METHOD OF TREATING PLANTS OR PLANT TISSUES

A method is provided for treating plants or plant tissues (including cuttings, roots, bulbs, corns, tubers, rhizomes and seeds) in order to induce a desired tissue morphology and/or a desired physiological state by applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to produce an at least partial inhibition of the formation of effector gibberellins in said plant. Effects obtained include dwarfing, stem and shoot and/or root (radicle) growth retardation, flowering, improved fruit quality, inhibiting fruit ripening, improving fruit set, controlling weed growth, inducing male sterility, retarded bud break and tillering.
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METHOD OF TREATING PLANTS OR PLANT TISSUES

This invention relates to a method of treating plants or plant tissues (including cuttings, roots, bulbs, corns, tubers, rhizomes and seeds) in order to induce a desired tissue morphology and/or a desired physiological state.

Numerous phytoactive substances are known which are used in agricultural and horticultural practice in order to promote desired physiological effects in higher plants. Such effects include promotion of flowering, weed control, inhibition of stem elongation (dwarfing), improvement of hardiness, promotion of rooting and inhibition of root or shoot growth in germinating seeds. Many available phytoactive substances have undesirable side effects and may give rise to toxic residues which tend to pollute the environment.

Naturally occurring gibberellins have found extensive use in agriculture and horticulture and can, for example, be used as components of compositions for promoting flowering. The so-called effector gibberellins (GAs), including \( \text{GA}_1 \) and \( \text{GA}_3 \), produce stem elongation in many plants and while this may in certain circumstances be desirable, often the converse is the case and it is preferable to retard stem elongation in order to produce dwarf plants. The control of flowering and stem elongation of higher plants is economically desirable for a number of reasons, including, but not restricted to enhancing earliness of flowering, ensuring uniformity of flowering, increasing the number of flowers produced, and reducing the height of the plant, thereby making it more resistant to falling over, or breakage, and also making it easier to train (i.e. orchard trees).
The literature makes it apparent that certain members of the gibberellin class of molecules will effectively promote flowering in many, but not all higher plants. However a major drawback to the use of those gibberellins which have been reported to promote flowering is the increased shoot and stem growth (elongation) caused by application of such gibberellins. Further, these overt side effects may make the plant more susceptible to being damaged, or falling over (being lodged) as a result of rain, hail or snow or simply as a result of sheer overgrowth. Additionally, it is known that application of many gibberellins to woody angiosperm species is known to be deleterious to next year's flowering. That is to say application of a gibberellin in order to enhance fruit set or fruit quality may inhibit the following year's flower crop.

Reducing the shoot growth in a flowering plant is extremely useful in many circumstances. First it makes the plant more resistant to adverse weather conditions in the field, such as wind, rain, hail and snow. Secondly, it makes the plant more compact, more stocky, and more resistant to falling over (technically known as "lodging") as a result of the aforementioned weather conditions and/or as a result of heavy fruit or seed or grain production. Thirdly, in orchard situations a more compact nature of the shrub or tree is extremely valuable for a variety of reasons, including ease of tending the tree, picking the fruit, applying other treatments and reducing the necessity to prune the tree or shrub. Also, shoot growth resulting either from the presence of high levels of endogenous gibberellins, or induced by gibberellins applied to the plant, can compete with growth and development of fruit, seed or grain, thereby reducing the final yield.
A high concentration of endogenous effector gibberellins can be undesirable in plants which have been subjected to conditions likely to cause physiological damage. Thus the presence of effector gibberellins such as gibberellins GA$_4$ and/or GA$_3$ in recently transplanted trees and woody shrubs can give rise to reduced hardiness resulting in diminished survival.

We have now developed a procedure for promoting a desired tissue morphology and/or physiological state in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

The use of C-16,17-dihydro gibberellins for this purpose has not hitherto been suggested. In fact in their review of the activities of number of gibberellins, including seven C-16,17-dihydro gibberellins, Brian et al (Phytochemistry (1967), 6, pp. 1475-1499) concluded that "None of the compounds listed in the tables proved inhibitory in our tests, Many were inactive in all four tests". Furthermore Brian et al contains no indication that C-16,17-dihydro gibberellins might possess useful growth inhibiting or florigenic properties.

As will be described in more detail below, the desired tissue morphology and/or physiological states can be promoted according to the invention without the often undesired effects (including shoot or stem elongation) associated with the application of so-called "effector" gibberellins.

Although the precise mechanism of action of C-16,17-dihydro gibberellins when applied in accordance with the invention is not known, it is believed that they produce an at least partial inhibition of formation of effector gibberellins in the plant. It is theorized
that this is a result of an at least partial inhibition of gibberellin 3β-hydroxylase activity in the plant. Gibberellin 3β-hydroxylase is a naturally occurring enzyme which mediates the interconversion of certain gibberellins in plant by hydroxylating them at position C-3. Thus many plants obtain their endogenous effector gibberellins by conversion from precursors in the biosynthetic pathway.

For example many plants convert gibberellin GA\textsubscript{20} to gibberellin GA\textsubscript{1} and/or gibberellin GA\textsubscript{20} to gibberellin GA\textsubscript{3} and then to gibberellin GA\textsubscript{3}.

Use of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor in accordance with the invention is believed to inhibit formation of endogenously produced effector gibberellin GA\textsubscript{1} and/or GA\textsubscript{3}, and will also inhibit their formation from certain exogenously applied gibberellins. The invention is thus particularly applicable to the treatment of plants which obtain their endogenous effector gibberellins by conversion from precursors by hydroxylating them at position C-3 by a pathway which involves a gibberellin 3β-hydroxylase.

It may also be applicable to situations where the desired morphology can be obtained by blocking 3β hydroxylation of active gibberellins and their production.

Thus according to one aspect of the present invention, there is provided a method for promoting a desired tissue morphology and/or physiological state in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to produce an at least partial inhibition of formation of effector gibberellins (e.g. gibberellins A\textsubscript{1} and/or A\textsubscript{3} among others).
While inhibition of formation of effector gibberellins is presently believed to contribute to the beneficial effects obtainable by applying a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor in accordance with the invention, the invention is not intended to be limited to any particular theoretical explanation of the observed results. Thus the invention according to preferred aspects thereof may be defined in terms of the macroscopic effects obtained, such as, for example, enhanced induction of flowering, improving fruit quality, inhibiting ripening of fruit, improving fruit set, controlling growth of weeds and other effects.

Thus according to a further aspect of the invention, there is provided a method for promoting flowering in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to induce flowering.

The finding that C-16,17-dihydro gibberellins or C-16,17-dihydro gibberellin precursors can promote flowering without inducing significant stem elongation, and indeed can promote flowering while often retarding stem elongation, is considered to be particularly surprising, because the extensive literature on the known physiological effects of available gibberellins shows numerous instances where shoot growth is enhanced with or without promotion of flowering, but never where promotion of flowering is achieved together with a reduction, inhibition or retardation of shoot growth.
The invention further provides:

a method for improving fruit quality in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

a method for inhibiting ripening of fruit of a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

a method for improving fruit set in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

a method of controlling growth of weeds in an area of land which comprises applying to said land area a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

a method for retarding bud break in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

a method for retarding shoot growth (with or without promoting flowering) in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

a method for promoting tillering and/or bud release in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.
a method for inducing male sterility in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

The C-16,17-dihydro gibberellins and C-16,17-dihydro gibberellin precursors useful in carrying out methods of the invention may be characterised by the following general formulae Ia, Ib, Ic, Id and Ie:
wherein A, B, C, D, E and F independently represent hydrogen atoms or hydroxyl groups and the dotted line represents one optional double bond either between the carbon atoms in positions 1 and 2 or between the carbon atoms in positions 2 and 3.

16,17-dihydro GA₅ is a particularly preferred compound for use in accordance with the invention, particularly for use as a growth inhibitor. Also 16,17-dihydro GA₃ is a particularly preferred compound for use in accordance with the invention, particularly where a flowering-promoting effect is desired. Specifically 16,17-dihydro GA₃ has a flowering-promoting effect similar to that obtainable with GA₃, but essentially without the growth promotion effects associated with the latter compound.

In certain applications using compounds of formula Ia, one optional proviso is that where B, C and D represent hydrogen and A represents hydroxy, a double bond is present between the carbon atoms in positions 1 and 2. Another optional proviso is that where A, B, C and D represent hydrogen and E represents hydroxy, a double bond is present between the carbon atoms in positions 1 and 2 or 2 and 3.

16,17-dihydro gibberellins for use in accordance with the invention may be produced by hydrogenating the corresponding 16,17-dehydro gibberellin, e.g. with Pd/H₂.

In the above formulae (wherein Formula Ie represents a typical 16,17-dihydro gibberellin precursor based on kaurenoic acid) the ent-gibberellane skeleton may be numbered as follows.
Examples of compounds which may be used in accordance with the invention include C-1,2-didehydro, C-16,17-dihydro gibberellins, for example C-16,17-dihydro GA$_3$. Other examples include C-16,17-dihydro GA$_{20}$; C-16,17-dihydro, 2,3 dehydro GA$_9$; C-16,17-dihydro GA$_{12}$; C-16,17-dihydro GA$_{15}$ and C-16,17-dihydro GA$_{53}$.

Further examples include the C-2,3 didehydro derivatives of C-16,17-dihydro GA$_3$; of C-16,17-dihydro GA$_{20}$ (this compound being C-16,17-dihydro GA$_5$), of C-16,17-dihydro GA$_{12}$, of C-16,17-dihydro GA$_{15}$ and of C-16,17-dihydro GA$_{53}$.

Most preferably, the C-16,17-dihydro gibberelin used in accordance with the invention is C-16,17-dihydro GA$_5$ of Formula IIa or IIb.

In the above formulae, the 16-exo compound has the 16-R configuration and the 16-endo compound has the 16-S configuration.
The 16,17-dihydro gibberellins used in accordance with the invention include compounds having one or more of the following structural features:

A. 2,3 unsaturation (i.e. as in 16,17-dihydro GA$_5$)

B. 1,2 unsaturation (i.e. as in C-1,2-dehydro 16,17-dihydro GA$_9$)

C. substitution with one or two hydroxy groups at one or more of C-1, C-11, C-12, C-13 and C-15

D. substitution with three hydroxy groups at one or more of C-1, C-11, C-12, C-13 and C-15

E. substitution with four hydroxy groups at one or more of C-1, C-11, C-12, C-13 and C-15.

Examples include

16,17-dihydro GA$_5$;
16,17-dihydro C-2,3-dehydro GA$_9$;
2,3-dehydro, C-12-hydroxy, 16,17-dihydro GA$_5$ and
2,3-dehydro, C12,15-dihydroxy 16,17-dihydro GA$_5$.

