RIP (ribosomal inactivating protein, sequence of a complete RIP gene, saporin 6:GenBank ID SOSAP6, Accession No. X15655) or barnase (Genbank Accession M14442) under the control of a specific embryogenesis, seed development or germination promoter.

2. Construction of a CRE Gene under the control of a Tetracycline-responsive element (TRE).

3. Third plasmid is a 35S promoter upstream of a fusion of a Tet Repressor Gene, reverse TetR (reverse tetracycline repressor), found in Escherichia coli bacteria, with the activation domain of another protein, VP16, found in the Herpes Simplex Virus (termed rtTA).

Upon application of tetracycline or its derivatives such as doxycycline the rtTA becomes activated and results in expression of the CRE recombinase and consequently activation of the death gene.

Another set of plasmids that are used is based on only two sets of plasmids:

1. a gene which expression results in an altered plant phenotype linked to a transiently active promoter and an operator that is upstream to the promoter and is responsive to an activator.

2. A second gene that encodes the activator specific for the operator from the first plasmid which is activated upon induction. Plasmid sequences are:

1. The death gene used is RIP (ribosomal inactivating protein, sequence of a complete RIP gene, saporin 6:GenBank ID SOSAP6, Accession No. X15655) or barnase (Genbank

Accession M14442) under the control of a specific embryogenesis, seed developmentor germination promoter and upstream to the promoter a TRE sequences.

2. A constitutive promoter upstream of a rtTA gene.

Upon application of tetracycline or its derivatives such as doxycycline the rtTA becomes activated and results in activation of the death gene.

Similar experimental setups are repeated with both plasmid sets explained above and the efficiency of sterility is calculated and evaluated as explained with the first plasmid set.

EXAMPLE 11

Generation and evaluation of the sterility property in A. Palmeri or A. tuberculatus transformed with sterility genes under specifically regulated promoter

A. Palmeri or A. tuberculatus sterile line is being produced using 2 plasmids:

1. Plasmid encoding for a disrupter protein under a promoter that is active in the embryo or seed, which makes it sterile where the gene promoter is under the control of a specific operator sequence responsive to repression by a repressor protein.

2. A repressor protein, whose gene is under the control of a constitutive promoter. When binding to a specific chemical the repressor can bind the operator from the first plasmid and inhibit the expression of the disrupter protein. Plasmid sequences are:

1. RIP gene (ribosomal inactivating protein, sequence of a complete RIP gene, saporin 6:GenBank ID SOSAP6, Accession No. X15655) or barnase (Genbank Accession M14442) under the control of a specific embryogenesis, seed development or germination promoter with a TetO that is responsive to reverse tetracycline repressor.

2. Construction of a reverse tetracycline repressor gene under the control of a constitutive promoter.

Upon tetracycline application the reverse tetracycline repressor binds tetracycline and leads to repression of disrupter gene.

Evaluation of the efficiency of sterility in the transformed line is conducted as described in Example 10. The evaluation includes two stages:

1. Comparing the total seed number and weight between the groups.

2. Comparing the fraction of emerged seedlings out of 50 seeds sown. The experimental setup for the second stage is illustrated in the table below:

Table 7

Female plants	Pollen source	Seeds count and weight	Seedling emergence estimation in progeny (as calculated from the number of seedlings emerge out of 50)
5 female plants F	5 males of the transformed line without tetracycline treatment M_{T-tet}	N_{seeds}(Fx M_{T-tet}) – seed count W_{seeds}(Fx M_{T-tet}) - total seed weight	$N_{seedlings}$ (Fx M_{T-tet}) - Number of seedlings
5 female plants F	5 males of the transformed line with tetracycline treatment M_{T+tet}	N_{seeds}(Fx M_{T+tet}) – seed count W_{seeds}(Fx M_{T+tet}) - total seed weight	$N_{seedlings}$ (Fx M_{T+tet}) - Number of seedlings
Efficacy of Sterility by number of seeds or seedlings = 1 - N(Fx M_{T-tet})/ N(Fx M_{T+tet})			

An alternative set of plasmids that are used are based on the Tet OFF system:

1. Plasmid encoding for a disrupter protein under a promoter that is active in the embryo or seed, which makes the plant sterile where the gene promoter is under the control of a specific operator sequence responsive to activation by an activator protein.

2. An activator protein, whose gene is under the control of a constitutive promoter. Upon specific chemical binding to this activator it becomes non-active and can no longer activate the transcription of the first plasmid.

Plasmid sequences are:

1. RIP gene (ribosomal inactivating protein, sequence of a complete RIP gene, saporin 6:GenBank ID SOSAP6, Accession No. X15655) or barnase (Genbank Accession M14442) under the control of a dual regulation with a specific embryogenesis, seed developmentor germination promoter and a TRE sequence.

2. Construction of a tetracycline transactivator protein tTA gene (composed of fusion of one protein, TetR (tetracycline repressor), found in Escherichia coli bacteria, with the activation domain of another protein, VP16 under the control of a constitutive promoter.

Upon application of tetracycline or its derivatives such as doxycycline the tTA becomes repressed and results in loss of activation of the disrupter gene and recovery of sterility.

Similar experimental setups are repeated with this plasmid set and the efficiency of sterility is calculated and evaluated as explained with the first plasmid set.

EXAMPLE 12

Generation and evaluation of the susceptibility to EPSPS inhibitor in A. Palmeri or A. tuberculatus transformed with antisense RNA under specifically regulated promoter

As in Example 10 with the use of an antisense RNA against EPSP synthase replacing the disrupter gene. EPSP synthase antisense sequence that is conserved across multiple Amaranthus species is used, e.g., corresponding to nucleotide positions 590-802 (antisense) of KF5692111.

Induced EPSPS inhibitor susceptibility will be examined following application of both tetracycline for activation of EPSPS antisense expression and application of EPSPS inhibitor (ROUNDUP, 360 g/l SL, MONSANTO is sprayed according to manufacturer instructions – 720 g/ha) for selection.

EXAMPLE 13

Generation of A. Palmeri or A. tuberculatus sterile hybrid line transformed with dual complementary male and female plant genetic recombinations systems

A. Palmeri or A. tuberculatus sterile line is being produced by crossing between two homozygous transformed plants. The male and female plants are each transformed with a plasmid encoding a disrupter gene controlled by a transiently active promoter, the gene and promoter being separated by a blocking sequence flanked on either side by specific excision sequences (such as lox or frt excision sequences). In addition the plasmid contains a second gene that encodes a genetic recombination enzyme (such as cre recombinase or flp flippase) specific for the excision sequences in the opposite sex (namely, the recombination enzyme of the female plant cut the excision sequence in the male and vice versa). These recombination enzymes are under the control of a promoter that is active post seed germination stage. The transformed plasmid both in the male and in the female homozygous lines are inserted to the same genomic locus position.

The following plasmid is transformed into the female plant:

Plasmid encoding a barnase or RIP gene under the control of a specific embryogenesis or germination promoter whereas the gene and promoter being separated by a blocking sequence flanked on either side by specific excision lox sequences and a second gene encoding for a flippase recombination enzyme under a promoter that is active post seed germination.

The following plasmid is transformed into the male plant:

Plasmid encoding a barnase or RIP gene under the control of a specific embryogenesis or germination promoter whereas the gene and promoter are being separated by a blocking sequence flanked on either side by specific excision frt sequences and a second gene encoding

for a cre recombinase recombination enzyme under a promoter that is active post seed germination.

Lines are being selected such that both insertions to both male and female are on the exact same genomic position.

Only upon crossing between these male plants with these female plants both recombination events by flp and cre are occurring thus yielding pollen that have a barnase or RIP gene under the control of a specific embryogenesis or germination promoter.

EXAMPLE 14

Evaluation of the sterility property in A. Palmeri or A. tuberculatus hybrid line transformed with dual complementary male and female plant recombinase/flippase systems

Evaluation of the efficiency of sterility in the transformed line is conducted as described in Example 10. The evaluation includes 2 stages: 1. Comparing the total seed number and weight between the two compared groups 2. Comparing the fractions of emerged seedlings out of 50 seeds sown. The experimental setup is illustrated in the table below:

Table 8

Female plants	Pollen source	Seeds count and weight	Seedling emergence estimation in progeny (as calculated from the number of seedlings emerge out of 50)
5 female plants F	4 natural male plants M	N_{seeds}(FxM) – seed count W_{seeds}(Fx M) - total seed weight	$N_{seedlings}$(FxM) - Number of seedlings
5 female plants F	4 hybrid male plants M_{hyb}	N_{seeds}(Fx M_{hyb}) – seed count W_{seeds}(Fx M_{hyb}) - total seed weight	$N_{seedlings}$ (FxM_{hyb}) - Number of seedlings
Efficacy of Sterility by number of seeds or seedlings = 1- (N(FxM_{hyb})/N(Fx M))			

EXAMPLE 15

Achieving reduction of A. palmeri or A. tuberculatus population by application of sterile pollen in growth room

A. palmeri or A. tuberculatus seeds are germinated on soil and seedlings are transferred and transplanted into pots. At flowering time two plots are being established, each of size 4 x 4 m, each containing together 5 female and 4 male plants.

The two plots are located in separated growth rooms in order to avoid pollen cross contamination. Sterile pollen generated as described in Example 10, 11 or 13 is dispersed on one of the plots. The application procedure is one application per day for 5 consecutive days. The plants continue to grow for 14 days and then harvested. Seed biomass is measured for each plant

and the number of seeds per 0.1 g is being counted and the total number of seeds per plant is being estimated and recorded. In addition, from each female plant, 100 seeds are taken. The seeds are planted in trays of 30 x 30 cm. Emerged seedlings are counted at the age of 14 days and the emergence rate is calculated for both groups. The reduction in the emergence proportion between the group pollinated with sterile pollen and the control group reflects the estimation for the reduction in A. palmeri or A. tuberculatus population size due to the treatment per one reproduction cycle.

