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(19)

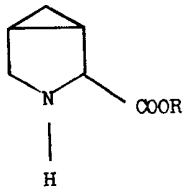
(54) METHOD OF STERILIZING MALE ANTHERS OF ANGIOSPERMS

(71) We, SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V., a company organized under the laws of The Netherlands, of 30, Carel van Bylandtlaan, The Hague, The Netherlands, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method of sterilising male anthers in angiosperms, especially in cereal crops, and to a composition therefor.

To obtain high-yielding F_1 hybrid seeds, seed-breeders cross-pollinate carefully selected parents, an operation which is very time-consuming and requires highly-skilled staff, especially in the case of small-grain cereals which have hermaphroditic flowers and normally self-pollinate. Generally speaking a small-grain cereal hybrid can only be obtained if self-pollination is completely avoided and in practice this is achieved by removing the male anthers from the cereal flowers by hand. Accordingly, if the flowers could be treated in such a way that the male anthers were sterilised without affecting female fertility, then this would represent a considerable advance in the seed-breeding industry.

The present invention provides a method of sterilising male anthers in a plant which is an angiosperm, which comprises applying to the plant, in an amount and at a growth stage of the plant such that sterilisation occurs, a heterocyclic coupound having the following general formula:



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wherein R represents a hydrogen atom, an alkali metal ion, one equivalent of an alkaline earth metal ion, an ammonium ion optionally substituted by one or more alkyl groups, or an alkyl group of up to 10 carbon atoms, preferably up to 4, carbon atoms. When R represents a metal ion this is preferably a sodium or potassium ion. When R represents an alkyl-substituted ammonium ion, this preferably has up to 6 carbon atoms in the or each alkyl group.

Preferred compounds of the general formula I are 2-carboxy-3,4-methanopyrrolidine and 2-methoxycarbonyl-3,4-methanopyrrolidine.

The heterocyclic compound may be applied to the plant at one of a number of stages in the development of the plant; if the plant is a grass-like plant, it is suitably treated during the period between the 2-node stage of the plant and just before emergence of the ear, i.e., during the stem extension period of the plant.

The process of the invention is preferably applied to large-grain cereals, for example maize or sorghum, or, especially, to small grain cereals, for example wheat, barley, oat, rye, rice or millet.

Preferably, the process according to the invention is carried out by applying the active compound in the form of a composition which in addition to the compound also comprises a carrier or a surface-active agent or both a carrier and a

surface-active agent. The invention also provides a composition which comprises a compound of the general formula I together with a solid carrier and/or a surface-active agent, or together with a liquid carrier and a surface-active agent.

We have also found that when a heterocyclic compound of the general formula I is applied to a plant, it causes a depression in the vegetative growth of the plant. Our co-pending Application No. 7912836 (Serial No. 1567154) (our internal reference K 1348 GBR/II) describes and claims a method of depressing the vegetative growth of a plant, which comprises applying to the plant, in an amount such that depression of growth results, a compound of the general formula I. It may be useful to achieve sterilisation of the male anthers and also depression of vegetative growth of an angiosperm, by applying only one chemical at a suitable stage in the growth of the plant.

The dosage of heterocyclic compound applied during the process according to the invention may vary over a wide range, for example in the range of from 0.05 kg/ha to 2.00 kg/ha. Compositions containing 50 to 1,500 ppm of heterocyclic compound are very suitable for this purpose.

The invention also provides a method of producing F_1 hybrid seed, which comprises cross-pollinating a plant which has been treated by the sterilisation method according to the invention, with a second plant of a different strain.

A carrier may be inorganic or organic and of synthetic or natural origin. It may be a solid or a liquid. Any of the materials usually employed in formulating agricultural compositions may be used as a carrier.

Suitable solid carriers are natural and synthetic clays and silicates, for example natural silicas, such as diatomaceous earths; magnesium silicates, for example, talcs; magnesium aluminium silicates, for example, attapulgites and vermiculites; aluminium silicates, for example, kaolinites, montmorillinites and micas; calcium carbonates; calcium sulphate; synthetic hydrated silicon oxides and synthetic calcium or aluminium silicates; elements, for example, carbon and sulphur; natural and synthetic resins, for example, coumarone resins, polyvinyl chloride and styrene polymers and copolymers; solid polychlorophenols; bitumen; waxes, for example, beeswax, paraffin wax, and chlorinated mineral waxes: and solid fertilizers, for example superphosphates.

Examples of suitable liquid carriers are water, alcohols, for example, isopropanol, glycols; ketones, for example, acetone, methyl ethyl ketone, methyl isobutyl ketone and cyclohexanone; ethers; aromatic hydrocarbons, for example, benzene, toluene and xylene; petroleum fractions, for example, kerosine, light mineral oils; chlorinated hydrocarbons, for example, carbon tetrachloride, perchloroethylene or trichloroethane. Compounds which are normally gaseous but which have been compressed to form a liquid may be used. Mixtures of different liquids are often suitable.