As indicated above, application of a C-16,17-dihydro gibberellin in accordance with the invention can be effective to promote flowering without producing simultaneous stem elongation. In fact promotion of flowering with simultaneous reduction, inhibition or retardation of shoot growth has been observed.

Thus according to a further aspect of the invention there is provided a method of promoting flowering in a higher plant which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin effective to induce flower formation.
Preferably the C-16,17-dihydro gibberellins are as defined above.

The C-16,17-dihydro gibberellins used in accordance with the invention may be applied in the form of free acids or as salts or esters thereof. Suitable salts and esters include the sodium and potassium salts and the C_{1-4} carboxylic acid esters.

The gibberellins may be used in accordance with the invention alone or with other plant growth regulators, for example chemical thinning agents.

Further the method of the invention may be carried out in the open, i.e. in the field, or in a glasshouse environment.

Particularly in connection with that aspect of the invention concerned with promotion of flowering, the C-16,17-dihydro gibberellins may be applied under photoperiod and temperature conditions which are inductive, marginally inductive, or non-inductive of flowering. Flowering may be promoted under any of these conditions while at the same time reducing unneeded or unwanted shoot growth.

The application of C-16,17-dihydro gibberellins in accordance with the invention may desirably be carried out in autumn, so as to improve cold hardiness or retard the next season's bud break, or in early spring, or late spring or early summer, either prior to normal flower initiation or during early stages of flower differentiation, or during early stages of floral development. Good results have been obtained at all of these stages. Although multiple applications of the C-16,17-dihydro gibberellin may be made, significantly improved flowering, with concomitant shoot length reduction, can often be achieved with a single application.
The method of application of the C-16, 17-dihydro gibberellin is not thought to be particularly critical and may be accomplished, for example, by spraying a solution or suspension of the C-16, 17-dihydro gibberellin to whole plants, or by application to seeds or roots or bulbs, corms or rhizomes, together with a suitable carrier. The addition of conventional adjuvants such as wetting agents and dispersants may prove to be beneficial in some agronomic situations.

Only small quantities of C-16, 17-dihydro gibberellin need be applied in accordance with the invention. The precise dose will depend upon the desired tissue morphology or physiological state which is desired to be induced and the plant species. Thus experiments have shown that in certain species, e.g. lettuce, root length is inhibited at concentrations of 16, 17-dihydro GA5 in the range of 10^{-10} to 10^{-7} M, but is promoted at concentrations of 10^{-6} M and higher.

For a given species, the required dosage and treatment regime can readily be determined by carrying out appropriate experiments, e.g. along the lines of those described herein.

As a general guide dosage rates of from 0.1 to 1000 micrograms of dihydro GA per gram of actively growing plant tissue, especially from 2 to 100 micrograms of dihydro GA per gram of actively growing plant tissue have been found to give useful results, and for stimulating flowering, satisfactory results have been obtained with as little as 2 micrograms per plant.

The amount of dihydro gibberellin or dihydro gibberellin precursor applied in accordance with the invention may also be expressed in terms of a proportion of the weight of fresh or dry plant tissue.
Expressed in this way the applied amount is preferably up to 1000 micrograms/gram fresh weight, especially from 1 to 1000 micrograms/gram fresh weight. Most preferably, the amounts applied are from 2 to 1000 micrograms/gram fresh weight, especially from 2 to 500 micrograms/gram fresh weight. Optimally, the applied amounts are from 2 to 333 micrograms/gram fresh weight, especially from 2 to 100 micrograms/gram fresh weight. (For most plant species, the ratio of fresh:dry weights is 10:1-6:1).

Dihydro gibberellins may be formulated for use in accordance with this invention at concentrations up to 5000 ppm ($1.5 \times 10^{-12}$M). Most preferably the minimum concentration is preferably 0.1 ppm (when applied as a seed soak or soil drench, lower concentrations may be used as detailed below). A preferred concentration range is 1-1000 ppm.

Concentrations of from 200 ppm, preferably from 5-350 ppm of the C-16,17-dihydro gibberellin will give satisfactory results, especially when applied as a foliar spray. With certain species (for example oilseed rape), application rates of from 10 to 100 times higher than those mentioned above may be required. Lower concentrations have been found to be effective when used as a seed soak or soil drench, for example concentrations in the range of $10^{-12}$ to $10^{-7}$ molar, although preferably the minimum concentration is at least $10^{-10}$M.

Although the method of the invention can be carried out using a C-16,17-dihydro gibberellin as the sole plant growth modifying agent, other plant growth regulators such as cytokinins or even shoot elongation-promotive gibberellins such as gibberellin A$_1$ or gibberellins A$_3$ may be additionally used. Thus, for example,
gibberellins $A_1$ or gibberellin $A_3$ or other gibberellins such as
the 3β-hydroxylated gibberellins $A_4$ and $A_7$ may be usefully
included in the treatment in order to counteract an excessively
intense shoot growth reduction caused by application of the
C-16,17-dihydro gibberellin.

The application of C-16,17-dihydro gibberellins in accordance with
the invention can be used to produce advantageous effects which can
manifest themselves in many different ways. Particularly, it has been
found to be possible to obtain many of the desirable physiological
effects hitherto produced by applying other gibberellins, but without
producing excessive shoot growth, excessive overgrowth of the stem and
diminished flowering the next year in woody angiosperms.
Specific examples of effects obtainable in accordance with the invention include the following:

(1) thinning of wine and table grapes without inhibition of the following year's flowering

(2) increased flowering in wine grapes

(3) improvement of the fruit quality in cherries, while reducing shoot growth.

(4) production of parthenocarpic fruit without inhibition of the following year's flowering and without increased shoot growth which results from known treatments with \( \text{GA}_3 \). To achieve this it is desirable to include an additional gibberellin such as \( \text{GA}_4 \).

(5) promotion of flowering in woody angiosperms so as to prevent bienniality without causing increased vegetative shoot elongation, or with concomitant reduction of shoot growth.

(6) maintaining green fruit and inhibiting ripening (on the tree) in citrus and other fruits. This effect is achievable without the negative side effects (for example increased shoot growth and reduced next year's flowering) resulting from known treatments involving the use of \( \text{GA}_3 \).
(7) increasing fruit set without inhibition of next year's flowering (as is caused by known treatments using GA₃ or GA₄/₇) with a reduction of vegetative shoot growth, thereby improving the allocation of photosynthate to the developing fruit.

(8) as a fruit thinning agent (at relatively high doses) without inhibiting next year's flowering and with a reduction in shoot growth of adjacent shoots, therefore improving the allocation of photosynthate to developing fruit.

(9) increasing fruit yield brought about by favouring and enhancing the redistribution of photosynthate to the fruit or grain head (this effect would primarily result from the reduction of vegetative growth) and is useful for a range of crops including strawberries, cereal, legumes, and fruit trees such as apples and pears.

(10) induction of male sterility particularly in the production of hybrid corn, wheat and sorghum seed.

(11) restoration of male fertility in varieties having diminished male fertility or male sterile lines.

(12) weed control. Promotion of flowering of longday weeds, either prematurely (e.g. in early spring or autumn or early winter) or in mid-season. This would cause longday weeds to flower, but not bolt and the weeds would then be shaded by the crop plants normal growth.
weed control e.g. by promoting germination of weed seeds, but retarding their subsequent growth, or by breaking bud dormancy, but allowing for only a very reduced weed growth. Application of C-16,17-dihydro gibberellins in accordance with the invention inhibits the early growth of weed seedlings such as wild oats by yielding a young seedling with a very reduced shoot growth (see Photographs 1 - 5) and in fact even yielding a toxic effect which would be unable to compete with the main crop (the main crop already being established). Alternatively, the main crop would be sown deeply enough so as to avoid the influence of the applied C-16,17-dihydro gibberellin. The resulting weed seedlings with slowed root growth would also be more prone to drought.

(14) weed control by prevention of flowering of shortday annual weeds under marginally inductive long nights, without the negative side effects that would be expected from the use of gibberellin A₃ on an accompanying longday or day neutral crop plant.

(15) priming or stimulation of uniform and more complete germination (by seed soak) without the overt elongation of the germinating seedling that is observed to occur with a gibberellin such as GA₃ or GA₄/7 mixture
(16) promotion of rooting in hard-to-root varieties e.g. where high endogenous gibberellin levels prevent rooting. Application of C-16,17-dihydro gibberellins in accordance with the invention may be accompanied by the application of an auxin such as indolebutyric acid or NAA.

(17) the treating of peonies, via the rhizome, to get more floral branches, but without the excessive elongation which is known to be induced by use of GA$_3$ (see M.R. Evans, W.O. Anderson, H.F. Wilkins [1990] Temperature and GA$_3$ Effects on emergence and flowering of potted Paeonia lactifolia. Hort. Science 25:923-924).

(18) the treating of cauliflower to alter the timing of flower (curd) development (e.g. advance curd development), but without causing untoward elongation of the base of the curd, as may occur with use of GA$_3$ or GA$_4/7$ (see R. Booij and reference cited therein [Effects of gibberelic acids on time of maturity and on yield and quality of cauliflower. Netherlands J. of Agric. Science 38:641-651 (1990)].

(19) in the malting of barley grain significant amounts of stored assimilate are diverted into the developing root and shoot of the germinating grain. This is wasteful and is considered a loss by the brewing and malting industries. It is presently controlled in some countries by the use of bromate ion, the safety of which can now be questioned, followed by application of
GA$_3$, the latter stimulating $\alpha$-amylase production over and above that obtained by use of the malted grain alone, with or without bromate ion. Influencing (retarding) the allometric distribution of stored assimilate from the starchy endosperm of the grain into the root and shoot can be accomplished by imbibing the seed in the presence of low levels (ca. $10^{-5}$ to $10^{-10}$ M) of C-16,17-dihydro gibberellins. This may then be followed by treatment with GA$_3$ to induce $\alpha$-amylase production (the $\alpha$-amylase breaks down starch to sugar).

(20) prevention of precocious germination (sprouting) on the seed head for grain crops by antagonizing the production of bioactive effector gibberellins.

(21) increasing cold hardiness by application of dihydro gibberellins. The production of excessive shoot growth in late summer and fall which would be tender and frost susceptible, is reduced, by antagonizing production of bioactive endogenous effector gibberellins.