Table 9

Female plants	Pollen source	Seeds count and weight	Population size reduction estimation (as calculated from the number of seedlings emerge out of 100 seeds)
5 female plants	4 male plants	N_{seeds}(FxM) – seed count W_{seeds}(FxM) - total seed weight	N (FxM) – Number of emerged seedlings
5 female Plants	4 male plants + sterile pollen	N_{seeds}(Fx(M+ M_s)) – seed count W_{seeds}(Fx(M+ M_s)) - total seed weight	N (Fx(M+ M_s)) - Number of emerged seedlings
Expected population size reduction per year = 1- N (Fx(M+ M_s)) / N (FxM)			

EXAMPLE 16

Achieving reduction of A. palmeri or A. tuberculatus population by application of sterile pollen in controlled field conditions

Sterile pollen is generated as described in Example 10, 11 or 13 and collected as described in Example 1. Two groups of 8 A. palmeri plants composed of 4 male plants and 4 females plants are transplanted in the field. Each group is arranged in 2 rows of four plants in alternating order of female and male. The distance between each plant is 1m. The distance between the location of the 2 groups is 1km. The two groups are treated similarly and are watered on a daily basis. One group is used as control group (C) to estimate the native population growth without any application of non-native pollen. The second group (T) is pollinated both with the native pollen and with additional sterile pollen that was generated as described in Examples 10, 11, or 13. At the beginning of the flowering time a pollination treatment is being applied to group T. The treatment is given in 4 applications in intervals of 3 days, each application is given once a day (at morning hours). All plants are harvested after seed maturation and seeds are being collected manually. Seed biomass is measured for each plant and the number of seeds per 0.1 g is being counted and the total number of seeds per plant is being estimated and recorded.

In addition, from each female plant, 100 seeds are taken. The seeds are planted in trays of 30 x 30 cm. Emerged seedlings are counted at the age of 14 days and the emergence rate is calculated for both groups. The reduction in the emergence proportion between the group pollinated with sterile pollen and the control group reflects the estimation for the reduction in A. palmeri or A. tuberculatus population size due to the treatment per one year.

Table 10

Female plants	Pollen source	Seeds count and weight	Population size reduction estimation (as calculated from the number of seedlings emerge out of 100 seeds)
4 females plants	4 male plants	N_{seeds}(FxM) – seed count W_{seeds}(FxM) - total seed weight	N (FxM) – Number of emerged seedlings
4 females Plants	4 male plants + sterile pollen	N_{seeds}(Fx(M+ M_s)) – seed count W_{seeds}(Fx(M+ M_s)) - total seed weight	N (Fx(M+ M_s)) - Number of emerged seedlings
Expected population size reduction per year = 1- N (Fx(M+ M_s)) /			

EXAMPLE 17

Achieving reduction of A. palmeri or A. tuberculatus population by application of sterile pollen from a natural seedless strain in growth room

Pollen is collected from naturally occurring seedless strain of A. palmeri or A. tuberculatus. This pollen is used as described in Example 15 to evaluate the efficacy of the sterility achieved.

EXAMPLE 18

Achieving sterility in A. Palmeri or A. tuberculatus by applying pollen harvested from tetraploid A. Palmer strain

Generation of A. Palmeri or A. tuberculatus tetraploid plants is achieved by treatment of 0.25% aqueous solution of colchicine on growing buds of seedling thrice daily for three consecutive days. Pollen from these plants is harvested and collected.

This pollen is used as described in Example 15 to evaluate the efficacy of the sterility achieved.

EXAMPLE 19

Achieving sterility in A. Palmeri or A. tuberculatus by applying pollen pre-treated with irradiation

Pollen from naturally occurring A. Palmeri or A. tuberculatus plants is harvested and collected. The pollen is treated by UV, X-ray or gamma irradiation. This pollen is used as described in Example 15 to evaluate the efficacy of the sterility achieved.

EXAMPLE 20

Achieving reduction of A. palmeri and A. tuberculatus populations by application of mixture of sterile pollen in a controlled field conditions

Sterile pollen is generated as described in Examples 10, 11, 13, 17, 18 or 19 and collected as described in Example 1 both from A. palmeri male plants and from A. tuberculatus male plants. The pollen from both species is mixed together and the treatment is with this mixture. The field experimental setup is similar to the one described in Example 16 except that instead of having in each group 8 A. palmeri plants (composed of 4 females and 4 males plants) each group contains 4 A. palmeri plants (2 females and 2 males) and 4 A. tuberculatus plants (2 females and 2 males). At the beginning of flowering time one group is being treated with the pollen mixture 1 application per day for 4 times in intervals of 3 days.

The effect of pollen treatment on the population size of both species is estimated similarly to the way described in example 16.

Table 11

Female plants	Pollen source	Population size reduction estimation (as calculated from the number of seedlings emerge out of 100 seeds)
2 A. palmeri + 2 A. tuberculatus	2 A. palmeri + 2 A. tuberculatus	N_p (FxM) – Number of A. palmeri emerged seedlings N_t (FxM) – Number of A. tuberculatus emerged seedlings
2 A. palmeri + 2 A. tuberculatus	2 A. palmeri + 2 A. tuberculatus + mixture of sterile pollen	N_p (Fx(M+ M_s)) - Number of A. palmeri emerged seedlings N_t (Fx(M+ M_s)) - Number of A. tuberculatus emerged seedlings
Expected population size reduction per year =1– $N_{p/t}$ (Fx(M+ M_s)) / $N_{p/t}$ (FxM)		

EXAMPLE 21

Generation and evaluation of induced EPSPS inhibitor susceptibility following A. Palmeri or A. tuberculatus transformation with AlcR based Ethanol inducible death gene

A. Palmeri or A. tuberculatus EtoH inducible line is being produced using a plasmid encoding for AlcR based EtoH inducible promoter linked to a barnase gene or a RIP gene. In this example there is no repression or tissue specific promoter. The promoter is activated after EtoH spraying and therefore, the seeds do not develop.

A. palmeri transformation is carried out as previously described in Pal A., et al 2013 to A. tricolor, supra. A stable transformed line that highly expresses the desired plasmids is selected for further stages.

Pollen collected from this line are examined in a similar protocol as explained in Example 4 except that seeds are sprayed with EtoH instead of the herbicide used in that example to evaluate the efficiency of death following EtoH application.

EXAMPLE 22

Generation and evaluation of induced death following A. Palmeri or A. tuberculatus transformation with AlcR based Ethanol inducible EPSPS antisense RNA

As in Example 21 with the use of an antisense RNA against EPSP synthase replacing the disrupter gene. EPSP synthase antisense sequence that is conserved across multiple Amaranthus species is used, e.g., corresponding to nucleotide positions 597-809 (antisense) of FJ861243.1.

Induced EPSPS inhibitor susceptibility will be examined following application of both EtOH for activation of EPSPS antisense expression and application of EPSPS inhibitor (ROUNDUP, 360 g/l SL, MONSANTO is sprayed according to manufacturer instructions – 720 g/ha) for selection.

EXAMPLE 23

Demonstration of seed production via artificial pollination in A. palmeri

A. Palmeri seeds were germinated on paper and the seedlings were transferred into small pots. After the plants reached a height of about 20 cm they were transferred again into larger pots. When plants began flowering, they were closely monitored daily to identify their sex at an early stage. Immediately after sex identification the females and males were separated and placed in different locations (~6 m apart) outside on September-October in Israel.

Pollen was collected at early morning from A. palmeri male plants using paper tubes (12 cm in length and a diameter of ~1 cm). Each such paper tube was placed on a single male spike. Pollen was released by gently tapping on the paper tube. Each paper tube was used to pollinate an A. palmeri female spike by placing it (with the pollen inside) on one spike and gently tapping it (tapping procedure was repeated several times at intervals of ~10 minutes to enhance pollination). The procedure of artificial pollination was repeated for several days (2-3 times) for each spike and the entire experiment was repeated 3 times – overall 8 spikes (first experiment – 2 spikes, second experiment – 2 spikes, third experiment - 4 spikes were pollinated and 7 spikes served as controls with no application of pollen (first experiment – 2 spikes, second experiment – 2 spikes, third experiment - 3 spikes). The total number of seeds formed (15-20 days post initial pollination event) from each spike and their weights were measured and the results are depicted in Table 12 below:

Table 12

# Exp	# of control spikes	# of pollinated spikes	Control seeds Avg. sample weight (g)	Pollinated seeds Avg. sample weight (g)	Fold Change Pollinated/Control	P-value
1	2	2	0.07	0.18	2.52	0.06
2	2	2	0.05	0.14	2.77	0.15
3	3	4	0.041	0.145	3.67	0.0078
Combined data	7	8	0.052	0.155	2.96	2.36E-5

As can be seen from the table artificial pollination significantly increase the amount of seeds formed.

To evaluate the quality of the seeds that were obtained, average seed weight was calculated and compared to average seed weight of seeds that were collected directly from the field. Results demonstrated that natural seeds and seeds obtained from artificial pollination had a similar weight (see FIG. 1).

EXAMPLE 24

Inhibition of seed development and demonstration of weed control by reduced seed germination in A. palmeri by applying X-ray irradiated pollen in growth room

A. Palmeri seeds were germinated on paper and the seedlings were transferred into small pots. After the plants reached a height of about 20 cm they were transferred into larger pots. When plants began flowering, they were closely monitored daily to identify their sex at an early stage. Immediately after sex identification the females and males were separated and placed in different growth rooms in order to avoid pollination. One female plant with relatively many flowering spikes was transferred into a growth chamber (conditions of 30°/22°C, photoperiod 16/8 day/night) where the pollination experiment was conducted.