A surface-active agent may be an emulsifying agent, a dispersing agent or a wetting agent; it may be non-ionic or ionic. Any of the surface-active agents usually applied in formulating agricultural compositions may be used. Examples of suitable surface-active agents are the sodium or calcium salts of polyacrylic acids and lignin sulphonic acids; the condensation products of fatty acids or aliphatic amines or amides containing at least 12 carbon atoms in the molecule with ethylene oxide and/or propylene oxide; fatty acid esters of glycerol, sorbitan, sucrose of pentaerythritol; condensates of these with ethylene oxide and/or propylene oxide; condensation products of fatty alcohols or alkyl phenols, for example *p*-octylphenol or *p*-octylcresol, with ethylene oxide and/or propylene oxide; sulphates or sulphonates of these condensation products; alkali or alkaline earth metal salts, preferably sodium salts, of sulphuric or sulphonic acid esters containing at least 10 carbon atoms in the molecule, for example, sodium lauryl sulphate, sodium secondary alkyl sulphates, sodium salts of sulphonated castor oil, and sodium alkylaryl sulphonates, such as sodium dodecyl benzene sulphonate; and polymers of ethylene oxide and copolymers of ethylene oxide and propylene oxide.

The compositions of the invention may be formulated as wettable powders, dusts, granules, solutions, emulsifiable concentrates, emulsions, suspension concentrates and aerosols. Wettable powders are usually compounded to contain 25, 50 or 75%w of active compound and usually contain, in addition to solid inert carrier, 3—10%w of a dispersing agent and, where necessary, 0—10%w of stabilizer(s) and/or other additives, such as penetrants or stickers. Dusts are usually formulated as a dust concentrate having a similar composition to that of a wettable powder but without a dispersant, and are diluted in the field with further solid carrier to give a composition usually containing 0.5—10%w of active compound.

Granules are usually prepared to have a size between 10 and 100 BS mesh, and may be manufactured by agglomeration or impregnation techniques. Generally, granules will contain 0.5—25%w active compound and 0—10%w of additives, such as stabilizers, slow-release modifiers and binding agents. Emulsifiable concentrates usually contain, in addition to the solvent and, when necessary, co-solvent, 10—50% w/v active compound, 2—20% w/v emulsifiers and 0—20% w/v of appropriate additives, such as stabilizers, penetrants and corrosion inhibitors. Suspension concentrates are compounded so as to obtain a stable, non-sedimenting, flowable product and usually contain 10—75%w active compound, 0.5—15%w of dispersing agents, 0.1—10%w of suspending agents, such as protective colloids and thixotropic agents, 0—10%w of appropriate additives, such as defoamers, corrosion inhibitors, stabilizers, penetrants and stickers, and water or an organic liquid in which the active compound is substantially insoluble; certain organic solids or inorganic salts may be dissolved in the carrier to assist in preventing sedimentation or as anti-freeze agents for water.

The compositions of the invention may contain other ingredients, for example, protective colloids, such as gelatin, glue, casein, gums, cellulose ethers, and polyvinyl alcohol; thixotropic agents, e.g., bentonites, sodium polyphosphates; stabilizers, such as ethylene diamine tetraacetic acid, urea, triphenyl phosphate; and stickers, for example, non-volatile oils.

Aqueous dispersions and emulsions, for example compositions obtained by diluting a wettable powder or an emulsifiable concentrate according to the invention with water, also lie within the scope of the present invention. The said emulsions may be of the water-in-oil or of the oil-in-water type, and may have a thick "mayonnaise"-like consistency.

The following Examples illustrate the invention. The terminology used to describe the growth stages in the development of cereal plants is that given in "Plant Pathology", Volume 3, 1954.

EXAMPLE 1.

Tests were carried out with 2-methoxycarbonyl-3,4-methanopyrrolidine on spring wheat plants, variety "Calibri".

The test compound was applied to the plants at 2 stages of plant development, namely:

Stage 7:
2-node stage, i.e., just before the ear has become discernible as a separate organ.

Stage 10:
mid-boot stage, i.e., where the ear is formed and is visible as swelling in the enveloping leaf sheath.

Groups of plants (20 pots, 3 plants per pot) at development Stages 7 and 10 were sprayed with the test compound formulated as a 100 ppm or 500 ppm solution in water with added wetting agent (TRITON X—155 (Trade Mark) at a concentration 0.1%). Control tests with untreated plants and also with plants sprayed with water and wetting agent only were carried out. At ear emergence a transparent bag was placed over the ears in order to prevent cross-pollination from untreated ears. The wheat plants were allowed to set seed and the average number of wheat grains per ear were counted, the results of these counts being shown in Table I below:

TABLE I

Experiment Number	Concentration of Test compound (ppm)	Average number of grains/ear from treatment of wheat plants at	
		Stage 7	Stage 10
Untreated control	–	36.3	36.3
1	0	34.3	32.8
2	100	3.4	0.5
3	500	0	0

From these results it will be seen that the test compound caused almost complete male sterility at 100 ppm and complete sterility at 500 ppm.