(22) increasing drought-hardiness by providing a more compact plant shoot by antagonizing production of bioactive gibberellins. Root growth would be proportionately less affected due to differential retention in the shoot. Plants will then have more efficient water use and be able to better withstand transplanting.
"safening" of a plant for subsequent sprays with herbicides. In this case, the main crop plant would be sprayed with the C-16,17-dihydro gibberellin several days to weeks before the proposed herbicide treatment. The dihydro gibberellin would retard growth of the crop plant, thereby rendering it more resistant to the herbicide.

retarding growth of both vegetative and floral parts of amenity grasses, and even inhibiting flowering of short-day induced amenity grasses, thereby providing a more useful grass for lawns, parkways and golf courses.

retarding bud break of both floral and vegetative buds thereby allowing for a delayed and more uniform bud break after, for example, the damage of frost has passed, or in the case of potted plants, to allow for staggered, or delayed bud break, with uniformity of bud break being brought about, where necessary, by subsequent use of an effector gibberelin such as GA1, GA3, GA4 or GA7.

promoting of tillering, especially in grain crop, and in amenity grasses, i.e. turf grasses

total prevention of flowering

increased prostrate growth form

promotion of male flowers (cone buds) in pinaceae.

reduction in seed set/production in Graminal species.
Species which may be treated in accordance with the invention include wheat, barley, oats, maize, sorghum, amenity grasses, native grasses and other members of the Gramineae,

*Spathiphyllum*, *Zantedeschia* and other members of the Araceae,
dicotyledenous crop plants such as cotton, sunflower, oilseed rape, soybean, field pea, native flowering plants,
woody angiosperm trees and shrubs (including azealea, grape and shade trees and orchard trees),
and gymnosperm amenity trees and seed orchard trees.
The invention will now be described in more detail with particular reference to the following examples.

EXAMPLE 1 - PROMOTION OF FLOWERING IN LolioM TEMULENTUMN

Plants of the species *Lolium temulentum* were grown under both non-inductive short day (SD) and one marginally inductive long day (LD) conditions.

Plants in each set were treated on 4th July 1990 with one of the following treatments and control plants received no gibberellin

<table>
<thead>
<tr>
<th>Set</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>None (control)</td>
</tr>
<tr>
<td>LD</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>SD</td>
<td>endo-dihydro GA5</td>
</tr>
<tr>
<td>LD</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>SD</td>
<td>GA3</td>
</tr>
<tr>
<td>LD</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>SD</td>
<td>GA5</td>
</tr>
<tr>
<td>LD</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

Each treatment consisted of applying the stated gibberellins as a microdrop to the leaf at a rate of 1 to 25 micrograms per plant.

The results are set out in graphical form in the attached Figure 1 from which it can be seen that endo-dihydro GA5 alone is effective in promoting flowering, without producing the stem extension observed following application of GA3 or GA5.
EXAMPLE 2 - PROMOTION OF FLOWERING IN XANTHIUM

Plants of the species genus *Xanthium* were grown under marginally inductive short day (SD) conditions.

Plants were treated with one of the following three treatments and control plants received no gibberellin

<table>
<thead>
<tr>
<th>Set</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None (control)</td>
</tr>
<tr>
<td>B</td>
<td>endo-16,17-dihydro GA5</td>
</tr>
<tr>
<td>C</td>
<td>GA5</td>
</tr>
<tr>
<td>D</td>
<td>endo-16,17-dihydro,15β-OH GA5</td>
</tr>
<tr>
<td>E</td>
<td>endo-16,17-dihydro GA5 + 10μg GA3</td>
</tr>
</tbody>
</table>

Each treatment consisted of applying the stated gibberellins as a microdrop in ethanol at a rate of 5 to 50 micrograms per plant, within which range an optimal dose of the stated dihydrometer could be found. However a very high dose inhibited flowering.

The results on *Xanthium* are set out in graphical form in the attached Figure 2 and shows that 16,17-dihydro GA5 can be used to promote flowering under marginally inductive short day (long night) conditions. In the experiments using GA3 and 16,17-dihydro GA5 in combination, the flowering-promoting effect of 16,17 dihydro GA5 was enhanced by addition of an optimal amount of GA3.

Similar results in plants of the genus *Pharbitis*, also grown under marginally inductive SD conditions were obtained, with D above, and also 16,17-dihydro GA3 being especially effective.
EXAMPLE 3 - GERMINATION OF BARLEY SEEDS

Barley seeds were germinated on endo-C16,17-dihydro GA₅ solutions and harvested after either 72 or 96 hours. 72 h results are expressed as shoot or root weight per 50 seeds. 96 h results are expressed as shoot or root weight per 10 seeds.

The results in Figures 3 and 4 show that diversion of stored carbohydrate into root and shoot is significantly diminished, which is of practical advantage to the brewer/malter.

EXAMPLE 4 - RETARDATION OF SHOOT GROWTH IN TAN-GINBOZU DWARF RICE

Uniconazole-treated rice plants of the dwarf variety Tan-ginbozu were treated with the following gibberellins:

- GA₅, GA₂₀ or GA₉
- endo-16,17-dihydro GA₅
- exo-16,17-dihydro GA₅
- exo-16,17-dihydro GA₉

Treatment rates varied from 0.01 to 1000 ng per plant for endo and exo-16,17-dihydro GA₅, from 0.1 to 100 ng/plant for GA₅ and from 0.1 to 1000 ng for GA₉ and GA₂₀.

The results are shown in Figures 5, 6, and 7, 7A 8 9 and 10 from which it can be seen that for both endo- and exo-16,17 dihydro GA₅, essentially no stem elongation effect is observed (c.f. GA₅ and GA₂₀), and further that both of endo- and exo-forms of dihydro GA₅ will significantly reduce (Figures 5,6,7 and 7A) the GA₅- or GA₂₀-induced growth promotion in the rice seedling. The exo-form of dihydro GA₅ is the least growth promotive at high doses (Figures 5, 7, 7A, 9 and 10) and also is significantly more growth inhibitory when
tested versus GA\textsubscript{5} (Figure 5), GA\textsubscript{20} (Figures 6, 7A) and GA\textsubscript{9} (Figure 9) although the relative effects of exo- vs. endo-forms varies with dose of the dihydro gibberelin and according to which of dihydro gibberelin is used. GA\textsubscript{5} and GA\textsubscript{20} are proven precursors to the effector gibberellins, GA\textsubscript{3} and GA\textsubscript{1} respectively.

The effect of 2,3-dehydro C-16,17-dihydro GA\textsubscript{9} was also tested against GA\textsubscript{9} on uniconazole-treated dwarf rice cv Tan-ginbozu, as were endo- and exo- forms of 16,17-dihydro GA5 (Figures 8, 9 and 10).

The results show:

I. 2,3-dehydro, C-16,17 dihydro GA\textsubscript{9} alone (Figures 8, 9 and 10)

2,3-dehydro, C-16,17 dihydro GA\textsubscript{9} alone is quite active, but still less active than GA\textsubscript{9} alone (e.g. roughly 100 ng of the dihydro required to yield same growth promotion produced by 30-50 nanograms of GA\textsubscript{9}).

II. 2,3-dehydro, C-16,17-dihydro GA\textsubscript{9} vs GA\textsubscript{9} (Figure 8)

A. at lower concentrations of the dihydro GA\textsubscript{9} derivative there is a modest growth promotion, relative to GA\textsubscript{9} alone.

B. at higher concentrations of the dihydro GA\textsubscript{9} derivative there is modest to significant (statistically significant) inhibition of growth, relative to GA\textsubscript{9} alone (but there is still very good growth).
C-16,17-dihydro GA$_5$ versus GA$_9$ also gave a modest, but highly significant growth retardation (relative to GA$_9$ alone) at the higher doses of GA$_9$ (Figures 9 and 10).

Relatively speaking, C16,17-dihydro GA$_5$ appears to be a better antagonist than C-2,3 dehydro 16,17 dihydro GA$_9$ of GA$_9$-promoted growth in rice, possibly because the 38-hydroxylase enzyme requires that the C-13 hydroxyl group be present on the dihydro GA molecule for good "recognition".

EXAMPLE 5 - CANE GROWTH IN CHARDONNAY GRAPE

The growth (February 5th - February 20th 1991) of Canes of *Vitis vinifera*, variety "Chardonnay", in response to a single application of 16,17-dihydro GA$_5$ on February 8th 1991, microdrops applied to tip and to each of 9 potential flower buds at each node. Average of 6 (usually) or 5 canes.

From the results in Figure 9 it can be seen, compared to the controls both the new internode growth and growth of pre-existing internodes showed a strong and significant (P<0.05) negative log-linear correlation with the applied dose of 16,17-dihydro GA$_5$.

Dissection of two of the buds (Nos. 3 and 7 from the tip) has shown no inhibition of flower bud numbers by the treatment with 16,17-dihydro GA$_5$.

It can thus be concluded that application of GA$_5$ to *Vitis vinifera* cv Chardonnay significantly retarded cane growth without inhibiting flowering.
EXAMPLE 6 - RETARDATION OF STEM ELONGATION IN OILSEED RAPE

The degree of stem bolting in oilseed rape (w canola) in response to applied GA$_1$, GA$_5$, and 16,17-dihydro GA$_5$ was determined.

From the results in the accompanying Figures 12 and 13 it can be seen that both GA$_1$ and GA$_5$ produced the expected promotion or stem elongation typical of effector gibberellins.

Dihydro GA$_5$ however produced a significant reduction (Figures 12 and 13) in the degree of stem elongation (stem bolting) while still promoting (Figure 12) floral stage development almost as well as GA$_5$ (GA$_1$ did not promote floral stage development) flowering.

EXAMPLE 7 - BUD BREAK - M POHUTAKAWA

The effect of C-16,17-dihydro GA$_5$ (specifically C-16,17-dihydro GA$_5$) on bud break was assessed on a shrub of the Myrtaceae family (Metrosideros Pohutakawa). Retardation of bud break in fruit trees, shrubs and forest trees is useful to prevent damage from late frosts, and to synchronise flowering in grapes, and in potted shrubs for the florist trade.
The results were as follows:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proportion of Buds Broken Dormancy at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 10</td>
</tr>
<tr>
<td>Control</td>
<td>42%</td>
</tr>
<tr>
<td>1 ppm C-16,17-dihydro GA₅</td>
<td>21%</td>
</tr>
<tr>
<td>10 ppm C-16,17-dihydro GA₅</td>
<td>34%</td>
</tr>
<tr>
<td>100 ppm C-16,17-dihydro GA₅</td>
<td>21%</td>
</tr>
</tbody>
</table>

Thus C-16,17-dihydro GA₅ effectively retarded bud break relative to controls.

EXAMPLE 8 - HERBICIDAL EFFECT ON WILD OATS

Durum wheat infested with wild oats was treated with C-16,17-dihydro GA₅ at a treatment rate of 33 ppm and 100 ppm.