Pollen was collected at early morning from A. palmeri male plants using paper tubes (12 cm in length and a diameter of ~1cm). Each such paper tube was placed on a single male spike. Pollen was released by gently tapping on the paper tube. Eight such paper tubes with fresh pollen were collected and divided into two sets of 4. Each set of 4 paper tubes was placed in a 15 cm petri dish. One petri dish was irradiated by X-ray radiation of 300 Gy (overall the duration of the radiation was 80 minutes) while the other petri dish was placed for that time in

similar conditions only without radiation and served as a control with non-irradiated pollen. About 2 hours after pollen collection it was used to artificially pollinate 8 spikes of a female A. palmeri plant. These 8 spikes were divided into 4 pairs where the height of the branch origin of each such pair was approximately the same. Each paper tube was used to pollinate an A. palmeri female spike by placing it (with the pollen inside) on one spike and gently tapping it (tapping procedure was repeated several times in intervals of ~15 minutes to enhance pollination). Pollination was conducted such that one spike from each pair was pollinated with the irradiated pollen and the other with non-irradiated pollen (overall 4 pairs were pollinated). Additional 2 empty paper tubes with no pollen inside were placed on additional 2 spikes in order to serve as a "no-pollen" control. The paper tubes were removed from the spikes after about an hour. 18 days after pollination the top 12 cm of each of the 10 spikes was cut and seeds were harvested. Total seed weight and total seed count per spike were measured and seed morphology was examined. The results are depicted in Table 13, below.

Table 13

Sample	Total Seed Weight (gr)	Number of Seeds	Average Seed Weight (mgr)
Regular pollen #1	0.0769	214	0.359
Regular pollen #2	0.0777	221	0.352
Regular pollen #3	0.0936	317	0.295
Regular pollen #4	0.0589	227	0.259
Irradiated pollen #1	0.0173	181	0.096
Irradiated pollen #2	0.0193	183	0.105
Irradiated pollen #3	0.0152	134	0.113
Irradiated pollen #4	0.0067	105	0.064
No-pollen	0.0011	1	NA
No-pollen	0	0	NA
Average value for regular pollen	0.076775	244.75	0.316417252
Average value for irradiated pollen	0.014625	150.75	0.094571738
t-test p-value	**0.00018**	**0.022**	**0.00015**

Seeds were examined under the microscope and for each sample pictures were taken for a random assortment of seeds with representative appearance (See FIG. 2). In general, the seeds obtained from the artificial pollination with the irradiated pollen looked thin, partly empty and their color was light brown while the ones obtained from the regular pollen looked more filled having a darker brown/black color.

Germination assay was conducted in order to estimate the different germination levels between the seeds obtained by artificial pollination with the irradiated pollen versus the ones obtained from artificial pollination with regular pollen.

Thirty seeds were taken from each of these 8 samples. Each set of 30 seeds was placed in a 6 cm petri dish on a towel paper with 7.5 ml tap water for the germination test. These petri dishes were sealed with parafilm and were placed in a growth chamber in 34/25 °C 16/8h day/night conditions for 16 days. After 16 days emerged seedlings were counted and germination rate was calculated for each sample. A comparison was conducted between the seeds obtained from irradiated pollen and the ones obtained from regular pollen. While the average germination rate obtained from the regular pollen was approximately 72 % none of the seeds obtained from artificial pollination with irradiated pollen germinated (p value of 2.43E-05).

The results are summarized in Table 14, below.

Table 14

Sample	Germination Rate (%)
Regular pollen #1	73.33333
Regular pollen #2	70
Regular pollen #3	86.66667
Regular pollen #4	56.66667
Irradiated pollen #1	0
Irradiated pollen #2	0
Irradiated pollen #3	0
Irradiated pollen #4	0
Average value for regular pollen	**71.66667**
Average value for irradiated pollen	**0**
t-test p-value	**2.43E-05**

The same experiment was conducted with an additional female plant in a similar manner only with 2 samples of X-ray irradiated pollen vs. 2 samples of non-irradiated pollen controls and a single “no-pollen” control. The results are depicted in Table 15 below.

Table 15

Sample	Total Seed Weight (gr)	Number of Seeds	Average Seed Weight (mgr)
Regular pollen #1	0.0486	247	0.197
Regular pollen #2	0.0401	202	0.199
Irradiated pollen #1	0.0192	173	0.110
Irradiated pollen #2	0.0138	170	0.081
No-pollen	0.0065	5	NA

Average value for regular pollen	0.04435	224.5	0.198
Average value for irradiated pollen	0.0165	171.5	0.096
t-test p-value	**0.031**	0.143	**0.020932284**

Seeds were examined under the microscope and for each sample pictures were taken for a random assortment of seeds with representative appearance (See FIG. 3). In general, the seeds obtained from the artificial pollination with the irradiated pollen looked thinner, partly empty and their color was lighter brown relative to the ones obtained from the regular pollen, which looked more filled, having a darker brown/black color.

A germination test was conducted as described above. The germination rates obtained are provided in Table 16 below.

Table 16

Sample	Germination Rate (%)
Regular pollen #1	56.66667
Regular pollen #2	16.66667
Irradiated pollen #1	0
Irradiated pollen #2	0
Average value for regular pollen	**36.66667**
Average value for irradiated pollen	**0**
t-test p-value	**0.21**

Overall, the results indicate that upon application of X-ray irradiated pollen, the seeds that are formed display seed development arrest with reduced number, weight and altered morphology and furthermore these seeds are devoid of their ability to germinate.

EXAMPLE 25

Evaluation of A. palmeri weed control efficiency by artificial pollination with UV irradiated pollen in growth room

A. Palmeri seeds were germinated on paper and the seedlings were transferred into small pots. After the plants reached a height of about 20 cm they were transferred into larger pots. When plants began flowering, they were closely monitored daily to identify their sex at an early stage. Immediately after sex identification the females and males were separated and placed in different growth rooms in order to avoid pollination. One female plant with relatively many

flowering spikes was transferred into a growth chamber (conditions of 34 °/25 °C, photoperiod 16/8 day/night) where the pollination experiment was conducted.

Pollen was collected at early morning from A. palmeri male plants using paper tubes (10 cm in length and diameter of ~1cm). Each such paper tube was placed on a single male spike. Pollen was released by gently tapping on the paper tube. Six such paper tubes with fresh pollen were collected and divided into two sets of 3. Each set of 3 paper tubes was placed in a 15 cm petri dish. Each such paper tube was cut and opened carefully and was organized and placed with pollen exposed from the upper direction. One petri dish was put into UVITEC cross-linker machine for irradiation by UV-C (wave length of 254nm) with energy of 2 joules. Total radiation time was 10 minutes. During this time the other petri dish was placed in similar conditions only without the irradiation treatment. After the irradiation procedure ended the opened paper tubes were re-attached to a cylindrical shape and each one of them was used to pollinate an A. palmeri female spike (in total 6 spikes) by placing it (with the pollen inside) on one spike and gently tapping it (tapping procedure was repeated several times in intervals of ~15 minutes to enhance pollination). These 6 female spikes were originally divided into 3 pairs where the height of the branch origin of each such pair was approximately the same and pollination was conducted such that one spike from each pair was pollinated with the irradiated pollen and the other with non-irradiated pollen (overall 3 pairs were pollinated). The paper tubes were removed from the spikes after about an hour. 17 days after pollination, the top 10 cm of each of the 6 pollinated spikes plus additional 2 non-artificially pollinated spikes (that served as a "no-pollen" control) were cut and seeds were harvested. Total seed weight and total seed count per spike were measured and the results are depicted in Table 17 below.

Table 17

Sample	Total Seed Weight (gr)	Number of Seeds	Average Seed Weight (gr)
Regular pollen #1	0.0506	157	0.000322
Regular pollen #2	0.0927	263	0.000352
Regular pollen #3	0.0447	108	0.000414
Irradiated pollen #1	0.0078	12	0.00065
Irradiated pollen #2	0.0315	48	0.000656
Irradiated pollen #3	0.0053	7	0.000757
No-pollen	0	0	
No-pollen	0	0	
Average value for regular pollen	0.062666667	176	
Average value for	0.014866667	22.33333	

irradiated pollen			
t-test p-value	0.050404957	0.031884	

Overall, the results indicate that upon application of UV irradiated pollen a reduction in the number of seeds obtained is demonstrated compared to application of regular pollen.

EXAMPLE 26

Evaluation of A. palmeri weed control efficiency by artificial pollination with gamma irradiated pollen in growth room

The experiment was conducted similar to Example 24 (X-ray) with the difference that the pollen is irradiated by gamma irradiation with the following radiation intensities:100, 300 and 500 Gy and compared to regular (non-irradiated) pollen as a control. The size of the paper tubes that were used for pollen collection and for artificial pollination was 6cm in length. 4 paper tubes were used for each condition: non-irradiated pollen, 100 Gy, 300 Gy and 500 Gy. Additionally, 3 empty paper tubes were used in order to estimate the background level of seed production without pollination. 16 days after the artificial pollination stage, the pollinated spikes were cut and seeds were harvested. In order to evaluate the efficiency of the treatments, total seed weight, seed number and average weight per seed in each sample were measured and the average values for each treatment were compared.

The results are depicted in Table 18, below.