In order to establish that female fertility has not been substantially impaired, male sterile plants produced by treatment with the test compound were cross-pollinated with untreated viable pollen. The seeds produced from this cross-pollination were allowed to germinate and the germination results established that the seed was 100% viable.

EXAMPLE II.

Tests were carried out with 2-carboxy-3,4-methanopyrrolidine on wheat plants, variety "Dove".

The test compound was applied to the plants at 2 stages of plant development during the stem extension period of the plant but before ear emergence, namely:

Stage 8:
when the last leaf is just visible.

Stage 9:
when the ligule of the last leaf is just visible.

Groups of plants (20 pots, 3 plants per pot) at development Stages 8 and 9 were sprayed with the test compound formulated as a 72 ppm or 144 ppm solution in water with added wetting agent (NONIDET P—40 (Trade Mark) at a concentration 0.1%). Control tests with untreated plants and with plants sprayed with water and wetting agent only were also carried out. At ear emergence a transparent bag was placed over the ears in order to prevent cross-pollination from untreated ears. The wheat plants were allowed to set seed and the average number of wheat grains per ear were counted, the results of these counts being shown in Table II below:

TABLE II

Experiment Number	Concentration of Test compound (ppm)	Average number of grains/ear from treatment of wheat plants at	
		Stage 8	Stage 9
Untreated control	–	36.3	36.3
1	0	34.3	32.8
2	72	0	0
3	144	0	0

These results clearly establish the sterility of the male anthers. In subsequent cross-pollination tests, it was confirmed that the female fertility was unaffected.

EXAMPLE III.

Tests were carried out with 2-carboxy-3,4-methano pyrrolidine on spring barley, variety Imber.

The test compound was applied to the plants at development Stages 8 and 9 in the way described in Example II, except that the test compound was applied as a 100 or 200 ppm solution in water. The results are given in Table III below:

TABLE III

Experiment Number	Concentration of Test compound (ppm)	Average number of grains/ear from treatment of barley plants at	
		Stage 8	Stage 9
Untreated control	–	21.2	21.2
1	100	0.25	16.25
2	200	0	7.95

The results clearly establish the sterilizing effect of 2-carboxy-3,4-methanopyrrolidine on barley.

EXAMPLE IV.

Tests were carried out with 2-carboxy-3,4-methanopyrrolidine on spring wheat, variety Sappo.

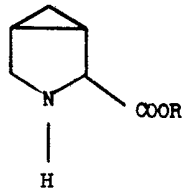
Groups of plants (10 pots, 2 plants per pot) at development Stages 8 and 9/10 were sprayed with the test compound formulated as a 50 or 100 ppm solution in water with added wetting agent (NONIDET P. 40 (Trade Mark) at a concentration 0.1%). Control tests with plants sprayed with water and wetting agent only were also carried out. At ear emergence a transparent bag was placed on the ears in order to prevent cross-pollination from untreated ears. The wheat plants were allowed to set seed and the average number of wheat grains per ear were counted, the results of these counts are given in Table IV below:

TABLE IV

Experiment Number	Concentration of Test compound (ppm)	Average number of grains /ear from treatment of wheat plants at	
		Stage 8	Stage 9/10
Untreated control	–	41.6	41.6
1	50	4.1	38.9
2	100	0.1	22.1

WHAT WE CLAIM IS:—

1. A method of sterilising male anthers in a plant which is an angiosperm, which comprises applying to the plant, in an amount and at a growth stage of the plant such that sterilisation occurs, a heterocyclic compound having the following general formula



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wherein R represents a hydrogen atom, an alkali metal ion, one equivalent of an alkaline earth metal ion, an ammonium ion optionally substituted by one or more alkyl groups, or an alkyl group of up to 10 carbon atoms.

2. A method as claimed in claim 1, in which the compound of the general formula I is 2-carboxy-3,4-methanopyrrolidine or 2-methoxycarbonyl-3,4-methanopyrrolidine.

3. A method as claimed in either claim 1 or claim 2, in which the plant is a cereal plant.

4. A method as claimed in claim 3, in which the plant is a small-grain cereal plant.

5. A method as claimed in either claim 3 or claim 4, which comprises treating the plant during the period after the 2-node growth stage of the plant and before the emergence of the ear.

6. A method of producing F_1 hybrid seed which comprises cross-pollinating a plant which has been treated by a method as claimed in any one of claims 1 to 5, with a second plant of a different strain.

7. F_1 hybrid seed produced by a method as claimed in claim 6.

8. A composition which comprises a compound of the general formula I as defined in claim 1, together with a solid carrier and/or a surface-active agent, or together with a liquid carrier and a surface active agent.

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