The results are shown in Figure 14 from which it can be seen that the wild oats were retarded significantly. The Durum wheat was retarded (a desirable effect) but appeared normal and dark green and flowered normally although a day or two delayed. Evidence was also seen of a yellowing and toxicity in the wild oat infestation.
EXAMPLE 9 - PROMOTION OF MALE CONE BUDS

A. C-16,17 dihydro GA5 was applied at pre-bud swell stage of development in 1991 to Douglas fir clonal propagules (used for seed production). Male flowers (conebuds) were assessed in spring, 1992 for endo- or exo- C-16,17 dihydro GA5 applied.

The results were as follows:

1. endo-C-16,17 dihydro GA5
   0.0,13.0,0.2,0.0 = 1.88 male conebuds/ treated propagule

2. Control
   0.0,8.0,0.0,0.0 = 1.0 male conebuds/ treated propagule

3. exo-C-16,17 dihydro GA5
   4.0, -, 1.0,0,0, = 0.7 male conebuds/ treated propagule

4. Control
   0.0,4.0,1.0,0,0, = 0.8 male

B. C-16,17 dihydro GA5 was applied at vegetative bud break stage of development in 1991 male flowers (conebuds) were assessed in spring, 1992 for endo- or exo- C-16,17 dihydro GA5 applied to Douglas fir clonal propagules (used for seed production in seed orchards).

Treatment branches were chosen for the likelihood that male flowers (conebuds) would be produce (e.g. low branches).

There were almost no female flowers (conebuds produced).

The results were as follows

1. endo-C-16,17 dihydro GA5
   20, 6, -, -, -, -, = 4.33 male conebuds/ treated propagule
   ( - - = branch died)

2. Control
   13, 2, -, -, -, - = 2.5 male conebuds/ treated propagule
3. exo-C-16,17 dihydro GA5
   0, 10, 0,0,0,.- = 1.4 male cone buds/treated propagule
   (0 = no male flowers (cone buds))

4. Control
   0,0,0,0,0,.-,0, = 1.0 male cone buds/ treated propagule

   There were essentially no female flowers (cone buds) produced on
   the chosen branches of these propagule.

EXAMPLE 10 - INHIBITION SEED SET OR PRODUCTION/INDUCTION OF TILLERING

Sprays of exo- dihydro GA5 were made up in 1% activator
(surfactant) at each of 0, 100 ppm and 330 ppm, and sprayed to "drip
off" to each of:

   oat (domestic -- *Avena savasti*, common oat)
   wild oat (weed) -- *Avena fatua*
   barley (*Hordeum vulgarias*)

The plants were planted in trays, emergence occurred within 3 to
5 days of planting.

Photoperiod was 16 hours, temperature regime was 24°C during the
16 hr day, and 18°C during the 8 hr night. These are "long days"
under which growth is rapid, and flowering will normally occur.

Figures 15 to 18 showing plant height (cm) are attached for each
species.

It can be seen that the highest dosage (330 ppm) gave a growth
(final height) of only 35 to 40 cm. Height on the 330 ppm treatment
was obtained by measuring the length of the longest/tallest leaf. The
appearance was one of "lawn grass", and no flowering occurred (based
on observations on day 70, when seed counts were made on other treatments (see Fig. 18), and apices were dissected out on the 330 ppm treatment to see if flowering had been initiated).

But when sprayed at 330 ppm gave no flowering at all (plants were dissected, no floral apices were apparent).

Observations were made as follows:

Day 49 --

Inflorescence heads observed on a few barley and wild oat plants for the first time (control plants only).

Domestic oat plants did not show inflorescences, but there was a swelling that could be felt, indicating that the inflorescence is "present in the boot" (again, control plants only).

100 ppm plants were shorter than controls, with no visible inflorescence heads.

330 ppm plants were more stunted than the 100 ppm plants.

Day 56 --

For control plants inflorescence heads were observed on all species, with wild oats being the most developed.

100 ppm dihydro GA5 (exo-) inflorescence heads were observed on all species, but size of inflorescence head and of the plant per se was reduced, relative to Control.
330 ppm dihydro GA5 (exo-) -- no inflorescence heads observed on any species, over all size of plant is very reduced.

Day 61 --

Control plants of all species had normal head development, with heads filled or filling with seeds. Wild oat are most advanced, and lowest seeds are beginning to ripen.

100 ppm dihydro GSA5 (exo-) shorter than control (see Figures), but some seed set had obviously occurred. Head size is reduced in comparison with Control.

330 ppm dihydro GA5 (exo-) show no heads, very little growth (see Figures). All plants have wide (wider than controls) dark green leaves.

Day 70 --

Harvest and Seed count.

I. Inhibition of seed production

Control plants looked "normal", with average size heads, filled with seed.

100 ppm dihydro GA5 (exo-) treated plants were reduced in size, and a number of glumes (common name is "houses") were
empty (e.g. either sterile, or aborted -- see bar graph showing reduced number of seeds/plant for the 100 ppm treatment).

330 ppm dihydro GA5 (exo-) showed very reduced height, no floral development, hence no seeds (see bar graph).

In essence, 330 ppm exo- dihydro GA5 yielded semi-prostrate plants that gave an appearance of lawn grass.

Not only was seed set/production prevented, but flowering was prevented (presumably due to absence of endogenous "effector gibberellin" caused by the dihydro GA5 blocking the biosynthetic 3β-hydroxylation "activating" step (e.g. blocking GA20 → 1 or GA20 → GA5 → GA3).

This experiment also shows that the dose is related to the desired effect, since 100 ppm only partially reduced seed production (see Fig.18), whereas 330 ppm completely eliminated seed production by preventing flowering.

II. The promotion of tillering

The promotion of tillering (producing of lateral buds, which form additional [tiller] shoots in grain species by application of the exo- form of C-16,17 dihydro GA5 was assessed.

Additional tiller shoots may be practically useful for additional grain yield if the tillers can be produced early on, and thus allow for extra spike and seed production/plant.

Gibberellin A3, a known "effector" of elongation growth to species of Graminae, including commercially important grain species,
is known to enhance apical dominance, thereby reducing or preventing tillering (see M.A. Harrison and P.B. Kaufman, 1980, Plant Physiology 66:1123-1127 and references cited therein).

Therefore it is surprising that a gibberellin such as C-16,17 dihydro GA5 (exo- isomer), when applied to a grain species (such as barley, the example given) would actually promote tillering (see Tables 1 and 2), rather than inhibiting it as does applied GA3.

However, a possible explanation is that applied C-16,17 dihydro GA5 will inhibit the production of endogenous "effector" gibberellin-A1 and gibberellin A3, thereby allowing the main culm to lose apical dominance control of its lateral buds, which then begin to grow out and develop, yielding 1 or more additional tiller shoots on each plant.

EXAMPLE 11 - CO-APPLICATION OF DIHYDRO GA5 WITH ETHEPHON

C-16,17 dihydro GA5 (exo- form) was dissolved in ethanol, water added as was Activator surfactant (0.1%) to make a final concentration of 330 ppm in 10% ethanolic solution.

Spray was to "drip-off" and three treatments were used:

weekly beginning at 21 days after sowing, ending 5 weeks (5 applications) later.
as above, but plus 40 milli-molar ethephon (an ethylene releasing compound) which also retards growth in barley and promotes tiller formation

one application of C-16,17 dihydro GA5 at Zadoks Growth Stage 43 + ethephon as noted above

The results are shown in the attached Table 1 (plant height and internode length) and Table 2 (production of early and late tillers and flowering).

With regard to tiller production it is apparent from Table 2 that use of C-16,17 dihydro GA5 (exo-form) significantly promotes the number of early sterile tillers (2.8) relative to control (0.7), and very significantly promotes the number of late tillers (pre-flowering at time of assessment), 7.8 or 7.1 tillers (5 applications of C-16,17 dihydro GA5 or one application of C-16,17 dihydro GA5 + ethephon, respectively, relative to control (1.6 tillers).

That the early tillers are sterile is undoubtedly due to the high dose used. A lower dose/frequency of C-16,17 dihydro GA5 should promote tillering while still retarding shoot growth, but with good seed set (for example see Fig. 18; 100 ppm dosage).

In Table 2 treatment with 5 applications of C-16,17 dihydro GA5 significantly reduced fertile florets to 3 per spike (control was 34 or 42), and significantly promoted sterile florets to 50 per plant (control was 11 or 13).

Treatment with 1 application of C-16, 17 dihydro GA5 + ethephon very effectively reduced number of florets to 1 per spike, and increased sterile florets to 57 per spike.
Hence, Table 2 shows evidence of very reduced fertility (virtual lack of seed production) from 5 times application of C-16,17 dihydro GA\textsubscript{5}, and an exceptionally reduced fertility with one application of C-16,17 dihydro GA\textsubscript{5} + Ethephon.

This would yield a herbicide effect by effectively limiting the production of seed in weeds in the Graminae Order, and would thus have practical uses in crops of dicotyledenous species (such as rapeseed) whereby production of weed seed (such as wild oats) could be eliminated.

Height reduction by C-16,17 dihydro GA\textsubscript{5} is also shown in Table 1, either by 5 applications of the dihydro GA\textsubscript{5} alone, or by one application of dihydro GA\textsubscript{5} + ethephon. Both were very significant retarders of height, and data from Table 1 could be useful as an example of plant growth retardation by dihydro GA\textsubscript{5}. 
EXAMPLE 12 - INHIBITION OF SEED SET IN BARLEY

Barley was grown in autumn 1991 in high intensity light supplemented heated glasshouses.

Treated plants received:

exo- isomer of C-16,17 dihydro GA5 at:

- 0 (Control) -- 0 ppm
- $1.0 \times 10^{-4}$ (minus 4) molar spray -- 35 ppm
- $3.3 \times 10^{-4}$ (minus 4) molar spray -- 116 ppm
- $1.0 \times 10^{-3}$ (minus 3) molar spray -- 350 ppm

applied initially to the barley plants at the three-to-four leaf stage.

In some experiments only one application was given, in other experiments 3 applications were given, the 2nd application being applied one week after the initial application, the 3rd application being applied two weeks after the initial application.

There are two replicate experiments. The repeat experiment was staggered in time, but applications began at the same approximate stage (e.g. three to four leaf stage). Hence, variability due to replicate experiments may be due to random variation, or to differences in weather (e.g. overcast for 10 days in one trial, those days being at a different stage of development in the repeat trial).

The index of sterility (male and/or female) is seed yield/plant, expressed in each of weight of kernels (grams) produced per plant and volume of grain produced per plant.
Similarly, the efficacy as a herbicide treatment (e.g. prevention of seed production) is seed yield/plant, expressed in each of weight of kernels (grams) produced per plant and volume of grain produced per plant.