Table 18

Sample	Total Seed Weight (gr)	Number of Seeds	Average Seed Weight (mgr)
Regular pollen #1	8.27E-02	231	3.58E-01
Regular pollen #2	6.03E-02	212	2.84E-01
Regular pollen #3	7.98E-02	234	3.41E-01
Regular pollen #4	6.82E-02	219	3.11E-01
Irradiated pollen (100Gy) #1	6.64E-02	231	2.87E-01
Irradiated pollen (100Gy) #2	7.51E-02	270	2.78E-01
Irradiated pollen (100Gy) #3	8.84E-02	291	3.04E-01
Irradiated pollen (100Gy) #4	3.29E-02	107	3.07E-01
Irradiated pollen (300Gy) #1	2.91E-02	157	1.85E-01
Irradiated pollen (300Gy) #2	3.72E-02	241	1.54E-01
Irradiated pollen (300Gy) #3	2.74E-02	183	1.50E-01
Irradiated pollen (300Gy) #4	3.18E-02	246	1.29E-01

Irradiated pollen (500Gy) #1	1.35E-02	96	1.41E-01
Irradiated pollen (500Gy) #2	6.90E-03	80	8.63E-02
Irradiated pollen (500Gy) #3	7.90E-03	106	7.45E-02
Irradiated pollen (500Gy) #4	4.90E-03	120	4.08E-02
No-pollen # 1	-	2	-
No-pollen # 2	-	6	-
No-pollen # 3	-	14	-
Average value for regular pollen	7.27E-02	224	0.32
Average value for irradiated pollen (100 Gy)	6.57E-02	224.75	0.29
Average value for irradiated pollen (300 Gy)	3.13E-02	206.75	0.15
Average value for irradiated pollen (500 Gy)	8.30E-03	100.5	0.09
t-test p-value (100 Gy versus regular pollen)	6.05E-01	9.86E-01	1.45E-01
t-test p-value (300 Gy versus regular pollen)	**3.17E-04***	4.72E-01	**1.45E-04***
t-test p-value (500 Gy versus regular pollen)	**2.34E-05***	**1.59E-05***	**1.02E-04***

***P-value < 0.001**

The data in the table demonstrates a significant decrease in total seed weight and weight per seed following pollination with the gamma irradiated pollen (300Gy and 500Gy) relatively to the ones obtained by regular pollen. In addition, seed number was also decreased significantly following the 500Gy irradiation treatment.

In addition, seed morphology was examined and compared to evaluate seed development. To that end seeds were examined under the microscope and for each sample pictures were taken for a random assortment of seeds with representative appearance (See FIG. 4). In general, the seeds obtained from the artificial pollination with the irradiated pollen looked thinner, partly empty and their color was lighter relative to the ones obtained from the regular pollen, which looked more filled, having a black color.

An additional repeat was conducted on a separate plant with conditions of regular (non-irradiated) pollen, 100 Gy and 300 Gy with one sample for each. It yielded a very similar trend. As shown in Table 19 below and in FIG. 5:

Table 19

Sample	Total Seed Weight (gr)	Number of Seeds	Average Seed Weight (mgr)
Regular pollen	1.23E-01	229	5.39E-01
Irradiated pollen (100Gy)	1.74E-01	337	5.16E-01
Irradiated pollen (300Gy)	5.56E-02	259	2.14E-01
No-pollen # 1	-	0	-

Overall, the results indicate that upon application of gamma irradiated pollen, the seeds that are formed display seed development arrest with reduced number, weight and altered morphology.

EXAMPLE 27

Evaluation of A. palmeri weed control efficiency by artificial pollination with chromosomally aberrant pollen in growth room

A. Palmeri Seeds are germinated for 8 hours at a temperature of 34 °C in distilled water. Thereafter seeds are soaked in solutions with 3 different colchicine concentrations:0.1%, 0.5% 1% with or without the addition of 1% DMSO. (Chen et al., 2004, Castro et al., 2003, Soo Jeong Kwon et al., 2014, Roselaine Cristina Pereira1 et al.,). The soaking procedure is conducted for 4 or 20 hours at 34°C. Finally, the seeds are washed and seeded in a 6cm petri dish on a towel paper with 7.5ml tap water. The petri dishes are sealed with parafilm and are placed in a growth chamber at 34/25°C 16/8h day/night conditions. One week later, seedlings are transferred into germination beds. Samples are taken to evaluate their chromosome set. The plants are then grown until reaching the flowering stage. Male plants with various chromosomal abnormalities (e.g., polyploidy, tertraploidy) are selected for an additional examination. Pollen is collected from these plants and tested for its ability to germinate in-vitro and to fertilize. Selected pollen is applied onto A. Palmeri diploid female plants. Total seed weight, seed number, seed morphology and seed germination are examined in comparison to seeds obtained from pollination with regular diploid pollen as explained in Examples 24-26.

Example 28

Achieving reduction of A. palmeri or A. tuberculatus population by application of sterile pollen in controlled field conditions

Sterile pollen is generated as described in Example 17, 18, 19 24, 25, 26 or 27 and collected as described in Example 1. Experiment is conducted similarly to Example 16 to evaluate weed control efficiency.

Example 29

Inhibition of seed development in A. palmeri by applying X-ray irradiated pollen in a growth room and in a net-house

A. Palmeri seeds were sown and one month later the experiment was conducted. Male plants were grown in a phytotron appartus at 28 °C /22 °C 16 h/8 h day/night cycles. At morning hours pollen was collected from males using paper tubes. The pollen was X- ray irradiated inside the paper tubes at different dosages: 150, 300, 450 and 550 Gy (XRAD-320, precision XRAY). Additional paper tubes with pollen inside served as control that did not undergo the irradiation procedure. The experiment contained 3 female A. palmeri plants. Two females were placed in a phytotron appartus at 34 °C/28 °C, 16 h/8 h day/night cycles and one female plant was placed in a net-house during summer times in Israel under natural conditions.

The artificial pollination procedure was done by placing paper tubes on female spikes for half an hour with tapping every ~10-15 minutes followed by an additional 30 min that the paper tubes remained on the spike.

Sixteen days following artifical pollination, spikes were harvested and seeds were extracted and analyzed. Results were averaged over 3 female plants with overall 11 samples for non treated, 10 samples of regular pollen control, 11 samples of pollen irradiated at 150 Gy, 12 samples of pollen irradiated at 300 Gy, 12 samples of pollen irradiated at 450 Gy as well as 11 samples of pollen irradiated at 550Gy.

Results demonstrated a dose dependent response where an increase in radiation intensity resulted in a statistically significant reduction in average weight per seed. Seed number was not statistically significantly different between different samples indicating that irradiated pollen maintained its ability to fertlize the female weed ovule. Additionally, morphology of the seeds that were obtained following irradiation were altered and suggested that seed development was inhibited and seeds could not complete their growth.

Table 20 – Reduction in average weight per seed following artificial pollination with X-ray-irradiated pollen

Sample	Average weight per seed (mg)	SDE	t-test vs. control
Control	0.43	0.033576	
X-ray 150	0.34	0.028387	0.02236303*
X-ray 300	0.19	0.019295	5.39066E-07*
X-ray 450	0.10	0.010786	4.19033E-10*
X-ray 550	0.09	0.011624	2.62169E-09*

* p value < 0.05

Table 21 – Number of seeds obtained following artifical pollination

Sample	Average number of seeds*	SDE	t-test vs. control
Control	303.87	57.07	
X-ray 150	380.53	55.21	0.33
X-ray 300	351.68	44.20	0.48
X-ray 450	291.66	52.03	0.87
X-ray 550	205.61	35.77	0.19

*Seed were photographed, and seed count was conducted using ImageJ

Example 30

Demonstration of competitiveness of X-ray-irradiated pollen and demonstration of weed control by reduced seed weight and germination in A. palmeri in a growth room

A. palmeri male plants were grown in a phyttron appartus at 28 °C/22 °C 16 h/8 h day/night cycles. Pollen was collected into a paper at morning hours from 11 males. Overall 660 mg of pollen was collected.

Pollen was divided to 4 Eppendorf tubes with 150 mg in 3 Eppendorf tubes each for the various irradiation intensities (150/300/450 Gy, XRAD-320, precision XRAY) and 210 mg of pollen served as control and was kept untreated.

Mixes of 1:1 control:irradiated samples were prepared by mixing 22.5 mg of regular pollen with the same amount of irradiated pollen – total of 45 mg. Also mixes of 1:3 samples comprising 11.25 mg of regular pollen with 33.75 mg of irradiated pollen with a total of 45 mg were prepared. Pollen was distributed into paper tubes with 15 mg of pollen into each paper tube per spike.

Two females were grown in a phytotron appartus under conditions of 34 °C /28 °C, 16 h/8 h day/night cycles. Each female was artificially pollinated using paper tubes with 15 mg of pollen.

Two replicas of the following treatments were used per each female. Treatment groups included: Non treated, Control, 150 Gy, 300 Gy, 450 Gy. In addition,1:1 mixes that included 150 Gy: Control, 300 Gy: Control and 450 Gy: Control. As well as 3:1 mixes that included 150

Gy : Control and 300 Gy : Control. The artificial pollination procedure was conducted for 30 min by placing the paper tubes on female spikes and tapping every several minutes.

Sixteen days after the artificial pollination seeds were harvested.

Results demonstrated that irradiation of pollen prior to artificial pollination resulted in a statistically significant reduction in average weight per seed (Table 22). Additionally, the morphology of the seeds that was obtained following irradiation was altered and suggested that seed development was inhibited and seeds could not complete their development. Furthermore, in Table 23 there is evidence demonstrating that the seeds obtained following pollen irradiation have lost their ability to germinate.

Table 22 – Reduction in average weight per seed following artificial pollen with irradiated pollen

	Average weight per seed (mg)	SDE	t-test versus control
Control	0.45	0.048	
X-Ray-150	0.07	0.006	1.05E-04*
X-Ray-300	0.05	0.005	7.73E-05*
X-Ray-450	0.07	0.011	1.28E-04*

* p value < 0.05

The germination assay was conducted in order to estimate the different germination levels between the seeds obtained by artificial pollination with the irradiated pollen versus the ones obtained from artificial pollination with regular pollen.

Fourty representative seeds were taken from each of these 4 samples. Each set of 40 seeds was placed in a 9 cm petri dish on a towel paper with 9 ml tap water for the germination test. These petri dishes were sealed with parafilm and were placed in a growth room in 35 °C /27 °C 16/8h day/night conditions. After 3 days emerged seedlings were counted and germination rate was calculated for each sample. A comparison was conducted between the seeds obtained from irradiated pollen and the ones obtained from regular pollen. While the average germination rate obtained from the regular pollen was approximately 69 %, none of the seeds obtained from artificial pollination with pollen that was irradiated by 300 or 450 Gy germinated and only 2.5 % of the seeds obtained via artificial pollination with pollen that was irradiated by 150 Gy germinated (Background seed contamination level in the experiment was 2 % on average, therefore this is in the range of the background).

Table 23 – Seeds obtained following pollen irradiation lose their ability to germinate

	Average % Germination Rate
Control	68.7
150 Gy	2.5
300 Gy	0
450 Gy	0

Background seed contamination level in the experiment = 2%

Additionaly, seeds were separated to two groups according to their weight using an air blower apparatus. Low weight was indicative of developmentally arrested seeds, whereas normal seed weight was indicative of normally developed seeds. Morphology of developmentally arrested seeds was different from normal seeds with lighter brown color versus a black color and "shallow" appearance versus full seed morphology. As can be seen in Table 24 the rate of normal or aborted seeds obtained was in close proximity to the expected rate of normal or aborted seeds suggesting that the pollen after irradiation maintains its competitiveness. It is also apparent that an increase in irradiation intensity results in reduction in competitiveness.

Table 24 - Rate of normal and aborted seeds as observed and expected

	Avg % Normal seeds	Avg % Aborted seeds	EXPECTED % Normal seeds	EXPECTED % Aborted seeds
Control	84%	16%		
X-Ray-150	2%	98%		
X-Ray-150:control 1:1	47%	53%	43%	57%
X-Ray-150:control 3:1	25%	75%	23%	77%
X-Ray-300	1%	99%		
X-Ray-300:control 1:1	53%	47%	42%	58%
X-Ray-300:control 3:1	30%	70%	22%	78%
X-Ray-450	3%	97%		
X-Ray-450:control 1:1	62%	38%	43%	57%

Background seed contamination level in the experiment = 2%

Example 32

Inhibition of seed development and demonstration of weed control in A. palmeri by applying X-ray-irradiated pollen in a growth room

A. palmeri male plants were grown in a phyttron appartus at 28°C /22°C 16h/8h day/night cycles and in a net-house during fall in Israel under natural conditions. Pollen was collected from males in both locations into paper at morning hours and mixed together. Pollen was divided into Eppendorf tubes and irradiated with X-Ray irradiation intensities of 20, 50, 75, 100, and 150 Gy (XRAD-320, precision XRAY). Non-irradiated pollen samples served as control.

Two females were grown in a growth room under conditions of 32 °C/26 °C, 16 h/8 h day/night cycles. Each female was artificially pollinated using paper tubes with 20 mg of pollen. Two replicas of each of the above irradiation treatments were used per each female.

Fourteen days following artificial pollination spikes, were harvested and seeds were extracted and analyzed.

Results demonstrated that irradiation of pollen with a dose higher than 50 Gy prior to artificial pollination, resulted in statistically significant reduction in average weight per seed (Table 25). Additionally, morphology of the seeds that were obtained following irradiation was altered and suggested that seed development was inhibited and that seeds could not complete their development.

Table 6 - Reduction in average weight per seed following artificial pollen with irradiated pollen

	Average weight per seed (mg)	SDE	t-test versus control
Control	0.26	0.014	
X-Ray-20	0.24	0.013	0.158034
X-Ray-50	0.21	0.014	0.026459*
X-Ray-75	0.14	0.006	0.000583*
X-Ray-100	0.12	0.010	9.34E-05*
X-Ray-150	0.08	0.009	1.82E-05*

* p value < 0.05

Fourty representative seeds were taken from each of these treatments. Each set of 40 seeds was placed in a 9 cm petri dish on a towel paper with 9 ml tap water for the germination test. These petri dishes were sealed with parafilm and were placed in a growth room in 35 °C /27 °C 16/8h day/night conditions. After 6 days emerged seedlings were counted and germination rate was calculated for each sample. A comparison was conducted between the seeds obtained from irradiated pollen and the ones obtained from regular pollen. No seeds germinated in any of the seeds obtained following artifical pollination with irradiated pollen while in the control sample there was germination rate of 7.5%. Low germination rate in the control might be a result of seed dormancy.

Table 6A

	% Germination rate
Control	7.5%
X-Ray-20	0
X-Ray-50	0
X-Ray-75	0
X-Ray-100	0
X-Ray-150	0

Example 33

Demonstration of weed control in A. palmeri by X-ray Irradiated pollen treatment under competitive conditions with male A. palmeri in net-house

Male A.palmeri plants were placed in a phytotron appartus at 28 °C/22 °C, 16 h/8 h day/night cycles and in a net-house during summer times in Israel under natural conditions. Pollen was collected into paper from A. palmeri male plants in the morning and was X-ray irradiated by dose of 300 Gy (XRAD-320, precision XRAY).

Five female A. Palmeri and 1 male A. Palmeri were grown separately in a net-house during summer in Israel under natural conditions. The male was placed in the middle and the 5 female plants were placed surrounding it at a distance of 75 cm (between each female and the central male). Four spikes per female were examined in this experiment: 2 spikes were artificially pollinated with the irradiated pollen and 2 spikes served as control and were exposed only to the pollen that was shed by the male plant. The male A. Palmeri plant remained in the net-house for 1 week following the artificial pollination procedure to provide competing natural pollination conditions and was then removed from the net house. Sixteen days after removal of the male from the net-house, the examined spikes were cut and seeds were harvested, weighed and sorted by the seed blower.

Results depicted in Table 26 display an average reduction of 69 % in normal seed production upon one treatment with irradiated pollen. Additionally, the percentages of normal seeds out of the total number of seeds was on average 11 % whereas 89 % of the total number of seeds were aborted.

Table 26 – Weed control of A. palmeri with a single X-RAY-irradiated pollen treatment

	# Normal Seeds In Control	# Normal Seeds with Artificial Pollination with irradiated pollen	% Normal seed reduction	Normal Seeds/ Total Seeds
P1-set1	296	76	74.3	0.13
P1-set2	257	50	80.5	0.08

P2-set3	44	16	63.6	0.05
P2-set4	36	8	77.8	0.02
P3-set5	287	83	71.1	0.08
P3-set6	174	46	73.6	0.05
P4-set7	241	150	37.8	0.13
P4-set8	395	139	64.8	0.14
P5-set9	691	184	73.4	0.17
P5-set10	1476	378	74.4	0.27
Average			69.1	0.11

Further analysis displayed in Table 27 presents results suggesting that the irradiation treatment resulted in a uniform population of seeds with reduced weight that has a statistically significant reduced standard deviation compared to naturally occurring aborted seeds (Levene test – p.value = 0.027). This result suggests that the irradiation treatment blocks development of seeds at an early stage and that the development arrest occurs equally in all seeds.

Table 27 – aborted seeds obtained following artificial pollination with irradiated pollen have significantly reduced weight that is more uniform compared to naturally occurring aborted seeds

		Natural pollination only	Natural + Single artificial pollination treatment with irradiated pollen	t-test	Levene's test
Normal seeds	Average normal seed weight	0.352	0.351	9.62E-01	
	SD	0.057	0.067		
Aborted seeds	Average aborted seed weight	0.068	0.040	9.26E-06*	
	SD	0.013	0.006		0.027*

* p value < 0.05

Example 34

Inhibition of seed development and demonstration of weed control in A. palmeri by applying Gamma irradiated pollen in a greenhouse

Experiment is conducted similar to Example 32 with gamma irradiation intensities of: 20, 50, 75,100, 125, 150, 450, 600, 800, 1000, 1200, 1600 and 2000 Gy.

Sixteen days following artificial pollination spikes are harvested and seeds are extracted and the efficiency of the different treatments for weed control is evaluated by comparing average weight per seed, seed morphology and germinability between the different treatments and control.

Example 35

Inhibition of seed development and demonstration of weed control in A. palmeri by applying XRAY irradiating pollen in a greenhouse

Experiment was conducted similar to example 4 with XRAY irradiation with doses of: 20, 50, 75, 100, 125, 150, 450, 600, 800, 1000 or 1200 Gy (XRAD-320, precision XRAY).

Sixteen days following artificial pollination, spikes are harvested and seeds are extracted and the efficiency of the different treatments for weed control is evaluated by comparing average weight per seed, seed morphology and germinability between the different treatments and control.

Example 36

Inhibition of seed development and demonstration of weed control in A. palmeri by applying Beta-irradiated pollen in a greenhouse

The experiment is conducted similarly to Example 32 with beta radiation in a linear accelerator with doses of: 1000, 1500and 2000 Gy.

Sixteen days following artificial pollination spikes are harvested and seeds are extracted and the efficiency of the different treatments for weed control is evaluated by comparing average weight per seed, seed morphology and germinability between the different treatments and control.

Example 37

Achieving pollen with special sterility property in A. Palmeri or A. Tuberculatus by UV irradiation and evaluation of weed control in a greenhouse

The experiment is conducted as in Example 32 with the difference that the pollen is irradiated by UV-C (wave length of 254nm) with energies of: 0.025, 0.05, 0.1, 0.3, 0.5, 0.8, 1, 1.2, 1.5 and 2 Joules.

Sixteen days following artificial pollination, spikes are harvested and seeds are extracted and the efficiency of the different treatments for weed control is evaluated by comparing average weight per seed, seed morphology and germinability between the different treatments and control.