### Test #A, cv. Leduc Barley

**Single Spray**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>0.92a</td>
<td>0.77a</td>
<td>0.64ab</td>
<td>0.33b</td>
<td>0.0108</td>
</tr>
<tr>
<td>Kernel Volume (ml)</td>
<td>4.75</td>
<td>4.00</td>
<td>3.25</td>
<td>2.50 NS</td>
<td>0.5699</td>
</tr>
</tbody>
</table>

Note: Different letters Connote Significant Difference Between Treatments
NS = No Significant Difference Between Treatments

### Test #B, cv. Leduc Barley (Repeat of Test #A)

**Single Spray**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>1.09a</td>
<td>1.19a</td>
<td>0.99a</td>
<td>0.44b</td>
<td>0.0157</td>
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<tr>
<td>Kernel Volume (ml)</td>
<td>5.00</td>
<td>5.50</td>
<td>5.00</td>
<td>2.31 NS</td>
<td>0.0897</td>
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</tbody>
</table>

Note: NS = No Significance Between Treatments
Different letters Connote Significant Difference Between Treatments

### Test #C, cv. Leduc Barley

**Three Spray Applications**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>0.71a</td>
<td>0.35b</td>
<td>0.10c</td>
<td>0.00c</td>
<td>0.0015</td>
</tr>
<tr>
<td>Kernel Volume (ml)</td>
<td>3.25a</td>
<td>1.31b</td>
<td>0.38b</td>
<td>0.00b</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

Note: Different Letters Connote Significant Difference Between Treatments
Test # D, cv. Leduc Barley (Repeat of Test # C)  
Three Spray Applications

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>1.54a</td>
<td>1.23a</td>
<td>0.56b</td>
<td>0.44b</td>
<td>0.0160</td>
</tr>
<tr>
<td>Kernel Volume (ml)</td>
<td>7.33a</td>
<td>5.50ab</td>
<td>2.63b</td>
<td>2.25b</td>
<td>0.0424</td>
</tr>
</tbody>
</table>

Note: Different letters Connote Significant Difference Between Treatments

Test # E, cv. Jackson Barley  
Single Spray

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>1.54a</td>
<td>1.54a</td>
<td>1.65a</td>
<td>0.88b</td>
<td>0.0001</td>
</tr>
<tr>
<td>Kernel Volume (ml)</td>
<td>8.50</td>
<td>9.75</td>
<td>9.25</td>
<td>5.50 NS</td>
<td>0.0898</td>
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</tbody>
</table>

Note: NS = No Significance Between Treatments  
a vs b = Significant Difference Between Treatments

Test # F, cv. Jackson Barley (Repeat of Test # E) Single Spray

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>0.50</td>
<td>0.35</td>
<td>0.29</td>
<td>0.24 NS</td>
<td>0.9363</td>
</tr>
<tr>
<td>Kernel Volume (ml)</td>
<td>1.01</td>
<td>0.76</td>
<td>0.50</td>
<td>0.13 NS</td>
<td>0.4702</td>
</tr>
</tbody>
</table>

Note: NS = No significance Between Treatments
Test # G, cv. Heartland Barley
Single Spray

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>1.60a</td>
<td>1.23b</td>
<td>0.80c</td>
<td>0.20d</td>
<td>0.0001</td>
</tr>
<tr>
<td>Kernel Volume (ml)</td>
<td>6.75a</td>
<td>5.23a</td>
<td>3.50b</td>
<td>1.00c</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Note: Different letters connote significant difference between treatments.

Test # H, cv. Heartland Barley (Repeat of Test # G) Single Spray

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>35 ppm</th>
<th>116 ppm</th>
<th>350 ppm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Wt (grams)</td>
<td>0.61ab</td>
<td>1.00a</td>
<td>0.89a</td>
<td>0.27b</td>
<td>0.0312</td>
</tr>
<tr>
<td>Kernel Volume (ml)</td>
<td>3.03</td>
<td>4.50</td>
<td>4.00</td>
<td>1.40 NS</td>
<td>0.0634</td>
</tr>
</tbody>
</table>

Note: NS = No significance between treatments
Different letters connote significant difference between treatments

CONCLUSIONS:

Reduced seed production (e.g. sterility) occurs with increased
dose of the exo-isomer of C-16,17 dihydro GA5.

In fact, in one test (# C, Leduc Barley), complete sterility (no
seed production occurred).
EXAMPLE 13 - INHIBITION OF FLOWERING; PROSTRATE GROWTH

Dihydro GA5 (exo- form) was sprayed 5 times to each of:

- oat (domestic)
- wild oat (weed)
- barley

When sprayed at 100 ppm dihydro GA5 gave reduced height (Figs. 15-17), but normal or near-normal seed set (Fig 18).

When sprayed at 350 ppm gave no flowering at all (plants were dissected, no floral spicules were apparent).

In essence, 350 ppm exo- dihydro GA5 yield prostrate plants that gave an appearance of lawn grass.

Not only was seed set/production prevented, but flowering was prevented (presumably due to absence of endogenous "effector gibberellin" caused by the dihydro GA5 blocking the biosynthetic 3β-hydroxylation "activating" step (e.g. blocking GA20 ---->GA1 or GA20---G A5---GA3).
EXAMPLE 14 - HERBICIDAL EFFECT - WILD OATS

Plant growth (wild oat plant growth) biomass data from the Summer 1991 trial (see Photographs 1 - 5) noted below provides additional evidence of a "herbicial type of effect" of exoC-16,17 dihydro GA5 when applied to wild oat:

<table>
<thead>
<tr>
<th>Wild Oat</th>
<th>Control</th>
<th>100 ppm</th>
<th>300 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straw Weight/plant</td>
<td>7.87 g</td>
<td>5.39 g</td>
<td>6.37 g</td>
</tr>
<tr>
<td>Straw Weight/3 square meters)</td>
<td>2259 g</td>
<td>1192 g</td>
<td>1166 g</td>
</tr>
<tr>
<td>No. Plants/plot (Surviving plants)</td>
<td>287</td>
<td>221</td>
<td>183</td>
</tr>
</tbody>
</table>

The above results demonstrate:

A. a reduction in surviving wild oat plants within the treated plots for a single spray of exo- dihydro GA5, 300 ppm being more efficacious than 100 ppm.

B. a reduction in biomass of wild oat straw (e.g. everything except the seed) by 68% of control for 100 ppm, and 81% of control for 300 ppm per plant.

C. a reduction in biomass per unit area (e.g. per acre, per hectare) to ca. 50% of control, 100 and 300 ppm being about equal
The above effects on wild oat are very much more pronounced than on durum wheat, for which the results are given below:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>100 ppm</th>
<th>300 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straw Weight/plant</td>
<td>5.92 g</td>
<td>4.69 g</td>
<td>4.68 g</td>
</tr>
<tr>
<td>Straw Weight/plot</td>
<td>870 g</td>
<td>737 g</td>
<td>660 g</td>
</tr>
<tr>
<td>No. Plants/plot</td>
<td>147</td>
<td>157</td>
<td>141</td>
</tr>
</tbody>
</table>

(Surviving plants)

EXAMPLE 15 - AN EFFECT OF DIFFERENT DOSAGE ON RETARDING THE STEM ELONGATION OF BARLEY CV LEDUC


Barley grown in autumn 1991 in high intensity light supplemented heated glasshouses. Treated plants received:

exo- isomer of C-16,17 dihydro QA5 at:

0 (Control) -- 0 ppm

1.0 x 10^{-4} (minus 4) molar spray -- 35 ppm

3.3 x 10^{-4} (minus 4) molar spray -- 116 ppm

1.0 x 10^{-3} (minus 3) molar spray -- 350 ppm

applied initially to the barley plants at the three-to-four leaf stage.
In some experiments only one application was given, in other experiments 3 applications were given, the 2nd application being applied one week after the initial application, the 3rd application being applied two weeks after the initial application.

The results are shown in the accompanying Figs 19 to 26.

Fig 19 shows the response to a single spray application (Leduc x 1), Fig 21 showing the response to 3 spray applications.

For extended height (mm) especially, dose response differences are apparent for both the single spray (Leduc x 1) and especially for the 3X spray (Leduc x 3).

Similarly to the top of the auricle (the leaf which shelters the grain head) the 3 times application (Leduc x 3) shows a very marked (and significant) difference with differing dose, 116 ppm and 350 ppm, especially being quite growth retardive.

Thus, the figures show:

- a dose response from 0 to 350 ppm (higher dose is most growth retardive)
- a frequency response (higher frequency is most growth retardive).
Table 1. Plant height and internode lengths following applications of ethephon and EDHGA5* separately and together to greenhouse grown Bonanza barley

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
<th>Internode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n+</td>
<td>Height</td>
<td>Peduncle</td>
<td>p-1</td>
<td>p-2</td>
<td>p-3</td>
<td>p-4</td>
<td>n</td>
<td>p-5</td>
<td>n</td>
<td>p-6</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>100a</td>
<td>28.3a</td>
<td>17.0a</td>
<td>12.3ab</td>
<td>11.1b</td>
<td>7.4c</td>
<td>21</td>
<td>5.3a</td>
<td>8</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Activator</td>
<td>23</td>
<td>101a</td>
<td>24.8b</td>
<td>17.7a</td>
<td>13.2a</td>
<td>12.2a</td>
<td>8.7a</td>
<td>22</td>
<td>6.0a</td>
<td>8</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Ethephon</td>
<td>22</td>
<td>67b</td>
<td>9.5d</td>
<td>6.7bc</td>
<td>11.1bc</td>
<td>11.9ab</td>
<td>8.4ab</td>
<td>22</td>
<td>5.1ab</td>
<td>6</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Ethephon + Activator</td>
<td>24</td>
<td>65b</td>
<td>10.0d</td>
<td>6.8bc</td>
<td>9.9c</td>
<td>11.1bc</td>
<td>8.6a</td>
<td>24</td>
<td>5.1ab</td>
<td>13</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>EDHGA5 (weekly)</td>
<td>26</td>
<td>57c</td>
<td>16.3c</td>
<td>7.7b</td>
<td>4.8d</td>
<td>3.5d</td>
<td>2.8d</td>
<td>26</td>
<td>2.1c</td>
<td>7</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>EDHGA5 (weekly)+ Ethephon</td>
<td>23</td>
<td>45d</td>
<td>8.6d</td>
<td>5.5c</td>
<td>4.6d</td>
<td>3.8d</td>
<td>3.0d</td>
<td>23</td>
<td>2.1c</td>
<td>2</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>EDHGA5 (once)+ Ethephon</td>
<td>21</td>
<td>67b</td>
<td>10.2d</td>
<td>7.8b</td>
<td>10.6c</td>
<td>10.6c</td>
<td>7.5bc</td>
<td>17</td>
<td>4.2b</td>
<td>4</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

| pvalue                   | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.4052   |
| LSD(0.05)                | 6      | 2.6    | 1.5    | 1.5    | 2.0    | 0.9    | 1.1    | n.s.     |