Example 38

Inhibition of seed development and demonstration of weed control in A. tuberculatus by applying X-ray irradiated pollen in a net-house

A. tuberculatus seeds were sown and grown until reaching flowering. Male and female A. tuberculatus were grown separately in a net-house during fall times in Israel under natural

conditions. Pollen was collected into paper from A. tuberculatus male plants in the morning and treated by X-Ray at different radiation doses of 50, 150, 300 and 450 Gy (XRAD-320, precision XRAY) as well as pollen that was not irradiated and served as control.

An artificial pollination procedure was done by placing paper tubes with 20 mg pollen on A. tuberculatus female spikes for 30 min hour with tapping every ~10-15 minutes followed by an additional half an hour that the paper tubes remained on the spike.

Fourteen days following artificial pollination, spikes were harvested and seeds were extracted and analyzed. Results demonstrated that irradiation of pollen prior to artificial pollination resulted in a statistically significant reduction in average weight per seed (Table 28). Additionally, morphology of the seeds that were obtained following irradiation was altered and suggested that seed development was inhibited and that seeds could not complete their development.

Table 28 - Reduction in average weight per seed following artificial pollination with irradiated pollen

	average weight per seed (mg)	stdev	ttest versus control
Control	0.13	0.003917	
50	0.11	0.000326	3.63E-02*
150	0.06	0.004523	4.15E-03*
300	0.07	0.005946	7.55E-03*
450	0.07	0.001343	2.62E-03*

* p value < 0.05

Example 39

Inhibition of seed development and demonstration of weed control in A. tuberculatus by applying gamma irradiated pollen in a greenhouse

A. tuberculatus seeds are sown and grown until reaching flowering. Pollen is collected from male plants using paper tubes. Pollen is gamma irradiated at different doses: 20, 50, 75, 100, 125, 150, 300, 450, 600, 800, 1000 or 1200 Gy. Additional paper tubes served as control with non-irradiated pollen.

Artificial pollination procedure is done by placing Paper tubes on A. tuberculatus female spikes for half an hour with tapping every ~10-15 minutes followed by an additional half an hour that the paper tubes remained on the spike. Sixteen days following artificial pollination spikes are harvested and seeds are extracted and analyzed.

Example 40

Inhibition of seed development and demonstration of weed control in A. tuberculatus by applying Gamma irradiated pollen in net-house

A. tuberculatus seeds were sown and grown until reaching flowering. Male and female A. tuberculatus were grown separately in a net-house during fall times in Israel under natural conditions. Pollen was collected into paper from A. tuberculatus male plants in the morning and irradiated by 300 Gy gamma irradiation (Biobeam GM 8000). Pollen was divided into paper tubes, each paper tube with 20 mg pollen. Each A. tubercultus female plant was treated with the following treatments: Blank (1 repeat per plant X 2 plants), Control (2 repeats per plant X 2 plants), 300 (2 repeats per plant X 2 plants). Sixteen days after pollination seeds were harvested, weighed and analyzed.

Table 29 – Reduction in average weight per seed following artificial pollination with irradiated pollen

Sample	Average weight per seed (mg)	SDE	t-test vs. control
Control	0.24	2.68E-02	2.46E-04*
Gamma 300 Gy	0.06	6.19E-04	

* p value < 0.05

Results demonstrated that irradiation of pollen prior to artificial pollination resulted in a statistically significant reduction in average weight per seed (Table 29). Seed number was not different between different samples indicating that irradiated pollen maintained its ability to fertlize the female weed ovule (Table 30). Additionally, the morphology of the seeds that were obtained following irradiation was altered suggesting that seed development was inhibited and seeds could not complete their development.

Table 30 – Number of seeds obtained following artifical pollination

Sample	Average number of seeds*	SDE	t-test vs. control
Control	1243	76	
X-ray 300	1307	108	0.596

*Seed were photographed, and seed count was conducted using ImageJ

Additionally, 40 representative seeds were taken from each of these treatments. Each set of 40 seeds was placed in a 9 cm petri dish on a towel paper with 9 ml tap water for the germination test. These petri dishes were sealed with parafilm and placed in a growth room in 32 °C/26 °C 16 h/8 h day/night conditions. After 3 days emerged seedlings were counted and

germination rate was calculated for each sample. The results appear in Table 31. It can be seen that seeds obtained via artificial pollination with irradiated pollen lost their ability to germinate.

Table 31: Germination test results of seed obtained via artificial pollination with regular pollen vs. irradiated pollen

	Germination rate		
Sample	Seeds from Plant #1	Seeds from Plant #2	t-test vs. control
Control	0.325	0.25	0.0166*
X-ray 300	0	0	

* p value < 0.05

Example 41

Inhibition of seed development and demonstration of weed control in A. tuberculatus by applying X-ray-irradiated pollen in a greenhouse

The experiment is conducted similar to Example 40 with X-ray irradiated with intensities of: 20, 50, 75, 100, 125, 150, 450, 600, 800, 1000 or 1200 Gy (XRAD-320, precision XRAY).

Sixteen days following artificial pollination, spikes are harvested and seeds are extracted and the efficiency of the different treatments for weed control is evaluated by comparing average weight per seed, seed morphology and germinability between the different treatments and control.

Example 42

Inhibition of seed development and demonstration of weed control in A. tuberculatus by applying particle irradiated pollen in a greenhouse

The experiment is conducted similar to Example 40 with particle radiation from a linear accelerator with doses of: 1000, 1500 and 2000 Gy. Sixteen days following artificial pollination, spikes are harvested and seeds are extracted and the efficiency of the different treatments for weed control is evaluated by comparing average weight per seed, seed morphology and germinability between the different treatments and control.

Example 43

Reduction of A. palmeri or A. tuberculatus population by application of sterile pollen in a controlled field conditions

Pollen is generated as described in Example 19, 24-27 or 29-42 and collected into paper. Two groups of 8 A. palmeri plants composed of 4 male plants and 4 females plants are transplanted in the field. Each group is arranged in 2 rows of four plants in alternating order of female and male. The distance between each plant is 1 m. The distance between the location of the 2 groups is 100 m. The two groups are treated similarly and are watered on a daily basis.

One group is used as control group (C) to estimate the native population growth without any application of non-native pollen. The second group (T) is pollinated both by the native pollen (shed by the males) as in the control group and with additional treated pollen that was generated as described in Examples 29-42. At the beginning of the flowering time, a pollination treatment is being applied to group T. The treatment is given in 4 applications in intervals of 1 week, each application is given once a day (at morning hours). All plants are harvested after seed maturation and seeds are collected manually. Seed biomass is measured for each plant and the number of seeds per 0.1 g is counted and the total number of seeds per plant is being estimated and recorded.

In addition, from each female plant, 100 seeds are taken. The seeds are planted in trays of 30 x 30 cm. Emerged seedlings are counted at the age of 7 days and the emergence rate is calculated for both groups. The reduction in the emergence proportion between the group pollinated with sterile pollen and the control group reflects the estimation for the reduction in A. palmeri or A. tuberculatus population size due to the treatment per one year.

Table 32

Female plants	Pollen source	Seeds count and weight	Population size reduction estimation (as calculated from the number of seedlings emerge out of 100 seeds)
4 females plants	4 male plants	N_{seeds}(FxM) – seed count W_{seeds}(FxM) - total seed weight	N (FxM) – Number of emerged seedlings
4 females Plants	4 male plants + sterile pollen	N_{seeds}(Fx(M+ M_s)) – seed count W_{seeds}(Fx(M+ M_s)) - total seed weight	N (Fx(M+ M_s)) - Number of emerged seedlings
Expected population size reduction per year = 1- N (Fx(M+ M_s)) / N (FxM)			

Example 44

Reduction of A. palmeri and A. tuberculatus populations by application of mixture of treated pollen in a controlled field conditions

Pollen is generated as described in Examples 29-42 and collected into paper both from A. palmeri male plants and from A. tuberculatus male plants. The pollen from both species is mixed together and the treatment is with this mixture. The field experimental setup is similar to the one described in Example 12 except that instead of having in each group 8 A. palmeri plants (composed of 4 females and 4 males plants) each group contains 4 A. palmeri plants (2 females and 2 males) and 4 A. tuberculatus plants (2 females and 2 males). At the beginning of flowering time one group is treated with the pollen mixture 1 application per day for 4 times in intervals of 1 week.

The effect of pollen treatment on the population size of both species is estimated similarly to the way described in Example 43.

Table 33

Female plants	Pollen source	Population size reduction estimation (as calculated from the number of seedlings emerge out of 100 seeds)
2 A. palmeri + 2 A. tuberculatus	2 A. palmeri + 2 A. tuberculatus	N_p (FxM) – Number of A. palmeri emerged seedlings N_t (FxM) – Number of A. tuberculatus emerged seedlings
2 A. palmeri + 2 A. tuberculatus	2 A. palmeri + 2 A. tuberculatus + mixture of sterile pollen	N_p (Fx(M+ M_s)) - Number of A. palmeri emerged seedlings N_t (Fx(M+ M_s)) - Number of A. tuberculatus emerged seedlings
Expected population size reduction per year =1– $N_{p/t}$ (Fx(M+ M_s)) / $N_{p/t}$ (FxM)		

Example 45

Reduction of A. palmeri and A. tuberculatus populations by application of sterile pollen in a controlled field conditions in the process of integrated weed management

The experiment is conducted similar to the experiment conducted by Norseworthy et al., 2016 (Norsworthy et al., Weed Science 2016 64:540-550). Each Plots size contain 20 soybean rows with a 1-m spacing between rows on raised beds. 2 plots were placed with a distance of 100m between plots. Three Glyphosate treatments of 870 g ha-1 (Roundup PowerMax, Monsanto Company, St. Louis, IL) are given during the experiment: 1. Two to 3 weeks prior to planting 2. At V2 soybean stage 3. At V7 soybean stage. Soybean is seeded at 30 seed m-1 row each year.