+n = the number of samples taken for each parameters to the right of this number.
EDHGA5 = exo-C-16,17-dihydro GA5
Table 2. Tillering and flowering following applications of ethephon and ADHGA5 separately and together to greenhouse grown Bonanza barley.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n*</th>
<th>Fertile No.</th>
<th>Sterile No.</th>
<th>DW g-plant¹</th>
<th>Total DW g-plant¹</th>
<th>Late DW g-plant¹</th>
<th>n</th>
<th>Maturity ZGS</th>
<th>Fertile No.</th>
<th>Sterile No.</th>
<th>DW g-plant¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>1.4a</td>
<td>0.7cd</td>
<td>1.6</td>
<td>1.6c</td>
<td>0.1</td>
<td>29</td>
<td>90ab</td>
<td>34ab</td>
<td>13cd</td>
<td>36.1</td>
</tr>
<tr>
<td>Activator</td>
<td>23</td>
<td>1.1a</td>
<td>0.5d</td>
<td>1.4</td>
<td>0.3c</td>
<td>0.0</td>
<td>25</td>
<td>91a</td>
<td>42a</td>
<td>11d</td>
<td>40.3</td>
</tr>
<tr>
<td>Ethephon</td>
<td>22</td>
<td>0.5b</td>
<td>0.9bcd</td>
<td>1.0</td>
<td>6.5a</td>
<td>1.3</td>
<td>12</td>
<td>91a</td>
<td>21bc</td>
<td>29b</td>
<td>10.6</td>
</tr>
<tr>
<td>Ethephon + Activator</td>
<td>24</td>
<td>0.5b</td>
<td>0.6cd</td>
<td>0.8</td>
<td>5.0b</td>
<td>1.3</td>
<td>13</td>
<td>91a</td>
<td>22b</td>
<td>25bc</td>
<td>11.4</td>
</tr>
<tr>
<td>EDHGA5 (weekly)</td>
<td>26</td>
<td>0.4b</td>
<td>1.2b</td>
<td>1.2</td>
<td>3.8b</td>
<td>0.5</td>
<td>11</td>
<td>87b</td>
<td>3cd</td>
<td>50a</td>
<td>5.2</td>
</tr>
<tr>
<td>EDHGA5 (weekly) + Ethephon</td>
<td>23</td>
<td>0.0d</td>
<td>2.8a</td>
<td>1.1</td>
<td>7.8a</td>
<td>0.8</td>
<td>1</td>
<td>91a</td>
<td>1d</td>
<td>57a</td>
<td>0.1</td>
</tr>
<tr>
<td>EDHGA5 (once) + Ethephon</td>
<td>21</td>
<td>0.5b</td>
<td>1.0bc</td>
<td>1.1</td>
<td>7.1a</td>
<td>1.2</td>
<td>13</td>
<td>91a</td>
<td>24b</td>
<td>29b</td>
<td>11.1</td>
</tr>
</tbody>
</table>

| p value                          | 0.0001 | 0.0001 | 0.0001 | 0.0013 | 0.0001 | 0.0001 |
| LSD(0.05)                        | 0.3     | 0.4    | 1.4    | 3      | 18     | 14     |

n = the number of samples taken for each parameter to the right of this number.
EDHGA5 = exo-C-16,17-dihydro GA5
EXAMPLE 16 - MECHANISM OF ACTION OF C-16,17 DIHYDRO GA5 IN RETARDING
THE GROWTH OF HIGHER PLANTS

Stable isotope-labeled $[^2\text{H}2]$ gibberellin A20 was applied to
dwarf rice cv. Tan-ginbozu (100 ng/plant) in the presence and absence
of C-16,17 dihydro GA5 (also applied at 100 ng/plant).

The gibberellins noted above were applied in microdrops to the
shoot of the young rice plant, and 72 hours later the plant shoots
were harvested, and extracted for analysis of $[^2\text{H}2]$ GA1 levels by
gas chromatography-mass spectrometry. Stable isotope-labeled $[^2\text{H}2]$
GA1 was added as an internal standard in order to quantify the levels
of $[^2\text{H}]$GA1, which was the major metabolite of the applied
$[^2\text{H}]$GA20.

Results

Table I. Height to the Second Leaf Sheath, in mm, of the Rice
Plant in Response to Application of Deuterated GA20, or deuterated
GA20 + C-16,17 dihydro GA5,

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf Sheath Height (Length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. $[^2\text{H}2]$ gibberellin A20 applied (100 ng/plant)</td>
<td>25.25 mm +/- 0.98 (*/- is P=0.01 confidence range)</td>
</tr>
<tr>
<td>B. As above in A., but with C-16,17 dihydro GA5 (100</td>
<td>18.50 mm +/- 0.76 (*/- is P=0.01 confidence range)</td>
</tr>
</tbody>
</table>
Table II. Actual Growth of the Rice Plant in Response to Application of Deuterated GA20, or Deuterated GA20 + C-16,17 dihydro GA5, Relative to Control (No GA Applied) Rice Plants, in mm and as a Percentage of Growth (Delta Growth above Control Growth).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Response Rel. to Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. [²H₂] gibberellin A20 applied (100 ng/plant)</td>
<td>16.45 mm of growth above control = 100%</td>
</tr>
<tr>
<td>B. As above in A., but with C-16,17 dihydro GA5 (100 ng per plant)</td>
<td>9.70 mm of growth above control = 41% Growth Reduction</td>
</tr>
</tbody>
</table>

Table III. Amount of [²H₂]GA1 present in the Rice Plant When Harvested at Hour 72 After Application to the of the Rice Plant of 100 ng of Deuterated GA20, or of 100 ng of Deuterated GA20 + 100 ng of C-16,17 dihydro GA5.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Picograms of [²H₂] GA1 Present per Rice Plant at Hour 72 After Application of [²H₂]GA20</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. [²H₂] gibberellin A20 applied (100 ng/p[plant])</td>
<td>3.40 pg/rice plant = 100%</td>
</tr>
<tr>
<td>B. As in A., above, but with C-16,17 dihydro GA5 (100 ng per plant)</td>
<td>2.15 pg/rice plant = 37% Reduction in [²H₂]GA1</td>
</tr>
</tbody>
</table>
Thus, application of 100 ng of C-16,17 dihydro GA5 significantly reduced the growth that should have been effected by application of 100 ng of $[^2\text{H}_2] \text{GA20}$. This retarding effect constituted a 41% reduction in height growth (delta height).

Further, assessment of the level of "effector" gibberellin A1 (e.g. $[^2\text{H}_2] \text{GA1}$) that was extractable from the rice plant 72 hrs after application of $[^2\text{H}_2] \text{GA20}$ showed that the deuterated GA1 levels were reduced by 37%, relative to plants to which only the deuterated GA20 had been applied.

CONCLUSIONS:

The proportion by which shoot growth was reduced by application of C-16,17 dihydro GA5 (41% reduction) is almost the same as the proportion by which extractable levels of deuterated GA1 have been reduced (37%).

This evidence, together with evidence shown in Example 4 (see earlier) is indicative that the mechanism of action of C-16,17 dihydro Gaa5 in retarding shoot growth, at least, is due to a partial blockage of the $3\beta$-hydroxylation step (e.g. GA20 $\longrightarrow \longrightarrow$ GA1 in the example given for rice) by application of C-16,17 dihydro GA5.
It is also reasonable to conclude that growth retardation, and possibly many other desirable effects brought about by application of C-16,17 dihydro GA\textsubscript{5} and other C-16,17 dihydro gibberellins has the same mechanism of action, most notably inhibition of 3β-hydroxylation.

Many higher plant species utilize gibberellin A\textsubscript{1} (GA\textsubscript{1}) or GA\textsubscript{3} as "effectors" of shoot growth. A biosynthetic precursor of GA\textsubscript{1} or GA\textsubscript{3} is gibberellin A\textsubscript{10}. Gibberellin A\textsubscript{20} is thus metabolized to GA\textsubscript{1} by 3β-hydroxylation, and to GA\textsubscript{3} via GA\textsubscript{5}, this time by 3β-hydroxylation of GA\textsubscript{5}.

The evidence of this example is that a dwarf rice plant which has been induced to grow by application of GA\textsubscript{20} can have this growth significantly reduced by simultaneous application of C-16,17 dihydro GA\textsubscript{5}, and that the mechanism of this growth retardation most likely involves an inhibition of the 3β-hydroxylation of GA\textsubscript{20} \( \rightarrow \) GA\textsubscript{1}, the latter gibberellin being the "effector" of shoot elongation.
EXAMPLE 17 - IMPROVEMENT OF FRUIT QUALITY - CHERRIES

Five levels of C-16,17 dihydro GA5 (0, 3.3, 10, 33.3 and 100 ppm) were compared with a similar range of gibberellin A3 levels. Application was made by spraying in aqueous solution (+ surfactant) to drip-off to fruitbearing branch units in late June, 1991.

RESULTS

I. Higher levels of GA3 significantly promoted shoot growth, whereas no level of C-16,17 dihydro GA5 had this undesired effect (see below).

II. Fruit weight at harvest was increased by both GAs, although most effectively by GA3 (data not shown).

III. Fruit colouring was delayed by both GAs, but most effectively by high levels of GA3 (data not shown).

IV. Fruit firmness was improved, and post harvest "pitting" (a physiological disorder) was reduced by both GAs, although most effectively by GA3 (see Tables below).
Table I. Effects of C-16,17 dihydro GA5 and GA3 on Shoot Elongation of Cherry Branches

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Branches</th>
<th>Shoot Elongation in cm (values with the same letters do not differ significantly at P= 0.05)</th>
<th>Mean Branch Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibberellin A3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21</td>
<td>0.690 B,C</td>
<td></td>
</tr>
<tr>
<td>3.33 ppm</td>
<td>22</td>
<td>4.091 B</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>17</td>
<td>0.412 C</td>
<td></td>
</tr>
<tr>
<td>33.3 ppm</td>
<td>23</td>
<td>4.065 B</td>
<td></td>
</tr>
<tr>
<td>100 ppm</td>
<td>22</td>
<td>8.773 A</td>
<td></td>
</tr>
<tr>
<td>C-16,17 dihydro GA5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21</td>
<td>0.690 B,C</td>
<td></td>
</tr>
<tr>
<td>3.33 ppm</td>
<td>17</td>
<td>0.053 D,C</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>22</td>
<td>2.175 B,C</td>
<td></td>
</tr>
<tr>
<td>33.3 ppm</td>
<td>10</td>
<td>0.580 C</td>
<td></td>
</tr>
<tr>
<td>100 ppm</td>
<td>22</td>
<td>0.977 B,C</td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSIONS:

These results with C-16,17 dihydro GA5, although preliminary, are important because they indicate that it may be possible to separate (by using C-16,17 dihydro GAs) the shoot growth promotion that has traditionally been found to occur after GA3 application, from the more desirable effects of increased fruit weight, delaying of fruit colouring, enhanced fruit firmness, and a reduction in fruit pitting that can be brought about by the use of C-16,17 dihydro GA5.