One plot receives no additional treatment whereas the other plot is artificially pollinated with pollen that is treated as in Examples 19, 24-27 or 29-42. The artificial pollination procedure is repeated for 10 times in intervals of 1 week.

2 weeks following the last treatment Palmer plants that survived are harvested. Harvested plants are placed in bags and dried for 2 weeks before threshing. Collected seeds are separated from plant tissue and seed production is determined. Additionally, soybean is harvested. All grain from each plot is weighed.

The effect of pollen treatment on A. palmeri seed production as well as soybean yield is determined.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to

be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

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Terminator patent - US 5,723,765

Reverse sterility patents - AU621195, US 5,808,034

WHAT IS CLAIMED IS:

1. A method of producing pollen that reduces fitness of at least one Amaranthus species of interest, the method comprising treating the pollen of plants of an Amaranthus species of interest with an irradiation regimen selected from the group consisting of:

(i) X-ray radiation at an irradiation dose of 20-1600 Gy;

(ii) gamma radiation at an irradiation dose of 20-2000 Gy;

(iii) particle radiation; and

(iv) UV-C radiation at an irradiation dose of 100μJ/cm^2 -50J/cm^2, with the proviso that when said weed is A. palmeri, when said irradiation is X-ray the irradiation dose is not 300 Gy and wherein when said irradiation is gamma irradiation the irradiation dose is not 100, 300 and 500 Gy and wherein when said radiation is UV-C the dose radiation is not 2 J/cm^2.

2. The method of claim 1, wherein said particle irradiation dose is 20 – 5000 Gy.

3. The method of claim 1 or 2, wherein said pollen is a harvested pollen.

4. The method of claim 1 or 2, wherein said pollen is a non-harvested pollen.

5. The method of claim 4, further comprises harvesting the pollen following said treating.

6. The method of any one of claims 1-5, wherein said Amaranthus species of interest comprise only male plants.

7. The method of any one of claims 1-6, wherein said plants are grown in a large scale setting.

8. The method of claim 7, wherein said large scale setting essentially does not comprise crops.

9. Harvested pollen obtainable according to the method of any one of claims 1-8.

10. A method of Amaranthus control, the method comprising artificially pollinating a Amaranthus species of interest with the pollen of claim 9.

11. The method of claim 10, wherein said pollen and said Amaranthus species of interest are of the same species.

12. The method of claim 10, wherein said pollen and said Amaranthus species of interest are of different species.

13. The method of any one of claims 10-12, wherein said artificially pollinating is effected in a large scale setting.

14. The method of any one of claims 10-13, wherein said pollen is herbicide resistant.

15. The method of claim 14, wherein said pollen is coated with said herbicide.

16. The method of any one of claims 10-15, wherein said artificially pollinating results in reduced average seed weight of at least 1.2 lower than that of the average seed weight of a plant of the same developmental stage and of the same species fertilized by control pollen.

17. A method of producing pollen for use in artificial pollination, the method comprising:

(a) providing the pollen of claim 9; and

(b) treating said pollen for use in artificial pollination.

18. A composition-of-matter comprising the pollen of claim 9, said pollen having been treated for use in artificial pollination.

19. A kit comprising a plurality of packaging means, each packaging different species of pollen wherein at least one of said different species of pollen is the pollen of claim 9 or the treated pollen of claim 18.

20. The kit of claim 19, wherein all of said different species of pollen are of the Amaranthus genus.

21. The kit of claim 19, wherein a portion of said different species of pollen are of the Amaranthus genus.

22. The composition of claim 18 or kit of claim 19-21, wherein a treatment of said treated pollen is selected from the group consisting of coating, priming, formulating, solvent solubilizing, chemical treatment, drying, heating, cooling and irradiating.

23. The method or composition or kit of any one of claims 1-22, wherein said Amaranthus species of interest is selected from the group consisting of a biotic stress or abiotic stress resistant Amaranthus.

24. The method or composition or kit of claim 23, wherein said Amaranthus species of interest is a herbicide resistant Amaranthus.

25. The method or composition or kit of any one of claims 1-23, wherein said pollen is of an herbicide susceptible Amaranthus.

26. The method or composition or kit of claim 25, wherein said herbicide susceptible Amaranthus is susceptible to a plurality of herbicides.

27. The method or composition or kit of any one of claims 1-26, wherein said pollen reduces productiveness of said Amaranthus species of interest.

28. The method or composition or kit of claim 27, wherein reduction in said productiveness is manifested by:

(i) inability to develop an embryo;
(ii) embryo abortion;
(iii) seed non-viability;
(iv) seed that cannot fully develop; and/or
(v) seed that is unable to germinate; and/or
(vi) reduced or no seed set.

29. The method or composition or kit of any one of claims 1-28, wherein said pollen is non-genetically modified pollen.

30. The method, composition or kit of claim 29, wherein said non-genetically modified pollen is produced from a plant having an imbalanced chromosome number.

31. The method or composition of any one of claims 1-28, wherein said pollen is genetically modified pollen.

32. The composition or kit of any one of claims 18-31, further comprising at least one agent selected from the group consisting of an agricultural acceptable carrier, a fertilizer, a herbicide, an insecticide, a miticide, a fungicide, a pesticide, a growth regulator, a chemosterilant, a semiochemical, a pheromone and a feeding stimulant.

33. The method or composition of any one of claims 1-18 and 22-32, wherein said at least one Amaranthus species of interest comprises a plurality of Amaranthus species of interest.

34. The method, composition or kit of any one of claims 1-32, wherein said Amaranthus species of interest is A. palmeri.

35. The method, composition or kit of any one of claims 1-32, wherein said Amaranthus species of interest is A. tuberculatus.

36. The method, composition or kit of any one of claims 1-35, wherein said irradiation is X-ray with an irradiation dose which is not 300 Gy.

37. The method, composition or kit of any one of claims 1-35, wherein said irradiation is gamma irradiation with an irradiation dose which is not 100, 300 and 500 Gy.

38. The method, composition or kit of any one of claims 1-35, wherein said irradiation is UV-C irradiation with an irradiation dose which is not is not 2 J/cm^2.

39. The method, composition or kit of any one of claims 1-37, wherein said Amaranthus species is A. palmeri and the X-ray irradiation dose is of 50-350 Gy.

40. The method, composition or kit of any one of claims 1-37, wherein said Amaranthus species is A. tuberculatos and the X-ray irradiation dose is of 20-200 Gy.

41. The method, composition or kit of any one of claims 1-37, wherein said the X-ray irradiation dose is 20-500 Gy.

42. The method, composition or kit of any one of claims 1-37, wherein said Amaranthus species is A. palmeri and the gamma irradiation dose is of 200-1200 Gy.

43. The method, composition or kit of any one of claims 1-37, wherein said Amaranthus species is A. tuberculatos and the gamma irradiation dose is of 50-600 Gy.

44. The method, composition or kit of any one of claims 1-37, wherein said the gamma irradiation dose is 50-1500 Gy.

45. The method, composition or kit of any one of claims 1-37, wherein said the particle irradiation dose is 20-5000 Gy.

46. The method, composition or kit of any one of claims 1-37, wherein said the UV-C irradiation dose is 1 mJ/cm^2-10 J/cm^2.

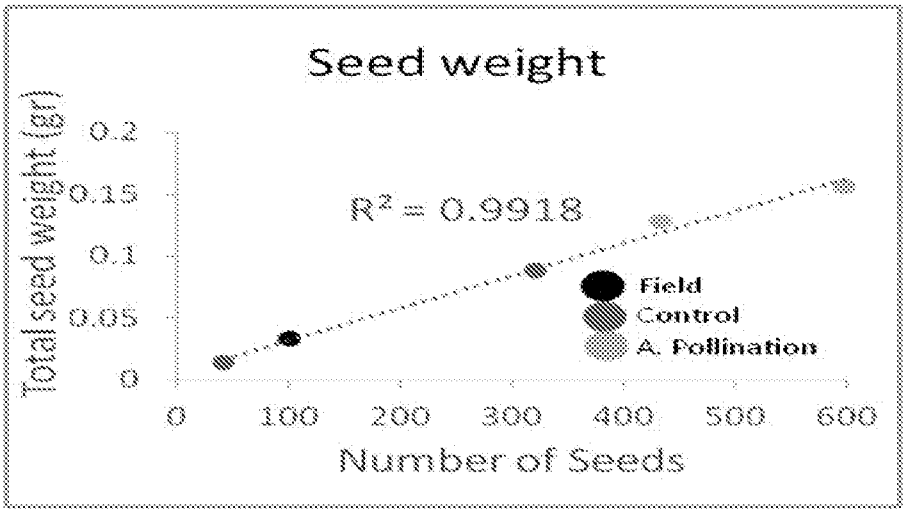
Seed weight
Total seed weight (gr)
0.2
0.15
0.1
0.05
0
R² = 0.9918
Field
Control
A. Pollination
0
100
200
300
400
500
600
Number of Seeds