Thus, only 100 ppm GA3 significantly promoted shoot growth, although 33.3 ppm and 3.33 ppm GA3 sprays tended to do so, as did 10 ppm C-16,17 dihydro GA5.
Table II. Effects of C-16,17 dihydro GA5 and GA3 Sprays on Quality (Average Fruit Firmness) of Lambert Cherry Fruit

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Fruit</th>
<th>Mean Measure of Fruit Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibberellin A3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>154</td>
<td>70.7143 G</td>
</tr>
<tr>
<td>3.33 ppm</td>
<td>196</td>
<td>76.2270 D</td>
</tr>
<tr>
<td>10 ppm</td>
<td>208</td>
<td>77.9399 C</td>
</tr>
<tr>
<td>33.3 ppm</td>
<td>172</td>
<td>81.6221 A</td>
</tr>
<tr>
<td>100 ppm</td>
<td>223</td>
<td>79.8969 B</td>
</tr>
<tr>
<td>C-16,17 dihydro GA5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>154</td>
<td>70.7143 G</td>
</tr>
<tr>
<td>3.33 ppm</td>
<td>222</td>
<td>73.7950 F</td>
</tr>
<tr>
<td>10 ppm</td>
<td>153</td>
<td>75.2941 E</td>
</tr>
<tr>
<td>33.3 ppm</td>
<td>158</td>
<td>74.1020 F</td>
</tr>
<tr>
<td>100 ppm</td>
<td>172</td>
<td>74.5827 E,F</td>
</tr>
</tbody>
</table>

Although GA3 was most effective in increasing fruit firmness, C-16,17 dihydro GA5 at all levels was significantly better than Controls (0 levels), thereby indicating that a higher dosage may hold promise of even greater fruit firmness, but w/o causing an increase in shoot elongation.
Table III. Effects of C-16,17 dihydro GA5 and GA3 Sprays on Quality (Average Level of Pitting after a Period of Cold Storage) of Lambert Cherry Fruit

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Fruit</th>
<th>Mean Measure of Fruit Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gibberellin A3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>154</td>
<td>0.977727 A</td>
</tr>
<tr>
<td>3.33 ppm</td>
<td>196</td>
<td>0.75266 D</td>
</tr>
<tr>
<td>10 ppm</td>
<td>208</td>
<td>0.68269 D</td>
</tr>
<tr>
<td>33.3 ppm</td>
<td>172</td>
<td>0.44767 E</td>
</tr>
<tr>
<td>100 ppm</td>
<td>223</td>
<td>0.45516 E</td>
</tr>
<tr>
<td><strong>C-16,17 dihydro GA5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>154</td>
<td>0.97727 A</td>
</tr>
<tr>
<td>3.33 ppm</td>
<td>222</td>
<td>0.88964 B,C</td>
</tr>
<tr>
<td>10 ppm</td>
<td>153</td>
<td>0.85621 C</td>
</tr>
<tr>
<td>33.3 ppm</td>
<td>158</td>
<td>0.94558 A,B</td>
</tr>
<tr>
<td>100 ppm</td>
<td>172</td>
<td>0.86047 C</td>
</tr>
</tbody>
</table>

*Scale = 0-3 where 0 = none, 1 = slight, 2 = moderate, 3 = severe.

Although GA3 was most effective in decreasing fruit pitting, C-16,17 dihydro GA5 at three levels was significantly better than Controls (0 level), thereby indicating that a higher dosage may hold promise of yielding a greater reduction in fruit pitting, but w/o causing an increase in shoot elongation.
CLAIMS

1. A method for promoting a desired tissue morphology and/or physiological state in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to produce an at least partial inhibition of the formation of effector gibberellins in said plant.

2. A method for promoting a desired tissue morphology and/or physiological state in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to produce an at least partial inhibition of gibberellin 3β-hydroxylase activity in said plant.

3. A method according to Claim 1 or Claim 2 wherein said desired tissue morphology or physiological state is selected from at least one of

(i) dwarfing

(ii) stem and shoot and/or root (radicle) growth retardation

(iii) flowering

(iv) improved fruit quality

(v) inhibiting fruit ripening

(vi) improving fruit set

(vii) controlling weed growth

(viii) inducing male sterility

(ix) retarded bud break

(x) tillering
4. A method for promoting flowering in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to promote flowering.

5. A method according to Claim 4 wherein flowering is promoted (a) with a simultaneous retardation of stem or shoot elongation or (b) with a nil or negligible effect on stem or shoot elongation.

6. A method for retarding stem or shoot growth in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to promote flowering.

7. A method for improving fruit quality in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

8. A method for inhibiting ripening of fruit of a higher plant plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

9. A method for improving fruit set in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.
10. A method of controlling growth of weeds in an area of land which comprises applying to said land area a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

11. A method for inducing male sterility in a higher plant, which comprises applying to the plant an effective amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

12. A method for retarding bud break in a higher plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor effective to promote flowering.

13. A method for inhibiting formation of effector gibberellins in a plant, which comprises applying to the plant an amount of a C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor.

14. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor has a formula selected from formulae Ia, Ib, Ic, Id and Ie.
wherein A, B, C, D, E and F independently represent hydrogen atoms or hydroxyl groups and the dotted line represents one optional double bond either between the carbon atoms in positions 1 and 2 or between the carbon atoms in positions 2 and 3.
15. A method according to any preceding claim wherein the gibberellin precursor is selected from C-16,17-dihydro steviol, C-16,17-dihydro ent-kaurene, C-16,17-dihydro ent-kaurenoic acid, C-16,17-dihydro-17-hydroxy kaurenoic acids, C-16,17-dihydro-16,17-dihydroxy kaurenoic acids and C-16,17-dihydro kaurenoic acids.

16. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor is C-2,3 dehydro, or C-1,2 dehydro.

17. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor has a hydroxy substituent in the 17-position.

18. A method according to any of Claims 1 to 16 wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor has a hydroxy substituent in both the 16- and the 17-positions.

19. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor has the hydroxylation pattern and stereochemical structure of GA5.

20. A method according to Claim 14 wherein said C-16,17-dihydro gibberellin is selected from C-16,17-dihydro gibberellin A₃ and C-16,17-dihydro gibberellin A₅.
21. A method according to any preceding claim wherein said gibberellin is selected from:

C-16,17-dihydro GA₃;
C-16,17-dihydro GA₂₀;
C-16,17-dihydro,2,3 dihydro GA₉;
C-16,17-dihydro,1,2 dihydro GA₉;
C-16,17-dihydro GA₁₂;
C-16,17-dihydro GA₁₅;
C-16,17-dihydro GA₅₃;
the C-2,3 dehydro derivative of C-16,17-dihydro GA₁₂;
the C-2,3 dehydro derivative of C-16,17-dihydro GA₁₅;
the C-2,3 dehydro derivative of C-16,17-dihydro GA₅₃;
the C-1,2-dehydro derivative of C-16,17-dihydro GA₂₀;
the C-1,2-dehydro derivative of C-16,17-dihydro GA₁₂;
the C-1,2-dehydro derivative of C-16,17-dihydro GA₁₅; and
the C-1,2-dehydro derivative of C-16,17-dihydro GA₅₃.

22. A method according to claim 20 wherein said gibberellin is C-16,17-dihydro GA₃.

23. A method according to any of claims 1 to 22 wherein said C-16,17-dihydro gibberellin or gibberellin precursor is applied in the form of a free acid or salts or esters thereof.
24. A method according to claim 23 wherein said salts and esters are selected from sodium and potassium salts and the \( \text{C}_{1-4} \) carboxylic acid esters.

25. A method according to any preceding claim wherein said C-16,17-dihydro gibberellin or gibberellin precursor is applied together with another plant growth regulators.

26. A method according to any preceding claim wherein said C-16,17-dihydro gibberellin or gibberellin precursor is applied by spraying a solution or suspension thereof to whole plants, or by seed application, together with a suitable carrier.

27. A method according to any preceding claim wherein said C-16,17-dihydro gibberellin or gibberellin precursor is applied at a rate of from 2 to 100 micrograms per gram fresh weight of actively growing plant tissue.

28. A method according to any preceding claim wherein said C-16,17-dihydro gibberellin or gibberellin precursor is applied at a concentrations of from 2-600 ppm, preferably from 5-450 ppm.

29. A method according to any preceding claim wherein the plant species is a floriculturally, agronomically, or horticulturally useful monocot or dicot, or gymnosperm, including woody ornamental or fruiting shrubs, or woody ornamental or fruiting trees, or a conifer of the Gymnospermae Order.
30. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor is applied prior to natural floral initiation.

31. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor is applied after natural floral initiation, but during early stages of floral differentiation.

32. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor is applied during early stages of floral development.

33. A method according to any preceding claim wherein the C-16,17-dihydro gibberellin or C-16,17-dihydro gibberellin precursor is applied to dry, imbibed or imbibing seeds so as to overcome a natural requirement for low temperature, particularly to obviate vernalization).

34. A method according to any preceding claim wherein the plant species is a member of the Gramineae.

35. A method according to claim 34 wherein the plant species is wheat or barley.

36. A method according to any of Claims 1 to 33 wherein the plant species is oilseed rape.
37. A method according to any of Claims 1 to 34 wherein the plant species is a member of the Araceae.

38. A method according to any of Claims 1 to 31 wherein the plant species is any monocotyledenous or dicotyledenous plant with a natural requirement for cold to promote flowering.

39. A method according to any preceding claim wherein the gibberellin is applied as a seed soak in an appropriate aqueous or organic solution yielding an uptake by the seed of from 1 picogram to 10 micrograms per seed.

40. A method according to any preceding claim wherein the gibberellin is applied at a rate of 10 nanograms to 100 milligrams per plant for herbaceous plants (the effective dose depending on species and size of plant), 10 micrograms to 500 milligrams per plant for shrubs, and at a rate of 1 milligram to 20 grams per plant for trees.

41. A method according to any preceding claim wherein the gibberellin is at a concentration up to 5000 ppm.

42. A method according to any preceding claim wherein the gibberellin is at a concentration of from 1 to 3000 ppm.

43. A method according to any preceding claim wherein the gibberellin is at a concentration in the range from 1-1000 ppm.
44. A method according to any preceding claim wherein the gibberellin is applied at a concentration in the range from 5-1000 ppm as a foliar spray and/or as a soil drench.

45. A method according to any preceding claim wherein the gibberellin is applied at a concentration in the range from 5-350 ppm as a foliar spray and/or as a soil drench.

46. A method according to any of Claims 1 to 43 wherein the gibberellin is applied as a seed soak at a concentration in the range of $10^{-12}$ to $1.5 \times 10^{-2}$ molar.

47. A method according to any of Claims 1 to 43 wherein the gibberellin is applied as a seed soak at a concentration in the range of $10^{-12}$ to $10^{-7}$ molar.

48. A method according to any preceding claim wherein the gibberellin is applied at a rate of up to 1000 micrograms/gram fresh weight.

49. A method according to any preceding claim wherein the gibberellin is applied at a rate of from 1 to 1000 micrograms/gram fresh weight.

50. A method according to any preceding claim wherein the gibberellin is applied at a rate of from 2 to 1000 micrograms/gram fresh weight.
51. A method according to any preceding claim wherein the gibberellin is applied at a rate of from 2 to 500 micrograms/gram fresh weight.

52. A method according to any preceding claim wherein the gibberellin is applied at a rate of from 2 to 333 micrograms/gram fresh weight.

53. A method according to any preceding claim wherein the gibberellin is applied at a rate of from 2 to 100 micrograms/gram fresh weight.

54. A composition comprising an effective amount of C-16,17-dihydro GA5 together with an agriculturally or horticulturally acceptable diluent or carrier.

55. A composition according to Claim 54 said composition being substantially free of gibberellins which are C-16,17-dehydro.

56. A composition according to Claim 54 or Claim 55 wherein said gibberellins or gibberellin precursors and/or concentrations are as defined in any of Claims 14 to 24 and 39 to 53.
Fig. 1

- □ = DIHYDRO GA5 (ENDO)
- × = SD/LD CONTROL
- ○ = GA3
- △ = GA5

SHORT DAY (NON-INDUCTIVE)

FLOWERING SCORE

FLOWERING SCORE

ENDO DIHYDRO GA5

GA5 OR GA3

ENDO DIHYDRO GA5

GA5

GA3

CONTROL (SHORT DAY ONLY)

CONTROL - 1 LONG DAY ONLY

STEM LENGTH (mm)

STEM LENGTH (mm)
**Fig. 2**

**Legend**

- **A**
- **B**
- **C**
- **D**
- **F**

**Xanthium-a Short-Day Requiring Plant**

- Stage 6.33
  - 16h Dark Controls

- Stage 5.33

- **ENDO-16,17-Dihydro, 15βOH GA$_5$**

- Stages 1.0 to 3.0, 10h Dark Controls, Variations Depending on Each Experiment

- **16,17 Dihydro GA$_5$ + GA$_3$**
  - At 10µg

- **GA$_5$**

- **GA$_3$ Alone, at 10µg**

- **GA$_3$ Alone at 33µg**
Fig. 5

- ◆ + 1 ng EXO-16,17-DIHYDRO GA5
- ◇ + 1 ng ENDO-16,17-DIHYDRO GA5
- ▲ + 10 ng EXO-16,17-DIHYDRO GA5
- △ + 10 ng ENDO-16,17-DIHYDRO GA5

2ND LEAF SHEATH LENGTH (mm)

GA DOSE PER PLANT IN µg OR (ng)

APRIL 15/91
Fig. 7A

- 10 ng EXO GA$_5$
- 10 ng ENDO GA$_5$
- 100 ng EXO GA$_5$
- 100 ng ENDO GA$_5$

2ND LEAF SHEATH LENGTH (mm)

GA DOSE PER PLANT IN µg OR (ng) MAY 10/91

- GA$_{20}$ ALONE
- ENDO-16,17-DIHYDRO GA$_5$
- EXO-16,17-DIHYDRO GA$_5$
CONCLUSION: 2,3 DEHYDRO, 16,17-DIHYDRO GA_9 ANTAGONIZES GA_9-PROMOTED GROWTH (FIG.10), BUT NOT AS EFFECTIVELY AS DOES 16,17-DIHYDRO GA_5 (FIGS.8&9)

\[
\begin{align*}
\circ &= 2,3\text{-DEHYDRO}, 16,17\text{-DIHYDRO GA}_9 \\
\bullet &= GA_9 \\
\triangledown &= GA_9(10^{-2})+a \\
\triangle &= GA_9(10^{-3})+a \\
\end{align*}
\]

GA_9 AT VARIOUS DOSES

(2,3-DEHYDRO, 16,17-DIHYDRO GA_9 ALONE

(0.01\mu g GA_9, + 2,3-DEHYDRO,
16,17-DIHYDRO GA_9 AT 1.0\mu g
OR AT 0.1\mu g

(0.001\mu g GA_9 + 2,3-DEHYDRO,
DIHYDRO GA_9 AT 0.1\mu g PER PLANT
OR AT 0.01 \mu g PER PLANT

a = 2,3-DEHYDRO, 16,17-DIHYDRO GA_9

Fig.8
Fig. 11

GROWTH OF CONES OF VITIS VINIFERA CV CHARDONNAY

CHARDONNAY CANE ELONGATION (NORMALISED FOR PRE-EXISTING LENGTH)

NEW INTERNODE GROWTH

0.33 μg

CONTROL

0 μg

1.0 μg (NB)

33 μg

10 μg

3.3 μg

0 μg

CONTROL

1.0 μg (NB)

3.3 μg

10 μg

33 μg

NB 1.0 μg TREATMENT HAD 3 TERMINAL BUDS "ARRESTED" OR DEAD

THE CIRCLED NUMBERS INDICATE THE NUMBERS OF INTERNODES

NEW INTERNODE

3.7 4.3 4.7 3.2 5 5

PRE EXIST INTERNODES

6.2 6.7 6.7 6.7 6.8 6.7
Fig. 13
WESTAR CANOLA (13 HR. DAY)

APEX HEIGHT (cm) INCREASE

DAYS POST GA APPLICATION
Fig. 14

ERROR BARS REPRESENT 95% CONFIDENCE LEVEL

HEIGHT TO Apex (cm)
18 DAYS POST APPLICATION

DURUM WHEAT WILD OATS
CONTROL 100 PPM 300 PPM CONTROL 100 PPM 300 PPM

+ EXO-16,17-DIHYDRO GIBBERELLIN A5

SUBSTITUTE SHEET
Fig. 15

HORDLUM VULGARIS (BARLEY)

PLANT HEIGHT (cm)

DAYS OF APPLICATION EXO-DIHYDRO GA5

CONTROL

100 PPM

330 PPM

DAYS POSTEmergence

21 28 35 42 49 56 63 70

SUBSTITUTE SHEET
Fig. 16

AVENA SAVASTI (COMMON OAT)

DAYS OF APPLICATION
EXO-DIHYDRO GA5

100 PPM
330 PPM

PLANT HEIGHT (cm)

DAYS POST EMERGENCE

0 20 40 60 80 100 120

21 28 35 42 49 56 63 70

CONTROL

SUBSTITUTE SHEET
Fig. 17

AVENA FATUA (WILD OAT)

PLANT HEIGHT (cm)

DAYS OF APPLICATION EXO-DIHYDRO GA5

CONTROL

100 PPM

330 PPM

DAYS POST EMERGENCE

21 28 35 42 49 56 63 70

SUBSTITUTE SHEET
Fig 18

EXO - DIHYDRO GA5

# OF SEEDS PER PLANT

WILD OAT

100 PPM

330 PPM

CONTROL

BARLEY

100 PPM

330 PPM

CONTROL

TAME OATS

100 PPM

330 PPM

COMMON OAT

SUBSTITUTE SHEET
Fig. 21

21/28
3 × SPRAY OF EXO-DIHYDRO GA₅
BARLEY, LEDUC × 3

TOP AURICLE HEIGHT (mm)

0 2 4 6 8 10
0 50 100 150 200 250 300 350 400 450 500

CHECK
10-4
3.3 × 10-4
10-3

Fig. 22

EXTENDED HEIGHT (mm)

0 2 4 6 8 10
0 100 150 200 250 300 350 400 450 500 550 600

CHECK
10-4
3.3 × 10-4
10-3
Fig. 23  SINGLE SPRAY OF EXO-DIHYDRO A5  
BARLEY HEARTLAND

Fig. 24

[Graphs showing changes in top auricle height and extended height over weeks after treatment, with different treatment levels indicated.]
Fig. 25  SINGLE SPRAY OF EXO-DIHYDRO GA5
BARLEY, JACKSON

Fig. 26
LEFT TO RIGHT

CONTROL WHEAT
CONTROL OATS
300 ppm C-16,17-DIHYDRO GA$_5$ WHEAT
300 ppm C-16,17 DIHYDRO GA$_5$ OATS

PHOTOGRAPH 1
FOREGROUND - 300 ppm C-16,17-DIHYDRO GA₅
BACKGROUND - CONTROL

PHOTOGRAPH 2
LEFT - CONTROL OATS
RIGHT - 300 ppm C-16,17 - DIHYDRO GA₅ OATS

PHOTOGRAPH 3
LEFT - 300 ppm C-16,17-DIHYDRO GA₅
RIGHT - CONTROL

PHOTOGRAPH 4
LEFT - CONTROL WILD OATS
RIGHT - 300 ppm C-16,17-DIHYDRO GA_5 WILD OATS

PHOTOGRAPH 5
A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl. A01N 45/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A01N 45/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
DERWENT DATABASE: FILE CASA, WPAT: KEYWORDS: GIBBERELL; DIHYDROGIBBERELL; GA3, GA5, GA9, GA20, PRECURSOR, HYDROLASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>The Merck Index, Eleventh Edition (1989), Merck &amp; Co., Inc. No. 4314</td>
</tr>
<tr>
<td>D,A</td>
<td>Phytochemistry (1967) Vol 6, pp 1475-1499, Brian et al</td>
</tr>
</tbody>
</table>

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

Special categories of cited documents:

*A* document defining the general state of the art which is not considered to be of particular relevance to the claimed invention

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*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

*Q* document member of the same patent family

Date of the actual completion of the international search
26 November 1992 (26.11.92)

Date of mailing of the international search report
30 Nov 1992 (30.11.92)

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200
WODEN ACT 2606
AUSTRALIA

Authorized officer

Facsimile No. (06) 2853929

Telephone No. (06) 2832491