FIG. 1

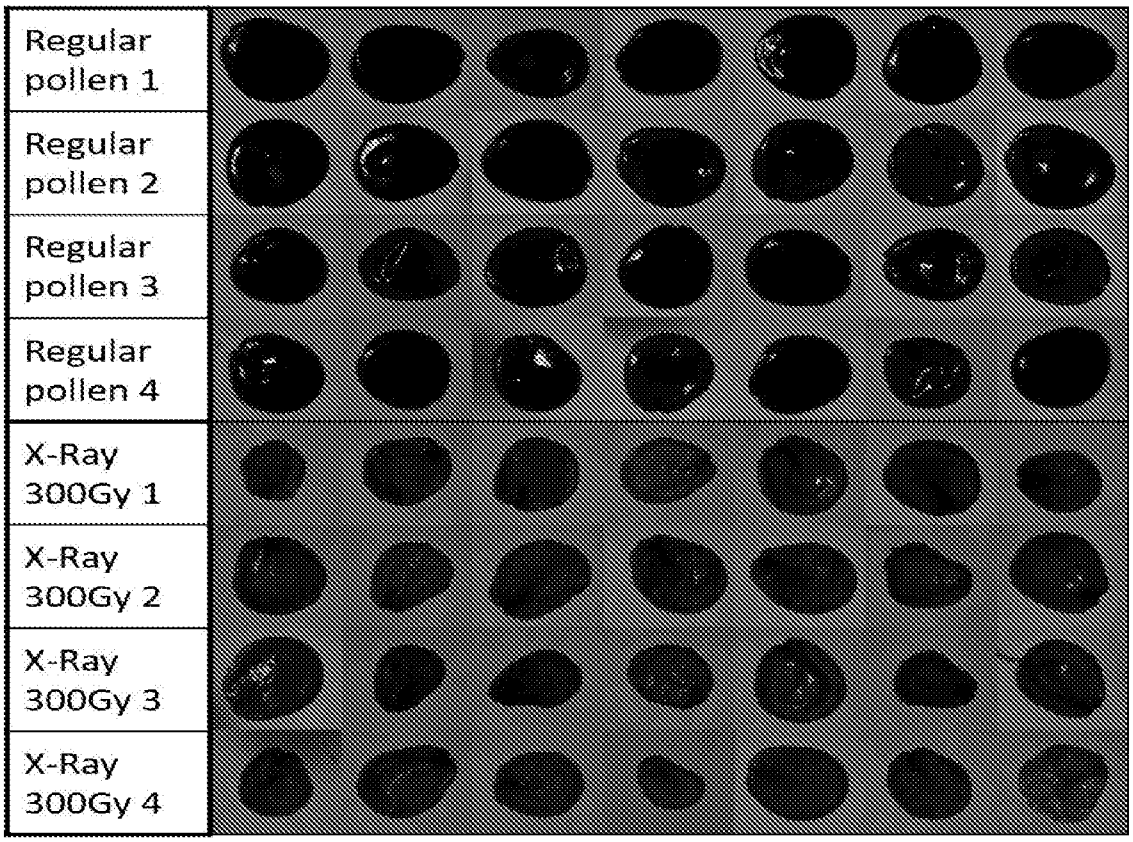
Regular pollen 1
Regular pollen 2
Regular pollen 3
Regular pollen 4
X-Ray 300Gy 1
X-Ray 300Gy 2
X-Ray 300Gy 3
X-Ray 300Gy 4

FIG. 2

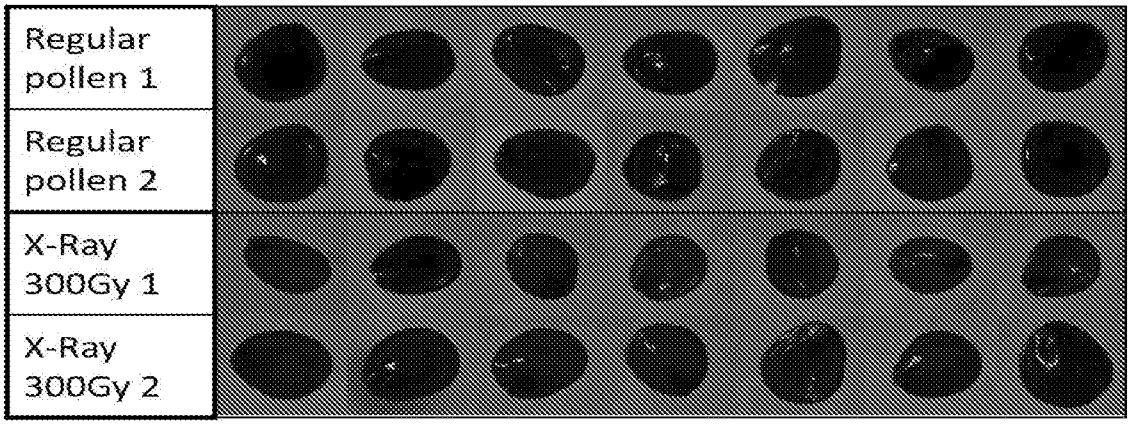
Regular pollen 1
Regular pollen 2
X-Ray 300Gy 1
X-Ray 300Gy 2

FIG. 3

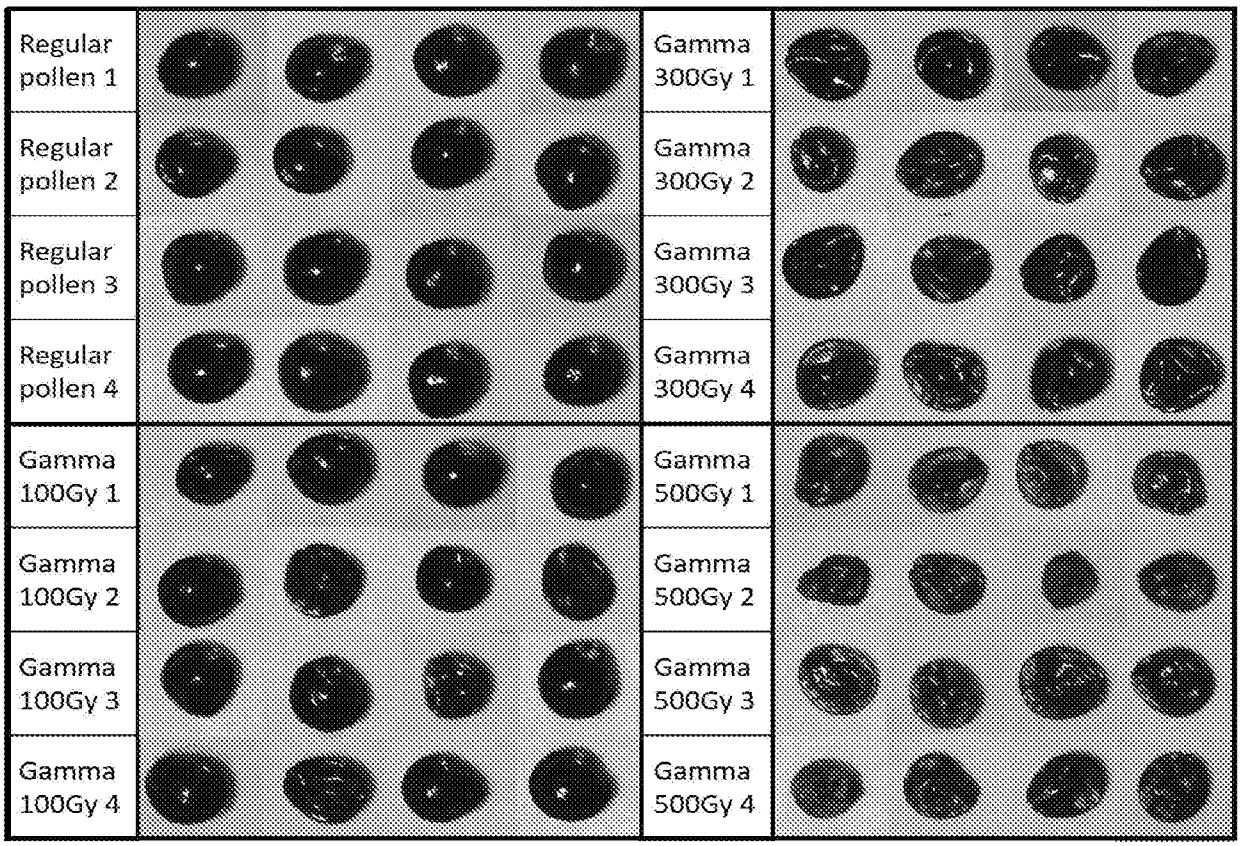
Regular pollen 1
Regular pollen 2
Regular pollen 3
Regular pollen 4
Gamma 300Gy 1
Gamma 300Gy 2
Gamma 300Gy 3
Gamma 300Gy 4
Gamma 100Gy 1
Gamma 100Gy 2
Gamma 100Gy 3
Gamma 100Gy 4
Gamma 500Gy 1
Gamma 500Gy 2
Gamma 500Gy 3
Gamma 500Gy 4

FIG. 4

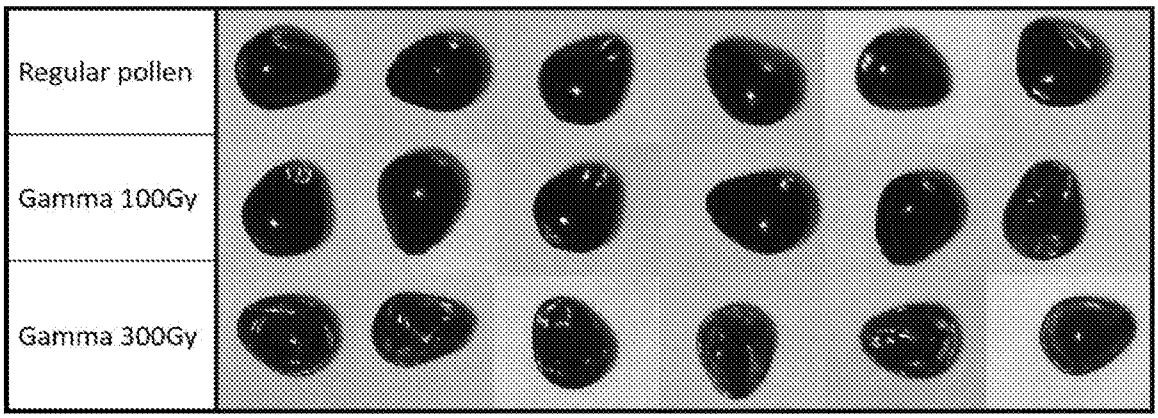

FIG. 5